STUDIES ON CERTAIN PLANT VOLATILES ATTRACTING THE SHOT HOLE BORER, *Euwallacea fornicatus* (EICHHOFF) (SCOLYTIDAE: COLEOPTERA) INFESTING TEA

A THESIS SUBMITTED TO THE BHARATHIAR UNIVERSITY
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN ENTOMOLOGY

BY
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CERTIFICATE

This is to certify that the thesis, entitled **"Studies on certain plant volatiles attracting the shot hole borer, *Euwallacea fornicatus* (Eichhoff) (scolytidae:Coleoptera) infesting tea"** is a record of original research work done by ***Mr. Sachin. P. James*** , in the Division of Entomology, United Planters' Association of Southern India Tea Research Foundation (UPASI TRF), Tea Research Institute, Valparai - 642 127, Coimbatore Dist., India as a full-time Research Scholar during the period of study from 2004 to 2007 under my guidance and supervision for the award of the Degree of Doctor of Philosophy in Entomology. I further certify that this research work has not previously formed the basis for the award of any other Degree or Diploma or Associateship or Fellowship or other similar title to any candidate of this or any other University.

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DECLARATION

I do hereby declare that the thesis entitled **"Studies on certain plant volatiles attracting the shot hole borer,** ***Euwallacea fornicatus*** **(Eichhoff) (Scolytidae:Coleoptera), infesting tea"** submitted to the Bharathiar University, Coimbatore - 641 046, for the award of the Degree of Doctor of Philosophy in Entomology, is a record of original and independent research work done by me during 2004 to 2007 under the supervision and guidance of ***Dr. R. Selvasundaram,*** Sr. Entomologist, Division of Entomology, United Planters' Association of Southern India Tea Research Foundation (UPASI TRF), Tea Research Institute, Valparai - 642 127, Coimbatore Dist., India and it has not previously formed the basis for the award of any other Degree, Diploma, Associateship, Fellowship or other similar title to any candidate in any University.

Signature of the candidate

(Sachin. P. James)

....................**Dedicated to my Parents and to my Grandparents in heaven**

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Of course, despite all the assistance provided by others, I alone remain responsible for the content of the following, including any errors or omissions which may unwittingly remain. bove all, I pray the **lmighty** for all the blessings throughout my life.

Sachin.P.James

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ABBREVIATIONS USED IN THIS THESIS

a	alpha
b	beta
BHT	butylated hydroxyl toluene
CO_2	carbon dioxide
cm	centi metre
Co-I	co-injection
CVT	cross vane trap
Dia.	diametre
DCM	dicloromethane
DHS	dynamic headspace
EAG	electro antennogram
ETL	economic threshold level
FVT	funnel vane trap
GC-MS	gas chromatograph with mass spectrum detector
GC-FID	gas chromatograph with flame ionisation detector
ha	hectare
h	hour(s)
IPM	integrated pest management
ISD	internal standard
kV	kilo volt
m	metre
g	micro gram
l	micro litre
m	micro metre
mm	milli metre
mg	milli gram
ml	milli liter
mV	milli volt
min.	minutes
MSL	mean sea level
MFT	multiple funnel trap

%	per cent
PET	polyethylene tube
RBD	randomised block design
RH	relative humidity
Rt	retention time
RS	rubber septa
RiS	ring septa
s	seconds
SBT	sticky board traps
SEM	scanning electron microscope
SHB	shot hole borer
SPSS	special purpose statistical software
sp.	species
spp.	species (plural)
SD	standard deviation
SE	standard error
UPASI	United Planters' Association of Southern India
UV	ultraviolet
v	volume
w	weight

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Introduction

INTRODUCTION

Tea is one of the popular beverages all over the world due to its special aroma, flavor and health benefits. The crop plant belongs to C*amelliaceae* and is perennial in nature. All the cultivated tea plants belong to two distinct taxa, viz., *Camellia sinensis* (L.) O. Kuntze the short leaved "China" plants and *Camellia assamica* (Masters) Wight, the broad leaved "Assam" cultivar. The "Cambod" variety, a subspecies of the latter, is classified as *C. assamica* spp. *lasiocalyx* (Planchon ex watt) Wight (Wight, 1959). "China", "Assam", "Cambod" 'jats' and a large number of their hybrids are exploited commercially in majority of the tea plantations. It is believed that many wild species of *Camellia* have also contributed to the present day hybrid population of cultivated tea plants. This crop is predominantly grown in Asia followed by Africa and to a very small extend in Europe, South America and Australia. India is the largest producer and consumer of black tea in the world. More than 520,000 ha is under tea cultivation in India. Majority of the tea plantations are situated in the north eastern and southern region of the country. Tea plantations in south India are spread over the slopes of Western Ghats of Wynaad, Central Travancore, High Ranges, Nilgiris, Anamallais and Chikmagalur of Karnataka (Fig.1)

Like any other plantation crop, tea is also affected by an array of pests mainly arthropods. More than one thousand species of pests have been reported to affect different parts of tea plants. Insects and mites are the major group of pests attacking the tea plants. Being a perennial crop and grown as monoculture, the tea ecosystem provides a stable favorable environment and undisturbed food supply to the pests. Insect pests which are predominant in the southern tea growing areas of the country include shot hole borer, thrips and tea mosquito. Apart from these some leaf eating caterpillars are also reported as minor pests which account for a very low economic damage and often controlled by the cultural operations carried out in tea plantations.

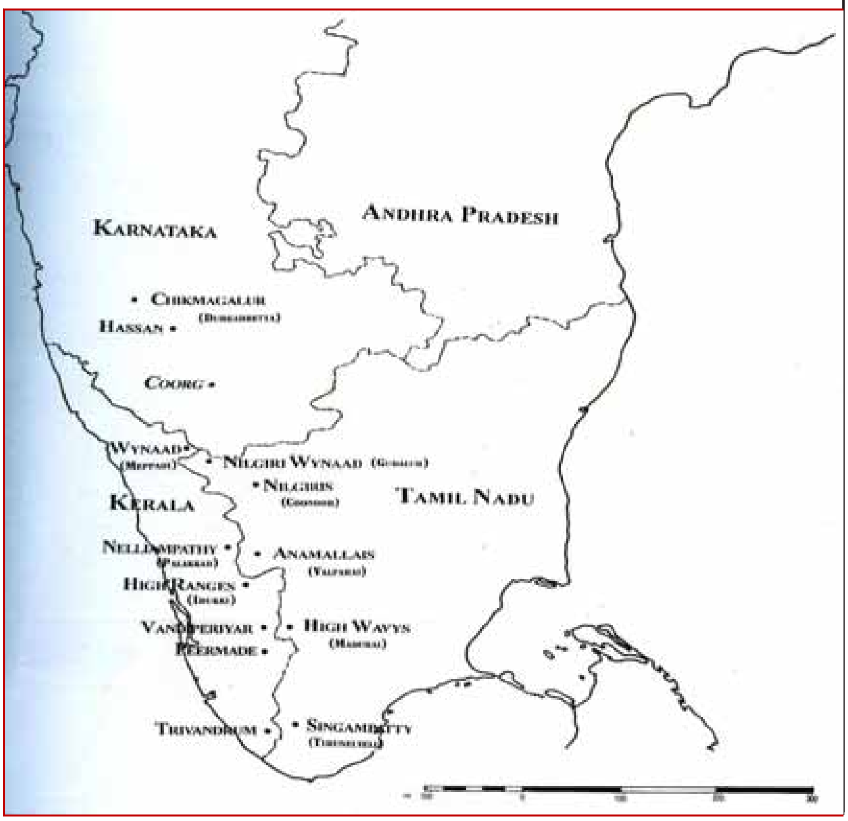


Fig.1. Tea growing areas of southern India

Among the pests, the shot hole borer (SHB), *Euwallacea fornicatus* (Eichhoff) (Scolytidae: Coleoptera) (= *Xyleborous fornicatus*) is a serious pest of tea in south India. Even though the occurrence of SHB of tea is reported in Indonesia, Malaysia, Taiwan, Philippines, New Guinea, Hawai, Fiji and New Hebrides, it gained major pest status only in Sri Lanka and south India. The life history and control measures of this beetle had been described by several authors (Gadd, 1941a&b; Muraleedharan, 1986a ; 1997; Muraleedharan and Radhakrishnan, 1989; 1994).

Female beetles are black, 2.0 to 2.5 mm long with strongly sclerotised body, well developed wings and mouthparts. In males, eyes, wings and mouthparts are atrophied and therefore they are unable to fly or bore into hard wood. Both these functions are carried out by the females. Newly emerged, creamy white/yellow adult beetles turned into light brown and then to characteristic black colour in six to twelve days. Scolytid beetles belonging to the tribe *Xyleborini* have symbiotic relationship with ambrosia fungus. In the case of *E. fornicatus* the symbiotic fungus is *Fusarium bugnicourtii* (Bray ford). The fungal spores are carried by the beetles in special organs called mycangia located in the buccal cavity of head (Parthiban and Muraleedharan , 1996). Spores of the ambrosia fungus borne by the female beetles adhere to the walls of the stem galleries. On germination of spores, the grubs and adults feed on the fungus. The female, a few days after emergence from the parental gallery, establishes its own gallery measuring 1.5 mm diameter on the branches of tea bush. There are two types of galleries, *viz.,* circular and longitudinal and more often both type of galleries observed in the same stem or branch which is called as mixed gallery (Plate. 1). Almost all females are fertilized by the less numerous males and mating takes place inside the parental gallery. Unmated females also lay eggs and such unfertilized eggs develop into males. Females start to lay eggs after a pre-oviposition period of six to eight days. Eggs are laid singly and are creamy white in colour, oval in shape and measures about 0.5 to 0.6 mm long. They hatch in four to six days and the newly emerged grubs (larvae) are also creamy white. They are about 3 mm long and start feeding on the fungus growing inside the stem galleries as mentioned earlier. There are three larval instars and the larval stage is completed in 16 - 18 days. Pupation takes place inside the same gallery. Adults emerge within seven to nine days and remain in the parental gallery for some time. They also feed on the fungus and the male: female ratio is found to 1: 8 (Muraleedharan, 1991a).

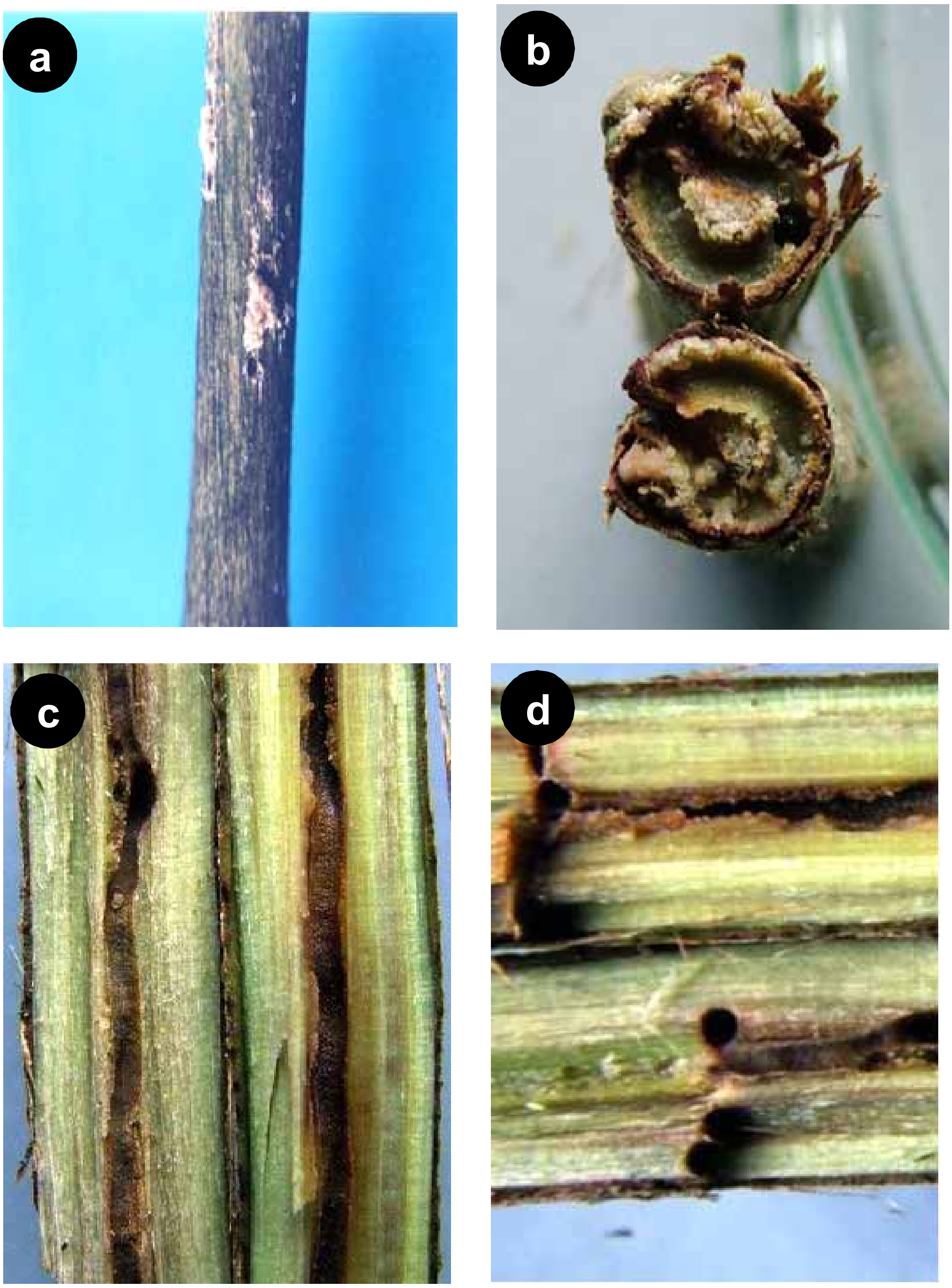


Plate.1.Different types of SHB galleries

a-Infested stem; b-circular gallery; c-longitudinal gallery; d-Mixed gallery

Adult beetles and their life stages are multivoltine (Plate 2) Incidence of SHB attains its peak during certain months in the Anamallais, population reached high levels during April/May, July, October and December (Muraleedharan , 1991 a & b). In Vandiperiyar region, the trend of population dynamics was similar, except a peak in December which is not very distinct. Adult beetles are active during day time, especially at noon. Calnaido (1965) reported that their speed of flight ranged between 0.3 to 0.6 cm/sec and maximum duration of flight was less than an hour.

At present, infestation by the SHB is known from most of the tea growing areas of south India, but its depredations are more pronounced in the mid elevation tea areas (< 1250 m above MSL) of Anamallais (Coimbatore District), Vandiperiyar and Peermade (Idukki District), Nelliampathy (Palakkad District) Wynaad (Wyanaad District) and Nilgiri (Gudalur Taluk of Nilgiri District). SHB infestation causes not only the loss in economic yield but also intensify the capital loss by weakening of bushes, branch breakage and debilitation of bushes. During the past one decade, management of this pest is mainly achieved by adopting certain cultural, chemical and biological control measures (Selvasundaram *et al.,* 2001). Achievement of control is moderate due to the peculiar habitat of these beetles. Escalating concern on the adverse effect of usage of pesticides on the environment and the presence of residues in made tea have precipitated a strong decision for limited and discriminate use of pesticides. This led to the development of an integrated pest management strategy in tea. To meet the expanding demand for safer tea, search for more effective and alternative procedures to control the pest based on sound biological principles is warranted.

The use of attractant trap has long been practiced by the entomologists in general and applied entomologist in particular (Nordlund *et al.,* 1981). Though the progress from a suction trap to more sophisticated trap was rapid and one of the greatest advantages of attractant trap is the specificity to the target insect. In course of time, many investigations had been made on different orders, genera and species that have attraction towards their own species (sex pheromones), or to other species (kairomones). The first isolation and identification of a pheromone was reported by Butenandt *et al.,* (1959) which sparked a busting activity in chemical ecology.

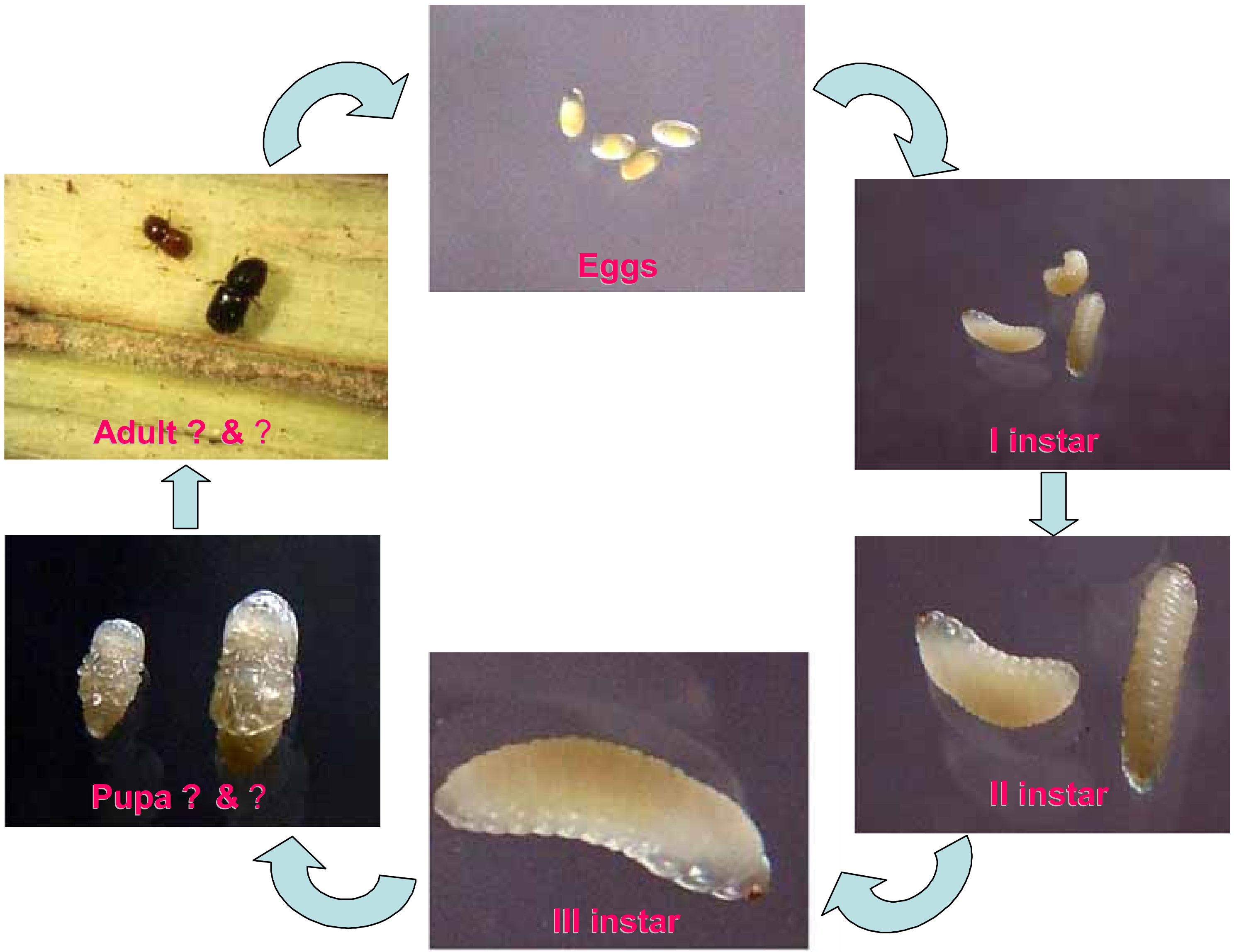


Plate 2. Life history of shot hole borer

The field of chemical ecology rapidly improved with the advent of recent chemical technologies particularly for the past thirty years. Chemical communication is generally accepted as an area of chemical ecology, considering a broad definition for communication. Organisms interact with the same and with different species where the communication may be different in nature. Law and Rignier (1971) proposed the term semiochemicals (Gk.*Semeon*, a mark or signal) for the chemicals that mediate interactions between organisms. Semiochemicals are sub divided into two groups i.e., pheromones and allelochemics on the basis of interactions, particularly, interspecific or intraspecific. Pheromone was first proposed by Karlson and Butenandt (1959) but their definition of the term pheromone was restricted to animals. Nordlund and Lewis (1976) broadened the definition as "a substance secreted by an organism to out side that causes a specific reaction in a receiving organism of the same species". Pheromone was then classified on the basis of type of interaction mediated such as sex pheromone, alarm pheromone and epidietic pheromone. The term allelochemics were first proposed by Whittaker (1970a&b) and he described them as chemicals which mediate interspecific interactions. At present, the four types of allelochemics recognized are allomone, kairomone, synomone and apnuemone.

Brown (1968) and Lewis *et al.,* (1975) described allomones as substance produced or acquired by an organism which in contact with an individual of the same or different species in the natural context which evokes a behaviour physiological response that is adaptabily favourable to the emitter but not to the receiver.

Kairomones are chemicals beneficial for the receiver rather than to the emitter in interspecific interactions. The term kairomone (Gk. *Kairos*, opportunistic) was proposed by Brown *et al.,* (1970). Synomones are chemicals which mediate mutualistic interactions and was first proposed by Nordlund and Lewis (1976). Apnuemones are chemicals which mediate interaction between the individuals of different species originate from a non living material (Nordlund and Lewis, 1976).

As scientists learned more about the system involved in chemical communication, more awareness came out of the complexity of the system, which is due to the number of factors, such as, (i) perception of a semiochemical by an organism (ii) dosage serving as another type of semiochemical in interspecific interactions or as a hormone (iii) physiological readiness of the organism to respond (iv) role of differing semiochemicals (v) difficult to demonstrate the advantage especially with kairomones and (vi) the need for appropriate environmental conditions which adds to the complexity of the various systems.

Selvasundaram *et al., (*2001) have recorded that partially dried cut stems of a jungle plant, *Montanoa bipinnatifida* C.Koch. *(Compositae)* attracted shot hole borer beetles in the field (Plate 3). *M. bipinnatifida*, an exotic plant species introduced from Mexico (Matthew, 1991), is a shrub which grew erect measured about 5 m and bloom with white flowers during the months of October to December. In India, the plant is naturalized at an elevation less than 1200-1800 m above MSL. Placing of the partially dried stems in the field has helped in trapping the beetles. It has been evident that the volatiles emanating from the partially dried stems of *M. bipinnatifida* were responsible for attracting the SHB beetles.

Present work is aimed to develop a protocol to extract, isolate and identify the attractants from the partially dried stems of *M. bipinnatifida* to construct an efficient attractant trap for SHB. Laboratory and field evaluations were carried out with these compounds singly and in combinations to monitor their efficiency in trapping of SHB beetles. Optimization of dispenser, suitable attractant blends and their optimum amount required per dispenser for efficient trapping were also carried out. Determination of suitable trap, position and height were evaluated. Field trials on the most effective trap with the most promising blend were carried out to determine the number of traps required per hectare. Using all these findings, large scale field experiments were conducted at two different locations to generate information on the efficiency of attractant trap which can further improve the integrated pest management schedule for SHB.

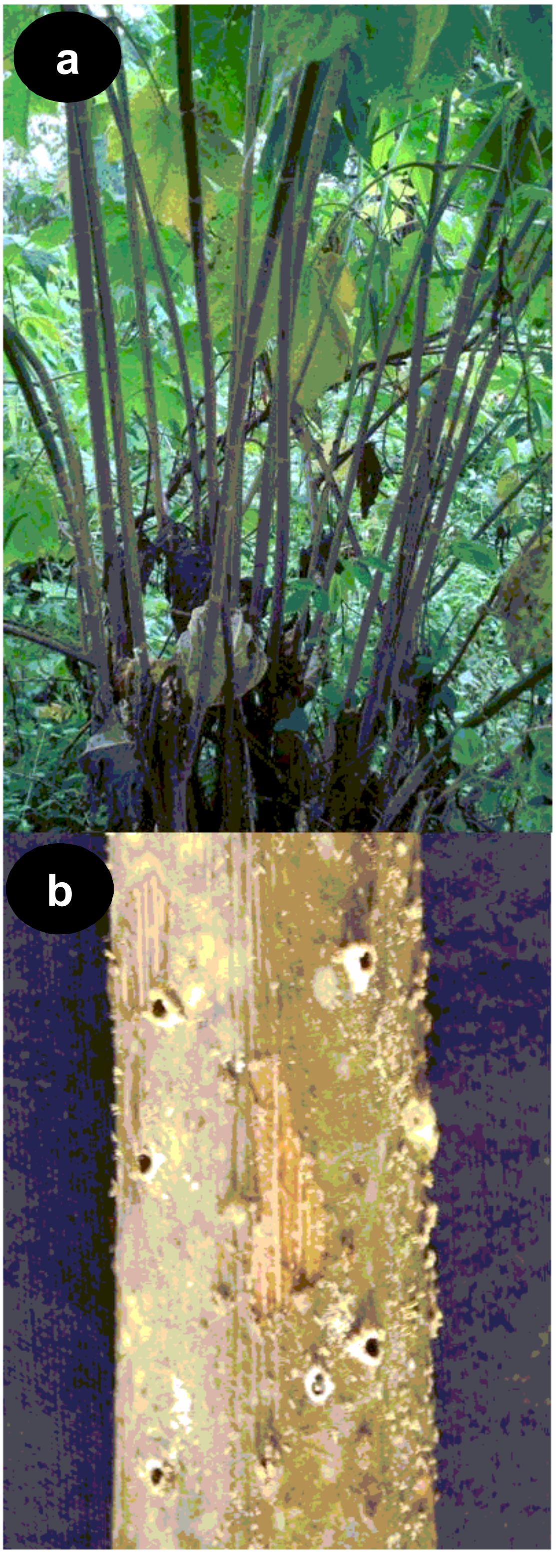


Plate 3. ***Montanoa bipinnatifida*** **plant**

a. ***Montanoa*** **plant; b.SHB infested partially dried cut stem**

Review of literature

REVIEW OF LITERATURE

Unlike the biology, ecology and chemical control strategies, availability of literature on naturally occurring semiochemicals/synthetic pheromones, interaction and behavioural studies of SHB are rather scanty. Literature pertaining to the present subject matter is reviewed elaborately. Besides the reports on SHB and relevant literatures related with the present subject in other crops is also given due importance.

Tea was first experimented by Mr. Robert Kyd during 1780 in the northern region of the country while in the southern region by Dr. Christie during 1832. However, commencement of large scale planting was done in north India during 1834 and followed by south India in 1839 (Griffiths, 1967). Since tea is grown as monoculture, it provides a congenial microclimate as well as continuous food supply for a large number of pests. Banerjee (1981) reported that number of pests infesting tea in any geographic region depends on the length and time for which tea is grown in that region. All over the world, more than 1,000 species of animals including arthropods, nematodes and rodents have been recorded in tea ecosystem (Chen and Chen, 1989).

Knowledge of tea pests starts with the contributions of Green (1890), Cotes (1895) and Watt and Mann (1903). Apart from the traditional reports, region specific literature updated the present data base. Among them some of the contributions reported from north east India (Andrews, 1920, 1921; Das, 1963, 1965; Hainsworth, 1952), southern India (Rau, 1954, 1955; Rao, 1970 a&b, 1976; Muraleedharan, 1983, 1986c, 1991a), Sri Lanka (Cranham, 1966; Danthanarayana, 1967; Hutson, 1932, 1933), Bangladesh (Ali, 1990), Indonesia and Malaysia (Dammermann, 1929; Dharmadi, 1979; Kalshoven, 1950), China (Anon, 1974), Japan (Minamikawa and Osakabe, 1979), Turkey (Alkan, 1957), Taiwan (Shiraki, 1919; Sonan, 1925), Mauritius (Ramlogun, 1971), USSR (Dzhashi, 1972, 1975), and Africa (Benjamin, 1968; Lavabre, 1970; Laycock and Templer, 1973; Rattan, 1992). Tea in Australia is comparatively free from pests, though there are reports on several potential pests (Hobman, 1980).

In southern India more than 300 species of arthropods are reported in tea. Important pests belong to the order Acarina, Thysanoptera, Coleoptera, Lepidoptera and Hemiptera (Muraleedharan, 1992). As mentioned earlier SHB beetles are considered as an important pest infesting tea which falls within an ecological group of the Scolytidae known as the ambrosia beetles. Ambrosia beetles are characterized by their utilization of sapwood of physiologically stressed and recently dead trees for colonization and by the use of symbiotic fungi as their sole food source (Omroa Bhagwandin, Jr., 1993). *E. fornicatus* forms an ectosymbiotic relationship with the fungus *Fusarium bugnicourtii* Brayford (Parthiban and Muraleedharan, 1992) which they transport into the host tree in a specialised organ called mycangia located in the buccal cavity of head (Parthiban and Muraleedharan, 1996). First mention of *Xyleborus fornicatus* was in 1868 when the female beetle was described by Eichhoff from a specimen collected in Ceylon. Unfortunately there is no record either of the locality or of the host plant from which Eichhoff's type specimen was secured. Since it was at this time that the tea industry was established in the island, it is possible that Eichhoff's specimens would have been obtained from the tea plant. Even though *Xyleborus fornicatus* described in 1868 by Eichhoff, this beetle was not reported as a pest of tea until 1892 (Rao, 1971). It was estimated that in south India the crop loss due to SHB infestation was 8.6% at which the SHB infestation level was 15 % (which has been derived as ETL based on crop loss studies) (Muraleedharan and Selvasundaram, 1996). SHB male and female beetles are having difference in their morphology. Adult female beetle is large in size with strong mouth parts which helps to bore to make galleries, body covered with tiny hairs and capable of flying whereas male beetles possess atrophied mouth parts, body covered with long hairs and incapable of flight (Parthiban, 1992). It was also found that the behaviour of both male and females are entirely different. Females are more interested in host searching, making new galleries, nourishing the young ones and giving parental care to the galleries whereas males are engaged with insemination and cleaning the galleries (Parthiban, 1992).

At present, infestation by the SHB is known from all the tea growing areas of south India, but its depredations are more pronounced in the middle elevation tea areas (< 1250m above MSL). The life history and control measures of this beetle had been described by several authors. Several insecticides were evaluated as post pruning operations and midcycle application in the past few decades and many of them were found effective against SHB (Devadas *et al.*, 1989; Muraleedharan, 1997; Selvasundaram *et al.*, 1999). An integrated management strategy involving cultural operations like rejuvenation/hard pruning, the use of biological fungal pathogen, manipulation of agronomic practices and application of chemicals has been recommended against SHB management (Selvasundaram *et al.,* 2001).

Semiochemicals, that evoke both behavioural and physiological responses in insects, play a major role in the insect pest management programme in several crop plants. Scolytid beetles attack and breed in live or recently dead wood plants, often causing death to all the parts of the host plant (Atkinson and Equihau, 1986). Certain members of the scolytids are monophagaous while others are polyphagous with a definite preference of young or old trees of host species, thin or thick barked portions of the trunk or root, branches or cones. Majority of ambrosia beetles are polyphagous and breed in wide range of host trees. *E. fornicatus* of tea is a polyphagous beetle, mainly attacks pencil thick stems of tea and has reported a wide range of host plants belonging to 36 families (Danthanarayana, 1968).

Recently, Selvasundaram *et al.* (2001) reported that the partially dried cut stems of *M. bipinnatifida* (C. Koch) (Compositae: Asteraceae), with 25-30 mm thickness and 90 cm long attracted a large number of shot hole borer beetles. *M. bipinnatifida* an exotic plant species introduced from Mexico (Matthew, 1991), is a shrub which grows erectly for about 5 m and blooms with white flowers during the months between October and December. In India, the plant is naturalized at a height of 1200-1800 m MSL. Selvasundaram *et al.* (2001) recommended the placing of 400 partially dried cut stems of *M. bipinnatifida* per hectare of tea to trap the beetles as a part of the integrated strategy for the management of SHB. Various authors have advanced evidence that a principal factor in the logs to attack by ambrosia beetles is a volatile chemical which serves as a guiding cue (Chapman, 1962, 1963; Francia and Graham 1967; Graham and Werner 1956).Meyer and Norris (1967 a & b) reported that *Scolytus multistriatus* (Marsh.) was attracted to air containing volatiles from either elm logs or bark extractives and were attracted in the laboratory to certain oxidative products of lignin indicating that *S. multistriatus* may utilize compounds in orienting to drying or decaying hardwood trees. From the reports of Selvasundaram *et al.,* (2001) it was evident that there are some volatile compounds which emits from the partially dried cut stems are responsible for the attraction of shot hole borer. If the identity of the attractants can be established, this may have potential value in field trapping of shot hole borer beetles which is conceivable and assist the present day integrated control methods.

Method involved in behaviour modifying chemical starts with extraction, identification and quantification of volatile chemicals. Historically, volatile semiochemicals were extracted by solvent and steam extraction and the same methods is used for isolation of semi- and non volatiles also (Millar and Sims, 1998). Major disadvantages for these extraction methods are firstly, the extracts are complex mixtures of volatile and nonvolatile compounds and numerous fractionation steps required to isolate pure compounds and secondly, the profile of volatiles obtained is often not representative of the blend released by the intact living organism (Lorbeer *et al.,* 1984; Teranishi *et al.,* 1993; Takeoka *et al.,* 1988; Tollsten and Bergstrom, 1988). To outwit these problems most of the recent work has focused on collection of volatiles onto an activated adsorbent emitted from the system (insects/plants) into the airspace inside a closed container (Shani,1990; Heath and Manukian, 1992; Plaza *et al.,* 2000). A variety of adsorbants have been used and among them the most versatile and commonly used are Porapak Q (Ethyl vinyl benzene - divinyl benzene - copolymer), Tenax GC (2, 6-diphenyl-*p*-phenylene oxide polymer), and activated charcoal (Millar and Sims, 1998). Identification and quantification of the volatile compounds were usually done using GC-MS and GC-FID (Cork *et al.,* 1991; David *et al.,* 1996).

Scanning electron microscopic study of the insect antenna is very important for the behavioural studies. Most of the olfactory sensilla are located in the paired antennae pointing out from the forehead on an insect. The classification of sensillum types in insects are based on the morphology and supported by studies of the ultra structure and electro physiology. Schneider (1964) classified the insect sensilla into ten different types which is widely followed universally till date.

A compound cannot be properly termed as a behaviour modifying chemical until its synthetic release rate and behavioral response is determined at levels that correspond to the natural biologic condition (Byers, 1988). Behavioural response of an insect towards a volatile chemical was usually tested in the laboratory using olfactometers, wind tunnels and electro antennogram. Liendo *et al.* (2005) proved through olfactometric and EAG experiments that *Steirastoma breve* (Coleoptera: Cerambycidae) olfactory behavior is highly influenced by odour emitted by cocoa plants. Behavioural bioassay measure is a change in behaviour in response to the test material and it is generally of three types of equipments *viz.,* static cages/arenas, olfactometers or wind tunnels (Baker and Carde, 1984). Wind tunnel is used for the free flow of air mixed with stimulus against the test insects. In 1970's English zoologist John Kennedy was the first to develop a wind tunnel to study insect's orientation, upwind movement and tracking of sex pheromone (Bakthavatsalam, 2005). Baker and Linn (1984) proved wind tunnel as the most effective apparatus for evaluating the responses of flying insects to semiochemicals of various types. These have been used successfully to determine the role of various components of pheromone blends in eliciting behavioral responses and thus elucidate the complete blend (Carde and Hagaman, 1979, Downham *et al.,* 1999). Horizontal wind tunnels have proved to be very useful for identification of proper sex pheromone. Hill and Roelofs (1981) identified three chemical components from the salt marsh caterpillar moth, *Estigmene acrea*, using a horizontal flight tunnel.

Electro antennogram, developed by Schneider (1957) is extremely useful for detecting various attractive components in fractionated extracts or individual compounds. In general, insect antennae possess olfactory sensillae, which contain receptor cells that respond to the behaviour modifying compounds. Schneider (1957) showed that by inserting microelectrodes into the base and tip of an insect's antenna it was possible to record a slow depolarization across the antenna in response to stimulation by volatile compounds. After the invention of EAG many studies have been executed on various insects. In tea, Han and Chen (2002) conducted studies on tea aphids, *Toxoptera aurantii* towards the volatiles of uninfested and infested tea shoots.

When a single component of volatile is presented, either it does not elicit behaviour response to source location/upwind flight or only elicits response at very high dosages that too only in a very small numbers (Linn *et al.,* 1986). It is mandatory that all the volatiles identified have to be tested individually and as blends to determine their role in evoking behavioural response. Bhasin *et al.* (2000) determined the sensitivity of *Culicoides impunctatus* to individual components by testing the nine host kairomones where only three compounds found to evoke significant behavioural activity. Many insects respond only to semiochemicals over a certain concentration range or require exposure to a defined blend (Suckling and Karg, 1999). In scolytid beetles, except for some individual pheromone compounds, a blend of chemicals especially monoterpenes are found to the cue for attraction (Sun *et al.,* 2003; 2004; Poland *et al.,* 2003). In many instances, monoterpenes served as synergists with host volatiles and pheromones (Byers, 1992; Byers *et al.,* 1990, 1998; Millar and Borden, 2000; Nadir *et al.,* 2003).

Dose response studies are very important for the development of a expected behavioural response towards the attractant which will happen only at an optimum dose (Baker and Haynes, 1989; Hall *et al.,* 1984; Kennedy *et al.,* 1981; Schofield *et al.,* 1995). Relationship between host monoterpenes and host selection behavior is strongly dose dependent (Coyne and Lott, 1961). A long-range orientation function against a semiochemical indicated if the compound had both a low threshold and a wide range of concentrations which elicited an electrophysiological response under EAG. Conversely, a short-range function was indicated for a semiochemical if the electrophysiological activities were observed only with a relatively high threshold concentration and over a narrow range of concentrations (Byers, 1989). Hence, dose-response range appears to be a valid indicator of a compound's role in distance orientation.

When a volatiles blend is reconstructed based on the results of an aeration analysis, the reconstructed blend has to put into or onto a release substrate/dispenser (Millar and Sims, 1998). Different kinds of dispensers have been evaluated such as sachets made from polymer film, polyethylene (PE) vial caps and tubes, rubber septa, hollow fibers, and other materials are utilized with respect to consistency and predictability in release rate (Butler and McDonough,1981; Kauth and Darskus, 1980; Urhama, 1982; Verkoc *et al*., 1988; Weatherston *et al.,* 1985; Yamamoto, 1982 a&b). Dispensers based on polymers or laminated materials have the ability to protect the components from UV radiation, which can otherwise lead to degradation and/or isomerization (Jones, 1998). Micro particle dispensers are now widely used for the controlled release in mating disruption studies (Stipanovic *et al.,*2004*)* and new strategies are still emerging.

In addition to the chemical composition, the amount of substance released/rate of release from the dispenser is of great importance in semiochemical based systems for control or monitoring of insect species. An ideal dispenser should have a constant release rate during the whole experimental period of the target insect (Byers, 1988). Ebeler *et al.* (1988) and Millar and Sims (1998) reported that the matrix effects on the release of volatiles are critically important because the volatile profile released may be substantially different from that of measured initially in the aeration method and that may be due to interactions with the release substrate.

According to Millar and Sims (1998) in practice, temperature and age are the most important factors affecting the release rate of lures. Length of the period with a constant release rate depends not only the temperature but also the dosage of the attractant in the dispenser (Hohansson *et al.,* 2001). Hillbur *et al. (*2000) found that the male pea midges have varied behavioural response towards different doses of pheromones extracted from female glands in wind tunnel. Pine shoot beetle was relatively less attracted by higher concentrations of synthetic pheromone components and they were also not as likely to fly directly to the pheromone source (Byers *et al.,* 1985). Kawasaki (1984) demonstrated a narrow optimum range of concentrations of *Plutella xyostella* pheromone.

Studying the behaviour of insect in the field especially towards attractant is very important in semiochemical based insect control method. Many studies had been carried out in the laboratory and field on the behaviour of scolytid beetles like *Dendroctonus pseudotsugae* and *Ips sexdentatus* (Atkins, 1965; Jactel, 1993). Flight activity of the elaterid beetles, *Agriotes lineatus* and *A.obscurus* were studied by Crozier *et al.* (2003). Judenko (1958 a&b) described the behaviour of shot hole borer under laboratory conditions and in the field to an extent. Calnaido (1965) reported the flight and dispersal of SHB under field conditions but there is no report on their behaviour and flight activity towards kairomones.

Like pheromone traps, kairomone baited traps also require intensive development of trap design, deployment strategy and lure formulation (Hoffmann *et al.,* 1996). Many types of traps have been developed and their influence on trapping efficiency has been investigated for monitoring/control of many insects (Jones, 1998; Lindgren *et al.,* 1983). Several designs have been tested successfully against bark and wood-boring beetles, primarily the scolytidae (De Groot and Nott, 2001). In olden days, sticky traps have been commonly used for trapping scolytid beetles (Browne 1978; Birch 1979; McLean and Borden 1979). Though these traps are efficient, the difficulties in removing captured insects as well as cleaning and reapplying sticky coverings make them prohibitively labor-intensive. Therefore, non-sticky traps of various designs have been come into existence (Bakke and Saether 1978; Chapman and Kinghorn 1958; Furniss 1981; Klimetzek and Vite, 1978; Moser and Browne, 1978) particularly omni directional traps with vertical cross vanes and a funnel leading into a collection bottle at the bottom (Hines and Heikkenen, 1977; De Groot and Zylstra, 1995) or traps that have an additional collection bottle above the vanes, to capture ascending insects (Wilkening *et al.,* 1981). Omni directional traps that mimic the cylindrical shape and silhouette of a tree trunk and they were introduced in the late 1970's. In early 1980's, Scandinavian drainpipe trap (Bakke *et al.,* 1983) and the multiple-funnel trap (Lindgren, 1983) were also experimented for their trapping efficiency. Hardware cloth insect screening, various other materials coated with a sticky material are widely used in research and trapping programs for scolytid beetles (Browne 1978; McLean and Borden 1979). Multiple funnel trap developed by Lindgren (1983) was widely accepted for the trapping scolytid beetles due to its efficiency and less impact on non target species. Borden *et al.* (1986) observed that capture of the beetles in the trap was doubled by increasing the height of conventional lindgren funnel traps. But in the case of *Rhyzopertha dominica* (Coleoptera: Bostrichidae), trap capture was not significantly different from lindgren four unit to eight-unit funnel traps (Edde *et al.,* 2005). Except a study conducted by Judenko (1958b) on the flight and dispersal of shot hole borer of tea using sticky material coated on cardboard, no other studies were carried out till date on SHB trapping.

Apart from trap design, factors that may affect efficacy of semiochemical traps include trap height, time, dosage of attractant per trap and habitat (Barak *et al.,* 1991; Boucher *et al.,* 2001; Carde and Elkinton, 1984; De Groot and De Barr, 1998; Muirhead-Thomson, 1991). Consideration of these factors would enhance consistency and efficiency of trapping of SHB. Trap catches are higher within and above the crop canopy especially for the European corn borer, *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae) (Cork *et al.,* 2003) Similarly, insect pests associated with tree crops are frequently trapped in highest numbers within the tree canopy (Bartlet *et al.,* 1994). Nevertheless, David and Horsburgh (1989) reported huge number of leaf roller, *Platynota flavedana* Clemens (Lepidoptera: Tortricidae) were trapped outside the apple tree canopy while the large number of sibling species *P.idaeusalis* Walker (Lepidoptera: Tortricidae) were caught within the tree canopy. Edde *et al.* (2005) proved that traps placed closer to the vegetation canopy in the forest habitats and placed 1 to 2 m high from the canopy in open habitats are reported to be efficient in trapping of *Rhyzopertha dominica* (Coleoptera: Bostrichidae).

Trap catches are likely to vary depending on many parameters especially blend ratio of the components of the lure and environmental conditions (Kumar and Shivakumara, 2003). Dosage of attractant per trap is also very important in semiochemical studies. Campion and Bettany (1974) described the optimum range of female sex pheromone dose for cotton leaf worm *Spodoptera littoralis*. Knight and Light (2004) demonstrated the significant difference in the codling moth, *Cydia pomonella* catch baited with different doses of ethyl or propyl ester. Wakamara *et al.* (1994) found that 24 g of the pheromone of *Euproctis pseudoconspersa,* tea tussock moth had a similar effect to 80 and 240 g which proved that an upper limit on the quantity/dosage of attractant to be optimized for the maximal attractiveness in field.

Trap density per hectare, in other words number of traps required per hectare, is an important criterion to be achieved for cost effectiveness and efficient trapping. To gain the best possible trapping by mass trapping at lowest cost, a series of trails has to be carried out to determine the optimal tarp density per hectare (Yongma *et* *al.,* 2005). Trematerra (1993) found out the optimal density of trap is twelve per hectare for the mass trapping of *Synathedon myoapaeformis* (Lepidoptera: Sesiidae) where as Faccioli *et al.* (1993) reported that more than 10 trap per hectare is unnecessary in the case of *Cossus cossus* moth. Ranga Rao *et al.* (1991) found that there is no significant improvement in trap catch achieved in the case of *Spodoptera litura* moth infecting ground nut plant, *Arachis hyopogaea* when more then four traps per hectare was installed.

Numerous field trapping studies have been performed using behaviour modifying chemicals on different scolytid beetles especially in bark beetles (Bakke *et al.,* 1983; Browne, 1978; Byers, 1989; Carde and Elkinton, 1984; Tilden *et al.,* 1979; Christopher *et al.,* 2006). Normally after developing a through knowledge and data individually on the trap design, its position, attractant lure, dosage per trap and trap density, an integrated short and long term field studies to be conducted to evolve the results for adoption in the tea plantations. Incorporating all the experimental findings is inevitable not only for the successful development of an attractant trap but also to incorporate with the IPM system based on the economic feasibility and assisting the existing biological control measures against SHB to sustain the crop productivity.

Scope of the present study

SCOPE OF THE PRESENT STUDY

The review of literature presented in the thesis revealed that considerable work has been done on the bioecology and chemical control of shot hole borer of tea and a lacuna on the biological control especially on the behaviour modifying chemicals. In view of these attempts were made to investigate the behaviour modifying chemicals involved in attraction and to develop an eco friendly method of management, which will form a component of IPM strategy.

For the last one decade management of this pest was mainly achieved by adopting certain cultural, chemical and biological control measures. Achievement of control was moderate due to the peculiar habitat of these beetles. Recent studies have shown that partially dried cut stems of a jungle plant, *Montanoa bipinnatifida* C.Koch. (Compositae) attracted shot hole borer beetles in the field. Placing of the partially dried stems in the field has helped in trapping the beetles and reducing the incidence. It was evident that the volatiles emanated from the partially dried cut stems of *M. bipinnatifida* were responsible for the attraction of the beetles. During the period of investigation the following aspects were studied.

Attractant chemicals emitted from partially dried cut stems of *M. bipinnatifida* was isolated using Dynamic Head Space technique and the attractant chemicals were identified and quantified using GC-MS and GC-FID. The compound identity was confirmed by comparing with mass spectrum library, retention time and co-injection with standards. Chemical which was not available synthetically was isolated from its natural source and tested.

All the compounds identified was procured and tested for its ability to attract the beetles in the laboratory. Wind tunnel and electro antennogram studies were carried out to find the important compounds involved in attraction. Attempts were made to find out the dose response of SHB towards all the compounds.

All the compounds were mixed in different proportion and also the proportions present naturally in the stem were tested in the laboratory. Among the identified compounds four compounds which showed good response was used for the development of attractant lure. Double, triple and multiple blends prepared using these four compounds were tested in the laboratory and field.

Studies on different types of dispensers were carried out to determine the suitable dispenser for shot hole borer in the laboratory and field using gravimetrical method. Release rate of all the volatile compounds and the most promising multiple blends, effect of fixers on the attractant and release rate of the blend was carried out in all types of tested dispenser. Effect of temperature on the rate of release of blend from the most promising dispenser polyethylene tube (PET) was done in the laboratory.

Observations were made on the behaviour of beetle outside the gallery and the behavioural response towards the attractant trap was studied. Attempts were made in the laboratory and field to find the optimal quantity of attractant needed per trap for the efficient trapping of SHB beetles and the durability of dispenser in the field.

Extensive field studies were carried out to find out the best trap for trapping shot hole borer. Fine tuning of the best trap design was also followed by the trap determination. Attempt were also made to find out the suitable trap position and height in the field during the investigation.

Studies on the number of traps required per hectare for the efficient trapping of SHB in the field was done with different trap spacing. After incorporating all the finding a pilot scale trapping experiment was conducted in the field.

Large scale field study was conducted in two different locations and attempts were made to find out suitable time for trap placement in the field by interpreting the trap catch through out the year which was correlated with the percentage infestation in the field and mean rain fall during the study period.

The observations obtained from this study will help to develop an attractant trap which can be further incorporate into the IPM control strategy of shot hole borer.

Materials & Methods

MATERIALS AND METHODS

All the experiments were carried out over a period of three years, from 2004 to 2007 at the UPASI (United Planters' Association of Southern India) Tea Research Institute (UPASI-TRI) , Valparai 642 127, Coimbatore District, Tamil Nadu. Laboratory experiments were executed at UPASI- TRI and scanning electron microscopic (SEM) studies were done at the Indian Institute of Technology, Chennai. Field experiments were executed at the UPASI Tea Experimental Farm and also in Nullacathu and Sheikalmudi estates located at Valparai during 2004-07. In order to confirm the results on the efficiency of attraction with respect to the volatile blend combinations, a study was conducted in Arnakal estate at Vandiperiyar, Idukki District, Kerala. Details of the laboratory and field experiments carried out with synthetic compounds matched with volatile compounds identified from the partially dried stems of *Montanoa bipinnatifida* are described here under. Efficiency of individual volatiles and their blends on the attraction of SHB beetles under laboratory conditions are also elaborated in this section. Relevant information on the experimental setup, experimental design, materials used in these investigations and methodology adopted are described in detail.

2.1. Scanning electron microscopic studies on the beetle antenna

General external morphology, structure and types of sensillae on the antenna of shot hole borer (SHB) were studied using SEM. Head of 15 SHB beetles were clipped off separately, placed in a solution of 70% ethanol and 2% formaldehyde for 24 hours and then serially dehydrated with 80, 90, and 100% ethanol for eight hours, individually. Dissected antennae were dried using a critical point drier and finally gold coated (70 nm) prior to SEM studies (QUNATA 200 ICON analytical make, Italy). The gun and column sections were under high vaccum than the specimen chamber where the pressure ranged from 0.08 to 30.0 torr and the resolution was as high as 30 kV. Microscopic photographs were taken and analysed for the antennal morphology and type of sensillae on it.

2.2. Extraction, isolation, identification and quantification of emitted volatiles from cut stems of *Montanoa bipinnatifida*

Cut stems of *M. bipinnatifida* collected from one year old plants, of five feet height and diameter of 25 to 30 mm were placed in the tea field as described by Selvasundaram *et al.* (2001) until the notice of first attack of the beetle on stems. These attacked stems were removed and brought to the laboratory for the extraction of volatiles. Stems were cut into 30 cm long pieces and kept inside the cylindrical glass volatile extraction unit fabricated for this work (60 cm height with 25 cm diameter). The lid of the extraction unit was provided with an inlet and outlet apertures and a clip to keep the materials in air tight condition. Inlet aperture was connected to an aerator and the outlet was connected to an adapter loaded with 80 mg of activated Porapak Q medium (80-100 mesh, activated at 200°C for 24 h in a laboratory oven). Air was passed through activated charcoal filter to the chamber using a small aerator where the flow rate was set at 400 ml/min for 48 h. Volatiles released by the partially dried cut stems of *M. bipinnatifida* were adsorbed by Porapak Q medium (Plate 4a) and washed with purified 3.0 ml of elutent, dichloromethane HPLC Grade (DCM). Samples were then concentrated to 25 l under a gentle stream of inert gas, nitrogen.

The concentrated sample was subjected to Gas Chromatographic–Mass Spectroscope (GC-MS) analysis immediately for the identification of volatiles. Analysis was carried out in Perkin-Elmer GC equipped with MS detector (Autosystem XL.GC; Turbo mass Gold MS) (Plate 4b). Column used for the elution was HP Innowax capillary polyethylene glycol column with 60 m length, 0.25 mm internal diameter with 0.25 m thickness. Injector temperature was set at 200°C and interface temperature at 220°C. Oven temperature was maintained at 40°C for 2 minutes and programmed to rise 2°C per minute up to 200°C. The system was supported by Turbo mass software with NIST library. Eluted compounds were compared using the NIST library the peaks of respective compounds were confirmed with the retention times of authentic standards and with mass spectra. Extracted volatiles were

Plate 4a. Dynamic head space (DHS) volatile extraction unit.

Plate 4b. Gas Chromatograph with Mass spectroscopy

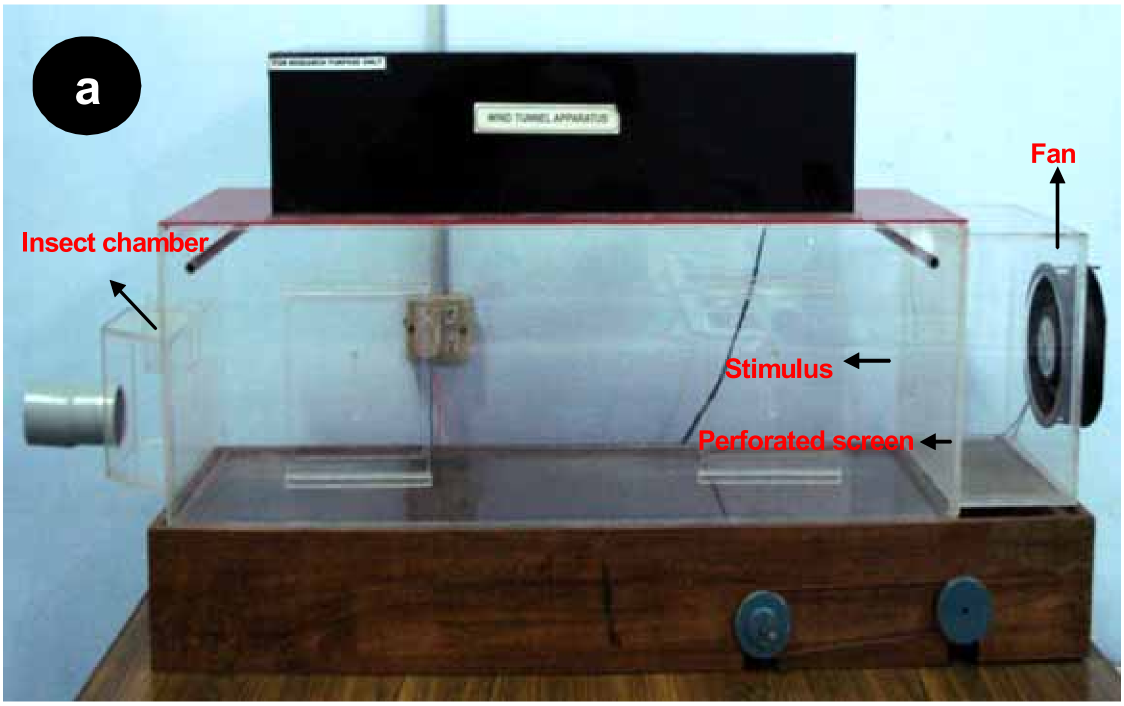
combined with known quantity of reference standards and injected into GC-MS (spiking test/co-injection) to confirm the enhancement of relevant peak areas.

Quantification of volatiles was carried out in GC-FID. Partially dried cut stems of *M. bipinnatifida* with 30 cm length and weight of 250 g were kept in the glass extraction unit and the volatiles emitting from the cut stems were collected for 48 h on Porapak Q medium, then desorbed using 3.0 ml of dichloromethane. Into the elutent, 20 l of a 10^{-4} g/ml of 4-methylpentan 1-ol was added as internal standard. The extract was concentrated to 25 l under a gentle stream of nitrogen, 1 l of concentrated sample was immediately injected into Perkin-Elmer GC equipped with FID detector (Clarius 500). GC conditions were, carrier gas nitrogen at a column flow rate of 2 ml/min and column was polyethylene glycol (DB-FFAP, Hewlett Packard), 30 m length, 0.25 mm internal diameter and 0.25 m thickness, with a temperature programme of 2 minutes for 40°C to 200°C at 5°C rise/min, split less injection with a volume of 1 l. Quantification was performed after the calculation of standard curves and response factors for each compound.

2.2.1. Screening of attractant chemicals in the laboratory - Bioassay in wind tunnel

Screening of the identified attractant volatiles for their efficiency in attracting SHB beetle was carried out under laboratory conditions using wind tunnel and electro antennogram (EAG) (Plate.5). From GC-MS study it was evident that, a complex mixture of seven volatile compounds was emitted from the cut stems of *M. bipinnatifida.* All the seven compounds were tested in the laboratory. Out of seven volatile compounds, six synthetic volatile compounds were procured from M/s. Sigma-Aldrich, USA. (-)-Germacrene-D, which was not commercially available, was isolated as per the protocol described by Rostelien and his co-workers (2000). (-)-Germacrene D was isolated from Ylang - Ylang (*Cananga odorata* Hook) an essential oil and the purity of the compound was found to be 92% through analysis using gas chromatograph equipped with DB wax column (Rostelien *et al.*, 2000).

Role of each identified compound in attraction was determined in the laboratory prior to the field experiments using wind tunnel and EAG. All the individual synthetic/isolated volatile compounds were tested individually and as blends which were made by mixing authentic/isolated standards of all the seven volatile



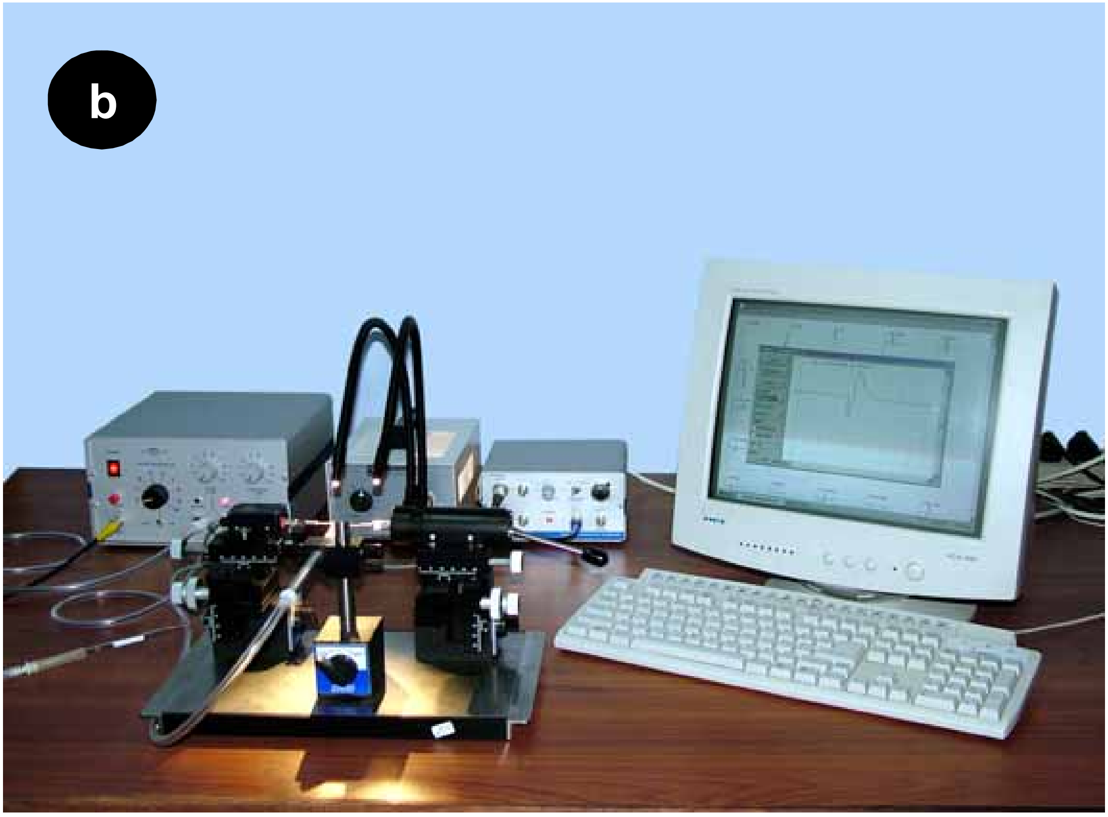


Plate 5.Instruments used for laboratory bioassay
a. Wind tunnel apparatus b. Electro antennogram

compounds in the maximum possible combinations. Blends were prepared by (i) mixing all the identified volatile compounds together in accordance with their natural ratio in the partially dried stem of *M. bipinnatifida,* (ii) avoiding any one of the compounds from the first blended mixture on the basis of highest concentration and (iii) increasing or decreasing the quantum of any one of the compounds from the mixture and keeping all other compounds at a constant level. Compounds which haven't showed any significant response or have not contributed any significant increase in the behavioral response were given less importance.

2.2.2. Wind tunnel studies on individual volatile compounds

Wind tunnel was fabricated with transparent, rectangular plexiglas (acrylic sheet) measuring 100 x 30 x 30 cm length, width, and height, respectively. Plexiglas duct at one end was provided with a small push type circular fan and at the other end with an insect releasing chamber (7 by 7 cm diameter) attached to the wall of the large rectangular tunnel. When the fan was switched on, it conducted air to the screen which was perforated for the smooth flow of air. Compound plume was scavenged from tunnel through exhaust tube. Inside the tunnel the temperature was 23 ± 2°C, RH 70 ± 5%, and illumination 720 lux; wind speed was regulated at 0.5 ml/s. Dispenser filled with attractant (attractant lure) was placed near the perforated screen (opposite to the insect release chamber) on a platform inside the tunnel at a height of 12 cm. Dispenser without volatile chemical was served as control.

SHB beetles collected from the field were used for the laboratory studies. Prior to the experiment, beetles were tested for sufficient flight exercise to ensure their subsequent response to the semiochemicals (Graham, 1959; Bennett and Borden, 1971; Salom and Mclean, 1990). Apart from that, beetles were screened and scrutinized for the presence of all tarsi, both antennae and for an overall healthy appearance. Beetles were acclimatized to the tunnel conditions for 30 minutes in order to ensure maximum response to the experiment. A total of five hundred beetles in batches of 100 were introduced into the tunnel. Behavioural response of beetles to the volatile compounds was observed for a period of one hour and data was recorded. Percentage of attraction was calculated on the basis of number of beetles attracted within the total number of insects tested. Of the total length of 100 cm wind tunnel, beetles which moved more than 75 cm towards the attractant source was scored as attracted beetles and which was less than 75 cm scored as not attracted. A fresh set of 100 beetles was used for each replicate and the used insects were not used again. Test solution (volatile compounds) used for all the studies were 50 l and each treatment was replicated five times. For all the wind tunnel studies, the experimental setup and methodology adopted were similar unless otherwise mentioned.

Behavioural responses of the beetles inside the wind tunnel towards the seven identified volatile compounds were tested individually. Concentration of the compound tested was 1 g/ml of DCM.

2.2.3. Dose response study using wind tunnel

Before testing the volatiles individually or in combination at different ratios in the field, each volatile compound was tested in the laboratory using wind tunnel at different dosages. Dose response experiments were carried out using all the seven compounds at a concentration of 10, 1, 10^{-1}, $10^{-2,}$ and 10^{-3} g/ml. Serial dilution of each compound in DCM was used to make 10 - 10^{-3} g/ml (w/v) solutions. Odour presentation was performed from the lowest to highest concentration of test volatile. Materials and the methodology adopted were same as described in section 2.2.2. After completing each test, wind tunnel was cleaned with acetone to avoid the influence of the previously tested chemical.

2.2.4. Bioassay studies with different volatile blends and different ratios of blended components

Volatile compounds namely, (+)-a-pinene,(-)-b-phellandrene,(+)–trans-caryophyllene and (-)-germacrene-D were combined together as double, triple and multiple component blends and tested in wind tunnel. Details of the blends are given in Table 3.

Among the blends, a multiple blend that showed enormous attraction of SHB beetle was tested at different ratios. Different ratios of this particular multiple blend was prepared by varying the quantity of the blended components. Prepared blends were individually diluted with DCM (1 g/ml) and filled in PET dispenser was used as attractant source.

2.2.5. Wind tunnel studies on rate of release of volatile blend from different dispensers

Three types of dispensers *viz.*, polyethylene tube (PET), rubber septa (RS) made of natural rubber and ring septa (RiS) were tried for consistency and predictability in release rate (Plate 6). Release rate from each dispenser was determined by gravimetric method. Dispensers with attractant kept in the wind tunnel were weighed at five days interval up to thirty days. Before filling the attractant, each dispenser was kept in DCM overnight and oven dried. Weighing was done using an analytical balance with an accuracy of measurement of 10 g and corrected for the weight changes using control dispenser. Dispensers with attractant (volatile compounds) were weighed at regular interval and change in weight of control dispenser was subtracted from the weight of its paired attractant containing dispenser. Percentage of attractant remaining in each dispenser was computed and plotted against the days. Release rate was regressed with the number of days and derived a regression model to predict the release rate of volatiles against increasing number of days. Role of an antioxidant fixer, butylated hydroxyl toluene (BHT - added 1% equivalent to the attractant) for the controlled release rate of attractant from the dispenser was also performed. Experiment was conducted for a period of thirty days and the study was repeated three times.

2.2.6. Rate of release of attractant at different temperature

Rate of release of the multiple blend (+)-a-pinene,(+) – trans-caryophyllene,(-)-b -phellandrene and (-)-germacrene-D at a ratio of 10:1:0.1:3 (hereafter will be mentioned as standard blend) from polyethylene tube dispenser with antioxidant at different temperature regimes *viz.,* 15, 20, 25 and 30°C was also attempted using a BOD incubator (Model CALTAN). Except for the varying temperatures, methodology adopted for determining release rate was the same as described in the section 2.2.5. To correlate the release rate of attractant from PET under field conditions, PET with attractant and without attractant (control) were kept in the field and weighed regularly at an interval of five days for a period of thirty days.

2.2.7. Wind tunnel studies with different dispensers and optimum quantity of attractant per dispenser

A wind tunnel study was carried out to evaluate the dispensers for trapping of SHB beetle which was further confirmed through field studies. Dispensers *viz.,* RiS,

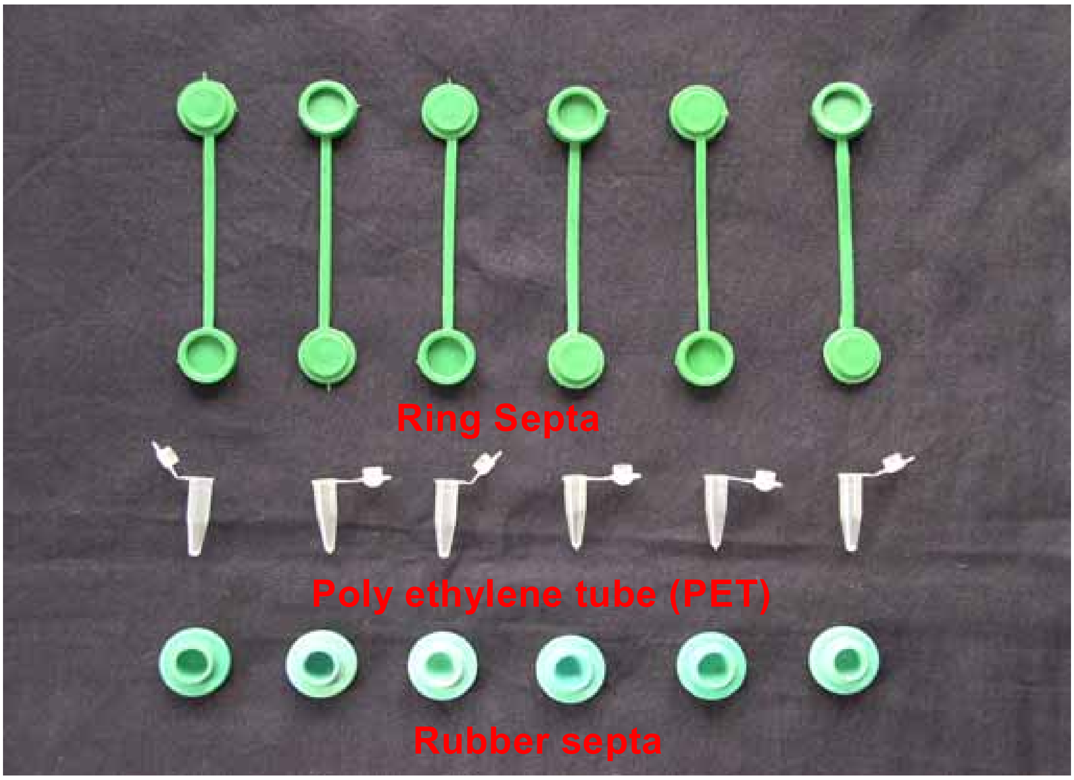


Plate 6. Different types of dispensers

RS and PET, filled with the standard blend was used as attractant source. Number of insects and experimental conditions were similar to that of the previous experiment on individual compounds (Vide: sect. 2.2.2).

The optimum quantity of attractant required in a dispenser was standardized through wind tunnel studies using different dosage of standard blend *viz.,* 25, 50, 75, 100, 125, 150, 175, 200 and 225 l per PET dispenser. All other experimental conditions were identical to the experiment on different dispensers.

2.3. Electro antennogram studies on *M. bipinnatifida* volatiles

In the EAG, voltage difference can be measured between the tip and the base of the antenna when it was exposed to an odour of biological significance to that insect. EAG measures micro voltage fluctuations between the tip and base of the antenna at the time of stimulus by amplifying the signal. Beetle was anaesthetized by exposing to CO_2 for five seconds and the head was clipped off. Antenna was excised from the head under the microscope with fine scissors and tweezers. Basal segment of the antenna was cut off, inserted into the ground electrode and the recording electrode was sleeved over the tip of the antenna. Odour presentation was organized as the lowest concentration first (Visser, 1979; Park *et al.*, 2000; Han *et al.,* 2001; Han and Chen, 2002). Ten microlitres of each solution was applied to a piece of filter paper (5 x 60 mm Whatman®, No. 4 ash less) and inserted into a glass Pasteur pipette (15 cm long). Tip of the glass Pasteur pipette was inserted about 3 mm into a small hole in the wall of a stainless steel tube (15 mm diameter, 15 cm long) directed over the antennal preparation. An air stimulus controller (model CS-05b, Syntech®, The Netherlands) was used for air and odour delivery with a constant air flow of 120 ml/min. Charcoal-filtered and humidified air was passed over the antenna through the open end of the glass tube positioned 15 mm from the antenna. During odour stimulation air at 2 ml/s was passed through the pasteur pipette into the main air flow for one second. A minimum period of two minute interval was maintained between two successive stimulations. EAG responses to a standard stimulus, Hexen-1-ol (that evoked detectable EAG but is chemically unrelated to those volatile compounds of *Montanoa* plant), was recorded first followed by five observations. Same pattern was continued for all the EAG studies and the quantity of test compounds applied on filter paper (stimulus) was 10 l through out the study. The mean of two EAG responses recorded for the standard stimulus was used for normalization of the responses to the test volatiles recorded.

All the synthetic/isolated chemicals of the volatile compounds identified from the partially dried stems of *Montanoa* were tested individually in the EAG to confirm the role of each chemical in the attraction of beetles.

2.3.1. EAG studies on the dose response of SHB against individual volatile compounds and different volatile blends

Dose response studies of all the seven volatile compounds were performed in EAG. Preparation of different doses of the attractant compound was described in section 2.2.3**.** Concentration of each compound was 1 g/ml of DCM.

Compounds which elicited good electro antennographic response was further mixed to make different blends and tested in EAG. Details of the blends tested are given in the table 6. Experimental conditions, antenna preparation method, stimulus application and quantity of stimulus used had already been elucidated in the section 2.3.

2.3.2. EAG studies on different ratio of blended compounds

Besides the studies on determination of suitable blend, an experiment was undertaken to find out the suitable ratio of each component in the selected blend. All the components involved in the blend were mixed at different ratio and tested. Details of the tested blends are given in the figure.

2.3.3. Optimisation of the quantity of blend in a dispenser

A study was conducted to find out the optimal quantity of blend required per dispenser. PET dispensers were filled with different quantity of standard blend *viz.*, 25, 50, 75, 100, 125, 150, 175, 200 and 225 l. Each dispenser containing attractant washed with 5 ml of dichloromethane and from the solution 10 l of each solution was applied to a piece of filter paper was used as stimulus containing dichloromethane solution.

2.4. Field evaluation of attractant chemicals

Experimental plots were laid out in one of the estates in the Anamallais, where the field was prone for SHB infestation. Fields in forth year pruning cycle were chosen for conducting field experiments. Experiments were carried out on the parameters such as behaviour of beetles in the field, determination of suitable trap design, trap height, position, ability of volatile compounds in attracting SHB individually as well as blend, suitable dispenser, quantity of attractant per dispenser, durability of dispenser and number of traps required per hectare. Pilot scale trapping experiment, based on the results obtained from the field studies for a period of three months was also conducted. This study was followed by a large scale study for a period of two years in one of the estates in the Anamallais. Experiment at a different location, Vandiperiyar - Idukki District, to confirm the efficiency of the developed attractant trap was studied for a period of one year. All the field experiments were done in randomized block design (RBD), standard blend was used as attractant source and replicated five times unless otherwise mentioned.

2.4.1. Behavioural studies of SHB beetles under field conditions

To generate information on beetle behaviour outside the galleries and towards the attractant trap was done which would not only contribute some information for developing an efficient trap but also for advocate the position of trap. Observations on the behaviour of beetles were restricted to five highly infested tea bushes. All bushes were in fourth year of pruning cycle and each bush measured 3-4 feet height with a minimum canopy diameter of 2-3 feet. Observations were made regularly between 10.00 am to 1.00 pm when the flight activity of beetle was maximum especially in the months of April, June, October and December (Muraleedharan, 1991a). Every 30 minutes interval, both the galleries on the stems and the soil surface were examined. Flight activity and behaviour of the beetles towards the attractant trap was also observed.

2.4.2. Field studies on different trap designs

Four different types of traps were tested for their comparative efficacy in trapping the beetles using the standard blend. Traps tested in the field were 1) multiple funnel trap (MFT), 2) funnel vane trap (FVT), 3) cross vane trap (CVT) and 4) sticky board traps (SBT) (Plate 7). Five unit multiple funnel traps have funnel size of 25 cm diameter with a transparent plastic collection jar at the bottom of the last funnel. An odourless sticky material, poly isobutane was coated inside the collection jar and the top funnel was protected from rain and sunlight by placing a plastic plate of 30 cm diameter. Differential gap between plastic plate and the first funnel was 15 cm. The attractant compound was kept in polyethylene tube dispenser attached to the first funnel using an adhesive tape (Plate 8a). Cross vane trap had two vanes at right angles to each other and trimmed to fit inside a pan leaving 1/3 part of the vane exposed above the pan. Pan and cross vanes were made up of transparent film sheet (30 x 15 cm) and the pan was coated with the odour less sticky material poly isobutane inside. Perforations were made in the centre of the vane for placement of the attractant material. When compared to a cross vane trap the only difference in the funnel vane trap was that instead of a pan, the vanes were trimmed to fit into a funnel (25 cm diameter) attached to a collection cup at the bottom. Sticky board (size 12 x 12 inch made of polymer frill sheet) was coated with the sticky material poly isobutane. A hole was made at the centre of the board for keeping the attractant. Each type of trap had five replicates and all the traps were hung from a vertical wooden stand equipped with a horizontal top arm. Location of each type of trap was changed at random to avoid position effect. Attractant was changed once a month and beetles captured in each type of trap were counted at seven days interval.

2.4.3. Trial on varying number of funnels in MFT

To determine the optimum number of funnels to be used in a MFT, an experiment was conducted in the field. Traps were designed with one to seven funnels and tested for their efficiency in trapping SHB beetles in the field. The only difference among the traps was the number of funnels. Number of replication, randomization of trap, replacement of standard blend and observation taken on beetle catch was same as section 2.4.2.

2.4.4. Field studies on suitable height and position of trap in the field

MFT traps placed at three different positions (i) in the walking lanes, (ii) between the rows and (iii) within the row (Plate 9). Similarly, different heights *viz.,* (i) top level (collection bottle kept at 15 cm above the plucking table), (ii) middle level

Plate 7. Different types of traps tested in the field

Plate 8. Traps at different heights in the field

a. Lure attached to the 1st funnel, b-Top level, c-Middle level, d- Low level

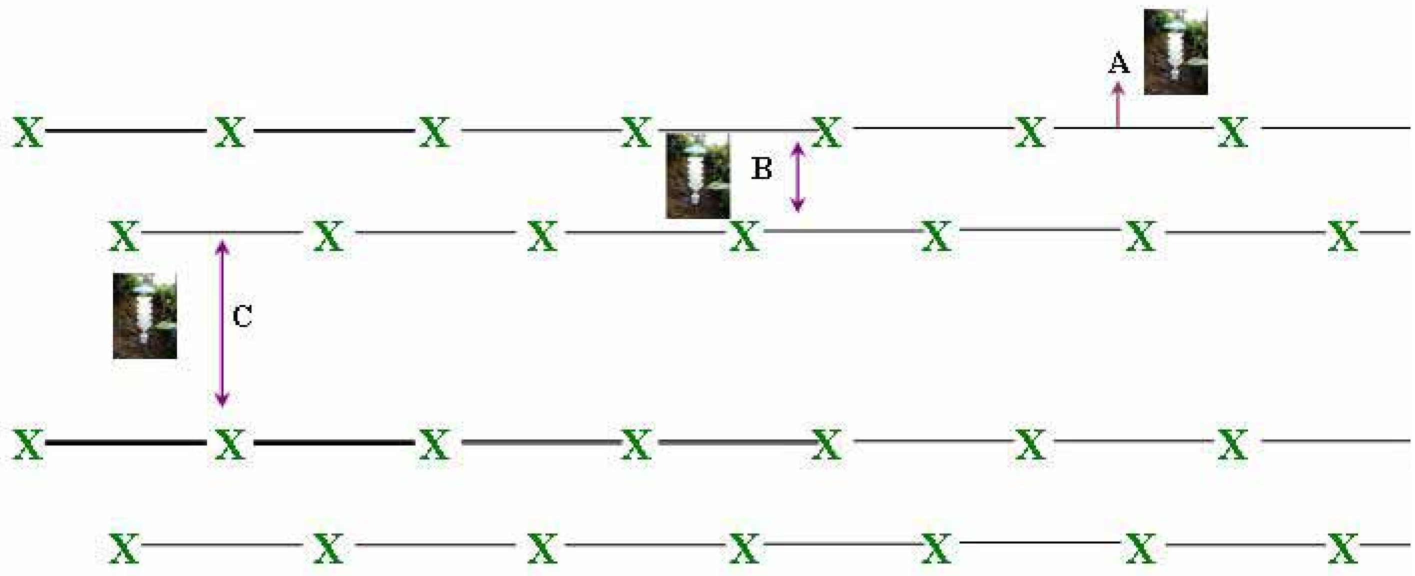


Plate 9. Position of trap in tea field

X= position of tea plant; A-With in the row; B- Between the rows; C-At the walking l (collection bottle kept at 15 cm below the plucking table) and (iii) low level (collection bottle kept at 15 cm height from the ground level) were evaluated (Plate 8b,c&d). Determination of correct position of the MFT trap and its height were ascertained on the basis of number of beetles trapped. In this experiment, multiple funnel trap with five funnels were used. Each treatment was replicated five times and the study was repeated three times.

2.4.5. Field studies on suitable dispenser

Studies were also carried out to select suitable dispenser under the field conditions. Ring septa, rubber septa and polyethylene tube were evaluated and the best dispenser was selected on the basis of number of beetles attracted. Five unit multiple funnel trap was used for trapping the beetles and each treatment replicated five times. Renewal of the attractant was done once in a month during the period of study and which was repeated three times and the number of beetle caught per trap was recorded and analysed. Treatment details were presented in the results.

2.4.6. Efficacy of individual attractant compounds and blends

Synthetic compounds identical to the volatile compounds isolated from the partially dried stems of *Montanoa* were tested individually in the field using five unit MFT. Chemicals *viz.,* (+)-a-pinene, (-)-b-pinene, (-)-b-phellandrene, (R)–(+)-limonene, (-)-Iso-caryophyllene, (+)–trans-caryophyllene and (-)-germacrene-D were evaluated under field conditions. Study was repeated three times and each treatment replicated five times.

Based on the laboratory study conducted using different doses of volatile compounds, the chemicals most preferred by the beetles were used for this study. Among the seven chemicals identified from *Montanoa,* four chemicals *viz.,* (+) - a-pinene, b -phellandrene, (+) – trans-caryophyllene and (-) -germacrene-D which elicited enormous behavioural response in laboratory studies were blended together as double, triple and multiple component blends and tested in the field for their ability to attract beetles. All the treatments were replicated three times and the study was repeated three times.

2.4.7. Different ratios of highly responsive blend

To assess the effects of varying ratios of the components in the highly responsive blend (standard blend), an experiment was conducted using five unit MFT with different blends mixed with BHT filled in PET dispenser. Details of blends were given in the results.

2.4.8. Field evaluation of optimum quantity and time for replace the attractant blend

Optimal quantity of the blend to be used in a single dispenser was tested under natural conditions. Standard blend with BHT at 125, 150, 175, 200 and 225 l per dispenser was tested in the field and trap catch was assessed. All the studies were conducted using five unit MFT with PET dispenser.

Durability of dispenser with attractant/optimum time for the replacement of attractant lure was calculated on the basis of trap catch of SHB beetles. After placing the attractant in a trap, beetle catch was observed every day. Polyethylene tube containing standard blend was used as source of attraction and it was kept in the field for about 40 days without replacing the attractant. From this particular study, durability (optimum time for replace) the dispenser was determined.

2.4.9. Number of traps required per hectare area

A study was conducted to optimize the number of traps required per hectare. Experimental site consisted of four hectare of tea field which was infected by SHB. Percentage infestation of SHB beetle was assessed by collecting 100 tea stem cuttings/ ha (1-1.5 cm diameter and 20 cm long) at random and counting the number of infested cuttings from the sample as described by Muraleedharan, (1991b) to minimize the influence of the SHB population on trap catch. Before assessing the percentage infestation, 4 hectare block was divided into four one hectare experimental blocks and it was found that difference in per centage infestation among the blocks are ± 5%. Traps were installed at different distances to workout the cost effectiveness. Distance between the traps tested were i) 50 x 50 m (9 traps/ha) ii) 25 X 25 m (16 traps/ha), iii) 20 x 20 m (25 traps/ha) and iv) 15 x 15 m (36 traps/ha) . All the traps were hung from a vertical wooden post with a horizontal arm. Number of trap catches was recorded every week between 8.00 to 10.00 am. All the treatment blocks were randomized as mentioned earlier to avoid position effects and influence of population near the trap vicinity at the time of replacing the attractant. The experiment was conducted for a period of four months and the attractant was replaced three times at an interval of one month.

2.5. Pilot scale bioassay studies on SHB attractant trap

A pilot scale field bioassay incorporating all the findings obtained from the above experiments was carried out in the field. The most suitable blend in PET dispenser with five unit MFT was used for trapping SHB. Twenty five traps (20 x 20 spacing) were installed at the middle level (15 cm below the canopy) in the walking lanes hanging on vertical wooden posts. Attractant was replaced once in a month and the area taken for study was one hectare with high SHB infestation. Number of beetles trapped were removed and counted at weekly intervals and the study was conducted for a period of three months. Percentage infestation was calculated as described by Muraleedharan *et al.* (1991b) during every time of replacement of attractant and number of adult female flyers per stem was noted.

2.6. Large scale field evaluation of attractant trap

Another study was undertaken in the Valparai, Coimbatore District by integrating the results of laboratory and field experiments for two consecutive years (2005 and 2006). In order to confirm the results further, one more large scale study was conducted in Vandiperiyar, Idukki District, for a period of one year (2006-07). These study areas consisted of 2 hectares with 40% SHB beetle infestation. Percentage infestation of SHB in the trial plot was assessed monthly once. Simultaneously, data on rain fall also recorded. Twenty five traps were used per hectare and the polyethylene vial with 150 l of standard blend [(+)-a-pinene, (+)-trans-caryophyllene, (-)-b-phellandrene and (-)-germacrene-D at a ratio of 10:1:.01:3 with BHT] was used as attractant source. Traps were kept in the middle level of the bush at a spacing of 20 X 20 m (25 traps/ha) in the walking lanes and the trapped beetles were counted and removed from the trap periodically. Attractant was replaced once in a month and the mean number of beetles trapped per month was tabulated

2.7. Statistical analysis

Data generated from the different studies were subjected to statistical analysis (standard error, standard deviation, Duncan's multiple range test and linear regression) where ever possible and results presented accordingly using special purpose statistical software (SPSS Ver.7.5)..

Results

RESULTS

Results of all the laboratory experiments using scanning electron microscope, wind tunnel, electro antennogram and field studies on behaviour of the beetle, experiments on suitable trap design, trap height, position, attraction studies with individual compounds, volatile blends, optimum ratio of components in the blend, suitability of dispensers, rate of release of blend from the dispenser, optimum quantity of blend in a dispenser, its durability and number of traps required per hectare are described below in detail, under the respective sections. Results of the pilot and large scale field experiments conducted at two different locations are also presented.

3.1. Scanning electron microscopic (SEM) studies of beetle antenna

Shot hole borer antenna is typically club shaped with four segments and the basal segment is firmly attached between the compound eyes at the base of mandibles (Plate 10). In both the sexes the terminal antennal segment (club) is fully distributed with tufts of long and short sensillae trichoidea. In female beetles, the sensillae are more numerous when compared to that of males and are distributed in three rows on the highly flattened club. In male, the number of long trichoidea are more whereas in female antenna short, slightly curved trichoidea are abundant. In the remaining antennal segments distribution of sensillae are meager.

3.2. Extraction, isolation, identification and quantification of volatiles emitted from cut stems of *Montanoa bipinnatifida*

Analysis of the volatiles collected from the partially dried cut stems of *Montanoa bipinnatifida* indicated the presence of seven important volatile compounds (Fig. 2). Among these compounds, (+) a- pinene was distributed relatively in higher quantity

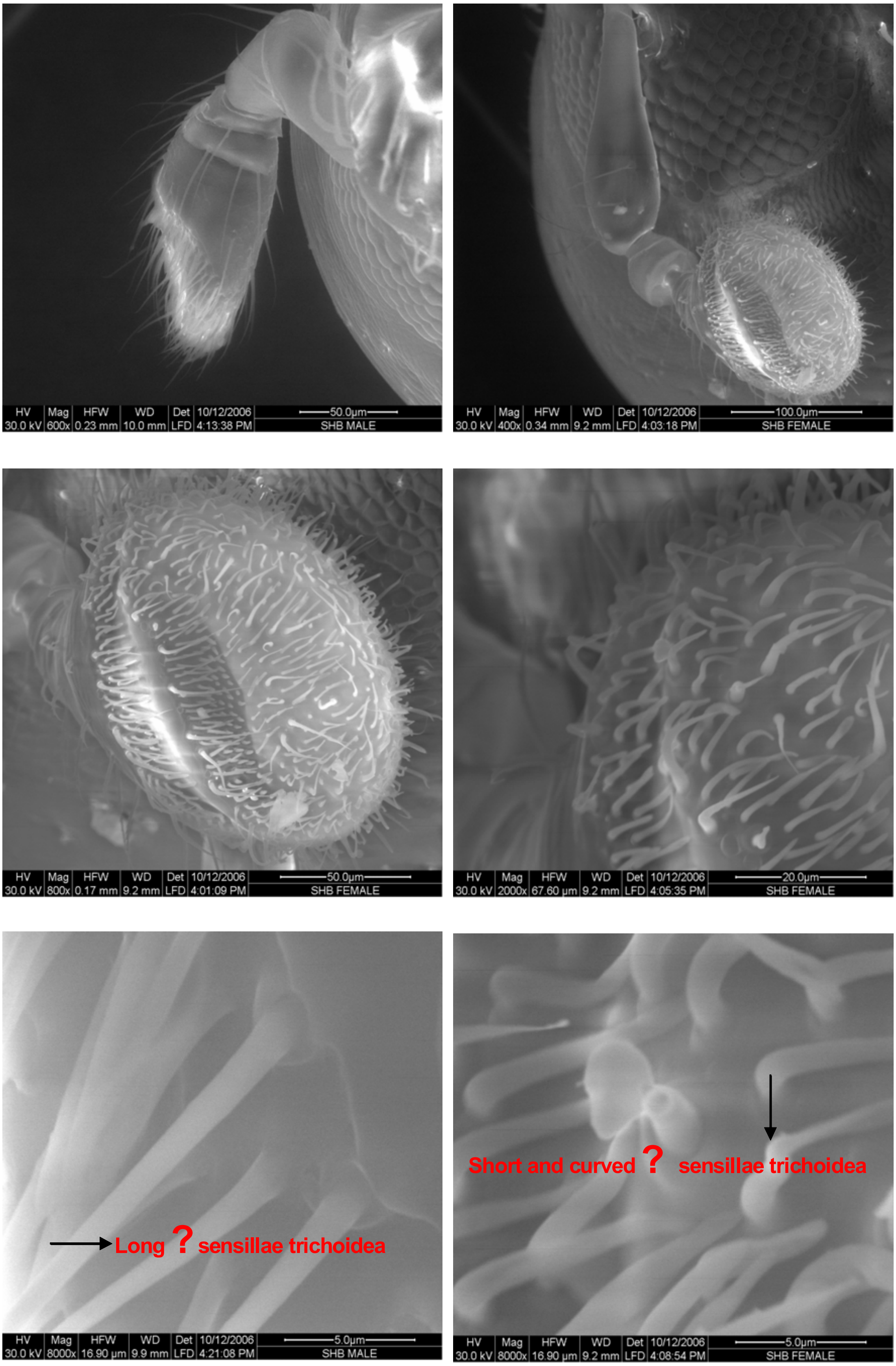


Plate 10. SEM of shot hole borer beetle antennae

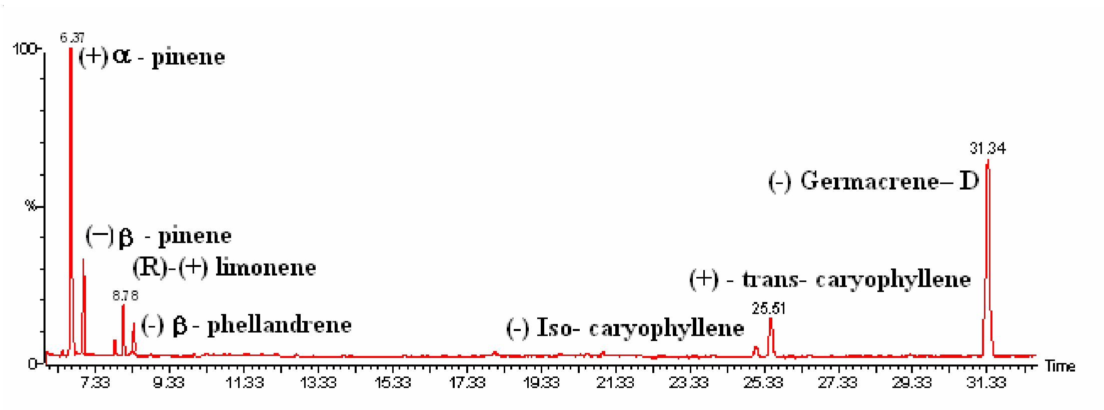


Fig.2. GC-MS chromatogram of partially dried stems of *Montanoa bipinnatifida*

while the quantity of other six volatiles ranged from 0.8 x 10^{-6} to 3.2 x 10^{-6} g. The volatile compounds were identified on the basis of their retention time, samples co-injected with the authentic standard (spiking test) and by comparing the mass spectra with the available synthetic compounds. The concentrations of these seven constituents in the stem are presented in the Table 1. All the chemical compounds, identified in GC-MS, were procured from synthetic suppliers, except (-)-germacrene-D. Method of identification and determination purity of the procured samples are presented in Table 2.

3.3. Screening of attractant chemicals in the laboratory

All the identified chemicals were tested in the laboratory individually and as blends using wind tunnel and electro antennogram. The results of different experiments are given below.

3.3.1. Experiment conducted with wind tunnel on individual compounds

Four compounds, (+) - a - pinene, (+) - trans-caryophyllene, (-) - b -phellandrene and (-) germacrene-D elicited behavioural response in SHB to a great extend. In the wind tunnel experiment, the percentage of SHB beetles attracted was as high as 47.6± 8.8% when a - pinene was used as test material followed by trans-caryophyllene where it was 44.8 ± 12.2% (Fig. 3). (-)-Germacrene-D and (-) - b -phellandrene elicited significantly lesser response than the other two compounds. Germacrene-D registered 29.6 ± 9.3% attraction while (-) - b -phellandrene resulted in 26.6 ± 6.8% attraction. The percentage of attraction to other three compounds *viz.,* a -pinene, (R)– (+) limonene and iso-caryophyllene was very low. However, the response was significantly more when compared to control stimulus (Fig. 3).

3.3.2. Dose response study using wind tunnel

Dose response studies in the wind tunnel experiment showed that the mean per cent attraction of SHB beetles to (+) - a - pinene and (-) germacrene-D were directly proportional to the quantity (Fig.4). Attraction of beetles to a - pinene gradually increased from the dosage of 0.001 g/ml and reached at a maximum of 53.2% at

Table 1. Volatile compounds and their relative quantity in the partially dried stems of *Montanoa bipinnatifida*

Compound	Amount [a]
(+)-a- pinene	9.8×10^{-6}
(-)-b- pinene	2.3×10^{-6}
(R) -(+) limonene	1.9×10^{-6}
4-Methyl 1-pentanone (ISD)	2.9×10^{-6}
(-)-b- phellandrene	1.2×10^{-6}
(-) Iso- caryophyllene	0.8×10^{-6}
(+) - trans- caryophyllene	2.1×10^{-6}
(-)-germacrene-D#	3.2×10^{-6}

[a] Units: g/25 l concentration of resulting extract in GC-FID; ISD-internal standard; Compounds listed in the order of elution

Table 2. Method of identification and purity of authentic standards of volatile compounds emitted from partially dried stems of *M. bipinnatifida*

Compound [a]	Method of identification	Authentic standard purity
(+)-a- pinene	Rt, Ms, Co-I	98 %
(-)-b- pinene	Rt, Ms, Co-I	99%
(R)-(+) limonene	Rt, Ms, Co-I	97 %
(-)-b- phellandrene	Rt, Ms, Co-I	95 %
(-)-Iso- caryophyllene	Rt, Ms, Co-I	98 %
(+) - trans-caryophyllene	Rt, Ms, Co-I	92 %
(-)-germacrene-D*	Rt, Ms	92%

[a] Compounds are in the order of elution; Rt- confirmed by retention time; Ms-confirmed by mass spectral library; Co-I- confirmed by co-injection (spiking test) of authentic compound. * isolated from natural source

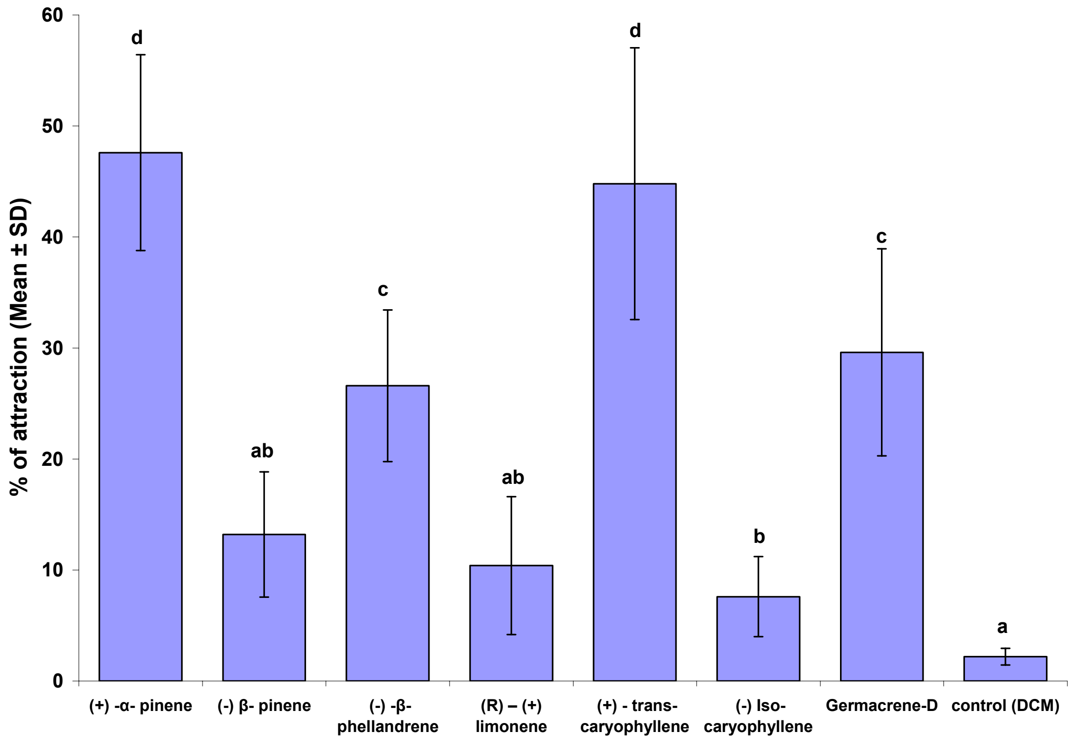


Fig.3. Attraction of SHB to volatile compounds of *M. bipinnatifda* in the wind tunnel

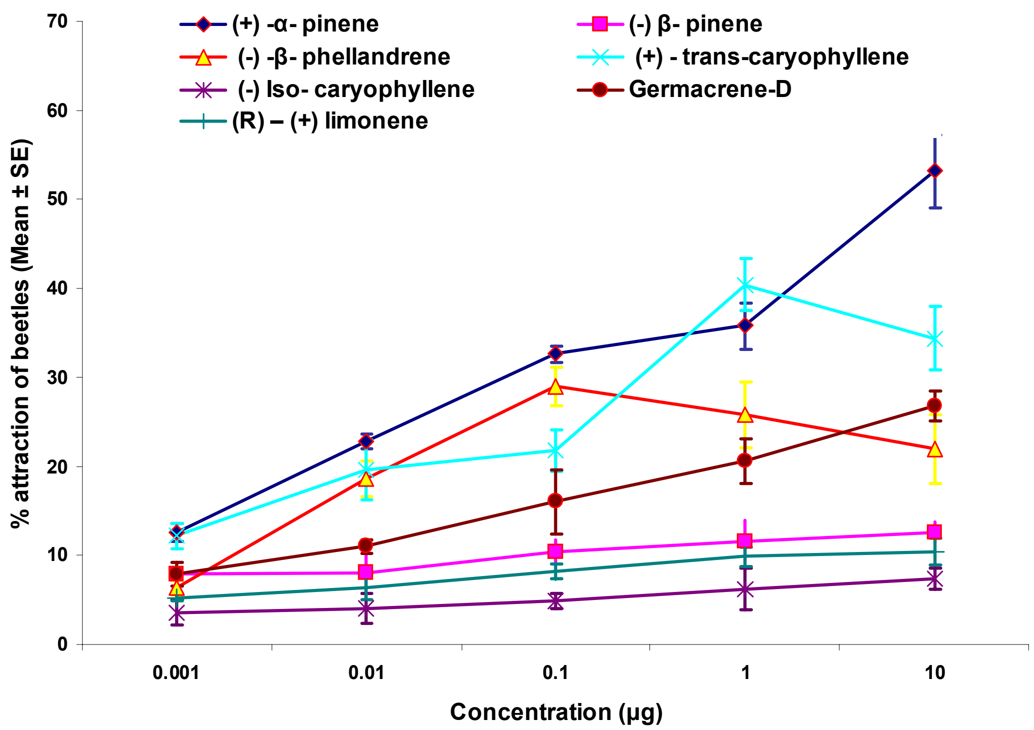


Fig. 4. Dose response of SHB to individual volatile compounds in wind tunnel

10 g/ml. Similar trend was observed with germacrene-D. In the case of trans-caryophyllene the percentage of attraction showed an increasing trend up to 1 g/ ml and thereafter declined whereas, the ability of (-)-b- phellandrene to attract SHB declined at 0.1 g/ml itself. Even at high dosage, (+) a-pinene, (R)-(+)-limonene and (-)-iso-caryophyllene did not elicit any significant response in SHB beetles (Fig. 4).

3.3.3. Bioassay with different volatile blends and different ratios of blended components

The volatile blends tested in wind tunnel are presented in Table 3. Blends were categorised into double, triple and multiple (four) component blends. The concentration of each blend was determined on the basis of dosage studies conducted in wind tunnel. Among the double component blends, the blend of (+) - a - pinene and (+) - trans-caryophyllene at a rate of 10: 1 attracted significantly more number of SHB beetles. The triple blend of (+) - a - pinene, (+) - trans-caryophyllene and (-) - b - phellandrene at 10: 1: 0.1 exhibited significantly enhanced efficacy by attracting more number of beetles. The efficiency of the multiple blend of (+) - a - pinene, (+) - trans-caryophyllene (-) - b - phellandrene and (-)-germacrene-D at a ratio of 10:1:0.1:3 was significantly superior to others in the wind tunnel (Table 3).

When the ratio of Germacrene-D increased from one to three, the attraction of beetles also simultaneously increased but declined when the ratio was increased to four (Fig. 5). On the other hand, increase in the ratio of trans-caryophyllene resulted in a decrease in attraction to SHB. It is evident that the optimum ratio of the four components in the multiple blend was 10:1:0.1:3. Increased concentration of (-) - b - phellandrene had a negative impact on SHB attraction while increasing the level of (+) - a - pinene beyond ten parts did not significantly enhance the attraction of beetles (data not presented in the figure)

3.3.4. Efficiency of different dispensers on the rate of release of the multiple blend and the blended compounds

The rate of release of multiple blend with fixer from the polyethylene tube (PET) dispenser was stable and low compared to rubber septum and ring septum (Table 4).

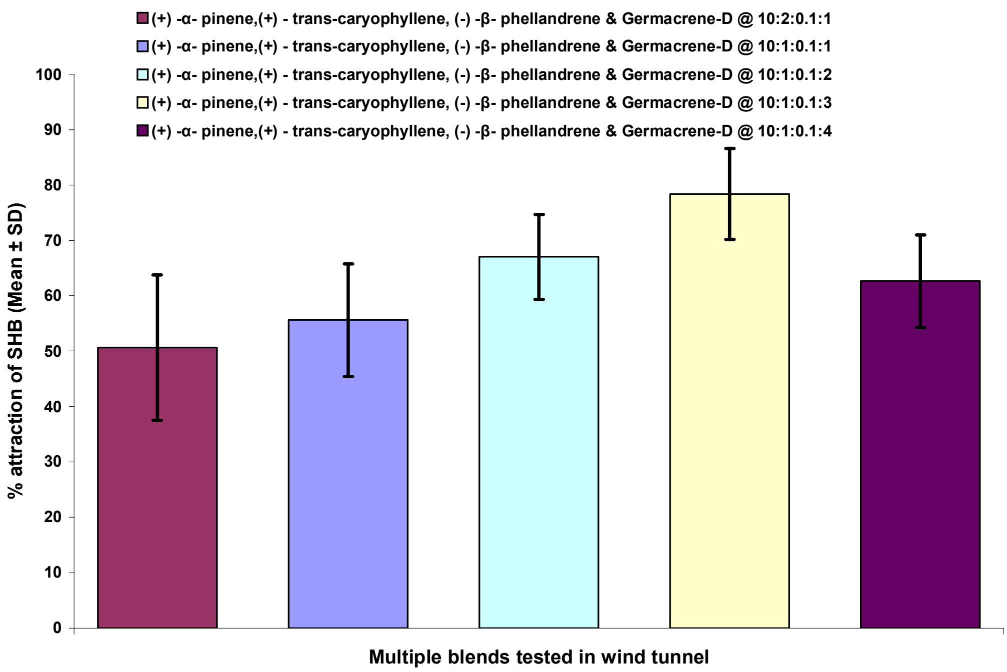


Fig. 5. Attraction of SHB in wind tunnel to different ratios of blended compounds in the multiple blend

Table 3. Response of SHB beetles to different blends in the wind tunnel

Compounds in the blend	Ratio of compounds	% attraction* in wind tunnel
Double blend		
I. (+) - a-pinene and (+)-trans-caryophyllene	10:1	57.7 ± 7.64
II. (+)-a-pinene and (-)-b-phellandrene	10: 0.1	47.3 ± 8.33
III. (+)-a-pinene and (-)-germacrene-D	10:3	55.3 ± 6.11
Triple blend		
I. (+)-a-pinene, (+)-trans-caryophyllene and (-) -b- phellandrene	10: 1: 0.1	69.3 ± 6.66
II. (+)-a-pinene, (+)-trans-caryophyllene and (-)-germacrene-D	10: 1: 3	56.7 ± 6.11
III. (+)-a-pinene, (-) -b-phellandrene and (-)-germacrene-D	10: 0.1:3	65.3 ± 4.93
Multiple blend		
I. (+)-a-pinene, (+)-trans-caryophyllene (-) -b- phellandrene and (-)-germacrene-D	10: 1: 0.1: 3	79.3 ± 5.03
C.D. at P = 0.05:		7.9

* Mean ± SD of five replications; Blends which attracted significant number of beetles were only showed in the table

Table 4. Rate of release of individual compounds and most effective blends from different dispensers

Volatile compounds/blends	Ring septum		Rubber septum		Polyethylene tube	
	Release rate* (mg/day)	r^2 (range)	Release rate* (mg/day)	r^2 (range)	Release rate* (mg/day)	r^2 (range)
(+)-a-pinene	1.21 ± 0.54	0.778-0.756	1.28 ± 0.71	0.804-0.791	1.13 ± 0.32	0.804-0.786
(-) b-phellandrene	1.25 ± 0.52	0.731-0.714	1.05 ± 0.74	0.926-0.907	1.01 ± 0.29	0.961-0.949
(+) - trans-caryophyllene	1.20 ± 0.54	0.926-0.901	1.05 ± 0.65	0.951-0.929	0.61 ± 0.23	0.951-0.946
(-) -germacrene– D	1.17 ± 0.37	0.958-0.936	0.83 ± 0.29	0.982-0.958	0.68 ± 0.16	0.983-0.971
Multiple blend I						
(+) a-pinene, (+) - trans-caryophyllene (-) b-phellandrene, (-) -germacrene– D @ 10:1:0.1:3	1.51 ± 0.38	0.652-0.616	1.34 ± 0.71	0.792-0.759	1.22 ± 0.38	0.795-0.784
Multiple blend II						
(+) a- pinene, (+) - trans- caryophyllene (-) b-phellandrene, (-) -germacrene– D @ 10:1:0.1:3 with BHT	1.46 ± 0.36	0.817-0.801	1.11 ± 0.53	0.901-0.889	0.72 ± 0.28	0.914-0.901

*Values of rate of release represented in the tables are the mean of five replicates. r^2 value represents the highest and lowest values obtained from the experiments repeated five times

Release rate ranged from 1.13 to 0.68 mg/day when the PET dispenser was used for different individual volatile whereas it was 1.17 to 1.25 mg/day for ring septum and 1.28 to 0.83 for rubber septum. Rate of release of multiple blend with BHT from PET dispenser was 0.72 mg/day whereas it was 1.22 mg/day without BHT. Gravimetric studies on the residual amount of compounds present in each type of dispenser after 30 days revealed that retention of all the volatile compounds were higher in the polyethylene tube than that in ring and rubber septum (Fig. 6- 8).

3.3.5. Rate of release of multiple blend from PET at different temperature

Multiple blend, at a ratio of 10:1:0.1:3, under different temperature regime had a fairly good release (Table 5). When temperature increased from 15 to 30°C, a concurrent increase in the release rate of the blend was observed. As days passed, the rate of release of the blend from the dispenser decreased. Release rate attained a maximum of 0.84 mg/day at 30°C (Fig. 9). Field studies under fluctuating temperatures is also confirmed the above findings.

3.3.6. Use of different dispensers and optimization of quantity of blend per dispenser

The mean percentage attraction to the polyethylene tube filled with multiple blend (attractant lure) was significantly higher followed by rubber septum and ring septum (Fig. 10). As the age of the attractant lure increased from 0 to 30 days, attraction of beetles declined gradually. On the 30th day, polyethylene tube attracted 46% of beetles, whereas ring and rubber septa recorded 23.2 and 35.4 % of attraction, respectively.

Increase in the quantity of blend proportionately increased the mean percentage attraction of beetles up to 150 l/dispenser and further increase in quantity of attractant did not show any significant increase in the catch of beetles (Fig.11).

3.4. Electro antennogram studies using individual compounds

Well defined and reproducible EAG responses were obtained when SHB beetle antenna was stimulated with the individual volatiles and the data were significantly higher with respect to (+) - a - pinene and trans caryophyllene. EAG response was almost identical when (-) - b- phellandrene and (-) germacrene-D were used as test

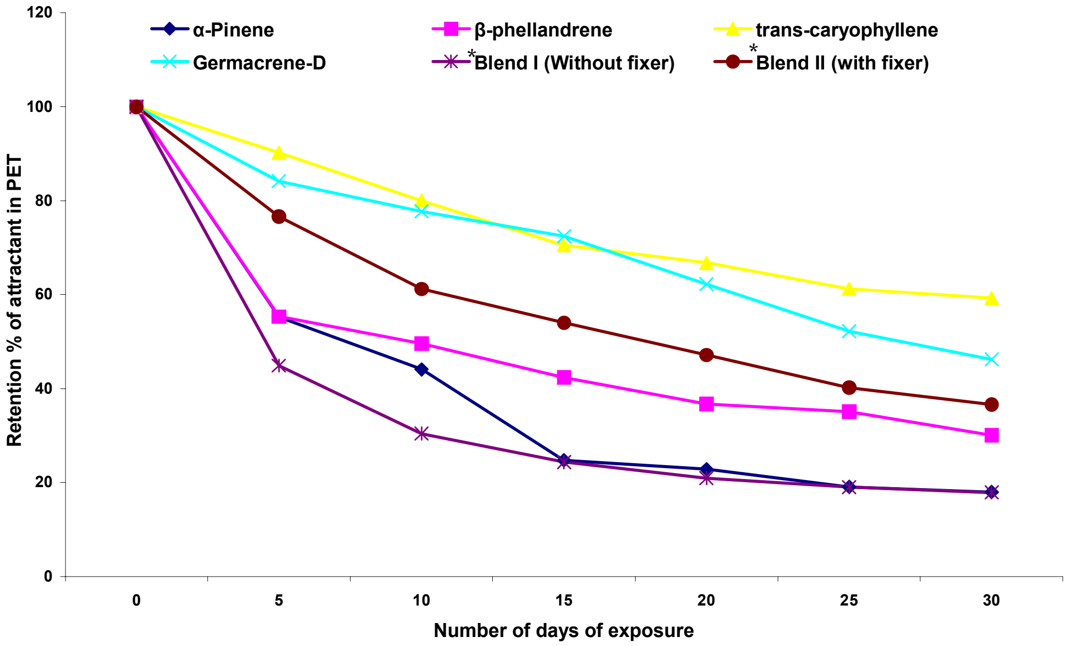


Fig.6. Retention of attractant in PET after exposure

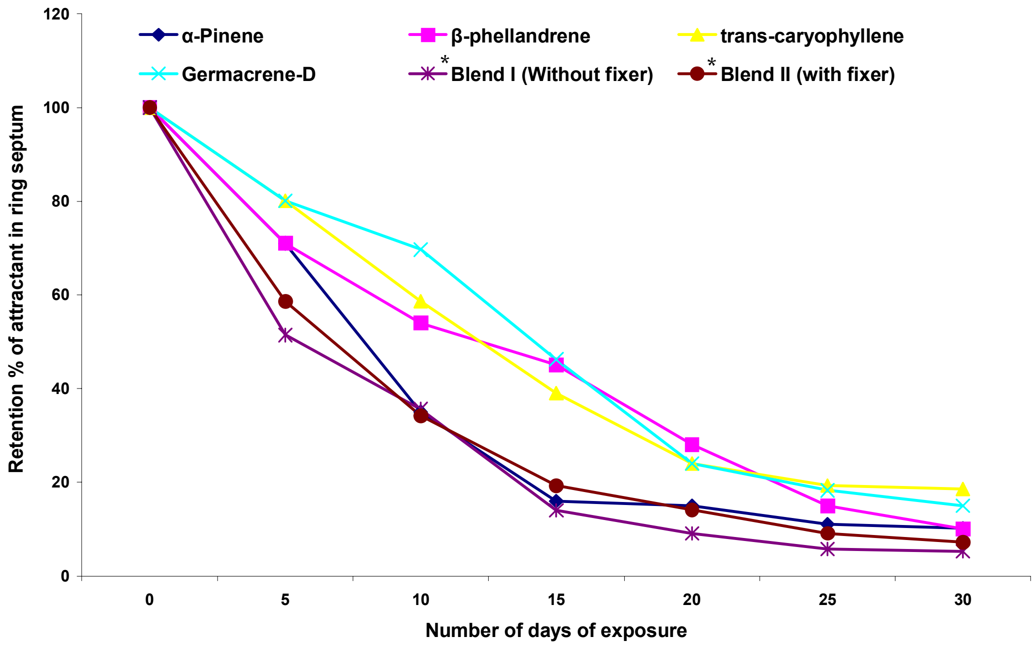


Fig.7. Retention of attractant in ring septa after exposure

* Blend - Multiple blend of (+)-a-pinene,(+)– trans-caryophyllenene,(-)- b -phellandrene and (-)-germacrene-D at a ratio of 10:1:0.1:3

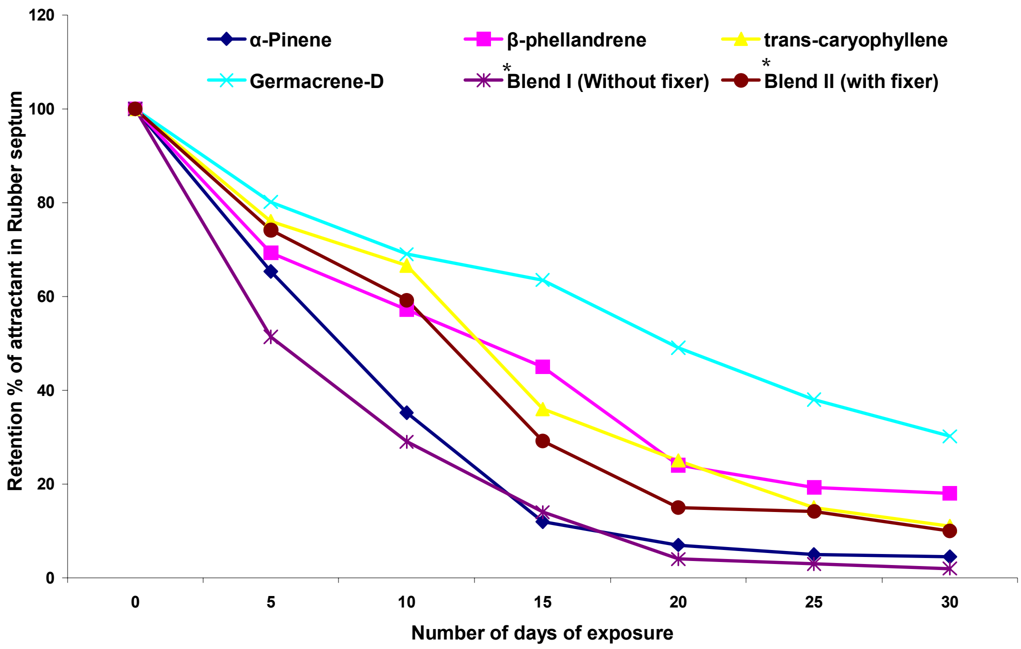


Fig.8. Retention of attractant in rubber septa after exposure

* Blend - Multiple blend of (+)-a-pinene,(+)– trans-caryophyllenene,(-)- b -phellandrene and (-)-germacrene-D at a ratio of 10:1:0.1:3

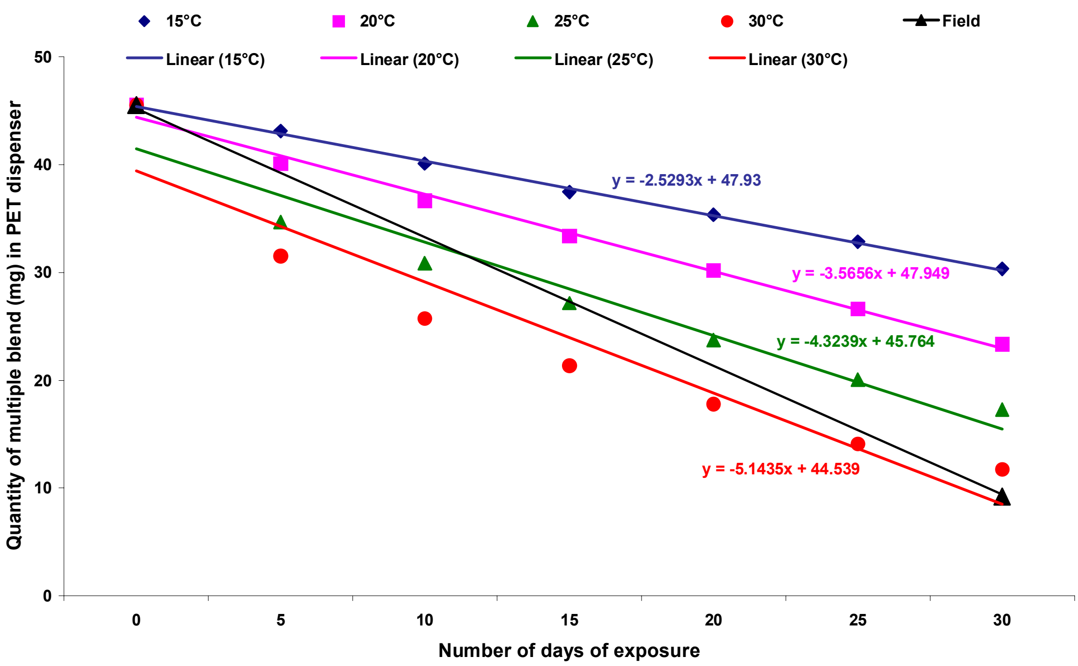


Fig. 9. Effect of temperature on the retention of multiple blend in PET

Table 5. Effect of temperature on the rate of release of multiple blend* from polyethylene tube dispenser (Mean ± SD)

Temperature	Rate of release (mg/day)	r^2 (range)
15°C	0.30 ± 0.03	0.986–0.996
20°C	0.46 ± 0.03	0.996–0.999
25°C	0.66 ± 0.05	0.958–0.999
30°C	0.84 ± 0.02	0.913–0.996

*Blend of (+) - a- pinene, (+) - trans-caryophyllene, (-) -b-phellandrene and (-)-germacrene-D @ of 10:1:0.1:3 with BHT

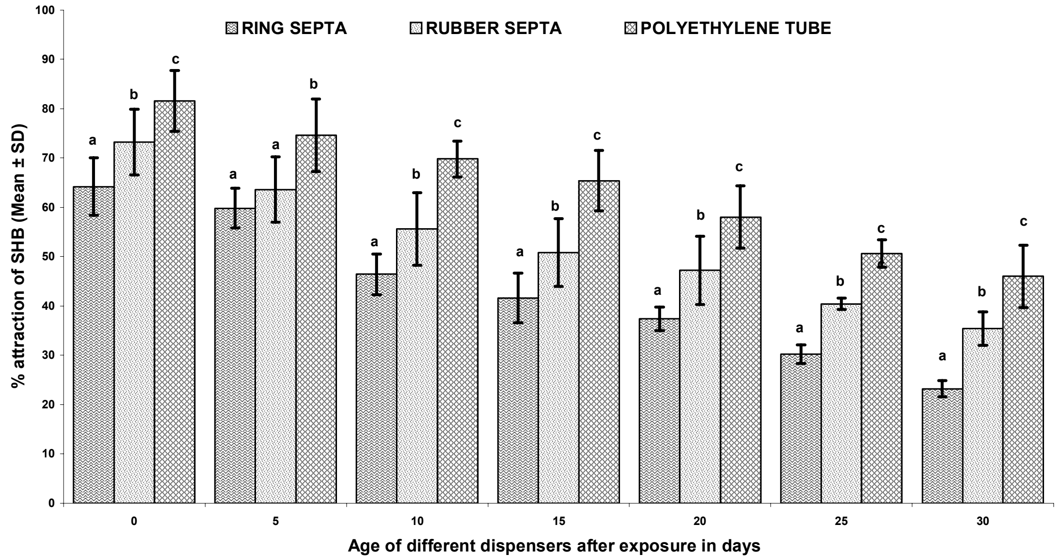


Fig. 10. SHB attraction to different types of dispensers and duration of its attractiveness in wind tunnel

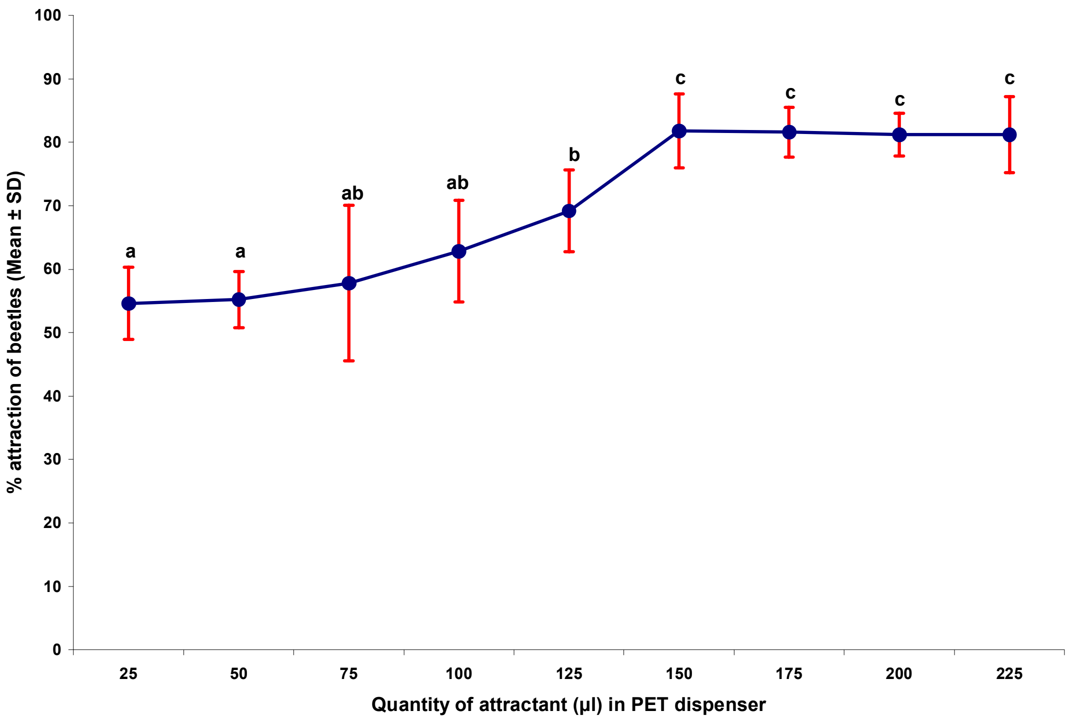


Fig. 11. Wind tunnel studies on the influence of quantity of attractant on SHB attraction

stimuli. On the other hand, EAG response was poor when (-) - b- pinene, (R) – (+) limonene and (-) Iso- caryophyllene were tested as stimuli (Fig. 12).

3.4.1. Dose response study with EAG

Dose response study with EAG confirmed the results of wind tunnel experiment Increased concentration of (+) - a - pinene and (-) germacrene-D elicited higher response than any other volatile compound tested (Fig.13). Saturation was not observed even at the higher dose of 10 g/ml of (+) - a - pinene and (-) -germacrene-D. Maximal antennal response/saturation level against (+) - trans-caryophyllene was at 1.0 g/ml while it was 0.1 g/ml for (-) - b - phellandrene (Fig.13). EAG response was poor against the remaining three compounds. In the present study (+) - a - pinene, (+) - trans-caryophyllene, (-) - b -phellandrene and (-) germacrene-D elicited significant behavioural response in SHB.

3.4.2. EAG studies with volatile blends

Among the blends tested, multiple blend of (+) - a - pinene, (+) - trans-caryophyllene (-) - b - phellandrene and (-)-germacrene-D at a ratio of 10:1:0.1:3 elicited significantly higher EAG response followed by the triple blend, (+) - a - pinene, (+) - trans- caryophyllene and (-) - b - phellandrene at a ratio of 10:1:0.1 (Table 6).

3.4.3. EAG studies on varying ratios of blended compounds

The combination of (+) - a - pinene, (+) - trans-caryophyllene (-) - b - phellandrene and (-)-germacrene-D at a ratio of 10:1:0.1:3 elicited significantly higher antennal response of 3.69 ± 0.2 mV. (Fig. 14). Result obtained in this study was comparable to that from wind tunnel experiments (Fig.5). When the amount of Germacrene-D was increased from one to three parts, the response also gradually increased. When the ratio was again changed from three to four parts, the EAG response significantly declined. On the other hand, increasing the concentration of trans caryophyllene decreased the EAG response. Study proved that ratio of 10:1:0.1:3 for the four components in multiple blend elicited maximum response in SHB. Increase in the ratio of (-) - b - phellandrene was found to depress the EAG response whereas an increase in (+) - a - pinene above ten parts has not enhanced the response, hence results are not shown in the figure (Fig. 14).

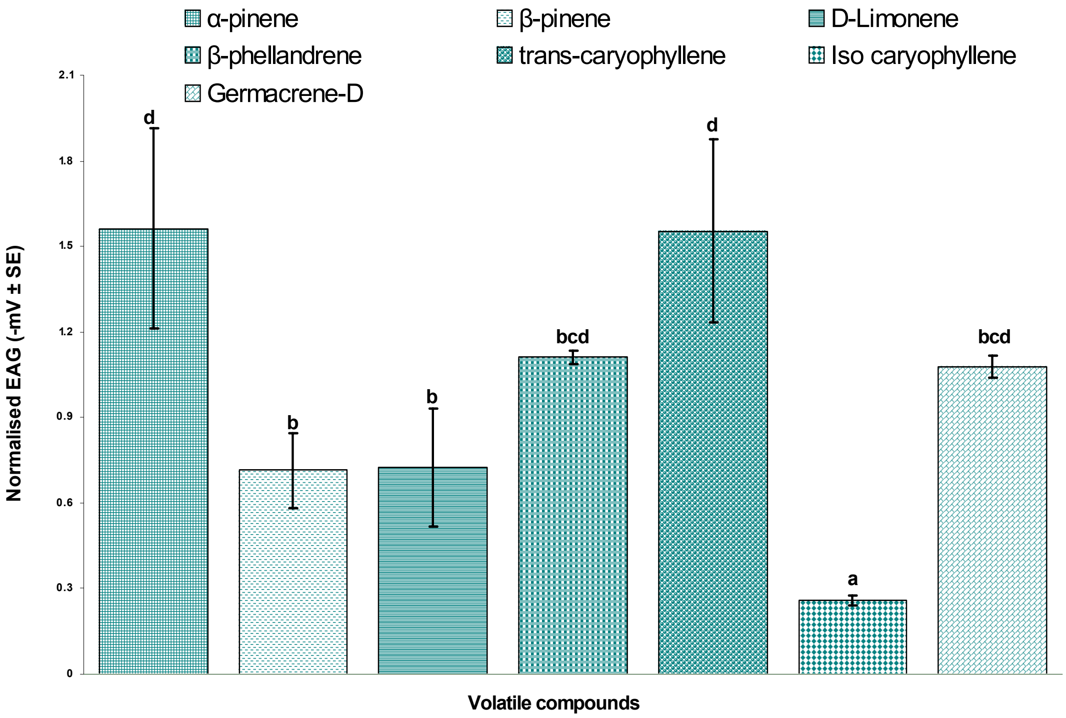


Fig. 12. EAG response of SHB to individual volatile compounds of *M. bipinnatifida*

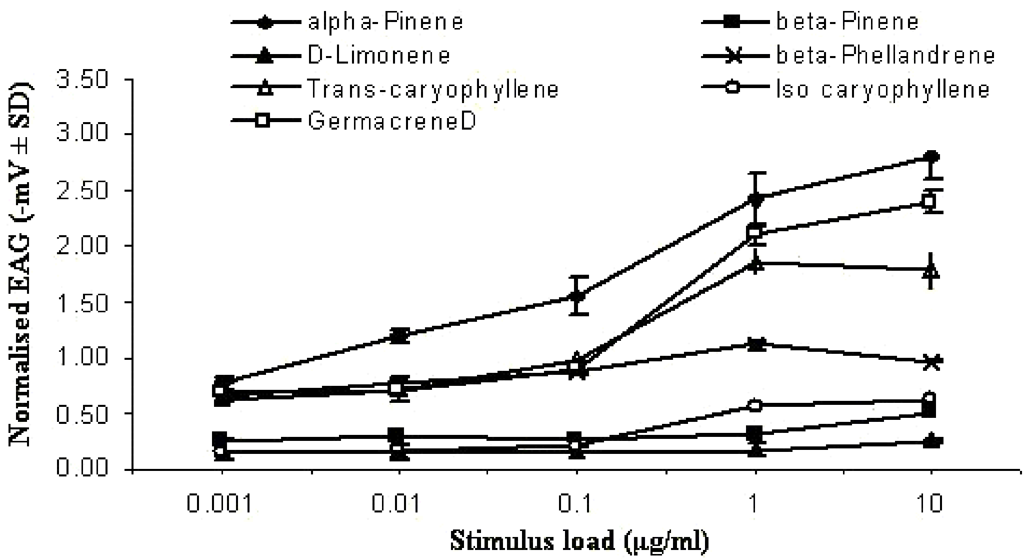


Fig. 13. Dose response of SHB to individual volatile compounds in EAG

Table 6. Response of SHB beetles to blends in electro antennogram

Compounds in the blend	Ratio of the compounds	EAG response (in mV)
Double blend		
I. (+)-a-pinene and (+) - trans-caryophyllene	10:1	1.74 ± 0.07
II. (+)-a-pinene and (-) -b- phellandrene	10: 0.1	1.63 ± 0.10
III. (+)-a-pinene and (-) -germacrene-D	10: 3	1.68 ± 0.11
Triple blend		
I. (+)-a-pinene, (+) - trans-caryophyllene and (-) -b-phellandrene	10: 1: 0.1	2.66 ± 0.30
II. (+)-a-pinene, (+) - trans-caryophyllene and (-)-germacrene-D	10: 1: 3	2.15 ± 0.17
III. (+)-a-pinene, (-) -b-phellandrene and (-)-germacrene-D	10: 0.1: 3	1.96 ± 0.05
Multiple blend		
I. (+)-a-pinene, (+) - trans-caryophyllene (-) -b- phellandrene and (-)-germacrene-D	10: 1: 0.1: 3	3.74 ± 0.20
C.D. at P = 0.05:		0.30

Ratio: Proportion of different components in the blend; Blends which attracted significant number of beetles are only shown in the table

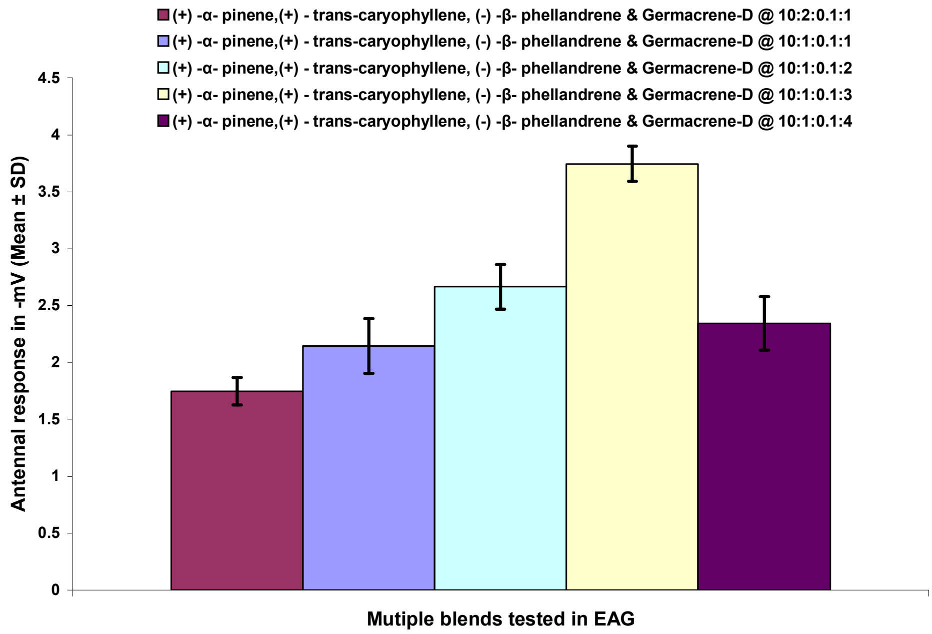


Fig. 14. EAG response of SHB to different ratios of blended compounds in the multiple blend

3.4.4. EAG response on varying quantity of blend

There was significantly higher response to 150, 175, 200 and 225 l of multiple blend (Fig. 15). Even though quantity of blend in PET dispenser increased from 150 l to175, 200 and 225 l, the EAG response achieved were similar. It was evident that increase in the amount of attractant per dispenser beyond 150 l had no special impact on the EAG response. This study again confirmed the results on wind tunnel experiment (Fig. 10).

3.5. Evaluation of attractant chemicals in the field

Results of the studies carried out on the behaviour of SHB in field and its response against volatile compounds individually as well as in blends executed in the field using trap are given below.

3.5.1. SHB behaviour in the field

Majority of the beetles came out from their galleries only during 10.00 am to 1.00 pm. Female beetles appeared out side the gallery more frequently than the males. Movements of black coloured male and female beetles on tea stem were quick when compared to those of the newly emerged yellow coloured male and female beetles. Adult beetles walked on the stems and branches at a height of 3 -30 cm above ground level for a period of 30-45 minutes. During their movements, some of them entered old galleries, spent 20-40 minutes inside in the gallery and came out; on certain occasions, beetles entering an old gallery didn’t come out. It was interesting to note that most of the female beetles entered old galleries instead of making new galleries. On several occasions, beetles which started making new galleries fell on the ground and failed to relocate their new gallery under construction even after searching for 30-45 minute. Subsequently, they started to make a new gallery again at a different place on the stem. Beetles which did not climb back immediately on to the bush went down into the litter, started to dig the soil for some time and then climbed back to the bush. Beetles which didn’t find a suitable place died in the soil. Newly emerged beetles also followed the same pattern of movement.

Out of the forty three beetles observed, sixteen fell down on the ground and ten of them climbed back on to the bush. Beetles moved in a zigzag manner over a

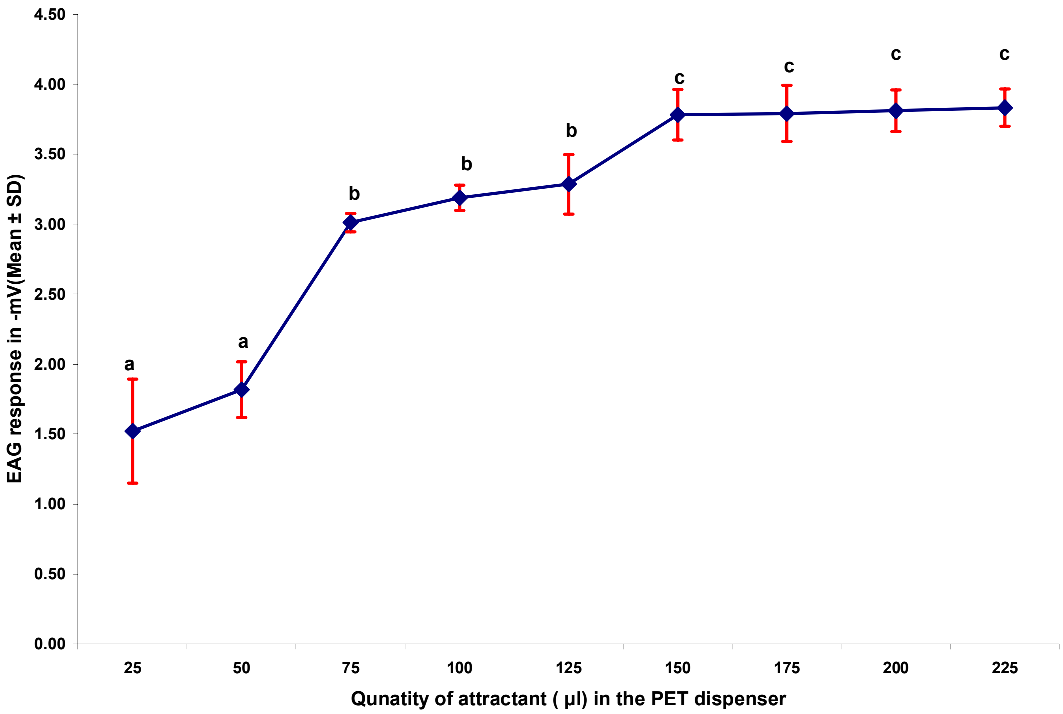


Fig. 15. EAG response of SHB to different quantities of blend per dispenser

distance ranging from 2 - 30 cm. On many occasions, beetles were found roaming solitarily. During the study, fifteen beetles were found flying away from one bush to another and it was noticed that when the surface of the stem was very wet, beetles didn't come out of the gallery.

It was observed that majority of the beetles flew to a distance of 1 – 3 m in one leap and reached a height of 1 m. Almost on all the occasions female beetles flew and landed on nearby bush. Three out of fifteen beetles flew to a distance of 10-12 m at a height of 1-3 m indicated that these beetles can reach new clearings from an adjacent tea fields. It was found that beetles which could fly were caught in the attractant trap. Beetles which could not fly walked on the stem and tried to reach the nearby attractant trap which was kept within the bush row. Beetles, while searching for the attractant source, slipped in to the funnel wall and get trapped. Beetles took off vertically upward in a slow fluttering fashion. Strong wind could sweep away the flying beetles laterally to a considerable distance and perhaps they were blown off to the nearby fields. Beetles in flight were attracted by the trap in the trap vicinity, but occasionally crossed the trap. They remained stationary in the air for a few seconds, approached the trap and fell into the trap. On most of the occasions, beetles were trapped in the first funnel where the attractant was kept. On a few instances, beetles were found landing on the surface of the trap and started searching for the source of attractant for a period 4-5 minutes. Some times, they flew away from the trap but again flew back to the vicinity of trap and finally got trapped into the funnel.

3.5.2. Evaluation of different trap designs

Among the four different traps tested in the field, multiple funnel trap (MFT) was found most suitable and the number of beetles trapped was significantly high (Fig. 16). MFT caught significantly more number of beetles than the other three types irrespective of their position and the population density of SHB in the field.

3.5.3. Optimising the number of funnels in a trap

Multiple funnel trap was further fine tuned by optimizing the number of funnels. The capture efficiency increased as the number of funnel increased from one to five.

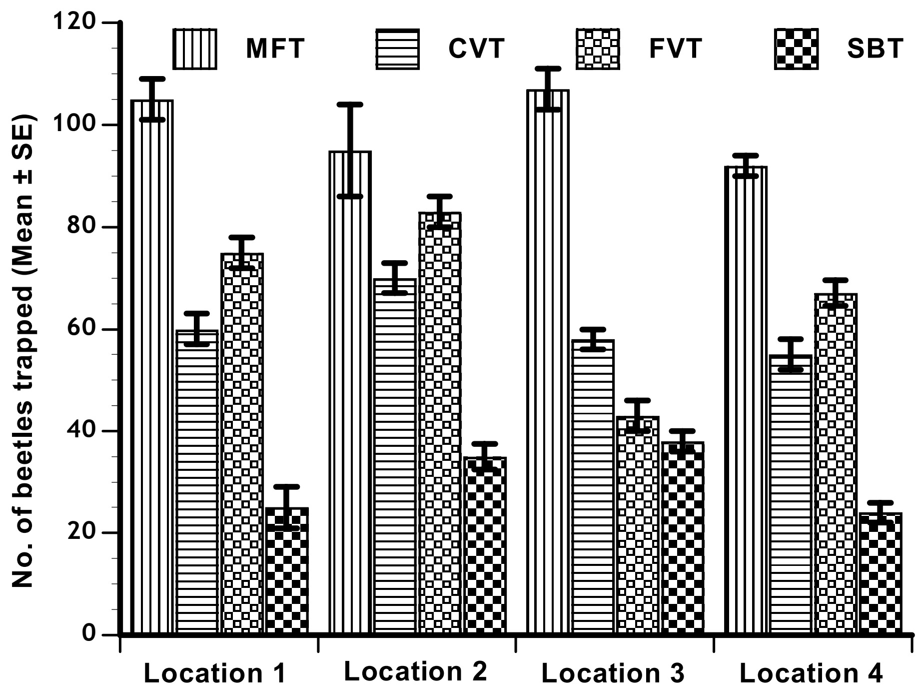


Fig. 16. Number of SHB captured in different types of traps in the field

Further increase in the number of funnels showed no significant impact on trapping efficiency (Fig.17). Based on the results, traps with five funnels were used for further field studies.

3.5.4. Influence of trap height and position in the level of attraction

Mean number of beetles captured in multiple funnel traps placed at the middle level (15 cm below the canopy) and low level (15 cm above the ground) were not significantly different from each other (Fig.18). Traps placed 15 cm above the canopy (top level) also trapped the beetles efficiently but inferior to other two trap heights. It may be concluded that the suitable position for the installation of multiple trap was either at the low or middle level in the tea field. Like the trap height, position of the traps in field also played a significant role in SHB trapping. Among the three positions evaluated, traps installed in the walking lanes trapped higher number of beetles (143.7 ± 16.9) when compared to other trap positions (Fig. 19).

3.5.5. Field studies on suitable dispenser

The polyethylene tube dispenser containing multiple blend with BHT trapped significantly higher number of beetles in the field (Table 7). Number of beetles trapped per day per trap using ring and rubber septum dispensers were lower to that of polyethylene tube dispenser. When the same multiple blend was used in the field without the fixer BHT, the trap catch declined drastically in all the three types of dispensers. Mean capture of beetles/day using three different dispensers filled with volatiles individually also showed same trend on trap catch (Table 7).

3.5.6. Field evaluation of individual attractant compounds and blends

Mean trap catch was significantly higher in (+) - a - pinene followed by (+) - trans-caryophyllene, (-)-germacrene-D and (-) - b - phellandrene when they were tested individually under identical field conditions. Mean trap catch of SHB beetles by the attractants, (-) - b - pinene and (R) – (+) limonene was significantly higher than that of (-) Iso- caryophyllene (Table 8).

Significantly higher trap catch was recorded for the multiple blend [(+) - a - pinene, (+) - trans-caryophyllene (-) - b - phellandrene and (-)-germacrene-D at a rate of 10:1:0.1:3] (Table 9). Even though triple blend [(+) - a - pinene, (+) - trans-

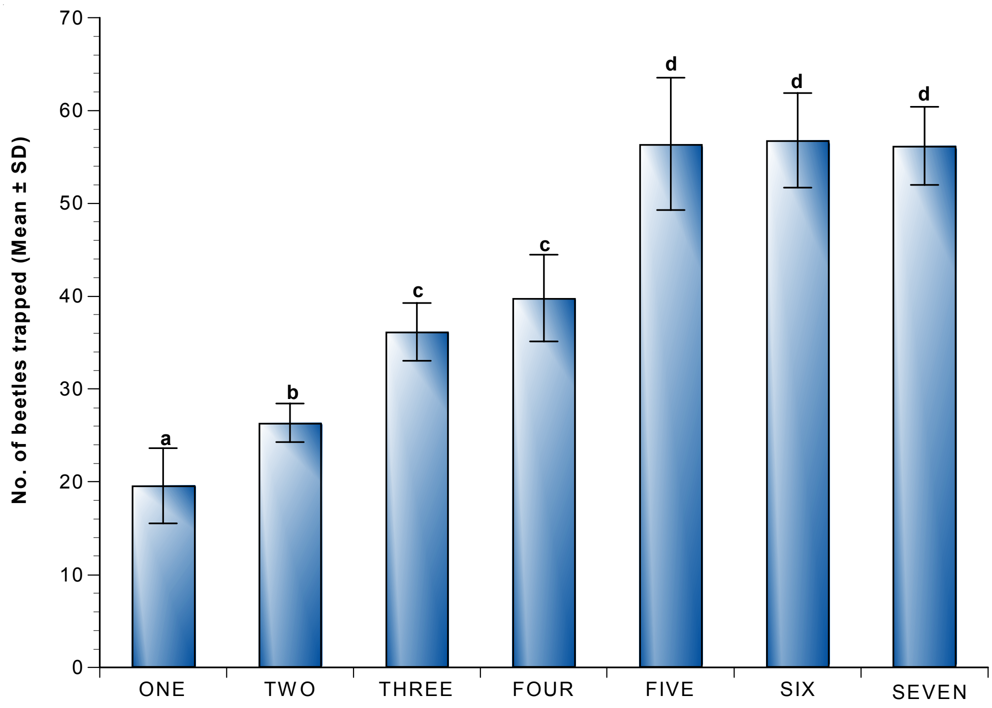


Fig. 17. Effect of number of funnels in the MFT on the SHB catch

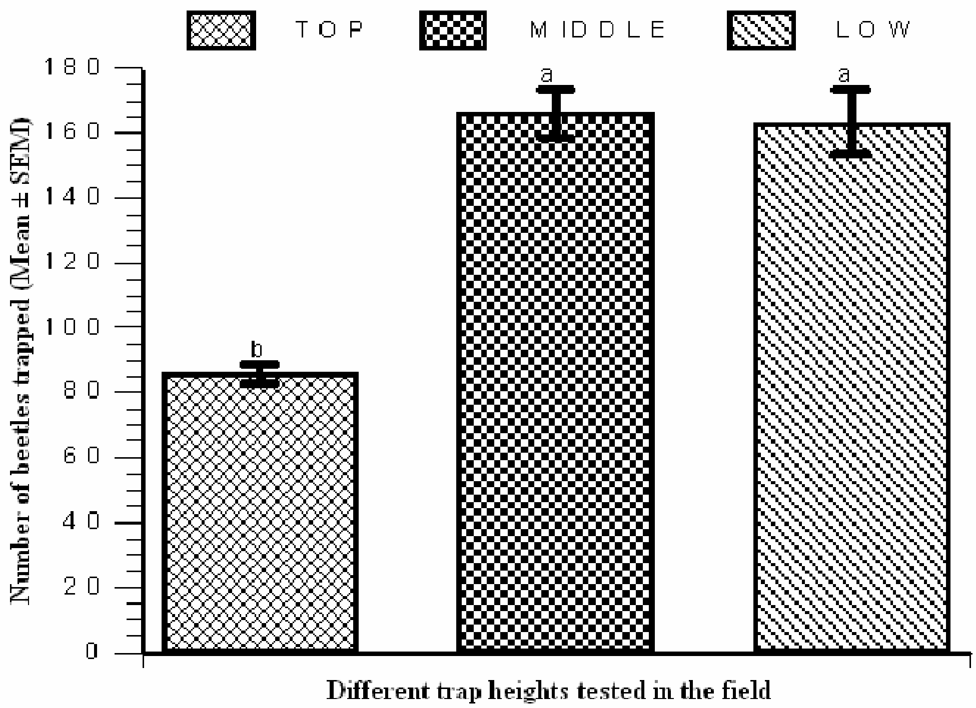


Fig. 18. Effect of trap height on shot hole borer catch

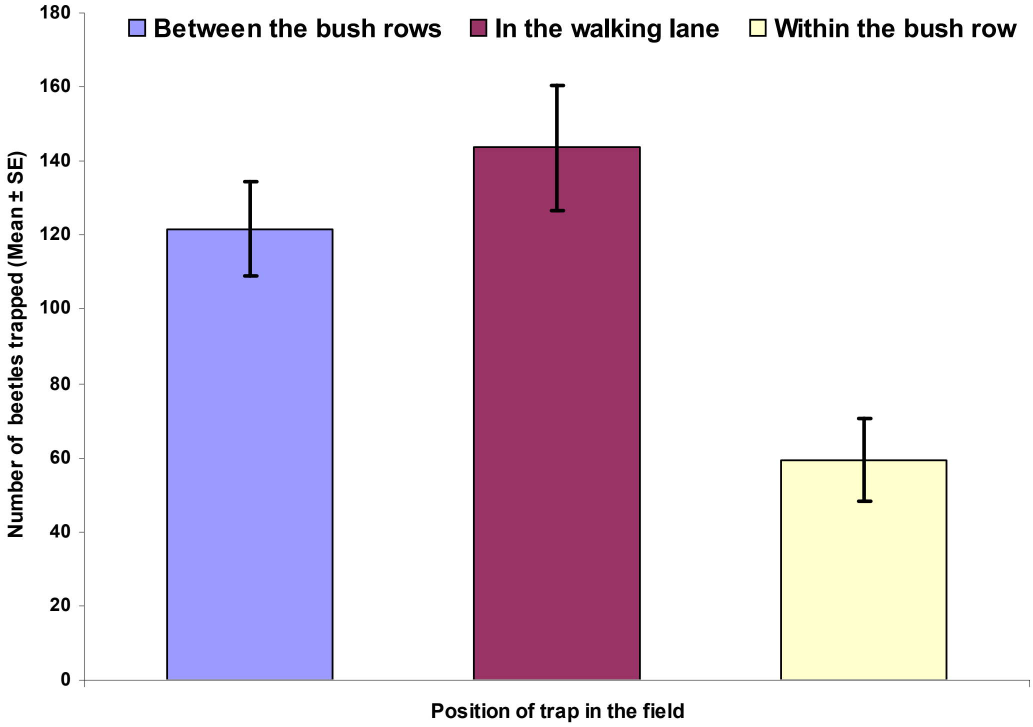


Fig. 19. Effect of position of trap on SHB catch

Table 7. Effect of different dispensers on trap catch

Volatile compounds/blends	Mean ± SD* beetles/trap/day		
	Ring septum	Rubber septum	Polyethylene tube septum
(+)-a-pinene	0.66 ± 0.09a	0.81 ± 0.16b	1.32 ± 0.27b
(-)-b-phellandrene	0.41± 0.10a	0.38 ± 0.10a	0.44 ± 0.12a
(+) - trans-caryophyllene	0.93 ± 0.11a	0.73 ± 0.12a	1.03 ± 0.20b
(-)-germacrene– D	0.76 ± 0.23b	1.17 ± 0.22b	1.09± 0.39ab
Multiple Blend I (+)-a- pinene, (+) - trans- caryophyllene (-)-b- phellandrene, (-)-germacrene– D @ 10:1:0.1:3	2.26 ± 0.46b	2.99 ± 0.20c	3.44 ± 0.36c
Multiple Blend II (+)-a-pinene, (+) - trans- caryophyllene (-)-b- phellandrene, (-)-germacrene– D @ 10:1:0.1:3 with BHT	4.73 ± 0.53c	4.99 ± 0.21d	6.15 ± 0.53d
C.D. at P = 0.05:	0.58	0.30	0.65

* Mean ± SD in the same column followed by a different letter are significantly different at 5% level (Duncan's multiple range test)

Table 8. Number of beetles trapped by individual volatile compounds of *M.bipinnatifida*

Volatile compound	Mean ± SE beetles*
(+)-a- pinene	38.4 ± 6.89
(-)-b- pinene	16.9 ± 3.54
(R)-(+) limonene	17.2 ± 3.53
(-) b- phellandrene	25.0 ± 3.99
(-) Iso- caryophyllene	8.4 ± 1.77
(+) - trans- caryophyllene	34.5 ± 6.00
(-)-germacrene– D	30.0 ± 5.79

* Meanof five replicates and MFT with five funnel was used as trap

Table 9. Mean number of beetles attracted to different blends in the field traps

Blend composition	Ratio of compounds	Mean number of beetles ± SE/trap		
		I replacement*	II replacement	III replacement
Double blend				
I. (+)-a-pinene and (+) - trans-caryophyllene	10:1	79.0±4.3	65.7± 5.4	80.3± 3.9
II. (+)-a-pinene and (-) -b-phellandrene	10:0.1	68.0±5.3	57.7±10.8	69.3± 4.7
III. (+)-a-pinene and (-)-germacrene-D	10:3	73.7±7.4	64.0± 7.9	75.3±10.3
Triple blend				
I. (+)-a-pinene, (+) - trans-caryophyllene and (-) -b- phellandrene	10: 1: 0.1	133.7 ± 8.9	124.3 ± 14.6	143.0± 11.4
II. (+)-a-pinene, (+) - trans-caryophyllene and (-)-germacrene-D	10: 1: 3	95.0 ± 6.8	96.0 ± 8.8	109.7± 9.6
III. (+)-a-pinene, (-) -b-phellandrene and (-)-germacrene-D	10: 0.1:3	91.7 ± 8.6	94.3 ± 13.1	101.0 ± 10.2
Multiple blend				
I. (+)-a-pinene, (+) - trans-caryophyllene (-) -b- phellandrene and (-)-germacrene-D	10: 1: 0.1: 3	176.7 ± 13.1	162.7 ± 11.1	168.3± 13.0

* Treatments were randomised during every replacement of PET lure at an interval of 30 days

caryophyllene and (-) - b - phellandrene at a rate of 10:1:0.1] also trapped large number of beetles, it was lower than that caught in multiple blend. In all the three studies results were identical and indicated that multiple blend was the best for trapping shot hole borer under field conditions.

3.5.7. Different ratios of highly responsive blend

The mean trap catch for the multiple blend was significantly higher followed by 10:1:0.1:2 ratio (Table 10). Increase of (-)-germacrene-D from three to four parts led to a decrease in trap catch, as the against expectation. Increase in the ratio of the trans-caryophyllene in the blend from one to two parts (10:2:0.1:3) also decreased the mean trap catch. The trend was same in all the experiments (Table 10).

3.5.8. Optimization of blend quantity per dispenser and its durability

Among the six volumes tested, the mean number of beetles trapped in 150 l of multiple blend was highest (Fig. 20). In all other treatments, the number of beetles trapped was almost the same while it was significantly inferior to that of 150 l. The mean number SHB caught per trap did not increase significantly even when the volume was increased from 150 l.

Since the polyethylene tube dispenser was more attractive to the beetles than the other two dispensers, the former was selected for field study. Data revealed that the number of beetles attracted was inversely proportional to the number of days it was kept in the field. As the attractant lure became older the number of beetles trapped also declined (Fig. 21). However, even after 25- 30 days the trap catch was quite acceptable. Thereafter trap catch declined sharply and became unattractive after 35-40 days. From this study, it was evident that the attractant had to be changed after 30 days (Fig. 21).

3.5.9. Field bioassay studies on the required number of traps per hectare

Studies on the number of traps required for the efficient catching of SHB revealed that installation of trap at 20 x 20 m spacing had the highest trap catch. Placement of traps at 15 x 15 m spacing (36 traps per hectare), 25 x 25 m spacing (16 traps per hectare) and 50 x 50 m spacing (9 trap per hectare) attracted lesser number of beetles (Fig. 22).

Table 10. Effect of different ratio of the blended compounds in multiple blend on shot hole borer attraction in the field

Volatile compounds	Ratio of the of compounds	Mean number of beetles± SE/trap				
		I replacement	II replacement	III replacement	IV replacement	V replacement
I. (+) -a - pinene, (+) - trans-caryophyllene (-) -b- phellandrene and ((-)-germacrene-D	10: 2: 0.1: 1	130.6 ± 8.9a	126.0 ± 5.9a	104.2 ± 7.1a	124.0 ± 7.4a	100.8 ± 4.0a
II. (+) - a- pinene, (+) - trans-caryophyllene (-) -b- phellandrene and (-)-germacrene-D	10: 1: 0.1: 1	136.0 ± 5.9ab	134.8 ± 8.2ab	111.4 ± 2.7a	136.4 ± 5.4ab	110.6 ±9.9 ab
III. (+) - a- pinene, (+) - trans-caryophyllene (-) -b- phellandrene and (-)-germacrene-D	10: 1: 0.1: 2	154.0 ± 9.5c	148.0 ± 9.8c	131.4 ± 2.7b	143.8 ± 5.6b	123.0 ± 9.8 bc
IV. (+) - a- pinene, (+) - trans-caryophyllene (-) -b- phellandrene and (-)-germacrene-D	10: 1: 0.1: 3	176.2 ± 5.6d	184.6 ± 3.3d	164.6 ± 10.3c	187.4 ± 9.9d	150.8 ± 6.9d
V. (+) - a- pinene, (+) - trans-caryophyllene (-) -b- phellandrene and (-)-germacrene-D	10: 1: 0.1: 4	148.6 ± 7.8bc	142.6 ± 8.8bc	138.2 ± 5.7b	162.0 ± 9.8c	133.6 ± 5.9 cd
C.D. at P = 0.05:		14.1	12.8	15.5	16.7	19.3

Figures in the same column followed by a different letter are significantly different at 5 % level (Duncan's multiple range test). Treatments were randomised during every replacement of lure

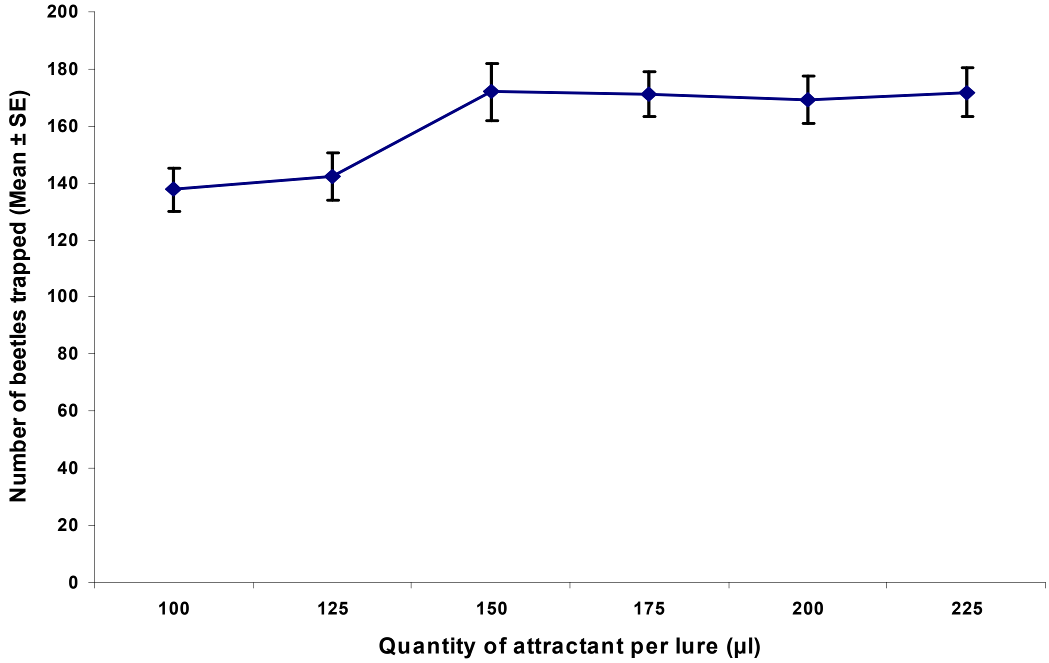


Fig.20. Influence of quantity of attractant on SHB trapping

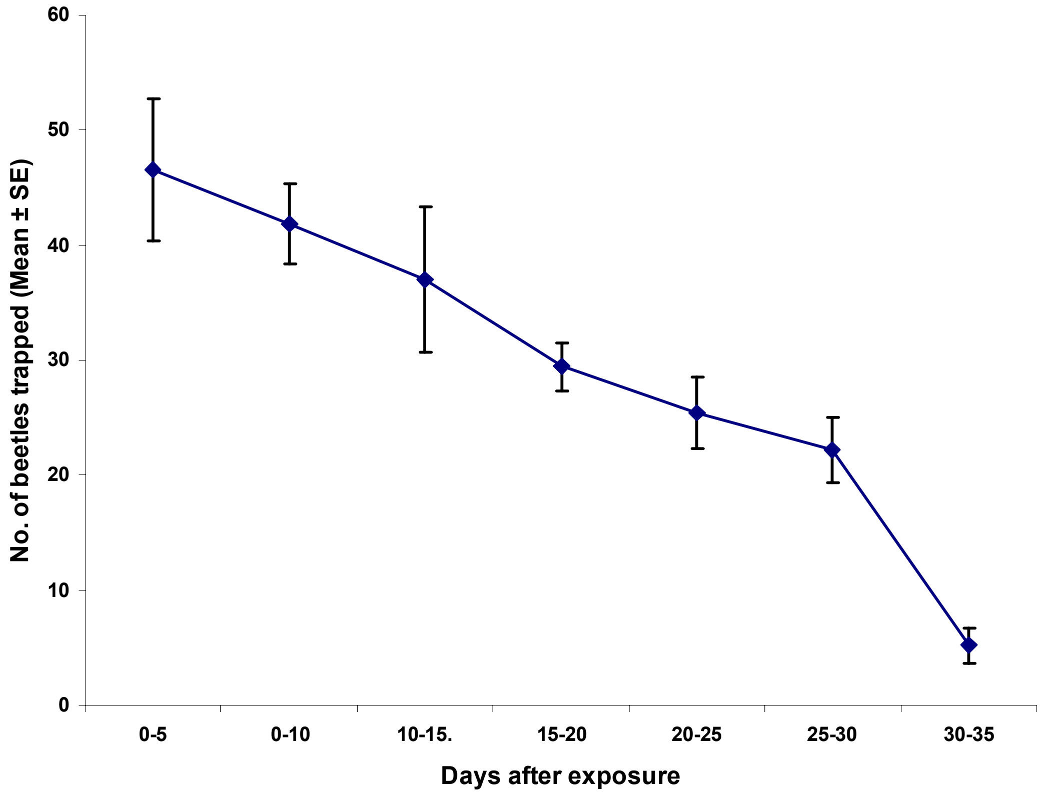


Fig.21. Duration of attractiveness of PET dispenser to SHB in the field

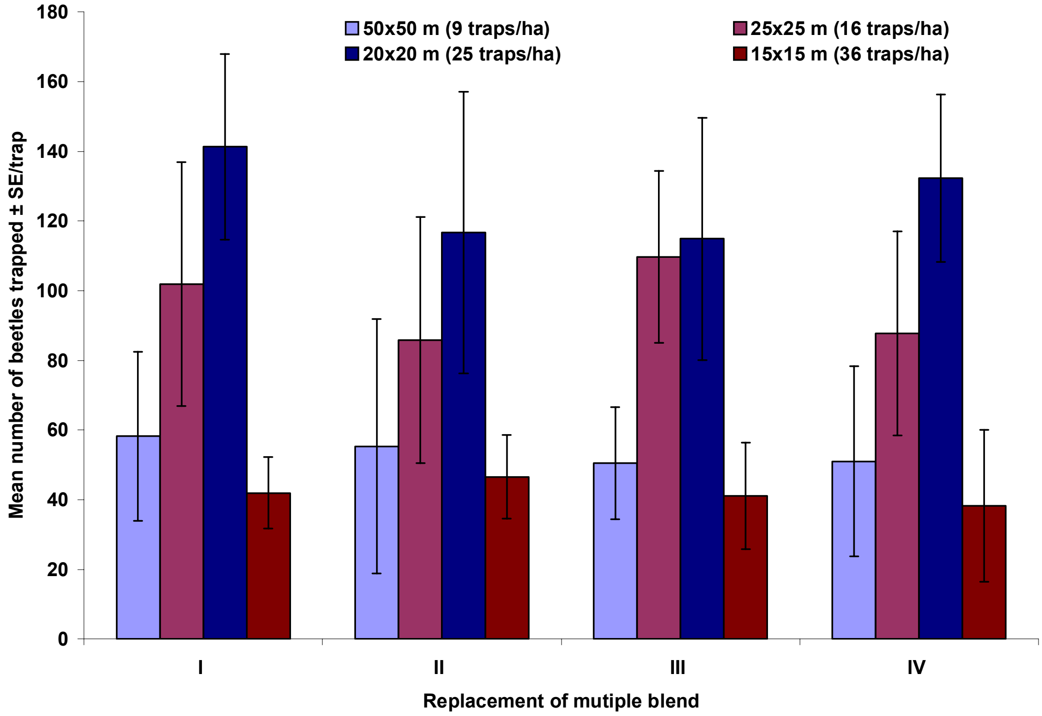


Fig.22. Influence of the number of traps/ha on SHB catch

3.6. Pilot scale bioassay studies on SHB attractant trap

The pilot scale experiment using multiple blend in MFT proved efficient trapping of SHB beetles. When the poly ethylene tube was filled with 150 l of standard attractant blend [(+) - a - pinene, (+) - trans-caryophyllene, (-) - b - phellandrene and (-)-germacrene-D at 10:1:.0.1:3] with fixer BHT in the field, the mean trap catch was high for 30 days (Fig. 23). During the experimental period, trapping of beetle during the month of installation of the trap was more when compared to the subsequent periods. From the assessment of SHB infestation in the treatment plots, there was a significant reduction in the number of adult female flying beetles when compared to the pre treatment infestation and a decline in the SHB infestation was also noted.

3.7. Large scale field evaluation of attractant trap

The large scale field study conducted at Valparai confirmed the results of pilot scale study. The trappings were continued for a period of two years (Fig. 24). When the infestation percentage increased in the field, there was concurrent increase in SHB trap catch. Mean trap catch increased from October to December and again from March to June under in the Anamallais (Fig. 24). Rain fall had a negative impact on trap catch.

The field trial conducted at a different location (Vandiperiyar) showed results similar to that obtained in Valparai. However, in Vandiperiyar region trap catch was more during September to December and for March to June (Fig. 25). Since both of the tested locations showed no significant variation in the trap catch, it may be concluded that the attractant trap can be used in all locations.

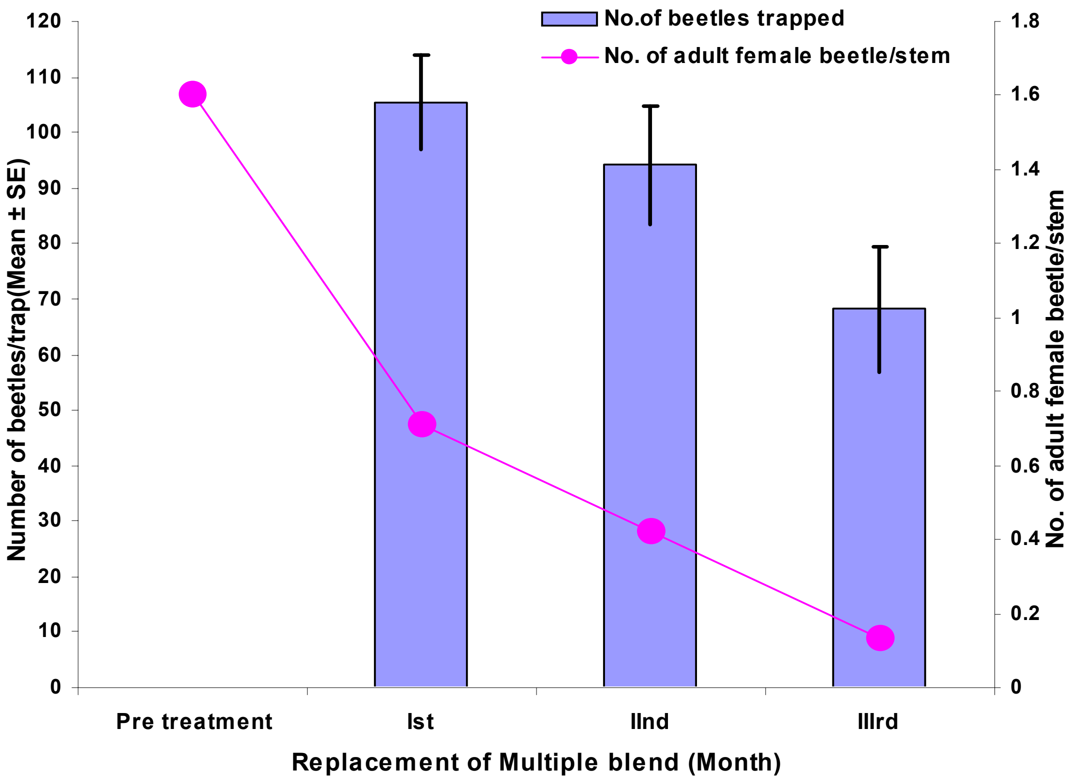


Fig.23. Pilot scale SHB trapping in the field

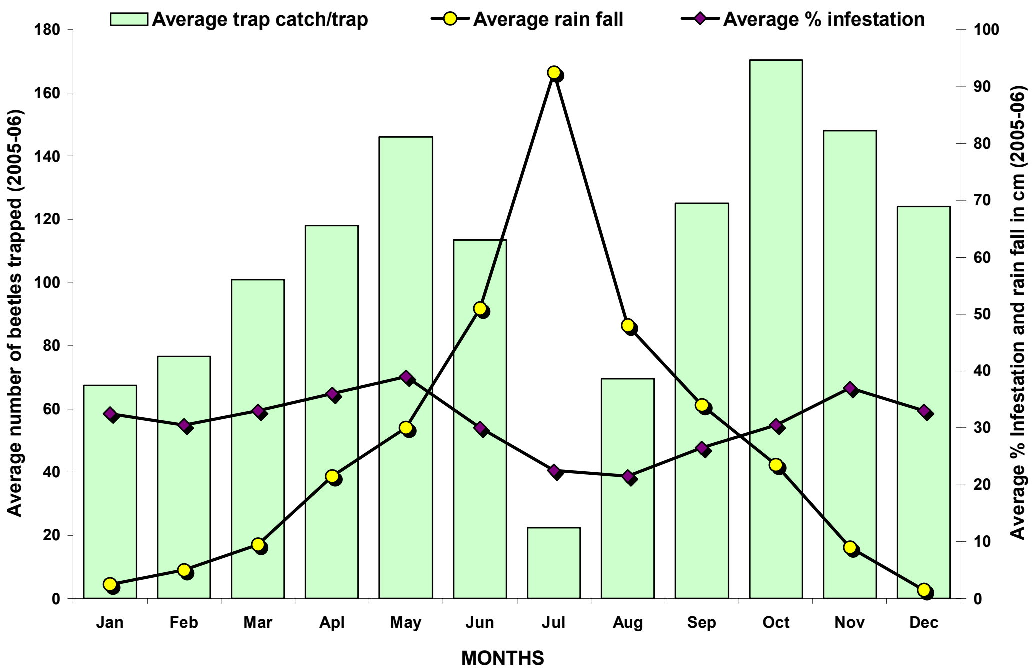


Fig.24. SHB trapping and weather conditions in the Valparai (2005 & 06)

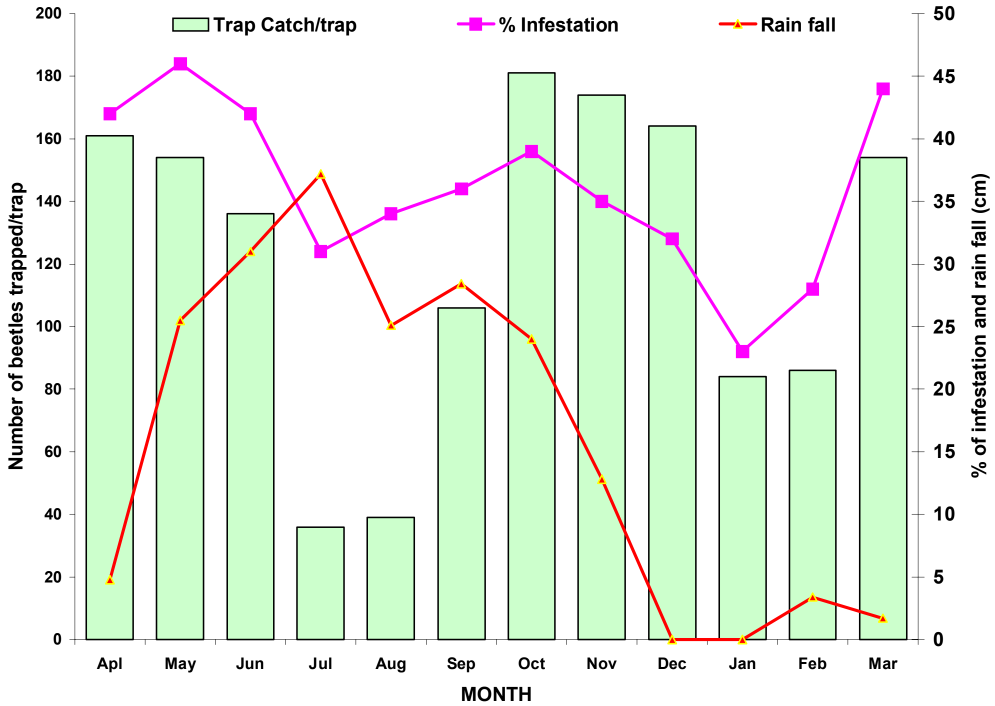


Fig.25. SHB trapping and weather conditions in Vandiperiyar (2006-07)

Discussion

DISCUSSION

In order to understand the chemical communication systems in insect in a better way and to maximize their potential for practical utility for pest management, the volatile semiochemicals emitted by insects and their host /associated plants must be analyzed both qualitatively and quantitatively (Heath and Manukian, 1992). Isolation, identification and quantification of the volatile compounds emitted from the partially dried stems of *Montanoa bipinnatifida* indicated the presence of four mono-terpenes and three sesquiterpenes. Many studies have demonstrated the ability of these monoterpenes and sesquiterpenes to elicit behavioural response in different insect families (Wibe *et al.*, 1998; Byers *et al.*, 2004; Miller and Borden, 1990; Rostelien *et al.*, 2000 and Sun *et al.*, 2004).

Antennal sensillae play a major role in eliciting behavioural response towards these semiochemicals. Schneider (1964) classified the insect sensillae into ten types based on their morphology. Female antennal club had more number of sensillae than that of males. This explains the wandering behaviour of female beetles of *Euwallacea fornicatus* in search of new areas in the host for making galleries and to establish the fungus, *Fusarium bugnicourtii,* for their emerging larvae. Indeed, female beetles are more exposed to the environmental conditions which need more number of sensillae for detection of different stimuli.

Influence of kairomones and allomones on the behaviour of several scolytid beetles have been reported (Byers, 1989; Vite and Baader, 1990; Wood, 1982). However, no information is available on the pheromones or kairomones of *Euwallacea fornicatus*, the shot hole borer infesting tea. Among the volatiles identified in the present study, (+)-a-pinene is known to be a common attractant for scolytid beetles (Klimetzek and Francke, 1980; Sun *et. al*., 2004) while (-)-b-phellandrene is reported as a kairomone for the beetle *Ips pini* (Millar and Borden, 1990). (-)-germacrene –D, a sesquiterpene, is reported as an attractant for the lepidopteran pest *Heliothis virescens* (Rostelien *et al.,* 2000). However, sesquiterpene, trans-caryophyllene alone has not been so far reported as an attractant for scolytid beetles. But, it was reported that (-)-trans -b- caryophyllene released from mountain ash has reduced the response of scolytid beetle *Pityogenes bidentatus* towards its aggregation pheromone (Byers *et al.,* 2004). In the present study, incorporation of trans-caryophyllene into the triple blend significantly enhanced the attraction of beetles both in the laboratory and field. Another sesquiterpene, (-) germacrene –D was reported to attract *Heliothis virescens* and on the other hand it acts as a masking substance in the case of the cerambycid beetle, *Monochamus alternatus* (Yamasaki *et al.,* 1997). In the present study, inclusion of (-) germacrene –D in the triple blend enhanced the attraction of beetle in the wind tunnel experiment and its presence was found essential to evoke behavioural response. Among the seven compounds tested in the wind tunnel, the response was high only towards four compounds. This proves the ability of beetles to discriminate among the different stimuli and in the presence of these compounds, they showed enhanced behavioural responses. Variation in the response and/or difficulty in eliciting beetle response to semiochemicals in wind tunnel experiments had been reported in many scolytids (Byers, 1988; Akers and Wood, 1989). However, the success in elucidating behavioural response of scolytid beetles in wind tunnel experiment had also been reported in many occasions (Birch and White, 1988; Bartelt *et al.,* 1990 and Domek *et al.,*1990). Study of insect behavior in a wind tunnel under controlled conditions is less complicated than the studies under field conditions wherein various abiotic factors interfere and influence the behaviour of the beetles. Although simulated conditions in the laboratory do not mimic those in nature, still it is possible to generate meaningful results. Wind tunnel studies using individual compounds enabled to narrow down the number of compounds from seven to four and it was helpful to arrive at a more efficient volatile blend for SHB.

Relationship between the monoterpenes and host selection behavior of scolytid beetles are strongly dose dependent. Lower concentrations can incite tunneling behaviour whereas higher concentrations act as repellant (Coyne and Lott, 1961; Raffa and Smalley, 1995). In SHB various degrees of attraction were achieved with respect to all the seven compounds at different concentrations. Dose response studies in the wind tunnel showed that a progressive response of *E. fornicatus* to (+)-a-pinene at its corresponding increase in concentration while (-)-b- phellandrene and (+) - trans- caryophyllene exhibited quadratic relationship. This indicated the ability of SHB to perceive (+)-a- pinene plumes from longer distances, even at lower concentrations. But behavioural response to the stimuli of (-)-b- phellandrene, (+) - trans- caryophyllene and (-)-germacrene-D was more at a closer range.

In general, insects respond to semiochemicals up to a certain concentration or require exposure to a well defined blend (Suckling and Karg, 1999). Blend of chemicals are the rule except in the case of sex pheromones of Lepidoptera and Coleoptera (Harris and Foster, 1991). In wind tunnel experiment on SHB, when a single component was exposed, the insect neither elicited enormous attraction towards the source nor showed upwind flight. It was proved that a multiple blend only elicited enormous behavioural response in SHB beetles compared to other tested blends. When the multiple blend was presented, beetles showed specific behaviour like hovering near the attractant lure or landing on the attractant lure wherein the mean percentage of attraction was two times higher than that of (+)-a- pinene tested individually. Mozuraitis and his co-workers (2002) reported the synergetic ability of germacrene-D and proved that the sesquiterpene acted as an oviposition stimulant in *Heliothis virescens*. In the present study, addition of germacrene-D along with triple blend exhibited synergism up to a particular level and its further increase had a negative impact. This finding coincided with the earlier findings of Lofstedt *et al.* (1991) and Witzgall *et al.* (1996). In multiple blend, when (-)-b-phellandrene concentration was increased from 0.1 parts to higher levels, mean percentage of attraction declined sharply and a similar trend was observed in the case of trans-caryophyllene also.

In addition to the chemical composition, the quantity of blend released from the dispenser is an important criterion in semiochemical based control/monitoring of insect species (Johansson *et al*., 2001). Among the dispensers polyethylene tube (PET) was the most suitable for trapping SHB in the field and the mean percentage of attraction achieved using PET was higher over the period of time. Eventhough, the efficiency of attraction gradually declined, satisfactory attraction of SHB was recorded upto 30 days in laboratory.

Matrix effects on release of volatiles are critically important in attracting insects (Ebeler *et al.*, 1988). When the volatile blend is reconstructed on the basis of dynamic headspace analysis (DHS), and put into a dispenser, the profile of volatiles released was substantially different from that measured initially in DHS, due to the interactions of compounds with the dispenser material (Millar and Sims, 1998). Oxidation of attractant blends can be restricted by the addition of antioxidants like butylated hydroxyl toluene (BHT) or butylated hydroxyanizole (BHA) (Millar and Sims, 1998). In the present study it was found that the PET dispenser with the antioxidant, BHT showed an optimum release rate and efficiently attracted the beetles.

Rate of release of multiple blend from PET lure was influenced by temperature and these results substantiate the earlier studies by Bradley *et al.* (1995) and Van der Kraan and Ebbers (1990) on the release rate of pheromone compounds at different temperature from polymeric and laminar dispensers. When the rate of release of the multiple blend from PET in the field was fitted into the linear regression model derived from the residual amount of blend at different temperatures, it was found the release rate of the blend and the residual amount recorded in the field fell between the values of these parameters recorded at 25 and 30°C. Since the tea ecosystem experiences a mean maximum temperature ranging between 25 and 30°C in most of the months, the expected release rate of the multiple blend from PET dispenser would be more or less similar to that observed between 25 and 30°C.

There is a report on dosage dependence of pheromones on the net up-tunnel velocity (behavioural response) of the oriental fruit moth (Harris and Foster, 1991). In SHB, there was a gradual increase in the mean percentage of attraction to an optimum level and further increase in quantity didn't show any significant increase in attraction. This coincides with the observation that increased load of pheromone compound, cis-9-trans-11-tetradecadien-1-yl acetate resulted gradual increase in trap catch of *Spodoptera littoralis* over a 20-day test period, while higher loadings resulted in little increase in trap catch (Campion *et al.*, 1974).

EAG studies of SHB beetles with the individual volatiles substantiated the wind tunnel results again confirming the ability of beetles to discriminate between the stimulus of different monoterpenes and sesquiterpenes. Compounds with significant EAG responses played an important role in attraction and was confirmed further from the field studies. Wallin and Raffa, (2000) explained that the concentration of compound is important in attraction studies especially in the case of kairomones. Dose response curves constructed from EAG's of SHB to the identified compounds revealed the differential threshold saturation level of each odour which was an indication for its role in behavioural modification and attraction.

Quantitative electrophysiological studies on the effects of 1-octen-3-ol on haemotophagous insects had shown the dose-dependent increase in EAG amplitude, where receptor saturation was reached at higher doses (Hall *et al.*, 1984; Schofield *et al.*, *1995*). In the present study, saturation was not attained towards (+)–a- pinene even at the higher tested dose. This revealed the ability of SHB to perceive a wide range of dosages of the respective compound which could mediate its behaviour. It was reported that, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) showed discrimination in EAG response to a- pinene and b- pinene stimulus which they interpreted as the insect's ability to differentiate different molecules of monoterpenes (Baehrecke *et al.*, 1989). However, *E. fornicatus* responded electro physiologically to all doses of (+)–a- pinene, while the response at these doses to (-)-b–pinene was negligible suggesting that shot hole borer was capable of discriminating not only among the volatile compounds but also to their isomeric forms.

EAG response in SHB to multiple blend was higher than that towards double and triple blends. Components of multiple blend at a specific ratio only elicited significant antennal response and change in this ratio led to decrease in the EAG response. This indicates the importance of the presence of all the four components in the blend at a specific ratio for enormous response. Eventhough the quantity of (-)-b–phellandrene used in the blend was very small, its presence enhanced the EAG response significantly.

Data generated on the quantity of blend per dispenser with EAG substantiated the results from wind tunnel experiment. Although marginal decrease in the response was noted at higher dosages, it could be attributed to the receptor saturation at higher doses. Similar results were reported in the case of *Plutella xylostella,* where higher dose of pheromone decreased insect activity (Macaulay *et al.*, 1986).

Field studies on the behaviour of beetles contributed valuable information for the efficient trapping of SHB. Although the behaviour of beetles on tea bushes was conducted under laboratory conditions, it was assumed that such movements would be similar to the behaviour under natural conditions. Activity of beetle in the field was noticed only during day time which is in agreement with the observations of Calnaido (1966). Majority of the SHB beetles flew only short distances except in few cases and these flyers were categorized as the individuals of inoculums to adjoining uninfected tea areas (Judenko, 1958b). Majority of female beetles flew above the level of tea bushes (Judenko, 1958b) but in the present study the number of SHB beetles which flew over the bushes was less when compared to those flew in the walking lane and this observation was strengthened by the higher number of beetle caught in the trap installed in the walking lane. Gadd (1941b, 1944) and Cranham (1963) felt that the flying capacity of beetles was low and they could probably fly only over very short distance and this observation is confirmed by the present study where the distance covered by the majority of the beetles in a single flight was very short. Based on the studies using sticky traps Judenko (1958b) concluded that beetles dispersed over a long distance only with the help of air currents.

Trap design and positioning in the field have profound influence on trap catch (Carde and Elkinton, 1984; Muirhead-Thomson, 1991). Trap designs significantly affected field trapping of SHB as well. Multiple funnel trap was more effective since it hardly allowed the beetles to escape and also due to increased trapping area (effective interception area). Beetles when attempting to land on trap hit a funnel and repeatedly contacted other funnels and fell into the collecting jar. The principle of this mode of action is identical to that of window flight traps (Chapman and Kinghorn, 1955). In the case of other trap designs like sticky board trap and cross vane trap apart from the trapping efficiency, the impact on non target organisms was more. Other difficulties like removing the captured insects as well as cleaning and repeated application of sticky coatings, which are labour-intensive in large scale operations were also encountered. Different trap designs have varying efficiencies of trapping of bark beetles because of different effective interception areas (Safranyik *et al.,* 2004). This is translated into the differences in the mean number of SHB beetles in different trap types tested in the present study.

It is possible that, the differences in the number of SHB capture in multiple funnel traps with different number of funnels was due to the enhanced exposed portions of the trap through which beetles could gain unhindered access into traps. Eventhough, MFT with six and seven funnels traps have more identical openings area than five-unit MFT, the observed mean capture of SHB was not significantly different which indicated that no significant improvement in trapping efficacy could be achieved beyond the optimum trap openings area. Results in the present study are in line with the observations made on a scolytid pest of stored grains, *Rhyzopertha dominica* (Edde, *et al.* 2005) in which trap catch was not significantly different in four and eight unit funnel trap.

Trap height and its position in the field are factors which affected the efficiency of trap catch (Barak *et al.,* 1991; Boucher *et al.,* 2001, Carde and Elkinton, 1984; David and Horsburgh, 1989; De Groot and DeBarr, 1998; Muirhead-Thomson, 1991). MFT traps installed at the middle and low levels were more efficient for trapping SHB. In several insect species, the trap catches are high within and above a crop canopy. In European corn borer, *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae), traps located 0.1 m below the canopy caught significantly more than those located at 0.5 m above (Barlet *et al.,* 1994). Among the positions tested MFT traps placed in walking lanes were more effective. David and Horsburgh (1989) found that maximum number of the moths of leaf roller, *Platynota flavedana* Clemens (Lepidoptera: Tortricidae) were trapped outside the apple tree canopy. The reasons for more attraction of SHB in middle and low levels are mainly due to the slow flight behaviour and the position on the plant in which it makes the galleries. SHB galleries are more profound on pencil thick stems just above the pruning cut which was always in the middle region of the plant. More over at top level, the influence of abiotic factors like temperature and wind speed was more when compared to that of other levels, which may affect the rate of release of the blend from PET dispenser and thereby the trap catch.

Rate of release of attractant from different dispensers highlighted the variation in the ability to attract. Polyethylene tube dispenser containing multiple blend with BHT trapped significantly higher number of beetles and addition of antioxidant enhanced the attraction through the controlled release. This is similar to the results of study on *Plutella xylostella* pheromone dispenser (Macaulay *et al.,* 1986).

Field studies using individual volatile compounds re-confirmed the laboratory results where higher SHB attraction was obtained with four compounds. All combinations made without (+)-a-pinene didn't show any significant attraction. From this observation it was proved that (+)-a-pinene was a critical compound for the trapping of SHB. When (+)-a-pinene was mixed with one of the other three compounds and presented as double blend, it attracted more number of beetles than (+)-a-pinene alone. And among the double blends of (+)-a-pinene with (+)-trans-caryophyllene attracted more number of beetles. This indicated the importance of (+) - trans-caryophyllene in the blend. When the third compound, (-)-b-phellandrene was added in a small quantity to the most successful double blend, increase in the attraction was high confirming the synergetic effect of (-)-b-phellandrene in the blend and (-)- b - phellandrene is well known for its synergistic effect with pheromones of *Ips latidens* and *Ips pini* (Miller and Borden, 1990). Eventhough most efficient triple blend attracted significant number of beetles, addition of germacrene- D to this blend enhanced the mean attraction which justified the presence of (-)-germacrene-D in the blend.

Trap catch at the field level was influenced by an array of factors including the ratio of a compound in the blend (Kumar and Shivakumara, 2003). When the ratio of trans-caryophyllene increased from one to two parts in the multiple blend, a decline in attraction was observed. This indicated that increase of this particular compound beyond one part in the multiple blend had a negative effect whereas the increase of germacrene-D from one to three parts had a positive effect.

De Groot and De Barr (1998) described that among the factors which affected efficiency of pheromone baited traps, dosage of pheromone per trap is very important. Maximum SHB attraction was achieved when 150 l of multiple blend and a further increase in quantity of blend per trap didn't show any further significant enhancement in attraction. Campion *et al.,* (1974) reported that increase in the quantity of pheromone compound of *Spodoptera littoralis* from 10 to 500 g resulted in a progressive increase in trap catch but further increased loadings between 500 and 5000 g resulted only in a moderate increase in trap catch.

There was practically no SHB catch in the trap after 35 days of exposure which must be due to the exhaustion of the multiple blend through evaporation and the change in rate of release. Present study proved that release rate decreased rapidly to sub optimum levels with the extended time, which was clear from the reduced SHB catch. This is similar to the results of work done by Hormeyr and Burger (1995) on polyethylene vial pheromone dispenser for the false codling moth, *Cryptophlebia leucotreta* which maintained an acceptable rate of release of pheromone during first four weeks, after which there was a rapid and permanent reduction.

Number of traps required per hectare is also an important factor for efficient trapping of SHB on a large scale and its economics. The efficient and cost effective way of installing the trap would be at a spacing of 20 x 20m distance (25 traps per hectare). Traps installed at larger spacing were not found effective because all the female beetles are not good flyers, the chances of reaching the trap from a long distance was limited. On the other hand, traps installed at lower spacing also resulted in low trap catch because as the number of traps per hectare increased, the probability of SHB encounter with trap increased but the mean trap catch per trap decreased. Trematerra (1993) considered three trap densities six, twelve and twenty four traps/ hectare, in mass trapping of tea tussock moth, *Synathedon myoapaeformis* and concluded that 12 traps/hectare was optimal, effective and economical. It can be concluded that optimum number of trap required per hectare is 25 which would be cost effective when an IPM schedule would be adopted for SHB management.

Pilot scale study on efficient trap with multiple blend warrants the replacement of PET dispenser at an interval of 30 days. This observation was strengthened by the decline in mean trap catch per trap. Field experiment conducted for a period of two years in Valparai region and one year at Vandiperiyar region helped to conclude the time and period of attractant trap installation in these two tea growing regions. Rain fall had adversely affected the trap catch. Eventhough the beetles were hiding inside the gallery when the tea stems are wet, enormous beetle activity was noticed outside the gallery when there was a break in rain for three to four hours. Population fluctuations observed in Vandiperiyar and Valparai coincided with the observation made by Muraleedharan (1986 a&b). He had reported that there was no correlation among the beetle population, temperature and rainfall. In the case of attractant trap, it was found that rain fall resulted in the variation of trap catch. Based on the results, it can be proposed that suitable time for the installation of trap would be between September and December as well as March and June in tea fields at Valparai region. In Vandiperiyar region the installation of attractant traps except in the month of July and August would give the desired trap catch.

Based on the results of the current investigation, it is evident that the developed attractant trap can be incorporated into the integrated pest management programme of shot hole borer.

Summary

SUMMARY

Methodology for extraction, isolation, identification and quantification of volatiles emanated from the partially dried cut stems of *Montanoa* were standardized using DHS aeration technique, GC-MS and GC-FID.

Volatile chemicals released from the partially dried cut stems were identified on the basis of standard procedures (Comparison of retention time with the authentic standards, co-injection with the standards (spiking test) and by comparison with the mass spectral library).

GC-MS studies revealed that seven important volatile compounds (four monoterpenes and three sesquiterpenes) were released from the partially dried stems of *M. bipinnatifida*: They are 1). (+) - a-pinene 2) (-) - b-pinene 3) (R) - (+)-limonene 4) (-)-b-phellandrene 5) (-)-Iso-caryophyllene 6)(+)- trans-caryophyllene and 7) (-) -germacrene-D.

GC-FID studies reveled that the compounds, (+)-a-pinene and (-)-germacrene-D were present in higher quantity.

Wind tunnel and electro antennogram studies authenticated that all the synthetic/ isolated compounds particularly (+)-a-pinene and trans-caryophyllene evoked higher response in SHB followed by (-)-b-phellandrene and (-)-germacrene-D.

Dose response studies in wind tunnel experiment showed that the mean percentage attraction of SHB beetles to (+)-a-pinene and (-)-germacrene-D were higher when compared to other compounds. EAG studies confirmed the results.

Among the blends tested, a multiple blend of four components (+)-a-pinene, (+) – trans-caryophyllene, (-)-b-phellandrene and (-)-germacrene-D) was found superior to all other tested blends.

Studies on optimization of the ratio of components in the multiple blends proved that (+)-a-pinene,(+)-trans-caryophyllene, (-)-b-phellandrene and (-)-germacrene-D at a rate of 10: 1.0: 0.1: 3 was optimum which was proved from the results of EAG and wind tunnel experiments.

Observations were made on the behaviour of beetle outside the gallery and the behavioural response towards the attractant trap in natural condition was studied.

Polyethylene tube (PET) was found to be a suitable dispenser for releasing the attractant. Addition of a fixer, BHT to the standard blend helped to regulate the rate of release of blend from the dispenser which increased both the attraction in laboratory and the SHB trapping in field.

Rate of release of multiple blends from PET at different temperature regimes showed that the rate of release and temperatures are directly proportional to each other. It was also proved that the residual amount of multiple blends recorded in the field coincided with the values of residual amount recorded in controlled conditions.

Optimization studies in wind tunnel and EAG on the quantity of multiple blend required per dispenser proved that 150 ml is ideal for SHB trapping. PET dispenser has an ability to withstand without decreasing its capacity to attract SHB drastically up to 30 days.

Among the field tested trap designs, multiple funnel trap (MFT) was found to be the best trap for SHB and experiment on the number of funnel required per trap revealed that MFT with five funnels was optimum for efficient trapping of SHB.

Studies on different trap heights in the field showed that traps installed at 15 cm below the tea bush canopy (middle level) and 15 cm above from the ground (low level) was suitable for the SHB trapping.

Among the three trap positions tested the most suitable position for the attractant trap installation in the field was in the walking lanes.

Optimization on the trap density per hectare revealed that 25 - five unit MFT traps placed at a spacing of 20 x 20 m was ideal. Multiple blend of 150 ml quantity with BHT in a PET dispenser was found to be the best lure for SHB trapping.

Pilot scale study using the proven attractant blend and traps placed at the established trap height and position was found to be more appropriate.

Large scale field studies conducted at two different locations proved the efficiency of the newly developed attractant trap for SHB.

In Valparai region the suitable time for installing the attractant trap is during September to December and from March to June whereas in Vandiperiyar region installation of traps except in the month of July and August is suggested.

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* Referred abstracts only/not seen the originals