

**Antifungal activity and Antennal ablation on *Plutella xylostella*
(Lepidoptera: Plutellidae) against *Zingiber officinale* and its effects
on mating and ovipositional proclivity**

Soundarya, M.

(15PZO009)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education

for Women , Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Zoology

April, 2017

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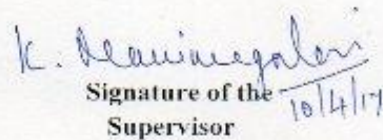
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10/4/2017

**Signature of the
Head of the Department**


10/4/17

**Signature of the
Supervisor**

ACKNOWLEDGEMENT

The author owes her sincere thanks and gratitude to **Padmashri Dr. P. R. Krishna Kumar**, Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for all the amenities provided for the conduct of the project.

The author place on her gratitude and heartfelt thanks to **Dr. (Mrs.) Premavathy Vijayan, M.Sc., M.Ed., Dip.Spl.Edn., M.Phil., Ph.D.** Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for extending all possible help towards the completion of the study.

The author feel greatly indebted and thankful to **Dr. (Mrs.) S.Kowsalya, M.Sc., M.Phil.,Ph.D.** Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her continued interest and valuable guidance in the conduct of this project work.

The author is greatly indebted and thankful to **Dr. (Mrs.) A. Parvathi, M.Sc., Dip. Ed., M.Phil., (Madras), Ph.D. (Bharathiar)**, Dean, Faculty of Science, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her continued interest and constant support in the conduct of project work.

The author expresses her deep sense of gratitude and special thanks to **Mrs. E. Santhi, M.Sc., Dip. Ed., M.Phil.**, Associate Professor and Head of the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for the constant support given during the period of investigation.

The author is highly grateful to her guide **Dr. (Ms) K. Manimegalai, M.Sc., M.Phil., (Madras), Ph.D.,(Avinashilingam)**, Associate Professor, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for the guidance rendered at every stage of the dissertation. Without her dynamic guidance, valuable suggestions, untiring help, meticulous efforts and enduring support, this study would never have seen the light of the day.

I take this opportunity to thank the **Staff members** and **Lab Assistants** in the Department of Zoology for their help to carry out the study.

I express thanks to my beloved parents **Mr. A. Murugesan** and **Mrs. M. Thulasi mani** for their motivation, encouragement and loving care.

I express my profound and deep sense of gratitude to my **friends** for their constant support and sustained interest and involvement throughout the study.

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I. Introduction

Plants are used medicinally in different countries and are a rich source of many potent and powerful drugs (Gislene et al., 2000). Although large number of plant species has been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Jantan et al., 2003; Srinivasan et al., 2001). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

Plants are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. Tiwari et al., (2011).

Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhoea, and tuberculosis as well as increase in resistance by several bacteria to various antibiotics and the increasing cost of prescription drugs, for the maintenance of personal health (Levy, 1998; Van den Bogaard et al., 2000; Smolinski et al., 2003). A large number of the plants are claimed to possess the antibiotic properties in the traditional System and are also used extensively by the tribal people worldwide.

Although modern biomedicine to a significant degree employs synthetic drugs as therapeutic agents, plants still occupy a prominent place in contemporary pharmacy, either as sources of pharmaceutical drugs in the form of isolated plant compounds, as sources of

precursors to drugs, or as sources of compounds that have served as models for synthetic or semisynthetic drugs. It has been estimated that about one-half of all drugs in current use are natural compounds or derivatives. (Iwu, 2002). It is however important to realize that despite the many advances of biomedicine, the progress afforded residents of first world countries is beyond the reach of the majority of the world's population. For the majority of people, many of whom live in miserable poverty, crude plants preparations are still the main form of medicine. In acknowledgment of this situation, the World Health Organization (WHO) is actively promoting the development of traditional medicine (Anonymous, 2002).

Ginger has a very long history of use, both as a spice and as a medicinal plant, and is mentioned in ancient Sanskrit texts and in classical Buddhist, Arabic, Greek and Roman literature (Govindarajan, 1982a). It was used widely in Europe by the tenth century (Vaughan and Geissler, 1997) and was first exported from Jamaica, where it became a significant agricultural crop, in 1547 (Mabberley, 1997). It is now cultivated in many tropical and subtropical regions including India, Africa, China, the West Indies and Australia, with the annual world production estimated at 100,000 tons in 2000 (Bartley and Jacobs, 2000; Evans, 2002).

The secondary metabolites found in the rhizome of ginger that are of primary interest can broadly be divided into volatile compounds and nonvolatile phenolic compounds, the major ones of which have pungent properties. It is generally considered that the pharmacological activity of ginger rhizome resides with compounds from these classes, in particular the non-volatile pungent phenolic compounds. The term oleoresin, when applied to ginger, refers to the volatile oil, the pungent compounds and other compounds extracted by means of solvents. (Connell, 1969; Govindarajan, 1982a).

Ginger rhizome is used in several traditional systems of medicine, including Traditional Chinese Medicine, Ayurveda and Western herbal medicine (WHO, 1989; Williamson, 2002). Rhizome or root part ginger is extensively employed in medicine for the management of the different diseased condition like nausea, vomiting, motion sickness, gastrointestinal ulcers, diabetes, fever, arterial tension, rheumatoid arthritis, dry mouth/ xerostomia, cancer, migraine headache, sore throat, minor respiratory ailments.

The horizontally solid underground stem / rhizome of this plant has proved to be one of the most extensively used culinary agent and spice in daily home cooking practice (Naveena et al.,2004;Lantz et al.,2007).Despite of its use as flavoring agent, ginger is also appreciated in ayurvedic, tibbe-e-unani (Srivastava and Mustafa,1989), allopathic (Fessenden et al.,2001), aromopethy (Shelly et al.,2004) and household medicines (Sloand and Vessey, 2001).Ginger rhizome can be employed in the form of fresh paste, ginger tea, dried powder and preserved slices (Ghorab et al.,2010).

Ginger can be available in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsules and syrup (Maxwell, 2008).The chief active constituents of ginger are Volatile oil, shogaols,Diarylheptanoids, gingerols, Paradol, Zerumbone, 1-Dehydro-(10) gingerdione, Terpenoids and Ginger flavonoids (Baliga et al.,2012). Shogaols and Gingerols are responsible for ginger pungency (Suekawa et al., 1984). Ginger has wide range of biological activities that are attributed to its active constituents (Sukla and Singh, 2007).

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “man- friendly medicines”.

The anti-inflammatory properties of ginger have been known and valued for centuries. The original discovery of ginger's inhibitory effects on prostaglandin biosynthesis in the early 1970s has been repeatedly confirmed. This discovery identified ginger as an herbal medicinal product that shares pharmacological properties with non-steroidal anti-inflammatory drugs. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse effects.

Cruciferous vegetables are vegetables of the family Brassicaceae with many genera, species, and cultivars being raised for food production such as cauliflower, cabbage, cress, bok

choy, broccoli, brussels sprouts and similar green leafy vegetables. Cruciferous vegetables are one of the dominant food crops worldwide. They are high in vitamin C and soluble fiber and contain multiple nutrients and phytochemicals. (Gibson AC).

Brassicaceae is a diverse family of 380 genera and over 3000 species of cultivated and wild plants (Heywood, 1993), which are characterized by the presence of glucosinolate secondary metabolites (Mithen, 1992). Domesticated plants exhibit allometric changes in potency of allelochemicals, which deter herbivores. By contrast, weed species may have evolved for millions of years under natural selection against various herbivores, and normally contain higher concentrations of defensive compounds than their domesticated relatives (Mithen, 1992; Harvey et al., 2003).

Cauliflower, *Brassica oleracea* var. *botrytis*, is an herbaceous annual or biennial vegetable plant in the family Brassicaceae grown for its edible head. The head is actually a mass of abortive flowers. Cauliflower plants are shallow rooted with a small, thickened stem. Cauliflower is a cool season crop and grows best in well draining, organic soil at a pH of 6.5. A high amount of organic matter in the soil will help to hold moisture. Cauliflower is less cold hardy than its relatives and it should be planted after the last frost as sub-freezing temperatures are likely to damage the plant. It is best grown as a fall crop in cooler areas. Delahaut and Newen house (1997).

Plutella xylostella is considered a specialist on Brassicaceae and it relies on some of the glucosinolates for host location, oviposition stimulation and feeding initiation (Thorsteinson 1953; Gupta and Thorsteinson, 1960; Sarfraz et al., 2006). Earlier reports suggest that certain wild food plants of *P. xylostella* affected some of its developmental and reproductive parameters (Muhamad et al., 1994; Begum et al., 1996; Idris and Grafius 1996; Shelton and Nault 2004; Kahuthia-Gathu, 2007; Sarfraz et al., 2010). *P. xylostella* is highly migratory and the primary source of populations in western Canada is attributed to influxes from southerly regions (Doddall et al., 2004; Soroka and Hopkinson, 2010). Similar migrations have been reported in the UK (Chapman et al., 2002), Japan (Honda, 1992), Australia (Goodwin and Danthanarayana, 1984) and New Zealand, South Africa, Chile and Argentina (Talekar and Shelton 1993).

Diamondback moth is an oligophagous insect and feed on plants that contains mustard glycosides (Thorsteinson, 1953). It's also feeds on large number cruciferous plants which considered to be weeds diamondback moth maintains itself on these weeds only in the absence of more proved cultivated host (Talekar and Shelton, 1993). The host range of *P. xylostella* is limited to crucifers because they contain mustard oils and their glycosides (Gupta et al., 1960). The mating takes place after few hours of emergence and only once in their life span of but male more than one time (Wang et al., 2005). The average Duration of life span was 8-10 in field condition. (Patil and Pokharkar, 1971) The newly hatch larvae are pale brown head while the fully grown caterpillar one light green measuring 10 mm (Chelliah and Srinivasan, 1986). The larvae soon after hatching make hole from side of the leaves and start mining. The entry was marked by very small dot like scratched area. (Nirmala Devi and Desh Raj, 1995). Fourth instar also feed like third but before pupation it minimizes the feeding and latterly stop covered into pre-pupa. Jayarathnam (1977) reported that were only four instars. At the slightest disturbance the larvae wriggled actively and dropped down the leaf suspending themselves by silken threads (Bhalla and Dubey, 1986).

The pre-pupal stage are few hours it secrete the silicon thread and covered the body completely and convert into pupa. The mature pupa are 6 mm long and of light brown colour (Bhalla and Dubey, 1986). Adults are grey or brownish in colour it distinguishes by having three pale triangular markings along the margin of the wings. Moth prefer to rest under the leaves and in protective plant structure. In field condition moth are more active and fly around the plants searching for the mate on place to deposit the eggs of *P. xylostella*.

The diamondback moth, *P. xylostella* (L.), is a serious pest of crucifer crops in Canada and throughout the world. It uses glucosinolates as host recognition cues for oviposition (Ratzka et al., 2002; Renwick et al., 2006; Sun et al., 2009; Badenes-Perez et al., 2013). Although small numbers of diamondback moth may survive the winter if conditions are ideal, populations on the Canadian prairies usually do not survive the winter months (Doddall, 1994). Field populations of *P. xylostella* also exploit wild cruciferous plants (Omar et al., 1994), and these plants may maintain *P. xylostella* populations in temperate regions, especially in the winter and early spring seasons before cruciferous crops are widely cultivated (Talekar and Shelton 1993).

Accumulating evidence indicates that the final decision to oviposit or not by *P. xylostella* is influenced by multiple factors, including leaf surface waxes, chemosensory stimulation, and abiotic factors (Justus and Michell 1996; Spencer 1996; Renwick et al., 2006; Caputo et al., 2006).

Host plant resistance is one of the effective strategies of integrated pest management programs that can reduce the initial infestations by *P. xylostella* and therefore minimize the usage of insecticides. The three essential modalities of host plant resistance are antixenosis, antibiosis, and tolerance (Painter 1951). However, the short generation time and high fecundity of the diamondback moth allow it to become a significant pest of oilseed crops in this region. Though insecticides remain the primary defense against the diamondback moth, insecticide resistance has become a problem in many diamondback moth populations (Shelton et al., 1993). As a result, alternative methods of insect control, including plant-based resistance, are being investigated.

One of the most promising examples of plant-based resistance is the reduced survival of larvae on glossy lines of *Brassica oleracea* (Lin et al., 1983; Eigenbrode and Shelton, 1990; Eigenbrode and Pillai, 1998). The leaf-wax characteristics of the glossy lines affect the ability of first-instar larvae to mine the leaf tissue, leaving larvae exposed for extended periods and increasing larval mortality due to abiotic factors and natural enemies (Eigenbrode et al., 1991b, 1995).

In phytophagous insects, the behavioral process leading to host location and acceptance for oviposition or feeding may include orientated movement from a distance in response to plant cues (Bernays and Chapman, 1994; Kennedy, 1965). Information on host selection behavior of DBM and response of females to various host plants are important for the development of IPM systems. Similarly host plants and ovipositional preferences of *C. carnea* play a vital role in the effective use of this predator to control DBM on different host plants.

Relationship between adult oviposition preference and offspring performance is a crucial issue for understanding the evolutionary ecology of host plant associations in herbivorous insects

(Thompson 1988). Adult females should, oviposit on host plants that insure the best survival of their offspring (Awmack and Leather 2002). Most of the Lepidopterans, as their first stadium larvae have limited mobility and are generally unable to move and locate a suitable food source if their mother oviposits on an unsuitable plant (Thompson and Pellmyr 1991; Zalucki et al., 2002; Rhainds et al., 2008).

The pest is notorious for its ability to rapidly evolve resistance to all classes of insecticides (Iqbal et al., 1996; Atumurarava and Furlong, 2011; Zhou et al., 2011) and it has already evolved field resistance to diamide insecticides in Thailand (Sukonthabhirom et al., 2011) and the Philippines (Edralin et al., 2011) following their introduction in the mid-late 2000s. Single tactic control strategies that rely on insecticides are doomed to failure and sustainable production requires the adoption of integrated pest management (IPM) programmes underpinned by natural enemies (Furlong et al., 2004, 2008b). Many studies have investigated intraplant oviposition site preferences by *P. xylostella* but no clear pattern of preference for oviposition site within plants has emerged. Studies have variously demonstrated a preference for the upper surface of leaves (Harcourt, 1957; Justus et al., 2000), the lower surface of leaves (Andrahennadi and Gillott, 1998; Marazzi and Stadler, 2004), or neither surface when female moths select an oviposition site (Badenes-Perez et al., 2011). In cabbage (*Brassica oleracea* L. Capitata), canola (*Brassica napus* L.), *Brassica juncea* (L.), Chinese cabbage (*Brassica rapa* L. Pekinensis), and *Brassica carinata* L., *P. xylostella* preferentially selected lower regions of the plant stem over leaves for oviposition (Reddy et al., 2004; Sarfraz et al., 2005).

Recent studies have shown that below-ground herbivores can induce defensive responses in plant foliage (van Dam et al., 2009) and that root and shoot tissues contain similar secondary compounds (van Dam et al., 2009). In the Brassicaceae, glucosinolates are the most prominent secondary compounds in both foliar and root tissue (Van Dam, 2009). Root herbivory can cause systemic changes in leaves and thus impact the behavior and performance of aboveground herbivores (Masters and Brown, 1992; Bezemer and van Dam, 2005; Van Dam, 2009).

The selective behavior of adult females is a key proximate mechanism shaping insect attacks on plants because oviposition choice may link food quality with offspring performance

(Price et al., 1990). Because natural selection favors mothers that have higher reproductive success, positive relationships between oviposition preference and offspring performance are expected (Thompson 1988; Mayhew 1997), particularly among moths and caterpillars, which exhibit a limited ability to disperse and exchange feeding sites (Thompson and Pellmyr 1991; Renwick and Chew 1994; Zalucki et al., 2002). Although there are many exceptions, offspring tend to survive better on preferred plant types, and adult females tend to lay more eggs on plant types conducive to offspring performance. (Gripenberg et al., 2010).

The oligophagous diamondback moth (DBM) *P. xylostella* L. (Lepidoptera: Plutellidae) is a highly destructive insect pest that specializes in feeding on plants in the family Brassicaceae, especially varieties of *B. oleracea* L. (Sarfraz et al., 2006; Furlong et al., 2013). Diamondback moth larvae can overcome the chemical defenses that usually occur in Brassicaceae, and the moths even rely on some plant glucosinolates and their metabolites for host location and oviposition stimulation. (Li et al., 2000; Ratzka et al., 2002; Sarfraz et al., 2006; Muller et al., 2010).

Young leaves of Brassicaceae contain higher concentrations of glucosinolates and protein than do intermediate and old leaves (Lambdon et al., 2003). Diamondback moth has shown some cases of positive relationships between preference and performance among plant types (Zhang et al., 2012); however, direct evidence for within-plant variability remains scarce (Badenes-Perez et al., 2014).

Olfaction plays a key role in moth-host plant interactions (Galizia and Rössler 2010). The antennae are the main olfaction organ in insects, and affect mating and oviposition behaviors. The paired antennae are endowed with a multitude of sensilla; the trichoid, basiconic, and coeloconic sensilla are considered major olfactory sensilla (Park et al., 2002). The trichoid sensilla have been shown to be responsible for detecting sex pheromones (Forstner et al., 2008; Krieger et al., 2009; Olsson and Hansson 2012; Xu et al., 2012; Gu et al., 2013) and plant odors (Shields and Hildebrand 2001; Maida et al., 2005; Anfora et al., 2014). The basiconic sensilla are sensitive to host plant chemicals (Laue 2000; Deng et al., 2012; Zhang et al., 2013), and the coeloconic sensilla may function as olfactory receptors for plant odors (Pophof et al., 2005; Frank et al.,

2010), and may be sensitive to humidity and temperature (Chen and Fadamiro 2008; Romani et al., 2009; Sun et al., 2011). Similar to previous reports (Chow et al., 1984; Yang et al., 2001). The trichoid, basiconic, and coeloconic sensilla are major sensilla on the antennae of the DBM.

The aim of the present study is to evaluate the phytochemical characterization of the petroleum ether, chloroform and ethanol extract of *Z. officinale* to check the antimicrobial activity against various human pathogens. In this study, we ablated the antennae of the Diamondback moth and it was treated rhizome extract in a standard way, with the aim of understanding the influences of antennal sensory inputs on the mating and oviposition behaviors of this economically important pest.

Objectives

In view of the above, the present study was undertaken with the following objectives

- To analyses the different phytochemicals in *Z. officinale* extracts.
- To evaluate the antifungal activity of *Z. officinale* extracts.
- To study the effect of antennal ablation on mating and ovipositional proclivity.

2. REVIEW OF LITERATURE

The literature pertaining to the study Antifungal activity and Antennal ablation on *P. xylostella* (Lepidoptera) against *Z. officinale* Roscoe and effects on mating and ovipositional proclivity is reviewed under the following headings.

2.1 Phytochemical analysis

2.2 Antifungal activity

2.3 Antennal ablation in insects

2.4 Mating behavior in insects

2.5 Ovipositional proclivity in insects

2.1 **Phytochemical analysis**

Koul (2008) examined the phytochemicals in plants may have the capacity to control pest. Plants based pest control agents have long been touted as alternatives to synthetic chemicals for integrated pest management.

Bhardwaj and Kaushik (2012) examined phytochemical and pharmacological studies in genus *Berberis* shows Pharmacological uses in traditional medicine system since ancient time. A number of pharmacological and clinical studies have been reported from different *Berberis* species which proves their importance as a medicinal plant. Berberine and berbamine alkaloids present in *Berberis* species have antioxidant, anti-inflammatory, hepto protective, anti-hyperglycemic and hypotensive properties as reported by various research groups (Koncic et al., 2010b).

Samejo et al. (2013) investigated phytochemical screening of *Tamarix dioica* Roxb. *Tamarix dioica* is used as a diuretic, carminative and for the treatment of hepatic and splenic inflammation. The aqueous extract along with dry powder of stems, flowers, leaves and roots of *T. dioica* were screened for the presence of phytochemicals showed the presence of steroids, phenols, tannins, terpenoids, flavonoids and saponins. While proteins, alkaloids, glycosides and amino acids were absent.

Amine et al. (2013) studied antimicrobial activity and phytochemical screening of *Arbutus unedo* L. The antimicrobial activities of water and methanol extract, and three phenolic fractions of the roots of *A. unedo* L. were investigated. Poor antibacterial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria were shown with water and methanol extract. However moderate antibacterial activity was shown by water extract and phenolic fractions against *Escherichia coli* and *S. aureus*, respectively. The phytochemical screening of roots of *A. unedo* revealed the presence of quinones, anthraquinones reducteurs compounds, anthocyanins, tannins and flavonoids. Quantitative analysis showed that the roots were strongly dominated by anthocyanins compounds followed by total flavonoids and flavones and flavonols.

Wong et al. (2015) experimented phytochemical screening and antimicrobial potentials of *Borreria* sps. Whole plant extracts of the *Borreria* sps were investigated for phytochemical screening and assessed for antimicrobial activity. Phytochemical analysis of *Borreria* sps extracts revealed the presence of phenolics, flavonoids and tannins. Among them, *Borreria laevicaulis* hexane extracts were found to be most effective showing the largest zone of inhibition against *Staphylococcus aureus* and *Candida albicans*. The studies indicated that the minimum inhibitory concentration of *B. laevicaulis* hexane extracts against *S. aureus* and *C. albicans* and the zone of inhibitions were significantly higher than nystatin.

Khanam et al. (2015) experimented *Eurycoma longifolia* phytochemical screening and determination of antimicrobial activity of methanol, acetone, ethyl acetate, chloroform and petroleum ether extracts of the stem and root of *E. longifolia*. The results revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, protein and cardiac glycosides in the extracts. Stem extracts were found to be a rich source of phytochemicals as compared to the root extracts. All the extracts exhibited dose dependent antimicrobial activity, however, highest antibacterial activity was observed against gram positive bacteria by both stem and root extracts. The preliminary studies on *E. longifolia* extracts exhibited their antimicrobial potential which could be exploited further as future antimicrobials for pharmaceutical treatment, natural therapies, food preservation and cosmetic applications.

Saikia et al. (2016) studied Phytochemical content and antioxidant activities of thirteen fruits of Assam, India and were studied for their phytochemical content and antioxidant activity. The highest total phenolic content was observed in black jamun followed by litchi, bogi jamun, amla, hog plum, panijamun and carambola. Amla showed the highest ferric reducing antioxidant potential value. The study fruit extracts showed presence of ascorbic acid, phenolic acids and flavonoids.

Ghasemzadeh et al. (2016) experimented changes in antioxidant and antibacterial activities as well as phytochemical constituents associated with ginger storage and polyphenol oxidase activity. This storage condition will provide greater stability to the concentration of the phytochemical constituents more similar to the fresh material.

Nagarajana et al. (2016) reviewed extraction of phytochemicals using in hydrotropic solvents indicates plant synthesizes phytochemicals with different properties to supply a pool of molecules for food, chemical and pharmaceutical applications. Hydrotrope is a one of the green solvents that enhances solubility of poorly water-soluble or hydrophobic compounds in aqueous solutions. This study critically reviews the extraction of phytochemicals using various hydrotropic solvents.

2.2 Antifungal activity

Wang and Ng (2002) experimented an anti-fungal protein designated alocaasin was isolated from the rhizomes of the giant taro *Alocasia macrorrhiza* reduced the activity of HIV-1 reverse transcriptase and exhibited weak hemagglutinating activity.

Tian et al. (2008) analyzed purification, characterization and molecular cloning of a novel mannose-binding lectin from rhizomes of *Ophiopogon japonicus* with antiviral and antifungal activities. A novel mannose-binding lectin (designated OJL) was purified from rhizomes of *Ophiopogon japonicas* actively against *Gibberella saubinetii* and *Rhizoctonia solani* and show inhibitory effect on herpes simplex virus type II. This novel lectin was a homo dimer consisting of approximately 12 k Da subunits linked by non-covalent bonds.

Supreetha et al. (2011) experimented antifungal activity of ginger extract on *C. albicans*. Plant derived products have been used for medicinal purposes for centuries. Traditional uses of plants have led to investigating their bioactive compounds, which have resulted in the detection of a significant number of therapeutic properties. Effect of ethanolic extract of ginger has pronounced inhibitory activities against *C. albicans*.

Al-Ja'fari et al. (2011) evaluated the composition and antifungal activity of the essential oil from the rhizome and roots of *Ferula hermonis*. Fractionation analysis of the essential oil from rhizome and roots of *F. hermonis* Boiss against several yeasts and filamentous fungi.

Bhargava et al. (2012) assessed chemical and phytochemical screening and evaluation of its antimicrobial activities. The study deals with antimicrobial activity of *Z. officinale* extract and their phytochemical composition. Their antimicrobial activity was tested against nine microorganisms that cause various diseases in human.

Rathee et al. (2012) examined phytochemical screening and antimicrobial activity of *Picrorrhiza kurroa*, an Indian traditional plant used to treat chronic diarrhea. The effects of methanolic and aqueous extracts of *P. kurroa* against some pathogenic bacterial and fungal strains viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* and *C. albicans*, *A. niger*, respectively, showed that the plant part can be used to treat infections caused by these bacteria and fungi. The aqueous and methanolic extracts showed antibacterial activity but the significant antimicrobial activity was shown by methanolic extract against *P. aeruginosa* and *S. aureus*. While moderate activity against *E. coli*, *B. subtilis* and *M. luteus*. The effectiveness of the crude extract confirmed its use in traditional medicine to treat skin, urinary tract, diarrheal infections and gastrointestinal infections.

Das et al. (2013) investigated antimicrobial and selected Zingiberaceae Species from Northeast India. Antimicrobial activity of crude rhizome oils from some important members of Zingiberaceae shows the inhibitory action on both bacteria and fungus. The plants used were *Curcuma amada*, *C. longa*, *Z. moran*, and *Z. zerumbet*. The rhizome oil of all the 24 extracts

exhibited potent antimicrobial activity against all pathogenic bacterial and fungal strains. Water extract of *Z. moran* was found to be the highest effective antimicrobial agent.

Sadiq et al. (2016) studied the antibacterial and antifungal potentials of the solvents extracts from *Eryngium caeruleum*, *Notholirion thomsonianum* and *Allium consanguineum*. Antifungal assay of these extracts shows, *N. thomsonianum* exhibited strong antifungal activity against *A. fumigatus*, *A. flavus* and *A. niger* respectively. The plant species were active against tested bacterial and fungal strains.

Rawal and Adhikari (2016) observed evaluation of antifungal activity of *Zingiber officinale* against *Fusarium oxysporum* f.sp. *lycopersici*. This study was conducted to determine the antimicrobial activity of dried ginger powder by using chloroform, ethanol, and acetone petroleum ether solvents, against *F. oxysporum* f. sp. *lycopersici*. The study showed the potent antimicrobial activity of the ginger extract against the pathogen by all tested solvents. Whereas, other solvents showed moderate to minimum antifungal activity. These findings suggest that some plant extracts tested possess antifungal activities against *F. oxysporum* f.sp. *lycopersici*.

2.3 Antennae ablation in insects

Qiu et al. (1998) analyzed chemoreception of oviposition inhibiting terpenoids in the diamondback moth *P. xylostella*. The terpenoids present in the extracts of the neem tree against the ovipositional behavior in diamondback moth were tested. Location of chemosensilla on prothoracic tarsi and ovipositor was examined by scanning electron microscopy. Electro physiological recordings from ovipositor and tarsal taste sensilla showed that distilled water produced distinct responses from one neuron. In tarsal sensilla, ethanol and drimane solutions produced responses from two neurons, one of which might be the water cell that fired at a reduced rate. A drimane significantly decreased the responses of tarsal chemoreceptors to a cabbage leaf extract, which is a possible sensory mechanism leading to behavioral avoidance of this compound.

Ryan and Sakaluk (2009) experimented the role of the antennae in mate recognition, copulation and mate guarding in decorated crickets. Antennae are important sensory organs in

insects because they enable the perception of chemical and tactile cues believed to be important in sex recognition and mating in various species. To examine the role of antennae in mating behavior of decorated crickets, *Gryllodes sigillatus*, they experimentally manipulated the chemosensory ability of males and females and altered the chemical cues present on the female cuticle. The result suggest that males rely heavily on their antennae to recognize conspecific females, because antennectomized and chemically ablated males were less likely to produce the courtship song upon contact with a female. Females, in contrast, did not seem to rely on their antennae for sex recognition, because antennectomized females frequently mounted males, although they took significantly longer to do so. Males lacking antennae almost never showed mate guarding behavior, while males with intact antennae almost always did. Collectively, these results suggest that sensory information provided by the antennae is critical to the mating success of both male and female crickets.

Soques et al. (2010) experimented age and mating status do not affect transcript levels of odorant receptor genes in male antennae of *Heliothis virescens* and *Heliothis subflexa*. In moth species, male response to female sex pheromones often is affected by moth age and mating status. Odorant receptors expressed in neurons within male antennae are critical for detecting the female pheromones. The expression level of these receptor proteins would be affected by age and mating status of male moths. They examined the expression levels of two Odorant receptor genes that are preferentially expressed in the male antennae of *H.virescens* and *H.subflexa*. Antennae were dissected from virgin males at 2 hour, 1 day, 2 day, 4 day, and 8 day and also dissected antennae from 4-day-old mated males. The study shows age had no effect on expression levels of either odorant receptors in either species, except for a small difference in *H. subflexa* expression between 2 h and 8-d-old virgin males. Furthermore, no effect of mating status on expression level of these odorant receptors in either species.

Piersanti et al. (2011) examined electrophysiological identification of thermo and hygro-sensitive receptor neurons on the antennae of the dragonfly *Libellula depressa*. Ultra structural investigations on Odonata antennal flagellum describe two types of sensilla styloconica, T1, T2. The styloconic sensilla are located in pits, at the bottom of deep cavities, and share common features typical of thermo-hygroreceptors. Odonata antennae are involved in hygroreception and

thermoreception, they carried out electrophysiological recordings from adult males and females of *L. depressa* L. After contact was established, the antenna was stimulated by rapid changes in temperature and humidity. The research showed the occurrence of a dry, a moist and a cold receptor neurons on the antennal flagellum of *L. depressa* stimulate the rapid changes.

Ernst et al. (2013) studied the structure and distribution of antennal sensilla in the centipede *Scolopendra oraniensis*. Scanning electron microscopic investigations of the antenna of adult *S. oraniensis* shows the presence of six types of cuticular sensilla: numerous slender sensilla trichodea, small sensilla microtrichodea with terminal pore, many slightly turned contorted sensilla with terminal pores, multiporous sensilla basiconica, hat like sensilla with terminalpores, and small sensory cones with terminalpores.

Sombkea and Ernst (2014) studied structure and distribution of antennal sensilla in *Oranmorpha guerinii* (Gervais, 1837). The study on the antennae of *O.guerinii* utilizing scanning electron microscopy revealed the presence of six sensillar types namely apical cones, sensilla trichodea, sensilla microtrichodea, sensilla chaetica, sensilla basiconica bacilliformia, and sensilla basiconica spiniformia. External structure and distribution of cuticular antennal sensilla are compared with data from other diplopod species.

Zhong et al. (2014) studied effects of various degrees of antennal ablation on mating and oviposition preferences of the diamondback moth, *P.xylostella* L. Experiments with various degrees of antennal ablation demonstrated that Diamondback moth antennae played a key role in the control of mating and oviposition. Based on the result, neither oviposition preference nor mating behaviors changed significantly when less than 1/4 of both antennae were removed. However, there was a significant behavioral change when the antennae were ablated by more than half. As the length of the antenna was shortened, the successful mating rate decreased and mating peak was delayed. An otherwise consistent host preference for oviposition was eliminated when both antennae were completely removed. The results indicate that antennal sensory information plays a critical role in the mating and oviposition behaviors of this economically important pest.

Ranieri et al. (2016) examined fine structure of antennal sensilla of the spittle bug *Philaenus spumarius* L. The antennae of *P. spumarius* consist of three segments: a basal scape, a pedicel and a flagellum composed of a basal enlargement and a long segment. The pedicel bears a single campaniform sensillum while the ampulla houses twelve coeloconic sensilla and three large basiconic sensilla. These latter sensilla show a smooth multiporous external cuticular wall and a total number of 27 sensory neurons per sensillum. The sensory peg of the double-walled sensilla is smooth at the base and distally has a grooved cuticular surface with pores organized in spoke channels between each ridge. Three sensory neurons enter the lumen while at the basal level, before entering the peg, a fourth sensory neuron is found. The single-walled sensilla show an aporous thick cuticular wall and two sensory neurons entering the sensillar lumen, with a third neuron ending at the sensillum base.

Rebora et al. (2016) investigated antennal sensilla of the stone fly *Dinocras cephalotes*. Adult males and females show a filiform antenna constituted of a scape, a pedicel and a flagellum composed of very numerous segments with no clear sexual dimorphism in the number and distribution of the antennal sensilla. The most represented sensilla are sensilla trichodea, with different length, whose internal structure reveal their mechano sensory function, sensilla chaetica, with an apical pore, with an internal structure revealing a typical gustatory function, porous pegs representing single-walled olfactory sensilla, digitated pegs with hollow cuticular spoke channels representing double-walled olfactory sensilla, pegs in pits for which they hypothesize a thermo-hygrosensory function. The diversity of described sensilla is discussed in relation to known biological aspects.

Wang et al. (2016) observed the structure and morphogenic changes of antennae of *Matsucoccus matsumurae* in different instars. To better understand the functioning and morphogenic changes of the antennae of *M. matsumurae* in different instars, the antennae are examined using light microscopy, scanning and transmission electron microscopy. The results showed that the antennae of *M. matsumurae* display three different styles in morphology and sensillar distribution in different instars. The antennae of first instar nymphs are relatively simple, including one campaniform sensillum, four smooth aporous trichoid sensilla, two intersegmental sensilla, two coeloconic sensilla, three multiporous pegs and four uniporous pegs.

The antennae of adult females and third instar male nymphs both possess similar antennae, and exhibit seven types of sensilla. Adult female antennae have in total 82 to 108 sensilla, whereas third instar male nymph antennae possess approximately 62 to 79 sensilla. Adult male antennae are the most developed, possessing 259 to 312 sensilla, including grooved aporous trichoid sensilla, knobbed seta sensilla 179 to 230 multiporous trichoid sensilla.

Rebora et al. (2017) reviewed antennal responses to volatile organic compounds in a stonefly. Recent investigation on the antenna of *Dinocras cephalotes* revealed two kinds of putative olfactory sensilla. The male and female antennae of *D. cephalotes* have been stimulated with a set of generic odours belonging to different functional groups, showed that Plecoptera can perceive olfactory cues through their antennal sensilla. Indeed, although many chemicals did not elicit any response, high EAG activity has been recorded in response to pentanoic acid, propanal, butyric acid, propionic acid, iso amylamine and ammonia.

2.4 Mating behavior in insects

Liang et al. (2007) investigated synthesis and assessment of attractiveness and mating disruption efficacy of sex pheromone microcapsules for the diamondback moth, *P. xylostella* (L.) Microcapsules of sex pheromone of the diamondback moth *P. xylostella* were synthesized through complex coacervation using gelatin and gum arabic as wall-forming materials. The encapsulated pheromone released from these microcapsules into the air was monitored over six weeks in the field. Results of a field trial showed that the attractiveness of microcapsules was superior to that of rubber septa loaded with the same amount of pheromone. The mating disruption efficiency of the pheromone treatment was estimated through comparing numbers of moths captured in pheromone-baited traps placed in pheromone treatment fields and pesticide treatment fields. The estimated mating disruption efficiency of the pheromone treatments ranged from 76.94% to 98.67% during the season. This study shows that pheromone microcapsules might provide a new method for *P. xylostella* control.

Johansson and Jones (2007) reviewed the role of chemical communication in mate choice. Chemical signals are omnipresent in sexual communication in the vast majority of living organisms. The traditional paradigm was that their main purpose in sexual behavior was to

coordinate mate and species recognition and thus pheromones were conserved in structure and function. They review the evidence for pheromones as indicators of mate quality and examine the extent of their use in individual mate assessment.

Lewis and Wedell (2007) examined effect of adult feeding on male mating behavior in the butterfly, *Bicyclus anynana*. Many species of lepidopterans supplement their nectar diet with foods rich in nitrogen and minerals, which are present only in trace amounts in nectar. Effect of adult diet on male reproduction in terms of mating rate and sperm production, although males fed on fruit produced larger spermatophores on their first mating compared to males fed sugar only. Males ejaculate larger spermatophores during their first mating, and produce spermatophores containing decreasingly fewer non-fertile sperm with number of matings performed. Males that produced more non-fertile sperm on their first mating had reduced life span possibly indicating a trade-off between sperm production and adult longevity.

Bengtsson et al. (2010) studied pheromone-based mating and aggregation in the sorghum chafer, *Pachnoda interrupta*. The aggregation behavior in *P. interrupta* appears is guided by a combination of pheromone and host volatiles. Females and males were extracted with hexane during the mating period.

Thomas (2011) observed detection of female mating status using chemical signals and cues. Males of many species choose their mate according to the female's reproductive status. However, females will also aim to regulate their mating activity so as to maximize their own fitness. Chemical cues and signals are used widely by males to discriminate between mated and unmated females, and explore the mechanisms by which female odour changes post-mating. There is substantial empirical evidence that mated and unmated females differ in their chemical profile, and this variation provides males with information on a females mating status.

Miyatake (2011) observed synchronized sex, mating system, and biological rhythm. The sterile insect technique is a method of eradicating insects by releasing mass-reared sterilized males into fields to reduce the hatchability of eggs laid by wild females that have mated with the sterile males. It requires mass-production of the target insect and maintenance of the quality of

the mass-reared insects. The most important factor is successful mating between wild females and sterile males depend on their synchronized copulation. The timing of male copulation attempts with receptivity of females is key to successful mating between released males and wild females. Therefore, focus on the mechanisms controlling the timing of mating in target insects and biological control projects, precise timing of the release of natural enemies to attack pest species is required because behavior of pests and control agents are affected by their circadian rhythms. Control of the biological clocks in sterile insects or biological control agents is required for advanced quality control of rearing insects.

Staples et al. (2013) observed the effectiveness of sterile insect programs depends on sterile males mating successfully and inducing reproductive failure in wild females. Thus, from the perspective of insect control, female mating failure involves mating with a mass-reared, sterilized male, which then results in female reproductive failure. First, at the pre-copulatory stage affect female mating failure with sterile males, such as differences between sterile and wild males in terms of male courtship success, male discrimination of females, pheromone production and dispersal. Emphasize studies with some degree of ecological realism and review certain factors that can affect female sexual development and choice, such as diet, age, and sex ratio. Second, at the post-copulatory stage functionally result in female reproductive failure, such as ejaculate transfer and control of female remating. Sterile insect technique operations strive to incorporate methods that increase wild female mating with sterile males so that ultimately population-wide reproductive failure is achieved in the target species.

Gordillo (2015) investigated lack of successful mating encounters in two-sex insect populations is a mechanism that might trigger reproductive allee effects. This study deals with the function models of ephemeral mating encounters through the expected density of pairs formed by individuals of both sexes at any time. When this function is incorporated in a general system of differential equations for a two-sex population the solutions exhibit the emergence of an allee effect for low population densities. Compared with current conceptual models for mate-finding allee effects, the proposed pairing function does not include a parameter that quantifies the allee effect strength, a feature that might be useful when information to parameterize allee effects is unavailable. The mating function is then used to numerically explore how mate-finding

allee effects are enhanced by their release of sterile males in theoretical models where (i) the initial sex ratio is skewed, (ii) sterile males are released in pulses and (iii) partial female remating is allowed.

Hoshi et al. (2016) studied mating disruption of a carpenter moth, *Cossus insularis* in apple orchards with synthetic sex pheromone, and registration of the pheromone as an agrochemical. Mating disruption of the carpenter moth, *C. insularis* with a synthetic version of its sex pheromone was tested for three successive years in apple orchards. Pheromone trap catches, percentage mating of tethered females and females enclosed with males in a mating cage and tree damage were measured in both the pheromone-treated and untreated control orchards. The attraction of male moths to pheromone traps at heights of 1.5, 3, and 5 m was strongly disrupted when the pheromone dispensers were placed at 1.5m height. Mating of tethered females placed at 1 m was completely inhibited, and the mating of tethered females at a height of 3 m was significantly reduced by the treatment in comparison to mating in an untreated control orchard. Similarly, mating of pairs of moths enclosed in mating cages was significantly reduced by the synthetic pheromone treatment in comparison to control. The percentage of damaged trees in the pheromone treated orchard also decreased significantly over the course of the experiment. These results suggested that mating disruption with the synthetic sex pheromone appears promising for reducing damage caused by *C. insularis* in apple orchards in Japan and a commercial mating disruption product has been developed and registered.

2.5 Ovipositional proclivity in insects

Spencer et al. (1996) experimented waxes enhance *P. xylostella* oviposition in response to sinigrin and cabbage homogenates. Oviposition by the diamondback on substrates treated with host stimuli waxes was quantified using continuous observations and endpoint bioassays. Paraffin mixture applied over cabbage homogenate or sinigrin caused an increase in oviposition compared to that on any single stimulus in choice tests. Addition of alkane over sinigrin made all sinigrin concentrations significantly more stimulatory than control. Given the ubiquity of waxes on plant surfaces and the interaction between waxes and host-specific chemical stimuli, waxes should be included when considering factors that significantly influence herbivore host acceptance.

Ulmera et al. (2002) studied the Diamondback moth, *P. xylostella* (L.) feeding and oviposition preferences on glossy and waxy *Brassica rapa* (L.) lines. Host plant resistance research to date indicates that *Brassica* plants expressing the glossy leaf wax characteristic show some resistance to diamondback moth, *P. xylostella* (L.). Oviposition and first-instar feeding preferences were depending on 'Glossy' and 'Waxy' near isogenic lines of *B. rapa* (L.). Although females did not discriminate between 'Waxy' and 'Glossy' plants for oviposition, there was a strong preference among first-instar larvae for 'Waxy' plants in the choice experiment. The experiment indicates that *B. rapa* expressing the glossy leaf wax characteristic shows some resistance to diamondback moth, similar to that observed previously with glossy *B. oleracea*.

Renwick et al. (2006) investigated isothiocyanates stimulating oviposition by the diamondback moth, *P. xylostella*. The two prominent volatile components were separated and identified by mass spectrometry as the isothiocyanates, iberin and sulforaphane. Subsequent bioassays of a range of isothiocyanates showed that iberin and sulforaphane were the most active of those tested. Other isothiocyanates with sulfur in the side chain were also active, whereas alkyl and phenyl isothiocyanates had only limited activity.

Silva and Furlong (2012) observed oviposition effects of host plant and herbivory. The effects of conspecific herbivore damage to foliage could be replicated by mechanical damage. When foliage was damaged, injured cabbage and canola plants were preferred for oviposition over intact conspecifics, whereas injured Chinese cabbage plants were less preferred than intact conspecifics. However, when root tissue was damaged, intact cabbage and canola plants were preferred over injured conspecifics, whereas moths did not discriminate between root-damaged and intact Chinese cabbage plants. Injury to upper leaves significantly affected the intra-plant distribution of eggs. In cabbage and canola plants, injury to leaf 6 significantly increased the number of eggs laid on this leaf, resulting in a significant decrease in the number of eggs laid on the lower foliage/stem of plants, whereas in Chinese cabbage plants it significantly decreased the number of eggs laid on leaf. The study demonstrates the complex and unpredictable interactions between *P. xylostella* and its host plants and provides a basis begin to understand observed distributions of the pest in *Brassica* crops.

Zhang et al. (2012) reviewed relationship between adult oviposition preference and larval performance of the diamondback moth, *P. xylostella*. Oviposition preference and offspring performance of the diamondback moth, *P. xylostella* on various wild and cultivated crucifer plants were analyzed. Intrinsic rate of increase, pupal weight and fecundity of the ensuing adults were regarded as an indicator of larval performance of *P. xylostella* among 18 species of wild and cultivated host plants.

Moreira et al. (2015) examined diamondback moth performance and preference for leaves of *B. oleracea* of different ages and strata. they measured leaf proteins, glucosinolates and fibre, as well as larval choice, developmental performance, and moth oviposition preference with regard to leaf age classes of three varieties of the main host plant *B. oleracea* L. Larvae consistently fit the prediction that specialist herbivores prefer and perform better on young, upper leaves that have the highest protein level, despite the highest content of defence compounds. Moths laid more eggs on fibrous and less nutritious leaves from the lower and senescent stratum. The ability of larvae to spread upwards over the plant to access the more nutritious leaf stratum is critical when eggs are preferentially laid on the protective low-quality leaves.

Lacewings are considered to be one of the most effective generalist predators (McEwen et al., 2001; New, 1975) feeding on eggs and young caterpillars, aphids, spidermites, scales, psylla, mealybugs, whiteflies, thrips, leaf hoppers, and other soft-bodied prey (Canard et al., 1984). Lacewings are highly predacious and cannibalistic as larvae (Nordlund, 1993) using their channeled mandibles to inject digestive enzymes into their prey and then to suck out body fluids. (Olkowski et al., 1991). Adult lacewings generally feed solely on nectar, pollen, and honeydew, although a few are predatory (Coppel and Mertins, 1977).

Ovipositional behavior sequences have been investigated for several insect species, including *C. obstrictus* (Wasser- man, 1985; Tisdale and Wagner, 1991; Renwick and Chew, 1994; Dmoch, 1998). The cabbage seedpod weevil has a rigid sequence of behaviors associated with oviposition (Kozłowski et al., 1983) The behavioral sequence is essential to successful oviposition; host plants with characteristics that change this sequence may consequently alter oviposition success which is reflected ultimately in differences in host-plant suitability.

Host selection for many insects is mediated by appropriate concentrations of key host plant chemicals (Thompson and Pellmyr, 1991; Honda, 1995). *P. xylostella* not only a range of glucosinolates and their hydrolysis products serve as oviposition stimulants (Reed et al., 1989; Renwick and Chew, 1994; Renwick et al., 2006), but they also stimulate larval feeding (Nayar and Thorsteinson, 1963).

3. MATERIALS AND METHODS

The methodology adopted in the present investigation is presented under the following headings.

3.1 Collection and Preparation of powder

Fresh *Z. officinale* Roscoe were collected from nearby market of Saibaba colony, Coimbatore. (BSI/SRC/5/23/2017/Tech/3448) after drying for four weeks, the *Zingiber officinale* Roscoe were ground in an electric pulverizer to get the powder collected from nearby market of Coimbatore, Tamilnadu, India. 10g of the powder was weighed using an electronic balance (Denver XS-210) and made into packets using Zero haze filter paper (A Grade, SD's). The powders were subjected to extraction (Harbourne, 1973; Vogel, 1978). Petroleum ether (60 – 80⁰C) extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity (Plate I). The rhizome extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40⁰C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use.

3.2 Phytochemical screening

Qualitative analysis

Preliminary screening of the extracts and identification was done by color tests adapting standard methods by Raman (2006)

Test for Alkaloids

- **Mayer's test**

A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream coloured precipitate.

Plate- I



Soxhlet Apparatus

Plate- II

(a) Zingiber officinale Roscoe



(b) Zingiber officinale

- **Wagner's test**

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

- **Hager's test**

A few ml of extract was treated with Hager's reagent (saturated aqueous solution of picric acid) and observed for the formation of prominent yellow precipitate.

Test for Flavonoids

- **NaOH test**

A small amount of extract was treated with aqueous NaOH and HCl was observed for the formation of yellow orange colour.

- **H₂SO₄ test**

A fraction of the extract was treated with concentrated H₂SO₄ and observed for the formation of orange colour.

Test for Steroids

- **Liebermann-Burchard test**

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H₂SO₄ was added and observed for the formation of dark pink or red colour.

Test for Terpenoids

- **Liebermann-Burchard test**

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H₂SO₄ was added and observed for the formation of dark green colour.

Test for Anthraquinones

- **Borntrager's test**

About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether.

The ether extract was further extracted with ammonia and formed pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

Test for Proteins

- **Ninhydrin test (Aqueous)**

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid and purple colour indicating the presence of protein.

- **Ninhydrin (acetone)**

Ninhydrin was dissolved in acetone and the extract was treated with ninhydrin and observed for the formation of purple colour.

- **Biuret test**

The extract was heated in distilled water and filtered. The filtrate is treated with 2% copper sulphate solution, to this added 95% ethanol and potassium hydroxide and observed for the formation of pink ethanolic layer.

Test for Phenols

- **Ferric chloride test**

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

- **Liebermann's test**

The extract was heated with sodium nitrite, added H_2SO_4 solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

- **Test for Quinones**

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Carbohydrates

- **Molisch's test for Carbohydrates**

Few drops of Molisch's reagent were added to each of the portion dissolved in distilled water, this was followed by addition of 1 ml of conc H₂SO₄ by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

- **Fehling's test for free reducing sugar**

About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

3.3. Anti fungal activity

Test microorganism

The fungal strains used were *Aspergillus fumigates* and *Aspergillus niger* (L).

Antifungal assay

The activities of the plant extracts on various fungal strains were assayed by agar well diffusion method.

Agar well diffusion method

Principle

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Reagents

1. Rose Bengal chloramphenicol agar medium

The commercially available (Hi media) Rose Bengal Chloramphenicol agar medium (32.15 g) was suspended in 1000 ml distilled water. The medium was dissolved completely by boiling and autoclaved at 15 lbs pressure (121⁰C) for 15 minutes.

Procedure

Rose Bengal chloramphenicol agar medium was prepared and poured on to the petriplates. A fungal plug was placed in the centre of the plate. Wells of 6 mm diameter was cut into the agar medium. Petroleum ether, chloroform and ethanol extracts was poured onto the wells in the plates. Nystatin was used as antifungal control. The antifungal effect was seen as crescent shaped zones of inhibition. (Schlumbaum et al., 1986).

Statistical Analysis

The antimicrobial data was assayed by standard deviation by means of three replicates.

3.4 Pest collection

P. xylostella larvae were collected from cauliflower from nearby markets of Coimbatore, Tamil Nadu, India. (Plate IV) The diamondback moth were reared automatically on cauliflower in green cage at room temperature. Larvae are generally found on the underside of the leaves, between the veins and in developing flower buds. They can burrow into the leaves, when they are small and making white tunnels. Later the caterpillars feed on the underside of the leaves and they do not eat the veins. They also feed the growing tips of the young plants, preventing them from developing further. Even in the winter season, larvae observed feeding in crucifer plants during daytime and maximum temperature is around 10⁰C.

Life stages of the diamondback moth are the egg, four larval instars, the pupa and adult. Eggs, larvae and pupae occur on the host. Adults occur on the host or on other plants adjacent to the crop. Adults are nocturnal in behavior. Eggs are laid singly or in groups of 2 to 8 on the upper or undersides of the leaves. They are frequently deposited in the hollows along the vein, on the young stems or on petioles. The small, flat, oval-shaped eggs are shiny yellow in colour when first laid and the eggs were hatched in 2 to 8 days. (Ho, 1965).

Role of antenna in *Plutella xylostella*

Olfaction plays a key role in moth-host plant interactions.(Galizia and Rössler 2010). The antennae are the main olfaction organ in insects, and affect mating and oviposition behaviors.

Plate - IV



***Brassica oleraceae*. var. *botrytis* L.**

The paired antennae are endowed with a multitude of sensilla; the trichoid, basiconic, and coeloconic are considered major olfactory sensilla. (Park et al., 2002). The trichoid sensilla have been shown to be responsible for detecting sex pheromones and plant odours (Forstner et al., 2008). The basiconic sensilla are sensitive to host plant chemicals and the coeloconic sensilla may function as olfactory receptors for plant odors, and may be sensitive to humidity and temperature (Romani et al., 2009).

3.5. Male antennal ablation

The antennae of newly emerged male moths were ablated to different lengths (1/4, 2/4, 3/4, and 4/4) were removed by using a sharp scalpel under a dissecting microscope, after anesthetization with chloroform and then it was treated with petroleum ether, chloroform and ethanol extract. A pair of moths (one female and one treated male) was placed in a ventilated container with access to a 10% sugar solution in distilled water. Each experimental group contained 5 pairs and the mating behaviors were observed at 30-min intervals every day until the natural death of the moths. Successful mating was recorded according to time taken for mating behavior and experiment was repeated two times in an insectary room. Ablated males were used as control.

Mating behavior

Couples were also checked randomly during the rest of the photophase in order to detect any mating occurring out of the period of observation. The approximate time males and females spent mating was recorded and only mating that lasted at least half an hour were considered successful as males need enough time to complete spermatophore transfer.

3.6. Female antennal ablation

A standard method was used in all ovipositional experiments. Prior to the start of each test healthy female adult were placed in temperature controlled cage. Ovipositional tests were conducted in oviposition beakers placed on laboratory benches. Before starting mating process, the antennae of female Diamond Back Moth were ablated to different lengths (1/4, 2/4, 3/4, 4/4) of were removed by using a sharp scalpel under a dissecting microscope, after anesthetization with chloroform and then it was treated with petroleum ether ,chloroform, ethanol. Females with ablated antennae were used as controls. Four beakers of alternating host plant species were

placed diagonally in the corners of a cage, into treated female moths were released. The plants were removed from the cages and the number of eggs laid on plant was determined after 24 hour and 48 hour. We performed this experiment two times. The number of eggs laid by the extract treated antennal ablated adult was calculated.

Oviposition preference

Ovipositional preference in *P. xylostella* is influenced by placing small macerated piece of cauliflower in each beaker. It stimulates the oviposition performance in diamondback moth after mating due to present of glucosinolates in host plants. Glucosinolates are secondary compounds that characterize in brassicaaceae family and it have important role in stimulating ovipositional preference in insects. Female moth were collected from the beakers after 24 hours and 48 hours and the number of laid on each cauliflower were counted.

Statistical analysis

The mating results were subjected to Analysis of Variance in (ANOVA) and the ovipositional results were subjected to standard deviation.

4. RESULT AND DISCUSSION

Plants are natural reservoir of medicinal agents almost free from the side effects normally caused by synthetic chemicals (Fennel *et al.*, 2004). The over use of synthetic drugs with impurities resulting in higher incidence of adverse drug reactions, has motivated mankind to go back to nature for safer remedies. Due to varied locations where these plants grow, coupled with the problem of different vernaculars names, the World Health Organization published standards for herbal safety to minimize adulteration and abuse (WHO, 1999). Some plant extracts and phytochemicals are known to have antimicrobial properties, which could be of great importance in the therapeutic treatments. Through phytochemical prospecting of the extracts, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities such as antimicrobial (Djipa *et al.*, 2000; Esquenazi *et al.*, 2002), antioxidant (Barreiros and David, 2006), antitumor and antiaphidic (Okuda *et al.*, 1989).

Microbes are considered as the mainstay and origin of multiple diseases. Microbial infiltration into the body tissues and blood lead to various diseases some of which are difficult to treat and extremely lethal. (Morens *et al.*, 2004). The etiology of various human infections may be related to bacteria, virus, fungi and protozoa. The exact chemical composition of a plant depends on its growing conditions, harvesting time, drying and extraction procedures etc. The clinical efficacy and pharmacological effects of a plant extract will depend strongly on the amounts of biologically active ingredients present, and these must be accurately measured if a herbal preparation is to be chemically standardized.

4.1. Phytochemical screening

In the present investigation, preliminary phytochemical screening of *Z. officinale* (R.) extracts showed presence of phytochemical constituents. The results confirmed the presence of constituents which are known to exhibit physiological activities. The Phytochemical characteristics of *Z. officinale* (R.) extract were investigated and summarized in table-1. The phytochemical study of chloroform extract of *Z. officinale* revealed the presence of alkaloids, terpenoids, and carbohydrate.

TABLE- I**Phytochemical constituents of Zingiber officinale Roscoe extracts**

S.No.	TEST	Zingiber officinale Roscoe			
		Pet. Ether	Chloroform	Ethanol	
1	Alkaloid	Mayer's Test	-	+	-
		Wagner's Test	-	-	-
		Hager's Test	+	-	+
2	Flavonoids	NaOH Test	-	-	-
		H ₂ SO ₄ Test	+	-	-
3	Steroids	Liebermann-Burchad Test	-	-	-
4	Terpenoids	Liebermann-Burchad Test	-	+	-
5	Anthraquinone	Borntrager's Test	-	-	-
6	Proteins	Ninhydrin (Aqueous) Test	-	-	-
		Ninhydrin (Acetone) Test	-	-	-
		Biuret Test	+	-	-
7	Phenols	Ferric Chloride Test	-	-	-
		Liebermann's Test	+	-	-
8	Quinones	Test for quinones	+	-	+
9	Carbohydrates	Molish Test	+	-	-
		Fehling's Test	-	+	-

+ Detected - Not detected

Alkaloids, Flavonoids, proteins, phenols, quinones and carbohydrate were observed in petroleum ether extract of *Z.officinale*. Presence of alkaloids and quinones were in ethanol extract of rhizome. Alkaloids are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen.(Sarker and Nahar, 2007). In nature, the alkaloids exist in large proportions in the roots of plants and often in combination with vegetable acids. Alkaloids have pharmacological applications as anesthetics and central nervous system stimulants (Madziga et al., 2010).

Flavonoids in plants can modulate the feeding behavior of insects, though mechanisms associated with these behavioral responses are not clearly understood. Flavonoids are important group of polyphenols widely distributed among the plant flora. (Kar, 2007). The compounds are derived from parent compounds known as flavans. These compounds are strong antioxidants that protect against cancer and cardiovascular disease. Its play major important role in blood clotting factors. Terpenes are among the most widespread and chemically diverse groups of natural products. Terpenoids have pharmacological applications as anti cancerous agent. Anthraquinone are derivatives of phenolic and glycosidic compounds. (Maurya et al., 2008 ; Firn, 2010).

Steroids show the analgesic for central nervous system activities. Plant steroids also referred to as 'cardiac glycosides' and are naturally occurring plant phyto constituents that have found therapeutic applications as arrow cardiac drugs (Firn, 2010). Saponins protect against hyper cholesterolemia and antibiotic properties. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa, 2007 and Mensah, 2008. Phytochemicals inhibit microorganisms, interfere with some metabolic processes or may modulate gene expression and signal transduction pathways (Kris-Etherton et al., 2002; Manson 2003; Surh 2003). Phytochemicals may either be used as chemotherapeutic or chemo preventive agents with chemo prevention referring to the use of agents to inhibit, reverse, or retard tumorigenesis. Chemo preventive phytochemicals are applicable to cancer therapy, since molecular mechanisms may be common to both chemoprevention and cancer therapy (Incalci et al., 2005; Sarkar and Li, 2006).

The results obtained in this study suggested that the identified phytochemical compounds of bioactive constituents responsible for the inhibiting the growth of microorganisms. The presence of these compounds has also been confirmed to have antimicrobial activity. Phytochemical screening and antimicrobial properties of *Z.officinale* using in vitro studies that might be useful to treat human infectious diseases, antibiotic resistant pathogens drug and discovery analysis. Similar result were observed in the study of phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia*. The results revealed from *E. longifolia* showed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, protein and cardiac glycosides in the extracts of petroleum ether, chloroform and ethanol. (Khanam et al., 2014).

Samejo et al. (2013) observed the phytochemical screening of *Tamarix dioica*. Phytochemical screening of this plant part reveals the presence of steroids, phenols, terpenoids, flavonoids and saponins and proteins, and amino acids were absent. Similar observation were made in the present study that *Z. officinale* extract showed the presence of alkaloids, flavonoids, terpenoids, phenols and quinones. Adeosun et al. (2016) experimented phytochemical, minerals and free radical scavenging profiles of *Phoenix dactilyfera* L. seed extract. The phytochemical results observed from these study showed the presence of alkaloids, flavonoids, anthraquinones, saponins, terpenoids, and tannins contents and similar results were observed in the present study of *Z. officinale*.

The observation were made in the present study of *Z. officinale* extract showed the presence of alkaloids, flavonoids, terpenoids, phenols, quinones, antraquinones and similarity results were observed in the phytochemical studies on *Pandanus tectorius*. (Andriani et al., 2015). Phytochemical analysis of some selected spices constituents of phytochemical were proved to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. (Harsha et al., 2013)

Phytochemical, pharmacological and ethnobotanical studies in mango ginger. The valuable medicinal plant with naturally enriched wide range of chemical constituents shows many phytochemical properties. Phytochemical properties of Curcuma amada against chronic diseases and glycosides, saponins, flavonoids, tannins and alkaloids have hypoglycemic activities and anti-inflammatory activities. (Jatoi et al., 2007).

4.2 Antifungal Activity

The antifungal activity of seed extracts were screened by agar well diffusion method. *A. fumigatus* and *A. niger* were used as fungal strains. Results are depicted in Table-II and these results revealed that all seeds have antifungal activity (Fig-I and II).

In *Z. officinale* rhizome, ethanol extract was found to have highest zone of inhibition against *A. fumigatus* ($18 \pm 1.63\text{mm}$) and *A. niger* ($18 \pm 0.81\text{mm}$) also showed similar effect of zone of inhibition. (Plate-V). Petroleum ether extract was more effective and obtained zone of inhibition against *A. fumigatus* ($16.6 \pm 1.24\text{mm}$) and highest zone of inhibition was observed in *A. niger* ($18.6 \pm 1.24\text{mm}$). Chloroform extract of *Z. officinale* possess zone of inhibition against *A. fumigatus* ($15.6 \pm 1.69\text{mm}$) and *A. niger* ($17.3 \pm 1.24\text{mm}$) (Plate-VI).

The antibacterial and antifungal potentials of the solvents extracts from *Eryngium caeruleum*, *Notholirion thomsonianum* and *Allium consanguineum* actively against the fungal strains of *A. fumigatus* and *A. niger*. In the present study extract of *Z. officinale* showed highest zone of inhibition and similar results were observed by Sadiq et al., 2016.

The preliminary studies on *E. longifolia* extracts exhibit moderate antimicrobial activity against fungal strains *A. fumigatus* and *A. niger*. (Khanam et al., 2015). In the present study similar observation were made in chloroform extract of *Z. officinale*.

The antimicrobial activity of rhizome extract of *Acorus calamus* actively inhibits the *A. niger* fungal strains. The inhibitory concentration of ethanol extract of *A. calamus* (19mm) observed by Kumar et al., 2014 and similar results observed in ethanol extract of *Z. officinale*. (18.1mm).

TABLE- II

Antifungal activity of Zingiber officinale Roscoe extracts against test organisms

Micro organisms	Extracts (Diameter of the zone of inhibition in mm)			
	Zingiber officinale Roscoe			
	Control	PE	CH	E
A. fumigatus	18.6±1.24	16.6±1.24	15.6±1.69	18±1.63
A .niger	23.5±1.27	18.6±1.24	17.3±1.24	18±0.81

Values are mean inhibition zone (mm) ± S.D.

Cont : Control PE : Petroleum Ether CH: Chloroform E : Ethanol

Figure I

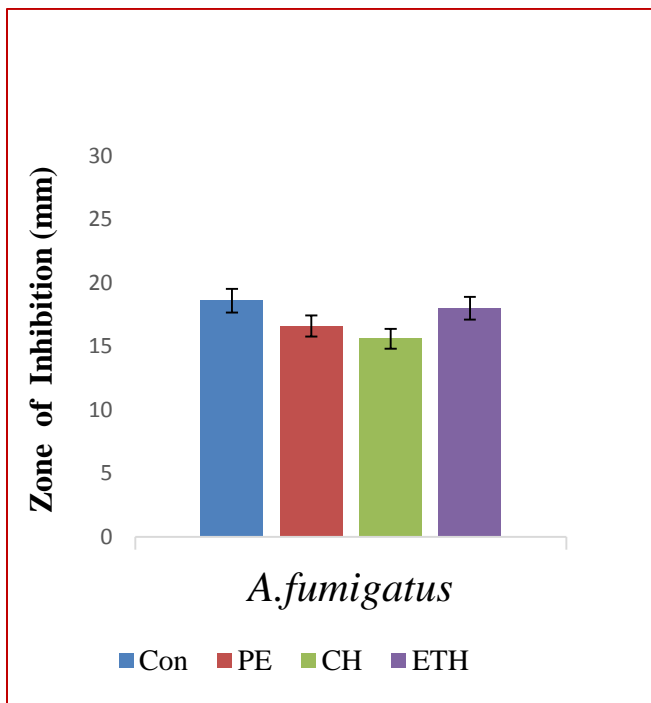
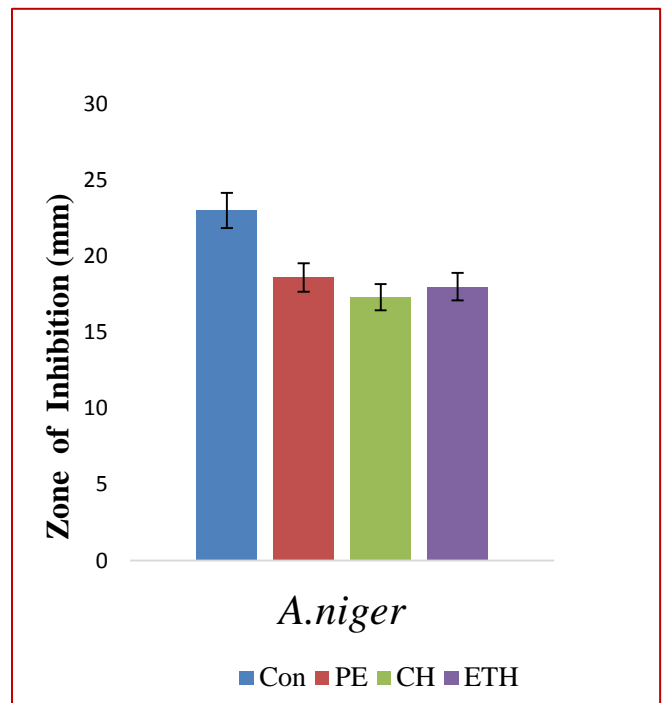


Figure II



Antifungal activity of Z. officinale Roscoe extracts against A. fumigatus

Antifungal activity of Z. officinale Roscoe extracts against A. niger

Plate VI

**Antifungal activity of *Z.officinale* Roscoe extracts
against *A.fumigatus***



a) Petroleum ether extract

b) Chloroform extract



(c) Ethanol extract



Plate VI

**Antifungal activity of *Z.officinale*
Roscoe extract against *A.niger***



a) Petroleum ether extract

b) Chloroform extract



c) Ethanol extract



The antimicrobial activity of dried ginger powder with the extract of chloroform, ethanol, acetone and petroleum ether solvents, strongly against *Fusarium oxysporum* f. sp. *Lycopersici* also. Chloroform extract of ginger showed highest zone of inhibition against tested pathogen. Whereas, other solvents showed moderate to minimum antifungal activity. These findings suggests that some plant extracts tested possess antifungal activities against *F. oxysporum* f.sp. *lycopersici*.(Rawal and Adhikari, 2016). But the present study observation showed moderate activity to against *A.fumigatus* and *A. niger*.

In vitro study, ginger paste showed inhibition zone better than ethanol, but cold ethanolic ginger extract showed the maximum inhibition zone at 24 hrs. In the present study showed that the ethanolic extract of ginger powder has pronounced inhibitory activities against *A. niger*, *A.fumigatus*. Supreetha *et al.*, (2011).

4.3 Effect of antennal ablation in mating and ovipositional behavior

Antennae are important peripheral sensory organs and play an important role in seeking and locating food, suitable habitat and reproductive partners. Antennal sensilla are the basic functional unit of antennae and are classified as chemoreceptors, mechanoreceptors, thermo receptors, or hygroreceptors. (Gullan and Cranston, 2004).

Chemoreception is essential for insects to find a mate, locate foods and oviposition sites (Leal, 2013). The sensilla on insect's antennae are not randomly distributed. Zacharuk (1985) observed that pattern may reflect the impact of many interacting selection pressures in which size of the individual, developmental stages, sex, feeding habits and habitats are of considerable significance (Chapman, 1982). Trichoid sensilla present in the antennae of *P. xylostella* play major important role for detecting sex pheromones. However, the basiconic and coeloconic sensilla were more abundant on female than male antennae and this sexual dimorphism is thought to be associated with plant odor detection (Laue, 2000).

The antennal ablation experiments in *P. xylostella* were showed changes in mating and ovipositional behavior. The total number of eggs laid on *B. olearaceae* was significantly

decreased with antennal removal. The number of successful mating decreased almost linearly with shortening of antennae. The time taken for duration of mating also decreased with shortening of antennae. Most of the antennal ablated males and females showed no movement. The complete removal of antennae in both sex showed no mating process and most of them were died within a few hours. It is because the antennas play an important role in detecting sex pheromones which induce the stimuli in both male and female. The extract treated antennal ablation study showed decreased mating and oviposition behavior compared to the untreated moths.

The result observed in this study showed successful mating in control. The successful mating rate were reduced as very low in 4/4 antennae ablation compared to 1/4. The observation of the table III, IV, V and VI showed the mating behavior in diamondback moth with shortening antennae.

The sex pheromone communication system between male and female insects is important for mating behavior. The DBM uses a male aphrodisiac pheromone which is involved in successful mating, but has been proven to be less important than the female sex pheromone (Chow et al., 1986). Mating behavior of moths with ablated antennae were similar to the oviposition behavior of moths with ablated antennae. Mating behavior did not significantly change when less than 1/4 of the antennae were removed, but changed significantly when antennae were ablated by more than half and mating rate was lower (Plate V).

Under natural conditions, the ability of DBM females to select cruciferous host plants for oviposition is dependent on the perception of glucosinolates (Renwick *et al.*, 2006; Sun 2009). Plant studies have found that females prefer to oviposit on specific cruciferous plant species (Yu 1998; Jiang 2001; Badenes-Perez *et al.*, 2005). This preference was altered in a predictable manner in our antennal ablation experiments and reducing antenna length resulted in a gradual, but consistent, statistically significant reduction in ovipositional preference. (Table VII) Complete antennal ablation resulted in no ovipositional preference. Gradual reduction in host preference with decreasing antennal length occurs, the ability to detect plant odors becomes weaker.

TABLE -III**Mating behavior in *P. xylostella* with antennal ablated male.**

	First pair	Second pair	Third pair	Fourth pair	Fifth pair
1/4 removed	0.957 a	1.043 a	2.123 a	3.343 a	3.340 a
2/4 removed	0.920 b	0.840 b	0.957 b	1.020 b	1.540 b
3/4 removed	0.540 c	0.850 b	0.903 c	0.650 c	0.980 c
4/4 removed	0.003 d	0.003 c	0.003 d	0.003 d	0.003 d
S.E.D	0.005	0.009	0.011	0.012	0.164
F	5.73 *	4.26 ns	<1	<1	<1

TABLE -IV**Mating behavior in *P. xylostella* with antennal ablated Female.**

	First pair	Second pair	Third pair	Fourth pair	Fifth pair
1/4 removed	0.977 a	1.533 a	2.003 a	3.013 a	3.973 a
2/4 removed	0.903 b	0.900 b	0.960 b	1.020 b	0.853 c
3/4 removed	0.650 c	0.620 b	0.710 c	0.960 c	1.020 b
4/4 removed	0.003 d	0.003 c	0.003 d	0.003 d	0.003 d
S.E.D	0.007	0.006	0.008	0.008	0.012
F	<1	5.84 *	<1	<1	<1

TABLE -V

Mating behavior in *P. xylostella* with antennal ablated male treated with *Z.officinale* Roscoe extract

	First pair	Second pair	Third pair	Fourth pair	Fifth pair
1/4 removed	0.910 a	0.870 a	1.057 a	1.033 a	0.760 a
2/4 removed	0.780 b	0.800 b	0.650 b	0.550 c	0.003 c
3/4 removed	0.540 c	0.460 c	0.003 c	0.677 b	0.337 b
4/4 removed	0.003 d	0.003 d	0.003 c	0.003 d	0.003 c
S.E.D	0.004	0.011	0.009	0.006	0.011
F	10.33*	<1	<1	5.47 *	4.20 ns

TABLE -VI

Mating behavior in *P.xylostella* with antennal ablated female treated with *Z.officinale* Roscoe extract

	First pair	Second pair	Third pair	Fourth pair	Fifth pair
1/4 removed	0.680 b	0.870 b	1.010 a	1.260 a	2.370 a
2/4 removed	0.720 a	0.907 a	0.967 b	1.010 b	1.040 b
3/4 removed	0.670 b	0.550 c	0.003 c	0.600 c	0.003 c
4/4 removed	0.003 c	0.003 d	0.003 c	0.003 d	0.003 c
S.E.D	0.010	0.008	0.008	0.008	0.007
F	<1	<1	1.50 ns	<1	<1

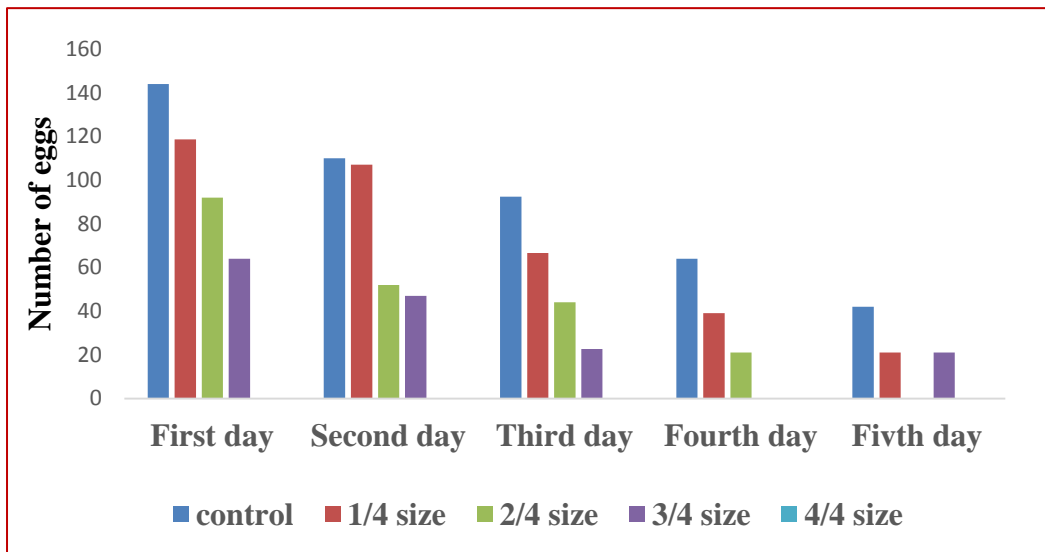
TABLE -VII

Ovipositional proclivity of Diamondback moth females with different antennal lengths

S.No	Control	1/4	2/4	3/4	4/4
First day	144±0.81	118.6±0.47	92±0.81	64±0.81	-
Second day	110±0.81	107±0.81	55±0.81	47±0.81	-
Third day	92.3±1.24	66.6±1.24	44±1.41	22.6±0.47	-
fourth day	64±1.41	39±0.81	21±0.81	-	-
Fifth day	42±0.81	21±0.81	-	21±0.81	-

Values are mean (number of eggs.) ± S.D.

Figure III



Ovipositional proclivity of diamondback moth with different antennal lengths

Plate –VII

Early stages of cocoon formation



Four instar larva

Cocoon Formation



Plate -VIII

Adults of *P. xylostella*

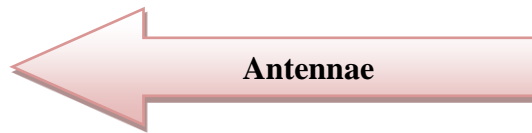
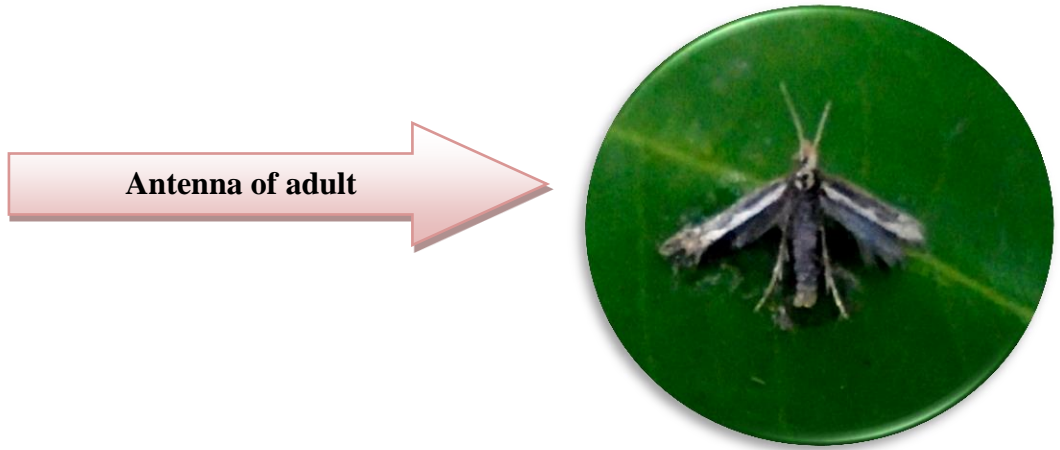


Plate- IX

Mating duration study in *P.xylostella*



a) Control



b) Antennal ablation in *P.xylostella*

Plates-X



a) Mating study in *P.xylostella*



b) Ovipositional proclivity in *P.xylostella*

We observed a significant reduction in the total number of eggs laid by moths with antennal ablation. (Plate VI) Therefore, we concluded that the reduction in number of eggs laid by moths with shortened antennae was predominantly due to failure to locate a mate. However, other mechanisms may be involved in this process.

Ryan and Sakaluk (2009) showed the antenna of the decorated crickets, *Gryllodes sigillatus* are important sensory organs in insects because they enable the perception of chemical and tactile cues believed to be important in sex recognition and mating in various species. Males lacking antennae almost never showed mate-guarding behaviour, while males with intact antennae almost always showed guarding and behavior. Collectively, these results suggest that sensory information provided by the antennae is critical to the mating success of both male and female crickets.

Similar observation was also made in the study of chemical ablation and surgical removal of the antennae in *T. oceanicus*. The removal antennae affected male propensity to court, suggesting that chemical cues are more important than tactile cues in sex recognition. Chemically ablation of the antennae in *T. oceanicus* using zinc sulphate suppresses courtship behavior of males. (Balakrishnan and Pollack, 1997).

The antennal structure, sensilla number and topography were different between males and females and were probably linked to the use of antennae during the searching behavior of *Cotesia plutellae* in mating. The ablation experiment of antennae showed reduction in mating success rate. Ablation experiments in *Cotesia plutellae* showed that antennae were essential in host detection for its ovipositional behavior. Antennal ablated females laid low number of eggs in *C. plutellae*. (Roux *et al.*, 2005).

Yang *et al.* (2001) reported, the trichoid sensilla were the most abundant, the mean number of the sensilla were significantly more abundant in the male than in the female antennae. Triichoid sensilla play major role in mating behavior and it stimulate the mating behavior in males. Similar results have been reported for *Talponia batesi* and *Spodoptera frugiperda*. (Castrejón-Gómez and Carrasco, 2008).

The sensilla on antenna detect information of the complex external world and transmit it into the insect's nervous system in which the information is integrated and the insect executes the appropriate behavior responses to the situation (Hansson and Stensmyr 2011).

5. SUMMARY

- The study entitled Antifungal activity and Antennal ablation on diamondback moth *P. xylostella* (Lepidoptera: Plutellidae) against *Z. officinale* Roscoe and its effects on mating and ovipositional proclivity was undertaken in the laboratory.
- Preliminary screening of the phytochemical screening was done by colour tests adapting standard methods to identify the constituents for petroleum ether, chloroform and ethanol extracts of *Z. officinale* Roscoe.
- Alkaloids, terpenoids, carbohydrate were present in chloroform extract of *Z. officinale* Roscoe. In petroleum ether extract, alkaloids, flavanoids, phenols, quinones, carbohydrate and proteins were present. Presence of alkaloids and quinones were observed in ethanol extract.
- The antifungal activity of *Z. officinale* Roscoe extracts was screened by agar well method and *A. fumigatus* and *A. niger* was used as fungal strains.
- In antifungal activity, Ethanol extract of *Z. officinale* Roscoe exhibited maximum zone of inhibition against *A. fumigatus* (18 ± 1.63 mm) followed by petroleum ether extract (16.6 ± 1.15 mm).
- Ethanol extracts of *Z. officinale* Roscoe exhibited highest zone of inhibition (18 ± 0.81) against *A. niger* followed by petroleum ether extracts (18.6 ± 1.24).
- Chloroform extract of *Z. officinale* Roscoe showed minimum zone of inhibition against *A. fumigatus* (15.6 ± 1.69 mm) and *A. niger* (17.3 ± 1.24 mm).
- A pilot study was conducted to explore the effects of *Z. officinale* Roscoe tested against mating and ovipositional behaviour of diamondback moth *P. xylostella*. The effectiveness of doses vary from plant to plant on basis of toxicity.
- *Z. officinale* Roscoe were collected, powdered, sieved and were subjected to soxhlet extraction using petroleum ether, chloroform and ethanol as solvents in increasing order of polarity.
- Larva of *P. xylostella* was treated with petroleum ether, chloroform, ethanol extract.
- Observations were made to every 24 hours, 48 hours, 72 hours and 96 hours to record adult emergence.
- Antennal ablation experiments were made in *P. xylostella* with different lengths.

- Observations were made at every 24 hours to record mating duration time and ovipositional proclivity in antennal ablated males and females. Antennal ablated diamondback moths with untreated moths are used as control.
- The data on mating behaviour were also subjected Analysis of variance. (ANOVA).
- From the Analysis of variance, the mating duration of the diamondback moth were varies according to the antennal ablation length and the ovipositional proclivity also varies according to the length of ablation.

CONCLUSION

- The presence of phytochemicals in the extract of *Z.officinale* may have been responsible for the activity possessed by the plant extracts.
- Phytochemical screening and antimicrobial properties of *Z.officinale* using in vitro studies that might be useful to treat human infectious diseases, antibiotic resistant pathogens drug and discovery analysis.
- However, further studies are required to identify the specific factors responsible for this strong ovipositional response of *P.xylostella* towards the damaged plants. The result demonstrated that diamondback moth oviposition preference can be altered by a reduction in antennal sensory input and that host preference can be eliminated by total antennal ablation.

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