**A THESIS**

**FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

# Biological Characteristics of *Bradysia procera* and Insecticidal Activities of Constituents from *Syzygium aromaticum* Bud and *Illicium verum* Fruit against *B. procera*

# 인삼줄기버섯파리의 생물학적 특성 및 정향과 팔각회향 유래 화합물들의 살충활성

**By**

**Tae-kyun Hong**

**Major in Interdisciplinary Program in Agricultural Biotechnology**

**Department of Agricultural Biotechnology**

**The Graduate School of Seoul National University**

**Febrary, 2018**

**A THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

# Biological Characteristics of *Bradysia procera* and Insecticidal Activities of Constituents from *Syzygium aromaticum* Bud and *Illicium verum* Fruit against *B. procera*

# 인삼줄기버섯파리의 생물학적 특성 및 정향과 팔각회향 유래 화합물들의 살충활성

**UNDER THE DIRECTION OF ADVISER YOUNG-JOON AHN**

**SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL**

**OF SEOUL NATIONAL UNIVERSITY**

**BY**

**Tae-kyun Hong**

**Major in Interdisciplinary Program in Agricultural Biotechnology**

**Department of Agricultural Biotechnology**

**APPROVED AS A QUALIFIED THESIS OF TAE-KYUN HONG**

**FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

**BY THE COMMITTEE MEMBERS**

| | | |
|---|---|---|
| **Chairman** | **Dr. Si Hyeock Lee** | _____ |
| **Vice Chairman** | **Dr. Young-Joon Ahn** | _____ |
| **Member** | **Dr. Soon-Il Kim** | _____ |
| **Member** | **Dr. Il-Kwon Park** | _____ |
| **Member** | **Dr. Kwang Pum Lee** | _____ |

# Biological Characteristics of *Bradysia procera* and Insecticidal Activities of Constituents from *Syzygium aromaticum* Bud and *Illicium verum* Fruit against *B. procera*


## ABSTRACT

The fumdamental information of biology and the insecticidal activities of constituents identified in *Syzygium aromaticum* bud and *Illicium verum* fruit methanol extract and hydrodistillate against third instar larvae of *Bradysia procera* and its possible mode of action. In addition, phytotoxicity and control efficacy of *S. aromaticum* bud-derived materials are also examined.

The female of *B. procera* laid approximately 160 eggs and average egg period was 6 days, approxemately. An average weight of first to third instar larva were 1.3, 8.1 and 81.0 μg, respectively. An average length and width of first to third instar larvae were 608, 1,670, 4,330 and 140, 510, 792 μm. The male forth instar larva of the weight, length and width were 145 μg, 7,224 and 875 μm, and female were 166 μg, 8,081 and 1,017 μm, respectively. The larval periods of female and male were 16.1 and 15.3 days, respectively. The pupal weigh, length and width of male and female were 99 μg, 3,972, 1,109 μm and 106 μg, 4,681 and 1,175 μm, respectively. Adult male and female emerged from pupa on 6.7 and 7 days on after pupation, and their longevities are 5.3 and 6.8 days, respectively. Comparison of develpopmental characteristics and lifecycles of *B. procera* among ginseng and garlic stem, there wa no significant difference, whereas survival rate was significant higher on ginseng stem than on garlic stem supplied. Seasonal occurrences of *B. procera* adult population observed mainly

three peaks, mid- and late-June and August. The damage symptoms to ginseng were described.

The toxicity and mechanism of action of constituents from *S. aromaticum* bud and *I. verum* fruit against third-instar larvae and eggs of *B. procera*. Also, phytotoxicity, control efficacy and anti-oviposition of *S. aromaticum* bud methanol extract and hydrodistillate in ginseng fields were examined.

In a filter-paper mortality bioassay, methyl salicylate ($LC_{50}$, 5.26 μg/cm$^{-2}$) from *S. aromaticum* bud and estragole ($LC_{50}$, 4.68 μg/cm$^{-2}$) from *I. verum* fruit were the most toxic compound. Egg hatch of methyl salicylate and estragole were inhibited 97 % at 11.7 μg/cm$^{-2}$ and 95 % at 30 μg/cm$^{-2}$, respectively. These constituents were consistently more toxic in closed versus open containers, indicating that toxicity was achieved mainly through the action of vapor. The mechanism of larvicidal action of methyl salicylate, 2-nonanone, eugenol, and eugenyl acetate of from *S. aromaticum* bud constituents might be due to interference with the octopaminergic system. α-Pinene and α-Copaene might act on acetylcholinesterase. In addition, estragole from *I. verum* fruit was a potent acetylcholinesterase (AChE) inhibitor, and the cyclic AMP level induced by this compound was slightly lower than that induced by octopamine alone. This finding indicates that estragole might act on both the AChE and octopaminergic receptors. *trans*-Anethole was a weak AChE inhibitor.

In phytotoxicity test, *S. aromaticum* bud-derived materials observed any phytotoxic symptoms. In control efficacy test, bud hydrodistillate revealed 56 and 54 % control efficacy at 0.484 g/m$^{-2}$ on 7 and 14 DAT (day after treatment) in Yeoju area, respectively. In Icheon area, 61 and 52 % control efficacy at 0.484 g/m$^2$ on 7 and 14

DAT respectively. The anti-ovipostion effect of bud hydrodistillate observed 51 and 44 % at 0.484 $g/m^{-2}$ on 7 and 14 DAT, respectively. The anti-oviposition of hydrodistillate was similar or more effective to that of clothianidin, dichlorvos, emamectin benzoate and thiamethoxam but was lower than that of cypermethin.

In field evaluation of *S. aromaticum* bud-dervided materials and its insecticide mixtures of four insecticides against *B. procera*, almost of single treatments of bud-derived materials were observed significantly different of control efficacy and anti-oviposition effect compared with insectides mixtures. However, bud hydrodistillate + cypermethrin mixture showed 94 and 93 % control efficacy and 95 and 93 % anti-oviposition effect on 7 and 14 DAT, respectively.

Futher studies with possible applications of *S. aromaticum* bud and *I. verum* fruit derived products as potential larvicides and ovicides for the control of *B. procera* populations are warranted.

**Key words:** *Panax ginseng*, *Bradysia procera*, *Syzyzium aromaticum*, *Illicium verum*, Botanical insecticide, Mode of action.

**Student number:** 2009-30980

**TABLE OF CONTENTS**

# CHAPTER I

# The Biology of *Bradysia procera* (Diptera: Sciaridae) and its Seasonal Occurrence and Symptoms of Damage Caused in the Field

## 1.1. Rearing Method and Investigation of the Developmental Characteristics of *Bradysia procera* and its Life cycle in the Laboratory Condition

## 1.2. Distribution and Seasonal Occurrence Pattern of *Bradysia procera* and Its Damage Caused in Ginseng fields

# CHAPTER II

# Larvicidal and Ovicidal activities and possible mode of action of *Syzygium aromaticum* Bud and *Illicium verum* fruit derived Constituents to Ginseng fungus gnat, *Bradysia procera* (Diptera: Sciaridae)

## 2.1. Larvicidal and Ovicidal Activities and possible mode of actions of Constituents Identified in *Syzygium aromaticum* Bud and *Illicium verum* Fruit against *Bradysia procera*

## 2.2. Field Evaluation of *Syzygium aromaticum* bud derived Materials against *Bradysia procera* on *Panax ginseng* fields

# LIST OF TABLES

# LIST OF FIGURES

# INTRODUCTION

Ginsengs have been consuming for health foods and herbs for a long time and, nowadays, its consumption is rapidly increasing due to interest of wellness. Therefore, the cultivation area of ginsengs is also expanding, and it became an important source of income. In ginseng fields, insect pests have been reported fourteen species (Kim, 1994; Shin et al., 2008). Among these species, ginseng fungus gnat, *Bradysia procera* increasing damage on ginseng fields in South Korea, currently. Kim (1994) reported that ginseng was damaged by unidentified dipteral larvae, which tunnel into the ginseng stem. Shin et al. (2008) described that ginseng stem damage caused by *Bradysia procera* (formerly *Phytosciara procera*). The larvae feed on the peduncle and stem of ginseng even root and they may transport organism as soft rot, *Erwinia carotovora* subsp. *Carotovora* (shin et al., 2008). Especially, *B. procera* the most abundant insect pest, occurring throughout the growing period in ginseng fields.

For the control of *B. procera*, synthetic insecticides have been used for several decades. Clothianidin and thiamethoxam are used for the control of *B. procera* larvae. Cypermethrin and emamectin benzoate are the most routinely used adulticides. The synthectic insecticide for the control of *B. procera* is limited by adversal effects such as disrupting natural biological control systems (DeBach and Rosen, 1991), development of resistance (Ahmad et al., 2002; Kranthi et al., 2002; Nauen and Denholm, 2005), residue and environmental and human health concerns (Brown, 1978; Hayes and Laws, 1991). These problems have highlighted the need for the development of new strategies for selective control of insect pests.

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). Many of them are large

free from harmful adverse effects (Hedin, 1997), often biodegrade to nontoxic products, and have little or no harmful effects on nontarget organisms or the environment (Koul et al, 2008). Much more effort has, therefore, been focused on plant-derived materials as potential sources of commercial insect-control agents or as lead compounds (Jacobson and Crosby, 1971; Elliott, 1977; Arnason et al., 1989; Isamn, 1995; Hedin et al., 1997).

Inspite of impressive recent advances in extraction technology, separation techniques, and analytical and spectroscopic instrumentation, it has been eximated that only 5 to 15 % of the total 250,000 existing species of higher plants have been systematically surveyed for the presence of bioactive compounds (Balandrin et al., 1993). However, naturally occurring compounds potentially useful as new insect control agents have not been relatively looked over. In Korea, much concern has been focused on the bioactivity of oriental medicinal plants and/or native plants since late 1980's, on the basis that plant-derived substances served as the sources of insect-control agents. Screening program of Korea Research Institute for Chemical Technology for insect-control agents from synthetic or natural compounds has been well established (Ahn et al., 1995). Little work has been done to consider their potential to manage insect pests despite excellent pharmacological actions of plants (Tang and Eisenbrand, 1992; Namba, 1993).

This paper describes the fundamental information of ecology and biology of *Bradysia procera*. In addition, an assessment is made of the larvicidal and ovicidal activities and possible mode of action of constituents from *Syzygium aromaticum* bud and *Illicium verum* fruit methanolic extract and hydrodistillate agaist the larvae. Also, phytotoxicity, control efficacy and anti-oviposition effects of *Syzygium aromaticum* bud-derived-materials and its insecticides mixure with four chemical insecticides in ginseng fields were evaluated.

# LITERATURE REVIEW

### 1. Fungus gnat – *Bradysia* Spp.

Winnertz (1867) percieved Sciaridae as a distinct family and presented four genera as follow: *Trichosia*, *Cratyna*, *Corynoptera* and *Bradysia*. In 1912, developed the first extensive taxonomic key for North American species placing them in the Sciarinae (Johannsen, 1912), however, a subfamily of Mycetophillidae. The sciarids and mycetophilids were grouped together based on morphological features by taxonomists (Harris et al., 1996). After cytological analysis method is developed, In 1949, White divided the suborder Nematocera into four groups placing the families Sciaridae and Cecidomyiidae by themselves based on their highly anomalous chromosome cycles (White, 1949). In 1966, The Sciaridae amended by Steffan who placed several species, which had formerly been recognized as species Sciara, in the genus *Bradysia* (Steffean, 1966).

The Fungus gnat, genus of *Bradysia* was represented by sixty-five species in North America and is the largest genus in the family in North America and Europe (Steffan, 1966). Twenty and sixty-five genus of *Bradysia* was occurred (Johansen, 1912; Shaw and Fisher, 1952; Vilkalmaa 2014; Wheeler, 1971) in New York and Finland, respectively as well as Asia, Brazil, South Africa and Sweden (Hellqvist, 1994; Menzel et al., 2003; Hurley et al., 2010; Heller et al., 2015). It also is found and distributed worldwide.

The taxonomy of fungus gnats is commonly distinguished on the morphology of the adults. Steffan (1966) and Price et al., (1993) presents morphological characters of *Bradysia* as follow: the maxillary palpi are consist of three-segment, usually with

sensory pit, protibiae with preapical, ventral, unilateral comb separated from general tibial vestiture by triangular bare area, metatibiae with two subequal spurs, one pair of wing, posterior wing Y-shape veins bare and possessed long antennae and legs.

Male of *Bradysia* spp. generally emerge 1 day prior to females; an approximate 24 hour pre-oviposition period follows female emergence during which mating occurs. Adult are lived 3 day approximately, males usually living longer than females which die soon after oviposition (Steffan, 1966; Kennedy, 1976). Species of *Bradysia* are multivoltine, the number of eggs deposited per female by female-producing *B. impatiens* averages of 111, whereas male producing females deposit an average of 153 eggs (Carson 1945). Similarly, overall fecundity of female *B. impatiens* are reported as averaging 150 (Carson 1945), 142 (Kennedy 1974), and 100 eggs (Perondini et al., 1986), whereas Wilkinson and Daugherty (1970) observed an average of only 75 eggs per female. The average length and width of the oblong *B. impatiens* egg is 189 and 98 μm respectively (Carson, 1945). The egg stage is followed by four larval instars and an obtect pupa. The presence of a shiny black head capsule on an otherwise translucent or white vermiform body distinguishes fungus gnat larvae from other immature dipteran.

The study of the morphology of the first larval instar of *B. tritici* reveals sufficient cuticular detail to distinguish individual segments (Bischof et al., 1985). Twelve denticle belts observed on the ventral side of the larvae are assumed to demarcate segment borders. The three thoratic segments may be differentiated by specific denticle patterns within these belts. Dorsal and ventrolateral sensillae patterns in combination with mechanoreceptor positions in relation to the denticle belts allow further identification of individual segments.

Developmental times among *Bradysia* spp. has been reported. Development from

egg to adult in *B. impatiens* has been measured in three studies at three different temperatures. Steffan (1966) observed an average developmental time of 16.3 days at 20 ℃, Wilkinson and Daugherty (1970) 19.9 days at 23.9 °, and Kennedy (1974) 15 days for male and 16 days for females at 25 °. The variation of developmental time of *B. coprophilar* has been reported, 24 to 32 days at unknown temperature (Hungerford, 1916), 18 to 23 days at 18 ℃ (Thomas, 1931), and 27 to 33 days at 23 ℃ (Smith and Stocking, 1936). This variation may reflect differing geographic isolates, food source, temperature, photoperiod, or experimental method (Kennedy, 1971; Steffan, 1974; Wessel, 1989).

Kennedy (1976) described that fungus gnat adults as generally aphagous, however, they have been reported elsewhere as feed on nectar (Mercier, 1911), a sodium arsenate and molasses solution (Hungerford, 1916), and organic ooze (Steffan, 1966). Larvae primarily feed on fungi (Mercier, 1911; Thomas, 1931; Kennedy, 1974; Anas and Reeleder, 1988; Gardiner et al., 1990; Harris, 1995) and can be cannibalistic (Steffan, 1966; Wilkinson and Daugherty, 1970; Harris et al., 1995). Fungus gnat larvae are known to feed on animal excrement, decaying and living plant tissues, and fungal hypae and fruiting bodies, including cultivated mushrooms (Anas and Reeleder, 1987; Anas and Reeleder, 1988, Springer, 1995; Barraclough and Londt, 1996). In nurseries, the larvae feed on decaying and healthy plant roots, causing a reduction in plant vigor (Kennedy, 1974; Springer, 1995a; Springer, 1995b). Feeding wounds may provide infection sites for various pathogenic microorganisms (Springer 1995b). Leath and Newton (1969) reported that alfalfa plants injured by *Bradysia* spp. larvae prior to inoculation of *Fusarium* spp. had a greater mortality than uninjured plants. Similarly, feeding wounds of fungus gnat larvae on pine seedling may increased infection of *F.*

*circinatum* and other pine pathogens. In ginseng plant, fungus gnat larvae were mediated soft rot disease by *Erwinia carotobora* (Shin et al., 2008).

**2. Agricultural importance**

Fungus gnats occur in all area of the mushroom crops are grown, occur throughout the worldwide. Adults generally aphagous, however, the larvae feed on organic matter, fungi, algae and damage roots of cuttings or small plants by tunneling through stems. The larvae commonly infests mushroom, glasshouse ornamental, and forestry nurseries (Gouge and Hague, 1995; White et al., 2000; Shen et al., 2009**;** Hurley et al., 2010).

Larval fungus gnats feed and can directly damage mushroom crop. Species of fungus gnats, especially of the genera *Bradysia* and *Lycoriella*, are among the main pests of *Agaricus blazei* in Brazil (Eira, 2003; Menzel et al., 2003). Larvae of those insects cause yield losses up to 75 % (Eira 2003). Also, the larvae attack the roots of various field crop plants in addition to greenhouse-grown plants (Ellisor, 1934; Metcalf et al., 1962; Fawzi and Kelly, 1982). Soybeans damaged by larvae of *B. coprophila* reach maturity; however, these plants easily lodge and produce fewer seed than undamaged plants (Graham and McNeill, 1972). Hamlen and Mead (1979) considered 5 to 10 larvae per pot-grown plant to represent a moderate infestation; however, such thresholds should be adjusted by plant age as seedlings appear to be particularly susceptible to damage (Coquillett, 1985; Edwards and Williams, 1916). Lead and Newton (1969) reported 90 % of alfalfa seedlings killed at densities of less than one *Bradysia* sp. larva per seedling.

In Korea, observed twenty-one host plants (Lee et al., 2001) and identifed thirteen

fungus gnat *Bradysia* species; *B. agretis*, *B. alpicola*, *B. aprica*, *B. atracornea*, *B boitsovoensis*, *B. chlorocornea*, *B. difformis* (= *impatiens*), *B. hilariformis*, *B. longimentula*, *B. peraaffinis*, *B. procera*, *B. sachalinensis*, *B. trispinifera* (Park et al., 1999; Kim et al., 2000; Shin et al., 2012; Shin et al., 2013; Shin et al., 2015). Among the fungus gnats, especially *B. agretis* and *B. procera* causing damage to crops in raising seedling and ginseng field (Park et al., 1999; Kim et al., 2000; Shin et al., 2015) respectively.

## 3. Control measures and strategies

### 3.1. Cultural controls

Fungus gnats prefer to deposit their eggs in substrates that are moist and high in organic matter (Harris, 1993; Landis, 1996). Therefore, eliminating excess moisture is the most important control method. Harris (1993), van Tonder (1994) and Landis (1996) discussed various methods to control fungus gnats in forestry nurseries. Over-watering must be prevented and adequate drainage provided. Nurseries must be cleared of weeds, moss and algae regularly. Placing stone chip or ash under nursery tables will decrease the dampness and presence of weeds, moss and algae. Watering from the bottom of the pot can be useful but make sure not to allow water to puddle for long periods of time as this can lead to root rot. Hungerford (1916) reported that a layer of sand also can be added over the top of potting media to inhibit infestation by fungus gnats. Minimize or eliminating organic matter on the top surface of the plant to reduce breeding, mating and feeding sites. Ginseng peduncle topping methods can be potential cultural control methods. Among the five different topping methods evaluated, the cumulative fungus gnat damage to ginseng was low (0.8 %) under partial peduncle

topping and removal of only flower buds, with fungus gnat control effects of 82 % and 86 %, respectively, compared to conventional topping (Lee et al., 2010).

### 3.2. Physical control

Other methods of control include the use of screening vents as barrier to the fungus gnats and the install of an attractant light trap with sticky traps (Thomas, 1931; Harris et al., 1996). However, these methods do not reduce population immediately (Boiteau, 2002), which often impractical and light trap may attract undesirable pests into nursery.

Growtones™ (Growstone, Inc., Albuquerque, NM, USA) aggregates, which are processed from 100 % recycled glass and are used as substrate in hydroponic systems (Evans, 2011) have been used as physical barriers and shown to reduce fungus gnat adult emergence over time and may also reduce egg-laying or egg survivability although this depends on the thickness of the Growtones™ layer (Raudenbush et al., 2014).

### 3.3. Biological control

Biological control of fungus gnat is needs because there has been an increase in insecticides resistance (White and Gribben, 1989) and environmental pollution problems induced. Biological controls should be introduced within an integrated pest management context. Scouting, accurate identification, establishing action thresholds, maintenance of records and the correct release strategy are needed (Landis, 1996).

Various biological control methods are used against fungus gnats. Biological control methods included entomopathogenic nematodes (Hungerford, 1919**;** Bovien, 1937; Hudson, 1974; Linquist and Piatkowski, 1993; Gouge and Hague, 1994; Gouge

and Hague, 1995a, b; Harris et al., 1995), parasitoids (Hellqvist, 1994), predatory mites (Ali et al., 1999; Enkegaard and Brødsgaard, 2000), entomopathogenic bacteria (Osborne et al., 1985) and fungi (Huang et al., 1992). These biological control method have been used and tested in various environmental condition, however no research on control of fungus gnats in nurseries and fields of South Korea has been reported.

### 3.3.1. Nematodes

Researchers suggest that entomophatogenic nematodes attack the larval and adult stage of fungus gnats (Poinar, 1992; Grewal et al., 1993; Gaouge and Hague, 1995).

The nematode *Steinernema feltiae*, the species most used to control fungus gnat (Richardson and Grewal, 1991), the infective third juvenile of *S. feltiae* enter into the larvae through natural opening (anus and mouth), penetrated the gut. Also, *S. feltiae* associated with toxic bacterium *Xenorhabdus*, which is release from oesophageal vesicles into the host insect, resulting that it caused septicemia and death. The nematodes then feed on the septicemic tissue, reproduce and when the food reserves in the host cadaver are depleted, new infective juveniles leave to find new host (Poinar, 1992; Grewal and Richardson, 1993; Gouge and Hague, 1995).

The nematodes as *S. feltiae,* have a greater control efficacy against fungus gnats than others. Gouge and Haugue tested the efficacy of various nematodes against six fungus gnat species. As a result, *S. feltiae* was showed most control efficacy. All six fungus gnat species were infested by *S. feltiae*. The establishment rates of *S. feltiae* on the sciarid host *B. paupera*, were investigated by Gouge and Haugue (1995). Infection by *S. feltiae* occurred three hours after invasion, adults developed after 27 hours and new infective juveniles were produced after 48 hours. The establishment rate thus

varies depending on the nematode and host species. Hay and Richardson (1995) assessed the susceptibility of larvae of the mushroom sciarid fly, *Lycoriella solani* to 16 isolates comprising five species of the entomopathogeic nematode (Nematoda: Steinernema). Among these species, *S. feltiae* was most virulent, however *S. kraussei* was the least. Similarly for control of *B. corprophila* on rooted poinsettias, *S. feltiae* was significantly more effective than *S. carpocapsae*.

Another entomopathogenic nematode, *Tetradonema plicans* was first observed in Kansas by Hungerford (1919) parasitizing sciarids which were identified as *B. coprophila*. Ferris and Ferris (1966) parasite is found in all stages of the host fly, who hypothesized that juvenile nematodes enter the host and develop to sexually mature adults in the hemocoel in about 8 days. Hudson (1974) showed that *T. plicans* was pathogenic, specific root gnat, and tested it highly effective in reducing fungus gnat in greenhouse as a biological control agent. Also, Peloquin and Platzer (1993) demonstrated that *T. plicans* caused 74 and 80 % population reduction of sciarid in four months. In these results, *S. feltiae* and *T. plicans* are the favoured nematodes to use for the control of fungus gnats.

### 3.3.2. Predatory mites

Predatory mites have been used as biocontrol agents against fungus gnat. *Hypoaspis miles* (Acari: Hypoaspidae), *Lasioseius fimetorum* (Acari: Podocinidae) and *Geolaelaps* sp. nr. *aculeifer* (Acari: Laelapidae) have been used for control fungus gnats.

The rapid and easy rearing of *H. miles* and its high tolerance to starvation, contribute to its success as a biocontrol agent. *H. miles* have determined that all larval

stages of *B. pauper* are attacked, but the smaller larvae or early instars are more readily consumed, whereas, the eggs and pupae are generally untouched (Wright and Chamber, 1994). In six-glasshouse on cyclamen and poinsettia, 55 per mite pot were controlled against *Bradysia* spp (Chamber et al., 1993). Effectiveness of the *H. miles* in conjuction with two IGRs (methoprene and dimilin) for control of the *L. solani* was evaluated by Ali et al (1999). The IGRs had no negative effect on *H. miles*.

*Lasioseius fimetorum* feed various prey in laboratory condition. Enkegaard and Brødsgaard (2000) demonstrate that the mite consumed a few number of larvae of *B. paupera* and *B. triciti*, however immature drain flies, *Psychoda* spp. were not consumed.

*Geolaelaps* sp. nr. *aculeifer*, introduced inoculative at a rate of 125 mites per plant to cucumber plants in selective rows in a commercial greenhouse reduced peak fungus gnat numbers to about 20 % of those in untreated row. This result suggests that predatory mites can form an important component of an integrated pest management strategy.

### 3.3.3. Fungi

Entomopathogenic fungi have not been widely used as biocontrol agent against fungus gnat. *Erynia ithacensis* (Zygomycetes: Entomophthorales) was first recorded and described by Kramer (1981) from the blood sucking fly, *Symphoromyia hirta*. Huang et al. (1992) used the *E. ithacensis* to control fungus gnat, *Phoradonta flavipes* in a mushroom hothouse. Infested fungus gnat cadavers were inflated, cheese-like, and white to yellowish on pest-infested areas. Pest-infested areas were also sprayed with water to establish a non-uniform relative humidity (RH) distribution. Higher RH

occurs around the mushroom beds on the lower racks close to the ground (LG), while lower RH occurs on the upper racks near the ceiling (UC). The fungus gnat mortalities of LG were significantly higher than UC even though population densities of UC were all higher than LG (Huang et al., 1992). These results indicate that RH significantly influence fungus gnat mortalities. However, research is needed to investigate the option more thoroughly and then to decide whether levels of control can be obtained, that compete with those of the entomopathogenic nematodes and other biological agents.

### 3.3.4. Bacteria

*Bacillus thuringiensis* var. *israelensis* (Bti) is a well known biological control agent for various insect pests, including fungus gnats. Bti produces a protein crystal that is a highly effective larvicide for Diptera such as Culicidae (Goldberg and Margalit, 1977), Chironomidae (Garcia et al., 1980; Ali et al.,1981), Simuliidae (Gaugler and Finney, 1982), and Sciaridae (Osborne et al., 1985). After consumption of a Bti caused cell disruption and other physiological problems which caused the cuticle to disintergrate and the host insect die. Bti has proved to be a successful control agent for fungus gnat. Osborne et al., (1985) demonstrate that larvae affected by bti became flaccid or moribund within 24 hours. Also he discussed that when fungus gnats were exposed to Bti throughout their development from egg to pupa, 84 % of the individual in the control group were still alive after 28 days whereas 8 % of the individuals in the treated group were alive. During this period, the hemolymph chaged from a clear constency to opaque and white. Within 48 hours after treatment, the cuticle of the larvae loose all integrity and the larvae disintergrated, leaving only the head capsule intact. Also, Bti may vary depending in medium, moisture and other environmental variables (Osborne

et al.,1985). Cantwell and Cantelo (1984) reported that over 90 % control of *L. mali* by Bti. A dipteran strain of *B. thuringiensis* use by White (1999), demonstrate effective for the control of *L. auripila* on mushroom farm. Bti and Btk (*Bacillus thuringiensis* var. *kurstaki*) was also tested for its control efficacy against *B. coprophila*, but its insecticidal activity was lower than Bti.

3.3.5. Others

Hymenopterous parasites of fungus gnat include several species in the family Diapriidae (Nixon, 1957; Hellén, 1964). *Synacra paupera* (Hymenoptera: Diapriidae) has been recorded parasitoid of *Bradysia paupera*, a greenhouse insect pest in Europe at several times (Hellqvist, 1994; Macek, 1995). Hellqvist (1944) indicates that *S. paupera* could be a useful biological control agent, which ability to regulate fungus gnat populations in greenhouses will, however, probably differ according to the crop grown and cultural practices. This parasite oviposited and developed in larvae of *B. pauper* in 2$^{nd}$ to 4$^{th}$ larval instar. Infested larva by *S. pauper* lives until pupation at which time it is killed and the wasp itself pupates. The larval stage lasted for 8 to 10 days at 23 ℃, approximately. Infested pupae of *B. paupera* were distinguished as the anterodorsal region was dark brown and transparent, while the posteroventral region was creamcolored and turbid. Total duration of pupa period lasted for about 8 day at 23 ℃, and total developmental time from egg to adult observed 23 to 26 days.

The rove beetle, *Atheta coriaria* (Coleoptera: Staphylinidae) is a soil-dwelling predator that appears to be an effective control agents for greenhouse insect pest such as fungus gnats, *Bradysia* spp. (Carney et al., 2002). *A. coriaria* is a small beetle, approximately 3-4 mm in length and dark brownish color (Miller and Williams, 1983).

The larvae are white in color during the early instars and become yellowish-brown color as past time. Both the larva and adult feed on various stage such as eggs and larvae of a wide range of insect pest, including shorefly, western flower thrip and fungus gnat (Helyer et al., 2003). *A. coriaria* now used control for fungus gnat commercially from several suppliers (Jandrick et al., 2005).

### 3.4. Chemical insecticides

Negative environmental impacts and high costs are often associated with chemical control. However, at times, chemical control is not only feasible, but the only form of control available. Many chemical insecticides have been used against fungus gnats such as conventional insecticides and insect growth regulators (IGRs).

Conventional insecticides included aldicarb (oxime carbamate), bendiocarb (carbamate), chlorpyrifos, diazinon (organophosphate), methiocarb (carbamate), permethrin (synthetic pyrethroid), oxamyl (oxamic carbamate), mercaptothion (organophosphate), calcium oxalate and sinapic acid (Lindquist et al., 1985; Harris, 1993; van Tonder, 1994; Bartlett and Keil, 1997; White, 1997; Jess and Klipatrick, 2000; Ludwig and Oetting, 2001). Lindquist et al (1985) reported that compared four insecticides such as aldicarb, bendiocarb, oxamyl, diazinon and methoprene for the control of *B. coprophila*. Aldicarb and oxamyl were showed most control efficacy compared with others. Also, King (1990) indicated that diazinon, bendiocarb, acephate, and oxamyl revealed adult fungus gnats control efficacy. Calcium oxalate and sinapic acid showed 50 % control efficacy against *L. auripila* in mushroom farm (White, 1997).

IGRs have been one of the fastest developing group of insecticides since the 1960s (Harris and Waindle, 2002). Most IGRs primarily effect the development and metabolism against the juvenile stage of insects such as fungus gants. These IGRs included azadirachtin, cyromazine, diflubenzuron, methoprene, and triflumuron (Eicker and Ludick, 1993; White, 1997; White, 1999; Ludwig and Oetting, 2001; Smith, 2004).

Azadirachtin, initially found to be effect as a feeding deterrent towards the *Schistocerca gregaria* (Orthoptera: acrididae) (Butterworth and Morgan, 1968). Ludwig and Oetting (2001) reported that azadirachtin resulted in the most significant reduction of *B. coprophila* emergence in greenhouse experiment.

Cyromazine, was registered in 1988 (Clift and Terras, 1992). It is highly effective toward dipteran larvae, including species that possessed conventional insecticides resistance (Schalpfer et al., 1986). Cyromazine effect in the larvae results in anomalous sclerotisation and melanisation of the cuticle, followed by necrotic lesions in the cuticle and rupture of the body wall, and then die. Clift and Terras (1992) compared the efficacy of five different IGRs such as methoprene, cyromazine, diflubenzuron, flufenoxuron and teflubenzuron, found that methoprene and cyromazine were effective against sciarid larvae in French mushroom cultures. Furthermore, IGRs observed no significant phytotoxicity on different mushroom strains.

Eicker and Ludwick (1993) compared control efficacy of two IGRs, methoprene and diflubenzuron, against *L. auripila*. Also, It showed good result when applied as drench treatment (White, 1981). Methoprene, a juvenile hormone mimic, was the first of the insect growth regulators registered in 1986 in France for use mushroom industry

(Clift and Terras, 1992). It disrupts the process of morphogenesis in the insect, so methoprene-treated larvae will be unable to successfully change from pupae to adults.

Diflubenzuron, a chitin synthesis inhibitor, which first used against organophosphorus insecticide resistance strains of *L. auriplia* in 1975 (White, 1986) and registered in the United States on 1982 (Ware, 2010). The mechanism of action of diflubenzuron involves inhibiting the production of chitin which is essential for the development of the cuticle. Molting does not occur and the insects development until the exoskeletons are immoderately tight and then they die.

**4. Botanical pesticides**

Over several decades, the control of insect pests has been through the development of synthetic insecticides. Although, the synthetic insecticides are effective, their repeat use for decades has disrupted natural biological systems, led to resurgence of insect, resulted in the widespread development of resistance, had undesirable effects on non-target organisms, ground water contamination, and human health effect (Brown, 1978; Georghiou, 1983; Hayes and Laws, 1991). These problems have highlighted the need for the development of new and safe types of selected insect control alternatives (Arnason et al., 1989). Therefore, the use of eco-friendly and easily biodegradable natural insecticides of plant origin has received renewed important for insect pest control. Currently, numerous products of botanical origin, especially the second metabolites, have received considerable renewed attention as potentially bioactive agents used insects control, because they have evolved so as to protect the plant from attack by insects and microbial pathogens (Johns, 1974; Feeny, 1975; Harborne, 1993).

According to Wink (1993), roughly 30,000 plant secondary metabolites have been reported, but as probably less than 10 % of terrestrial plants have been phytochemically analyzed, the actual number of secondary metabolites likely exceeds 100,000.

Plant secondary metabolites are synthesized although three synthetic pathways. Terpenoids, phenolics, and nitrogen-based secondary metabolites are synthesized from mevalonate, shikimic acid, or amino acid, as precursors, respectively (Berenbaum, 1989; Kang, 1991; Harborne, 1993). Besides, these groups, insect growth regulators and insect behavior control agents are paid attention to as new types of insect control agents. Williams (1967) named these compounds "the Third Generation Pesticides".

The terpenoid, sometimes referred to as isoprenoid, are a class of naturally occurring chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. With more than 23,000 described compounds, terpenoids or terpenes are the largest group of natural products. Within this group, various interesting substances are already known to be present. Furthermore, presumably large numbers of efficacious terpenoids are yet to be discovered. Most are multicyclic structures which differ from one another not only in functional groups, but also in their basic carbon skeletons. Therefore terpenoic compounds are divided into limonoids, clerodane diterpenoids, triterpenoids and sesquiterpenoids.

Limonoid have in recently received particular attention from applied entomologist because of their excellent properties as insect control agents. Most well-known limonoids are azadirachtin, which demonstrated strong insect growth regulator and feeding deterrents by Butterworth and Morgan (1968).

The genus *Teucrium* is one of richest sources of clerodame diterpene, and the new natural products are conveniently reviewed (Piozzi et al., 1998). According to Erler et al (2008). *T. divaricatum* extract caused significant reduction of emerging adult and mushroom damage by larvae of *Megaselia halterata* (Diptera: phoridae).

Wu et al**.** (2001) was isolated six sesquiterpenoids lactones and one monoterpenoid from fruit of *Carpesium abrotanoides*, these terpenoids shown stomach-contact combination toxicity against 4$^{th}$ instar larvae of *Bradysia odoriphaga* (Diptera: Sciaridae).

Terpenoids are usually very expensive, because, like most secondary metabolites, they are produced by plants in relatively small amounts. In addition, due to their complex structure, most terpenoids can′t be obtained in a profitable way through chemical synthesis. Consequently, with bio-production being the only economic source for production proposes, terpenoids are to be considered ideal candidates for a biotechnological approach to improve production.

Phenolic compouns which has penolic functional group was contained in plant except algae. Phenolic compounds are divided into coumarins, tannins, quinines, and lignoids.

Khanikor and Bora (2011) reported that coumarin compound isolated *Angelica conyzoides* (Apiales: Apiaceae) had contact toxicity against *Exorista sorbillans* (Diptera: Tachinidae). Another phenolic compound isopimpinellin isolated *A. acutiloba* had insecticidal activities against *Drosophila melanogaster*. This compound AChE of adults of *D. melanogaster in vitro*, and inhibitory activity was strong. Moreover, the investigation of acute adulticidal activity *in vivo* and AChE inhibitions *in vitro* had a similar result (Miyazawa et al., 1997, 1998).

Lignoids are a large group of chemical compounds found in plants. The antifeeding effects of lignoids, inhibiting the larval growth, intoxication and effect juvenile hormone in insects have been noted (Miyazawa et al., 1994; Isman, 1995; Cabral et al., 1999). Also, Capinoresinol demonstrated by Cabral et al. (1999) that toxic to 4$^{th}$ larvae of *Oncopeltus fasciatus* and *Rhodnius prolixus*.

Nitrogen based compounds are divided into non-protein amino acid, amines, cyanogenic glycosides, glucosinolates, and alkaloids. Among these conpounds, chemical structure of non-protein amino acid is the most simple, and more than 400 non-protein amino acids have been identified. While they are found in a number of un-related families, they are particularly characteristic of legumes and occur mainly in the seeds. L-DOPA (3,4-dihydroxyphenyl-alanine) in *Mucuna* seeds shows antifeeding activity against southern armyworm, *Spodoptera eridania* (Lepidoptera: Noctuidae). This compound interferes with the activity of tyrosinase, an enzyme essential to the hardening and darkening of the insect cuticle (Harborne, 1993).

Another structurally simple class of nitrogenous toxins are the cyanogenic glycosides. Many of these cyanogenic plants are capable of releasing HCN in sufficient quantities to be toxic and, as a result, are often avoided by herbivores (Nahrstedt, 1985; Jones, 1998). They are toxic not as such but only when broken down enzymically with release of HCN or prussic acid. The primary site of action of HCN is on the cytochrome system; terminal respiration is inhibited, and oxygen starvation occurs at the cellular level to result in rapid death (Harborne, 1993).

Glucosinolates (Mustard oil glycosides) are closely related biosyntherically to cyanogenic glycosides and can also be toxic to insect herbivores, when they occur in sufficient amounts in plants. Especially, glucosinolates breakdown products are safer

biofumigants in pest control as they are considered to be fully biodegradable and less toxic. Insecticidal activity of several ITCs has been demonstrated, especially for aromatic compounds (Ahman, 1986; Borek, et al., 1995; Chew, 1988; Lichtenstein, et al., 1962, 1964; Seo and Tang, 1982; Wadleigh and Yu, 1988). Borek et al. (1995) demonstrated that aromatic ITCs are most toxic to the eggs of the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Matthiessen and Shackleton (2000) reported that methyl-ITCs are toxic to whitefringed weevil larvae, *Naupactus leucoloma* (Coleoptera: Curculionidae).

Alkaloids are the most abundant secondary metabolites contained in plant, and more than 10,000 compounds have been described (Harborne, 1993). Nicotine is one of the famous compound and naturally occurring insecticide, which derived from the tobacco plant *Nicotiana* (Schmeltz 1971; Soloway, 1976). Use of nicotine as an insecticide has been known for at least 200 years and is still in use today. It is believed to act by blocking acetylcholine receptors (Crobett et al., 1984). Because of high toxic to mammals, human and since the development of synthetic pyrethroids, nicotine has been declining use, primarily as fumigant in green houses against soft-bodied insects (Casanova et al., 2002).

Cevadine, as an alkaloid occurring in the seeds of *Schoenocaulon officinale*. Its active principles are extremely toxic to mammals, however, commercial preparations typically contained less than 1 % active gradient. The mode of action of cevadine is remarkably similar to that of pyrethrin. Cevadine affects the voltage-dependent sodium channels of nerve axons (Ohta et al., 1973; Levi et al., 1980; Hayes, 1982; Liebowitz et al., 1986; Garber and Miller, 1987; Bloomquist, 1996). Another botanical insecticide in declining use is ryania, obtained by grinding the wood of the Caribbean shrun *Ryani*

*speciosa* (Flacoourtiaceae). The powdered wood contained < 1 % ryanodine, an alkaloid that interferes with calcium release in muscle tissue and paralysis in muscles of insect. (National Research Council 2000; Weinzierl, R. A., 2000). It is used to a restricted extent by organic apple farmers for control of the codling moth, *Cydia pomonella*. (Weinzierl, R. A., 2000). Physostigmine (serine) is an alkaloid toxin contained calabar bean (*Physostigma venenosum*) (Stedman and Barger, 1925). After physostigmine was found to inhibit the acetylcholinesterase (Metcalf and March, 1950), derivates of physostigmine was paid attention to the development of the new pesticide. In 1952, Ciba-Geigy first developed the carbamate insecticide pyrolan (Kuhr and Dorough, 1976). DIMBOA (2,4-dihtdroxy-7-methoxy-3-one) isolated from maize inhibited the growth of *Ostrinia nubilaris* (Hübner) (Klun et al., 1967), and cocculolidine isolated from Cocculus trilobus leaves showed the strong insecticidal activity against *Nephotettox cincticeps* (Uhler) and *Callosobruchus chinensis* (Lucas) (Wada and Munakata, 1967).

**5. Mode of action of plant-derived materials**

Elucidation of mode of action of insecticidal natural products and insecticides is of practical importance for insect control because it provides useful information on the most appropriate formulation, delivery means, and resistance management. According to Ryan and Byrne (1988), biochemical, six terpenoids such as citral (aldehyde), pulegone (ketone), linalool (alchol), (-)-bornyl acetate (ester), cineol (ether) and gossypol (alcohol) showed inhibition activity of acetylcholinesterase (AChE) *in vivo* effects of insect paralysis and death. Certain *Mentha* species such as *M. aquatic* (water mint) inhibit AChE from bovine erythrocytes *in vitro* (Miyazawa et al., 1998). Certain

monoterpenoid (e.g. pulegone and methone) and sesquiterpene alcohols (e.g. elemol and viridiflorol) derived from essential oils of *Mentha* species competitively inhibit AChE *in vitro* **(**Miyazawa et al., 1997, 1998). As suggested by Isman (2001), this inhibitory action does not appear to correlate with overall toxicity of insects *in vivo*.

The octopaminergic nerveous system has been suggested as novel target site of the compounds by Enan (2001), who examined inhibition of $^{3}$H-octopamine binding in a cockroach nerve cord preparation in the presence of essential oil compound (e.g. *trans*-anethole and eugenol). Also, tymol isolated from *Thymus vulgaris*, block octopamine receptors by working though tyramine receptors cascade (Enan, 2005a; Enan 2005b).

Kostyukovsky et al (2002)., suggested that essential oil constutients were found to cause a increase in the levels of intracellular messenger, cAMP of abdominal epidermal tissue in cotton ballworm, *Helicoverpa armigera*. Formamidine pesticides such as chlordimeform target the octopaminergic nervous system, and the major difference between the amidines and other conventional pesticides lies in the potentially important behavioral and physiological effects at sublethal doses (Hollingworth and Lund, 1982). It has been reported that behavioral effects (feeding deterrence and repellency) of essential oils and their constituents are consistent with this mode of action (Hummelbrunner and Isman, 2001; Isman, 2001). However, very few studies on insecticide mode of action of plant-derived compounds have been done. Many plant extracts and their constituents do not cause knockdown effect. Knockdown effect is not a common characteristic of AChE inhibitors carbamates and organophosphates, but is the general symptom of arthropods affected by pyrethrins and pyrethroids (Miller and Adams, 1982). Therefore other mechanism rather than AChE inhibition might be involved in toxicity of most insecticidal natural products. Their

extract mode of action remains to be proven.

The insecticidal effects of certain plant extracts, essential oils, or their chemical constituents are likely by vapor action via the respiratory system, whereas many commercial synthetic insecticides have contact toxicity. Volatile compounds of plant extracts and essential oils are composed of alkaloid, alcohols, aldehydes, and terpenoids, particularly monoterpenoids (Visser, 1986; Coats et al., 1991; Isman, 2001).

Fumigant activity of plant-derived components against some fungus gnats (Sciaridae) has been reported (Park et al., 2006 a; Park et al., 2006 b; Chen et al., 2015; Yi et al., 2015). Park *et al*. (2006 a) demonstrated that allyl isothiocyante identified from horseradish (*Armoracia rusticana*) showed 100 % fumigant activity at 1.25 μL/L$^{-1}$ air against *Lycoriella ingenua* larvae. In addition, (-)-pulegone identified from *Schizonepeta tenuifolia*, was 6.7-fold more effective than dichlorvos (Park et al., 2006 b). Also, fumigant activity of phenylpropanoids has been demonstrated, such as methyleugenol, myristicin and safrole to *L. ingenue* by Yi et al (2015).

*trans*-2-Hexanal exists widely in green leaf volatile compounds (Tandon et al., 2000; Farag and Pare, 2002; Tapia et al., 2007, Takayama et al., 2012), which fumigant activity toward different developmental stages of *Bradysia odoriphaga* (Diptera: Sciaridae) (Chen et al., 2015).

**6. Insecticide mode of actions**

Studies on the mechanisms of insecticides or plant derived constituents are very important from several points of view. Firstly, such knowledge is needed to understand the side effects of these chemicals to human and other nontarget organisms. Secondly, it helps chemists to design additional chemicals with similar mode of action. Thirdly, it

could give scientists important leads as to the cause of resistance development in insects, particularly that involving target insensitivity, and thereby helps in designing countermeasures to avoid resistance or reverse the development of resistance. Finally, the knowledge gained by such studies yields valuable basic information on the nature of the target systems (i.e. the weakness of sensitive insects) in terms of physiological, biochemical, and biophysical knowledge of vital biological system (Matsumura, 1986). Most of the insecticides in current use act by interfering with the passage of impulse in the insect nervous system. Insects depend, like mammals, on an integrated nervous system which enables external stimuli to be translated into effective action.

A number of different chemicals have been implicated in transmission at various insect synapses including acetylcholine. Acetylcholine is the neurotransmitter at central nervous system (CNS) synapses in insects. In order for the nervous system to operate properly it is necessary that, once the appropriate message has been passed, excess acetylcholine should be removed from the synapse, both to prevent repetitive firing and to allow a succeeding message to be transmitted. This removal is effected acetylcholinesterase (AChE), which catalyses hydrolysis of the ester bond. Organophosphate and carbamate insecticides inhibit AChE. The result of this inhibition is that acetylcholine accumulates in the synapses, so that nerve function is impaired. This leads ultimately to the death of the insect. Enzyme kinetics analysis has identified insensitive forms of AChE in insecticide resistant strains, often apparently involving just one mutant form of the enzyme (Hemingway et al., 1986) or as in the housefly, a family of alleles each conferring a distinct pattern of sensitivity (Devonshire, 1987). Neurotoxicity of pyrethroids has been attributed to their activity on the nervous system. Ion channels are the primary target sites for several classes of natural and

synthetic pyrethroids (Bloomquist, 1996). The pyrethroids appear to be acting at virtually every part of the insect nervous system: on sensory neurons (Roeder & Weiant, 1946), on interneurons (Narahashi, 1971), on motor neurons (Yeager & Munson, 1945), and on neurosecretion (Singh & Orchard, 1983). Through *in vivo* and *in vitro* assays, several enzymes and cellular processes have been proposed as targets of pyrethroid modification: synaptic neurotransmitter release, voltage-dependent sodium channels, potassium channels, calcium channels, calmodium, peripheral benzodiazepine receptors, ATPase, nicotinic acetylcholine receptors, Na+/Ca2+ exchangers, receptors for gammaaminobutyric acid (GABA) (Rossignal, 1991; Bloomquist, 1996), acetylcholine-receptor complex (Kiss and Osipenko, 1991) and release of neurohormones (Singh and Orchard, 1983). Peripheral actions are action on the peripheral nervous system which in insects consists of sensory neurons and their axons, motor neurons, and their terminals, and all neurosecretory axons and neurohaemal organs that lie outside of the ventral nerve cord and paired ganglia. The central nervous system (CNS) is considered to be the ganglia, connectives, and commissures from the brain to the terminal abdominal ganglia.

The toxicological significance of ATPase inhibition by pyrethroids is wholly unknown. The Ca2+, Mg2+-ATPase are thought to be involved in sequestering calcium, so their inhibition should increase intracellular calcium. It was suggested by Clark and Matsumura (1982) this could account for the increase of spontaneous transmitter release by pyrethroids, but Salgado et al. (1983) showed that the increase of spontaneous transmitter release by pyrethroids was due to depolarization of the nerve terminals by pyrethroid-induced sodium influx. The GABA-gated channel is the proposed target in insects and mammals for several types of commercial and

experimental insecticides. Gammon and Casida (1983) reported that insecticidal isomers of the type II pyrethroids, deltamethrin, cypermethrin, and fenvalerate blocked the GABA-activated conductance in crayfish muscles, while permethin, resmethrin, and S-bioalethrin had no effect. GABA receptors are much less sensitive to pyrethroids than the sodium channels suggesting that their contribution to pyrethroid poisoning is normally secondary importance (Chalmers et al., 1987). A work on *Aedes aegypti* and *Drosophila melanogaster* demonstrated that cyclodiene insecticide resistanec is associated with change in GABA receptor/chloride ion channel (Ffrench-Constant et al., 1994). Bloomquist (1996) stated that endosulfan like pyrethroids block the GABA-gated chloride channel.

**7. *Syzygium aromaticum***

Cloves are the aromatic flower buds *Syzygium aromaticum* (=*Eugenia caryophyllata* L.) (Myrtaceae) (Srivastava and Malhotra, 1999) which collected in the maturation phase before flowering. This plant has been used spices and well known for its medicinal properties. *S aromaticum* is best known cuisine as *clavos de olor*, and often accompany cumin and cinnamon (Dorenburg and Karen, 2003) in Mexico. Additionally, the oil of *S.aromaticum* and its main constituents such as eugenol, have been described as useful antiseptic, analgesic and anaesthetic effects (Chaieb et al., 2007) and are used for its symptomatic relief of toothache (Leung, 1988) and to promote healing and also finds use in the fragrance and flavoring industries (Chaieb et al., 2007)

*S. aromaticum*-derived materials and constituents possess various biological activities such as antibacterial, fungicidal, antiviral and insecticidal activity. Joshi et al.

(2011) reported that the aqueous ethanolic extract of this plant was found to be anti-bacterial activity against *Salmonella typhi*. In addition, the aqueous diffusion of the plant that showed high activity against *Pseudomonas aeruginosa* (Saeed and Tariq, 2008). Also, Friedman et al. (2002) demonstrate that the essential oil of *S. aromaticum* exhibited bacterial activities against *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*.

The fungicidal activity of essential oil of *S. aromaticum* was demonstrated on several food-borne species. Ranasinghe et al. (2002) reported that essential oil of *S. aromaticum* showed antifungal activity against three fungal pathogen, *Colletotrichum musae*, *Lasio diplodia theobromae* and *Fusarium proliferatum* and fungicidal activity against *Fusarium graminearum* (Velluti, et al., 2004). Also, *S. aromaticum* revealed inhibition fungal activity against three fungi, *Candida albicans*, *Salmonella choleraesuis* and *Pseudomonas aeruginosa* by López et al (2005).

Generally, viruses are highly sensitive to the constituents of essential oils, and phenylpropanoids, monoterpinols and monoterpenals have shown in vitro antiviral activity (Chaieb et al., 2007). Hussein et al. (2000) reported that the *S. aromaticum* water extract exhibited anti-HCV (Hepatitis C virus) activity ($\geq$ 90 % inhibition).

Kurokawa et al. (1998) isolated and identified eugeniin from from extract of *E. caryophyllata*. Eugeniin exhibited anti-herpes simplex virus (HSV) in mice, also inhibited the viral DNA and protein synthesis, and effect DNA polymerase activity of HSV strains.

The biological activity of essential oil of *S. aromaticum* has been examined toward several insects. It was revealed acaricidal activity toward *Dermanyssus gallinae* and *Psoroptes cuniculi* (Kim et al., 2004; Fichi et al., 2007). Also, Kim et al. (2003)

reported that clove bud oil and their constituent as eugenol, were showed contact activity with treated fabric disc against *Tyrophagus putrescentiae*. A more study has examined that clove oil could be used as a novel fumigant against Japanese termite, *Reticulitermes speratus* (Park and Shin, 2005). *S. aromaticum* oil was revealed to inhibit the emergence of *Culex pipens* larvae (El Hag et al., 1999) and showed insecticidal activity toward *Pediculus capitis* (Yang et al., 2003). Insecticidal action of methanol extract from *S. aromaticum* flower bud possessed slow action, but over 93 and 87 % mortality was revealed at 4 day after treatment by filter paper diffusion method against *Sitophilus oryzae* and *Callosobruchus chinensis* repectively (Kim et al., 2003).

**8. *Illicium verum***

Fruit of *Illicum verum*, commonly called star anise, which mainly cultivated for perfume, medicines, and as a culinary spice in southern China as well as in Vietnam.

The flowers are seen from March to May, and the fruit ripen from September to October (Editorial Board of Florida of China, 2004). This fruits are collected before ripen, then sun dried. Star anise enhances licorice flavor in red cooked dishes as well as eggs simmered for a lengthy period in black tea (Rosengarten, 1969). In China, *I. verum* fruit is an ingredient of five spice powder (mixture of star anise, cinnamon, clove, fennel and pepper), containing all five flavors of sweet, sour, bitter, pungent, and salty. In other countries, star anise is featured in signature dishes, such as *Pho bo* soup in Vietnam (Chempakam and Balaji, 2008). It is also the secret ingredient in many Indian stews and curies.

In Asia countries, especially in China, *I. verum* has a long history as a medicinal

plant. Different formulations are used, including crude drug, powders and essential oils. The air-dried ripe star anise fruit was recorded in the Compendium of Materia Medica in Ming Dynasty (Li, 1596). Chinese Pharmacopoeia (2010 edit.) stated the properties of Anisi Stellati Fructus (Chinese name Bajiaohuixiang) as warming yang and dispelling cold, and regulating the flow *Qi* to relieve pain or common cold. The clinical indications include abdominal colic, vomiting, abdomen and lower back pain. Also, the crude fruit and its powders were used in traditional teas to treat nervousness, sleepless and as a sedative while distillated essential oil use for stomachaches (Wang et al., 2011). Chin and Keng (1990) reported that the oil of *I. verum* could be an antidote for a number of poisons and used to treat rheumatism.

In Japan, the essential oil is used for carminative and stomachic remedies (Namba and Tuda, 1993). In addition, the fruits of star anise are chewed for a stomachic and carminative in India (Ilyas, 1980). In other countries, such as Indonesia, Mexico and America, the star anise for the treatment of insomnia, in external applications after childbirth, alleviate colic babies and stomachaches (Burkill, 1966; Kasahara and hemmi, 1995; Wang et al., 2011).

The biological activity of *I. verum* fruit-derived materials has been investigated on several microorganisms and parasites. Hitoko et al. (1980) reported that powdered star anise seeds completely inhibited the fungal growth of three species of toxigenic *Aspergillus* strains such as *A. flavus*, *A. ochraceus* and *A. versicolor*. Dried extracts of star anise and fruits revealed antifungal activities. The star anise extract at a concentration of 16 mg/ml inhibited the growth of four dermatophytes and one saprophytes such as *Aspergillus niger*, *Candida albicans*, *Microsporum canis*, *Epiermophyton fluccosum* and *Trichophyton mentagrophytes*, while E. *fluccosum* and

*T. mentagrophytes* inhibited growth at 4 mg/ml (Yazdani et al., 2009). In addition, methanol extract of *I. verum* fruit showed antibacterial activity against *Eikenella corrodens* (Iauk et al., 2003). The fungicidal activity of essential oil of *I. verum* was demonstrated on plant pathogenic fungi. Huang et al. (2010) observed that the essential oil possessed inhibitory activity against eleven plant pathogens, which $IC_{50}$ values ranged from 0.07 to 0.25 mg/mL. Anethole, the main constituent of star anise oil, has been showed antibacterial and antifungal activity against twelve bacteria and fifteen fungi (De et al., 2002). Good insecticidal activity against *Lasioderma serricorne* adults was showed with methanol extract of *I. verum* fruit applied in a filter paper diffusion method (Kim et al., 2003). At a rate of 3.5 mg/cm$^2$, the methanol extract revealed fast acting, showing 100 % mortality 1 day after treatment. Ho et al. (1995) demonstrated that hexane extracts of *I. verum* fruit were toxic to eggs, larvae and adults of *Tribolium castaneum* and adults *Sitophilus zeamais*. The extracts also suppressed progeny production in both species.

Insecticidal activity of *trans*-anethole isolated from star anise against fruit fly, *Drosophila melanogaster* was revealed *in vitr*o by direct contact application and fumigation method (Miyazawa et al., 1993). The insecticidal activity of *trans*-anethole also showed against German cockroach, *Blattella germanica* (Chang and Ahn, 2002). *trans*-Anethole, present in anise oil from *Pimpinella anisum* L. (Umbelliferae), was found to be toxic to *D. melanogaster, Musca domestica* and third instar larvae of *Aedes aegypti* (Marcurs and Lichtenstein, 1979). It is also present in the leaves and pericarp extracts of *Zanthoxylum dipetalum* (Rutaceae) which had ovicidal activity against eggs of *Dacus dorsalis* (Marr and Tang, 1992).

## 9. Perspectives

The use of conventional insecticides such as organophosphate and carbamate insecticides will be restricted by recent pesticide regulatioin policies in the USA (USEPA, 2010) and EU (Anonymous, 2005). Until 2003, the US gorverment banned or severely restricted 64 insecticides belong to the categories of UN Severely Hazardous Pesticides Formulations (SHPF), US PIC (Prior Information Consent) and US PIC lists such as chlordimeform and ethyl parathion (USEPA, 2003). In spite of the widespread public concerns for long-term health and environmental effect of conventionally used pesticides, natural-based pesticides have not yet occupied much portion in the pesticide marketplace. Due to the improve condition for insect pests growth by environmental changes and limited use of chemical insecticides, insect pests are prevalent in ginseng farms, fungus gants in ginseng fields, especially. At the same time, consumer′s and peasant′s demand for new minimum risk insect pest control in ginseng products will be increasing.

In recent years, the emphasis to control the ginseng′s insect pests populations has shifted steadily from the use conventional chemicals toward more specific and eco-friendly materials, which are generally of botanical origin. Modern synthetic chemicals could provide immediate results for the control of insect; on the contrary, they bring irreversible environmental hazard, severe side effect, and pernicious toxicity to human being and beneficial organism. Therefore, use of eco-friendly and cost-free plant based products for the control of fungus gnats are inevitable. Also, considering these situations, botanical fungus gnat control products might replace some synthetic ones in the near future.

# CHAPTER I

# The Biology of *Bradysia procera* (Diptera: Sciaridae) and its Seasonal Occurrence and Symptoms of Damage Caused in the Field

## 1.1. Rearing Method and Investigation of the Developmental Characteristics of *Bradysia procera* and its Life cycle in the Laboratory Condition

## 1.2. Distribution and Seasonal Occurrence Pattern of *Bradysia procera* and Symptoms of its Damage caused in Ginseng fields

# 1.1. Rearing Method and Investigation of the Developmental Characteristics of *Bradysia procera* and its Life cycle in Laboratory

## INTRODUCTION

Fungus gnats, *Bradysia* spp. (Diptera: Sciaridae) are an important insect pests in various crops, glasshouse ornamentals, mushrooms, and forestry nurseries (Gouge and Hague, 1995; White et al., 2000; Shen et al., 2009**;** Hurley et al., 2010). Their larvae feed on organic matter, plants, and the spawn of fungi. Owing to such a wide spectrum of hosts, several species of this family have been recognized as pests in agricultural production. Among *Bradysia* species, the ginseng fungus gnat, *B. procera* Winertz (Diptera: Sciaridae) is an important insect pest affecting ginseng cultivation in South Korea. It was first identified in 1868 by Winertz and first reported as a pest was in ginseng in 1993 (Kim, 1994). Recently, *B. procera* has been a concern in the ginseng tillers, because of heavily infestations on 5-6 years old cultivated ginseng.

*Bradysia* species deposit an average of 75 to 272 eggs, which are followed by four instars of larvae and an obtect pupa (Thoman, 1931; Carson, 1945; Kennedy, 1974; Wilkins and Daugherty, 1970; Peronidi et al., 1986). The larvae have a shiny black head capsule and can be discriminated other immature larvae. Many researchers have reported that *B. impatiens* has an average developmental time of 16.3 to 33 days. However, relevant studies on *B. procera* are limited, especially on ecological and physiological research.

For the chemical control *B. procera*, only two insecticides have beeb registered (KRDA, 2016), however neither insecticide was determined to be effective in our

previous experiments because many farmers possibly abuse un-registered insecticides in ginseng farms (unpublished data). Moreover, chemicals have several side effects, such as ginseng root residue, environmental risk in human, animals, water contamination, and beneficial organisms. As insecticides have various risks, many researchers in other countries have tried to find other cultivation based, physical, and biological control methods for the management of the fungus gnat before mentioned. The establishment of the efficient management of *B. procera* populations requires accurateinformation regarding aspects such as species identification of species, rearing methods and developmental stages.

The present study aimed to determine fundamental information of ecologcal and physiological characteristics for the development of an effective rearing method of *B. procera* using ginseng stems and alternative food sources, their lifecycles under laboratory conditions, and morphological characteristics of the developmental stages.

## Materials and Methods

### 1.1.1. Tested insect

The tested insects were collected from a damaged by *B. procera* larvae in a 6-years old ginseng field, Yeoju, Gyeounggi in 2013. The collected larvae were immediately transferred to an insect rearing room without any insecticides. They were reared in polyethylene containers (5.5 cm diameter × 10.5 cm) containing moistened paper towels (Yuhan-Kimberly, Seoul, Korea) on the bottom with pieces of 6-years old ginseng stem (5 cm), and sealed with an original solid lid pierced with two small holes for ventilation.

### 1.1.2. Rearing method and investigation of the developmental characteristics of *Bradysia procera*

1.1.2.1. Eggs

A male–female adult *B. procera* pair was collected using a Hausherr's handheld, battery-powered aspirator MX-991 (Toms River, NJ, USA), and captured adults were directly added to polyethylene container (5.5 cm diameter × 10.5 cm) in the laboratory condition. After oviposition, 20 eggs were collected using a fine brush, and immediately placed in a petri dish placed under moistened (5% sucrose solution) no. 2 filter paper (4.25 cm diameter). The petri-dishes were kept at 25 ± 1℃ in an insect rearing room with 45 ± 1% relative humidity under a photoperiod of a 16:8 light and dark cycle. The periods of the egg stage and hatching rate were estimated under a binocular microscope every 24 h. These measurements were replicated three times. The weight and size of the eggs were measured using an ultra microbalance XP-2U (Mettler Toledo, Columbus, OH, USA) and microscope equipped

with a Ds-fi1 digital analyzer (Nikon, Tokyo, Japan) directly after oviposition. Hatching was determined when the larvae totally escaped from the eggshell. The survival rate of each stage was calculated from the formula: survival rate (%) = *SI* / *TI* × 100, where *SI* is the survival of individuals and *TI* is total number of individuals.

1.1.2.2. Larvae

*B. procera* larvae were collected as previously described. Following egg hatching, 100 individual first instar larvae were transferred, using a fine brush, to each insect breeding dish (5 cm diameter × 1.5 cm) and supplied with fresh 6-y old ginseng stems daily as their diet. Each larval period was analyzed every 24 h for evidence of molting (e.g., cast head capsule or exuviae). The body weight and size of the 10 larvae were measured after ecdysis less than 12 hours. Before assessment, each larva was anesthetized using a chilled plate. These measurements were taken three times.

1.1.2.3. Pupae

When mature larvae had spun cocoons, the larvae were placed onto a straw (0.5 cm diameter × 7 cm) filled with moistened (5% sucrose solution) paper towel to induce pupation. Soon after pupation, each pupa was transferred to an insect breeding dish (5 cm diameter × 1.5 cm) with an original solid lid with a fine mesh screen (200 mesh, 1.5 diameter). The pupal period was checked every 24 h for evidence of their complete emergence from the cocoon. The body weight and size of 10 male and female pupae were observed using the same method as described previously < 24 h after pupation. These measurements were replicated three times.

1.1.2.4. Adults

Adult *B. procera* were isolated soon after emerging from the pupal casing. After eclosion, each adult was captured with a Hausherr's handheld, battery-powered aspirator MX-991 (Toms River, NJ, USA) and transferred into a polyethylene container (5.5 cm diameter × 10.5 cm) with moist (5 % sucrose solution) paper towel placed at the bottom and sealed with an original solid lid pierced with two small holes to permit the ventilation. The body weight and size of ten male and female adults were observed using the same apparatus after eclosion less than 24 hours. Before assessment, each adult male and female was induced anesthetized using carbon dioxide gas.

The fecundity of *B. procera* adults were determined by counting the number of egg ovipositions. Two males and one female were captured with a Hausherr's handheld, battery-powered aspirator MX-991 (Toms River, NJ, USA) and directly placed a polyethylene container. The adults used in the experiments were less than 12 hour eclosion. After oviposition, the number of eggs were transferred onto black paper using a fine brush and counted under a binocular microscope (20 ×). This experiment was replicated ten times.

### 1.1.3. Experiments to identify an ingestible food source and determine its effect on the growth *B. procera* and comparison with ginseng stem

1.1.3.1. Alternative food source for rearing

This experiment was conducted to identify an alternative ingestible food source to the ginseng stem for rearing, and compare the growth of ginseng fungus gnat fed different diets. The 18 vegetables and fruits examined in this study are as follows: *Apium graveolene*, *Allium ascalonicum*, *Allium fistulosum*, *Allium sativum*, *Asparagus asparagoides*, *Brassica oleracea* var. *acephala*, *Brassica oleracea* var. *capitata*,

*Brassica oleracea* var. *italic*, *Brassica rapa* var. *glabra*, *Cucumis melo* var. *makuwa*, *Cucumis sativus*, *Ipomoea batatas*, *Malva verticillata*, *Oenanthe javanica*, *Panax ginseng*, *Lycopersicon esculentum*, *Solanum melongena*, and *Solanum tuberosum*. All vegetables and fruits were purchased from a commercial market.

The fresh vegetables and fruit were placed in three corners and the ginseng stem was placed in the other corner of an insect breeding box (7.2 × 7.2 × 10 cm) with moistened cotton wool at the bottom (Fig. 1). Fifty newly hatched larvae (< 12 h old) were placed in the center of the insect breeding box. The number of larvae was counted on the vegetables/fruits in each corner with consideration of eating marks. All treatments were replicated three times.

**Fig. 1.** Feeding choice experiment to determine an alternative food source for *Bradysia procera* larvae

1.1.3.2. Effects of *Allium sativum* and *Panax ginseng* stems on the development and life history of *Bradysia procera*.

This study was used to compare the growth and development of *B. procera* when supplied different food sources, such as garlic and ginseng stems.

The effect of different food sources on the growth and developmental time were examined at 25 ± 1℃ in an insect rearing room with 45 ± 1% relative humidity under a photoperiod of a 16:8 light and dark cycle. Two hundred eggs were collected from a laboratory colony with a fine brush, and each placed in a petri-dish (5 cm diameter × 1.5 cm) placed under moistened (5% sucrose solution) Whatman no. 2 filter paper (4.25 cm diameter). After the eggs hatched, the first larvae were each transferred to an insect breeding dish (5 cm diameter × 1.5 cm),with different diets of either a stem of garlic or ginseng. Each developmental stage was determined using the same methods described previously. The survival rate (SR) of each stage was calculated from the formula: SR = *SI* / *TI* × 100, where *SI* is the number of individuals surviving, and *TI* is total number of individuals. All experiments were replicated three times.

### 1.1.4. Data analysis

The fecundity and viability percentages were transformed to an arcsine square root value for analysis of variance (ANOVA). A Student's *t*-test was used for significant differences in larvae, pupae and adult for weight, length, width, and longevity (SAS, 2012).

## Results

### 1.1. Life cycles and biology of *B. procera* in laboratory condition

1.1.1. Eggs

Female *B. procera* laid an average of 167 (from 131 to 204) eggs on moistened paper towel (Fig. 2). The egg period was approximately 6 d. The eggs were oval and the first deposited eggs were a glossy yellow that became transparent as they reached the hatching period. The average weight, length, and width of eggs were estimated to be 1.1 μg, 472 μm, and 322 μm, respectively, and the egg survival rate was 92% (Table 1).

Table 1. Developmental characteristics of *Bradysia procera* eggs

| Parameter | Mean (± SE) | Range |
|---|---|---|
| Weigh of egg, μg | 1.1 ± 0.05 | 0.94 – 1.25 |
| Length of egg, μm | 427.2 ± 8.40 | 396 – 471 |
| Width of egg, μm | 259.5 ± 3.61 | 244 – 277 |
| Amount of spawning, Unit | 167.4 ± 8.06 | 131 – 204 |
| Eclosion rate, % | 91.5 ± 1.98 | 85 – 100 |
| Period of egg, Day | 5.9 ± 0.54 | 4.8 – 9.8 |
| Hatching rate, % | 91.5 ± 1.98 | 90 – 100 |

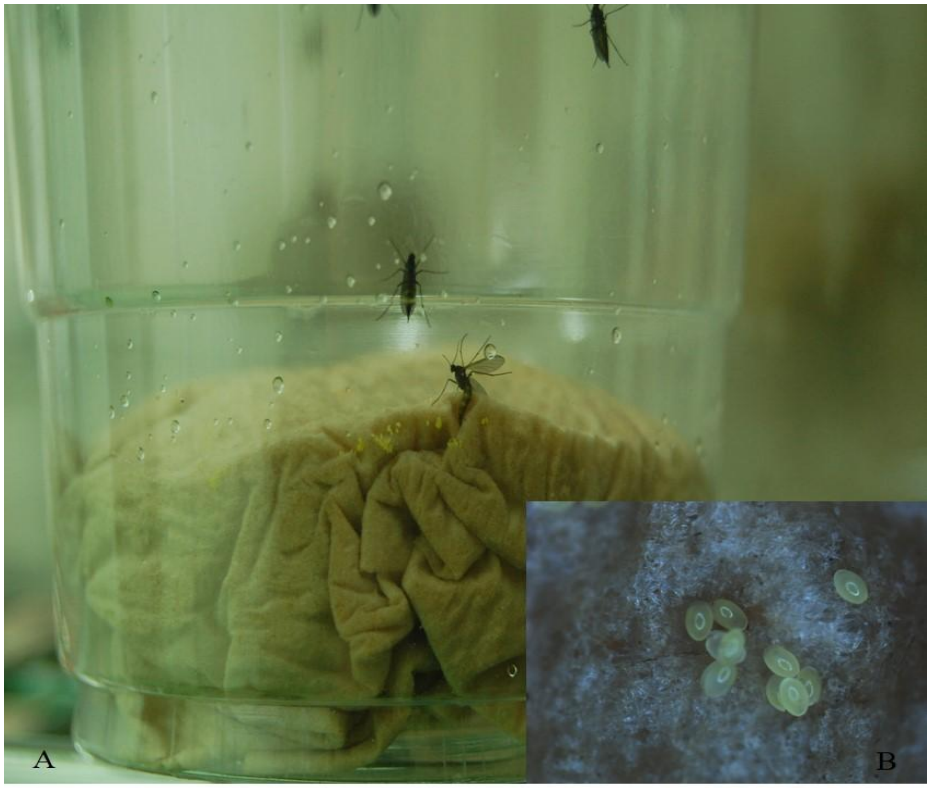


**Fig. 2.** Oviposition (A) of *Bradysia procera* and their eggs (B).

1.1. 2. Larvae

*Bradysia procera* has four larval instars excluding a pre-pupal stage, which is considered to be the terminal stage of the 4$^{th}$ instar larva. Morphology is similar in the four instar stages, with a soft body and sclerotic head capsule. Soon after hatching, the larvae moved from the eggshell to the ginseng stem (or food source), and started to feed directly. The larvae preferred moist areas. From the 4$^{th}$ instar larvae, males and females were mainly distinguished by size characteristics of the head and body. The female was generally larger than the male (Fig. 3). Both males and females comprise 14 brilliant milky-white segments that became brilliant yellowish as they reached the 4$^{th}$ instar and pre-pupa. The 1$^{st}$ to 4$^{th}$ larval period was approximately 17.5 d. Based on the measurements of the larval stage, peak

growth was observed during the last stage of the 2nd instar. Each larval stage was described as follows:


**Fig. 3.** Comparison of male and female body sizes of 4th instar larvae of *Bradysia procera*.

The body of the 1st instar larva was transparent to white with a brown head capsule immediately after eclosion (Fig. 4-A). Later the head capsule darkened to nearly black. The average weight, length, and width of the 1st instar larvae was 1.3 μg, 608 μm, and 138 μm, respectively. This period was approximately 3.1 d. The color of the body and head capsule of the 2nd instar larvae was similar to that of the 1st instar larvae (Fig. 4-B). The average weight, length, and width of the 2nd instar larvae were 8.1 μg, 1.7 mm, and 509 μm, respectively. The duration of the 2nd instar larva was approximately 3.7 d. The color of the body and head capsule of the 3rd instar larvae was similar to that of the 1st and 2nd instar larvae (Fig. 4-C). The average weight, length, and width of the 3rd instar larvae were 81 μg, 4.3 mm, and 792 μm,

respectively. The period of the 3rd instar larval stage was approximately 2.8 d. The body of 4th instar larvae was a brilliant yellowish color and the internal organs were clearly visible (Fig. 4-D). At the end of the larval stage, the larvae secreted mucus from the mouth. From the 4th instar larval stage, males and females could be distinguished by morphological characteristics and body sizes. The abdomen of males was blunt, whereas that of females was peaked (Fig. 5). Spiracles were observed on segments 4–10 during this stage (Fig. 6). Males were generally smaller than females. The average larval weight, length, and width of males were 145 μg, 0.9 mm, and 944 μm, respectively and those of females were 166 μg, 10 mm, and 1.1 mm, respectively (Table 2). The average period of the 4th male and female larvae was 5.7 and 6.5 d, respectively. More detailed information regarding the developmental characteristics of *B. procera* larvae is given in Table 2.

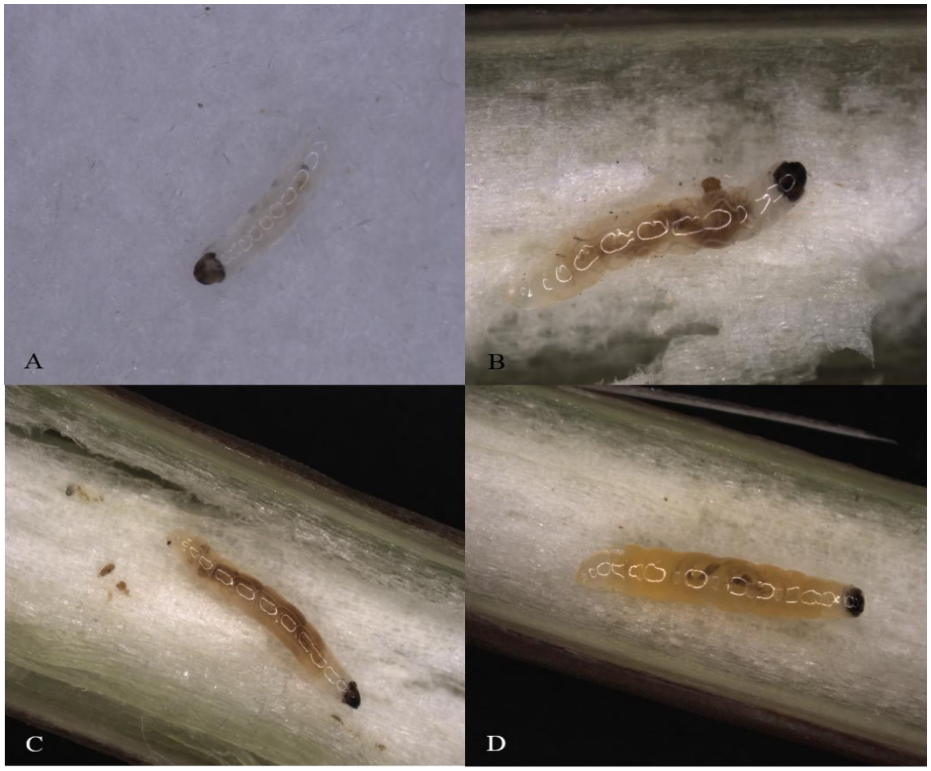


**Fig. 4.** Larvae of the ginseng stem fungus gnat, *Bradysia procera*. A: 1st instar larva, B: 2nd instar larva, C: 3rd instar larva, D: 4th instar larva.

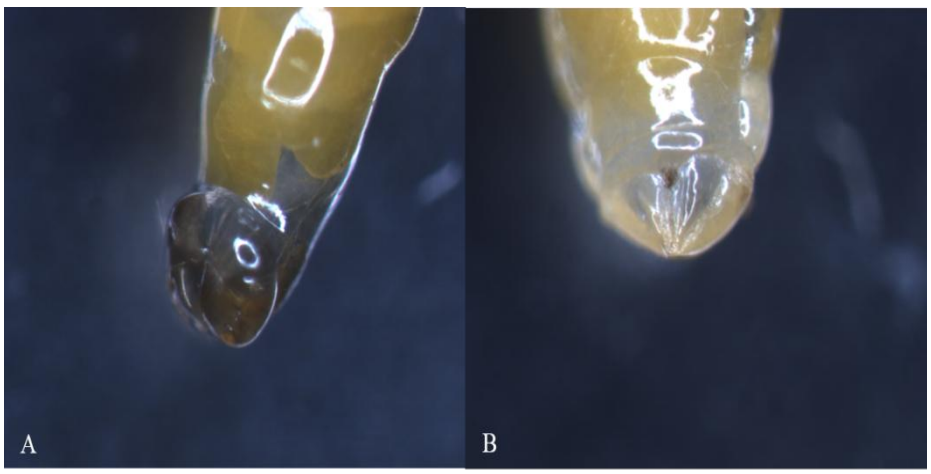


**Fig. 5.** Lateral view of the morphological characteristics of the 4th instar larvae of *Bradysia procera*. A: end of abdomen of a male, B: end of abdomen of a female

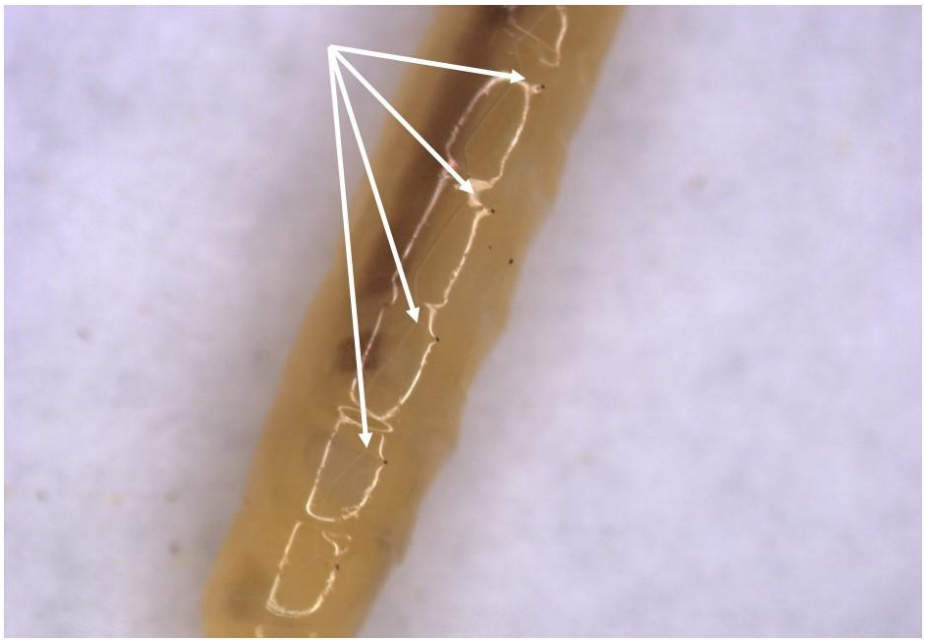

**Fig. 6.** Spiracles of the 4th instar larva of *Bradysia procera*

1.1.3. Pupae

The pupae were obtect, and yellow to dark gray in coloration, with increasing black pigmentation over time (Fig. 7). They comprised 14 segments, and the tergum and sturnanum were covered with several setae (Fig. 8). The pupae showed sexual dimorphism; males were generally much smaller and more slender than females. The average weight, length, and width of the male larvae were 99.1 μg, 4.0 mm, and 1.1 mm, respectively, and those of female larvae were 105.1 μg, 4.7 mm, and 1.2 mm, respectively. The average pupal period was 6.7 and 7.0 d for males and females, respectively; therefore, there were differences in the pupal stage between males and females (Table 2). Male pupae were distinguished from females by the presence of two dististylar sheaths, which comprised the ventral portion of the terminal segment of the abdomen (Fig. 9).


**Fig. 7.** Pupae of *Bradysia procera*. Pupation after 24 h (A), 72 h (B), and 144 h (C).

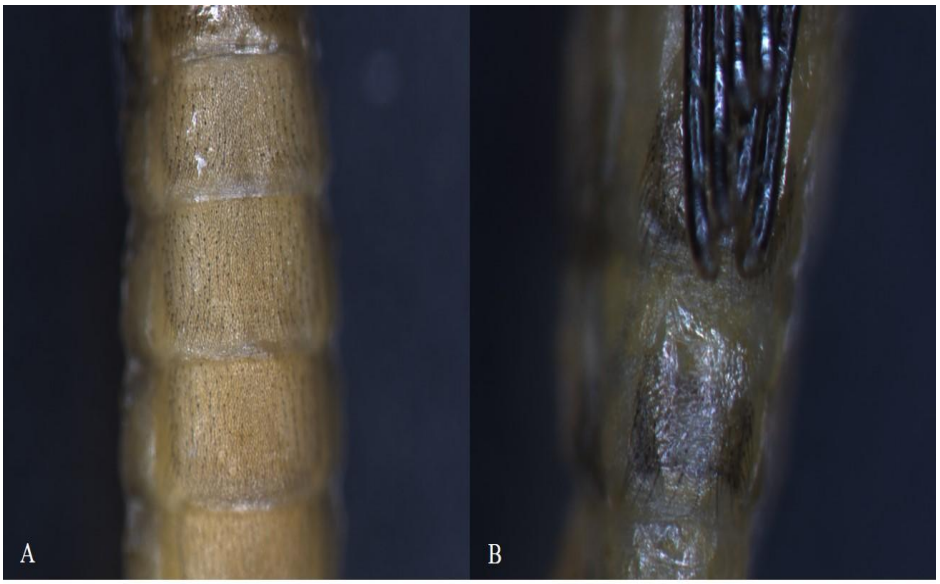


**Fig. 8.** Photomicrograph of setae from *Bradysia procera*. A: tergum, B: sturnanum.

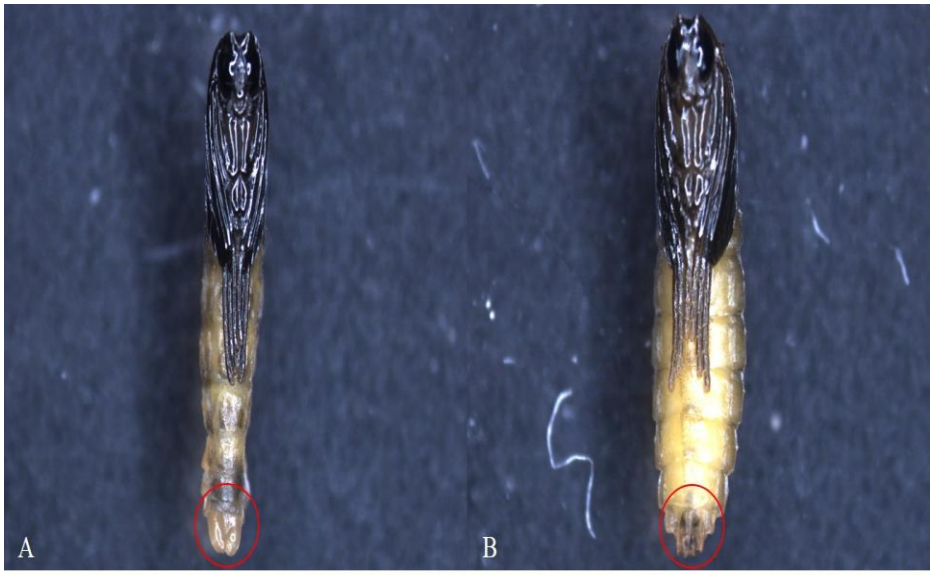


**Fig. 9.** Photograph of the terminal abdomen of male (A) and female (B) pupae

1.1.4. Adult

The adults were small and most of the body was dark-grey or black in color, and the sexes were similar in appearance; however, the abdomen of males was slender and terminated in a double hook-like copulatory organ, whereas the female abdomen was rounded and terminated with a more pointed oviposition organ (Fig. 10).

Female *B. procera* laid from average weight, length, and width of the male larvae were 81.9 μg, 3.6 mm, and 1.0 mm and female average weight, length and width were observed 134.3 μg, 4.8 mm, and 1.2 mm, respectively. The average larval period was 5.7 and 6.5 d for males and females, respectively. In general, males emerged before and lived longer than females (Table 2).

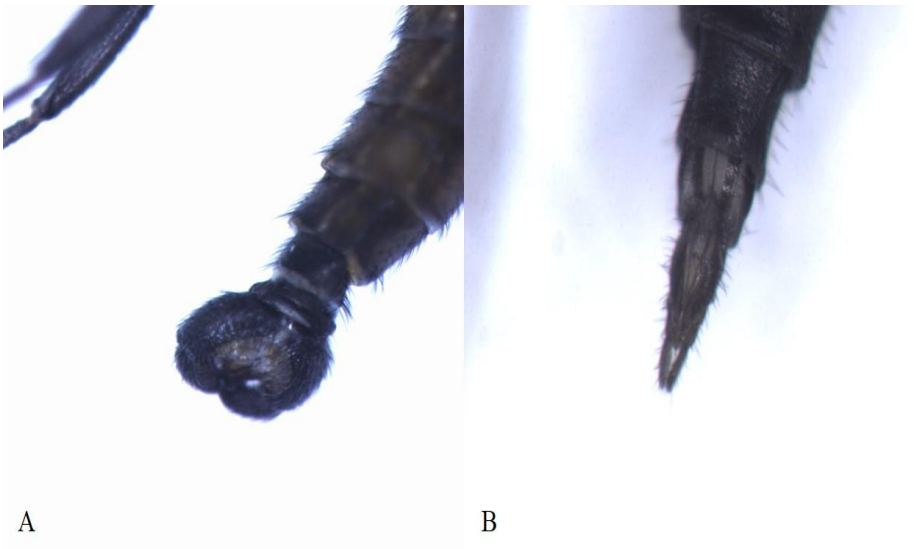


**Fig. 10.** Male (A) and female (B) adult terminalia of *Bradysia procera.*

Table 2. Developmental characteristic of each larval stages of *Bradysia procera*.

| Stage | | No. | Weigh (μg) | Length (μm) | Width (μm) | Period (day) (No.) |
|---|---|---|---|---|---|---|
| 1st larvae | | 10 | 1.3 ± 0.04 | 608.3 ± 12.91 | 138.2 ± 1.86 | 3.05 ± 0.06 (87) |
| 2nd larvae | | 10 | 8.1 ± 0.63 | 1669.6 ± 51.52 | 509.7 ± 10.97 | 3.72 ± 0.07 (79) |
| 3rd larvae | | 10 | 81.0 ± 4.76 | 4329.5 ± 129.23 | 792.02 ± 9.63 | 2.80 ± 0.09 (75) |
| 4th larvae | Male | 10 | 144.9 ± 4.92 | 9123.6 ± 174.75 | 944.8 ± 17.29 | 5.7 ± 0.15 (25) |
| | Female | 10 | 166.0 ± 6.72 | 10306.2 ± 135.43 | 1102.16 ± 20.61 | 6.5 ± 0.13 (38) |
| Pupae | Male | 10 | 106.1 ± 1.16 | 4372.7 ± 93.52 | 1109.1 ± 54.44 | 6.7 ± 0.10 (23) |
| | Female | 10 | 108.7 ± 0.91 | 4760.9 ± 98.39 | 1175.0 ± 42.06 | 7.0 ± 0.10 (31) |
| Adult | Male | 10 | 81.9 ± 2.06 | 3582.7 ± 104.75 | 1011.0 ± 28.79 | 5.3 ± 0.29 (19) |
| | Female | 10 | 134.3 ± 2.91 | 4812.1 ± 121.50 | 1164.9 ± 26.93 | 6.8 ± 0.11 (29) |

## 1.2. *Bradysia procera* larval feeding choices when presented with alternative food sources and effects on growth and survival

### 1.2.1. Evaluation of vegetables and fruits as ingestible food source on *B. procera*

The 1st instar larvae fed on *B. oleracea* var. *capitata*, *B. rapa* var. , and stems of *Allium sativa*, despite the presence of ginseng (Table 3). The number of larvae on the different foods differed significantly. The proportion of larvae on ginseng (*Panax ginseng*) was higher than on the other six vegetables and fruits. However, the proportions of larvae on garlic and cabbage were significantly larger than that on asparagus, broccoli, Chinese cabbage, and flowering cabbage (Fig. 11).

Table 3. Various vegetables and fruits for larval feeding choice experiment toward *B. procera*.

| Scientific name | Common name | Korean name | Used part | Index of digestion[a] |
|---|---|---|---|---|
| *Apium graveolene* | Celery | 셀러리 | Whole plant | + |
| *Allium ascalonicum* | Chive | 쪽파 | Whole plant | + |
| *Allium fistulosum* | Welsh onion | 파 | Whole plant | + |
| *Allium sativum* | Garlic | 마늘 | Stem | +++ |
| *Asparagus asparagoides*, | Asparagus | 아스파라거스 | Whole plant | + |
| *Brassica oleracea* var. acephala | Flowering cabbage | 꽃양배추 | Bud | + |
| *Brassica oleracea* var. capitata, | Cabbage | 양배추 | Leaf | ++ |
| *Brassica oleracea* var. italic | Broccoli | 녹색꽃양배추 | Bud | ++ |
| *Brassica rapa* var. glabra | Chinese cabbage | 배추 | Leaf | ++ |
| *Cucumis melo* var. makuwa | Oriental melon | 참외 | Fruit | + |
| *dSCucumis sativus* | Cucumber | 오이 | Fruit | + |

Table 3. (continued)

| Scientific name | Common name | Korean name | Used part | Index of digestion[a] |
|---|---|---|---|---|
| *Ipomoea batatas* | Sweet potato | 고구마 | Stem | + |
| *Malva verticillata* | Mallow | 아욱 | Stem | + |
| *Oenanthe javanica* | Japanese parsley | 미나리 | Stem | + |
| *Lycopersicon esculentum* | Tomato | 토마토 | Stem | + |
| *Solanum melongena* | Eggplant | 가지 | Fruit | + |
| *Solanum tuberosum* | Potato | 감자 | Tuber | + |
| *Panax ginseng* (control) | Korean ginseng | 인삼 | Stem | +++ |

[a] + : not digestion, ++ : slight digestion without debris, +++ : complete digestion with associated debris secretions.

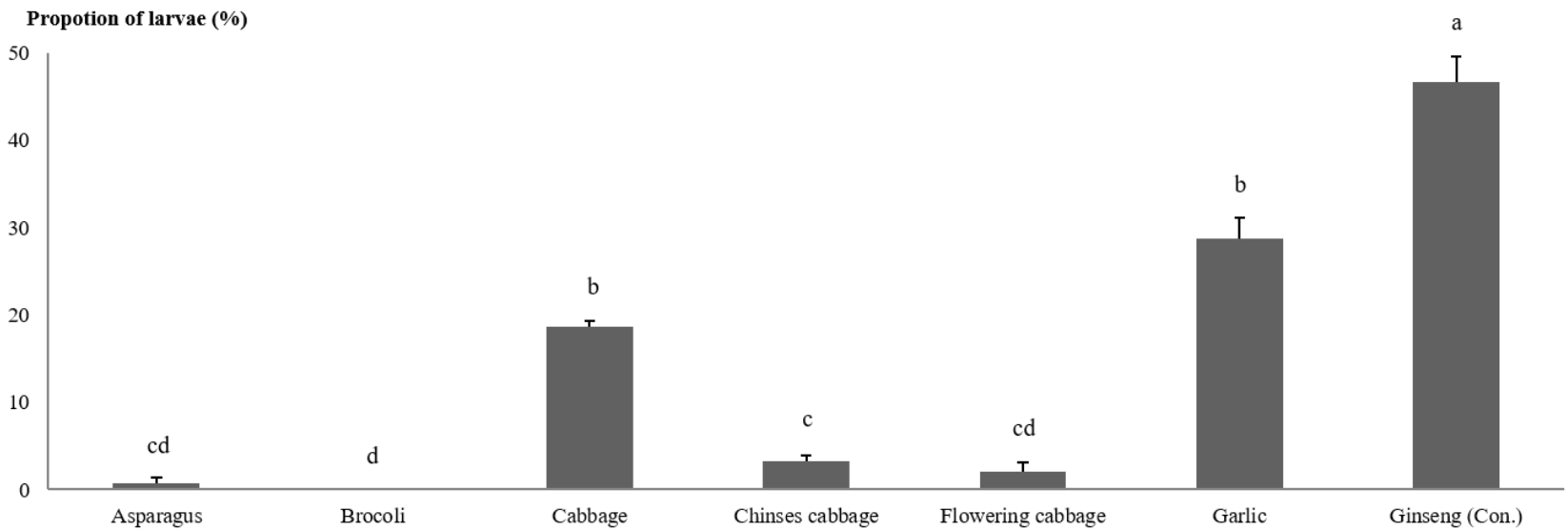


**Fig. 11.** Feeding choice experiments; proportion of *Bradysia procera* larvae on different diets. Bars in a single series marked with the same letter are not significantly different at adjusted $P = 0.05$ (Bonferroni method)

1.2.2. Comparison of the development characteristic and life cycle of *Bradysia procera* on ginseng stem and alternative food source

This study aimed to determine the developmental characteristics, mortality, hatching, and survival rate of *B. procera* fed *Allium sativum* and *Panax ginseng*. In each developmental stage of *B. procera*, there was no significant difference of the stem of *A. sativum* and *P. ginseng* on the larval length, width, and weight ($P = 0.05$). Comparing each larval stage, the body size and weight of the 3rd instar larva was shown to rapidly increase more than that of the other stages. The mean length, width, and weight for both groups of each stage of *B. procera* is summarized Table 4.

The development time of eggs, larvae, pupae, and adults fed two different diets was not significantly different. The period of *B. procera* male and female development when fed *A. sativum* stems was 27.7 and 29.6 d, respectively. Similarly, the period of male and female development when fed *P. ginseng* stems was 27.2 and 29.0 d, respectively. The egg survival rate was not significant different ($P = 0.106$); however, the survival rate of 1st, 2nd, and 4th instar larvae and of pupae was significant higher on the ginseng stem diet than on the garlic stem diet. The period of each stage of *B. procera* development and the survival rate is summarized in Table 5.

Table 4. Comparison of the length, width, and weight of each stage of *Bradysia procera* development fed *Allium sativum* and *Panax ginseng* stems

| Stages | Parameters | Diets | | *P* value[a] |
|---|---|---|---|---|
| | | Garlic | Ginseng | |
| 1st Larva | Length (μm) | 599.7 ± 5.61 | 606.7 ± 3.94 | 0.313 |
| | Width (μm) | 124.6 ± 1.97 | 126.5 ± 1.27 | 0.424 |
| | Weight (μg) | 1.2 ± 0.02 | 1.2 ± 0.03 | 0.452 |
| 2nd Larva | Length (μm) | 1632.4 ± 13.26 | 1636.9 ± 17.98 | 0.842 |
| | Width (μm) | 500.7 ± 5.52 | 507.3 ± 3.62 | 0.326 |
| | Weight (μg) | 7.6 ± 0.13 | 8.0 ± 0.17 | 0.068 |
| 3rd Larva | Length (μm) | 4263.9 ± 54.77 | 4334.4 ± 44.68 | 0.323 |
| | Width (μm) | 774.7 ± 11.30 | 792.7 ± 4.25 | 0.142 |
| | Weight (μg) | 79.9 ± 0.35 | 80.5 ± 0.28 | 0.189 |
| 4th Larva | Length (μm) | 9277.6 ± 100.99 | 9494.0 ± 102.69 | 0.138 |
| (M)[b] | Width (μm) | 954.4 ± 14.92 | 989.5 ± 19.79 | 0.162 |
| | Weight (μg) | 152.3 ± 3.07 | 149.9 ± 6.02 | 0.724 |
| 4th Larva | Length (μm) | 10904.1 ± 54.47 | 10987.9 ± 55.08 | 0.283 |
| (F)[c] | Width (μm) | 1008.7 ± 12.66 | 1020.9 ± 15.01 | 0.539 |
| | Weight (μg) | 160.8 ± 4.14 | 166.5 ± 4.40 | 0.349 |

Continued (Table 4).

| Stages | Parameters | Diets | | *P* value[a] |
|---|---|---|---|---|
| | | Garlic | Ginseng | |
| Pupa (M)[b] | Length (μm) | 3984.4 ± 78.92 | 3988.3 ± 40.24 | 0.965 |
| | Width (μm) | 1066.5 ± 14.29 | 1094.1 ± 40.39 | 0.522 |
| | Weight (μg) | 99.5 ± 3.03 | 104.9 ± 0.94 | 0.092 |
| Pupa (F)[c] | Length (μm) | 4676.62 ± 60.12 | 4825.6 ± 70.51 | 0.113 |
| | Width (μm) | 1178.1 ± 17.93 | 1198.3 ± 23.32 | 0.495 |
| | Weight (μg) | 108.8 ± 1.08 | 110.0 ± 1.08 | 0.161 |
| Adult (M) | Length (μm) | 3459.4 ± 82.67 | 3550.8 ± 93.92 | 0.468 |
| | Width (μm) | 1063.17 ± 8.56 | 1068.2 ± 12.29 | 0.736 |
| | Weight (μg) | 106.0 ± 1.02 | 108.6 ± 1.61 | 0.184 |
| Adult (F) | Length (μm) | 4639.0 ± 83.64 | 4751.9 ± 86.84 | 0.353 |
| | Width (μm) | 1131.9 ± 17.15 | 1153.8 ± 17.86 | 0.379 |
| | Weight (μg) | 128.2 ± 1.42 | 131.8 ± 1.65 | 0.104 |
| Egg | Length (μm) | 414.7 ± 5.79 | 419.6 ± 5.80 | 0.552 |
| | Width (μm) | 259.7 ± 3.94 | 260.8 ± 3.18 | 0.829 |
| | Weight (μg) | 1.0 ± 0.03 | 1.1 ± 0.03 | 0.131 |

[a]According a Student's *t*-test

[b]Male

[c]Female

Table 5. . Development period and survival rate of each stage of *Bradysia procera* fed *Allium sativum* and *Panax ginseng* stems

| Stage | | Development period (day) | | *P*-value[a] | Survival rate (%) | | *P*-value[a] |
|---|---|---|---|---|---|---|---|
| | | Garlic | Ginseng | | Garlic | Ginseng | |
| Egg | | 5.7 ± 0.11 | 5.8 ± 0.14 | 0.710 | 86.5 ± 3.10[b] | 93.0 ± 2.26[b] | 0.106 |
| 1st | | 3.2 ± 0.10 | 3.0 ± 0.09 | 0.088 | 72.0 ± 2.08 | 86.7 ± 1.45 | 0.004 |
| 2nd | | 3.8 ± 0.16 | 3.7 ± 0.18 | 0.578 | 66.0 ± 2.08 | 80.7 ± 2.33 | 0.009 |
| 3rd | | 3.1 ± 0.14 | 2.8 ± 0.13 | 0.229 | 59.0 ± 3.79 | 72.7 ± 2.19 | 0.035 |
| 4th | Male | 5.7 ± 0.12 | 5.6 ± 0.13 | 0.852 | 48.7 ± 2.73 | 68.0 ± 2.00 | 0.005 |
| | Female | 6.5 ± 0.13 | 6.3 ± 0.14 | 0.390 | | | |
| Pupa | Male | 6.4 ± 0.16 | 6.2 ± 0.14 | 0.432 | 41.3 ± 2.91 | 61.7 ± 0.67 | 0.002 |
| | Female | 6.5 ± 0.13 | 6.3 ± 0.14 | 0.390 | | | |

Continued (Table 5).

| Stage | | Development time (day) | | *P*-value[a] | Survival rate (%) | | *P*-value[a] |
|---|---|---|---|---|---|---|---|
| | | Garlic | Ginseng | | Garlic | Ginseng | |
| Adult | Male | 5.3 ± 0.10 | 5.4 ± 0.12 | 0.532 | 39.0 ± 2.00 | 53.0 ± 3.79 | 0.031 |
| | Female | 6.8 ± 0.17 | 7.0 ± 0.13 | 0.542 | | | |
| Total | Male | 27.7 ± 0.08 | 27.2 ± 0.22 | 0.075 | | | |
| | Female | 29.6 ± 0.33 | 29.0 ± 0.16 | 0.185 | | | |

[a] According a Student's *t*-test

[b] Hatching rate

## DISCUSSION

The results of this study on *B. procera* contributed to the knowledge of the biology and rearing methods of this species under laboratory conditions.

The life cycle of *B. procera* was 33.2 to 35.8 d 25℃ in this experiment. Similarly, Smith and Stocking (1936) estimated that the life cycle of *B. corprophila* at 23℃ lasted about 27 to 33 d, and 49 to 20 d at constant temperatures of 55 and 85℉, respectively (Wilkinson and Daugherty, 1970). Steffan (1966) reported that the life cycle of *B. impatiens* at 20℃ lasted about 16.3 d. Regarding *B. coprophila*, the life cycle lasted 24 to 32 d at an unknown temperature (Hungerford, 1916) and 18 to 23 days at 18℃ (Thomas, 1931). The developmental time for the *B. procera* larvae was proportionally longer than that for *B. impatiens*, studied by Wilkinson and Daugherty (1970), who demonstrated that the larval period of *B. impatiens* was 14.1 d. Similarly, results obtained by Choi et al. (1997) for *Lycoroella* sp. showed that the larval period was 14 to 14.5 d. Wetzel et al. (1982) reported that the larval period of *L. mali* was approximately 10 d, shorter than for another fungus gnat, *S. fenestrialis* (Pitcher, 1936).

The mean egg weight for *B. procera* was 1.1 μg, whereas the egg width (*ca*. 260 μm) and length (*ca*. 430 μm) were higher than those reported for *L. ingenua* (Lewandowski et al., 2004) and *Lycoriella* sp. (Choi et al., 1997). In addition, *B. impatiens* eggs were found to have an average length and width of 0.22 and 0.12 mm, respectively (Wilkinson and Daugherty, 1970).

The average of body length of *B. procera* larvae in this study ranged from 0.6 to 8.1 mm, increasing 13.5 times during development. In this study, the average body length of *B. procera* larvae reached 10 mm, whereas *B. impatiens*, *L. mali*, and *L. ingenua* were 3.0, 2.1, and 7.9 mm, respectively (Wilkinson and Daugherty, 1970;

Lewandowski, 2004; Kielbasa and Snetsinger, 1980).

The mean body weight of *B. procera* larvae ranged from 1.3 μg for the 1st larva to 166.7 μg, suggesting the body weight increased 128 times during development. Similar results for *B. paupera* larvae were presented by Berg (2000). The average body weight of *B. paupera* larva was 0.011 to 2.238 mg; whereas, *L. mali* larvae increased 1,417 times during the larval period (Lewandowski, 2004).

In this study, the mean average body weight of *B. procera* pupae was 106.1 μg and 108.7 μg, for male and female, respectively. Tung and Snetsinger (1973) reported that the body weight of *L. mali* was 0.46 and 1.18 mg for female and male pupae, respectively this variation may reflect differences in species development or experimental conditions, such as food source, temperature, and photoperiod.

Various species of *Bradysia* larvae feed on various crops, such as mushrooms, glasshouse ornamentals, and forestry nurseries (Gouge and Hague, 1995; White et al., 2000; Shen et al., 2009; Hurley et al., 2010). However, it has been observed that *B. procera* feed and damage ginseng only. Various rearing methods have resulted in the successful maintenance of laboratory colonies of *Bradysia* spp. (Hungerford, 1916; Smith-Stocking, 1936; Wilkinson and Daugherty, 1970; Hudson, 1974; Gillespie, 1986; Gardiner et al., 1990). Hungerford (1916) reared *B. corprophila* on autoclaved potato by adding yeast and dried blood fertilizer. Hudson (1974) used pots containing horse manure and dried blood within muslin cages to rear *B. puapera*. Smith and Stocking (1936) set up an agar culture method that was modified by other researchers, including Steffan (1966) and Kennedy (1973). Plastic containers with cotton pads and moistened filter paper were used with the addition of ground soybeans (Wilkinson and Daugherty, 1970). Gillespie (1986) established a medium composed of moist peat and

crushed kidney beans that supported fungal and bacterial growth, which was modified by Gardiner et al. (1990).

In the rearing method used in this study, 18 vegetables and fruits were used to maintain a laboratory colony of *B. procera*. The results indicated the larvae were consuming and possibly developing on *B. oleracea* var. *capitata*, *B. rapa* var. *glabra*, and *A. sativa* and *P. ginseng* stems. In particular, *B. procera* larvae totally consumed *A. sativa* stems, and there was no significant difference in the morphological and temporal development than those reared on *P. ginseng* stems. However, the survival rate of *B. procera* was higher on ginseng than on garlic stems.

# 1.2. Distribution and Seasonal Occurrence Pattern of *Bradysia procera* and Its Damage Caused in Ginseng fields

## INTRODUCTION

Fungus gnats, *Bradysia* spp. (Diptera: Sciaridae) are an important insect pest in various crops, mushrooms, glasshouse ornamentals, and forest nurseries (Gouge and Hague, 1995; White et al., 2000; Shen *et al*., 2009**;** Hurley et al., 2010; Lee et al., 2010). In the Republic of Korea (ROK), this genera has been observed on 22 host plants (Lee et al., 2001; Shin *et al*., 2015) and 13 *Bradysia* species have been identified: *B. agrestis*, *B. alpicola*, *B. aprica*, *B. atracornea*, *B. boitsovoensis*, *B. chlorocornea*, *B. difformis* (= *impatiens*), *B. hilariformis*, *B. longimentula*, *B. peraffinis*, *B. procera*, *B. sachalinensis*, and *B. trispinifera* (Park et al., 1999; Kim et al., 2000; Shin et al., 2012; Shin et al., 2013; Shin et al., 2015). Among these species, *B. agrestis* (Sasakawa) (Diptera: Sciaridae) and *B. procera* (Winnertz) (Diptera: Sciaridae) damage crops in seedlings and ginseng (Park et al., 1999; Kim et al., 2000; Shin et al., 2015), respectively. In addition, fungus gnat larvae cause secondary infections by spreading pathogens (Leath and Newton, 1969). For example, *B. agrestis* feeds on soft tissues of host plants, fungal colonies, or plant debris in soil and can transmit well-known fungal pathogens, such as *Fusarium* and *Phoma*, by injection into the inner tissue of host plants (Kim et al., 2009; Ludwig and Oetting, 2001). Owing to such a wide spectrum of hosts, and direct and indirect damage caused by *Bradysia* spp., they have been recognized as serious insect pests in agricultural production.

Ginseng (*Panax ginseng* C. A. Meyer) is the most valued of all medicinal herbs in Korea, China, and Japan (Choi, 2008). In 2009, the global ginseng market was estimated at

approximately 1,130 million USD, and South Korea at 613 million USD (Baeg and So, 2013). In addition, ginseng cultivation area and production, especially 6-y old ginseng, have been expanded owing to the increasing annual consumer demand (MAFRA, 2012; Baeg and So, 2013). In ginseng cultivation, especially 6-y old ginseng, fields are exposed to various plant diseases and insect pests owing to a long-term periods of cultivation (Lee et al., 2010). To control ginseng pests, growers rely on the use of pesticides; however, improper or unregistered pesticide application is restricted in ginseng fields owing to the risk of unwanted residues.

In ginseng fields, 14 insect pests have been reported: *Ectinus sericeus*, *Holotrichia diomorphalia*, *H. morose*, *H. titanis* and *Maladera orientalis* (Coleoptera), and *Glyllotalpa africana* and *Teleogryllus emma* (Orthoptera), *Agrotis tokionis*, *Mamestra brassiace* and *Ostrinia furnacalis* (Lepidoptera), *Bradysia procera* and *Hydrellia griseola* (Diptera), *Metcalfa pruinosa* and *Pseudococcus comstocki* (Homoptera) (Kim, 1994, Kim, 2014, Shin et al., 2015). Among these insect pests, *B. procera* is an important insect pest in ginseng cultivation owing to recent heavy infestations on 5–6 y old cultivated ginseng in South Korea. However, *B. procera* has not reported been reported as an insect pest of any crops other than ginseng; moreover, there is limited global information on this species.

To establish the efficient management of *B. procera* populations, accurate information of the target species is required, which included infestation symptoms and damage, and distribution and occurrence; however, previous data have lacked necessary information to understand the biology of *B. procera*.

The present study aimed to examine precipitation and environmental variables affecting the occurrence pattern of *B. procera*. The distribution and occurrence of *B. procera* in inseng fields and damage to ginseng fields in ROK during the growing period were investigated.

## Materials and Methods

### 1.2.1. Survey on the distribution of *Bradysia procera* and damage rate in ginseng fields in South Korea

Field surveys were conducted over 3 years (2012–2014) on the distribution of *B. procera* and their damage rate (%) at 6-y old randomly selected commercial ginseng production fields in ROK during June to September. The field surveys included 130 ginseng fields from 27 different locations distributed in 7 provinces. All ginseng fields were cultivated using standard agricultural practices, but pesticide applications were not investigated. Each field investigated was 66 $m^2$, which included a simple visual inspection where all peduncles, petioles, and stems were checked for larval infestation. Moreover, the peripheral areas of the ginseng fields were investigated, including nearby forests, streams, and transplanted soil types.

The percentage of damaged stems (PD) was calculated from the formula: $PD = (D / T) \times 100$, where $D$ is the number of damaged individuals, and $T$ is the total number of ginseng stems investigated.

### 1.2.2. Relationship between seasonal occurrence of *Bradysia procera* and environmental variables

Seasonal occurrence patterns of the fungus gnat adults were examined at two commercial ginseng production fields in Cheongju, Chungbuk Province (var. Jakyung, N 36 ° 43′21.5″, E 127 ° 21′21.0″) in 2012 and Yeoju, Gyeonggi Province (var. Cheonpoong, N 37 ° 18′31.6″, E 127 ° 33′15.8″) in 2013 during May to October. Each field was observed for fungus gnat damage the previous year (2011 [CJ] and 2012 [YJ]). Each field was 10,000

$m^2$ and 6,600 $m^2$, respectively, and both experimental plot scales were 825 $m^2$.

To examine the relationship between the abundance of fungus gnat adults and environmental variables (average temperature, precipitation, and relative humidity), stepwise multiple regressions were conducted to test the relative importance of all independent environmental variables. Stepwise multiple regressions were conducted using R version 3.3.2 (R Core Team 2016). We performed stepwise multiple regressions using the 'MASS' package. For collecting the adults at each field, yellow sticky flat traps (25 × 15 cm, n = 15 per plot, Green Agrotech, Ltd., Kyungsan, Korea) were established under shade net of 6 m in the middle of each plot, and the number of *B. procera* adults on the traps were counted at 7 d intervals and the traps were replaced with new ones from April to October. The environmental variables were obtained from the Korea Metrological Administration.

### 1.2.3. Symptoms of damage caused by *B. procera* larvae in ginseng field

The *B. procera* damage caused was observed in 6 y old ginseng fields using quadruple-clothed black and blue quadruple polyethylene mats and shading sheets (0.5 ha with about 1,500 Kan, var. Cheonpoong, N 37 ° 20′40.8″, E 127 ° 30′25.4″) in Yeoju, during June to September and until near harvest in mid-October 2013. This field was 5,000 $m^2$ and the experimental plot size was 66 $m^2$. The plot was maintained using standard agronomic practices and irrigated as needed, but insecticides were not applied in the experimental period. During the investigation, the experimental plot was visited at 2 wk intervals and each ginseng plant was checked for the presence or absence of damage by larval feeding on the aerial parts by visual inspection. The characteristic damage symptoms by larval infestation were carefully investigated and photographs were taken of the peduncles, stems, and roots of ginseng. Moreover, some of the damaged aerial parts and

roots were stored in plastic bags and transported in a cooler to the laboratory, and photographed with a digital camera (D40, Nikkon, Japan) equipped macro lens (AF-S VR, Nikon, Japan).

### 1.2.4. Data analysis

The percentage of damaged stem (DS) was calculated from the formula: $DS = (D / T) \times 100$, where $D$ is number of damaged individuals, and $T$ is the total number of ginseng stems investigated.

## Results

### 1.2. 1. Occurrence and distribution of *Bradysia procera* in Korea

Among ginseng fields surveyed in seven provinces in ROK, *B. procera* damage was found in six provinces; Gangwon-do (GW), Gyeonggi-do (GG), Chunchungnam-do (CN), Chunchungbuk-do (CB), Jeollabuk-do (JB), and Gyeongsangbuk-do (GB). However, no damage was found in Jeollanam-do (JN). Among 130 observed areas, 35 areas in 27 locations were observed to have damaging caused by *B. procera* larvae during 2012 to 2015 (Table 6).

The degree of damage by *B. procera* was estimated to range from 0.1 to 25.0%, with both the highest and lowest percentage occurring Cheolwon and Bongwha, respectively. Especially, in Cheolwon, the damage was observed in all areas every year and showed > 15% damage rate. In addition, extensive stem damage rate (> 20%) was observed, including nearby forest fields (FF) with nearby streams (NS) in Cheolwon (2013–2015) and Yangpyeong (2014 and 2015). However, intensive stem damage in Chuncheon, Pocheon, and Bongwha were observed at < 1 % (Table 6).

Table. 6 Survey on the occurrence and damage rate of the fungus gnat, *Bradysia procera*, in ginseng cultivation areas in South Korea.

| Province | Region (a/b)[a] | Location | Year | Survey date[b] | PD (%)[b] | SEF[c] |
|---|---|---|---|---|---|---|
| GW | Cheolwon (5/5) | Cheolwon-eup | 2012 | June 12 | 16.5 | FF |
| | | | 2013 | June 22 | 25.0 | FF, NS |
| | | | 2015 | July 11 | 15.3 | FF |
| | | Kimhwa-eup | 2014 | August 8 | 17.5 | FF, NS |
| | | | 2015 | August 21 | 23.5 | FF, NS |
| | Chuncheon (1/6) | Sabuk-myeon | 2012 | June 11 | 0.7 | CF |
| | Hoengsung (2/8) | Dunnae-myeon | 2012 | June 14 | 1.5 | CF |
| | | | 2013 | July 24 | 4.4 | FF |
| | Hongcheon (2/5) | Dong-myeon | 2013 | July 24 | 10.1 | CF, NS |
| | | Nae-myeon | 2014 | August 7 | 5.8 | CF, NS |
| | Hwacheon (1/4) | Sangseo-myeon | 2015 | July 11 | 11.5 | FF, PF |

Table. 6 (continued)

| Province | Region (a/b)[a] | Location | Year | Survey date | PD (%)[b] | SEF[c] |
|---|---|---|---|---|---|---|
| GW | Wonju (1/7) | Hojeo-myeon | 2012 | July 24 | 1.8 | FF |
| | Yanggu (1/7) | Dong-myeon | 2013 | September 3 | 2.8 | FF |
| | Yeongwol (1/7) | Jucheon-myeon | 2012 | July 4 | 2.4 | FF |
| GG | Icheon (3/11) | Baeksa-myeon | 2012 | May 30 | 1.5 | CF, NS |
| | | | 2013 | June 20 | 3.8 | CF, NS |
| | | | 2014 | July 14 | 12.5 | FF, PF |
| | Paju (1/4) | Jangdan-myeon | 2012 | September 10 | 7.8 | FF |
| | Pochun (1/5) | Yeongjung-myeon | 2012 | September 12 | 0.5 | FF |
| | Yangpyeong (2/6) | Cheongwoon-myeon | 2014 | September 11 | 20.0 | FF, NS |
| | | | 2015 | September 3 | 15.4 | FF, NS |
| | Yeoju (3/9) | Neungseo-myeon | 2014 | June 20 | 18.6 | CF, NS |

Table. 6 (continued)

| Province | Region (a/b)[a] | Location | Year | Survey date | PD (%)[b] | PFG[c] |
|---|---|---|---|---|---|---|
| GG | | Heungchun-myeon | 2014 | June 9 | 19.4 | FF |
| | | Neungseo-myeon | 2015 | August 17 | 12.1 | FF |
| | Yeonchun (2/9) | Shinseo-myeon | 2012 | June 25 | 7.8 | FF |
| | | Jung-myeon | 2012 | June 25 | 5.0 | FF |
| CN | Sejong (2/8) | Janggun-myeon | 2013 | July 29 | 1.8 | PF |
| | | | 2014 | July 14 | 2.1 | PF |
| | Seosan (1/4) | Buseok-myeon | 2012 | June 15 | 1.7 | PF |
| CB | Cheongju (2/8) | Ochang-eup | 2012 | August 7 | 16.8 | CF, NS |
| | | | 2014 | July 16 | 15.1 | PF, NS |
| | Chungju (1/3) | Sotae-myeon | 2014 | September 13 | 21.1 | FF |
| JB | Gochang (1/6) | Gochang-eup | 2013 | June 27 | 1.3 | RF |

| Province | Region (a/b)[a] | Location | Year | Survey date | PD (%)[b] | PFG[c] |
|---|---|---|---|---|---|---|
| GB | Bongwha (2/5) | Bongwha-eup | 2012 | August 28 | 0.7 | FF |
| | | | 2014 | July 2 | 0.1 | FF |
| JN | Yeongam (0/1) | Yeongam-eup | 2013 | July 15 | - | CF |
| | Haenam (0/2) | Hwasan-myeon | 2013 | July 16 | - | CF |

[a] a/b, Number of ginseng fields where the infested stem by *B. procera* larvae was observed (a) per total number of surveyed ginseng fields (b).

[b] Percentage of the damaged stem rate of ginseng stems (PD) (%) = (Number of damaged stems/Total number of surveyed stems) × 100.

[c] Peripheral factors in ginseng field s(PFG). CF: cultivated field; FF: forest field; NS: nearby stream; PF: paddy field; RF: reclamation field.

### 1.2. 2. Seasonal occurrence patterns of *Bradysia procera* in relation to weather conditions

The seasonal occurrence patterns of *B. procera* adults was observed on the yellow sticky flat traps in two experimental ginseng fields in 2012 and 2013. The fungus gnat adult showed three peaks per year, with the greatest populations in August in both fields. *B. procera* adults were first observed from late-May when the daily average temperature was above 20℃. In Cheongju, the first generation was observed in mid-June, and second and third generations were revealed mid to late July and late August. However, first, second and third generation peaks were revealed early June, mid-July, and mid- August, respectively, in Yeoju (Fig. 12).

The temporal variation of fungus gnat adults between Cheongju and Yeoju fields revealed significant differences in environmental variables (Table. 7). The seasonal occurrence of adults was related to the average temperature and relative humidity ($F_{3,19}$ = 9.37, $P < 0.001$) in the Cheongju field. However, the temporal pattern of fungus gnat adults in Yeoju showed significant differences in the average temperature only ($F_{1,24}$ = 9.07, $P$ = 0.006).

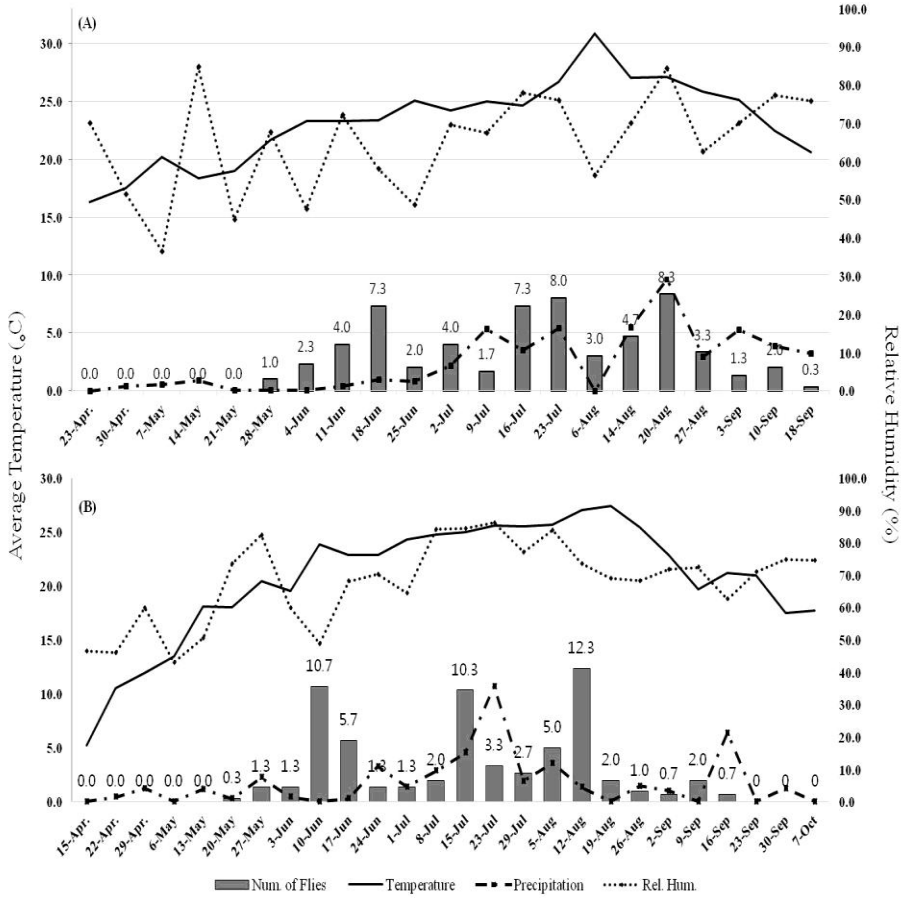


**Fig. 12.** Seasonal occurrences of *Bradysia procera* adults captured on yellow sticky flat traps during three seasons in four different areas

Table 7. Relationship between abundance of fungus gnat adult and selected independent variables determined using a stepwise multiple regression analysis.

| Site | (Intercept) | Avr. Temp | Precip. | Rel. Hum | Statistics | | |
|---|---|---|---|---|---|---|---|
| | | | | | adj. $r^2$ | *F* | *P* |
| Cheongju | $^{***}$-15.0048 | $^{***}$0.4967 | -0.0021 | $^{**}$0.1063 | 0.533 | 9.37 | <0.001 |
| Yeoju | -4.9312 | $^{**}$0.3536 | $^{\dagger}$N.E. | $^{\dagger}$N.E. | 0.244 | 9.07 | 0.006 |

Superscript asterisks indicate the significance of a *P* value ($^{**}$ <0.01, $^{***}$ <0.001). The table includes the parameter (beta, relative importance of the predictor) for each variable in the models as well as the significance level and adj. $r^2$ for the overall models.

$^{\dagger}$N.E. indicates that the given variable was not entered into the regression model.

### 1.2. 3. Damage caused by *Bradysia procera* larvae

The larvae feed on healthy plant tissue of the aerial part of ginseng plants, including peduncles, petioles, stems, and even tunnel into the roots.

In the initial period of infection by ginseng fungus larvae, ginseng peduncles were distinguished by color differences in late June to July. When compared to normal peduncles that showed a greenish color (Fig. 13-A). The, whereas, the damaged peduncles observed showed partial discoloration (Fig. 13-B). The discolored (or infested) peduncles had observed injury marks (Fig. 13-C) and larvae (Fig. 13-D) inside when it dissected.

During the medium period of injury in August, damaged petioles were crushed (Fig. 14-A) after total discoloration of the peduncles (Fig. 14-B), and during this period ecdysis molts and feeding marks on ginseng peduncles were observed (Fig. 14-C and D). Also, during this period, eggs were observed on the injured parts (caused by fungus gnats) of ginseng stems (Fig. 15) and secreted transparent mucus (Fig. 16) from larva covered the external entrance, which seemed to protect against predators and maintain the proper humidity for effective survival.

During the later period of infection in late August, the larval feeding resulted in an increased area of injured marks (Fig. 17-A) on entire stems, petioles, and leaves, which turned brown and withered (Fig. 17-B). This intensive damage caused ginseng to lose vitality, crush the petiole, and die (Fig. 17-C), and occasionally resulted in root decay (Fig. 17-D). Unlike in the summer, *B. procera* larvae were not observed in damaged peduncles and petioles in the harvest (or fall) season; however, the larvae occurred in the head part of ginseng roots (Fig. 18-A), old stems below the soil (Fig. 18-B), and sometimes inside the roots (Fig. 18-C).


**Fig. 13.** Initial damage symptoms infested by *Bradysia procera* larvae in ginseng peduncle.

A: normal peduncle, B: infested peduncle. C: injured peduncle, D: 1$^{st}$ larvae in peduncle.

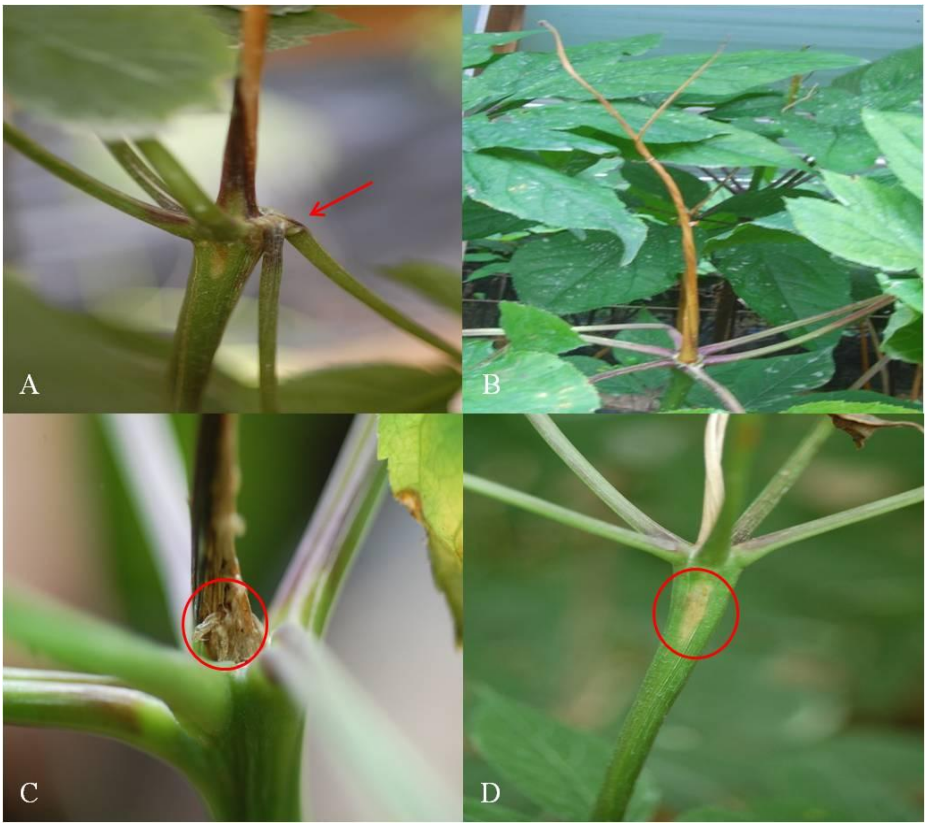


**Fig. 14.** The medium period of damage caused by *Bradysia procera* in August. A: crushed ginseng petiole, B: discoloration of peduncles, C: ecdysis molt of *B. procera* adults, D: feeding marks on ginseng stems.

**Fig. 15.** Oviposition on damaged ginseng peduncles by *Bradysia procera* adult.

**Fig. 16.** Mucus secretion by *Bradysia procera* larvae.


**Fig. 17.** Lateral damage caused by *Bradysia procera* larval infestations in ginseng stems and roots. A: expanded injury mark, B: discoloration and wilt of aerial parts, C: death of aerial parts, D: decayed ginseng roots.


**Fig. 18.** *Bradysia procera* larvae in damaged ginseng stems and roots. A: old stem, B: head part of ginseng root, C: invasion in ginseng root.

## DISCUSSION

The results of this study demonstrated seasonal occurrences and damage caused by *B. procera* in cultivated ginseng fields in ROK. The correct symptoms of damage, distribution, and occurrence of this fungus gnat in ginseng cultivation are important for the establishment of effective and sustainable management strategies. Fungus gnat, *Bradysia* spp., larvae are important insect pests in various crops such as bean, carrot, cucumber, lettuce, potato, soybean, and tomato (Kennedy, 1970, 1974; Hafidh and Kelly, 1982; Speyer, 1923; Wilkinson and Daugherty, 1970; Gillespi and Menzies, 1993). Globally, there is limited information on *B. procera*, such as biology and ecology in ginseng cultivation. Damage to ginseng crops was first reported in Yongin, Korea, in 1994. In this study, we first report *Bradysia procera* (Winertz) in ROK, with a description of its damage to peduncles, stems, and roots of the ginseng. Also, we examined the distribution, damage rate, and seasonal occurrence of *B. procera* in ginseng fields. *B. procera* larvae tunneled into the stem and fed on infected ginseng, and resulted in the discoloration and death of aerial parts such as petioles, leaves, and stems, and secondary infections. The intensive damage caused a loss of vitality even occasional root decay. The distribution of the fungus gnat was observed in most cultivated ginseng fields in Korea, excluding Jeollaman-do Province. Among 106 ginseng fields located in 36 locations surveyed for 3 y, the fungus gnat, *B. procera* occurred in a total of 35 ginseng fields (25.7%), in 21 regions (69.4%), and the average damage rate of *B. procera* in ginseng fields was 9.3%; however, Cheolwon locations showed and average damage rate of 19.6%. This result indicated that *B. procera* spread throughout most ginseng cultivation areas; however, it preferred shady and high humidity habitats with nearby forests and streams. Similarly, the occurrence of

*Phytosciara procera* damage to ginseng is more prevalent in areas with high humidity and heavy and frequent fogging (Lee et al., 2010). Also, in ginseng cultivation areas, adult *Phytosciara procera* (Diptera: Sciaridae) emergence increased in late July and early or late August to early September (Shin et al., 2008; Lee et al., 2010). Temporal variation in environmental variables, such as temperature and relative humidity, is important to *B. procera*. Therefore, microclimatic variables could be used to estimate *B. procera* density in ginseng fields. In particular, temperature and humidity are important for *Bradysia* spp. in terms of life cycle and development (Lee et al., 1998; Yuting, 2015). Also, insects are affected by various biotic and abiotic factors (Messenger, 1959), such as rainfall, soil moisture, temperature, humidity (or combined temperature and humidity), and locality. In our study, we demonstrated that the average temperature was related to the seasonal occurrence (Table 7) of *B. procera*, which was first captured when the daily average temperature was above 20℃ in May; however, seasonal occurrence patterns were inconsistent (Fig. 12). Therefore, the differences in occurrence patterns owing to the differences in environmental characteristic between the two sites is possibly a result of temporal variation and humidity.

This result was first recorded in ginseng fields in ROK, where *B. procera* damaged occurred in most ginseng cultivation areas. Our surveys revealed that three generation peaks of *B. procera* occurred at ginseng cultivation areas, and were especially affected by the average temperature. However, the present data could result in some uncertainties because of the short-term survey and limited data. In addition, because ginseng fields are moved every 6 y, research of other environmental variables would also be needed. Therefore, *B. procera* must be monitored and controlled as necessary during the ginseng growing season to avoid the serious damage that this insect pest can

cause.

# CHAPTER II

# Larvicidal and Ovicidal activities and Possible Mode of Action of *Syzygium aromaticum* Bud and *Illicium verum* Fruit Derived Constituents to Ginseng fungus gnat, *Bradysia procera* (Diptera: Sciaridae)

## 2.1. Larvicidal and Ovicidal Activities and Possible Mode of Actions of Constituents Identified in *Syzygium aromaticum* Bud and *Illicium verum* Fruit against *Bradysia procera*

## 2.2. Field Evaluation of *Syzygium aromaticum* bud derived Materials against *Bradysia procera* on *Panax ginseng* fields

# 2.1. Larvicidal and Ovicidal Activity of Constituents Identified in *Syzygium aromaticum* bud and *Illicium verum* fruit against *Bradysia procera*

## INTRODUCTION

The ginseng fungus gnat, *Bradysia procera* (formerly *Phytosciara procera*), is one of the most serious pests of economically inmortant ginseng plants (*Panax ginseng*) in the republic of Korea (ROK) (Shin et al., 2008). It was first identified from collections made in ginseng field in Paju (Gyeonggi, ROK) in 2005 (Shin et al., 2008). Since then, the fungus gnat have dispersed rapidly in the *P. ginseng* field. If not managed properly from the early growth stage of the plant, *B. procera* causes serious damage directly from larvae feeding on the peduncles and stems of *P. ginseng* and even the roots, and indirectly from adults spreading diseases such as soft rot, *Erwinia carotovora* subsp. *Carotovora* (Shin et al., 2008). Approximately 30% of *P. ginseng* damaged by *B. procera* are also infected by bacterial disease caused by soft rot (Shin et al., 2008). *Bradysia procera* had a 30–40 days per generation, and over two to three generations can occur annually in a *P. ginseng* field (Lee et al., 2010). The seasonal occurrence and economic importance of *B. procera* have been well described well by Shin et al (2008), although much of the biology of *B. procera* remains unknown. Controlling *B. procera* populations in the ROK has been achieved principally by the use of two commercial insecticides metaflumizone and spinetoram. These insecticides are registered for ginseng fields in the ROK (KCPA, 2017), although insecticides uses are limited in the *P. ginseng* cultivation because of widespread use of ginseng as a health food. Increasing public concern for the environmental effects of insecticides, human health

effects, residues in *P. ginseng*, and undesirable effects on nontarget organisms intensifies when conventional contact insecticides are used repeatedly. In addition, the number of approved insecticides will probably be reduced soon in the United States (US) (USEPA, 2017) and in the European Union (EU) as reregistration occur. The removal of those conventional insecticide products from markets due to the increase resistance or other concerns will have a serious impact on the proliferation of *B. procera*. Therefore, there is a pressing need for the development of selective control alternatives with novel target sites to establish an efficient management strategy and tactics for *B. procera*, particularly those with fumigant action, which allow effective application in *P. ginseng* plants that reach deep harborages missed by conventional spraying of many commercially available insecticides.

Plants may provide potential sources of insect pest control products largely because they are sources of bioactive secondary metabolites (SMs) that are perceived by the general public as relatively safe and posing less risk to the environment, and with minimal impacts to human and animal health (Sukumar et al., 1991; Isman, 2006; Koul et al., 2008; Pavela, 2016). The SMs act at multiple, novel target sites (Sukumar et al., 1991; Isman, 2006; Koul et al., 2008; Pavela, 2016), thereby reducing the potential for resistance (Chae et al., 2014; Yi et al., 2016).The major target sites of plant SMs include cholinergic system (e.g., acetylcholinesterase (AChE), nicotinic acetylcholine receptors), γ-aminobutyric acid (GABA) system (e.g., GABA-gated chloride channel), mitochondrial system (e.g., sodium and potassium ion exchange disruption, mitochondrial complex I electron transport inhibitor), and octopaminergic system (octopaminergic receptors, blockage of the octopamine receptors by working through tyramine receptors cascade) (Isman, 2006; pavela et al., 2016; Rattan, 2010).

As judged by these benefits of botanical insecticides, numerous papers are published annually (Isman and Grieneisen, 2014). Phytochemicals are regarded as potential sources to develop commercial insecticides as certain plant-derived preparations and their constituents meet the criteria as minimum-risk insecticides (Koul et al., 2008; Isman and Grieneisen, 2014; USEPA, 2015). Previous studies have shown that a methanol extract from the buds of the clove plant, *Syzygium aromaticum* (L.) Merrill and Perry (Myrtales: Myrtaceae), exhibited good toxicity to third-instar larvae and eggs of *B. procera*. However, no previous studies have investigated the potential use of *S. aromaticum* for managing *B. procera* for future commercialization. The plant extract possessed the toxicity to *Culex pipiens* larvae (EI Hag et al., 1999) and two stored grain beetle species (Ho et al., 1994), whereas the plant essential oil possessed the toxicity to *Pediculus capitis* (Yang et al., 2003), *Reticulitermes speratus* (Park et al.,2005), and two *Dermatophagoides* species (Kim et al., 2003), as well as repellency against *Anopheles dirus* mosquitoes (Trontokit et al., 2005). Traditional medicine uses of *S. aromaticum* have been well documented by Chaieb (2007) and Cortés-Rojas et al (2014). In addition, the methanol extract from the fruits of star anise plant, *Illicium verum* (Austrobaileyales: Schisandraceae), possessed good larvicidal activity against *B. procera*. However, no previous studies have investigated the potential use of *I. verum* for managing *B. procera*, despite insecticidal activity against *Drosophila melanogaster* [(Miyazawa, 1993), *Tribolium castaneum* eggs, larvae and adults and *Sitophilus zeamais* adults [Ho et al., 1995; Wei et al., 1995), *Blattella germanica* adults (Chang, 2002), *Lasioderma serricorne* adults (Kim et al.,2003), and *Callosobruchus chinensis* larvae and adults (Chaubey, 2008). Traditional use, chemistry, and pharmacological properties of *I. verum* have been well documented by Wang et al. (2011).

In this study, we aimed to assess the potential of *S. aromaticum* bud and *I. verum* fruit methanol extract, and *S. aromaticum* bud and *I. verum* fruit hydrodistillate and their constituents against third-instar larvae and eggs of *B. procera*, using the filter-paper contact and vapor-phase mortality bioassays. The results were compared with those of five insecticides clothianidin, thiamethoxam, cypermethrin, dichlorvos, and emamectin benzoate to assess their use as future commercial larvicides and/or ovicides. In addition, the route of larvicidal action was determined and compared with that of two fumigants dazomet and metam-sodium. Finally, the possible mechanism underlying the larvicidal actions of the phenylpropanoids (*trans*-anethole estragole, eugenol, 2-hephyl acetate and eugenyl acetate), terpenoids (methyl salicylate, α-copaene, caryophyllene oxide) and ketone (2-nonanone) against *B. procera* was elucidated by determining the acetylcholinesterase (AChE) inhibition and cyclic adenosine monophosphate (cAMP) levels.

## Materials and Methods

### 2.1.1. Instrumental analysis

The $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra were recorded in deuterochlorform on an AVANCE 600 spectrometer (Bruker, Rheinspettem, Germany) at 600 and 150 MHz, respectively, using tetramethylsilane as an internal standard. The chemical shifts are given in δ (ppm). A distortionless enhancement by polarization transfer spectra was acquired using Bruker software. The ultraviolet (UV) spectra were obtained in methanol on a Jasco 550 series spectrophotometer (Jasco, Tokyo, Japan), and the mass spectra were acquired on a JMS-DX 303 spectrometer (Jeol, Tokyo, Japan). Silica gel 60 (0.063–0.2 mm) (Merck, Darmstadt, Germany) was used for column chromatography. Merck precoated silica gel plates (Kieselgel 60 $F_{254}$) were used for analytical thin-layer chromatography (TLC). An Agilent 7890A gas chromatograph and an Agilent 6890N with a HP7973 mass selective detector (Agilent, Santa Clara, CA) were used for chemical analysis. A Waters Alliance 2695 high-performance liquid chromatograph (Waters, Milford, MA) equipped with a Waters 2998 photodiode array detector was used to isolate the active constituents.

### 2.1.2. Tested insect

The stock cultures of *B. procera*, originally collected from the stems from *P. ginseng* plants (5 years old) in the *P. ginseng* field in Yeoju (Gyeonggi, ROK) in mid-July of 2013, have been maintained in an insect rearing room without exposure to any known insecticide. They were reared in polyethylene containers (5.5-cm diameter × 10.5 cm) containing five pieces of stems (5 cm) from a fresh *P. ginseng* plant (5 years

old). The containers were held at 25 ± 1 ℃, 50–60 % relative humidity, and a 16:8 h light:dark cycle. Under these conditions, longevity of eggs, larvae, pupae, and adults was approximately 6, 16, 7, and 6 days, respectively.

To synchronize the developmental stages for bioassays, the adult flies were placed on five pieces of stems (10 cm) from fresh *P. ginseng* plants (5 years old) in polyethylene containers (5.5-cm diameter × 10.5 cm) with a Hausherr's MX-991 handheld battery-powered aspirator (Tom Rivers, NJ) and allowed to lay eggs for 24 h. After this time, the adults were removed and the infested stems were held at the same conditions as those described above.

**2.1.3. Chemical**

Fourty three compounds examined in this study were as follows: *p*-allylphenol obtained from BOC Science (Shirley, NY, USA), acetaldehyde (99.5 % purity), anethole (99.0 % purity), (*E*)-anethole (99.0 % purity), *p*-anisaldehede (98.0 % purity), borneol (97.5 % purity), camphene (95.0 % purity), δ-3-carene (90.0 % purity), 1, 4-cineol (≥95.0 % purity), caryophyllene oxide, α-copaene, *p*-cymene, cypermethrin, dazomet (99.9 % purity), eugenol, linalool, linalool oxide (95.0 % purity), β-myrcene (90.0 % purity), α-phellandrene (≥85.0 % purity), γ-terpinene (97.0 % purity), methyl salicylate, α-humulene, 2-nonanone, linalool, α-pinene (99.0 % purity) and eugenyl acetate (98.0 % purity) purchased from Sigma-Aldrich (St. Louis, MO, USA), α-terpinene (85.0 % purity), γ-terpineol (90.0 purity) and heptanone (99.0 % purity) purchased from Fluka (Buchs, Swizerland), β-caryophyllene (>90.0 % purity), 1,8-cineol (99.0 % purity) limonene (95.0 % purity), α-terpineol (>95.0 % purity), terpinolene (90.0 % purity) and (±)-Limonene purchased TCI (Tokyo, Japan), β-caryophyllene, α-pinene (90.0 % purity)

and terpinen-4-ol purchased from Wako (Osaka, Japan). clothianidin (99.9 % purity), dichlorvos (100 % purity), emamectin benzoate (99.7 % purity), thiamethoxam (99.6 % purity) and metam-sodium (99.5 % purity) were supplied by Sigma-aldrich and Supelco (Bellefonte, PA, USA), respectively. Tween-40 was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of reagent grade and available commercially.

**2.1.4. Plants and hydrodistillations**

2.1.4.1. Plant sources

The buds from *S. aromaticum* were purchased from the Boeun medicinal herb shop at Seoul Yangnyeongsi (Seoul, ROK) and air-dried fruits from *I. verum* were purchased from the Samrak medicinal herb shop (Seoul Yangnyeongsi, Seoul, ROK). The voucher specimens (SA-B01 and IV-FR01) were deposited in the Research Institute of Agriculture and Life Sciences at Seoul National University.

2.1.4.2. Hydrodistillation

Air-dried bud (600 g) of *S. aromaticum* and *I. verum* fruit (600 g) finely grounded and subjected to steam distillation at 100°C for 2 h using a Clevenger-type apparatus. The volatile oil was dried over anhydrous sodium sulfate and stored in a sealed vial at 4 °C until use. The yield of the *S. aromaticum* bud and *I. verum* fruit hydrodistillate were12.2 and 7.8 % based upon dried weight of bud and fruit, respectively.

2.1.4. 3. Extraction and isolation

2.1.4. 3.1. Isolation procedure of bud of *S. aromaticum*

The air-dried buds (500 g) from *S. aromaticum* were finely powdered, extracted with

methanol (2 × 3 L) at room temperature for 1 day, and filtered. The combined filtrate was concentrated to dryness by rotary evaporation at 40 ℃ to yield approximately 842 g of a dark brownish tar. The extract (120 g) was sequentially partitioned into hexane- (84.3 g), chloroform- (16.2 g), ethyl acetate- (4.1 g), butanol- (8.4 g), and water-soluble (7.0 g) portion for subsequent bioassay (Fig. 19). This fractionation procedure was repeated three times. The organic solvent-soluble portions were concentrated under a vacuum at 40 ℃, and the water-soluble portion was freeze-dried. To isolate the active constituents, 224.8–56.2 μg/cm$^2$ of each *S. aromaticum* bud-derived fraction were tested in a filter-paper mortality assay as described in the section 2.1. 8.

The hexane-soluble fraction (80 g) was the most biologically active fraction, and it was chromatographed on a 5.5 × 70 cm glass column (600 g silica gel). Separation was achieved with a gradient of hexane and ethyl acetate [(95:5 (2000 ml), 90:10 (1000 ml), 80:20 (2000 ml), and 50:50 (1000 ml) by volume] and then elution with methanol (1500 ml) to provide 28 fractions (each approximately 250 ml). The column fractions were monitored by TLC on the silica gel plate developed with a hexane and ethyl acetate (7:3 by volume) mobile phase. Fractions with similar $R_f$ values on the TLC plates were pooled. The spots were detected by spraying the plate with 2% sulfuric acid and then heating the samples on a hot plate. Active fractions 9–19 (H2) were pooled and rechromatographed on a 4 × 60 cm column (450 g silica gel) using hexane and ethyl acetate (90:10 (4000 ml) and 80:20 (2000 ml) by volume) and a final elution with methanol (1000 ml) to provide 28 fractions (each approximately 250 ml). The column fractions were monitored by TLC on silica gel plates developed with a hexane and ethyl acetate (9:1 by volume) mobile phase. Active fractions 6–15 (H22) were pooled. A high-performance liquid chromatography (Table 8) was performed to

separate the constituents from fraction H22. The column was a 4.6 mm i.d. × 250 mm Supelco discovery C18 column (Bellefonte, PA) with a mobile phase of acetonitrile and water (8:2 by volume) at a flow rate of 1 ml/min. The chromatographic separation was monitored using a UV detector at 231 nm (Fig. 21). Finally, active compound **1** (3.1 g) was isolated (Fig. 21) at a retention time of 1.42 min (Fig. 25).

2.1.4.3.2. Isolation procedure of fruit of *I. verum*

The air-dried fruits (6.0 kg) of *I. verum* were pulverized, extracted with methanol (2 × 3 liters) at room temperature for 1 day, and filtered. The combined filtrate was concentrated to dryness by rotary evaporation at 40℃ to yield approximately 528 g of a dark greenish tar. The extract (100 g) was sequentially partitioned into hexane- (44.7 g), chloroform- (6.2 g), ethyl acetate- (2.4 g), butanol- (13.3 g), and water-soluble (33.4 g) portions for subsequent bioassays (Fig. 20). This fractionation procedure was repeated three times. The organic solvent-soluble portions were concentrated under a vacuum at 40℃, and the water-soluble portion was concentrated at 50℃. To isolate the active constituents, 225–75 μg/cm$^2$ of each *I. verum* fruit-derived fraction was tested in a filter-paper contact + fumigant mortality bioassay (Yang et al. 2003).

The hexane-soluble fraction (40 g) was the most biologically active fraction, and it was chromatographed on a 5.5 × 70 cm silica gel (600 g) column. Separation was achieved with a gradient of hexane and ethyl acetate (99:1 (1500 ml), 90:10 (1000 ml), 70:30 (2000 ml), and 50:50 (1000 ml) by volume) and then elution with methanol (2000 ml) to provide 30 fractions (each of which reached approximately 250 ml). The column fractions were monitored by TLC on the silica gel plate developed with a hexane and ethyl acetate (7:3 by volume) mobile phase. Fractions with similar $R_f$

values on the TLC plates were pooled. The spots were detected by spraying the plate with 10% sulfuric acid and then heating the samples on a hot plate. Active fractions 1–14 (H1) were pooled and rechromatographed on a 4 × 60 cm silica gel (450 g) column by elution with hexane and ethyl acetate (100:0 (2500 ml) and 80:20 (2000 ml) by volume) and a final elution with 1000 ml of methanol to provide 21 fractions (each of which had a volume of approximately 250 ml). A preparative HPLC was used (Table 32) to separate the constituents from active fractions 1–16 (H11). The column was a 4.6 mm i.d. × 250 mm discovery C18 column (Supelco, Bellefonte, PA) with a mobile phase of acetonitrile and water (8:2 by volume) at a flow rate of 1.0 ml/min. The chromatographic separation was monitored using a UV detector at 254 nm (Fig. 22). Finally, active compound **1** (42.2 mg) was isolated (Fig. 26)at a retention time of 5.1 min (Fig. 24).

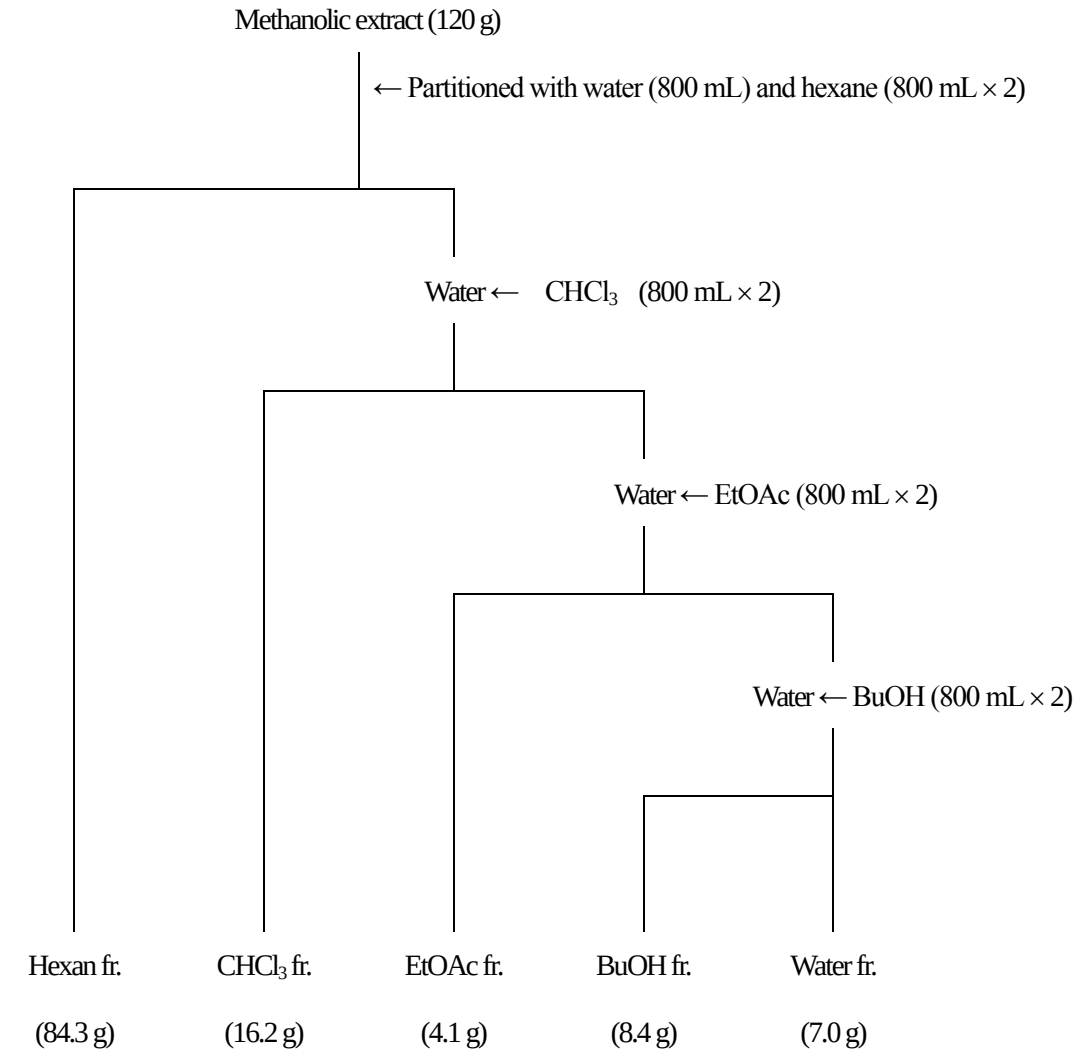


**Fig. 19.** Procedure of solvent partitions for methanolic extract of *Syzygium aromaticum* bud.

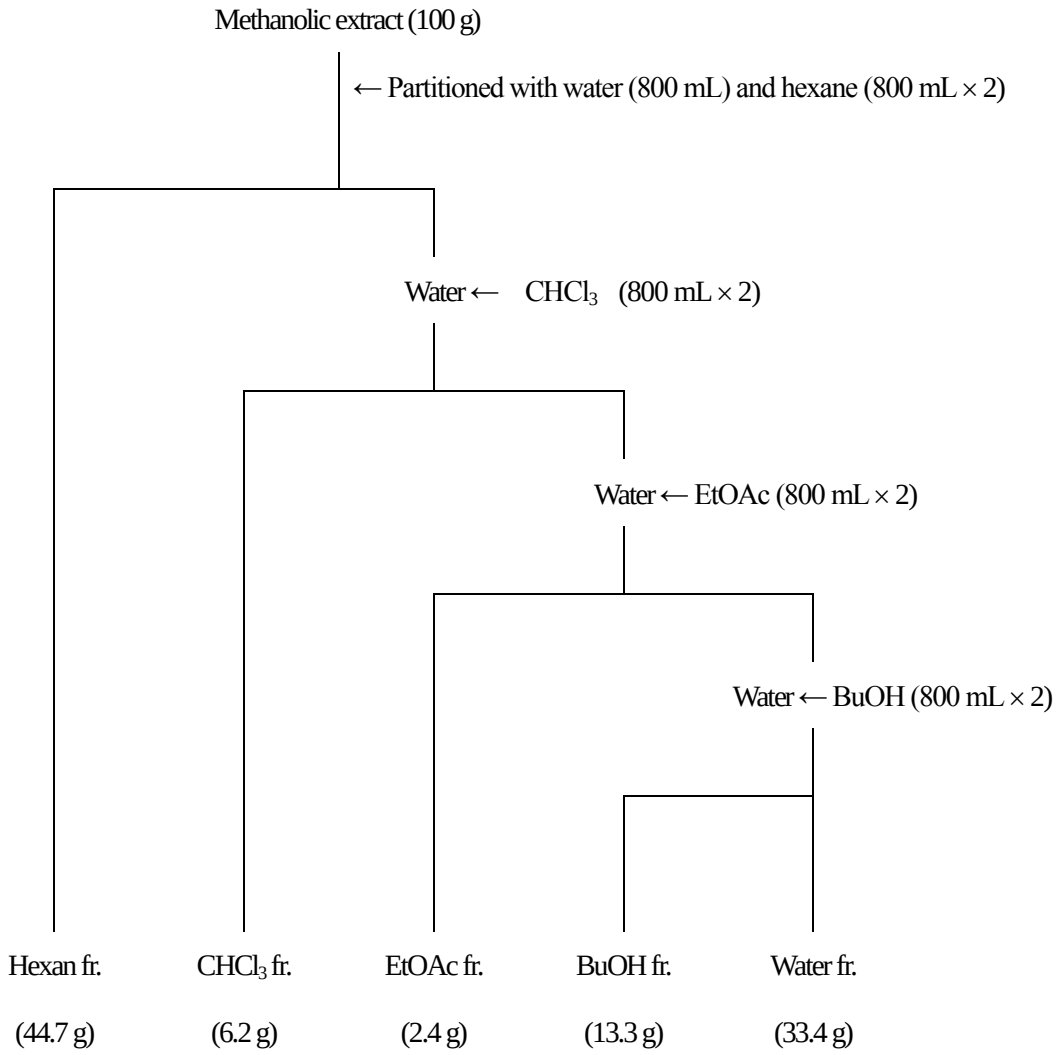


**Fig. 20.** Procedure of solvent partitions for methanolic extract of *Illicium verum* fruit.

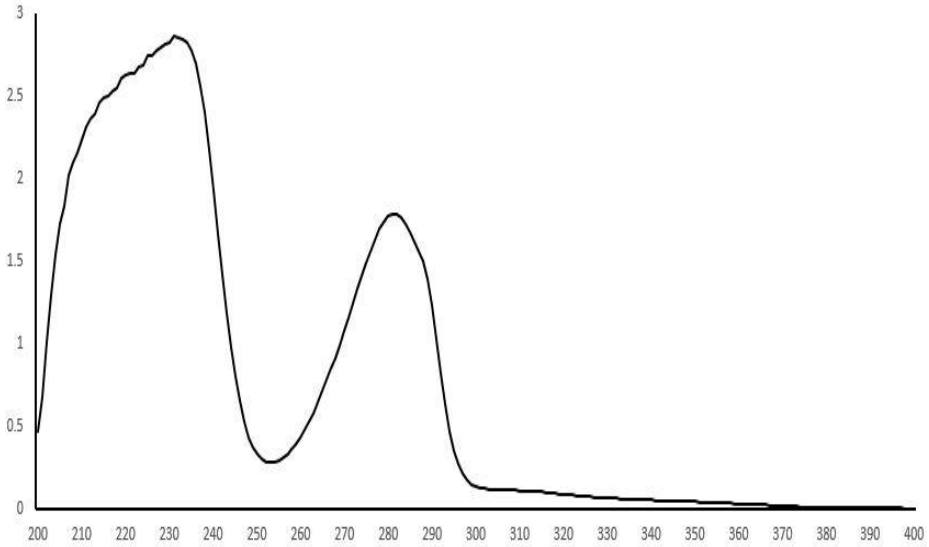


**Fig 21.** UV spectra of H221from *Syzygium aromaticum* bud.

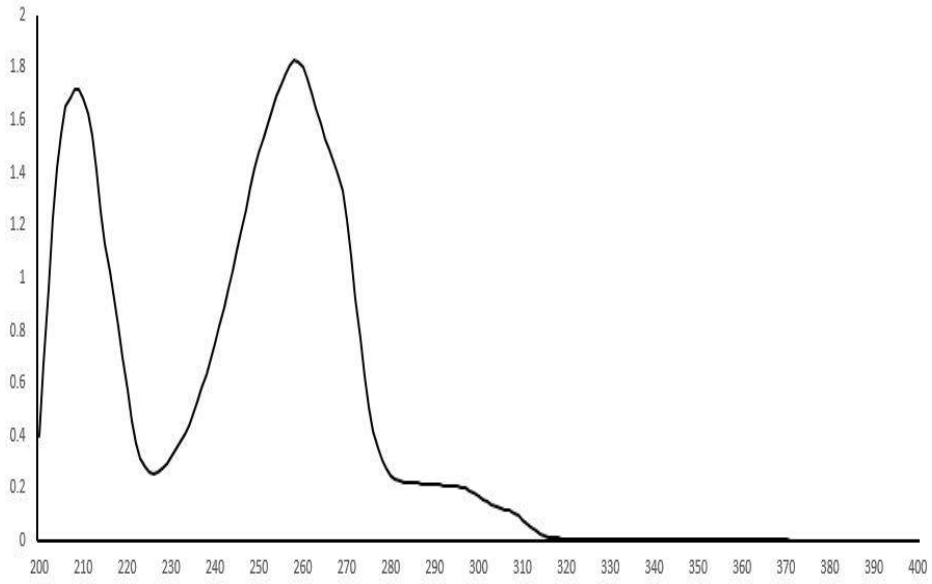


**Fig 22.** UV spectra of H111 from *Illicium verum* fruit

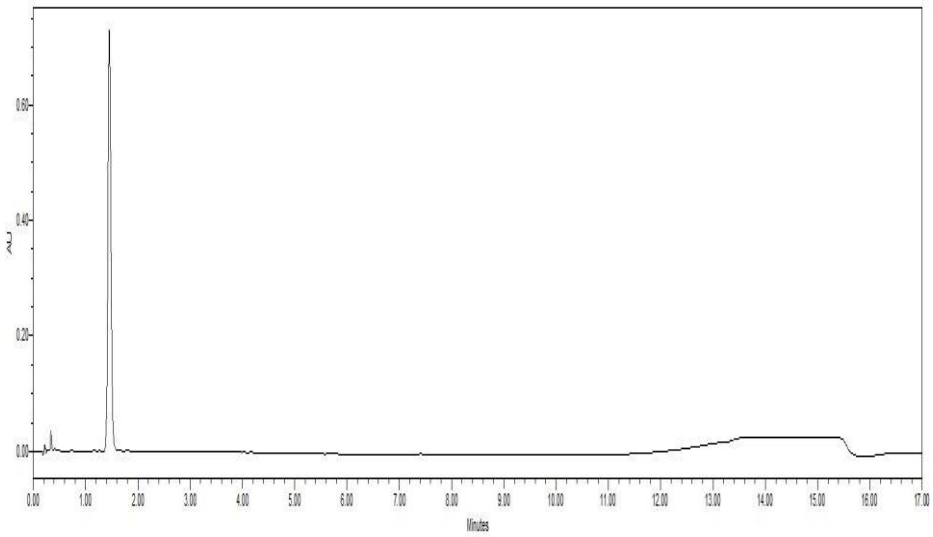


**Fig. 23.** HPLC chromatogram of H221from *Syzygium aromaticum* bud.

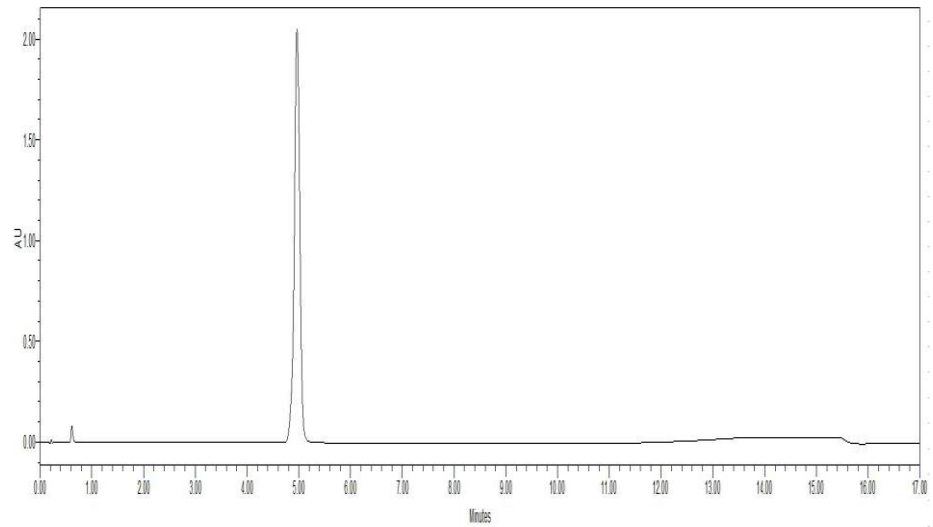


**Fig. 24.** HPLC chromatogram of H111 from *Illicum verum* fruit

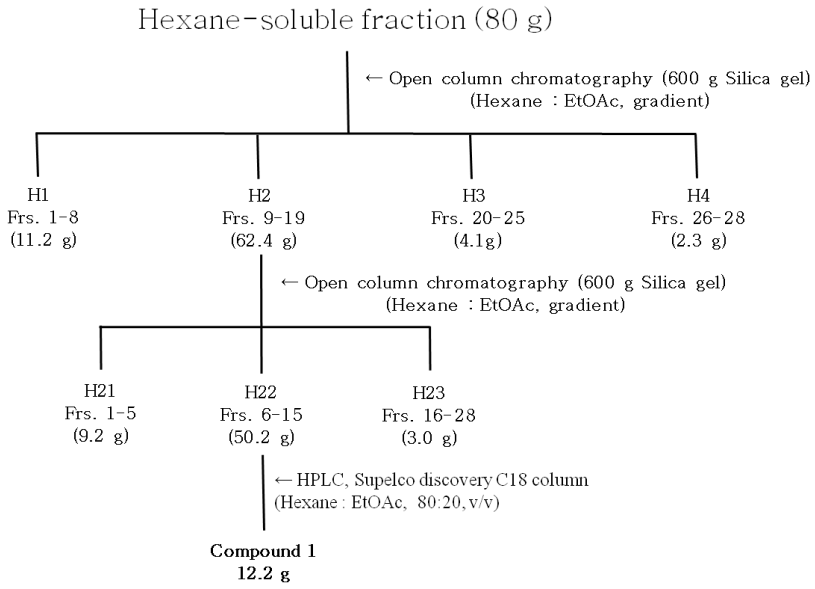


**Fig. 25.** Isolation procedures of *Syzygium aromaticum* hexane fraction.

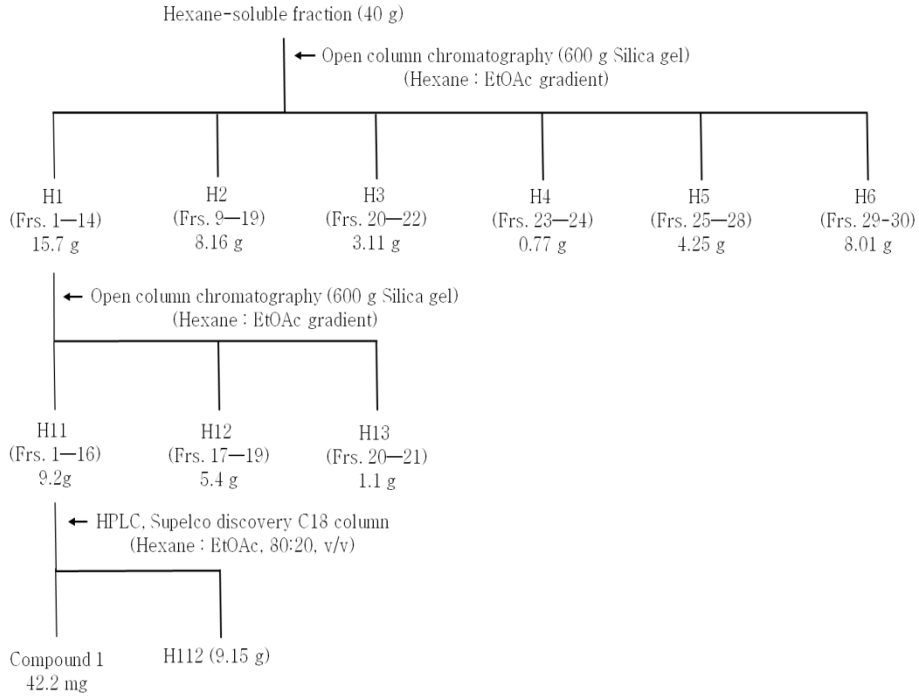


**Fig. 26.** Isolation procedures of *Illcium verum* fruit hexane fraction.

Table 8. The condition of HPLC used identification of larvicidal principles from H111 and H221

| | |
|---|---|
| **HPLC system** | |
| | Waters 2695 Alliance |
| **Column** | |
| | Supelco discovery C18 column |
| | Particle size: 5 ㎛ |
| | Column size: 4.6 mm i.d × 150 mm |
| **Solvent system** | |
| | Acetonitrile : Water = 8:2, 1 mL/min |
| **Detector** | |
| | Waters 2998 photodiode array detector |

**2.1.6. Gas chromatograph (GC)**

2.1.6.1. *S. aromaticum* bud

An Agilent 7890A gas chromatography (GC) system (Agilent, Wilmington, DE) equipped with a split injector and a flame ionization detection system was used to separate and detect the constituents of the SA-BHD. These constituents were separated with a 30 m × 0.25 mm i.d. ($d_f$ = 0.25 μm) DB-5 MS capillary column (Agilent J&W Scientific, Folsom, CA) and a 1 μl injection volume was used. The oven temperature was kept at 60 ℃ (5 min isothermal) and programmed to 260 ℃ at a rate of 5 ℃/min, then isothermal at 260 ℃ for 5 min. The linear velocity of the nitrogen carrier gas was 1.0 ml/min at a split ratio of 10:1. The constituents were identified by the coelution of authenticated samples after coinjection.

2.1.6.2. *I.verum* fruit

An Agilent 6890N gas chromatography (GC) system (Agilent, Wilmington, DE, USA) equipped with a split injector and a flame ionization detection system was used to separate and detect the constituents of the IV-FH. These constituents were separated with a 30 m × 0.25 mm i.d. ($d_f$ = 0.25 μm) DB-5 MS capillary column (J&W Scientific, Ringoes, NJ, USA). The oven temperature was kept at 60℃ (5 min isothermal) and programmed to 220℃ at a rate of 5℃/min, and then to 280℃ (that was held for 5 min at the final temperature) at 10℃/min. The linear velocity of the nitrogen carrier gas was 1.0 ml/min at a split ratio of 30:1. The constituents were identified by the coelution of authenticated samples after coinjection.

**2.1.7. Gas chromatography-mass spectroscopy (GC-MS)**

2.1.7.1. *S. aromaticum* bud

A gas chromatography-mass spectrometry (GC-MS) analysis was performed using an Agilent 6890N GC-MS system with a 5975C mass selective detector. The capillary column and temperature condition for GC-MS analysis were the same as those described above for GC analysis. A helium carrier gas was used at a flow of 1ml/min. The ion source temperature was 200 °C, and mass spectra were obtained in EI-scan mode at 70 eV electron energy. The sector mass analyzer was set to scan from 50 to 450 atomic mass units every 0.5 s. The chemical constituents were identified by comparing the mass spectra of each peak with those of the authentic samples in a mass spectrum library (Stein et al. ,2008).

2.1.7.2. *I. verum* fruit

A gas chromatography-mass spectrometry (GC-MS) analysis was performed using an Agilent 6890N GC-MS system with a 5975C mass selective detector. The capillary column and temperature conditions for the GC-MS analysis were the same as those described above for GC analysis. A helium carrier gas was used at a flow of 1 ml/min. The ion source temperature was 280℃, and mass spectra were obtained in EI-scan mode at 70 eV of electron energy. The sector mass analyzer was set to scan from 50 to 450 atomic mass units every 0.5 s. The chemical constituents were identified by comparing the mass spectra of each peak with those of the authentic samples in a mass spectra library (Stein et al. ,2008).

**2.1.8. A filter paper mortality bioassay**

The method by Yang et al. (2003) was used to evaluate the filter-paper contact + fumigant toxicity of *S. aromaticum* bud derivates (SA-BME, SA-BHD, and 15 compounds) and I. verum fruit derivates (IV-FME, IV-FHD, and 23 compounds ) to third-instar *B. procera* larvae. Based on the preliminary test results, 4–6 concentrations of the test materials in 1 ml of methanol-Tween 40-distilled water (DW) (0.1: 0.5: 0.9 by volume) were applied to 4.25 cm diameter Whatman no. 2 filter papers (Whatman, Maidstone, UK). The three insecticides clothianidin, thiamethxoam, and emamectin benzoate served as positive controls and were similarly prepared. The negative controls (i.e., no test material or insecticide) consisted of 1 ml of methanol-Tween 40-DW solution. After they were dried in a fume hood for 10 min, each treated filter paper was placed onto the bottom section of a 5 cm diameter × 1.5 cm insect breeding dish (SPL, Pocheon, Gyeonggi, ROK). Groups of 30 *B. procera* larvae were separately placed onto the treated filter papers. Each insect breeding dish was then

sealed with the original tight-fitting lid and wrapped with low-density polyethylene film (Cleanwrap, Kimhae, Gyeongnam, ROK). The organophosphorus insecticide (OP) dichlorvos was used to compare the larvicidal and acetylcholinesterase inhibitory activities of the test compounds.

The treated and control (methanol-Tween 40-DW only) larvae were held under the same conditions as those used for colony maintenance. The larval mortalities were determined at 24 post-treatment under a binocular microscope (20×). A larva was considered dead if its body and appendages did not move when it was prodded with a fine wooden dowel. All the treatments were replicated three times.

**2.1.9. Vapor-phase mortality bioassay**

The closed and open container treatment described by Kim et al. (2007) was used to determine if the lethal activity of the six (from *S. aromaticum* bud) and four (from *I. verum* fruit) test compounds which possessed potent larvicidal activity, against third-instar *B. procera* larvae was attributable to either contact or fumigant action. Approximately 1.5-fold quantities of the contact + fumigant $LC_{50}$ values of each test compound in 1 ml of methanol were applied to the filter papers as described in the section 2.1.8. After they were dried in a fume hood for 1 min, each treated filter paper was placed onto the bottom section of an insect breeding dish. Each dish was then sealed with another dish that had a fine wire screen covering a 4.5-cm diameter central hole. Groups of 30 larvae were placed on top of the wire screen containing two pieces of stems (2 cm) from fresh *P. ginseng* plants (5 years old), thereby preventing the direct contact of the *B. procera* larvae with the test compound. Each breeding dish was then sealed with the original tight-fitting lid (closed container treatment method) or sealed

with another lid with a 4.5-cm diameter central hole (open container treatment method) to investigate the potential vapor-phase toxicity of the test compounds. Two fumigants dazomet and metam-sodium served as positive controls and were similarly formulated. The negative controls (i.e., no test material or fumigant) consisted of 1 ml of methanol. Treated and control (methanol treatment only) larvae were held under the same conditions as those used for colony maintenance. The larval mortalities were determined at 48 h posttreatment as described in section 2.1.8. All the bioassays were replicated three times.

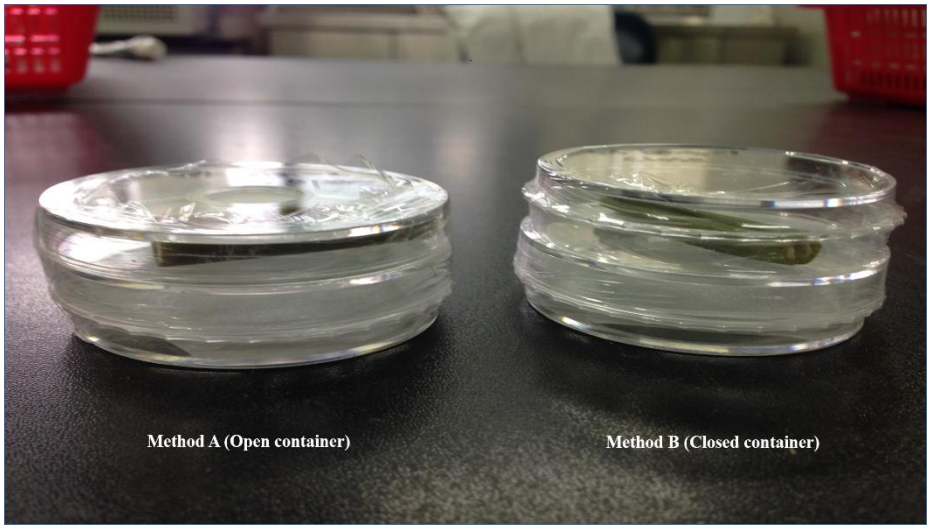


**Fig. 27.** Vapor phase mortality bioassay at open (A) and closed (B) container.

### 2.1.10. Egg-hatching inhibition bioassay

A 5-mg quantity of each test compound in 1 ml of Tween 40-methanol-distilled water was applied to the filter papers as described in section 2.1.8, producing a concentration of 375 μg/cm$^2$. If a material showed egg-hatching inhibitory activity, further bioassays were conducted at concentrations ranging from 187 to 2.9 μg/cm$^2$.

Clothianidin, thiamethoxam, cypermethrin, and emamectin benzoate served as positive controls and were similarly formulated. The negative controls (i.e., no test material or insecticide) consisted of 1 ml of Tween 40-methanol-distilled water solution only. After they were dried in a fume hood for 10 min, each treated filter paper was placed onto the bottom section of an insect breeding dish. Groups of 30 eggs (3 days old) were separately placed on the treated filter papers in each breeding dish using a fine brush and sealed with a lid, as described in section 2.1.8. The treated and control (Tween 40-methanol-distilled water treatment only) eggs were held under the same conditions as those used for colony maintenance. The egg-hatching inhibition was determined at 168 h posttreatment under a binocular microscope (20×). The egg-hatching inhibition was based on the number of unhatched eggs. All the treatments were replicated three times.

**2.1.11. Acetylcholinesterase inhibition assay**

Third-instar *B. procera* larvae were used in all the experiments. The whole bodies of 30 larvae were homogenized in 1 mL of ice-cold 0.1 M phosphate buffer (pH 8.0) using a glass tissue homogenizer. After it was filtered through cheese-cloth, the homogenate was centrifuged at 1,000 × *g* at 4 ℃ for 5 min. The supernatant was used directly as the enzyme source for AChE. The protein content was determined using a Bradford Protein Assay Kit with bovine serum albumin as the standard. The reaction mixture consisted of 10 µL of the crude enzyme preparation (4.75–4.89 µg protein equivalents), 100 µL of 0.1 M phosphate buffer, 20 µL of 3 mM DTNB in phosphate buffer (pH 7.0), and 4 µL of various concentrations of each test compound in ethanol. The reaction mixture was incubated at 30 ℃ for 5 min and 20 µL of 32 mM ATChI was then added to the mixture. After incubating for 30 min, the reaction was

terminated by adding 20 µL of 5 mM eserine salicylate. The optical density at 412 nm was determined using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA). The OP dichlorvos served as a positive control, and it was used in a similar manner. The results are expressed as the means ± standard errors (SEs) of triplicate samples from three independent experiments.

**2.1.12. Determination of the cyclic adenosine monophosphate level**

The *in vitro* octopamine-sensitive adenylate cyclase activity was investigated according to the methods by Pratt and Pryor (1986) and Perumalsamy et al. (2015). In brief, the whole bodies of 30 third-instar *B. procera* larvae were homogenized in 500 µL of 2 mM Tris-maleate buffer (pH 7.4) containing 0.8 mM ethylene glycol tetraacetic acid (EGTA). The adenylate cyclase activity was determined using a cAMP Biotrak Enzymeimmunoassay system according to the manufacturer's protocol. Each reaction mixture consisted of 80 mM Tris-maleate buffer, 5 mM theophylline, 2 mM $MgSO_4$, 0.5 mM adenosine triphosphate (ATP), 0.2 mM EGTA, 50 µL of whole-body homogenate (equivalent to 4.89 µg of protein), and 1 µL of the test compounds in Tris-maleate buffer containing 0.2% ethanol to a final volume of 100 µL. After incubating at 20 ℃ for 5 min, the reaction was initiated by adding ATP. An incubation was performed at 30 ℃ for 3 min in a shaking water bath. The reaction was terminated by boiling for 2 min. The assay tube was then cooled and centrifuged at 8,000 × *g* for 10 min. The 50 µL aliquots of the supernatants were assayed for their cAMP levels.

Polystyrene microplates (1 strip of 8 wells) coated with a goat anti-mouse polyclonal antibody were used. Fifty microliters of mouse monoclonal antibody solution was added to each well, with the exception of the blank wells. The wells were covered with

an adhesive strip and incubated at 25 ℃ for 1 h in a shaking incubator. After four washes with 400 μL of wash buffer, 50 μL of each test compound sample and a cAMP standard were added to each well. Control, blank, and zero standard wells were filled with 50 μL of RD5-55 buffer, which is a diluent. Fifty microliters of horseradish peroxidase-labeled cAMP were then added to each well. The plate that was covered with a new adhesive strip was incubated at 25 ℃ for 2 h on the shaker. After additional washing as described previously, 200 μL of substrate solution (an equal volume of stabilized hydrogen peroxide and stabilized chromogen) were added to each well. The test plate was incubated at 25 ℃ for 30 min on the benchtop in the dark. The reaction was then stopped by adding 100 μL of 2 *N* sulfuric acid to each well. The absorbance was recorded at 450 and 540 nm using the VersaMax microplate reader. The readings at 540 nm were subtracted from the readings at 450 nm. The cAMP concentrations were expressed as nmol μg protein$^{-1}$. The results are expressed as the means ± SEs of triplicate samples from three independent experiments.

**2.1.13. Data analysis**

The data were corrected for control mortality using Abbott's (1925) formula. The percentage of inhibition of egg hatching (PIH) was determined using the following equation: PIH = $[(C - T)/C] \times 100$, where $C$ is the control percentage hatch and $T$ is the treated percentage hatch (Yang et al. 2003). The mortality and hatching percentages were transformed to arcsine square root values for analysis variance (ANOVA). The concentration of the test compounds required to produce 50% inhibition of AChE activity ($IC_{50}$) was determined using a SAS 9.13 program (SAS Institute 2014). The $IC_{50}$ values and cAMP levels were subjected to ANOVA. The Bonferroni multiple-

comparison method was used to test for significant differences among the test materials (SAS Institute 2014). Student's *t*-test was used to test for significant differences in lethality between the two treatment methods (SAS Institute 2014). The means ± SEs of untransformed data are reported. The concentration-mortality data were subjected to a probit analysis (SAS Institute 2014). The $LC_{50}$ values for their treatments were considered significantly different from one another when their 95% confidence intervals (CIs) did not overlap. A compound with an $LC_{50} > 300$ µg/cm$^2$ was considered ineffective.

## Results

### 2.1. 1. Toxicity of *S. aromaticum* bud and *I. verum* fruit-derived materials to *B. procera* larvae and eggs

2.1.1. 1. Larvicidal activity of *S. aromaticum* bud–derived materials

Toxic effect on third instars of *B. procera* of *S. aromaticum* bud-derived materials (SA-BME and SA-BHD) and five insecticides (clothianidin, cypermethrin, dichlorvos, emamectin benzoate and thiamethoxam) were evaluated by the contact + fumigant mortality bioassay (Table 9). Responses varied with the material and application rate used. The SA-BME exhibited 100, 90 and 59 % mortality at application rates of 224.8, 149.9 and 74.9 μg/cm$^2$, respectively. The SA-BHD treatment resulted in 100, 97 and 67 % mortality at 224.8, 149.9 and 74.9 μg/cm$^{-2}$, respectively. The lethality of the SA-BME and SA-BHD at 224.8 μg/cm$^{-2}$ were almost similar to that of five insecticides at 0.075 μg/cm$^{-2}$.

Table 9. Toxicity of *Syzygium aromaticum* bud-derived materials and five insecticides against third instar of *Bradysia procera* using a filter paper mortality bioassay during a 24-h exposure.

| Materials | Dose (μg/cm$^{-2}$) | Mortality % (±SE)[a] |
|---|---|---|
| SA-BME[b] | 224.8 | 100 a |
| | 149.9 | 90 ± 1.9 c |
| | 74.9 | 59 ± 2.9 d |
| SA-BHD[c] | 224.8 | 100 a |
| | 149.9 | 97 ± 1.9 a-c |
| | 74.9 | 67 d |
| Clothianidin | 0.09 | 99 ± 1.1 a-c |
| | 0.045 | 56 ± 2.2 d |
| | 0.0225 | 21 ± 1.1 e |
| Cypermethrin | 0.09 | 98 ± 1.1 a-c |
| | 0.045 | 56 ± 2.2 d |
| | 0.0225 | 21 ± 1.1 e |
| Dichlorvos | 0.01125 | 100 a |
| | 0.005625 | 94 ± 1.1 bc |
| | 0.028125 | 50 ± 1.9 d |
| Emamectin benzoate | 0.359 | 99 ± 1.1 ab |
| | 0.179 | 51 ± 1.1 d |
| | 0.09 | 16 ± 1.1 e |
| Thiamethoxam | 0.09 | 99 ± 1.1 ab |
| | 0.045 | 50 ± 3.3 d |

[a]Means within a column followed by the same letter are not significantly different ($p = 0.05$, onferroni method).

[b]*Synzygium aromaticum* bud methanol extract

[c]*Synzygium aromaticum* bud hydrodistillate

2.1.1.2. Larvicidal activity of *I. verum* fruit-derived materials

Toxic effect on third instars of *B. procera* of *I. verum* fruit-derived materials (methanol extract and hydrodistillate) and five insecticides (clothianidin, cypermethrin, dichlorvos, emamectin benzoate and thiamethoxam) were evaluated by a filter paper mortality bioassay (Table 10). Responses varied with the material and application rate used. The fruit methanol extract (IV-FME) exhibited 99, 48 and 14 % mortality at application rates of 225, 112 and 56 $\mu g/cm^{-2}$, respectively. The fruit steam hydrodistillate (IV-FHD) treatment resulted in 100, 58 and 24 % mortality at 225, 112 and 56 $\mu g/cm^{-2}$, respectively. The lethality of the IV-FME and IV-FHD at 225 $\mu g/cm^{-2}$ were almost similar to that of five insecticides.

Table 10. Toxicity of *Illicium verum* fruit-derived materials and four insecticides against third instar of *Bradysia procera* using a filter paper contact mortality bioassay during a 24-h exposure

| Materials | Dose (μg/cm$^{-2}$) | Mortality % (±SE)[a] |
|---|---|---|
| IV-FME[b] | 224.8 | 99 ± 1.1 ab |
| | 112.4 | 48 ± 1.1 d |
| | 56.2 | 14 ± 2.2 e |
| IV-FHD[c] | 224.8 | 100 a |
| | 112.4 | 58 ± 1.1 d |
| | 56.2 | 24 ± 2.2 e |
| Clothianidin | 0.09 | 99 ± 1.1 ab |
| | 0.045 | 56 ± 2.9 d |
| | 0.0225 | 22 ± 1.1 e |
| Cypermethrin | 0.09 | 94 ± 2.2 bc |
| | 0.045 | 51 ± 1.1 d |
| | 0.0225 | 20 e |
| Dichlorvos | 0.01125 | 100 a |
| | 0.005625 | 93.3 c |
| | 0.028125 | 51 ± 1.1 d |
| Emamectin benzoate | 0.359 | 97 ± 1.9 a-c |
| | 0.179 | 52 ± 2.2 d |
| | 0.09 | 18 ± 1.1 e |
| Thiamethoxam | 0.09 | 99 ± 1.1 ab |
| | 0.045 | 50 d |
| | 0.0225 | 20 ± 5.8 e |

[a]Means within a column followed by the same letter are not significantly different ($p = 0.05$, Bonferroni method).

[b]IV-FME: *Illicium verum* fruit methanol extract.

[c]IV-FHD: *Illicium verum* fruit hydrodistillate.

2.1.1.3. Egg hatching inhibition of *S. aromaticum* bud–derived materials

The hatching inhibition effects of SA-BME and SA-BHD and five insecticides (clothianidin, cypermethrin, dichlorvos, emamectin benzoate and thiamethoxam) were evaluated by measuring egg hatch of the egg of *B. procera* using the filter paper contact mortality bioassay (Table 11). After a 7-day expose to SA-BME, egg hatch was inhibited 95, 73, and 14 % at 374.7, 187.3, and 93.7 μg /$cm^2$. The SA-BHD caused 98, 75, and 22 % inhibition of egg hatch at 374.7, 187.3, and 93.7 μg/$cm^{-2}$, respectively. The lethality of the SA-BME and SA-BHD at 347.7 μg/$cm^{-2}$ were almost similar to that of clothianidin, thiamethoxam (0.09 μg/$cm^{-2}$) and dichlorvos (0.01125 μg/$cm^{-2}$). However, emamectin benzoate (0.359 μg/$cm^{-2}$) at 0.09 and 0.359 μg/$cm^{-2}$, was lower than treated SA-BME and SA-BHD at 347.7 μg/$cm^{-2}$, respectively.

Table 11. Inhibition of egg hatch of *Syzygium aromaticum* bud-derived materials and three insecticides using a filter paper contact mortality bioassay during a 7-day exposure

| Materials | Dose (μg/cm$^{-2}$) | Egg hatch (%) (±SE)[a] | Inhibition of egg hatch (%) |
|---|---|---|---|
| SA-BME[b] | 347.7 | 4.5 ± 2.23 l | 95.2 |
| | 187.3 | 24.4 ± 2.94 g-j | 73.3 |
| | 93.7 | 78.9 ± 2.94 ab | 13.9 |
| SA-BHD[c] | 347.7 | 2.2 ± 2.22 l | 97.6 |
| | 187.3 | 23.3 ± 1.92 h-j | 74.5 |
| | 93.7 | 71.1 ± 2.22 bc | 22.4 |
| Clothianidin | 0.09 | 8.9 ± 2.22 j-l | 90.3 |
| | 0.045 | 24.4 ± 2.22 g-j | 73.3 |
| | 0.00225 | 46.7 ± 3.85 d-f | 49.1 |
| Cypermethrin | 0.09 | 16.7 ± 3.33 i-k | 81.8 |
| | 0.045 | 27.8 ± 2.22 f-i | 69.7 |
| | 0.00225 | 44.4 ± 4.44 d-g | 51.5 |
| Dichlorvos | 0.01125 | 5.6 ± 1.11 kl | 93.9 |
| | 0.005625 | 50.0 ± 1.92 c-e | 45.4 |
| | 0.028125 | 77.8 ± 1.11 ab | 15.1 |
| Emamectin benzoate | 0.359 | 22.2 ± 2.94 h-j | 75.8 |
| | 0.1795 | 36.7 ± 1.92 e-h | 60 |
| | 0.08975 | 63.3 ± 1.92 b-d | 30.9 |
| Thiamethoxam | 0.09 | 6.7 ± 1.92 kl | 92.7 |
| | 0.045 | 25.6 ± 2.94 f-i | 72.1 |
| | 0.00225 | 44.4 ± 4.01d-g | 51.5 |
| Control (Tween40-methanol-water only) | | 91.7 ± 1.35 a | |

[a]Means within a column followed by the same letter are not significantly different ($p = 0.05$, Bonferroni method).

[b]*Synzygium aromaticum* bud methanol extract

[c]*Synzygium aromaticum* bud hydrodistillate

2.1.1.4. Egg hatching inhibition of *I. verum.* fruit–derived materials

The hatching inhibition effects of IV-FME and IV-FHD and five insecticides (clothianidin, cypermethrin, dichlorvos, emamectin benzoate and thiamethoxam) were evaluated by measuring egg hatch of the *B. procera* egg using the filter paper contact mortality bioassay (Table 12). After a 7-day expose IV-FME, egg hatch was inhibited 94, 66, and 11 % at 348, 187, and 94 μg/cm$^{-2}$. The IV-FHD caused 100, 98 and 72 % inhibition of egg hatch at 348, 187, and 94 μg/cm$^{-2}$, respectively. The lethality of IV-FHD at 348 μg/cm$^{-2}$ was almost similar to that of three insecticides as clothianidin, dichlorvos and thiamethoxam.

Table 12. Inhibition of egg hatch of *Illicium verum* fruit-derived materials and five insecticides using a filter paper contact mortality bioassay during a 7-day exposure.

| Materials | Dose (μg/cm$^{-2}$) | Egg hatch (%) (±SE)[a] | Inhibition of egg hatch (%) |
|---|---|---|---|
| IV-FME[b] | 347.7 | 6 ± 1.1 i-j | 94 |
| | 187.3 | 33 ± 2.7 e-g | 66 |
| | 93.7 | 56 ± 1.1 ab | 11 |
| IV-FHD[c] | 347.7 | 0 jk | 100 |
| | 187.3 | 2 ± 1.1 jk | 98 |
| | 93.7 | 27 ± 1.9 fg | 72 |
| Clothianidin | 0.09 | 9 ± 2.2 h-j | 91 |
| | 0.0045 | 26 ± 2.9 fg | 73 |
| | 0.00225 | 47 ± 3.3 h-j | 51 |
| Cypermethrin | 0.09 | 17 ± 1.9 fg | 83 |
| | 0.0045 | 26 ± 2.9 de | 73 |
| | 0.00225 | 47 ± 3.3 g-i | 51 |
| Dichlorvos | 0.01125 | 6 ± 1.1 fg | 94 |
| | 0.005625 | 50 ± 1.9 de | 48 |
| | 0.028125 | 78 ± 1.1 bc | 19 |
| Emamectin benzoate | 0.359 | 22 ± 2.9 f-h | 77 |
| | 0.179 | 37 ± 1.9 ef | 62 |
| | 0.0895 | 63 ± 1.9 cd | 34 |
| Thiamethoxam | 0.09 | 7 ± 1.9 i-j | 93 |
| | 0.0045 | 26 ± 2.9 fg | 73 |
| | 0.00225 | 56 ± 7.3 d | 42 |
| Control (Tween40-methanol-water only) | | 96 ± 1.1 a | |

[a]Means within a column followed by the same letter are not significantly different ($p = 0.05$, Bonferroni method).

[b]IV-FME: *Illicium verum* fruit methanol extract.

[c]IV-FHD: *Illicium verum* fruit hydrodistillate.

2.1.1.5. Route of larvicidal action of *Syzyzium aromaticum* bud derived materials

Because of potent lethality of *S. aromaticum* bud derived materials in a filter paper contact bioassay system, the fumigant toxicity of these bud materials and two fumigants (dazomet and metam-sodium) to *B. procera* third instar larvae were investigated using the vapor-phase mortality bioassay in two formats (Table 13). Responses were dependent on treatment methods. After 48 h of exposure to 7.5 mg/cm$^2$, there was a significant difference ($P$ = 0.0001) in the lethal activity of SA-BME between exposure in a closed petri-dish, which resulted in 96 % mortality, and exposure in open petri-dish, which result 17 % mortality against *B. procera* larvae. Similar differences in the responses of larvae to SA-BHD in treatment A and B were likewise observed. Two fumigants exhibited strong fumigant action.

Table 13. Fumigant toxicity of *Syzygium aromaticum* bud-derived materials and two fumigant to third instar of *Bradysia procera* using open and closed test during a 48-h exposure

| Materials | Dose ($mg/cm^2$) | Mortality (%) (±SE) | | *P* value[a] |
|---|---|---|---|---|
| | | Closed container treatment method | Open container treatment method | |
| SA-BME[b] | 7.5 | 96±1.1 | 17±1.9 | 0.0001 |
| | 3.75 | 71±4.4 | 12±1.1 | 0.0003 |
| | 1.78 | 10±0.0 | 8±1.1 | 0.1161 |
| SA-BHD[c] | 7.5 | 98±0.0 | 21±1.1 | 0.0001 |
| | 3.75 | 72±2.2 | 18±1.1 | 0.0001 |
| | 1.78 | 18±2.2 | 10±1.9 | 0.0602 |
| Dazomet | 0.018 | 100 | 40±1.9 | 0.0001 |
| | 0.009 | 88±2.9 | 17±1.9 | 0.0001 |
| Metam-sodium | 0.018 | 99±1.9 | 40±1.9 | 0.0002 |
| | 0.009 | 88±2.2 | 28±2.2 | 0.0001 |

[a] According to a Student's *t*-test

[b] *Synzygium aromaticum* bud methanol extract

[c] *Synzygium aromaticum* bud hydrodistillate

2. 1. 1. 6. Route of larvicidal action of *Illcium verum.* fruit-derived materials

Because of potent lethality of *I. verum* fruit derived materials in a filter paper contact bioassay system, the fumigant toxicity of these fruit materials and two fumigant (dazomet and metam-sodium) to *B. procera* third instar were investigated using the vapor-phase mortality bioassay in two formats (Table 14). Responses were dependent on treatment methods. After 48 h of exposure to 892.2 μg /cm$^{-2}$, there was a significant difference ($P$ = 0.0003) in the lethal activity of IV-FME between exposure in a closed petri-dish, which resulted in 98 % mortality, and exposure in open petri-dish, which result 28 % mortality against *B. procera* larvae. Similar differences in the responses of larvae to IV-FHD in treatment A and B were likewise observed. Two fumigants exhibited strong fumigant action.

Table 14. Fumigant toxicity of *Illicium verum* fruit-derived materials and two fumigants to third instar of *Bradysia procera* using open and closed test during a 48-h exposure

| Materials | Dose ($\mu g/cm^{-2}$) | Mortality (%) (±SE) | | *P* value[a] |
|---|---|---|---|---|
| | | Closed container treatment method | Open container treatment method | |
| IV-FME[b] | 892.2 | 98 ±2.2 | 28 ± 2.2 | 0.0003 |
| | 713.76 | 68 ± 2.9 | 11 ± 2.9 | 0.0007 |
| IV-FHD[c] | 638.56 | 96 ± 2.2 | 19 ± 2.2 | 0.0002 |
| | 319.28 | 82 ± 2.9 | 9 ± 1.1 | < 0.0001 |
| Dazomet | 0.125 | 92 ± 1.1 | 11 ± 2.2 | < 0.0001 |
| | 0.0625 | 63 ± 1.9 | 6 ± 1.1 | 0.0010 |
| Metam-sodium | 0.125 | 100 | 26 ± 2.9 | < 0.0001 |
| | 0.0625 | 81 ± 2.2 | 9 ± 1.1 | < 0.0001 |

[a] According to a Student's *t*-test

[b] IV-FME: *Illicium verum* fruit methanol extract.

[c] IV-FHD: *Illicium verum* fruit hydrodistillate.

## 2.1. 2. Toxicity of *Syzygium aromaticum* bud and *Illicium verum* fruit constituents to *Bradysia procera* larvae and eggs

### 2.1.2.1. Chemical composition of *Synzygium aromaticum* bud hydrodistillate

*S. aromaticum* bud hydrodistillate was composed of five constituents by comparison of mass spectral data and by GC with authentic sample co-injection (Fig. 28). The five constituents, eugenol, β-caryophyllene, eugenyl acetate, cyclodecane and α-humulene comprised 86.8, 7.9, 3.7, 1.0 and 0.7 % of the hydrodistillate (Table 15), respectively. They constituted about 94 % of total hydrodistillate. *S. aromaticum* bud hydrodistillate GC-MS identified compounds were confirm by the co-injection with hydrodistillate. As a result of co-injection (Fig. 29), 4 compounds were identical which is present in the hydrodistillate of *S. aromaticum* bud.

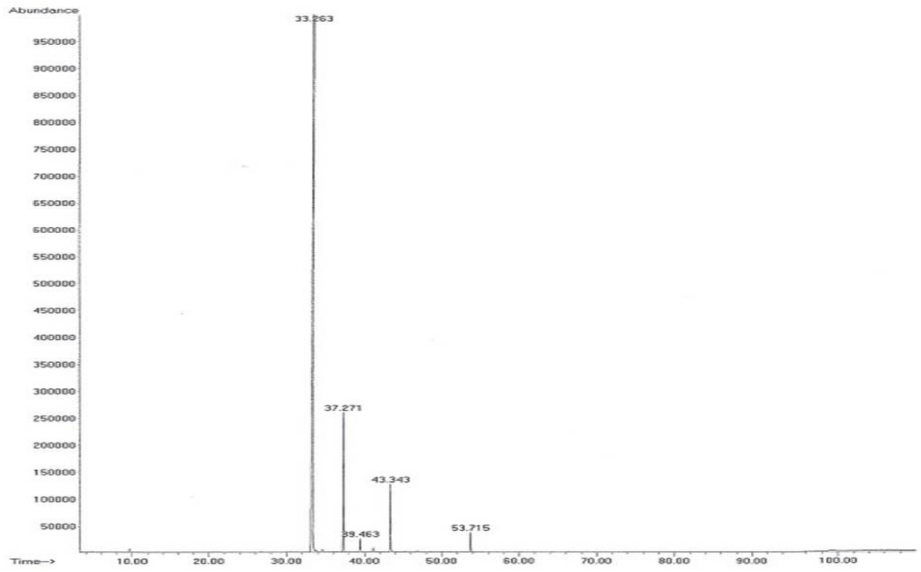


**Fig. 28.** GC-MS chromatogram of hydrodistillate from *Syzygium aromaticum* bud.

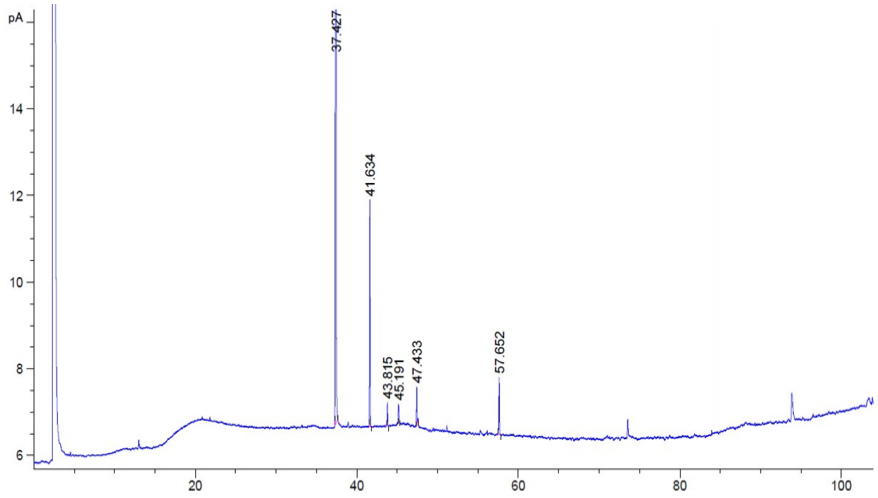


**Fig. 29.** Co-injection of identified compounds from hydrodistillate from bud of *Syzygium aromaticum*.

Table 15. Chemical constituents of *Syzygium aromaticum* bud hydrodistillate identified by gas chromatography and gas chromatography-mass spectrometry (GC-MS)

| Compound[a] | RT[b] (min) | % Area |
|---|---|---|
| Eugenol | 33.263 | 86.79 |
| β-caryophyllene | 27.271 | 7.85 |
| α-humulene | 39.463 | 0.69 |
| Eugenyl acetate | 43.343 | 3.67 |
| Cyclodecane | 53.715 | 1.00 |

[a]Tentative identifications from mass spectral data.

[b]Retention time.

21.2.2. Chemical constituents of *I. verum* fruit hydrodistillate

*I. verum* fruit hydrodistillate was composed of one major, thirteen minor constituents by comparison of mass spectral data and by GC with authentic sample co-injection (Fig. 30). The one major constituent was *trans*-anethole and comprised 91.6 % of the hydrodistillate (Table 16). *I. verum* fruit hydrodistillate GC-MS identified compounds were confirm by the co-injection with hydrodistillate. As a result of co-injection (Fig. 31), 12 compounds were identical which is present in the hydrodistillate of *I. verum* fruit.

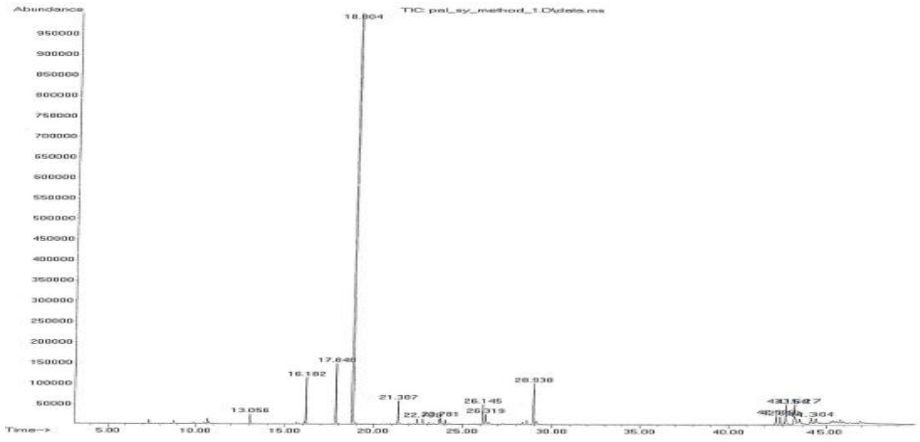


**Fig. 30.** GC-MS chromatogram of hydrodistillate from *Illicium verum*.

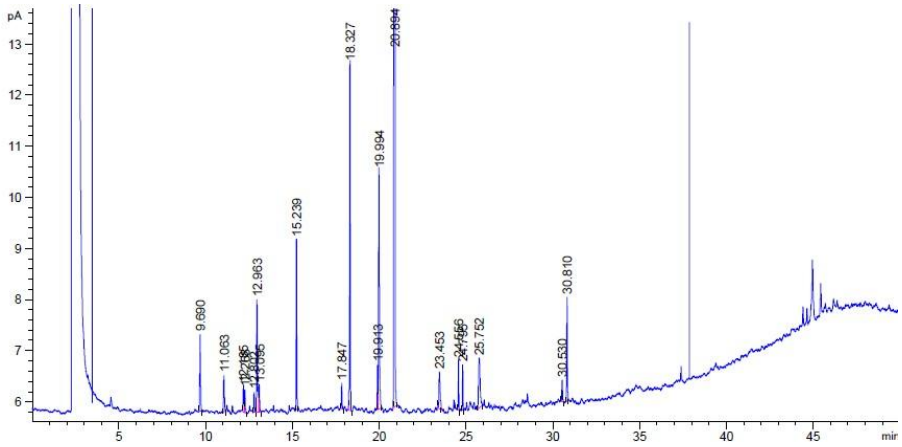


**Fig. 31.** Co-injection of identified compounds from hydrodistillate from fruit of *Illicium verum*.

Table 16. Chemical constituents of *Illicium verum* fruit hydrodistillate identified by gas chromatography and gas chromatography-mass spectrometry (GC-MS)

| Compound[a] | RT[b] (min) | % Area |
|---|---|---|
| α-pinene | 7.36 | 0.20 |
| α-phellandrene | 8.80 | 0.12 |
| δ-3-carene | 9.96 | 0.18 |
| Limonene | 10.68 | 2.04 |
| 1,8-Cineole | 10.79 | 0.35 |
| β-myrcene | 13.06 | 0.45 |
| Terpinen-4-ol | 15.66 | 0.15 |
| α-terpineol | 16.11 | 0.14 |
| Estragole | 16.19 | 0.46 |
| *p*-anisaldehyde | 17.85 | 1.08 |
| *trans*-anethole* | 18.81 | 91.60 |
| Unknown | 20.58 | 0.53 |
| β-caryophyllene | 22.46 | 0.35 |
| *trans*-α-bergamotene | 22.77 | 0.21 |

[a]Tentative identifications from mass spectral data.

[b]Retention time.

2.1.2.3. Bioassay-guided fractionation and isolation of active principles from *Syzygium aromaticum* bud

The fractions obtained from the solvent partitioning of the methanol extract from the *S. aromaticum* buds were tested for lethal activity against third-instar *B. procera* larvae by filter paper contact + fumigant application (Table 17). Significant differences ($F = 1644.18$; df = 11, 24; $P < 0.0001$) in lethal activity were observed among the fractions, and they were used to identify the peak activity fractions for the next purification step. After 24 h of exposure to 224.8 μg/cm$^2$, the hexane-soluble fraction was the most potent one, while the chloroform-soluble fraction exhibited weak lethal activity. No lethal activity was obtained using the ethyl acetate-, butanol-, and water-soluble fractions (Table 18). The mortality in the Tween 40-methanol-water-treated controls in this study was less than 2%.

Most active hexane soluble fraction was re-chromatographed using silica-gel column and was received four column fraction (H1-H4). All hexane fractions were assayed against third instars of *B. procera*. Of four fractions obtained, fraction H2 showed the strong activity (Table 19).

Table 17. Lethal activity of each solvent fraction derived from methanol extract of *Syzygium aromaticum* buds against third instars of *Bradysia procera* using a filter paper contact mortality bioassay during a 24 h exposure

| Materials | Dose, μg/cm$^{-2}$ | Mortality (%) (±SE)[a] |
|---|---|---|
| Methanol extract | 224.8 | 100 a |
| | 149.9 | 88 ± 1.1 bc |
| | 74.9 | 59 ± 1.1 d |
| Hexane-soluble fraction | 224.8 | 100 a |
| | 149.9 | 93 ± 1.9 b |
| | 74.9 | 80 ± 1.9 c |
| | 37.5 | 50 ± 1.9 d |
| Chloroform-soluble fraction | 224.8 | 36 ± 1.1 e |
| | 149.9 | 8 ± 1.1 f |
| Ethyl acetate-soluble fraction | 224.8 | 4 ± 1.1 g |
| Butanol-soluble fraction | 224.8 | 0 g |
| Water-soluble fraction | 224.8 | 0 g |

[a]Means within a column followed by the same letter are not significantly different ($P$=0.05, Bonferroni method).

Table 18. Toxicity of each solvent fraction-derived from methanol extract of *Syzygium aromaticum* bud against third instar of *Bradysia procera* using a filter paper contact toxicity bioassay during a 24 h exposure

| Materials | $LC_{50}$ (μg/cm$^{-2}$) (95%CL[a]) | Slope±SE | $\chi^2$ | *p*-value |
|---|---|---|---|---|
| Methanol extract | 47.45 (39.22-55.95) | 2.2±0.34 | 1.09 | 0.9933 |
| Hexane soluble fraction | 32.09 (26.69-37.69) | 2.3±0.34 | 0.95 | 0.9956 |
| Chloroform soluble fraction | 350.63 (280.94-418.04) | 2.1±0.34 | 0.79 | 0.9976 |
| Ethyl acetate soluble fraction | 770.02(640.85-904.82) | 2.3±0.34 | 0.95 | 0.9955 |
| Butanol soluble fraction | > 1,000 | | | |
| Water soluble fraction | > 1,000 | | | |

[a] CL denotes confidence limit.

Table 19. Lethality of hexane soluble sub-fraction derived from silica gel column chromatography against third instar of *Bradysia procera*

| Fraction | Mortality (%) (±SE) | | |
|---|---|---|---|
| | 224.8 (μg/cm$^{-2}$) | 449.6 (μg/cm$^{-2}$) | 674.4 (μg/cm$^{-2}$) |
| H1 | 9 ± 2.2 b | 12 ± 2.2b | 14 ± 1.1 b |
| H2 | 99 ± 1.1a | 100 a | 100 a |
| H3 | 9 ± 2.9 b | 10 ± 1.9 b | 13 ± 1.9 bc |
| H4 | 2 ± 1.1 b | 2 ± 1.1 c | 8 ± 1.1 c |

[a] Means within a column followed by the same letter are not significantly different (*P*=0.05, Bonferroni method)

Purification of the larvicidal active constituents from the H2 fraction was done by silica gel chromatography. At 224.8 μg/cm$^{-2}$, very strong lethal activity was observed in H22 (Table 20).

Table 20. Lethality of hexane soluble sub-fraction derived from silica gel column chromatography against third instar of *Bradysia procera*.

| Fraction | Mortality (%) (±SE) | | |
|---|---|---|---|
| | 224.8 (μg/cm$^{-2}$) | 449.6 (μg/cm$^{-2}$) | 674.4 (μg/cm$^{-2}$) |
| H21 | 0 b | 0 c | 0 b |
| H22 | 100 a | 100 a | 100 a |
| H23 | 2 ± 1.1 b | 3.3±0.0 b | 0 b |

[a]Means within a column followed by the same letter are not significantly different (*P*=0.05, Bonferroni method)

The filter-paper mortality bioassay-guided fractionation of the *S. aromaticum* buds led to an active compound that was identified through spectroscopic analyses, including UV, $^{1}$H-NMR, $^{13}$C-NMR, EI-MS and DEPT. The active constituent was characterized as the phenylpropanoids eugenol (4-allyl-2-methoxyphenol) (Fig. 35). Eugenol was obtained as pale brownish liquid and identified by spectroscopic analysis, including EI-MS, $^{1}$H-NMR (Fig. 32), $^{13}$C-NMR (Fig. 33), and DEPT (Fig. 34). The $^{13}$C NMR spectra showed 10 carbons in the molecule and including one oxynated methyl groups suggesting the molecular formula $C_{10}H_{12}O_2$. The interpretation of proton and carbon signals was largely consistent with those of Margoth et al (1983) and Kim (2002). Eugenol: pale brownish liquid; UV (EtOH) : $\lambda$max = 254. EI-MS ( 70 eV), *m/z* (rel. int) : 164 $[M]^+$ (100), 149 (31.0), 131 (20.3), 121 (14.1), 103 (20.2), 91 (15.0), 77 (20.0), 65 (7.3), 55 (10.9). $^{1}$H NMR (MeOD, 600 MHz) and $^{13}$C NMR (MeOD 150 MHz).

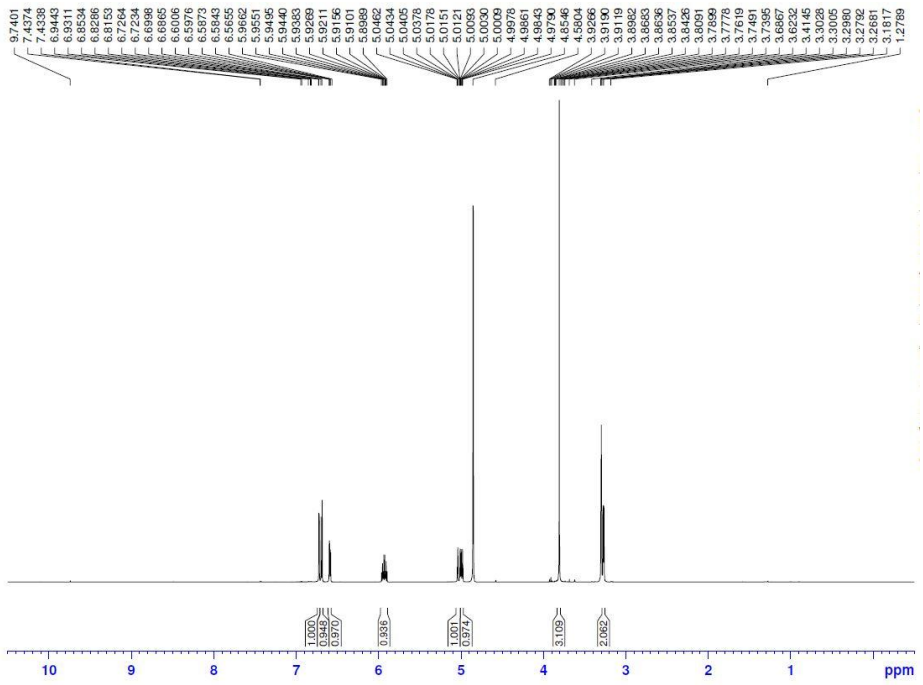


**Fig. 32.** $^{1}$H-NMR spectrum of active constituent

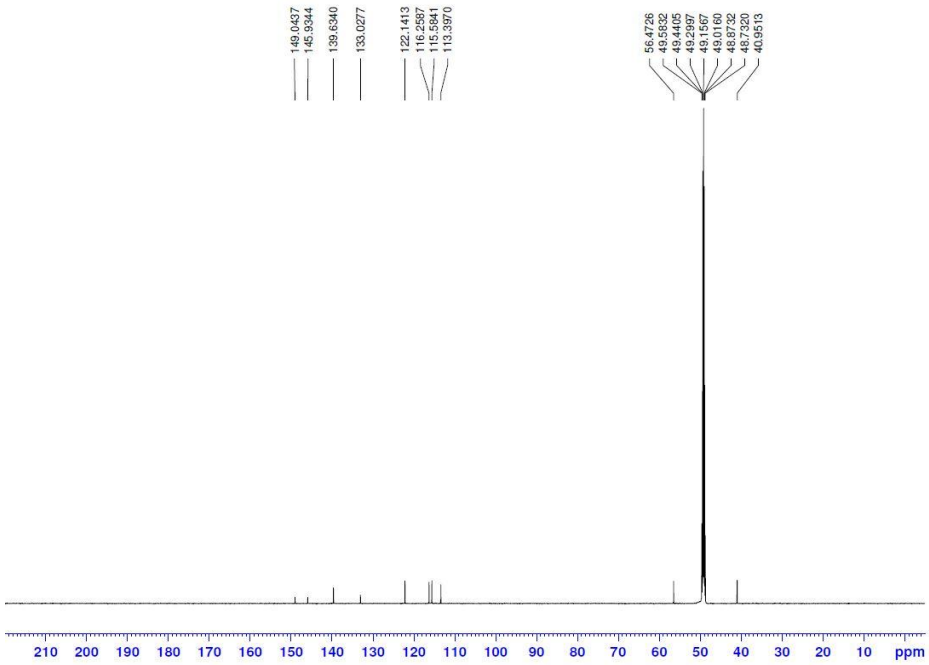


**Fig. 33.** $^{13}$C-NMR spectrum of active constituent

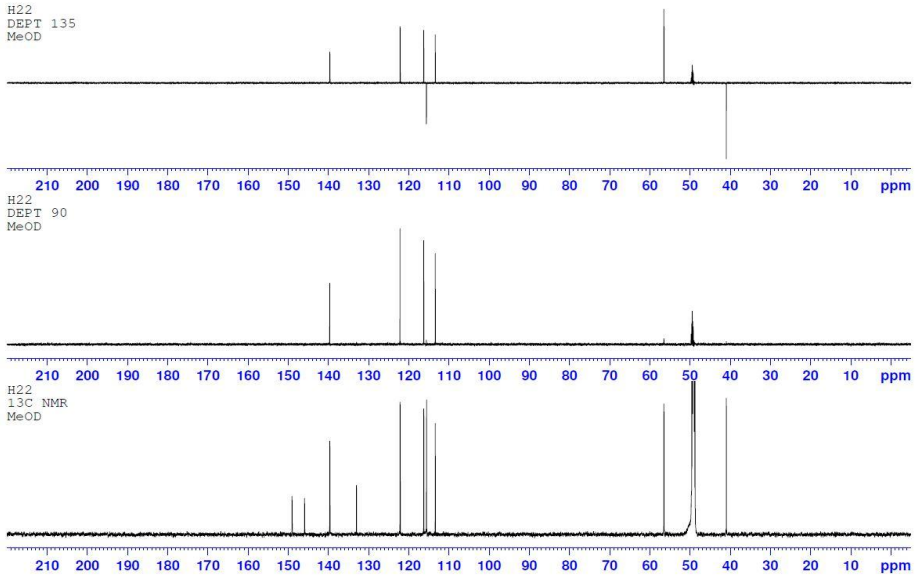


**Fig. 34.** DEPT spectrum of active constituent.

Structure of isolates from *S. aromaticum* bud, eugenol is given in Fig. 30.

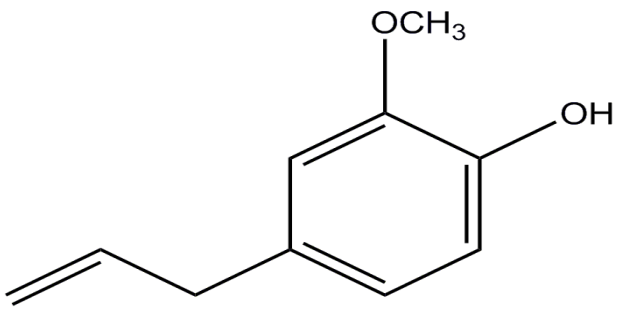


**Fig. 35.** Structure of isolated compound from *Syzygium aromaticum*.

2.1.2.4. Bioassay-guided fractionation and isolation of active principles from *I. verum* fruit

Fractions obtained from the methanol extract of *I. verum* fruits were bioassayed against third instar from *B. procera* by the filter paper contact application (Table 21). Significant differences in lethal activity in fractions of the extract were observed, and they were used to identify peak activity fractions for the next step in purification. Based on 24 h $LC_{50}$ values, hexane-soluble fraction was most toxic material (Table 22). Weak and no activity were observed in the chloroform-soluble fraction, ethyl acetate, butanol and water-soluble fraction, respectively. Mortality in the Tween 40-methanol treated controls was less than 2 %. Most active hexane soluble fraction was re-chromatographed using silica-gel column and was received six column fraction (H1-H6). All hexane fractions were assayed against third instars of *B. procera*. Of six fractions obtained, fraction H1 showed the strong activity (Table 23).

Table 21. Lethal activity of each solvent fraction derived from methanol extract of *Illicium verum* fruit against third instar of *Bradysia procera* using a filter paper contact mortality bioassay during a 24 h exposure.

| Materials | Dose, μg/cm$^{-2}$ | Mortality (%) (±SE)[a] |
|---|---|---|
| Methanol extract | 225 | 100 a |
| | 150 | 73 ± 1.9 b |
| Hexane-soluble fraction | 225 | 100 a |
| | 150 | 99 ± 1.1 a |
| | 75 | 46 ± 1.1 c |
| Chloroform-soluble fraction | 225 | 32 ± 2.2 c |
| | 150 | 9 ± 1.1 d |
| Ethyl acetate-soluble fraction | 225 | 7 d |
| Butanol-soluble fraction | 225 | 1±1.1 e |
| Water-soluble fraction | 225 | 0 e |

[a]Means within a column followed by the same letter are not significantly different ($P$=0.05, Bonferroni method).

Table 22. Toxicity of each solvent fraction-derived from methanol extract of *Illicium verum* fruit against third instars of *Bradysia procera* using a filter paper contact toxicity bioassay during a 24 h exposure.

| Materials | $LC_{50}$ (μg/cm$^2$) (95%CL[a]) | Slope±SE | $\chi^2$ | *p*-value |
|---|---|---|---|---|
| Methanol extract | 133.06 (123.22-140.30) | 6.4±1.09 | 5.78 | 0.762 |
| Hexane soluble fraction | 93.29 (86.25-100.71) | 5.1±0.54 | 57.83 | 0.001 |
| Chloroform soluble fraction | 363.56(346.60-381.08) | 16.0±1.54 | 1.63 | 0.001 |
| Ethyl acetate soluble fraction | 417.62 (398.99-438.70) | 15.5±2.36 | 25.03 | 0.009 |
| Butanol soluble fraction | >700 | | | |
| Water soluble fraction | >700 | | | |

[a] CL denotes confidence limit.

Table 23. Lethality of hexane soluble sub-fraction derived from silica gel column chromatography against third instar of *Bradysia procera*

| Fraction | Mortality (%) (±SE) | | |
|---|---|---|---|
| | 225 (μg/cm$^{-2}$) | 450 (μg/cm$^{-2}$) | 675 (μg/cm$^{-2}$) |
| H1 | 100 a | 100 a | 100 a |
| H2 | 6 ± 1.1 b | 8 ± 1.1 b | 13±1.9 b |
| H3 | 4 ± 1.1 b | 6 ± 1.1 b | 12±1.1 bc |
| H4 | 3 ± 1.9 b | 7 ± 1.9 b | 9 ± 2.2 bc |
| H5 | 1 ± 1.9 b | 6 ± 1.1 b | 9 ± 1.1 bc |
| H6 | 1 ± 1.1 b | 2 ± 0.0 b | 6 ± 0.0 c |

[a]Means within a column followed by the same letter are not significantly different ($P$=0.05, Bonferroni method).

Purification of the larvicidal active constituents from the H1 fraction was done by silica gel chromatography. At 168.8 μg/cm$^2$, very strong lethal activity was observed in H11 (Table 24).

Table 24. Lethality of hexane soluble sub-fraction derived from silica gel column chromatography against third instar of *Bradysia procera*.

| Fraction | Mortality (%) (±SE) | | |
|---|---|---|---|
| | 675 (μg/cm$^{-2}$) | 337.5 (μg/cm$^{-2}$) | 168.8 (μg/cm$^{-2}$) |
| H11 | 100 a | 100 a | 100 a |
| H12 | 8 ± 1.1 b | 3.3 ± 0.0 b | 0 b |
| H13 | 0 c | 0 c | 0 b |

[a]Means within a column followed by the same letter are not significantly different ($P$=0.05, Bonferroni method).

Direct contact mortality bioassay-guided fractionation of *I. verum* fruit methanol extract afforded one active principle identified by spectroscopic analyses, including UV, $^1$H-NMR, $^{13}$C-NMR, EI-MS and DEPT. The active constituent was characterized as the phenylpropene *trans*-anethole [1-methoxy-4-(1-propenyl) benzene] (Fig. 40). *trans*-Anethole was obtained as pale white liquid and identified by spectroscopic analysis, including EI-MS (Fig. 36), $^1$H-NMR (Fig. 37), $^{13}$C-NMR (Fig. 38), and DEPT (Fig. 39). The $^{13}$C NMR spectra showed 10 carbons in the molecule and one oxynated methyl group suggesting the molecular formula $C_{10}H_{12}O$. The interpretation of proton and carbon signals was largely consistent with those of Choo et al. *trans*-Anethole : colorless liquid ; UV

(MeOH) : $\lambda_{max}$ = 200. EI-MS (70 eV), *m/z* (rel. int.) : 148 $[M]^+$ (100), 147 (44), 133 (28.3), 121 (28.2), 117 (26.8), 105 (23.6), 77 (18.8), 51 (5.5). $^1$H NMR (MeOD, 600 MHz) and $^{13}$C NMR (MeOD, 150 MHz)

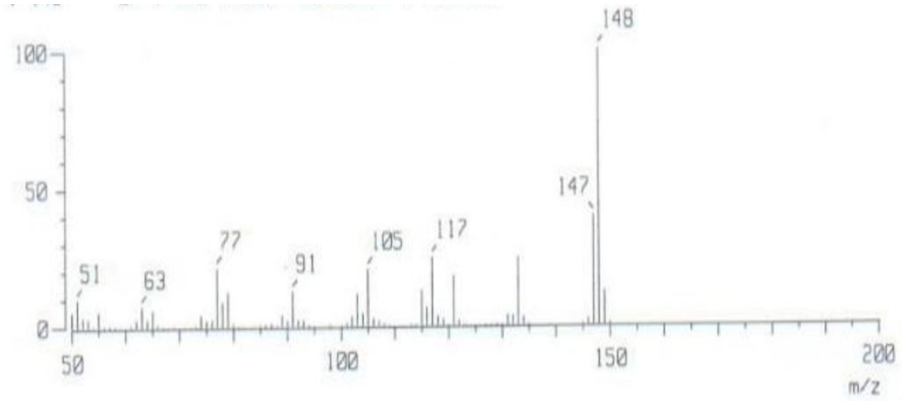


**Fig. 36.** EI-MS spectrum of active constituent.

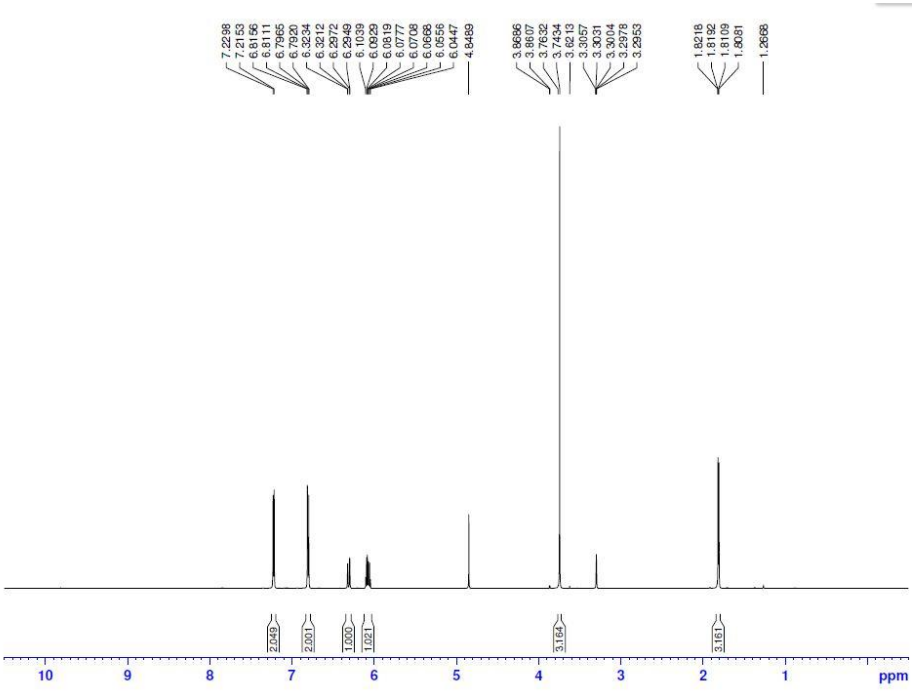


**Fig. 37.** $^1$H-NMR spectrum of active constituent.

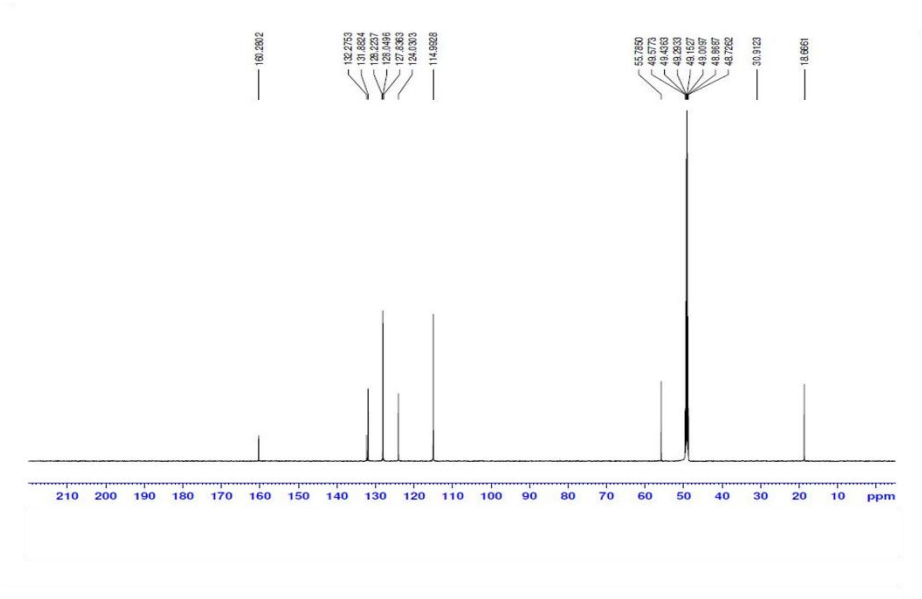


**Fig. 38.** $^{13}$C-NMR spectrum of active constituent.

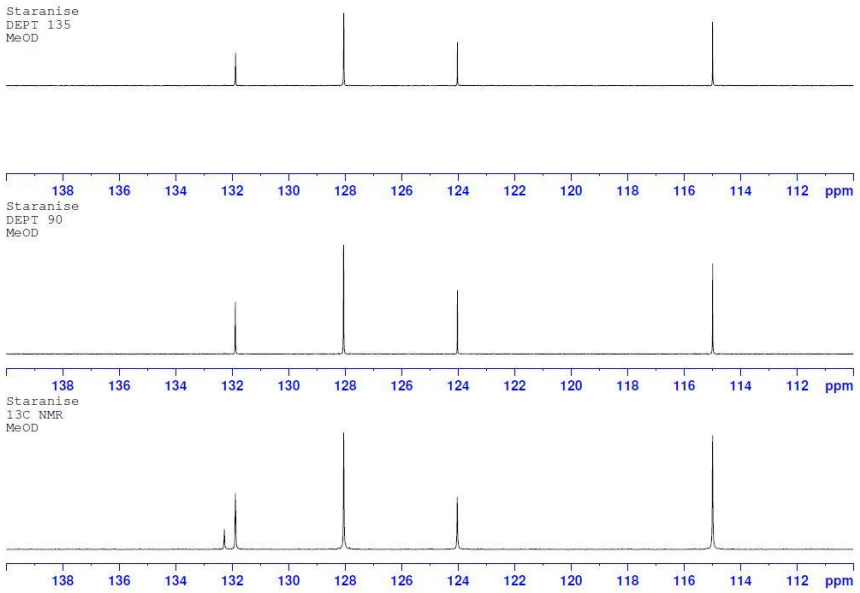


**Fig. 39.** DEPT spectrum of active constituent.

Structure of isolates from *I. verum* fruit, *trans*-anethole is given in Fig. 40.

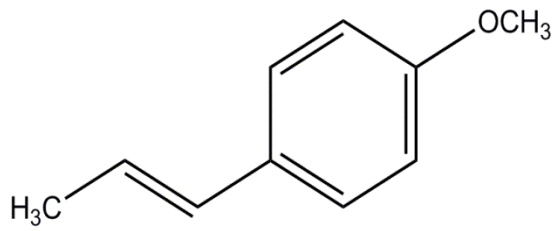


Fig. 40. Structure of isolated compound from *Illicium verum*.

2.1.2.5. Direct contact toxicity of *S. aromaticum* bud constituents

The toxicity of the SA-BME, SA-BHD, pure eugenol, 10 bud constituents identified, another five previously known bud essential oil constituents, and five commercial insecticides, which were used as positive controls, to third-instar *B. procera* larvae was likewise evaluated (Table 25). The responses varied with compound. Based on the 24 h $LC_{50}$ values, natural and pure organic eugenol had similar toxicities against *B. procera* larvae, indicating that the activity of the methanol-extracted eugenol was purely due to eugenol. Methyl salicylate (5.26 $\mu g/cm^{-2}$) was the most toxic compound and was 155, 150, 142, and 38 times less toxic than the insecticides thiamethoxam, clothianidin, cypermethirn, and emamectin benzoate, respectively. As judged by $LC_{90}$ values, methyl salicylate (21.15 $\mu g/cm^{-2}$) was 209, 209, 196, and 38 times less toxic than thiamethoxam, clothianidin, cypermethirn, and emamectin benzoate, respectively. Potent larvicidal activity was also produced by 2-nonanone, 2-heptyl acetate, 2-heptanone, eugenol, and eugenyl acetate ($LC_{50}$, 8.77–15.40 $\mu g/cm^{-2}$; $LC_{90}$, 31.92–33.04 $\mu g/cm^{-2}$). The $LC_{50}$ and $LC_{90}$ of SA-BME and SA-BHD were 45.96 and 34.40 $\mu g/cm^{-2}$ and 173.and 167.25 $\mu g/cm^{-2}$, respectively. The $LC_{50}$ and $LC_{90}$ of *p*-allylphenol, α-humulene, caryophyllene oxide, and α-pinene were between 138.15 and 178.62 $\mu g/cm^{-2}$ and between 304.62 and 669.91 $\mu g/cm^{-2}$, respectively. The $LC_{50}$ and $LC_{90}$ of α-copaene were 242.14 and 478.05 $\mu g/cm^{-2}$, respectively. No toxicity was found when testing the other four compounds.

Table 25. Toxicity of *Syzygium aromaticum* bud hydrodistillate constituents and four insecticides to third instar of *B. procera* using direct-contact mortality bioassay during 24 h exposure

| Material | Slope ± SE | $LC_{50}$ (μg $cm^{-2}$) (95% CL[a]) | $LC_{90}$ (μg $cm^{-2}$) (95% CL) | $\chi^{2b}$ | *P*-value |
|---|---|---|---|---|---|
| SA-BHD[c] | 1.9 ± 0.27 | 34.40 (27.57–41.93) | 167.25 ( 116.28–308.79) | 1.68 | 0.976 |
| β-Caryophyllene[*] | | > 300 | | | |
| Caryophyllene oxide | 2.2 ± 0.35 | 178.62 (150.37–211.40) | 669.91 (476.77–1242) | 2.60 | 0.920 |
| *ρ*-Allyl phenol | 3.7 ± 0.54 | 140.84 (126.68–155.71) | 311.85 (257.79–431.44) | 3.13 | 0.872 |
| α-Copaene | 4.5 ± 0.73 | 242.14 (222.48–263.65) | 468.05 (393.07–647.06) | 1.35 | 0.987 |
| *ρ*-Cymene | | > 300 | | | |
| Eugenol[*] | 3.0 ± 0.46 | 13.98 (12.16–15.80) | 37.62 (29.74–56.98) | 6.13 | 0.525 |
| Eugenyl acetate[*] | 3.0 ± 0.46 | 14.70 (12.86–16.63) | 39.52 (30.98–60.88) | 5.66 | 0.580 |
| 2-Heptanone | 2.4 ± 0.34 | 10.83 (9.17–12.68) | 37.63 (27.78–62.27) | 3.62 | 0.823 |

Table 25. (Continued)

| Material | Slope ± SE | $LC_{50}$ (μg $cm^{-2}$) (95% CL[a]) | $LC_{90}$ (μg $cm^{-2}$) (95% CL) | $\chi^{2b}$ | *P*-value |
|---|---|---|---|---|---|
| 2-Heptyl acetate | 2.7 ± 0.45 | 14.61 (12.60–16.72) | 43.34 (32.72–73.70) | 10.59 | 0.157 |
| α-Humulene* | 2.4 ±0.36 | 174.98 (148.97–204.39) | 594.85 (440.50–1001) | 5.99 | 0.541 |
| (±)-Limonene | | > 300 | | | |
| Linalool | | > 300 | | | |
| Methyl salicylate | 2.2 ± 0.34 | 5.26 (4.39–6.21) | 21.15 (14.42–35.14) | 1.31 | 0.988 |
| 2-Nonanone | 2.3 ± 0.34 | 9.14 (7.50–10.76) | 35.78 (25.10–56.20) | 3.79 | 0.804 |
| α-Pinene | 2.2 ± 0.35 | 172.77 (144.27–204.83) | 669.55 (473.32–1268) | 2.29 | 0.942 |
| Clothianidin | 2.7±0.42 | 0.035 (0.030–0.041) | 0.107 (0.082–0.169) | 3.22 | 0.864 |
| Cypermethrin | 2.7 ± 0.42 | 0.039 (0.034–0.045) | 0.116 (0.088–0.188) | 4.75 | 0.690 |

Table 25. (Continued)

| Material | Slope ± SE | $LC_{50}$ (μg $cm^{-2}$) (95% CL[a]) | $LC_{90}$ (μg $cm^{-2}$) (95% CL) | $\chi^{2b}$ | *P*-value |
|---|---|---|---|---|---|
| Dichlorvos | 4.6 ± 0.69 | 0.0028 (0.0025–0.0030) | 0.0053 (0.004–0.0069) | 0.82 | 0.997 |
| Emamectin benzoate | 2.1 ± 0.34 | 0.136 (0.109–0.162) | 0.560 (0.404–1.018) | 2.92 | 0.893 |
| Thiamethoxam | 2.6 ± 0.42 | 0.034 (0.029–0.039) | 0.106 (0.081–0.169) | 4.09 | 0.769 |

[*] Compounds identified in this study. The other compounds were reported by Yang et al (2003) and M. Hakki et al (2007).

[a] CL denotes confidence limit.

[b] Pearson's chi-square goodness-of-fit test.

[C] *Syzygium aromaticum* bud hydrodistillate

2.1.2.6. Direct contact toxicity of *Illicium verum* fruit constituents

The toxicity of the IV-FME, IV-FHD, pure *trans*-anethole, and 12 fruit constituents that were identified, another 11 previously known fruit essential oil constituents, and four insecticides (clothianidin, thiamethoxam, dichlorvos, and emamectin benzoate) were likewise evaluated on third-instar *B. procera* larvae (Table 26). The responses varied by compound. As judged by the 24 h $LC_{50}$ values, natural and pure organic *trans*-anethole had similar inhibitory activities, indicating that the activity of the methanol-extracted *trans*-anethole was purely due to *trans*-anethole. Estragole ($LC_{50}$, 4.68 μg/cm$^2$) was a 3-fold more potent compound than *trans*-anethole. This compound was 120, 114, and 32 times less toxic than the commercial insecticides thiamethoxam, clothianidin, and emamectin benzoate, respectively. Based on the $LC_{90}$ values, estragole (40.65 μg/cm$^2$) was 223, 213, and 74 times less toxic than thiamethoxam, clothianidin, and emamectin benzoate, respectively. The $LC_{50}$ and $LC_{90}$ values of α-phellandrene and camphene were 46.48 and 77.86 μg/cm$^2$ and 152.74 and 304.00 μg/cm$^2$, respectively. The $LC_{50}$ and $LC_{90}$ values of IV-FHD and IV-FME were 79.82 and 89.22 μg/cm$^2$ and 235.28 and 251.60 μg/cm$^2$, respectively. The $LC_{50}$ and $LC_{90}$ values of β-myrcene, α-pinene, and δ-3-carene were between 158.49 and 250.15 μg/cm$^2$ and between 514.17 and 1057.00 μg/cm$^2$, respectively. No toxicity ($LC_{50}$, > 300 μg/cm$^2$) was found when testing the other 16 compounds.

Table 26. Toxicity of *I. verum* fruit hydrodistillate constituents and five insecticides to third instar of *B. procera* using direct-contact mortality bioassay during 24 h exposure.

| Material | Slope ± SE | $LC_{50}$ (μg $cm^{-2}$) (95% CL[a]) | $LC_{90}$ (μg $cm^{-2}$) (95% CL) | $\chi^{2b}$ | *P*-value |
|---|---|---|---|---|---|
| Fruit hydrodistillate | 2.9 ± 0.46 | 89.22 (76.98–101.36) | 251.60 (195.65–397.39) | 2.237 | 0.946 |
| Estragole | 1.4 ± 0.22 | 4.68 (3.42–6.10) | 40.65 (24.21–103.84) | 2.411 | 0.934 |
| Natural *trans*-anethole | 2.1 ± 0.33 | 13.43 (11.06–16.02) | 55.39 (38.88–105.85) | 2.061 | 0.956 |
| Pure *trans*-anethole | 2.1 ± 0.33 | 13.77 (11.43–16.37) | 54.83 (38.84 –102.11) | 2.189 | 0.949 |
| α-phellandrene | 2.5 ± 0.35 | 46.48 (39.18–53.96) | 152.74 (117.30–236.44) | 2.423 | 0.933 |
| Camphene | 2.2 ±0.34 | 77.86 (63.91–92.11) | 304.00 (220.25–540.20) | 2.285 | 0.942 |
| β-myrcene | 2.5 ± 0.36 | 158.49 (134.34–183.56) | 514.17 (392.60–811.28) | 2.828 | 0.900 |
| α-pinene | 2.3 ± 0.36 | 165.76 (139.17–194.46) | 597.22 (436.76-1041) | 2.639 | 0.916 |
| δ-3-carene | 2.1 ± 0.34 | 250.15 (202.86–298.56) | 1057 (743.23–2024) | 2.584 | 0.921 |

Table 26. (Continued)

| Material | Slope ± SE | $LC_{50}$ (μg $cm^{-2}$) (95% CL[a]) | $LC_{90}$ (μg $cm^{-2}$) (95% CL) | $\chi^{2b}$ | *P*-value |
|---|---|---|---|---|---|
| Clothianidin | 1.9 ± 0.29 | 0.041 (0.033–0.049) | 0.191 (0.132–0.366) | 0.789 | 0.998 |
| Thiamethoxam | 1.9 ± 0.29 | 0.039 (0.031–0.047) | 0.182 (0.127–0.342) | 2.086 | 0.955 |
| Dichlorvos | 3.0 ± 0.45 | 0.0022 (0.0019–0.0025) | 0.006 (0.005–0.009) | 0.765 | 0.998 |
| Cypermethirin | 1.8 ± 0.29 | 0.041 (0.034–0.049) | 0.157 (0.116–0.259) | 1.472 | 0.9849 |
| Emamectin benzoate | 2.2 ± 0.34 | 0.146 (0.121–0.172) | 0.5486 (0.4044–0.9358) | 2.464 | 0.930 |

[a] The following 16 compounds were ineffective: acetadehyde, *p*-anisaldehyde, borneol, β-caryophyllene, 1,4-cineol, 1,8-cineol, *p*-cymene, limonene, linalool, linalool oxide, terpinen-4-ol, α-terpineol, α-terpinene, γ-terpinene, γ-terpineol, and terpinolene ($LC_{50}$, > 300 μg/$cm^{-2}$).

[b] CL denotes confidence limit.

[c] Pearson's chi-square goodness-of-fit test.

[d] *I. verum* fruit methanol extract.

[e] *I. verum* fruit hydrodistillate.

2.1. 2. 7. Intoxication symptoms

Typical intoxication symptoms in larvae from the both plants from test compounds were compared with those clothianidin, cypermethrin, dichlorvos, emamectin benzoate and thiamethoxam. Like five insecticides body of the test compound-treated dead larvae usually showed an extended and contracted shape (Fig. 41 and 42) without movement when prodded with a fine brush.

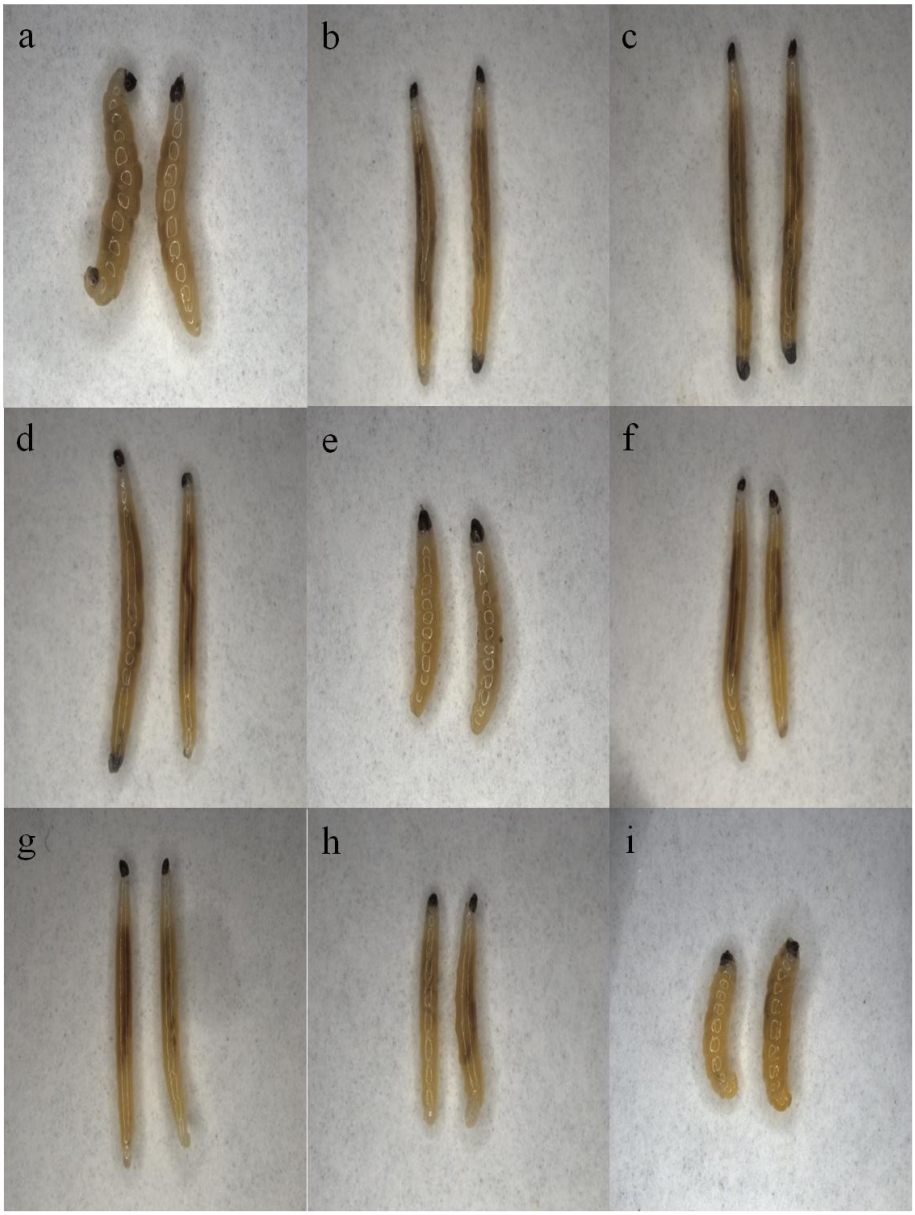


**Fig. 41.** Intoxication symptoms of *B. procera* larvae, a: Untreatment, b: Treated *S. aromaticum* bud methanol extract, c: *S. aromaticum* bud hydrodistillate, d: Eugenol, e: Control agent (clothianidin), f: Control agent (cypermethrin), g: Control agent (dichlorvos), h: Control agent (emamectin benzoate), i: Control agent (thiamethoxam), (bar =1 mm).

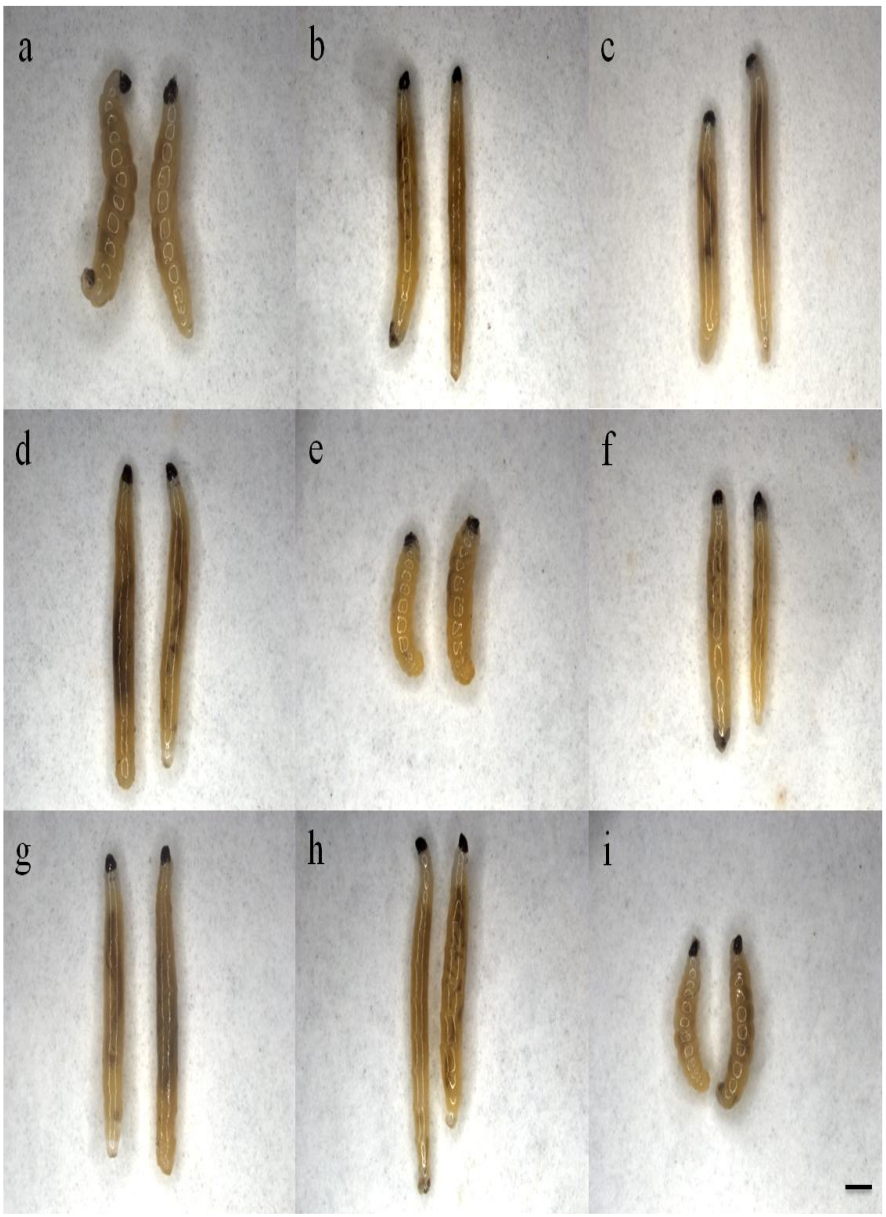


**Fig. 42.** Intoxication symptoms of *B. procera* larvae, a: untreatment, b: *Illicium verum* fruit methanol extract, c: *I. verum* fruit hydrodistillate, d: *trans*-anethole, e: Control agent (clothianidin), f: Control agent (cypermethrin), g: Control agent (dichlorvos), h: Control agent (emamectin benzoate), i: Control agent (thiamethoxam), (bar =1 mm).

2.1.2.8 Route of larvicidal action of *S. aromaticum* bud constituents

The lethal activity of the six selected test compounds and two fumigants against third-instar *B. procera* larvae were elucidated using the vapor-phase mortality bioassay in two formats (Table 27). After 48 h of exposure to 7.5 μg/cm$^2$ methyl salicylate, there was a significant difference ($P$ = 0.0023) in lethality between exposure in a closed container, which resulted in 100% mortality, and exposure in an open container, which resulted in 2% mortality against *B. procera* larvae. At 20 μg/cm$^2$, there was a significant difference ($P$ < 0.0001) in lethality of eugenol against *B. procera* larvae between exposure in a closed container (100% mortality) and exposure in an open container (0% mortality). Similar differences in the larval response to 2-heptanone, 2-nonanone, 2-heptyl acetate, and eugenyl acetate in closed versus open container treatments were also observed. The fumigants dazomet and metam-sodium also exhibited fumigant action.

Table 27. Fumigant toxicity of 12 constituents from *Syzygium aromaticum* bud to *Bradysia procera* third instars using vapor-phase mortality bioassay during a 48 h exposure

| Materials | Dose ($mg/cm^{-2}$) | Mortality (%) (±SE) | | *P* value[a] |
|---|---|---|---|---|
| | | Closed container treatment method | Open container treatment method | |
| Methyl salicylate | 7.5 | 100 | 2 ± 1.1 | 0.0023 |
| 2-Heptanone | 15 | 96 ± 2.9 | 2 ± 1.1 | 0.0035 |
| 2-nonanone | 15 | 93 ± 3.3 | 2 ± 1.1 | 0.0038 |
| Eugenol | 7.5 | 100 | 0 | < 0.0001 |
| 2-Heptyl acetate | 15 | 99 ± 1.9 | 9 ± 1.1 | < 0.0001 |
| α-Humulnene | 360 | 7 ± 1.9 | 0 | 0.0003 |
| Eugenyl acetate | 15 | 97 ± 1.9 | 14 ± 2.9 | 0.0002 |
| *p*-Allyl phenol | 150 | 100 | 26 ± 2.9 | < 0.0001 |
| Caryophyllene oxide | 360 | 4 ± 1.1 | 2 ± 1.1 | 0.2182 |
| α-Pinene | 360 | 7 ± 1.9 | 0 | 0.0413 |
| α-Copaene | 500 | 9 ± 1.0 | 1 ± 1.1 | 0.0452 |
| Dazomet | 0.125 | 90 ± 3.3 | 8 ± 2.9 | 0.0005 |
| | 0.0625 | 66 ± 4.0 | 3 ± 1.9 | 0.0141 |
| Metam-sodium | 0.125 | 99 ± 1.1 | 27 ± 3.9 | 0.0002 |
| | 0.0625 | 79 ± 2.2 | 10 ± 1.9 | < 0.0001 |

[a] According to Student's t-test.

2.1.2.9 Route of larvicidal action of *I. verum* fruit constituents

The fumigant toxicity of the IV-FME, IV-FHD, four compounds, and commercial fumigant metam-sodium (which was used as a positive control) against third-instar *B. procera* larvae were elucidated using the vapor-phase mortality bioassay in two formats (Table 28). After 48 h of exposure to 37.6 μg/cm$^2$ estragole, there was a significant difference ($P$ = 0.0002) in lethality between exposure in a closed container, which resulted in 97% mortality, and exposure in an open container, which resulted in 23% mortality against *B. procera* larvae. Similar differences in the larval response to IV-FME, IV-FHD, *trans*-anethole, α-phellandrene, and camphene in closed versus open container treatments were also observed.

Table 28. Fumigant toxicity of estragole, *trans*-anethole, α-phellandrene and camphene to *Bradysia procera* third instars using vapor-phase mortality bioassay during a 48 h exposure

| Materials | Dose ($\mu g/cm^{-2}$) | Mortality (%) (±SE) | | *P* value[a] |
|---|---|---|---|---|
| | | Closed container treatment method | Open container treatment method | |
| Estragole | 37.6 | 97 ± 0.9 | 23 ± 1.9 | 0.0002 |
| | 18.8 | 84 ± 1.1 | 18 ± 1.1 | < 0.0001 |
| *trans*-anethole | 53.6 | 91 ± 2.2 | 26 ± 1.1 | < 0.0001 |
| | 26.8 | 69 ± 2.9 | 1 ± 1.1 | 0.0041 |
| α-phellandrene | 371.84 | 100 | 17 ± 1.9 | < 0.0001 |
| | 185.92 | 77 ± 3.8 | 7 ± 1.9 | 0.0003 |
| Camphene | 778.6 | 88 ± 2.9 | 12 ± 1.1 | < 0.0001 |
| | 622.88 | 53 ± 3.3 | 4 ± 1.1 | 0.0002 |
| Metam-sodium | 0.125 | 98 ± 1.1 | 27 ± 1.9 | < 0.0001 |
| | 0.0625 | 76 ± 2.9 | 13 ± 1.9 | 0.0002 |
| Dazomet | 0.125 | 92 ± 1.1 | 11 ± 2.2 | < 0.0001 |
| | 0.0625 | 63 ± 1.9 | 6 ± 1.1 | 0.001 |

[a] According to a Student's *t*-test.

2.1.2.10. Egg-hatching inhibition of *S. aromaticum* bud constituents

The egg-hatching inhibition of all materials against *B. procera* eggs were evaluate using the filter-paper mortality bioassay (Table 29). There were significant differences ($F$ = 71.21; df = 39, 80; $P$ < 0.0001) in the egg-hatching inhibition among the treatments. After 7 days of exposure to SA-BME and SA-BHD, the egg hatching was inhibited by 94 and 98% at 375 μg/cm$^{-2}$ and 73 and 82 % at 187 μg/cm$^{-2}$, respectively. The PIHs of methyl salicylate, 2-heptanone, and 2-nonanone were 97, 90, and 85 at 11.7 μg/cm$^{-2}$ and 74, 47, and 37 at 5.9 μg/cm$^{-2}$, respectively. The PIHs of 2-heptyl acetate were 100 and 78 at 23.4 and 11.7 μg/cm$^{2}$, respectively, whereas the PIHs of eugenol and eugenyl acetate were 100 and 61 at 46.8 μg/cm$^{2}$ and 80 and 19 at 23.4 μg/cm$^{-2}$, respectively. The PIHs of *p*-allylphenol, α-copaene, and α-humulene were 62, 46, and 45 at 375 μg/cm$^{-2}$. β-Caryophyllene, caryophyllene oxide, *p*-cymene, limonene, linalool, and α-pinene did not affect egg hatching at 375 μg/cm$^{-2}$. The PIHs of clothianidin, thiamethoxam, and cypermethrin were 92, 94, and 90 at 0.09 μg/cm$^{-2}$, respectively, whereas the PIH of emamectin benzoate was 82 at 0.36 μg/cm$^{-2}$.

Table 29. Inhibition of egg hatch of *Syzygium aromaticum* bud derived constituents and five insecticides using filter paper contact mortality bioassay during a 7-day exposure

| Materials | Dose ($\mu g/cm^{-2}$) | Egg hatch (%) (±SE)[a] | Inhibition of egg hatch (%) |
|---|---|---|---|
| Methyl salicylate | 11.7 | 3.3 t | 96.4 |
| | 5.9 | 25.6 ± 2.94 n-q | 72.3 |
| | 2.9 | 40 ± 1.92 j-n | 56.6 |
| 2-Heptanone | 11.7 | 10.0 ± 1.92 r-t | 89.2 |
| | 5.9 | 52.2 ± 1.11 h-l | 43.4 |
| | 2.9 | 76.7 d-f | 16.9 |
| 2-Nonanone | 11.7 | 14.4 ± 1.11 q-s | 84.3 |
| | 5.9 | 62 ± 2.22 f-h | 32.5 |
| 2-Heptyl acetate | 23.4 | 0 u | 100 |
| | 11.7 | 22.2 ± 2.94 p-q | 75.9 |
| Eugenol | 46.8 | 0 u | 100 |
| | 23.4 | 20.0 ± 1.92 q-r | 78.3 |
| | 11.7 | 58.9 ± 1.11 g-i | 36.1 |
| | 5.9 | 87.8 ± 2.22 b-d | 4.8 |
| Eugenyl acetate | 93.7 | 10.0 ± 1.92 r-t | 89.2 |
| | 46.8 | 38.9 ± 2.22 r-t | 57.8 |
| | 23.4 | 80.0 ± 3.85 c-e | 13.2 |
| *p*-Allyl phenol | 375 | 37.8 ± 2.22 k-o | 59.0 |
| | 187 | 72.2 ± 1.11 e-g | 21.7 |
| α-Copaene | 375 | 53.3 ± 1.92 h-k | 42.2 |

Table 29 (continued).

| Materials | Dose ($\mu g/cm^{-2}$) | Egg hatch (%) (±SE)[a] | Inhibition of egg hatch (%) |
|---|---|---|---|
| α-Copaene | 187 | 86.7 ± 1.92 b-d | 6.0 |
| α-Humulene | 375 | 54.4 ± 2.94 h-j | 41.0 |
| | 187 | 90 ± 1.92 bc | 2.4 |
| β-Caryophyllene | 375 | 100 a | 0 |
| Caryophyllene oxide | 375 | 100 a | 0 |
| *p*-Cymene | 375 | 100 a | 0 |
| (±)-Limonene | 375 | 100 a | 0 |
| Linalool | 375 | 100 a | 0 |
| α-pinene | 375 | 100 a | 0 |
| Thiamethoxam | 0.09 | 5.6 ± 2.22 s-t | 94.0 |
| | 0.045 | 25.6 ± 1.11 n-q | 72.3 |
| | 0.025 | 43.3 ± 1.92 i-m | 53.0 |
| Clothianidin | 0.09 | 7.8 ± 1.11 s-t | 91.6 |
| | 0.045 | 24.4 ± 2.94 o-q | 73.5 |
| | 0.025 | 44.4 ± 4.01 i-l | 51.8 |
| Cypermethrin | 0.09 | 27.8 ± 1.11 r-t | 89.2 |
| | 0.045 | 27.8 ± 1.11 m-q | 69.9 |
| | 0.025 | 44.4 ± 4.01 i-l | 51.8 |
| Dichlorvos | 0.01125 | 4.4 ± 1.11 t | 95.1 |
| | 0.005625 | 76.7 ± 1.92 i-l | 50.6 |
| | 0.00281 | 76.7 ± 1.92 d-f | 16.9 |

Table 29 (continued).

| Materials | Dose ($\mu g/cm^{-2}$) | Egg hatch (%) (±SE)[a] | Inhibition of egg hatch (%) |
|---|---|---|---|
| Emamectin benzoate | 0.36 | 17.8 ± 2.22 o-q | 80.7 |
| | 0.18 | 36.7 l-p | 60.2 |
| | 0.09 | 62.2 ± 2.94 f-h | 32.5 |
| Control | | 92.2 ± 1.11 b | |

[a]Means within a column followed by the same letter are not significantly different. (*P*=0.05, Bonferroni method)

2.1.2.11. Egg-hatching inhibition of *I. verum* fruit constituents

The egg hatching inhibition of IV-FME, IV-FHD, two phenylpropanoids, and two terpenoids against *B. procera* eggs was compared with that of the three insecticides (Table 30). The responses varied according to the compound and the test concentration. There were significant differences ($F_{25, 52}$ = 94.34, $P$ < 0.0001) in the egg hatching inhibition among the treatments. After 7 days of exposure to IV-FME and IV-FHD, the egg hatching was inhibited by 94 and 100 at 375 μg/cm$^{-2}$ and 66 and 72 at 187 μg/cm$^{-2}$, respectively. The PIHs of estragole were 95, 67, and 21 at 30, 15, and 7.5 μg/cm$^{-2}$, respectively. The PIHs of *trans*-anethole were 92, 65, and 50 at 60, 40, and 30 μg/cm$^{-2}$, respectively. The PIHs of α-phellandrene and camphene were 94 and 84 at 300 μg/cm$^{2}$ and 81 and 73 at 150 μg/cm$^{-2}$, respectively. The PIHs of thiamethoxam and clothianidin were 92 and 93 at 0.09 μg/cm$^{-2}$, respectively, whereas the PIH of emamectin benzoate was 80 at 0.36 μg/cm$^{-2}$.

Table 30. Inhibition of egg hatch of *Illicium verum* fruit derived constituents and three insecticides using filter paper contact mortality bioassay during a 7-day exposure.

| Materials | Dose ($\mu g/cm^{-2}$) | Egg hatch (%) (±SE)[a] | PIH (%)[b] |
|---|---|---|---|
| Estragole | 30 | 4 ± 1.1 l | 95.3 |
| | 15 | 31 ± 2.2 f–i | 67.4 |
| | 7.5 | 76 ± 2.9 bc | 20.9 |
| (*E*)-anethole | 60 | 8 ± 1.1 kl | 91.9 |
| | 40 | 33 ± 3.3 e–i | 65.1 |
| | 30 | 48 ± 2.9 d–f | 50.0 |
| α-Phellandrene | 300 | 6 ± 1.1 l | 94.2 |
| | 150 | 18 ± 2.2 ij | 81.4 |
| | 75 | 33 ± 1.9 e–i | 65.1 |
| Camphene | 300 | 16 ± 1.1 jk | 83.7 |
| | 150 | 26 ± 2.9 h–i | 73.3 |
| | 75 | 44 ± 1.1 d–g | 53.5 |
| Dichlorvos | 0.01125 | 5.6 ± 1.1 l | 94.2 |
| | 0.00563 | 50.0 ± 1.9 d–f | 47.7 |
| | 0.002813 | 77.8 ± 1.1 b | 18.6 |
| Thiamethoxam | 0.09 | 8 ± 2.2 kl | 91.9 |
| | 0.045 | 24 ± 2.2 h–j | 74.4 |
| | 0.0225 | 53 ± 1.9 c–e | 44.2 |

Table 30 (continued).

| | | | |
|---|---|---|---|
| Clothianidin | 0.09 | 7 ± 1.9 l | 93.0 |
| | 0.045 | 24 ± 2.9 h–j | 74.4 |
| | 0.0225 | 54 ± 2.2 b-d | 43.0 |
| Cypermethrin | 0.09 | 16 ± 2.9 jk | 84 |
| | 0.045 | 29 ± 1.1 g–j | 70 |
| | 0.0225 | 42 ± 2.2 d–h | 56 |
| Emamectin benzoate | 0.36 | 19 ± 2.2 hj | 80.2 |
| | 0.18 | 48 ± 2.9 d–f | 50.0 |
| | 0.09 | 76 ± 2.9 bc | 20.9 |
| Control (Tween40-methanol-water only) | | 96 ± 1.1 a | |

[a] Means within a column followed by the same letter are not significantly different ($P$ = 0.05, bonfferoni test).

[b] Percentage of inhibition of hatch.

### 2.1.3. Acetylcholinesterase inhibition

2.1.3.1. *S. aromaticum* bud constituents

The *in vitro* AChE inhibitory activity of the 11 selected compounds and OP dichlorvos, which was used as a positive control, was evaluated using AChE from third-instar *B. procera* larvae (Fig. 43). Based on $IC_{50}$ values, there were significant differences ($F = 6062.99$; df = 11, 24; $P < 0.0001$) in the AChE inhibitory activity by the test compounds. α-Pinene and α-copaene were the most potent AChE inhibitors ($IC_{50}$, 26.9 and 27.7 mM) and were approximately 3 times less inhibitory than dichlorvos. 2-Heptyl acetate ($IC_{50}$, 49.3 mM) was significantly more pronounced at inhibiting AChE than either 2-heptanone or *p*-allylphenol (64.8 and 68.7 391 mM). The $IC_{50}$ of 2-c at inhibiting AChE than either 2-heptanone or *p*-allylphenol (64.8 and 68.7 391 mM). The $IC_{50}$ of 2-nonanone was 88.8 mM, while the $IC_{50}$ of the other five compounds was between 149.7 and 168.7 mM.

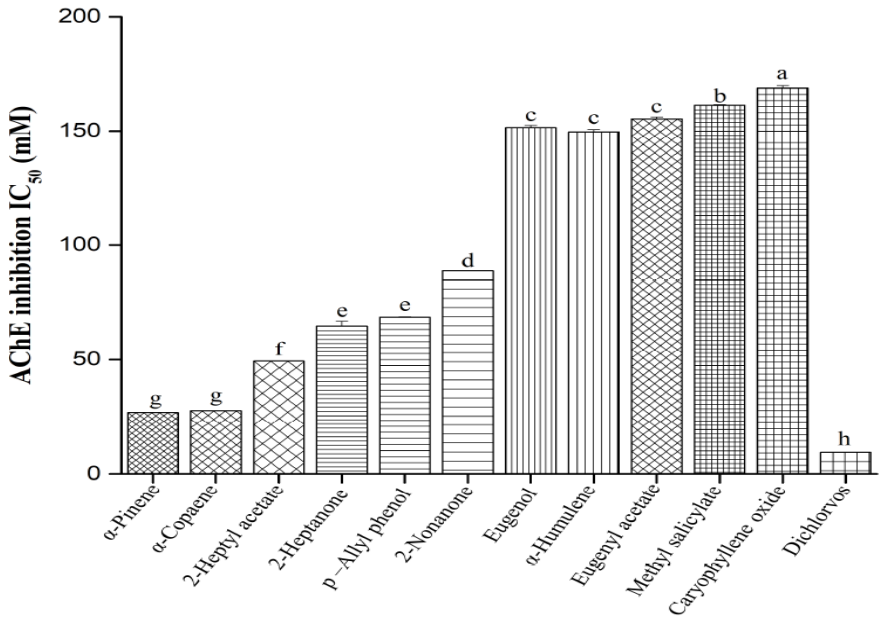


**Figure 43.** Inhibitory effect on acetylcholinesterase. Inhibition of acetylcholinesterase (AChE) extracted from third-instar *Bradysia procera* larvae by four phenylpropanoids, four terpenoids, two saturated unbranched ketones, one branched aliphatic ester, and organophosphorus insecticide dichlorvos, which was used as a positive control, was measured by acetylthiocholine iodide hydrolysis at 30 ℃ and pH 8.0. Each bar represents the mean ± standard error of triplicate samples of three independent experiments. * $P = 0.05$, according to Bonferroni multiple comparison method.

2.1.3.2. *I. verum* fruit constituents

The *in vitro* AChE inhibitory activity of the two phenylpropanoids and OP dichlorvos, which was used as a positive control, was evaluated using AChE from third-instar *B. procera* larvae (Fig. 44). As judged by the $IC_{50}$ values, there were significant differences ($F$ = 42297.5; df = 2, 6; $P < 0.0001$) in the AChE inhibitory activity of the test compounds. Estragole was a potent AChE inhibitor ($IC_{50}$, 16.4 mM) and was 1.7 times less pronounced at inhibiting AChE than dichlorvos. The $IC_{50}$ of *trans*-anethole was 162.7 mM.

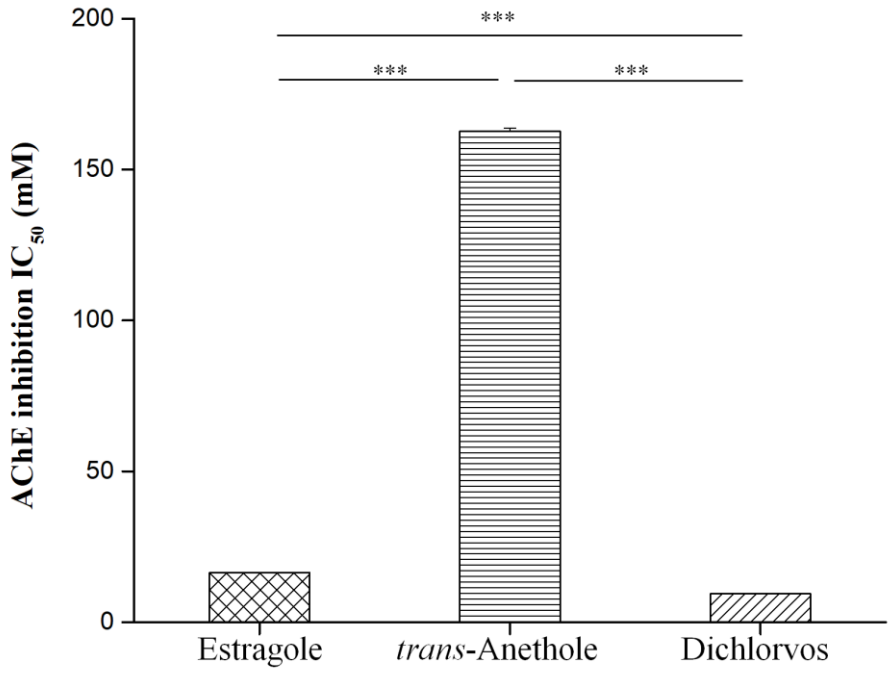


**Fig. 44.** Inhibitory effect on acetylcholinesterase. Inhibition of acetylcholineseterase (AChE) extracted from third-instar *Bradysia procera* larvae by two phenylpropanoids and the organophosphrous insecticide dichlorvos (which was used as a positive control) was measured by acetylthiocholine iodide hydrolysis at 30 ℃ and pH 8.0. Each bar represents the mean±standard error of triplicate samples of three independent. (****P* = 0.001, using Bonferroni multiple comparison test).

### 2.1.4. Effect on cyclic AMP production

2.1.4.1. *S. aromaticum* bud constituents

The effects of the 11 selected compounds on cAMP levels of whole-body homogenates from third-instar *B. procera* larvae were elucidated and compared with those induced by octopamine alone (Fig. 45). There were significant differences ($F$ = 87.19; df = 12, 26; $P < 0.0001$) in cAMP levels by the test compounds. At a concentration of 100 μM, the cAMP levels induced by caryophyllene oxide, 2-nonanone, eugenyl acetate, 2-heptyl acetate, α-copaene, and eugenol were significantly higher than that induced by octopamine.

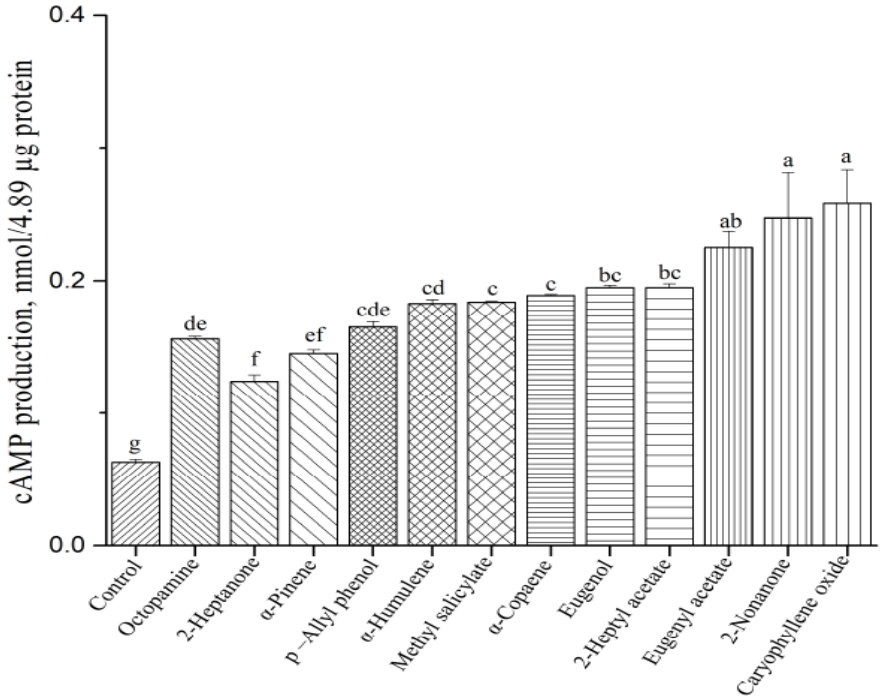


**Figure 45.** Effect on cyclic AMP levels. A whole-body homogenate from third-instar *Bradysia procera* larvae was assayed for adenylate cyclase activity, as described in 'Materials and Methods' section, in the presence of 100 µM of four phenylpropanoids, four terpenoids, two saturated unbranched ketones, and one branched aliphatic ester. The effects of the test compounds on cyclic AMP (cAMP) levels of the homogenate were compared with those induced by octopamine (100 µM) alone. Data were expressed as nmol/4.89 µg protein. Each bar represents the mean ± standard error of triplicate samples of three independent experiments. *$P$ = 0.05, according to Bonferroni multiple-comparison method.

2.1.4.2. *I. verum* fruit constituents

The effects of the two phenylpropanoids on the cAMP levels of whole-body homogenates from third-instar *B. procera* larvae were elucidated and compared with those induced by octopamine alone (Fig. 46). There were significant differences ($F = 708.57$; df = 3, 8; $P < 0.0001$) in the cAMP levels according to the test compounds. At a concentration of 100 μM, the cAMP levels induced by *trans*-anethole and estragole were significantly lower than that induced by octopamine.

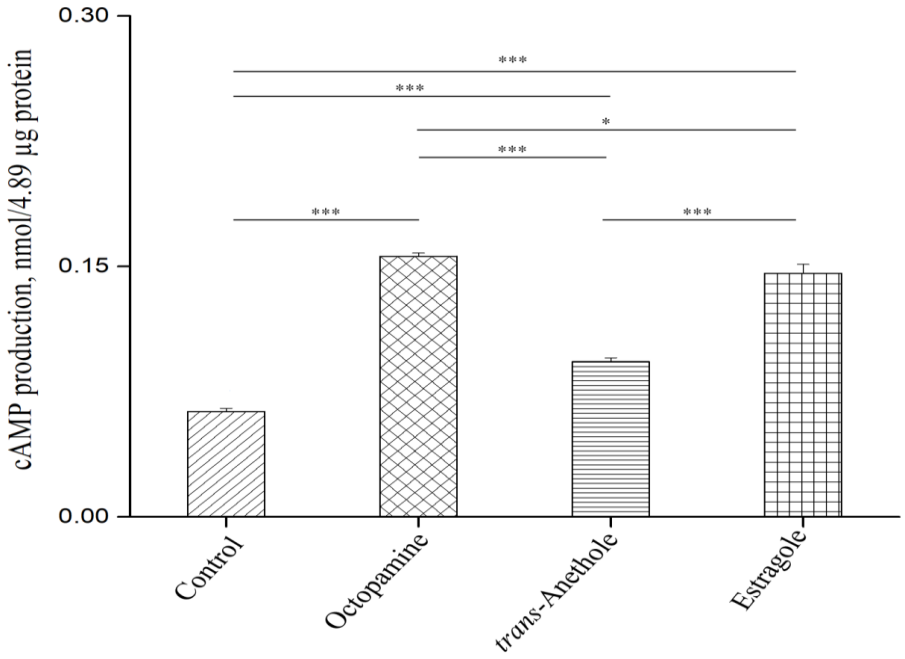


**Fig. 46.** Effect on cyclic AMP levels. A whole-body homogenate from third-instar Bardysia larvae was assayed for adenylate cyclase activity, as described in 'Materials and methods' section, in the presence of 100 µM of four phenylpropanoids, four terpenoids, two saturated unbranched ketones, one branched alipatic ester. The effects of the test compounds on cyclic AMP (cAMP) levels of the homogenate were compared with those induced by octopamine (100 µM) alone. Data were expressed as nmol/4.89 µg protein. Each bar represents the the mean±standard error of triplicate samples of three independent. (***$P < 0.001$; * $P < 0.05$, using Bonferroni multiple comparison method.

## DISCUSSION

Our current findings clearly indicate that *S. aromaticum* bud and *I. verum* fruit possess good toxicity against third-instar larvae and eggs of *B. procera*. Certain plant extracts, essential oils, and their constituents can be developed into products suitable for integrated pest management because they can be target-specific, biodegrade to nontoxic products, have few harmful effects on nontarget organisms, and be environmentally nonpersistent.(Sukumar et al., 1991; Isman, 2006; Koul et al., 2008; Pavela., 2016). They also can be used in conjunction with biological control (Isman and Machial, 2006; Miresmailli and Isman., 2006). The plant-derived preparations can be applied to the breeding or hiding places of *B. procera* in the same manner as conventional insecticides. Some plant preparations manifest insecticidal activity against *B. procera* and have been proposed as alternatives to the conventional insecticides. For example, neem, *Azadirachta indica*, seed derived materials have a variety of biological activities against various arthropod species without any adverse effects on most nontarget organisms, including natural enemies (Lowery, 1995). It has been reported that the shrubby sophora (*Sophora flavescens*) extract possessed highly insecticidal activities against *B. procera* larvae, and mixture of the *S. flavescens* extract and paraffin oil possessed ovicidal action and oviposition repellency (Seo et al., 2011). Various compounds, including phenolics, terpenoids and alkaloids, existed in plants, alone or in combination, contribute to behavioral efficacy such as repellence and feeding deterrence and physiological efficacy such as acute toxicity and developmental disruption against various insect species(Isman, 2006; Koul., 2008; Isman and Machial, 2006; Khater., 2012). Most promising botanical insect-control agents are plants in the families, Annonaceae, Apiaceae, Asteraceae, Canellaceae, Cupressaceae, Lamiaceae

(formerly Labiatae), Lauraceae, Meliaceae, Myrtaceae, Piperaceae, Poaceae, Rutaceae, and Zingiberaceae (Khater., 2012; Jacobson., 1989).

Elucidation of poisoning symptoms and the modes of delivery of natural insecticide products is of practical importance for insect control because it may give useful information on the most appropriate formulations and delivery means to be adopted for their future commercialization (Kim et al., 2006). Plant volatile constituents such as alkanes, alcohols, aldehydes and terpenoids (particularly monoterpenoids and sesquiterpenoids) primarily act as fumigants with additional contact action (Koul et al., 2008; Isman and Machial, 2006; Khater, 2012). Fumigant activity against *Lycoriella ingenua* larvae has been reported with some monoterpenoids carvacrol, *p*-cymene, and linalool and the phenylpropanoids methyleugenol, myristicin, and safrole (Yi et al., 2015).Similar results were also reported in the treatments with butylidenephtthalide (Kwon, 2002). It has been reported that *Henosepliachna vigintioctopunctata* was knockdowned accompanying intoxication of its central nervous system when acetylcholinesterase activities in the brain were inhibited (Kono et al., 1983). Furuno et al. (1994) reported a knockdown type death when *N. sericea* leaf oil was applied to adult of *D. pteronyssinus* and *D. farina*. Intoxication symptoms of *B. procera* larvae, *S. aromaticum* bud and *I. verum* fruit-derived materials showed knockout and straight shape, whereas, neonicotinoid insecticides (clothianidin and thiamethoxam) showed contracted. These compounds get in the way of information transfer between nerve cells by interfering with nicotinic acetylcholine receptors in the central nervous system, and eventually paralyzes the muscles of the insects (FAO, 2000; Bee and Pesticides, 2013).

In the current study, we used a filter-paper mortality bioassay to identify the

larvicidal and ovicidal constituents of *S. aromaticum* bud and *I. verum* fruit. These constituents were determined to be the phenylpropanoids methyl salicylate, eugenol, and eugenyl acetate and the saturated unbranched ketone 2-nonanone, and two phenylpropanoids trans-anethole and estragole, respectively. These constituents were highly effective against larvae and eggs of *B. procera*, although they were less effective than either dichlorvos, clothianidin, thiamethoxam, cypermethrin, or emamectin benzoate. Furthermore, these constituents were consistently more effective in closed versus open containers against *B. procera* larvae. Our finding indicates that the route of insecticidal action of the phenylpropanoids and ketone was likely by vapor action via the respiratory system. The dual contact + fumigant action of the phenylpropanoids and ketone, as demonstrated through the current filter-paper and vapor-phase mortality bioassays, is of practical importance because volatile constituents can easily reach deep refuges, resulting in good control. This original finding indicates that *S. aromaticum* bud- and *I. verum* fruit-derived preparations containing the active constituents described hold promise for the development of novel, effective naturally occurring larvicidal and ovicidal products for the control of *B. procera* populations. In addition, it is well acknowledged that the minor plant constituents may act as synergists, enhancing the effectiveness of the major constituents through various mechanisms for reducing the dose of polluting substances and the risk of developing resistance (Khater., 2012; Bekele and Hassanali., 2001; Miresmaili et al., 2006). For example, Miresmailli *et al* (2006) studied the effect of the chemical complexity of *Rosmarinus officinalis* essential oil on toxicity in *Tetranychus urticae*. They reported that a blend containing all 10 constituents of the oil is the most potent acaricide, and proposed that the minor constituents may act as synergists,

enhancing the effect of the major constituents. Therefore, an interaction between various constituents of SA-BHD and IV-FHD may contribute to enhancement of larvicidal or ovicidal activity of the oil against *B. procera*. Eugenol has been reported to possess the insecticidal and repellent activity against several insect pests attacking stored food products (Obeng-Ofori and Reichmuth, 1997; Ogendo et al., 2008). Estragole is reported to be toxic against three tephritid fruit fly species (*Ceratitis capitata* (Wiedeman), *Bactrocera dorsalis* (Hendel), and *Bactrocera cucurbitae* (Coquillett) (Chang et al., 2009). *trans*-Anethole is effective against the three tephritid fruit fly species (Chang et al., 2009), *Lycoriella ingenua* (Dufour) (*Park* et al. 2006), *B. germanica* (Chang and Ahn 2002), and the three *coleopteran stored-product insects Sitophilus oryzae* (L.), *C. chinensis* and *L. serricorne* (*Kim and Ahn* 2001). An investigation of intoxication symptom of natural insecticidal products are of practical importance for ginseng fungus gnat control because they may give useful information on the most appropriate formulation, delivery means, and resistant management approaches.

An investigation of the mechanisms of action of botanical insecticides can provide useful information for the development of biorational insect control alternatives with novel target sites and low toxicity. The target sites and mechanisms underlying the insecticidal actions of plant SMs have been well documented by Isman (2006) and Pavela. (2016) Certain terpenoids inhibit AChE from housefly and Madagascar roach (Grundy and still, 1985) and head louse (Picollo et al., 2008). Ryan and Byrne (1988) reported a relationship between insecticidal and electric eel AChE inhibitory activities of terpenoids, whereas no direct correlation between insect toxicity and AChE inhibition by terpenoids was also reported (Perumalsamy et al., 2015; Grundy and Still,

1985; Picollo et al, 2008). In addition, the octopaminergic and γ-aminobutyric acid receptors have also been suggested as novel target sites for some monoterpenoid essential oil constituents in the German cockroach (Picollo et al, 2008) and the cotton bollworm moth (Kostyukovsky, 2002) and the common fruit fly (Priestley, 2003), respectively. In the current study used a contact + fumigant mortality bioassay to identify the larvicidal and ovicidal constituents of *S. aromaticum* bud and *I. verum* fruit.

An investigation about the mechanisms of action for naturally occurring insect control agents can provide useful information for the development of biorational insecticides. Alternative *B. procera* control products with novel target sites, low toxicity to mammals and natural enemies, and little environmental impact are urgently needed. These potential new naturally occurring insecticides can be applied to ginseng plants in the same manner as conventional insecticides. Certain terpenoids were reported to inhibit AChE from houseflies and Madagascar roaches (Grundy and Still 1985) and head lice (Picollo et al. 2008). It has also been reported that certain flavonoids and fatty acids inhibit AChE from *Aedes aegypti* (L.) larvae (Perumalsamy et al. 2015). A relationship between the insecticidal and electric eel AChE inhibitory activities of terpenoids has been reported (Ryan and Byrne 1988), whereas no direct correlation between the toxicity and AChE inhibition by terpenoids has been reported (Grundy and Still 1985; Picollo et al. 2008; Perumalsamy et al. 2015). In addition, the octopaminergic receptor has been suggested as a novel target site for some monoterpenoid essential oil constituents in *B. germanica* (Enan 2001) and *Helicoverpa armigera* (Hübner) (Kostyukovsky et al. 2002) and for some fatty acids in larval *A. aegypti* (L.) (Perumalsamy et al., 2015). The γ-aminobutyric acid receptor has also been suggested as a novel target site for some monoterpenoid essential oil constituents

in *D. melanogaster* (Priestley et al. 2003). Our findings indicate that methyl salicylate, eugenol, and eugenyl acetate might act on octopaminergic receptor. The saturated unbranched ketones 2-heptanone and 2-nonanone were also potent larvicides and mild AChE inhibitors. The cAMP level induced by 2-nonanone was higher than that induced by octopamine. Similar results with 2- nonanone were observed with 2-heptyl acetate. In addition, estragole was a potent AChE inhibitor, and it was a highly toxic larvicide. The cAMP level induced by this phenylpropanoid was slightly lower than that induced by octopamine alone. Our findings indicate that estragole might act on both AChE and the octopaminergic receptor. *trans*-Anethole was also a highly toxic larvicide and a weak AChE inhibitor. The cAMP level induced by the phenylpropanoid was lower than that induced by octopamine alone. Results of the current study indicate that bioinsecticides derived from *S. aromaticum* bud containing eugenol, eugenyl acetate, methyl salicylate, and 2-nonanone and *I. verum* fruit containing estragole and trans-anethole could be useful as contact action fumigants to manage *B. procera* eggs and larvae. Further research is needed regarding the practical applications of *S. aromaticum* bud and *I. verum* fruit-derived preparations as novel insecticide products. In addition, detailed tests are needed to understand how to improve the insecticidal potency and stability of the constituents isolated from *S. aromaticum* bud and *I. verum* fruit for eventual product development.

# 2.2. Field Evaluation of *Syzygium aromaticum* bud derived Materials against *Bradysia procera* on *Panax ginseng* fields and Its Phytotoxicity

## INTRODUCTION

Ginseng fungus gnat, *Bradysia procera* (Diptera: Sciaridae) have become increasingly difficult insect pests on ginseng (*Panax ginseng* C.A Mayer) in ROK during the last few years. Their damage has been reported only ginseng field and has been widespread over the past years. Beside its infection by larvae assumed that transmit the infections of soft rot disease such as *Erwinia carotovora* subsp. *Carotovora* (shin et al., 2008). In a practically of the farming situation, control of this insect is have become increasingly difficult because of the lack of ecological informations and control methods. In general, management strategy depends premarilly on the use of chemicals.

For control of *B. procera*, several methods have been tried such as cultural and chemical methods (Shin et al., 2008; Lee et al., 2010; Seo et al., 2011). Initially, application of chemicals was focused on the control of *B. procera*, either killing or repelling them. Although chemicals effectiveness has been proven, however the appearance of resistance to insecticides on target insect, together public concern about the safety and availability of the insecticides have prompted the necessity to search for alternative insecticides that would be environmentally acceptable and less costly.

Plant-derived materials such as plant extracts, essential oils and their constituents can be alternative sources for insect control because of that they are selective, but also

they can biodegrade to nontoxic products and have no negative effects on nontarget organism and the environment (Isman, 2006; Jiang et al., 2012; Liu et al., 2012).

Clove bud, essential oil of *Syzygium aromaticum* has been reviewed for its insecticidal activities against various insect pests (Chaieb et al., 2007). However, these studies were based on the use of single plant extract or essential oil, which may not give as satisfactory results compared with chemical insecticides. In addition, many essential oils have been reported to exhibit phytotoxicity such as herbicidal effect including clove oil and its constituent eugenol (Tworkoski, 2002). Clove oil and eugenol was showed damaging to leaves of *Taraxacum officinale* by increasing cell membrane permeablility, which caused membrane damage (Tworkoski, 2002).

In this study was aimed at assessing the phytotoxicity of the bud methanol extract and bud hydrodistillate that comprise the bud of *S. aromaticum* on ginseng at leafing period, and evaluating the control efficacy and anti-oviposition effect of clove bud-derived materials and their mixures with insecticides against *B. procera* was then compared with those of four currently used commercial insecticides, clothianidin, cypermethrin, emamectin benzoate and thiamethoxam.

## Materials and Methods

### 2.2.1. Preparation of test materials

2.2.1.1. Plant extraction of *Syzygium aromaticum*

Air-dried bud of *S. aromaticum* (3.0 kg) pulverized and extracted with methanol (2 × 3 litres) at room temperature for 1 day and filtered respectively. The combined filtrate of *S. aromaticum* was concentrated under vacuum at 40℃ to yield *c* 420 g as a dark brownish tar.

2.2.1.2 Hydrodistillation

Air-dried bud (2 kg) of *S. aromaticum* finely grounded and subjected to steam distillation at 100℃ for 2 h using a Clevenger-type apparatus. The volatile oil was dried over anhydrous sodium sulfate and stored in a sealed vial at 4 ℃ until use. The yield of the hydrodistillate of *S. aromaticum* bud was 13.02 % based upon dried weight of materials.

2.2.1.3. Chemicals

Six chemicals examined in this study were as follows: Tween-40 was purchased from Sigma-aldrich (St Louis, MO, USA), cypermethrin EC, 5 % was obtained from Dongbangagro, Co., (Seoul, Republic of Korea). Clothianidin SC, 7 % was purchased from Hankooksamgong, Co., (Seoul, Republic of Korea). Emamectin benzoate EC, 2.15 % and thiamethoxam WG 10 % purchased from Syngenta Korea Ltd. All other chemicals were of reagent grade and available commercially.

### 2.2.2. Field experiments

2.2. 2.1. Phytotoxicity

The phytotoxicity of *S. aromaticum* bud derived materials (SA-BME and SA-BHD) were conducted in randomized block design on the 6-years old ginseng field with a quadrable-clothed black and blue quadrable polyethylene mats and shading sheets (var Jakyung, N 36°22′50.1″, E127°20′49.4″) during leafing period at experimental field of Ginseng resource research institute, KGC R&D Headquarter, Daejeon, Republic of Korea.

SA-BME and SA-BHD in 10 L of Tween-40-methanol-water (0.1: 0.5: 0.9 by volume) were foliar application three time at $9^{th}$ , $16^{th}$ and $23^{th}$ May 2013 using battery charged sprayer (pressure: 6.5kg/$cm^2$; triple nozzle: 1.2 mm) on experimental plot of ginseng field. Control received 10 L of Tween 40-methanol-water (0.1:0.5:0.9 by volume) only. Phytotoxicity on ginseng was assessed by visual observation at 1, 3, 7 day after treatment for leaf malformation, discoloration, eliminated petiole, leaf and stem. A visual classification of phytotoxicity was made and grade scale of 0–4 was devised, based on personal observations (Table 31). All experiments were three replicates.

Table 31. Classification of phytotoxicity on ginseng, var Jakyung, based on field observations.

| Scale | Description |
|---|---|
| 0 = | Green with no external symptoms of aerial part of ginseng |
| 1 = | Some part of leaves were discolored and tip burning |
| 2 = | Leaves were discolored, malformated and eliminated more than half |
| 3 = | Petioles, leaves and stems were eliminated |
| 4 = | Aerial part of ginseng was withering to death |

### 2.2.3. Field evaluations of *Syzygium aromaticum* derived materials against *Bradysia procera*

2.2.3.1. Control efficacy of *S. aromaticum* derived materials against *B. procera* on ginseng field

This studies were conducted at randomized block design on the 6-years old ginseng field in the 2013 (var Jakyung, N 37°18′41.5″, E127°33′23.1″, Yeoju and var Cheonpoong, N37°20′36.3″, E127°28′16.1″, Icheon) seasons in differential areas during June to August. Each treatment plots were consist of 82.5 $m^2$.

Before treatment, total numbers of un-damaged ginseng stems in experiment plots were recorded at one day before foliar application as a pre-treatment count. Treatment of first foliar application was conducted at 17$^{th}$ and 19$^{th}$ June in Yeoju and Icheon respectively. The treatments were three times with a week interval. The observation of damaged ginseng stems were recorded at 7 and 14 day after treatment (DAT) as post foliar application counts.

The treatments included *S. aromaticum* bud derived materials (SA-BME and SA-BHD) in 20 L of Tween-40-methanol-water (0.1:0.5:0.9 by volume) and recommended doses of clothianidin, emamectin benzoate and thiamethoxam in 19.99 L water, cypermethrin in 19.98 L water were foliar application three times with a week interval using battery charged sprayer (pressure: 6.5kg/$cm^2$; triple nozzle: 1.2mm) on experimental plot of ginseng. Control received 20 L of Tween 40-methanol-water (0.1:0.5:0.9 by volume) only. Each plot was separated by a gap of 3.6 m as buffer zone so that drifting of materials and insecticides during spraying was minimized. All experiments were five replicates.

2.2.3.2. Efficacy of *Syzygium aromaticum* bud derived materials and pesticide mixtures against *Bradysia procera* on ginseng field

This study was performed at the 6-years old ginseng field during June to August in Hwacheon (var Yeonpoong, N38°08′45.8″, E127°44′16.1″) on 2014, where infested ginseng field by *B. procera* larvae in previous year (2013). Experiment plots were laid out in randomized block design with five replicates. Each treatment plots were consist of 29.7 $m^2$.

Before treatment, total numbers of un-damaged ginseng stem in experiment plots were recorded at one day before foliar application as a pre-treatment count. Treatment of first foliar application was performed at 20$^{th}$ June. The treatments were three times with a week interval. The observations of damaged ginseng stem or peduncle were recorded at 7 and 14 day after treatment (DAT) as post foliar application counts.

A total twenty-six treatments were tested. The treatments included *S. aromaticum* bud derived materials (methanol extract and hydrodistillate), recommended doses of four insecticides and plant-derived materials with recommended doses of insecticides mixture in 20 L of Tween-40-methanol-water (0.1:0.5:0.9 by volume). Un-treatment received 20 L of Tween-40-methanol-water (0.1:0.5:0.9 by volume) only. Each plot was separated by a gap of 3.6 m so that transferring of materials and insecticides during spraying was minimized. The detail about treatments which were used during experiment was given in Table 32. All experiments were five replicates.

Table 32. Detail of various treatments as *Syzygium aromaticum* bud-derived materials, insecticides and *Syzygium aromaticum* bud-derived materials with insecticides mixture doses with foliar sprays to control *Bradysia procera*.

| No. | Test materials | Dose ($g/m^{-2}$) | Insecticides | Dose (g a.i/$m^2$) |
|---|---|---|---|---|
| T1 | SA-BME[a] | 0.484 | | |
| T2 | | 0.242 | | |
| T3 | SA-BHD[b] | 0.484 | | |
| T4 | | 0.242 | | |
| T5 | | | Clothianidin SC | 0.0097 |
| T6 | | | Cypermethrin EC | 0.0121 |
| T7 | | | Emamectin benzoate EC | 0.0026 |
| T8 | | | Thiamethoxam WG | 0.0121 |
| T9 | SA-BME[a] | 0.484 | Clothianidin SC | 0.0097 |
| T10 | | | Cypermethrin EC | 0.0121 |
| T11 | | | Emamectin benzoate EC | 0.0026 |
| T12 | | | Thiamethoxam WG | 0.0121 |
| T13 | | 0.242 | Clothianidin SC | 0.0097 |
| T14 | | | Cypermethrin EC | 0.0121 |
| T15 | | | Emamectin benzoate EC | 0.0026 |
| T16 | | | Thiamethoxam WG | 0.0121 |
| T17 | SA-BHD[b] | 0.484 | Clothianidin SC | 0.0097 |
| T18 | | | Cypermethrin EC | 0.0121 |
| T19 | | | Emamectin benzoate EC | 0.0026 |
| T20 | | | Thiamethoxam WG | 0.0121 |

Continued (Table 32).

| No. | Test materials | Dose ($g/m^{-2}$) | Insecticides | Dose ($g\ a.i/m^2$) |
|---|---|---|---|---|
| T21 | SA-BHD[b] | 0.242 | Clothianidin SC | 0.0097 |
| T22 | | | Cypermethrin EC | 0.0121 |
| T23 | | | Emamectin benzoate EC | 0.0026 |
| T24 | | | Thiamethoxam WG | 0.0121 |
| T25 | Control[c] | | | |
| T26 | Un-treatment | | | |

[a]SA-BME: *Syzygium aromaticum* bud methanol extract

[b]SA-BHD: *Syzygium aromaticum* bud hydrodistillate

[c]Treated Tween 40-methanol-water only

2.2.3.3. Anti-oviposition activity of *Syzygium aromaticum* against *Bradysia procera* in field condition

Because of the test materials possessed potential ovicidal activities as described at chapter 2, this study was used to determine whether the anti-oviposition activity of *S.aromaticum* bud against *B. procera* adult

This study was performed at randomized block design on the 6-years old ginseng field in the 2015 (var Jakyung, N38°12′15.8″, E127°17′20.1″, Cheolwon) during late May to July. Each treatment plots were consist of 82.5 $m^2$. Before treatment, the ginseng peduncles were removed for inducing oviposition at 3 days previous foliar application (29$^{th}$ May).

The treatments included *S. aromaticum* bud derived materials (SA-BME and SA-BHD) in 20 L of Tween-40-methanol-water (0.1:0.5:0.9 by volume) and recommended doses of clothianidin, emamectin benzoate and thiamethoxam in 19.99 L water, cypermethrin in 19.98 L water were foliar application three times with a week interval using sprayer on experimental plots of ginseng field. Control received 20 L of Tween 40-methanol-water (0.1:0.5:0.9 by volume) only. Each plot was separated by a gap of 3.6 m so that moving of materials and insecticides during spraying was minimized. Data on total number of un-damaged ginseng peduncles in experiment plots were recorded at one day before application (31$^{th}$ May) as a pre-treatment count and the observations of damaged peduncles were recorded at 7 and 14 day after treatment (DAT) as post foliar application counts. All experiments were five replicates.

2.2.3.4. Anti-oviposition of *Syzygium aromaticum* bud-derived materials and pesticide mixtures in ginseng fields against *Bradysia procera*

This study was performed at randomized block design on the 6-years old ginseng field during late-May to July in Cheolwon (var Yeonpoong, N38°16′39.1″, E127°22′47.4″) on 2016, where infested ginseng field by *B. procera* larvae previous year (2015). Each treatment plots were consist of 29.7 $m^2$.

Experiment was performed same manner and materials described before (1.2.3.3. and table 2, respectively). Before treatment, the ginseng peduncles were removed for inducing oviposition at 3 days previous foliar application. A total twenty six treatments were tested. The treatments included *S. aromaticum* bud derived materials (SA-BME and SA-BHD), recommended doses of four insecticides and plant-derived materials with recommended doses of insecticides mixture in 20 L of Tween-40-methanol-water (0.1:0.5:0.9 by volume). Un-treatment received 20 L of Tween 40-methanol-water (0.1:0.5:0.9 by volume) only. Each plot was separated by a gap of 3.6 m so that drifting of materials and insecticides during spraying was minimized. The detail about treatments which were used during experiment was described before (Table 22) Data on total number of ginseng peduncles in experiment plots were recorded at one day before application (30$^{th}$ May) as a pre-treatment count and 7 and 14 day after treatment (DAT) as post-treatment count. All experiments were five replicates.

### 2.2.4. Data analysis

Data were corrected for control mortality using the formula of Abbot (1925). The mortality percentages were transformed to arcsine square root values for analysis of variance (ANOVA). Means (±SE) of untransformed data are reported. Bonferroni multiple-comparison method was used to test for significant differences among the test materials and insecticides. The data on damage stem and anti-oviposition rate were calculated following formula. Damage stem (DSR) or Anti-oviposition rate (AOR) (%) = $N/T \times 100$, where $N$ is number of damaged stem (or peduncle) in experiment plot and $T$ is total number of stem (or peduncle) in experiment plot. Control efficacy (CE) (%) was calculated from the formula CE = (un-treatment DSR or AOR − DSR or AOR) / un-treatment DSR or AOR × 100.

## Results

### 2.2.1. The phytotoxicity of *Syzygium aromaticum* bud derived materials on ginseng field

Phytotoxic effect on ginseng of S. *aromaticum* bud derived materials were evaluated by foliar application in field condition. Responses varied with the material and application rate used. The phytotoxicity symptoms of aerial part of ginseng were not observed at 1, 3 and 7 days after treatment (DAT) of each dose of S. *aromaticum* bud derived materials (SA-BME and SA-BHD) in leafing period (Table 33)

Table 33. Phytotoxicity of *Syzygium aromaticum* bud-derived materials at 1, 3 and 7 days after treatment.

| Materials | Dose ($g/m^2$) | Scale of phytotoxicity[a)] | | |
|---|---|---|---|---|
| | | 1DAT[b)] | 3DAT[b)] | 7DAT[b)] |
| SA-BME[c] | 0.968 | 0 | 0 | 0 |
| | 0.484 | 0 | 0 | 0 |
| | 0.242 | 0 | 0 | 0 |
| SA-BHD[d] | 0.968 | 0 | 0 | 0 |
| | 0.484 | 0 | 0 | 0 |
| | 0.242 | 0 | 0 | 0 |
| Control (Tween 40-methanol-water) | | 0 | 0 | 0 |
| Un-treatment | | 0 | 0 | 0 |

[b]1DAT: 24th May, 3DAT: 26th May, 7DAT: 30th May.

[c]SA-BME: *Syzygium aromaticum* bud methanol extract.

[d]SA-BHD: *Syzygium aromaticum* bud hydrodistillate.

### 2.2.2. Toxicity of *Syzygium aromaticum* bud derived materials and four insecticides against *B. procera* in field condition.

Toxic effect on *B. procera* larvae of *S. aromaticum* bud derived materials (SA-BME and SA-BHD) and four commercial insecticides (clothianidin SC, cypermethrin EC, emamectin benzoate EC and thiamethoxam WG) were evaluated by foliar application in ginseng fields (Table 34). Responses varied with material and application rate used. In Yeoju experiment, the SA-BHD exhibited 56 and 45 % control efficacy at application rates of 0.484 and 0.242 g/m$^2$ on 7 day after treatment. After 14 days of treatment, 2 and 5 % were decreased in control efficacy at 0.484 and 0.242 g/m$^2$ respectively. In Icheon experiment, the SA-BHD showed 61 and 41 % control efficacy at application rates of 0.484 and 0.242 g/m$^2$ on 7 day after treatment and then 14 day after treatment observed 52 and 40 % control efficacy at 0.484 and 0.242 g/m$^2$. In these results, the control efficacy of the SA-BHD treatment at 0.484 g/m$^2$ was similar to that of two insecticides such as cypermethrin and emamectin benzoate EC. However, SA-BME lower than that four commercial insecticides.

Table 34. Control efficacy of *Syzygium aromaticum* bud derived materials and four insecticides against *Bradysia procera* in field condition

| Materials | Dose ($g/m^2$) | Control efficacy (CE) (%) (± SE)[a] | | | |
|---|---|---|---|---|---|
| | | Yeoju | | Icheon | |
| | | 7DAT | 14DAT | 7DAT | 14DAT |
| SA-BME[b] | 0.484 | 33 ± 2.4 de | 25 ± 0.4 e | 28 ± 2.93 de | 25 ± 4.4 de |
| | 0.242 | 21 ± 2.8 e | 11 ± 2.7 f | 23 ± 3.99 e | 20 ± 2.8 e |
| SA-BHD[c] | 0.484 | 56 ± 3.9 bc | 54 ± 2.7 cd | 61 ± 3.98 ab | 52 ± 3.2 bc |
| | 0.242 | 45 ± 2.5 cd | 40 ± 1.9 d | 41 ± 5.95 cd | 40 ± 0.8 cd |
| Clothianidin SC | 0.0097 | 77 ± 2.6 a | 75 ± 2.8 ab | 76 ± 3.97 a | 71 ± 4.9 a |
| Cypermethrin EC | 0.0121 | 65 ± 0.5 b | 61 ± 2.0 bc | 71 ± 4.44 ab | 62 ± 2.2 ab |
| Emamectin benzoate EC | 0.0026 | 60 ± 2.2 b | 56 ± 2.0 cd | 53 ± 1.39 bc | 42 ± 4.7 cd |
| Thiamethoxam WG | 0.0121 | 78 ± 0.9 a | 77 ± 0.6 a | 78 ± 0.80 a | 73 ± 0.6 a |
| Control (Tween 40-methanol-water) | | 1 ± 0.5 f | 2 ± 1.8 g | 1 ± 0.5 f | 1 ± 1.3 f |

[a]Means within a column followed by the same letter are not significantly different different ($P$=0.05, Bonferroni method)

[c]SA-BME: *Syzygium aromaticum* bud methanol extract

[d]SA-BHD: *Syzygium aromaticum* bud hydrodistillate

### 2.2.3. Field evaluation of *Syzygium aromaticum* bud derived materials, four insecticides and their mixtures against *Bradysia procera* in field condition.

Toxic effect on *B. procera* larvae of *S. aromaticum* bud derived materials (SA-BME and SA-BHD), four commercial insecticides (clothianidin SC, cypermethrin EC, emamectin benzoate EC and thiamethoxam WG) and bud derived materials with insecticides mixture were evaluated by foliar application in ginseng fields (Table 35). Responses varied with material and application rate used. The control efficacy of SA-BHD at 0.484 showed 59 and 52 % on 7 and 14 days after treatment respectively. The lethality of SA-BME revealed week activities compared with four insecticides. The control efficacy of four insecticides exhibited 52 to 80 and 68 to 72 % (0.0026–0.00121 g a.i/$m^2$) on 7 and 14 days after treatment respectively. The SA-BME with four insecticides mixture (clothianidin SC, cypermethrin EC, emamectin benzoate EC and thiamethoxam WG) were exhibited no difference compared with insecticides treated only. However, the lethality of SA-BHD (0.484 and 0.242 g/$m^2$) and cypermethrin EC (0.0121 g a.i/$m^2$) mixtures were increased comparing the single treatment of cypermethrin.

Table 35. Toxicity of *Syzygium aromaticum* bud derived materials, four insecticides and their mixtures against *Bradysia procera* larvae.

| No. | Test materials | Dose ($g/m^2$) | Insecticides | Dose (g a.i/$m^2$) | Efficacy (%) (±SE)[a] | |
|---|---|---|---|---|---|---|
| | | | | | 7 DAT | 14 DAT |
| T1 | SA-BME[b] | 0.484 | | | 22 ± 2.29 h | 11 ± 0.7 j |
| T2 | | 0.242 | | | 25 ± 4.15 h | 18 ± 4.4 j |
| T3 | SA-BHD[c] | 0.484 | | | 59 ± 2.26 fg | 52 ± 2.4 hi |
| T4 | | 0.242 | | | 43 ± 4.11 g | 41 ± 3.7 i |
| T5 | | | Clothianidin SC | 0.0097 | 80 ± 2.98 c | 72 ± 2.0 ef |
| T6 | | | Cypermethrin EC | 0.0121 | 74 ± 2.73 c-f | 70 ± 1.5 f |
| T7 | | | Emamectin benzoate EC | 0.0026 | 52 ± 2.89 g | 52 ± 2.5 hi |
| T8 | | | Thiamethoxam WG | 0.0121 | 78 ± 1.56 c–e | 72 ± 1.9 d–f |
| T9 | SA-BME[b] | 0.484 | Clothianidin SC | 0.0097 | 78 ± 1.03 cd | 66 ± 2.3 fg |
| T10 | | | Cypermethrin EC | 0.0121 | 85 ± 3.01 bc | 83 ± 2.2 bc |

Continued (Table 35).

| Test materials | | Dose (g/m$^2$) | Insecticides | Dose (g a.i/m$^2$) | Efficacy (%) (±SE)[a)] | |
|---|---|---|---|---|---|---|
| | | | | | 7 DAT | 14 DAT |
| T11 | SA-BME[b] | 0.484 | Emamectin benzoate EC | 0.0026 | 56 ± 3.0 g | 52 ± 2.6 hi |
| T12 | | | Thiamethoxam WG | 0.0121 | 82 ± 1.5 bc | 73 ± 1.0 c–f |
| T13 | | 0.242 | Clothianidin SC | 0.0097 | 85 ± 2.4 bc | 80 ± 1.3 c–e |
| T14 | | | Cypermethrin EC | 0.0121 | 89 ± 0.4 a-c | 82 ± 1.4 b–e |
| T15 | | | Emamectin benzoate EC | 0.0026 | 59 ± 2.1 fg | 57 ± 1.5 gh |
| T16 | | | Thiamethoxam WG | 0.0121 | 83 ± 2.4 bc | 65 ± 1.1 fg |
| T17 | SA-BHD[c] | 0.484 | Clothianidin SC | 0.0097 | 86 ± 1.8 a–c | 76 ± 1.7c–f |
| T18 | | | Cypermethrin EC | 0.0121 | 94 ± 2.5 a | 93 ± 0.2 a |
| T19 | | | Emamectin benzoate EC | 0.0026 | 60 ± 1.4 e–g | 52 ± 1.3 hi |
| T20 | | | Thiamethoxam WG | 0.0121 | 86 ± 2.3 a–c | 83 ± 0.5 b–d |

Continued (Table 35).

| Test materials | Dose (g/m$^2$) | Insecticides | Dose (g a.i/m$^2$) | Efficacy (%) (±SE)[a)] | |
|---|---|---|---|---|---|
| | | | | 7 DAT | 14 DAT |
| T21 SA-BHD[c] | 0.242 | Clothianidin SC | 0.0097 | 87 ± 2.0 a–c | 82 ± 1.1 b–d |
| T22 | | Cypermethrin EC | 0.0121 | 91 ± 2.3 ab | 89 ± 2.0 ab |
| T23 | | Emamectin benzoate EC | 0.0026 | 62 ± 2.1 d–g | 51 ± 2.3 hi |
| T24 | | Thiamethoxam WG | 0.0121 | 85 ± 2.6 a–c | 82 ± 1.3 b–e |
| T25 Control (Tween 40-methanol-water) | | | | 0 i | 0 k |
| T26 Un-treatment | | | | | |

[a]Means within a column followed by the same letter are not significantly different different ($P$=0.05, Bonferroni method).

[c]SA-BME: *Syzygium aromaticum* bud methanol extract

[d]SA-BHD: *Syzygium aromaticum* bud hydrodistillate

### 2.2.4. Effects of oviposition of *Syzygium aromaticum* bud derived materials and four insecticides against *B. procera* in field condition.

The effect of *S. aromaticum* bud derived materials and four commercial insecticides were evaluated by measuring anti-oviposition of the *B. procera* using the foliar application (Table 36). Responses varied with material and application rate used.

Based on 7 and 14 DAT in bud derived materials (methanol extract and hydrodistillate of *S. aromaticum* bud), SA-BHD at applicate rate of 0.484 g/m$^2$ was showed 51 and 44 % anti-oviposition efficacy. It was the most effective materials and was similar to that of clothianidin SC and thamethoxam WG at 7 and 14 DAT, respectively. Among four insecticides and plant derived materials, cypermethrin EC was revealed highest anti-oviposition efficacy (75 and 74 %) at 7 and 14 DAT, respectively.

Table 36. Anti-oviposition activity of *Syzygium aromaticum* bud derived materials and four commercial insecticides against *B. procera* in field condition.

| Materials | Dose ($g/m^2$) | Anti-oviposition efficacy (%) (± SE)[a] | |
|---|---|---|---|
| | | 7DAT | 14DAT |
| SA-BME[b] | 0.484 | 30 ± 2.0 de | 24 ± 1.4 c |
| | 0.242 | 21 ± 2.2 f | 16 ± 1.9 d |
| SA-BHD[c] | 0.484 | 51 ± 2.6 b | 44 ± 2.0 b |
| | 0.242 | 36 ± 0.8 cd | 30 ± 1.0 c |
| Clothianidin SC | 0.0097 | 42 ± 1.4 bc | 40 ± 1.2 b |
| Cypermethrin EC | 0.0121 | 75 ± 1.7 a | 74 ± 0.5 a |
| Emamectin benzoate EC | 0.0026 | 26 ± 1.7 ef | 25 ± 2.7 c |
| Thiamethoxam WG | 0.0121 | 41 ± 1.1 c | 41 ± 0.6 b |
| Control (Tween 40-methanol-water) | | 0 g | 0 e |

[a]Means within a column followed by the same letter are not significantly different different (*P*=0.05, Bonferroni method).

[b]SA-BME: *Syzygium aromaticum* bud methanol extract.

[c]SA-BHD: *Syzygium aromaticum* bud hydrodistillate.

### 2.2.5. Effects of oviposition of *Syzygium aromaticum* bud derived materials, four insecticides and their mixtures against *B. procera* in field condition.

The anti-oviposition effect on *B. procera* of *S. aromaticum* bud derived materials (methanol extract and hydrodistillate), four commercial insecticides (clothianidin SC, cypermethrin EC, emamectin benzoate EC and thiamethoxam WG) and bud derived materials and insecticides mixture were evaluated by foliar application on ginseng fields (Table 37). Among the plant derived materials, SA-BHD caused 53 and 49 % anti-oviposition efficacy at application rate of 0.484 g/m$^2$ on 7 and 14 DAT. However, SA-BME showed below 30 % anti-oviposition efficacy. The single treatment of three insecticides, clothianidin SC, emamectin benzoate EC and thiamethoxam WG were revealed below 45 % activity of anti-oviposition. However, cypermethrin EC was possessed 81 and 75 % efficacy of anti-oviposition at 7 and 14 DAT, respectively. The almost plant derived material and insecticide mixtures were showed similar activity to that of single treatment of insecticides, but potency of anti-oviposition of SA-BHD and cypermethrin EC mixture was increased compared with single treatment of SA-BHD and cypermethrin EC. It appeared 95 and 93 % anti-oviposition activity at application rate of 0.484 g/m$^2$ on 7 and 14 DAT, respectively.

Table 37. Anti-oviposition of *Syzygium aromaticum* bud derived materials, four insecticides and their mixtures against *Bradysia procera* larvae.

| No. | Test materials | Dose ($g/m^2$) | Insecticides | Dose (g a.i/$m^2$) | Efficacy (%) (±SE)[a] | |
|---|---|---|---|---|---|---|
| | | | | | 7 DAT | 14 DAT |
| T1 | SA-BME[a] | 0.484 | | | 28 ± 2.6 gh | 26 ± 1.1 f–h |
| T2 | | 0.242 | | | 21 ± 1.2 h | 18 ± 3.2 h |
| T3 | SA-BHD[b] | 0.484 | | | 53 ± 1.8 c | 49 ± 2.1 cd |
| T4 | | 0.242 | | | 36 ± 1.7 e–g | 33 ± 4.3 d–h |
| T5 | None | | Clothianidin SC | 0.0097 | 41 ± 0.8 c–f | 35 ± 1.3 d–g |
| T6 | | | Cypermethrin EC | 0.0121 | 81 ± 1.0 b | 75 ± 1.4 b |
| T7 | | | Emamectin benzoate EC | 0.0026 | 32 ± 1.3 fg | 21 ± 1.6 hg |
| T8 | | | Thiamethoxam WG | 0.0121 | 41 ± 2.2 c–f | 32 ± 1.1 d–h |
| T9 | SA-BME[a] | 0.484 | Clothianidin SC | 0.0097 | 44 ± 2.4 c–f | 35 ± 1.2 d–g |
| T10 | | | Cypermethrin EC | 0.0121 | 86 ± 0.7 b | 77 ± 1.7 b |

Continued (Table 37).

| No. | Test materials | Dose (g/m$^2$) | Insecticides | Dose (g a.i/m$^2$) | Efficacy (%) (±SE)[a)] | |
|---|---|---|---|---|---|---|
| | | | | | 7 DAT | 14 DAT |
| T11 | SA-BME[a] | 0.484 | Emamectin benzoate EC | 0.0026 | 34 ± 1.2 fg | 55 ± 6.7 c |
| T12 | | | Thiamethoxam WG | 0.0121 | 41 ± 4.5 c–f | 44 ± 8.9 c–e |
| T13 | | 0.242 | Clothianidin SC | 0.0097 | 40 ± 1.1 c–f | 32 ± 1.5 d–h |
| T14 | | | Cypermethrin EC | 0.0121 | 82 ± 1.3 b | 75 ± 1.5 b |
| T15 | | | Emamectin benzoate EC | 0.0026 | 36 ± 2.0 e–g | 30 ± 5.4 e–h |
| T16 | | | Thiamethoxam WG | 0.0121 | 39 ± 3.2 d–g | 29 ± 1.0 e–h |
| T17 | SA-BHD[b] | 0.484 | Clothianidin SC | 0.0097 | 50 ± 1.3 cd | 44 ± 1.8 c–e |
| T18 | | | Cypermethrin EC | 0.0121 | 95 ± 1.4 a | 93 ± 1.3 a |
| T19 | | | Emamectin benzoate EC | 0.0026 | 51 ± 1.3 cd | 48 ± 0.6 cd |
| T20 | | | Thiamethoxam WG | 0.0121 | 52 ± 1.6 c | 46 ± 1.8 c-e |

Continued (Table 37).

| No. | Test materials | Dose (g/m$^2$) | Insecticides | Dose (g a.i/m$^2$) | Efficacy (%) (±SE)[a)] | |
|---|---|---|---|---|---|---|
| | | | | | 7 DAT | 14 DAT |
| T21 | SA-BHD[b] | 0.242 | Clothianidin SC | 0.0097 | 47 ± 3.0 c–e | 39 ± 1.7 c–f |
| T22 | | | Cypermethrin EC | 0.0121 | 89 ± 1.8 ab | 80 ± 0.5 b |
| T23 | | | Emamectin benzoate EC | 0.0026 | 37 ± 1.8 e–g | 32 ± 2.5 d–h |
| T24 | | | Thiamethoxam WG | 0.0121 | 41 ± 1.4 c–f | 32 ± 2.4 d–h |
| T25 | Control (Tween 40-methanol-water) | | | | 0 i | 0 i |
| T26 | Un-treatment | | | | | |

[a]Means within a column followed by the same letter are not significantly different different ($P$=0.05, Bonferroni method).

[b]SA-BME: *Syzygium aromaticum* bud methanol extract.

[c]SA-BHD: *Syzygium aromaticum* bud hydrodistillate.

## DISCUSSION

Various compounds such as alkaloids, phenolics, and terpenoids, exist in plant and essential oils (Isman, 2006; Wink, 1993; Lawless, 2002). They jointly or independently contribute to a variety of biological activities, including ovicidal, antifeeding, repellent, and insecticidal activity against various insect species (Saxana, 1989; Desmarchelier, 1994; Isman 2000, 2001). Very little work has been done to consider its potential to manage fungus gnat, although *S.aromaticum* bud extract has been reported to be toxic to rice weevil, *Sitophilus oryzae* and pulse beetle, *Callosobruchus chinensis* (Kim et al., 2003).

While investigating the clove as a insecticide, it was also important to determine whether it was adverse effect to crop. Essential oil of clove, and its constituent eugenol, have been reported to have phytotoxic effects (Bainard et al., 2006; Boyd et al., 2006; Tworkoski, 2002; Waliwitiya et al., 2005; Walter et al., 2001). In this study, *E. caryophyllata* bud extract and hydrodistillate were not observed phytotoxicity on leafing period of ginseng. In field test with *S. aromaticum* bud extract and hydrodistillate against *B. procera* and their anti-oviposition, bud methanol extract revealed not effective compared with four commercial insecticides, while hydrodistillate was almost similar to that of clothianidin SC, cypermethrin EC and thiamethoxam WG at 0.484 g/$m^2$. Aditionally, hydrodistillate was similar control efficacy to that of clothianidin SC and thiamethoxam WG. This is first report of the control efficacy of the *S. aromaticum* bud hydrodistillate against *B. procera* population.

The curren findings clearly indicate that *S. aromaticum* bud hydrodistillate and insecticide mixtures (0.484 g/$m^2$ and 0.0121 g a.i/$m^2$) exhibited synergistic action

against *B. procera* and their oviposition. The original finding indicates that these mixture may hold promise for the development of novel and effective insecticide even against their eggs. Individual materials are active at high dose, which makes them high cost (uneconomical) and crop residue for practical use. Mansour et al. (2011) reported that some botanicals and commercial insecticides mixtures revealed larval toxic and developmental effect against the housefly, *Musca domestica*.

Investigations on the joint toxic action mechanism of binary mixture and the insecticides resistance mechanisms are of practical importance for insect control largely because they may give useful information on the most appropriate formulations to be adapted for therir future commercialization and for future resistance management (Kim et al., 2006; Perumalsamy et al., 2010). In conclusion, the binary mixtures of *S. aromaticum* bud derived materials and cypermethin EC could be useful management stragtegy in the control of *B. procera* population, particulary in the light of their activity against larvae and eggs. For practical use of mixtures of *S. aromaticum* bud derived materials and cypermethin EC as novel inseciticide to proceed, futher research is needed to establish their human safety and their ecotoxicity (honeybee, mammalian and crop residue). In addition, formulations for improving insecticidal potency and stability, thereby reducing cost, also need to be developed.

## CONCLUSSION

A correct damage symptoms, distribution and occurrence of this fungus gnat in ginseng cultivation are important for the establishment of effective and sustainable management strategy.

The fungus gnats, *Bradysia* spp., larvae are important insect pests in various crops such as bean, carrot, cucumber, lettuce, potato, soybean, tomato and mushroom (Kennedy, 1970, 1974; Hafidh and Kelly, 1982; Speyer, 1923; Wilkinson and Daugherty, 1970; Gillespi and Menzies, 1993). In worldwide, *B. procera* has lack of information such as biology and ecology in ginseng cultivation.

This study, we first report *Bradysia procera* (Winertz) in Korea, with a description of its damage to ginseng cultivation. Also, we examined to distribution, damage rate and their seasonal occurrence of *B. procera* in ginseng fields. The infested ginseng by fungus gnat larvae were tunneled into the stem and feeding, and revealed discoloration and defeated of aerial parts such as petioles, leaves and stems, and caused secondary infection of *B. procera* and other pests. The intensive damage caused to lose vitality and die even root decay, occasionally. The *B. procera* was distributed at ginseng field in South Korea, throughout almost ginseng cultivation area excluding Jeollaman-do province. Total 130 ginseng fields were surveyed during three years, the fungus gnat was occurred at a total 35 ginseng fields (26.9 %), which average damage rate was 9.3 %. However Cheolwon locations were average damage rate showed 19.6 %. At this result indicate that the *B. procera* spread throughout almost ginseng cultivation areas, however they prefer to shady and high humidity because of those habit nearby forest and stream. Similarly, the occurrence of *Phytosciara procera* (Diptera:

Sciaridae) damage to ginseng is more prevalent in areas with high humidity and heavily-and frequent fogging (Lee et al., 2010). Also, at ginseng cultivation area, adult of *Phytosciara procera* (Diptera: Sciaridae) emergence increased in late July and early or late August to early September (Shin et al., 2008; Lee et al., 2010).

Temporal variation in environmental variables such as temperature and relative humidity is important to *B. procera*. Therefore, microclimatic variables could be used to estimate *B. procera* density in ginseng fields. In particular, temperature and humidity are important for *Bradysia* spp. in terms of life cycle and development (Lee et al., 1998; Yuting, 2015). Also, insects are effected various biotic and abiotic factors (Messenger, 1959) such as rainfall, soil moisture, temperature, humidity (or combinded temperature and humidity) and locality. In our study demonstrated that average temperature was related with seasonal occurrence of *B. procera*, which first captured daily average temperature above 20 ℃ in May, however, seasonal occurrences pattern was showed inconsistency. Therefore, the difference in occurrence pattern due to the difference of environmental characteristic between two sites seems to be the cause of temporal variation and humidity.

This result was first record at ginseng fields in Korea that *B. procera* damaged in the almost ginseng cultivation areas. Our surveys were revealed that *B. procera* could occur three generation peaks at ginseng cultivation areas, especially they were effected average temperature. However, the present date can involve some uncertainties because of short-term survey and use of limited data. In addition, because of ginseng fields are migrated every 6 years, research of other environmental variables would also be need. Therefore, *B. procera* must be monitored and controlled as necessary during the growing season of ginseng to avoid the serious damage that this insect pest can

cause.

*Syzygium aromaticum* bud and *Illicium verum* fruit-derived products containing active constituents, particularly eugenol, eugenyla acetate, methyl salicylate, 2-nonanone, estragole and *trans*-anethole, could be useful as contact-action fumigants for the control of *B. procera* populations, provided that a carrier with a slow release of active material can be selected or developed. Further research is needed regarding the practical applications of *S. aromaticum* and *I. verum* fruit-derived preparations as novel insecticide products. This original finding indicates that *S. aromaticum* bud-derived preparations containing the active constituents described hold promise for the development of novel, effective naturally occurring larvicidal and ovicidal products for the control of *B. procera* populations. In addition, it is well acknowledged that the minor plant constituents may act as synergists, enhancing the effectiveness of the major constituents through various mechanisms for reducing the dose of polluting substances and the risk of developing resistance (Khater, 2012; Bekele and Hassanali, 2001; Miremailli et al., 2006). For example, Miresmailli et al.(2006) studied the effect of the chemical complexity of Rosmarinus officinalis essential oil on toxicity in *Tetranychus urticae*. They reported that a blend containing all 10 constituents of the oil is the most potent acaricide, and proposed that the minor constituents may act as synergists, enhancing the effect of the major constituents. Therefore, an interaction between various constituents of *S. aromaticum* bud hydrodistillate may contribute to enhancement of larvicidal or ovicidal activity of the oil against *B. procera*. Also, *I. verum* fruit-derived products, Specifically, it will be essential to establish safety profiles for these preparations in humans. *Illicium verum* and its essential oil as well as their major constituent anethole are generally recognized as safe (GRAS) and are used

widely in the food, brewery, and health supplement industries (Okuyama et al. 1993; Newberne et al. 1999; Wang et al. 2011; USFDA 2017), although the oral administration of a high dose (500 mg/kg) of the *I. verum* fruit ethyl acetate extract produced convulsions and lethal toxicity in mice (Okuyama et al. 1993). In addition, both plants, potential changes in the quality (e.g., color, flavor, odor, and texture) of ginseng plants treated with the essential oil products and their effects on residues in ginseng, non-target organisms including natural enemies and the environment must be examined. Lastly, detailed tests are needed to understand how to improve the insecticidal potency and stability of the constituents isolated from *S. aromaticum* bud and *I. verum* fruit for eventual product development.

## REFERRENCES

**A. Enkegaard and H. F. Brødsgaard.** 2000. Lasioseius fimetorum: a soil-dwelling predator of glasshouse pests? Biocon. **45**:285-293.

**Agrochemicals Use Guide Book**. Korea Crop Protection Association, Seoul, Republic of Korea (2017).

**Ahn, Y. J., S. I. Kim, H. K. Kim, and J. H. Tak. 2006.** Naturally occurring house dust mites control agents: development and commercialization, pp. 269–289. *In* M. Rai, M.C. Carpinella [eds.], Naturally occurring bioactive compounds. Elsevier, London, United Kingdom.

**Ahmad, M., Arif, M. I., Ahmad, Z., and Denholm, I.** 2002. Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. Pest. Manag. Sci. **58**: 203-208.

**Ahman, I.** 1986. Toxicities of host secondary compounds to eggs of the *Brassica* specialist *Dasineura brassicae*. J. chem. Ecol. **12**: 1487-1488.

**Ahn, Y. J., G. H. Kim and K. Y. Cho.** 1995. Bioassay system for insecticidal compounds In proceedings of third symposium on the biochemical methology fro research and development of the bioactive substances. The biochemical society of the Republic Korea, pp. 495-506

**Ahn, Y. J., S. B. Lee, H. S. Lee and G. H. Kim.** 1998. Inseciticdal and acaricidal activity of carvacrol and β- thujaplicine derived from *Thujopsis dolabrata* var. hondai sawdust. J. Chem. Ecol. **24**: 1-9.

**Ali, A., R. D. Baggs, and J. P.** 1981. Stewart Susceptibility of some Florida chrironomids and mosquitos to various formulations of *Bacillus thuringiensis* var. *israelensis*. J. Econ. Entomol. **74**: 672-677.

**Ali. O., R. Dunneb and P. Brennana.** 1999. Effectiveness of the predatory mite Hypoaspis miles (Acari: Mesostigmata: Hypoaspidae) in conjunction with pesticides for control of the mushroom fly *Lycoriella solani* (Diptera: Sciaridae). Exp. Appl. Acarol. **23**: 65–77.

**Anas, O., and Reeleder, R. D.** 1987. Feeding habits and maturity of *Bradysia* larvae as affected by different food sources. Phytoprotection. **68**: 131 pp.

**Anas, O., and Reeleder, R. D.** Feeding habits of larvae of *Bradysia coprophila* on fungi and plant tissue Phytoprotection. **69**: 73-78.

**Anonymous.** 2005. Commision Regulation (EC) No. 1048/2005 of 13 June 2005 amending Regulation (EC) No. 2032/2003 on the secong phase of the 10-year work programme referred to in Article 16(2) of Diretive 98/8/EC of the European Parliament and of the Council concering the placing of biocidal

product on the market. Official Journal of the European Union, 9.7.2005.

**Arnason, J. T., B. J. R Philogene, and P. Morand.** 1989. Insecticides of plant origin. 213 pp., ACS Sym. Ser. No. 387, AM. Chem. Soc., Washington, D. C.

**Baeg, In-ho and Seung-ho So.** 2013. The world ginseng market and the ginseng (Korea). J. Ginseng Res. **37**: 1–7.

**Bainard, L.D., M.B. Isman, and M.K.Upadhyaya.** 2006. Phytotoxicity of clove oil and its primary constituent eugenol and the role of leaf epicuticular wax in the susceptibility to these essential oils. Weed Sci. 54:833–837.

**Balandrin, M., A. D. Kinghorn, N. Farnsworth.** 1993. Plant-derived natural products in drug discovery and development, en Human medicinal agents from plants, Kinghorn, A., Balandrin, M. (eds). American Chemical symposium series 534, American Chemical society, New York, 2-12.

**Barraclough, D. A and Londt, J. G. H. 1996.** Diptera. In Insects of Southern Africa, eds C.H. Scholtz and E. Holm. University of Pretoria, Pretoria. pp. 283-321.

**Bartlett, G. R. and Keil, C. B. O.** 1997. Identification and Characterization of a permethrin resistance mechanism in populations of the fungus gnat *Lycoriella mali* (Fitch) (Diptera: Sciaridae). Pest. Biochem. Physiol. **58**: 173-181.

**Bee and Pesticides:** Commission goes ahead with plan to better protect bees. Retrieved 22, Nobember 2013.

**Bekele, J and Hassanali, A.** 2001. Blend effects in the toxicity of the essential oil constituents of *cimum kilimadscharicum* and *Ocimum kenyense* (Labiateae) on two post-harvest insect ests. Phytochemistry **57:**385–391.

**Berenbaum, M. R.** 1989. North American ethnobotanicals as sources of novel plant-based insecticides.pp. 11-14. in Insecticides of plant origins. Eds by J. T. Arnason, B. J. Philogene, and P. Morand. ACS sym. Ser. No. 387, Washington, D. C.

**Berg, M. P.** 2000. Mass-length and mass-volume relationships of larvae of *Bradysia pauper* (Diptera: Sciaridae) in laboratory cultures. Eur. J. Soil iol. **36**: 127-133.

**Binns E. S.** 1981. Sciarids as migrants. Mushroom journal. **108**: 415-421.

**Bischof, L., A. L. P. Peronidil and H. O. Gutzeit.** 1985. Morphology of the first instar larva of *Bradysia tritici* (Diptera: Sciaridae). Int. J. Insect Morphol. & Embryol. **14**: 193-198.

**Bloomquist, J. R.,** 1996. Ion channels as targets for insecticides. Annu. Rev. Entomol. **41**: 163-190.

**Borek, V., Elberson, L. R., McCaffrey, J. P., and Morra, M. J.** 1995. Toxicity of aliphaticand aromatic isothiocyanates to eggs of the black vine weevil (Coleoptera: Curculionidae). J. Econ. Entomol. **88**: 1192-1196.

**Boyd, N.S. and E.B. Brennan.** 2006. Burning nettle, common purslane, and rye response to a clove oil herbicide. Weed Technol. **20**:646–650.

**Brown, A. W. A.** Ecology of pesticides. John Wiley & Sons, New York

**Budavari S. O'Neil M. J., Smith A. and Heckelman P. E.** 1989. The Merck Index, Merck and Co Inc, Rahway, NJ USA .

**Burkill, I. H.,** 1966. A dictionary of the economic products of the Malay Peninsula, vol. 2. Government of Malaysia and Singapore. Ministry of agriculture and cooperatives, Kuala Lumpur. pp. 1244-1246.

**Butterworth, J. and Morgan, E.** 1968. Isolation of a Substance that suppresses Feeding in Locusts. Chem. Commun. (London). 1: 23-24.

**Cabral, M. M. O., A. Kelecom, and E. S. Garcia.** 1999. Effects of the lignan, pinoresinol on the moulting cycle of the bloodsucking bug *Rhodnius prolixus* and of the milkweed *Oncopeltus fasciatus*. Fitoterapia. **70**: 561-567.

**Cantwell, G. E and Cantelo, W. W. 1984.** Effectiveness of *Bacillus thuringiensis* var.

*israelensis* in controlling a sciarid fly, *Lycoriella mali*, in mushroom compost. J. Econ. Entomol. **77**: 473-475

**Carney, V. A., J. C. Diamond, G. D Murphy, and D. Marshall.** 2002. The potential of *Atheta coriaria* Kraatz (Coleoptera: Staphylinidae), as a biological control agent for use in greenhouse crops. Bulletin OILB/SROP. **25**: 37-40.

**Carson, H. L.** 1945. The selctive elimination of inversion dicentric chromatids during meiosis in the eggs of *Sciara impatiens*. Genetics. **31**: 95-113.

**Carson, H. L.** 1944. An analysis of natural chromosome variability in *Sciara impatiens*. J. morph. **75**: 11-59.

**Casanova, H., Ortiz, C., Peláez, C., Vallejo, A. and Moreno, M. E.** 2002. Insecticides formulations based on nicotine oleate stablized by sodium caseinate. J. Agric. Food. Chem. **50**: 6389-6394.

**Chae S. H, Kim S. I, Yeon S. H, Perumalsamy, H and Ahn, Y.J.** 2014. Fumigant toxicity of summer savory and lemon balm oil constituents and efficacy of spray formulations containing the oils to B- and neonicotinoid-resistant Q-biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. **107**:286–292.

**Chaieb, K., Zmantar, T., Ksouri, R., Hajlaoui, H., Mahdouani, K., Abdelly, C. and Bakhrouf, A.** 2007. Antioxidant properties of the essential oil of *Eugenia*

*caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. Mycoses. **50**: 403-406.

**Charlmers, A. E., T. A. Miller, and R. W. Olsen**. 1987. Deltamethrin: a neurophysiological study of the site of action. Pestic. Biochem. Physiol. **27**: 36-41.

**Chempakam, B., and Ballaji, S.** Chemistry of spices. CABI publishing, London, UK, pp. 319-330.

**Chen, C., Mu, W., Zhao, Y., Li, H., Zhang, P. Wang, Q., and Liu, F.** 2015. Biological activity of trans-2-hexenal against *Bradysia odoriphaga* (Diptera: Sciaridae) at different developmental stages. J. Insect sci. 1**5**: iev075.

**Chew, F. S. 1988.** Biological effects of glucosinolates. In H. G. Cutler (Ed.), Biologically active natural products: Potential use in agriculture (pp. 155–181). Washington, D.C.: American Chemical Society.

**Chin, W. Y., and Keng, H.,** 1990. An illustrated dictionary of Chinese medical herbs. Times editions, Singapore, and Eu Yang Sang Holdings, Malaysia pp. 96.

**Choi, K. H., H. C. Park., P. D. Kang., S. K. Kang and H. D. Sohn.** 1997. Developmental characteristics and life cycle of a sciarid fly (Lycoriella sp.) in indoor rearing. Korean J. Appl. Entomol. **36**: 77-82.

**Choi, K. T.** 2008. Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C. A. Meyer. Acta. Pharmacol. Sin. **29**: 1109-1118.

**Choo. E. J, Rhee. Y. H, Jeong. S. J, Lee. H. J, Kim. H. S, Kim. J. H, Kwon. T. R, Jung. J. H, Kim. J. H, Lee. H. J, Lee. E. O, Kim. D. K, Chen. C. Y and Kim. S. H.** 2011. Anethole Exerts Antimetatstaic Activity via Inhibition of Matrix Metalloproteinase 2/9 and AKT/Mitogen-Activated Kinase/Nuclear Factor Kappa B Signaling Pathways. Biol. Pharm. Bull. **34**: 41-46.

**Clark, J. M. and F. Matsumura**. 1982. Two different types of inhibitory effects of pyrethroids on nerve Ca2+ and Ca2++Mg2+-ATPase activity in the squid, *Loligo pealei*. Pestic. Biochem. Physiol. **18**: 180-190.

**Clift, A. D and Terras, M. A. 1992.** Evaluation of two insect growth regulators for insect pest control in the Austrailian mushroom industry. Plant. Prot. Quart. **7**: 59-61.

**Coats, J. R., L. L. Karr and C. D. Drewes.** 1991. Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In: Hedin, P. A. (Ed.), naturally occurring pest bioregulators. American Chemical society, Washington, DC, p. 305-316.

**Commission Regulation (EC) No. 1048/2005 of 13 June 2005.** Amending Regulation (EC) Np. 2032/2003 on the secong phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Off. J. Eur. Union.

**Coquillet, D. W.** 1895. A new wheat pest (Sciara tritici n. sp.). Insect Life. **7**: 406-408.

**Cortés-Rojas DF, Fernandes de Souza CR and Oliveira WP.** 2014. Clove (*Syzygium aromaticum*): a precious spice. Asian Pac. J. Trop. Biomed. **4**:90–96.

**Cu, J. Q.** 1990. GC/MS analysis of star anise oil. J. Essent. Oil Res. **2**: 91-92.

**D. B. Hay and P. N. Richardson.** 1995. Inter- and Intraspecific variation in infectivity of *Steinernema* spp. to larvae of the mushroom fly *Lycoriella solani.* entomol. exp. Appl. **77**: 11-15.

**DE, M., A. K., Sen, P., Banerjee, A. B.** 2002. Antimicrobial properties of star anise (*Illicium verum*). Phytother. Res. **16**: 94-95.

**DeBach, P. and D. Rosen.** 1991. Biological control by natural enemies. 2nd eds, Cambridge, Cambridge University Press.

**Devonshire, A. L.** 1987. Combating resistance to xenobiotics- biological and

chemical approach. Ford, M. G, Hollomon, D. W, Khambay. B. P. S. & Sawicki, R. M. [eds]. Ellis Horwood, Chichester. pp. 227-238.

**Desmarchelier, J. M.** 1994. Carbonyl sulfide as a fumigant for control of insects and mites In: Highley, E, E. J. Wright, H. J. anks, B. R. Champ (Eds), proceedongs of the sixth International Working Conference on Stored product protection, Canberra, Austria, April 1994. CAB International 1. 78-82. United Kingdom.

**Dorenburg, A and P. Karen.** 2003. The new American Chef: Cokking with the best flavors and techniques from around the world, John Wiley and Sons Inc.

**Editorial Board of Florida of China.** 2004. Secience Publishing House, Beijing.

**Edward, F. W. and C. B. Williams.** 1916. Sciara tritici Coq., a fly injurious to seedlings. Ann. App. Biol. **2**:257-262.

**EI Hag E. A., EI Nadi A. H., and Zaiton, A. A.** 1999. Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). Phytother. Res. **13**: 388-392.

**Eicker, A. and Ludick, E.** 1993. Methoprene for the control of *Lycoriella auripila* sciarid infestation of the cutivated mushroom Agaricus bisporus. Mushroom Res. **2**:19-24.

**Eira, A. F.** 2003. Cultivo do cogumelo medicinal Agaricus blazei (Murrill) ss Heinemann ou Agaricus brasiliensis. Aprenda Fácil Editora, Viçosa, MG

**Elliott, M.** 1997. Syntheic pyrethroids. Pp. 1-28., Synthetic pyrethroids. Ed. By M. Elliott. ACS symp. Ser. 42. Washington.

**Ellisor. L. O.** 1934. Notes on the biology and control of *Neosciara ocellaris* (Comstock) (Diptera: Sciaridae). Iowa State Coll. J. Sci. **9**: 25-36.

**Enan, E.** 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. C. **130**: 325-337.

**Enan, E.** 2005a. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Arch. insect biochem. Physiol. **59**: 161-171.

**Enan, E.** 2005b. Molecular response of *Drosophila melanogaster* tyramine receptor cascade to plant essential oils. Insect biochem. Mol. Biol. **35**: 309-321.

**Erler, F., Polat, E., Demir, H., Cetin., H and Erdemir, T.** 2008. Control of the mushroom phorid fly, *Megaselia halterata* (Wood), with plant extracts. Pest. Manag. Sci. **65**: 144-149.

**Expert Committee on Food Additives.** 1982. *Evaluation of Certain Food Additives*

*and Contaminants*. WHO Technical Report Series 683, WHO Press, Geneva, Switzerland.

**FAO Specifications and Evaluations for Agricultural Pesticides**: Thiamethoxam" (PDF). 21 June 2000.

**Farag, M. A., and P. W. Pare.** 2002. C6-green leaf volatiles trigger local and systemic VOC emissions in tomato. Phytochemistry. **61**: 545-554.

**Fawzi, T. H. and W. C. Kelly.** 1982. Cavityspot of carrots caused by feeding of fungus gnat larvae. J. Amer. Soc. Hort. Sci. **107**: 1177-1181.

**Feeny, P.** 1975. Biochemical coevolution between plants and their insect herbivores. pp. 3-19. in Coevelution of animals and plants eds. By Gilbert, L. E. and Raven, P. H. Texas Univ. Press

**Ferris, J. M., and Ferris, V. R.** 1966. Observation on *Tetradonema plicans*, an entomoparasitic nematode with a key to the genera of the family Tetradonematidae (Nematoda: Mermithoidea). Ann. Entomol. Soc. Am. **59**: 964-970.

**Fichi, G., G. Flamini, F. Giovanelli, D. Otranto., and S. Perrucci.** 2007. Efficacy of an essential oil of *Eugenia caryophyllata* against *Psoroptes cuniculi*. Exp. Parasitol. **115**: 168-172.

**Ffrench-Constant, R. H., J. C. Steichen and F. Shotkoski**. 1994. Polymerase Chain Reaction diagnostic for cyclodiene insecticides resistance in the mosquito *Aedes aegypti*. Med. Vet. Entomol. **8**: 99-100.

**Furuno T. Terada Y, Yano S. Uehar T and Jodai, S.** 1994. Activities of leaf oils and their components from Lauraceae trees against house dust mites. Mokuzai Gakkaishi. **40**: 78-87.

**Gammon, D. and J.E. Casida**. 1983. Pyrethroids of the most potent class antagonize GABA action on the crayfish neuromuscular junction. Neurosci. Lett. **40**: 163-168

**Garber, S. S., and Miller, C.** 1987. Single Na+ channels activated by veratridine and batrachotoxin. J. Gen. Physiol. **89**: 459-480.

**Garcia, R. B., B. Des Rochers, and W. Tozer.** 1980. Studies on the toxicity of *Bacillus thuringiensis* var. *israelensis* against organisms found in association with mosquito larvae. Proc. Calif. Mosq. Vector Control Assoc. **48**:33-36.

**Gardiner, R. B., W. R. Jarvis and J. L. Shipp.** 1990. Ingestion of Pythium spp. by larvae of the fungus gnat Bradysia impatiens (Diptera: Sciaridae). Ann. Appl. Biol. **116**: 205-212.

**Gaugler, R., and J. R. Finney.** 1982. A review of *Bacillus thuringiensis* var. *israelensis* (serotype 14) as a biological control agent of black flies (Simuliidae). Misc. Publ. Entomol. Soc. Am. **12**:1-17.

**Georghiou, G. P., and T. Saito.** 1983. Pest resistance to pesticides. Plenum Pub., New York.

**Gillespi, D. R. and M. J. Menzies.** 1993. Fungus gnats vector *Fusarium ozysporum* F. sp. radicis-lycopersici. Ann Appl. Biol. 123: 539-544.

**Gillespie, D. R.** 1986. A simple rearing method for fungus gnats *Corynoptera* sp. (Diptera: Sciaridae) with notes on life history. J. Entomol. Soc. of Br. Colum. **83**: 45-48.

**Gillespie, D. R. and Qhirung, M. J.** 1990. Biological control of fungus gnats, *Bradysia* spp. (Diptera: Sciaridae), and western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), in greenhouses using a soil dwelling predatory mite, *Geolaelaps* sp. nr. Acleifer (Canestrini) (Acari: Laelapidae). Can. Ent. **122**: 975-983.

**Goldberg, L. J. and Margalit, J.** 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens*. Mosquito News. **37**: 355-358.

**Gouge, D. H., and Hague, N. G. M.** 1995. Glasshouse control of fungus gnat *Bradysia paupera* on fuchsias by *Steinernema feltiae*. Fundam. Appl. Nematol. **18**: 77-80 (1995)

**Graham, C. L. and M. J. McNeill.** 1972. Soybean crown and root damage by *Bradysia coprophila*. J. Econ. Entomol. **65**:597-599.

**Grewal. P. S., M. Tomalak, C. B. O. Keil and R. Gaugler.** 1993. Evaluation of a genetically selected strain of *Steinernema feltiae* against the mushroom sciarid *Lycoriella mali*. Ann. Appl. Biol. **123**: 695-702.

**Greene, T., Rogers, S., Franzen, A and Gentry, R.** 2017. A critical review of the literature to conduct a toxicity assessment for oral exposure to methyl salicylate. Crit Rev Toxicol. **47:**98–120.

**H. S. Lee., T. S. Kim., H. Y. Shin., H. H. Kim., and K. J. Kim.** 2001. Host plant and damage symptom of Fungus gnat, Bradysia spp. (diptera: Sciaridae) in Korea. Korean J. Appl. Entomol. **40**: 149-153

**Hafidh, F. T. and W. C. Kelly.** 1982. Cavity spot of carrot caused by feeding of fungus gnat larvae. J. Amer. Soc. Hort. Sci. **107**: 1177-1181.

**Hakki, A., M. Ertas, Siegfrie, N., and Hubert, K.** 2007. Research on essential oil content and chemical composition of Turkish clove (*Syzygium aromaticum* L.). BioResources. **2**: 265-269.

**Hemingway, J ., C. Smith, K. G. I. Jayawardena and P. R. J. Herath.** 1986. Field and laboratory detection of the altered acetylcholinesterase resistance genes which confer organophosphate and carbamate resistance in mosquitoes (Diptera:Culicidae). Bull. Entomol. Res. **76**: 559-565.

**Harborne, J. B.** 1993. Introduction to Ecological Biochemistry, 4th ed. Academic Press, New York.

**Harris M.A.** 1993. Fungus gnat: They're more than just a nuisance. Grower talks. 56: 49-59.

**Harris M.A., Gardner W.A. and Oetting R.D.** 1996. A review of the scientific literature on fungus gnats (Diptera: Sciaridae) in the genus Bradysia. J. Entomol. Sci. **31**: 252–276.

**Harris, M.A.** 1995. Dissemination of the phytopathogen *Thielaviopsos basicola* by the fungus gnat *Bradysia coprophila* and biological control of tese pests by *Fusarium proliferatum* and steinernematid nematodes. Ph. D Diss., Univ. of Georgia. Thens, G. A. pp. 59.

**Harris, M.A., R. D. Oetting and W. A. Gardner.** 1995. Use of entomopathogenic nematodes and a new monitoring technique for control of fungus gnats, *Bradysia coprophila* (Diptera: Sciaridae), in floriculture. Biol. Control. **5**: 412-

418.

**Harris, S. and Waindle, M.** Insect growth regulators (IGRs) [Online]. Chase research gardens, Inc. Available: www.chaseresearchgardens.com/pdfs.igr.pdf .[14 April 2002]

**Hayes, W. J. Jr.** 1982. Pesticides studied in man. Williams & Wilkins, Baltimore, pp 672.

**Hayes, W. J. Jr., and E. R. Laws, Jr.** 1991. Handbook of Pesticides Toxicology. Academic press.

**Hedin, P. A. Hollingworth, R. M., E. P. Masler, J. Miyamoto and D. G. Thomson (eds),** 1997. Phytochemical for pest control, ACS symp. Ser. No 658, American Chemistry society, Washinton, DC, USA, 372.

**Hellén, W.** 1964. Die Ismarinen und Belytinen Finnland. Faunnica. **18**:1-68.

**Heller. K, Hippa. H and Vilkamaa. P.** 2015. Taxonomy of *Bradysia* Winnertz (Diptera, Sciaridae) in the Northern Holarctic, with the description of four new species. European Journal of Taxonomy. **122**: 1–15.

**Hellqvist. S.** 1994. Biology of *Synacra* sp. (Hym., Diapriidae),a parasitoid of *Bradysia paupera* (Dipt.,Sciaridae). J. Appl. Entomol. **117**:491–497.

**Helyer, N., Brown, K. and Cattlin.** A Colour handbook of biological control in plant protection. Manson Publishing, London. 224pp.

**Hitoko, H., S, Morozumi, T, Wauke, S, Sakai and H. Kurata.,** 1980. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Appl. Environ. Micorobiol. **39**: 818-822.

**Ho, S. H., Cheng, L. P. L., Sim, K. Y., and Tan, H. T. W.** 1994. Potential of cloves (*Syzygium aromaticum*) (L.) Merr. And Perry as a grain protectant against *Tribolum castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Postharvest. Biol. Thechnol. **4**: 179-183.

**Ho, S. H., Y. Ma, P. M. Goh and K. Y. Sim,** 1995. Star anise, *Illicium verum* Hook. F. as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Postharvest. Biol. Thechnol. **6**: 341-347.

**Hollingworth, R. M. and A. E. Lund.** 1982. Biological and neurotoxic effects of amidine pesticides. Pp. 189-227, in Insecticide Mode of Action. Ed. J. R. Coat. Academic Press, New York.

**Huang Y, J. Zhao, L. Zhou, J. Wang, Y. Gong, X. Chen, Z. Guo, Q. Wang, and W. Jiang.** 2010. Antifungal activity of the essential oil of *Illicium verum* fruit and its main component *trans*-anethole. Molecules **15**: 7558-7569.

**Huang Y, Zhen, B. and Li, Z.** 1992. Natural and induced epizootics of *Erynia ithacensis* in mushroom hothouse populations of yellow-legged fungus gnats. J. Invertebr. Pathol. **60**:524-258.

**Hudson, K. E.** 1974. Regulation of greenhouse sciarid fly populations using *Tetradonema plicans* (Nematoda: Mermithoidea). J. Invertebr. Pathol. **23**: 85-91.

**Hummelbrunner, L. A. and M. B. Isman.** 2001. Acute, sublethal, antifeedant and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep. Noctuidae). J. Agric. Food. Chem. **49**: 715-720.

**Hungerford, H. B.** 1916. *Sciara* maggots injurious to potted plants. J. Econ. Entomol. **9**: 538-549.

**Hungerford, H. B.** 1919. Biological notes in *Tetradonema plicans*, Cobb, a nematode parasite of *Sciara coprophila* Lintner. J. invertebr. Pathol. **5**: 186-192.

**Hurley B. P., B. Slippers B. D. Wingfield P. Govender J. E. Smith and M. J. Wingfield.** 2010. Genetic diversity of *Bradysia difformis* (Sciaridae: Diptera) populations reflects movement of an invasive insect between forestry nurseries. Biol Invasions. **12**:729–733

**Hussein, G., H. Miyashiro, N. Nakamura, M. Hattori, N. Kakiuchi and K. Shimotohno.** 2000. Inhibitory effects of Sudanese medicinal plant extracts on Hepatitis C Virus (HCV) Protease. Phytother. Res. **14**: 510-516.

**Ilyas, M.,** 1980. Spice in India. Econ. Bot. **34**: 236-259.

**Isman, M. B.** 2000. Plant essential oils for pest and disease management. Crop Prot. **19**: 603-608.

**Isman, M. B.** 2001. Pesticides based on plant essential oils for management of plant pests and diseases. Pp. 1-9, in International Symposium on Development of Natural Pesticides from Forest Resources. Korea Forest Research Institute. Seoul, Korea.

**Isman, M. B.** 1995. Leads and prospects for the development of new botanical insecticides. Rev. estic. Toxicol. **3**:1-20.

**Isman, M. B.** 2006. Botanical insecticides, deterrent, and repellents in mordern agriculture and as incresingly regulated world. Annu. Rev. Entomol**. 51**: 45-66.

**Isman, M. B and Grieneisen, M. L.** 2014. Botanical insecticide research: many publications, limited useful data. Trends Plant Sci. **19**: 140-145.

**Isman, M. B and Machial, C.M.** 2006 Pesticides based on plant essential oils: from

traditional practice to commercialization, in *Naturally Occurring BioactiveCompounds,* ed. by Rai M and Carpinella MC. Elsevier, London, pp. 29–44.

**J. S. Shin., D. H. Cho., H. S. Cho., H. Y. Kim and H. S. Lee.** 2008. A report on the damaged caused by *phytosciara procera*, ginseng stem fungus gnat. J. Ginseng Res. **32**: 275-278.

**Jacobson, M.** 1989. Botanical pesticides – past, present, and future, pp. 1–10. *In* J. T. Arnason, B. J. R. Philogene, P. Morand [eds.], Insecticides of plant origin. ACS Symposium Series 387, American Chemical Society, Washington, DC.

**Jacobson, M. and D. G. Crosby.** 1971. Naturally occuring insecticides. Marcel Dekker, New York, USA, 585pp.

**Jandrick, S., Murphy, G., Broadbent, B., Scott-Dupree, C. ad Harris, R.** 2005. Compatibility *of Atheta coriaria* with other biocontrol agents used in greenhouse production. IOBC/WPRS bulletin. **28**: 135-138.

**Jeon, Y. H., Lee, B. D., Yu, Y. H., Chang, S. P., Kim, S. G., Hwang, I., and Kim, Y, H.,** 2008. First description of crown gall disease on ginseng. Plant. Pathol J. **24**: 207-210.

**Jess, S and Klipatrick, M.** 2000. An intergrated appoach to the control of *Lycoriella*

*solani* (Diptera: Sciaridae) during production of the cultivated mushroom (*Agaricus bisporus*). Pest. Manag. Sci. **56**: 477-485.

**Jiang, C. H., Q. Z. Liu, S. S. Du, Z. W. Deng, and Z. L. Liu.** 2012. Essential oil composition and insecticidal activity of *Evodia lepta* (Spreng) Merr.Root barks from China against two grain storage insects. J. Med. Plants Res. 6: 3464-3469.

**Johannsen, O. A.** 1912. The fungus gnats of North America, Part IV. Maine Agric. Exp. Sta. Bull. **200**: 57-146.

**Jones, D. A.** 1974. Co-evolution and cyanogenesis. Pp. 213-242, in Taxonomy and Ecology. Ed. By Heywood, V. H. Academic press, London

**Jones, D.A.** 1988. Cyanogenesis in animal-plant interactions, pp. 151–165, in D. Evered and S. Harnett (eds.). Cyanide Compounds in Biology. John Wiley & Sons, Chichester, United Kingdom.

**Joshi, B., G. P. Sah, B. B. Basnet, M. R. Bhatt, D. S. K., Subedi, J. Pandey and R. Malla.** 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata*(Datiwan) and *Azadirachta indica* (Neem). J. Microbiol. Antimicrob. **3**: 1-7.

**Kalyanasundaram, M. and C. J. Babu.** 1982. Biologically active plant extracts as

mosquito larvicides. Ind. J. Med. Res. **76**: 102-106.

**Kalyanasundaram, M. and P. K. Das.** 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Ind. J. Med. Res. **82**: 19-23.

**Kang, J. S.** 1991. Evaluation of inhibitory effects of tropical plant tissues on the corn earworm, *Helothis zea* Boddie (Lepidoptera; Noctuidae). MS Thesis. Univ. of Missouri, Columbia.

**Kasahara, S., and Hemmi, S.,** 1995. Medical herb index in Indonesia, 2nd ed. PT Eisei Indonesia, Jakarta. pp. 10.

**Kennedy, M. K.** 1971. The significance of fungi in the ecology of *Bradysia impatiens* (Johansen) ( Diptera: Sciaridae). MS thesis, Cornell Univ. pp.66.

**Kennedy, M. K.** 1974. Kennedy, M. K. Survival and development of *Bradysia impatiens* (Diptera: Sciaridae) on fungal and non-fungal food sources. Ann. Entomol. Soc. Am. **67**: 756-749.

**Kennedy, M. K.** 1976. The interaction of *Bradysia impatiens* (Joh.) (Diptera: Sciaridae), a fungal host and the root systems of vascular plants. Ph.D. Diss., Cornell Univ., Ithaca, NY. 87 pp.

**Khanikor, B. and Bora, D.** 2011. Toxicity of essential oil compounds against *Exorista sorbillans* (Diptera: Tachinidae), a parasitoid of silkworm. **10**: 19807-19815.

**Khater, H. F. 2012.** Prospects of botanical biopesticides in insect pest management. Pharmacologia **3**: 641–656.

**Kim, D. E and J, Kim.** 2014. Occurrence and host plant of Metcalfa pruinosa (say) (Hemiptera: Flatidae) in Korea. J. Environ. Sci. Int. **23**: 1385-1394.

**Kim, E. H., H. K. Kim., D. H. Choi and Y. J. Ahn.** 2003. Acaricidal activity of clove bud oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae). Appl. Entomol. Zool. **38**: 621-266.

**Kim, H. H., H. H. Choo, H. S. Lee, S. R. Cho, H. H. Shin, C. G. Park and Y. M. Choo.** 2000. Occurences and damage of *Bradysia agretis* Sasakawa (Diptera: Sciaridae) in propagation house. Korean J. Appl. Entomol. **39**: 89-97.

**Kim, K. W.,** 1994. Surveys on ginseng damage by insect and other animal pests. Korean J. Appl. Entomol. **33**: 237-241.

**Kim, S. I., H. K. Kim. Y. Y. Koh, J. M. Clark, and Y. J. Ahn.** 2006. Toxicity of spray and fumigant products containing cassia oil to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). Pest Manag. Sci.

**62**: 768-774.

**Kim, S. I., J. H, Lee., J. H, Tak., and Y. J. Ahn.** 2004. Acaricidal activity of plant essential oils against Dermanyssus gallinae (Acari: Dermanyssidae). Vet. parasitol. **120**: 297-304.

**Kim, S. I., J. Y. Roh, D. H. Kim., H. S. Lee and Y. J. Ahn.** 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. J. Stored prod. Res. **39**: 293-303.

**King, A. I.** 1990. It is a shore fly or a fungus gnats? Greenhouse Grower. **8**: 38-39.

**Kiss, T. and O. Osipenko.** 1991. Deltamethrin depress acetylcholine-activated currents in snail neurons. Pestic. Sci. **32**: 392-395.

**Klun, J. A., C. L. Tipton, and T. A. Brindley.** 1967. 2,4-Dihydroxy-7-methoxy-3-one (DIMBOA), an active agent in the resistance maize to the European corn borer. J. Econ. Entomol. **60**:1529-1533.

**Koul O, Walia S and Dhaliwal GS.** 2008. Essential oils as a green pesticides: potential and constrains. Biopestic. Int. **4:**63–84.

**Kostyukovsky, M., A. Rafaeli, C. Gileadi, N. Demchenko and E. Shaaya.** 2002. Activation of octopaminergic receptors by essential oil constituents isolated

from aromatic plants: possible mode of action against insects pests. Pest. Manag. Sci. **58**: 1101-1106.

**Kramer, J. P.** 1981. A mycosis of the blood-sucking snipe-fly Symphoromyia hirta caused by Erynia ithacensis. Mycophathol. **75**: 159-164.

**Kranthi, K. R., Jadhav, D. R., Kranthi, S., Wanjari, R. R., Ali, S. S., and Russell, D. A.** 2002. Insecticide resistance in five major insect pests of cotton in India. Crop. Prot. **21**: 449–460

**Kurokawa, M., T. Hozumi, P. Basnet, M. Nakano, S. Kadota, T. Namba, T. Kawana and K. Shiraki.** 1998. Purification and Characterization of Eugeniin as an Antiherpesvirus Compound from Geum japonicum and *Syzygium aromaticum*. J. Pharm. Exp. Therap. **284**:728-735.

**L. Iauk, A. M. Lo Bue, I. Milazzo, A. Rapisarda and G. Blandino.** 2003. Antibacterial activity of medicinal plant extracts against Periodontopathic bacteria. Phytother. Res. **17**: 599-604.

**L. Ranasinghe, B. Jayawardena1 and K. Abeywickrama.** 2002. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M.Perry against crown rot and anthracnose pathogens isolated from banana. Letters Appl. Microbiol. **35**: 208-211.

**Landis, T. D.** 1996. Biocontrols for fungus gnats. Forest nursery notes. 1: 18-19.

**Lawless, J.** 2002. The encyclopedia of essential oils. London, UK: Thorsons.

**Leath K. T and Newton R. C. 1969.** Interaction of a fungus gnat, *Bradysia* sp. (Sciaridae) with *Fusarium* spp. on alfalfa and red clover. Phytopathol. **59**: 257-258.

**Lee, S. H, Shin, J. S., Hong, T. K., Ahn, Y. J., and D. H., Cho.,** 2010. Biological characteristic of Ginseng stem fungus gnat (*Phytosciara procera*) and its environmental-friendly control using modified topping of ginseng peduncles. J. Ginseng Res. **34**: 23-29.

**Lee, H. S., T. S. Kim., Shin, H. Y., H. H. Kim., and Kim, K. J.** 2001. Host plant and damage symptom of Fungus gnat, *Bradysia* spp. (diptera: Sciaridae) in Korea. Korean J. Appl. Entomol. **40**: 149-153

**Lee, H. S., Kim, K. J and Hyun-Uk. Lee.** 1998. Effect of temperature on the development of sciarid fly, *Bradysia* sp. (Diptera: Sciaridae). Korean J. Appl. Entomol. **37**: 171-178.

**Leibowitz, M. D., Sutro, J. B., Hille, B.** 1986. Voltage-dependent gating of veratridine-modified Na channels. J. Gen. Physiol. **87**: 25-46.

**Levi G, Gallo V, Raiteri M.** 1980. A reevaluation of veratridine as a tool for studying the depolarization-induced release of neurotransmitters from nerve endings. Neumchem. Res. **5**: 281-295.

**Li, S. Z.** 1596. Compedium of materia medica (Bencaogangmu). People's Hygiene Publication co., Beijing. pp. 1636-1639.

**Li, S., and Liu, S.** 2000. Chemical constituents of essential oil from Cenxi's *Illicium*. China condiment. **20**: 69-70.

**Lichtenstein, E. P., Morgan, D. G., and Mueller, C. H.** 1964. Naturally occurring insecticides in cruciferous crops. J. Agric. Food. Chem. **12**: 158-161.

**Lichtenstein, E. P., Strong, F. M., and Morgan, D. G.** 1962. Identification of 2-phenylethylisothio-cyanate as an insecticide occurring naturally in the edible part of turnips. J. Agric. Food. Chem. **10**: 30-33.

**Lindquist, R. K., Faber, W. R., and Casey M. L.** 1985. Effect of various soilless root media and insecticides on fungus gnats. Hort. Sci. **20**: 358-360.

**Liu, Z. L., Q. Z. Liu, S. S. Du, and Z. W. Deng.** 2012. Mosquito larvicidal activity of alkaloid and limonoids derived from *Evodia lepta* unripe fruit against *Aedes albopitus* (Diptera: Culicidae). Parasitol. Res. **111**: 991-996.

**Lowery, D. T and Isman, M. B.** 1995. Toxicity of neem to natural enemies of aphids. *Phytoparasitica.* **23**: 297–306.

**López, P., C. Sánchez, R. Batlle, and C. Nerín.** 2005. Solid- and Vapor-Phase Antimicrobial Activities of Six Essential Oils: Susceptibility of Selected Foodborne Bacterial and Fungal Strains. J. Agric. Food Chem. **53**: 6939-6946.

**Ludwig, S. W. and Oetting, R. D.** 2001. Evaluation of medium treatments for management of *Frankliniella occidentalis* (Thripidae: Thysanoptera) and *Bradysia coprophila* (Diptara: Sciaridae). Pest. Manag. Sci. **57**: 1114-1118.

**Mansour, S. A., R. F. A. Bakr, R. I. Mohamed and N. M. Hasaneen.** 2011. Larval acivity of some botanical extracts, commercial insecticides and their binary mixture against the the housefly, *Musca domestica*. The open toxicology journal. **4**: 1-13.

**Marcus, C. and E. P. Lichtenstein,** 1979. Biologically active components of anise: toxicity and interactions with insecticides in insect. J. Agri. Food. Chem. **27**: 1217-1223.

**Margoth suarez, Jorge Bonilla, Aura M. P. De Diaz and Hans Achenbach.** 1983. Dehydrodieugenols from *Nectandraa polita*. Phytochemistry. **22**: 609-610.

**Marr, K. L. and C. S. Tang,** 1992. Volatile inseciticidal compounds and chemical variability of Hawaiian *Zanthoxylum* (Rutaceae) species. Biochem. Syst. Ecol.

**20**: 209-217.

**Matthiessen, J. N., and Shackleton, M. A.** 2000. Advantageous attributes of larval whitefringed weevil, *Naupactus leucoloma*(Coleoptera: Curculionidae) for bioassaying soil fumigants, and responses to pure and plant-derived isothiocyanates. Bull. Entomol. Res. **90**: 349-355.

**Matsumura, F.** 1986. Advances in understanding insecticide modes of action. In: pesticide science and biotechnology, Sixth IUPAC congress of pesticides chemistry. pp. 151-159.

**Menzel. F, Smith J. E., Colauto N. B.** 2003. *Bradysia difformis* Frey and *Bradysia ocellaris* (Comstock): two additional neotropical species of black fungus gnats (Diptera:Sciaridae) of economic importance: a redescription and review.

**Mercier, L.** 1911. Sur les roles des insectes comme agents de propagation de l'ergot des graminees. Comp. Rend. Soc. Biol. (Paris). **70**: 300-302 (1911)

**Messenger, P. S.** 1959. Bioclimatic studies with insect. Annu. Rev. Entomol. **4**: 183-206.

**Metcalf, C. L., W. P. Flint and R. L. Metcalf.** 1962. Destructuve and usefuk insectL their habits and control. 4th edi. McGraw-Hill Book Co., New York NY. 1087 pp.

**Metcalf, R. L., and R. B. March.** 1950. Properties of acetylcholine esterase from the bee, the fly and the mouse and their relation to insecticide action. J. Econ. Entomol. **42**: 670-677.

**Miller T. A. and Adams M. E.** 1982. Mode of action of pyrethroids. In: Insecticide Mode of Acrion (Edited by Coats J. R.), Academic Press, New York. pp. 3-27.

**Ministry of Agriculture, Food and Rural affairs (MAFRA),** 2012. Ginseng Statistical Data (No. 11-1543000-000004-10). MAFRA. pp. 1-9.

**Miresmailli S, Bradbury, R and Isman, M. B.** 2006. Comparative toxicity of *Rosmarinus officinalis* L.essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Manag Sci **62:**366–371.

**Miresmailli, S and Isman, M. B.** 2006. Efficacy and persistence of rosemary oil as an acaricide against twospotted spider mite (Acari: Tetranychidae) on greenhouse tomato. J Econ Entomol. **99**:2015–2023.

**Miyazawa M., H. Watanabe and H. Kameoka.** 1997. Inhibition of acetylcholinesterase activity by monoterpenoids with a *p*-menthane skeleton. J. Agric. Food. Chem. **45**: 677-679.

**Miyazawa M., H. Watanabe, K. Umemoto and H. Kameoka.** 1998. Inhibition of acetylcholinesterase activit by essential oils of *Mentha* species. J. Agric. Food.

Chem. **46**: 3431-3434.

**Miyazawa, M., Ota, H., Ishikawa, Y., Kameoka, H,** 1993. An insecticidal compound from *Illicium verum*. Chem. Express. **8**: 437-440.

**Moawed, H. A. M.** 1998. Joint action of some plant extracts against the mosquito larvae of *Culex pipiens* and their physiological impact. M.Sc., Thesis, Damiatta Faculty of Science, Mansoura University, Damietta, Egypt.

**Mulla, M. S. and T. Su.** 1999. Activity and biological effects of neem products against arthropods of medical and veternary importance. J. Am. Mosq. Control Assoc. **15**: 133-152.

**Nahrstedt, A.** 1985. Cyanogenesis and the role of cyanogenic compounds in insects. Plant Syst. Evol. **150**:35-47.

**Nakamura, T., Okuyama, E., and Yamazaki, M.** 1996. Neurotropic components from star anise (*Illicium verum* Hook. F.). Chem. Pharm. Bull. **44**: 1908-1914.

**Namba, T.** 1993. The encyclopedia of Wakan-Yaku (traditional Sino-Japanese Medicines) with color picture I, Hoikusha, Osaka, Japan, pp. 606.

**Namba, T., and Tuda, T.,** 1993. Outline of pharmacognosy. A textbook. Nankodo, Tokyo. A textbook. Nankodo, Tokyo. pp. 257-309.

**Narahashi, T**. 1971a. Mode of action of pyrethroids. Bull. WHO. **44**: 337-345.

**National Research Council.** 2000. The future role of pesticides in US agriculture. Washinton, DC: Natl. Acad. Press. pp 301.

**National Toxicology Program.** 1983. Carcinogenesis studies of eugenol (CAS No. 97-53-0) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Program Tech Rep Ser **223**:1–159.

**Nauen, R. and Denholm, I.** 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Arch. Insect Biochem. Physiol. **58**: 200–215

**Newberne, P., R. L. Smith, J. Doull, J. I. Goodman, I. C. Munro, P. S. Portoghese, B. M. Wagner, C. S. Weil, L. A. Woods, T. B. Adams, C. D. Lucas, R. A. Ford. 1999.** The FEMA GRAS assessment of *trans*-anethole used as a flavouring substance. Food Chem. Toxicol. **37**: 789–811.

**Nixon, G. E. J.** 1957. Hymenoptera Proctotrupoidea. Diaprrodae subfam. Belytinae. Handbooks for the identification of Britich insects. **8**:1-107.

**Obeng-Ofori, D and Reichmuth, C.** 1997. Bioactivity of eugenol, a major component of essential oil of *Ocimum suave* (Wild.) against four species of

stored-product Coleoptera. Int J Pest Manag **43**:89–94.

**Ogendo, J. O., Kostyukovsky, M., Ravid, U., Matasyoh, J. C., Deng, A. L., Omolo, E, O., Kariuki, S. T and Shaaya, E.** 2008. Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. *J* Stored Prod Res **44**:328–334.

**Oh, I. C., T. Denk, and E. M. Friis.** 2003. Evolution of *Illicium* (Illiciaceae): mapping morphological characters on the molecular tree. Plant Syst. Evol. **240**: 175–209.

**Ohta, M., T. Narahashi, and R. F. Keeler.** 1973. Effects of veratrum alkaloids on membrane potential and conductance of squid and crayfish giant axons. J. Pharmacol. Exp. Ther. 184: 143.

**Okuyama, E., Nakamura, T., and Yamazaki, M.** 1993. Convulsants from star anise (*Illicium verum* Hook. F.). Chem. Pharm. Bull. **41**: 1670-1671.

**Osborne, L. S., Boucias, D. G. and Lindquist, R. K.** 1985. Activity of *Bacillus thuringiensis* var. *israelenesis* on *Bradysia coprophila* (Diptera: Sciaridae). J. Econ. Entomol. **78**: 922-925.

**Park, C. G., J. Yoo, M. Sasakawa, H. Y. Choo, H. H. Kim and H. S. Lee.** 1999. Notes on newly recorded insect pest, *Bradysia agretis* (Diptera: Sciaridae). Korean J. Appl. Entomol. **38**: 59-62.

**Park, I. K., and S. C, Shin. 2005**. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). J. Agric. Food Chem. **53**: 4388-4392.

**Park, I. K., Choi, K. S., Kim, D. H., Choi, I. H., Kim, L. S., Bak, W. C., Choi, J. W., and Shin, S. C. 2006a**. Fumigant activity of plant essential oils and components from horseradish (*Armoracia rusticana*), anise (*Pimpinella anisum*) and garlic (*Allium sativum*) oils against *Lycoriella ingenua* (Diptera: Sciaridae) Pest. Manag. Sci. **62**: 723-728.

**Park, I. K., Kim, L. S., Choi, I. H., Lee, Y. S., and Shin, S. C. 2006b.** Fumigant activity of plant essential oils and components from *Schizonepeta tenuifolia* against *Lycoriella ingenua* (Diptera: Sciaridae). J. Econ. Entomology. **99**: 1717-1721.

**Pavela R.** 2016. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects – a review. Plant Protect Sci. **52:** 229–241.

**Pavela, R. and G. Benelli.** 2016. Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci. **21**: 1000–1007.

**Pekka Vilkalmaa.** 2014. Checklist of the family Sciaridae (Diptera) of Finland. ZooKeys. **441**: 151–164.

**Peloquin, J. I and Platzer, E. G.**1993. Control of root gnats (Sciaridae: Diptera) by *Tetradonema plicans* Hungerford (Tetradonema: Nematoda) produced by a novel culture method

**Perondini, A. L. P., H. O. Gutzeit and L. Mori.** 1986. Nuclear division and migration during early embryogenesis of *Bradysia tritici* Coquillet (syn. *Sciara ocellaris*) (Diptera: Sciaridae). Int. J. Insect Morphol. & Embryol. **15**: 155-163.

**Perrucci, S., Macchioni, G., Cioni, P. L., Flamini, G., and Morelli, I.** 1995. Structure activity relationship of some natural monoterpenes as acaricides against *Psoroptes cuniculi*. J. Nat. Prod. **8**: 1261-1264.

**Perumalsamy, H., K. S. Chang, C. Park, and Y. J. Ahn.** 2010. Larvicidal activity of Asarum heterotropoides root constituents against insecticide-susceptible and resistant Culex pipiens and Aedes aegypti and Ochlerotatus togoi. J. Agric. Food Chem. **58**: 10001-10006.

**Piozzi, F., M. Bruno, and S. Rosselli.** 1998. Futher furoclerodanes from *Teucrium* genus. Heterocycles. **48**: 2185-2203.

**Pitcher, R. S. 1936.** Investigations on the insects ans allied pests of cultivated

mushrooms. VIII. Observations on the larval instars of *Sciara fenestralis* Zett. J. S. E. Agric Coll. **38**: 83-85.

**Price, J. F., L. S. Osborne, C. A. Nagle and E. McCord Jr.** Management of Fungus Gnats in ornamentals. http://edis.ifas.ufl.edu/ig125.

**Priestley, C. M., E. M. Williamson, K. A. Wafford, and D. B. Sattelle.** 2003. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human $GABA_A$ receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. Br. J. Pharmacol. **140**: 1363-1372.

**Radad, K, G. Gille and W.-D. Rausch.** 2003. Use of ginseng in medicine: perspectives on CNS disorders, Iranian J. Pharmacol. Ther. **3**: 30–40.

**R. A. Hamlen and F. W. Mead.** 1979. Fungus gnat larval control in greenhouse plant production. J. Econ. Entomol. **72**: 269-271.

**R Core Team.** 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

**R. J. Chamber, E. M. Wright and R. J. Lind.** 1993. Biological control of glasshouse sciarid flies (*Bradysia* spp.) with the predatory mite, *Hypoaspis miles*, on cyclamen and poinsettia. Biocontrol. Sci. Techn. **3**: 285-293.

**Richardson, P. N and Grewal, P. S.** 1991. Comparative assessment of biological (Nematoda: Steinernema feltiae) and chemical methods of control for the muchroom fly *Lycoriella auripila* (Diptera: Sciaridae). Biocontrol Science and Technology. **1**: 217-228.

**Rolli, E., M. Marieschi, S. Maietti, G. Sacchetti, and Renato Bruni.** 2014. Comparative phytotoxicity of 25 essential oils on pre- and post-emergence development of *Solanum lycopersicum* L.: Amultivariate approach. Ind. Crops. Prod. **60**: 280-290.

**Roeder, K. D. and E. A. Weiant.** 1946. The site of action of DDT in the cockroach.Science. **130**: 304-306.

**Rosengarten.** 1969. The book of spices. Livingston Publishing company, Wynnewood, Pennsylvania.

**Rossignol, D. P.** 1991. Analysis of pyrethroid binding by use of photoreactive analogue: possible role of GTP-binding proteins in pyrethroid activity. Pestic. Biochem. Physiol. **41**: 103-120.

**Ryan, M. F., and Byrne, O.** 1988. Plant-insect coevolution and inhibition of acetylcholinesterase. J. chem. Ecol. **14**: 1965-1975.

**S. Shin, S. Jung, K. Heller, F. Menzel, T. K. Hong, J. S. Shin, S. H. Lee, H. Lee and S. Lee.** 2015. DNA barcoding of Bradysia (Diptera: Sciaridae) for detection of the immature stages on agricutural crops. J. Appl. Entomol. **139**: 638-645.

**Saeed, S. and P. Tariq.** 2008. *In vitro* antibacterial activity of clove against gram negative bacteria. Pak. J. Bot. **40**: 2157-2160.

**Salgado, V. L., Irving, S. N. & Miller, T. A.** 1983. Depolarization of motor nerve terminals by pyrethroids in susceptible and *kdr*-resistant houseflies. Pestic. Biochem. Physiol. **20**: 100-114.

**Schalfer, T., Cotti, T. and Moore, J. L.** 1988. Cyromazine, a new insect growth regulator for leafminer control. Proc. 1986 Brit crop prot conf on pests and disease, BCPC, Alton, Hants, UK, pp. 193-198.

**Schmeltz, I.** 1971. Nicotine and other tobacco alkaloids. In: Jacobson M, Crosby DG (eds) Naturally occurring insecticides. Marcel Dekker, New York. pp 99–136.

**Seo, M. J., H. S. Shin., C. S. Gawk, S. H. Jo, H. S. Cho, J. S. Shin, D. H. Cho, Y. N. Youn and Y. M, Yu.** 2011. Environmental-friendly control of the ginseng stem fungus gnat (*Phytosciara procera*) in the ginseng field. The korean J. Pest. sci. **15**: 471-478.

**Seo, M. J., H. S. Shin., S. H. Jo, C. S. Gawk, Kwon, H. R., Park, M. W., Kim, S. H,**

**Yu, Y. M., and Youn Y. N.** 2011. Selection of environmental-friendly control agents for controlling the comstock mealybug [*Pseudococcus comstaki* (Wuwana), Pseudococcidae, Hemiptera]. The korean J. Pest. sci. **12:** 479-484.

**Seo, S. T., and Tang, C. S.** 1982. Hawaiian fruit flies (Diptera: Tephritidae): toxicity of benzyl isothiocyanate against eggs or 1st instars of three species. J. Econ. Entomol **75**: 1132-1135.

**Sexena, K.** 1989. Clinical features and management of poisonong due to potassium chloride. Med Toxicol Advese Drug Exp. **4**: 429-443.

**Shen, D. R., Zhang, H. R., Li, Z. Y., He, S. Y., and Zhang, T.** 2009. Taxonomy and dominance analysis of sciarid fly species (Diptera: Sciaridae) on edible fungi in Yunnan. Acta Entomol. Sin.

**Shin, J. S., D. H. Cho., H. S. Cho., H. Y. Kim., H. S. Lee.,** 2008. A report on the damaged caused *by phytosciara procera*, ginseng stem fungus gnat. J. Ginseng Res. **32**: 275-278.

**Shin, S. G., Lee, H., Lee, S.** 2012. Dark winged fungus gnats (Diptera: Sciaridae) collected from shiitake mushroom in Korea. J. Asia-pacif. Entomol. **15**: 174-181.

**Shin, S. G., S, Jung, F. Menzel, K. Heller, H, Lee., and Lee, S.** 2013. Molecular phylogeny of black fungus gnats (Diptera: Sciaroidea: Sciaridae) and the

evolution of larval habitats. Mol. Phylogenet. Evol. **66**: 833-846.

**S. Shin, S. Jung, K. Heller, F. Menzel, T. K. Hong, J. S. Shin, S. H. Lee, H. Lee and S. Lee.** 2015. DNA barcoding of Bradysia (Diptera: Sciaridae) for detection of the immature stages on agricutural crops. J. Appl. Entomol. **139**: 638-645

**Singh, G. J. P. and I. Orchard**. 1983. Action of bioresmethrin on the corpus cardiacum of *Locusta migratoria*. Pestic. Sci. **14**: 229-234.

**Smith, J. E.** Mushroom pests- Insecticide resistance [Online]. Available: http://www2.hri.ac.uk/preview.php?record=10105 [july 2004]

**Smith, R. L., Doull, J., Feron, V. J., Goodman, J. I., Munro, I. C., Newberne, P. M., Portoghese, P. S., Waddell, W. J., Wagner, B. M., Adams, T. B and McGowen, M. M.** 2001. GRAS flavoring substances 20. Food Technol. **55**:34-55.

**Smith-Stocking, H.** 1936. Genetic studies on selective segregation of chromosome in Sciara coprophila Lintner. Genetics. **21**: 421-443.

**Soloway, S. B.** 1976. Naturally occurring insecticides. Environ. Health. Perspect.

**Speyer, E. R.** 1923. Mycetophilid flies as pests of the cucumber plant in greenhouses. Bull. Entomol. Res. **13**: 255-260.

**Springer, T. L.** 1995a. Fungus gnat (Diptera: Sciaridae) feeding damage to legume seedlings. J. Kansas. Entomol. Soc. **68**: 240-242.

**Springer, T. L.** 1995b. Vulnerability of pature and range legumes to fungus gnats. Crop Sci. **35**: 534-536.

**Stedman, E., and G. Barger.** 1925. Physostigmine (eserine). Physostigmine (eserine). Par III. J. Chem. Soc. **127**: 247.

**Steffan, W. A.** 1966. A gereric revision of the family Sciaridae (Dipetera) of America north Mexico. Univ. of Calif. Publ. Entomol. **44** :1-77.

**Steffan, W. A.** 1974. Laboratory studies and ecological notes on Hawaiian Sciaridae (Diptera). Pac. Insects. **16**: 41-50.

**Stein, S., Y. Morokhin, D. Tcheckhovskoi, and W. G. Mallard.** 2008. NIST Standard Reference Database Number 69. *In* P. J. Linstrom, W. G. Mallard [eds.], NIST Chemistry WebBook. National Institute of Standards and Technology, Gaithersburg MD.

**Sukumar K, Perich MJ and Boobar L**. 1991. Botanical derivatives in mosquito control: a review. J. Am. Mosq. Control Assoc. **7:**210–237.

**Takayama, K., R. Jansen, E. J. van Henten, F. W. Verstappen, H. J.**

**Bouwmeester, and H. Nishina.** 2012 Emission index for evaluation of volatile organic compounds emitted from tomato plants in greenhouse. Biosyst. Eng. **113**: 220-228.

**Tandon, K., E. Baldwin, and R. Shewfelt.** 2000. Aroma perception of individual volatile compounds emitted from tomato plants in greenhouses. Postharvest. Biol. Thechnol. **20**: 261-268.

**Tang, W. and G. Eisenbrand.** 1992. Chinese drug of plant orisin. Springer, New York, pp. 1056.

**Tapia, T., F. Perich, F. Pardo, G. Palma, and A. Quiroz.** 2007. Idenification of volatiles from differently aged red clover (*Trifolium pratense*) root extracts and behavioral responses of clover root borer (*Hylastinus obscurus*) (Marsham) (Coleoptera: Scolytidae) to them. Biochem. Syst. Ecol. **35**: 61-67.

**Thangnam, T. S. and K. Kathiresan.** 1990. Synergistic effects of insecticides with plant extracts on mosquto larvae. Trop. Biomed. **7**: 135-137.

**Thangnam, T. S. and K. Kathiresan.** 1991. Mosquito larvicidal activity of marine plant extracts with insecticides. Botanica Marina. **34**: 537-539.

**Thomas, C. A.** 1929. A method for rearing mushroom insects and mites. Entomol. News. **40**: 222-225.

**Thomas, C. A.** 1931. Mushroom insects: their biology and control. Bull. Penn. Sta. School Agric. Exp. Sta. **270**: 3-42.

**Trongtokit, T., Rongsriyam, Y., Komalamisra, N., and Apiwathnasorn, C.** 2005. Comparative repellency of 38 essential oils against mosquito bites. Phytother. Res. **19**: 303-309.

**Tung P. S., and Snetsinger R.** 1973. New technique for culturing a mushroom-infesting sciarid, *Lycoriella mali* (Fitch). Melsheimer Entomol. Series. **11**: 1-4.

**Tworkoski, T.** 2002. Herbicide effects of essnetial oils. Weed Sci. **50**: 425-431.

**U.S. EPA.** 1996. Exemption of certain pesticide substances from federal insecticides, fungicide, and rodenticde act requirements. U.S.Environmental Protection Agency, Final Rule, 40 CFR 152.25 (g), May 6.

**U. S. EPA.** 2002. Organophosphate pesticides; schedule for actions on organophosphate, http://www.epa.gov/pesticides/op/actionops.htm. US Environmental Protection Agency.

**U. S. EPA.** 2003. Pesticides: regulating pesticides, http://www. epa.gor/oppfead1/international/piclist.htm.

**U. S. EPA.** 2004. Biopesticides-25b Minimum risk pesticides. http://www.epa.gov/oppbppd1/biopesticides/regtool/25b_list.htm.U.S.Environmental Protection Agency, Washington, DC.

**U. S. EPA.** Pesticides. 2010. reregistration; U.S Environmental Protection Agency: Washington, DC, 2010. https://archive.epa.gov/ pesticides/reregistration/web /html/ status.html

**[USEPA]. U.S. Environmental Protection Agency. 2017**. Reregistration and other review programs predating pesticide reregistration review. (https://www.epa.gov/pesticide-reevaluation/registration-and-other-review-programs-predating-pesticide-reregistration).

**[USFDA]. U.S. Food & Drug Administration. 2017.** Generally Recognized as safe (GRAS).(http://www.fda.gov/Food/Ingredients PakagingLabelin -g/GRAS/ucm2006850.htm).

**van Tonder, J.** 1994. Act against fungus gnats. Farmer's Weekly May 20.

**Velluti, A., V. Sanchis, A.J. Ramos, C. Turon and S. Marín.** 2004. Impact of essential oils on growth rate, zearalenone and deoxynivalenol production by *Fusarium graminearum* under different temperature and water activity conditionsin maize grain. J. Appl. Microbiol. **96**: 716-724.

**Visser, J. H.** 1986. Host odor perception in phytophagous insects. Annu. Rev. Entomol. **31**: 121-144

**Wada, K., and K. Munakata.** 1967. An insecticidal alkaloid, Cocculoidine from C*occulus trilobus* DC. Part I. The isolation and the insecticidal activity of Cocculolidine. Agric. Biol. Chem. **31**: 336-339.

**Wadleigh, R. W., and Yu, S. J.** 1988. Detoxification of isothiocyanate allelochemicals by gluta-thione transferasein three lepidopterous species. J. chem. Ecol. **14**: 1279-1288.

**Waliwitiya, R., M.B. Isman, R.S. Vernon, and A. Riseman.** 2005. Insecticidal activity of selected monoterpenoids and rosemary oil to *Agriotes obscurus* (Coleoptera: Elateridae). J. Econ. Entomol. **98**:1560–1565.

**Walter, J.F., J.C. Locke, and M.C.Normoyle.** 1997. Clove oil as a plant fungicide.U.S. Patent No. 5,679,351. U.S. Patent and Trademark Office, Washington, DC.

**Ware, W. G.** An introduction to insecticides. [Online]. Available: http://weedscience.siuc.edu/moa2005/download/Session%2013/insecticide%20MOA/George%20Ware.pdf[17 May 2010]

**Wang, G. W., W. T. Hu, B. K. Huang, L. P. Qin.** 2011. *Illicium verum*: a review on its botany, traditional use, chemistry and pharmacology, J. Ethnopharmacol. **136**: 10–20.

**Weinzierl, R. A.** 2000. Botanical insecticides, soaps, and oils. In biological and biotechnological control of insect pest, ed. J. E. Rechcigl, N. A. Rechcigl. pp 101-121. Boca Raton, FL: CRC Press.

**Wessel, M.** 1989. Trauermücken-Einflub von substraten auf entwicklund und eiablage. Zierpflanzenbau. **29**: 918-920.

**Wetzel, H. A., Wuest, P. J., Rinker, D. L. and Finley, R. J.** 1982. Significant insect pests of the commercial mushroom.In Pennsylvania State Handbook for commercial Mushroom Growers (WUEST P. J., BENGTSON G. D., Eds), pp 34-39, Pennsylvania State University, Pennsylvania.

**White, M. J. D.** 1949. Cytological evidence on the phylogeny and classification of the dipteral. Evo. **3**: 252-261.

**White, P. F.** 1981. Chemical control of the mushroom sciarid *Lycoriella auripila* (Winn.). Mushroom science, Proc. 11th Interna. Cong. Sci. and cultivation of edible fungi, Sydney, Austrailia

**White, P. F.** 1986. Effect of bendiocarb and diflubenzuron on mushroom cropping.

Ann. Appl. Biol. **108**:11-20.

**White, P. F.** 1997. The use of chemicals, antagonists, repellents and physical barriers for the control of *Lycoriella auripila* (Diptera: Sciaridae). Ann. Appl. Biol. **131**: 29-42.

**White, P. F.** 1999. Comparative effects of three insecnt growth regulator insecticieds and a dipteran-active strain of *Bacillus thruringiensis* on cropping of the cultivated mushroom *Agaricus bisporus*. Ann. Appl. Biol. **131**: 29-42.

**White, P. F. and Gribben D. A.** Variation in resistance to diazinon by mushroom sciarid *Lycoriella auripila*. Proceedings of Mushroom Science XII, Braunschweig Part II: 851-859.

**White, P. F., Smith, J. E. and Menzel, F.** 2000. Distribution of Sciaridae (Dipt.) species ingesting commercial mushroom farms in Biatain. Entomol. Mon. Mag. **136**: 207-209.

**Wilkinson, J. D. and D. M. Daugherty.** 1970. Comparative development of *Bradysia impatiens* (Diptera: Sciaridae) under constant and variable temperatures. Ann. Entomol. Soc. Am. **63**: 1079-1083.

**Williams, C. W.** 1967. Third-generation pesticides. Sci. Am. **217**: 13-17.

**Wink, M.** 1993. Production and application of phytochemicals from an agricultural perspective, pp. 171-213. In T. A. van Beek and Breteler (eds.), Phytochemistry and agriculture, Vol. 34 Clarendon, Oxford, UK.

**Winnertz, Johannes.** 1868. Acht neue Arten der Gattung Sciara. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Ö sterreich. (Verh. Zool. -bot Ges. Wien). **18**: 535-536.

**Wright, E. M and Chamber, R. J.** 1994. The biology of the predatory mite Hypoaspis miles (Acari: Laelapidae), a potential biological control agent of Bradysia paupera (Dip.: Sciaridae). Entomophaga. **39**: 225-235.

**Wu, H., H., Wu, Wang, W., Liu, T., Qi, M., Feng, J., Li, X., and Y. Liu.** 2016. Insecticidal activity of sesquiterpene lactones and monoterpenoid from the fruit of *Carpesium abrotanoides*. Ind. Crops. Prod. **92**: 77-83.

**Yamaguchi, I., and Fugimura, M.** 2005. Recent topics on action mechanisms of fungicides. J. Pestic. Sci. **30**: 67-74.

**Yang, Y. C., Lee, S. H., Lee, W. J., Choi, D. H. and Ahn, Y. J.** 2003. Ovicidal and adulticidal effect of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. J. Agric. Food Chem. **51**: 4884-4888.

**Geng, Y. J. Liu, R. Lv, J. Yuan, Y. Lin, X. Wang.** 2007. Separation and Purification

Technology. **57**: 237-241

**Yazdani. D, Rezazadeh, S. H., Amin, G. H. Zainal, A. M. A, Shahnani, S. and Jamalifar, H.,** 2009. Antifungal Activity of Dried Extracts of Anise (*Pimpinella anisum* L.) and Star anise (*Illicium verum* Hook. f.) Against *Dermatophyte and Saprophyte* Fungi J. Med. Plnat. **8**: 24-29.

**Yeager, J. F. and S. C. Munson.** 1945. Physiological evidence of a site of action of DDT in an insect. Science. **102**: 305-307.

**Yi C. G, Hieu T. T, Lee S. H, Choi B. R, Kwon, M and Ahn Y. J,** 2016. Toxicity of *Lavandula angustifolia* oil constituents and spray formulations to insecticide-susceptible and pyrethroid-resistant *Plutella xylostella* and its endoparasitoid *Cotesia glomerata*. Pest Manag. Sci. **72:**1202–1210.

**Yi, J. H., Perumalsamy, H., Sankarapandian, K., Choi, B. R., and Ahn, Y. J.** 2015. Fumigant toxicity of phenylpropanoids identified in *Asarum sieboldii* aerial parts to *Lycoriella ingenua* (Diptera; Sciaridae) and *Coboldia fuscipes* (Diptera: Scatopsidae). J. Econ. Entomol. **108**: 1208-1214.

**Yu, Y. H. and Ohh, S. H.,** 1993. Research on ginseng disease in Korea. Korean J. Ginseng Sci. **17**: 61-68.

**Yu, Y. H., Yi, Y. K., and Ohh, S. H.,** 1991. Stem rot of ginseng caused by *Erwinia*

*carotovor*a subsp. *Carotovora*. Korean J. Plant pathol. **7**: 183-187.

**Yuting. Y., Li. W., Xie. W., Wu. Q., Xu. B., Wang. S., Li. C and Zhang. Y.** 2015. Development of *Bradysia odoriohaga* (Diptera: Sciaridae) as affected by humidity: an age-stage, two-sex, life-table study. Appl. Entomol. Zool. **50**: 3-10.

# 국문초록

인삼줄기버섯파리의 생물학적 특징 및 정향 꽃봉오리와 팔각회향 유래 성분에 대한 살충/살란 활성, 이에 대한 작용기작에 대한 실험을 실시하였다.

인삼줄기버섯파리 암컷은 약 160개의 알을 산란하였으며 알 평균기간은 6일 이었다. 1령 유충과 3령 유충의 평균 무게는 각각 1.3, 8.1, 81 μg 이었으며 각 유충의 평균 길이는 608, 1670, 4,330 μm 및 140, 510, 792 μm로 조사되었다. 각 암컷과 수컷 기간은 16.1일과 15.3일 이며 암컷과 수컷의 번데기의 무게 길이 폭은 각각 99 μg, 3,972, 1,109 μm와 106 μg, 4,681, 1175 μm 였다. 수컷과 암컷은 번데기로부터 기간은 각각 6.7일과 7일이었으며 그들의 수명은 각각 5.3일과 6.8일로 조사되었다. 대체 먹이인 마늘과 인삼의 비교 실험에서는 무게, 폭, 크기의 차이는 없었으나 생존율에서 차이를 보였다.

인삼줄기버섯파리 년중 발생 실험에서 성충은 년간 3회, 6월 상순과 7월중순, 8월 하순에 발생 최성기를 보였다. 인삼줄기버섯파리 유충의 피해 증상으로는 최초 감염시 인삼 화경의 변색을 보이며 시간이 지날수록 인삼의 엽자루의 탈락과 화경이 전체적으로 변색되는 것을 관찰할 수 있었으며 피해정도가 심해짐에 따라 인삼이 시들거나 죽는 경우를 확인하였다. 수확 시기에는 인삼줄기버섯파리의 유충은 토양 내 인삼의 뇌두, 고죽내부 및 뿌리까지 들어가 월동하는 것을 관찰 할 수 있었다.

정향 꽃봉오리에서 유래물질(메탄올 추출물과 정유)과 분리한 3종의phenylpropanoids, 3종의 terpenoids, 그리고 1종의 ketone을 대상으로 한 필터페이퍼 살충 효과 실험에서는 methyl salicylate ($LC_{50}$, 5.26 μg/cm-$^2$)에서 가장 높은 활성을 보였으며 2-nonanone, eugenol, eugenyl acetate (8.77 –15.40 μg/cm-$^2$) 순으로 확인 되었다. 이 물질들은 대조약제로 사용 된 thiamethoxam, clothianidin 및 cypermethrin보다 낮은 효과를 나타내었다.

알부화 억제는 11.7 μg/cm-$^2$을 처리한 methyl salicylate, 2-nonanone, eugenol 에서 각각 97, 85, 40 %의 억제율을 보였으며 대조약제에서는 각각 90 % 에서 94 %의 억제율을 확인하였다. 정향 꽃봉오리 유래 성분들은 훈증 효과를 나타내었다. 정향 꽃봉오리에서 분리한 물질들의 작용기작은 methyl salicylate, 2-nonanone, eugeol, eugenyl acetate에서는 아마도 octopaminergic system에 관여할 것으로 판단되며 α-pinene과 α-copaene은 acetylcholine esterase의 inhibition에 관여하는 것으로 판단 된다.

팔각회향 열매유래 물질 (메탄올 추출물과 정유)과 에서 분리한 2종의 phenylpropanoids를 대상으로 한 필터페이퍼 살충 효과 실험에서는 estragole ($LC_{50}$, 4.68 μg/cm-$^2$)이 가장 높은 살충 활성을 나타내었으며 *trans*-anethole ($LC_{50}$, 13.43 μg/cm-$^2$)에서도 살충 활성을 나타내었다. 대조약제인 thiamethoxam, clothianidin 및 emamectin benzoate의 반수치사 농도는 0.039에서 0.146 μg/cm-$^2$로 조사되었다. 팔각회향에서 분리한 2종의 phenylpropanoids는 open container 보다 closed container에서 높은 활성을 나타내어 훈증 활성이 있는 것으로 조사 되었으며 알 부화 억제 실험에서는 estragole은 15와 30 μg/cm-$^2$ 처리 했을 시 95 %와 67 %의 알부화 억제율을 나타냈으며 *trans*-anethole의 경우 60과 30 μg/cm-$^2$ 처리 했을 시 92 %와 50 %의 알 부화 억제율을 나타내었다. 반면에 대조약제인 emamectin benzoate는 0.36 μg/cm-$^2$ 처리 했을 시80 %의 알 부화억제율을 나타내었다. 팔각회향에서 분리한 물질들의 작용기작에서는 Estragole은 acetylcholinesterase inhibitor와 cAMP의 유도체로서 작용을 하는 것으로 확인하였으며 반면에 *trans*-anethole은 약한 acetylcholinesterase 억제효과를 나타내는 것으로 확인 되었다.

인삼에 대한 약해 시험으로 정향 유래물질 (메탄올 추출물과 정유)은 인삼의 출아기와 생육기에 약해를 관찰 할 수 없었으며, 약효 시험의 경우 정향 꽃봉오리 정유는 처리 7, 14일 후 여주 인삼밭에 0.484 g/m$^2$ 처리 했을 때 각각 56 %와 54 %의 방제 효과를 이천 인삼밭에 처리 시 61 %과

52 %의 방제 효과를 확인하였다. 인삼줄기버섯파리의 anti-oviposition 시험 결과 정향의 정유는 대조약제인 clothianidin 액상수화제와 thiamethoxam 입상수화제, emamectin benzoate 유제보다 높은 anti-oviposition 효과를 나타냈지만 cypermethrin 유제보다 낮은 anti-oviposition 효과를 보였다.

정향 유래물질과 화학제의 혼합처리 실험에서는 단독으로 정향 유래 물질과 대조화학제를 처리한 처리구와 큰 차이가 없는 반면에 정유와 cypermethrin 유제를 혼합 처리한 경우 방제효과와 anti-oviposition 효과가 증가하는 것으로 나타났다.

이상의 결과로 정향 꽃봉오리 및 팔각회향 열매 유래 물질은 잠재적인 살유충제 및 살란제로 추후 인삼줄기버섯파리의 밀도경감에 도움이 될 것으로 사료된다.

**키워드**: 인삼, 인삼줄기버섯파리, 정향, 팔각회향, 천연살충제, 작용기작