INVESTIGATIONS ON CHEMICAL ECOLOGY AND BEHAVIOUR OF BANANA PSEUDOSTEM WEEVIL *Odoiporus longicollis* Olivier (COLEOPTERA: CURCULIONIDAE)

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G.K.V.K., BENGALURU - 560 065

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Thesis submitted to the

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in partial fulfillment of the requirements

for the award of the Degree of

DOCTOR OF PHILOSOPHY

in

AGRICULTURAL ENTOMOLOGY

BENGALURU **NOVEMBER, 2016**

Affectionately Dedicated to

My Beloved Parents,

My Chairman

Dr. A. K. Chakravarthy

& Co-chairman

Dr. (Mrs.) K. R. M. Bhanu

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BENGALURU 560 065

CERTIFICATE

This is to certify that the thesis entitled **"Investigations on chemical ecology and behaviour of Banana pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae)"** submitted in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **AGRICULTURAL ENTOMOLOGY** to the University of Agricultural Sciences, Bengaluru, is a record of *bona fide* research work done by **Ms. RANI, A. T., ID No. PALB 3011** during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
November, 2016

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Bengaluru

November, 2016 ***(RANI, A. T.)***

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RANI, A. T.

ABSTRACT

The Banana pseudostem weevil, *O. longicollis* is a monophagous pest of banana and plantains. To date, no reports are available regarding the chemical identification of pheromone of *O. longicollis*. Current study was undertaken to identify pheromone compounds for developing semio-chemical based management. The calling and mating pattern of *O. longicollis* was observed throughout the day. The peak mating period occurred during 6th and 9th hour of photophase when 100% of the pairs mated. The maximum percentage of calling females were observed between 6th to 10th hour photophase, coinciding with peak mating period. GC and GC-EAD analysis of male volatiles against male and female antennae followed a similar pattern of peaks at 5. 935RT, 6.005RT, 6.465RT and these peaks were identified as 1-Hexadecene (19.862), Hexadecane (19.992) and Diphenylamine (20.479), respectively through GC-MS. The results from EAG, Y-tube olfactometer and field cage trial of the different blend combinations of synthetic pheromone compounds suggested that the response of male and female weevils was higher to the blend of Diphenylamine: 1-Hexadecene: Hexadecane at 0.5:10:20 followed by 100:08:05. This blend evoked increased electrophysiological and behavioural responses with increased doses in both the sexes. The cross vane trap was found better for trapping adult weevils. Release of pheromone compounds occurred during the photophase. The quantity of pheromone compounds was higher in volatiles collected from only males than males + females and the maximum production was recorded between 02.00PM-04.00PM. The production of pheromone compounds depended on the presence of host plant and insects feeding on pseudostem released more volatiles than in the absence of food. The results from EAG and Y-tube olfactometer assay revealed that healthy and mechanically damaged pseudostem extracts elicited significantly higher response in males and females. The combination of male and plant volatiles are more attractive to weevils than to plant volatiles alone.

November, 2016

Department of Agricultural Entomology
CoA, UAS, GKVK, Bangalore – 65

Dr. A. K. Chakravarthy
(Major Advisor)

ಬಾಳೆಯ ಮೆದುಕಾಂಡಕೊರಕ, *ಓಡಿಯೋಪೊರಸ್ ಲಾಂಗಿಕಾಲಿಸ್* (ಕೋಲಿಯಾಪ್ಟೇರಾ: ಕುರ್ಕುಲಿಯೋನಿಡೆ) ದ ರಾಸಾಯನಿಕ ಪರಿಸರ ವಿಜ್ಞಾನ ಮತ್ತು ಅದರ ಸ್ವಭಾವಗಳ ಕುರಿತ ಅಧ್ಯಯನ

ರಾಣಿ, ಎ. ಟಿ.

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಬಾಳೆಯ ಮೆದುಕಾಂಡಕೊರಕವು *ಓಡಿಯೋಪೊರಸ್ ಲಾಂಗಿಕಾಲಿಸ್* ಒಂದೇ ಬಗೆಯ ಬಾಳೆಗಿಡವನ್ನು ತಿನ್ನುವ ಕೀಟವಾಗಿದ್ದು, ಇಲ್ಲಿಯವರೆಗೆ ಓ. *ಲಾಂಗಿಕಾಲಿಸ್* ಲಿಂಗಾಕರ್ಷಕದ ರಾಸಾಯನಿಕ ಗುರುತಿನ ಬಗ್ಗೆ ಯಾವುದೇ ವರದಿಗಳು ಲಭ್ಯವಿಲ್ಲ. ಸೆಮಿಯೋಕೆಮಿಕಲ್ ಆಧಾರಿತ ನಿರ್ವಹಣಾ ಪದ್ದತಿಯನ್ನು ಅಭಿವೃದ್ಧಿ ಪಡಿಸಲು ಲಿಂಗಾಕರ್ಷಕದ ಸಂಯುಕ್ತಗಳನ್ನು ಗುರುತಿಸುವ ಅಧ್ಯಯನವನ್ನು ಕೈಗೆತ್ತಿಕೊಳ್ಳಲಾಗಿದೆ. *ಓ. ಲಾಂಗಿಕಾಲಿಸ್* ಜೀರುಂಡೆಯ ಕರೆ ಮತ್ತು ಮಿಲನದ ಮಾದರಿಯನ್ನು ದಿನವಿಡೀ ಗಮನಿಸಲಾಯಿತು. ಗರಿಷ್ಠ ಶೇಕಡಾವಾರು (ಶೇ.೧೦೦) ಮಿಲನದ ಅವಧಿಯು ಬೆಳಕಿನ ೬ ಮತ್ತು ೯ ನೇ ಗಂಟೆ ಸಮಯದಲ್ಲಿ ಸಂಭವಿಸಿದೆ. ಕಾಕತಾಳೀಯವಾಗಿ ಅಧಿಕ ಪ್ರಮಾಣದ ಕರೆಹೆಣ್ಣು ಜೀರುಂಡೆಗಳನ್ನು ಗರಿಷ್ಠ ಮಿಲನದ ಅವಧಿಯಲ್ಲಿ, ಅಂದರೆ ಬೆಳಕಿನ ೬ ಮತ್ತು ೧೦ ಗಂಟೆ ಸಮಯದ ನಡುವೆ ಗಮನಿಸಲಾಯಿತು. ಗಂಡು ಮತ್ತು ಹೆಣ್ಣು ಜೀರುಂಡೆಗಳ ಕುಡಿಮೀಸೆ ಜೊತೆ ಗಂಡಿನ ಬಾಷ್ಪಶೀಲವನ್ನು ಅನಿಲ ವರ್ಣಚಿತ್ರಣ–ವಿದ್ಯುತ ಗ್ರಾಹಕ ಪ್ರಸರಣ ಪತ್ತೆಯ ವಿಶ್ಲೇಷಣೆಯ ಮೂಲಕ ವಿಶ್ಲೇಷಿಸಿದಾಗ ಎರಡೂ ಸಂದರ್ಭಗಳಲ್ಲಿ ಧಾರಣಾ ಸಮಯವು ೫.೯೩೫, ೬.೦೦೫ ಮತ್ತು ೬.೪೦೫ ನಿಮಿಷಗಳಷ್ಟಿದ್ದು ಒಂದೇ ಮಾದರಿಯ ಶೃಂಗವನ್ನು ಅನುಸರಿಸುತ್ತವೆ ಹಾಗೂ ಅನಿಲ ವರ್ಣಚಿತ್ರಣ – ತೂಕ ವರ್ಣಪಟಲ ಮಾಪಕ ವಿಶ್ಲೇಷಣೆಯ ಮೂಲಕ ಅನುಕ್ರಮವಾಗಿ ೧–ಹೆಕ್ಸಡೆಸೀನ್, ಹೆಕ್ಸಡೆಕೇನ್ ಮತ್ತು ಡೈಫಿನೈಲ್‌ಅಮೈನ್ ಎಂದು ಗುರುತಿಸಲಾಯಿತು. ಕೃತಕ ಲಿಂಗಾಕರ್ಷಕದ ಸಂಯುಕ್ತಗಳನ್ನು ವಿವಿಧ ಮಿಶ್ರಣ ಸಂಯೋಜನೆಯಲ್ಲಿ –ವಿದ್ಯುತ ಗ್ರಾಹಕ ಪ್ರಸರಣ ಪತ್ತೆ, ವೈ–ಟ್ಯೂಬ್ ಓಲ್‌ಫ್ಯಾಕ್ಟೋಮೀಟರ್ ಮತ್ತು ಕ್ಷೇತ್ರ–ಬಲೆ ಪ್ರಾತ್ಯಕ್ಷಿಕೆಗೆ ಒಳಪಡಿಸಲಾಯಿತು ಹಾಗೂ ಈ ಪ್ರಾತ್ಯಕ್ಷಿಕೆಗಳ ಫಲಿತಾಂಶಗಳ ಪ್ರಕಾರ ಗಂಡು ಮತ್ತು ಹೆಣ್ಣು ಜೀರುಂಡೆಗಳ ಪ್ರತಿಕ್ರಿಯೆಯು ಹೆಚ್ಚಿನ ಸಂಖ್ಯೆಯಲ್ಲಿ ೦.೫:೧೦:೨೦ ಅನುಪಾತದಲ್ಲಿಯೂ ನಂತರ ೧೦೦:೦೮:೦೫ ಅನುಪಾತದ ಉಪಚಾರದಲ್ಲಿಯೂ ಕಂಡುಬಂದಿದೆ. ಈ ಮಿಶ್ರಣವು ಎರಡೂ ಲಿಂಗಗಳಲ್ಲಿ ಹೆಚ್ಚಿನ ಪ್ರಮಾಣದ ಜೊತೆಗೆ ಹೆಚ್ಚಿನ –ವಿದ್ಯುತ ಗ್ರಾಹಕ ಪ್ರಸರಣವನ್ನು ಮತ್ತು ವರ್ತನೆಯ ಪ್ರತಿಸ್ಪಂದನೆಯನ್ನು ಹೆಚ್ಚಿಸಿತು. ಅಡ್ಡ ದಿಕ್ಸೂಚಿ ಎಂಬ ಅಂಟಿನ ಬಲೆಯು ವಯಸ್ಕ ಜೀರುಂಡೆಗಳನ್ನು ಹಿಡಿದು ಕೊಲ್ಲುವುದರಲ್ಲಿ ಉತ್ತಮವಾಗಿ ಕಂಡುಬಂದಿದೆ. ಜೀರುಂಡೆಗಳು ಬೆಳಕಿನ ಸಮಯದಲ್ಲಿ ಲಿಂಗಾಕರ್ಷಕವನ್ನು ಹೊರಸೂಸುವುದು ಕಂಡುಬಂದಿದೆ. ಗಂಡು ಜೀರುಂಡೆಗಳನ್ನು ಮಾತ್ರ ಒಂದೆಡೆ ಕೂಡಿಟ್ಟು ಅವುಗಳಿಂದ ಸಂಗ್ರಹಿಸಿದ ಬಾಷ್ಪಶೀಲ ವಸ್ತುವಿನಲ್ಲಿ ಲಿಂಗಾಕರ್ಷಕ ಸಂಯುಕ್ತಗಳ ಪ್ರಮಾಣವು ಗಂಡು ಮತ್ತು ಹೆಣ್ಣು ಜೀರುಂಡೆಗಳನ್ನು ಕೂಡಿಟ್ಟು ಸಂಗ್ರಹಿಸಲ್ಪಟ್ಟ ಬಾಷ್ಪಶೀಲಕ್ಕಿಂತ ಹೆಚ್ಚಾಗಿರುವುದು ದಾಖಲಾಗಿದೆ ಹಾಗೆಯೇ ಲಿಂಗಾಕರ್ಷಕದ ಗರಿಷ್ಠ ಉತ್ಪಾದನೆಯು ಮಧ್ಯಾಹ್ನ ೦೨:೦೦ ಮತ್ತು ೦೪:೦೦ ಗಂಟೆಯ ನಡುವೆ ಅಧಿಕವಾಗಿರುತ್ತದೆ.ಲಿಂಗಾಕರ್ಷಕ ಸಂಯುಕ್ತಗಳ ಉತ್ಪಾದನೆಯು ಆಹಾರ ಸಸ್ಯಗಳ ಉಪಸ್ಥಿತಿಯನ್ನು ಅವಲಂಭಿಸಿದೆ ಹಾಗೆಯೇ ಜೀರುಂಡೆಗಳು ಆಹಾರದ ಅನುಪಸ್ಥಿತಿಯಲ್ಲಿ ಬಿಡುಗಡೆಮಾಡುವ ಬಾಷ್ಪಶೀಲಕ್ಕಿಂತ, ಆಹಾರವನ್ನು ತಿನ್ನುವ ಸಂದರ್ಭದಲ್ಲಿ ಹೆಚ್ಚು ಬಾಷ್ಪಶೀಲವನ್ನು ಹೊರಸೂಸಲ್ಪಡುವುದು ಕಂಡುಬಂದಿದೆ. ವಿದ್ಯುತ ಗ್ರಾಹಕ ಪ್ರಸರಣ ಪತ್ತೆ ಮತ್ತು ವೈ–ಟ್ಯೂಬ್ ಓಲ್ ಫ್ಯಾಕ್ಟೋಮೀಟರ್ ವಿಶ್ಲೇಷಣೆಯ ಫಲಿತಾಂಶಗಳ ಪ್ರಕಾರ ಆರೋಗ್ಯಕರ ಮತ್ತು ಯಾಂತ್ರಿಕವಾಗಿ ಹಾನಿಗೊಳಗಾದ ಬಾಳೆದಿಂಡಿನ ಸಾರಗಳು ಗಣನೀಯವಾಗಿ ಗಂಡು ಮತ್ತು ಹೆಣ್ಣು ಜೀರುಂಡೆಗಳೆರಡರಲ್ಲಿಯೂ ಹೆಚ್ಚಿನ ಪ್ರತಿಕ್ರಿಯೆಯನ್ನು ಬಹಿರಂಗಪಡಿಸಿವೆ. ಜೀರುಂಡೆಗಳಿಗೆ ಕೇವಲ ಸಸ್ಯಾಧಾರಿತ ಬಾಷ್ಪಶೀಲಕ್ಕಿಂತ ಗಂಡು ಲಿಂಗಾಕರ್ಷಕ ಮತ್ತು ಸಸ್ಯ ಬಾಷ್ಪಶೀಲ ಸಂಯೋಜಿತ ಮಿಶ್ರಣವು ಹೆಚ್ಚು ಆಕರ್ಷಕವಾಗಿರುತ್ತದೆ.

ನವೆಂಬರ್, ೨೦೧೬

ಕೃಷಿ ಕೀಟ ಶಾಸ್ತ್ರ ವಿಭಾಗ
ಕೃ.ವಿ.ವಿ., ಜಿ.ಕೆ.ವಿ.ಕೆ, ಬೆಂಗಳೂರು–೬೫

ಡಾ. ಎ.ಕೆ. ಚಕ್ರವರ್ತಿ
(ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರು)

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I INTRODUCTION

Banana, *Musa* spp. is the fourth most important fruit crop, commonly grown in tropical and subtropical parts of the world. India is the largest producer in the world and banana occupies top position among total fruit production in India. It is cultivated in India in 0.822 million ha with production of 29.221 million MT (National Horticulture Board, 2014). In select countries, bananas used for cooking may be called plantains. *Musa* species are native to tropical Indo-Malaysia and Australia and are first domesticated in Papua New Guinea (Nelson *et al.*, 2006*).* They are grown in at least 107 countries (Anon., 2005), primarily for their fruit and to a lesser extent to make fiber, banana wine, banana beer and as ornamental plant.

Buddhist texts of 600 BC for the first time in history mention banana as a highly nutritive food. Travelagues of 327 BC mention that Alexander the Great discovered the taste of banana in the valleys of India. In the year 200 AD, China had an organized banana plantation. In 1502 AD, colonists started the first banana plantation in the Caribbean and in Central America. Thus, humans have used banana as a staple food for thousands of years. Also, it is interwoven with national heritage and culture. It is one of the oldest fruits known to mankind. In India, banana crop accounts for 2.8 per cent of agricultural GDP. It is an important crop for subsistence farmers and ensures year-round security for food or income. Most of the banana is produced on a small scale basis in different production systems (http://www.naushiebanana.com/Indian_banana_history.php). The phenomenal increase in production has been due to adoption of high density planting, use of tissue-cultured seedlings and drip irrigation which significantly improved productivity.

Like other fruit crops, banana too, is exposed to a wide variety of biotic and abiotic constraints. The incidence of insect pests is one of them. A total of 470 species of insects and mites were reported to infest banana (Ostmark, 1974). More than 180 species of insect pests have been recorded on banana world over (Simmonds, 1966), of which 30 are major (Wadhi and Batri, 1964). In India Banana is infested with 19 insect pests from planting to harvesting (Padmanaban *et al.*, 2001). Of these, the banana pseudostem weevil (BSW) or banana pseudostem borer, *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) is gaining importance as a serious pest causing heavy losses to the grower. It has been a threat to banana under garden-land cultivation and their occurrence in nontraditional areas of Tamil Nadu has been reported (Justin *et al.*, 2008). The weevil is becoming increasingly severe in South India particularly in Tamil Nadu and Kerala. Currently in India, this weevil is posing a serious threat to the banana cultivation in Andaman Islands, Uttar Pradesh, Bihar, West Bengal, Assam, Kerala, Tamil Nadu and Karnataka (Ravi and Palaniswami, 2002). BSW distribution and severity of infestation in South Karnataka was not related to geographical location but influenced by the varieties cultivated (Thippaiah *et al.*, 2010).

The banana weevil, *O. longicollis* for the first time is recorded from Kerala in India (Visalakshi *et al.*, 1989). The BSW is believed to have originated in South and South East Asia, which is also the centre of origin of the present day bananas and plantains. Banana pseudostem weevil is a monophagous pest (Visalakshi *et al.,* 1989; Valmayor *et al.,* 1994). Adult weevils are attracted by the volatiles released by the banana plants. Infestation of the weevil normally starts in five-month-old plants (Justin *et al*., 2008). Adult females of *O. longicollis* lay eggs inside the air chamber of the outer sheath of the pseudostem through holes made by its rostrum. Emerging grubs make extensive tunnels in the pseudostem for feeding and pupate inside the pseudostem to become adults. Early symptoms of the infestation are the presence of small pin-head-sized holes on the stem, exudation of a gummy substance from such holes and fibrous extrusions from bases of leaf petioles. Extensive tunnelling both in the leaf sheath and pseudostem occurs at the advanced stages of infestation. Rotting occurs due to secondary infection of pathogens and a foul odour is emitted. When the true stem and peduncle are tunnelled after flowering, the fruits do not develop properly, presenting a dehydrated condition with premature ripening of the bunch itself. In severely infested plantations, more than 20 % plants do not flower (Padmanaban and Sathiamoorthy, 2001). Although the weevil has been described as an important pest, there are no available data on plant loss and/or yield reductions. The severity of the loss is greater when infestation occurs at the early vegetative stage (5 months old). It is estimated that banana pseudostem weevil incurs 10-90 per cent yield loss depending on the growth stage of the crop and management efficiency (Padmanaban and Sathiamoorthy, 2001). The problem is generally noticed only when the damage is in the advanced stage and grubs are fully grown (Padmanaban *et al.,* 2001). The pest status of the banana weevil varies depending on agro-ecological conditions (Gold *et al*., 2001). The biology, ecology, seasonal incidence and chemical control of Banan psuedostem weevil has been previously studied in detail ((Anitha, 2000; Padmanaban *et al.,* 2001; Azam *et al.,* 2010; Thippaiah *et al.,* 2012; Priyadarshini *et al.,* 2014 and Khairmode *et al.,* 2015).

Owing to its restricted feeding habit and monophagy, *O. longicollis* may use specific host plant volatiles to find host for feeding and oviposition. Exploiting these Behaviourally active plant volatiles as a potential tool for monitoring and mass trapping purposes offers an ecofriendly management option. Many studies reported that use of pseudostem traps as well as pseudostem pieces with aggregation pheromone become less popular due to difficulties in maintenance of host tissue in pheromone baited traps for longer periods and becomes less efficient due to rapid dehydration leading to decrease in the attractiveness after few days (Gunawardena and Dissanayake, 2000b; Tinzaara *et al*., 2007). Decayed pseudostem pieces and its extract have attractant property against *O. longicollis.* Insect and mechanical damage induce changes in the volatiles profile of plants that may serve as better olfactory cues to locate hosts and conspecifics (Bolter *et al*., 1997 and Landolt *et al*., 1999). Hence, based on these reports, an attempt was made to screen pseudostem extracts of banana plants of different physiological states to determine which type of plant volatile is most attractive to *O. longicollis.*

Farmers adopt several control measures, but all have not been effective or validated or integrated with other practices. Owing to some of the drawbacks and also the improper handling of technologies like burning of infested plants, use of pseudostem traps, stem injection with moncrotophos, use of *Beauveria bassiana* and Entomopathogenic nematode on split banana stem. The endophytic Behaviour of the larvae and long life span of adults complicate the management of this pest and insecticides failed to reach them. Hence, alternative control methods like semio-chemical based pest management that is safe, specific, effective, efficient, cheap and environmentally safe are warranted.

Semiochemicals or insect Behaviour modifying chemicals, that include pheromones, have proved to provide effective control (Welter *et al.*, 2005). The use of aggregation pheromones, in combination with host volatiles, has led to the development of mass trapping as a control strategy for several pest weevil species (Alpizar *et al.,* 2002 and Weissling *et al.,* 1994). Literature also cites the successful management of weevil populations in cotton, coconut and sweet potato using aggregation pheromones (Dickerson *et al.,* 1987; Chinchilla *et al.*, 1996 and Downham *et al.,* 2001). Sordidin, the aggregation pheromone of *Cosmopolites sordidus* Germar, another important pest of banana, closely related to *O. longicollis* has been identified, synthesized and a commercial formulation has been developed and successfully used for the control of the pest (Jayaraman *et al.,* 1997 and Tinzaara *et al.*, 2000). Several attempts have been made to optimize and develop semiochemical based methods for the management of *O. longicollis* but these were not successful.

Information on the Behaviour of the *O. longicollis* is scanty. Hence, in order to understand the insect for efficient management, a detailed study on insect Behaviour was taken up. Studying the Behaviour of the insect may provide evidences for semiochemicals used for attraction and mate recognition that could be used to develop new control strategies (Martins *et al.,* 2013). Hence, studies were also carried out for identification of volatiles released by male banana pseudostem weevils. Gunawardena and Dissanayake (2000a) reported that male banana pseudostem weevil produced a pheromone, 2-methyl-4-heptanol which attracted both male and female banana weevils. Preliminary studies on *O. longicollis* by few workers reported the existence of female produced sex pheromone (Ravi and Palaniswami, 2002) and male produced aggregation pheromones (Prasuna *et al.,* 2008). But, to date, no reports are available providing information about the chemical identification of aggregation pheromone of *O. longicollis.* Considering these facts, a study was formulated "Investigations on chemical ecology and Behaviour of Banana pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae)". The study embraced following objectives:

1. To elucidate Behavioural responses of Banana pseudostem weevil related to pheromones
2. Chemical identification of pheromones of Banana pseudostem weevil
3. Laboratory and field evaluation using identified pheromone components

II REVIEW OF LITERATURE

The available literature pertaining to banana pseudostem weevil (BSW), *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) taxonomy, distribution, bioecology, calling and mating Behaviours, identification of pheromone components and bioassay, laboratory and field evaluation of pheromones are compiled and reviewed in this chapter, under the following headings.

2.1 Taxonomy

Curculionidae is one of the largest families of insects with approximately 44,000 species (Martins *et al*., 2013). The banana pseudostem weevil (Order: Coleoptera) belongs to Curculionidae, in the genus *Odoiporus* and the species *longicollis*. The insect has synonyms as *Calandra longicollis* Olivier and *Sphenophorus planipennis* Gyllenhal (http://eol.org/pages/521885/overview). The genus *Odoiporus* includes 7 species *viz.*, *Odoiporus gages* Csiki, E., 1936; *Odoiporus glabricollis Chevrolat*, L. A. A., 1882, *Odoiporus glabridiscus* Csiki, E., 1936, *Odoiporus longicollis* Marshall, G. A. K., 1930, *Odoiporus major* Heller, K. M., 1898, *Odoiporus planipennis* Chevrolat, L. A. A., 1882, *Odoiporus sulcicollis* Günther, 1936 (http://www.gbif.org/species/search?dataset). According to NCBI taxonomy the genus *Odoiporus* is classified in the family Curculionidae and subfamily Dryophthorinae, (http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser, https://species.wikimedia.org/wiki/*Odoiporus_longicollis*-wikispecies) whereas according to the Species 2000 & ITIS Catalogue of Life 2013 the genus *Odoiporus* is classified under the superfamily Curculionoidea and family Dryophthoridae.

2.2 Distribution

The banana pseudostem weevil, *O. longicollis* is recorded from Kerala, India, for the first time (Visalakshi *et al*., 1989). The BSW is believed to have originated in South and South East Asia, which is also the centre of origin of the present day bananas and plantains. This insect is found in India, China, Malaysia, Indonesia and Thailand and is a key pest of bananas and plantains, posing a threat to banana production systems in these countries (Valmayor *et al.,* 1994).

In recent years, severe incidence of weevil has been reported from different parts of India. It has been a threat to banana under garden-land cultivation and their occurrence in nontraditional areas of Tamil Nadu has been reported. The weevil is becoming increasingly severe in South India particularly in Tamil Nadu and Kerala (Justin *et al*., 2008). Pest density may vary from field to field. The weevil prefers plantains and highland bananas, particularly 'Pome' types (Padmanaban and Sathiamoorthy, 2001).

Banana pseudostem weevil is a serious and devastating pest of banana in many parts of the world. Currently in India, this weevil is posing a serious threat to the banana cultivation in Bihar (Tiwary, 1971), Tamil Nadu and Kerala (Padmanaban *et al*., 2001 and Visalakshi *et al*., 1989), West Bengal (Dutt and Maiti, 1970), Manipur (Prasad and Singh, 1988); Delhi (Batra, 1952); UP (Shukla and Kumar, 1970), Jammu and Kashmir (Tara *et al*., 2010 and Azam, 2007), Karnataka (Arun *et al.*, 2012) and the Andaman

Islands (Lefroy, 1909). It has been reported from Sikkim, Burma, China, Japan, Java, Sri Lanka (Shukla and Kumar, 1969) and Nepal (Tiwari *et al*., 2006). BSW distribution and severity of infestation in South Karnataka was not related to geographical location but influenced by the varieties cultivated (Thippaiah *et al.,* 2010).

2.3 Biology and generations

Considering the economic importance of *O. longicollis*, several studies have been conducted by researchers in the past to study biology in detail. Mating is observed at any time outside the pseudostem or in the space between the outer most and inner sheaths of the pseudostem. Frequent mating is observed and is polygamous. The females at the time of oviposition cut slits on the outer sheath of pseudostem, wide enough to permit entry of ovipositor for egg deposition in the air chamber of the leaf sheath (Dutt and Maiti, 1970). The pre-oviposition period is 15-20 days and the adult weevils mate throughout the day and night (Khairmode *et al*., 2015). One egg laid in one air chamber. Gravid females lay on an average 62 eggs in leaf sheath by inserting the ovipositor through ovipositional slits cut by the rostrum on the outer epidermal layer of the leaf sheath of the pseudostem down to the air chambers.

Eggs are yellowish white, cylindrical in shape with rounded ends and on an average measure about 3.14 mm in length and 1.1 mm in diameter. Incubation period varies from 3-5 days in summer and 5- 8 days in winter (Dutt and Maiti, 1972).

The larvae are apodous but not “C” shaped, usually yellowish white and the mouth parts are of biting and chewing type. Larvae are apodous, soft, fleshy, subcylindrical, wrinkled, bulged in the middle and pointed towards both ends covered with sparse brownish setae of different lengths. The grub passed through five instars to complete the larval period. The total larval period varied from 30 to 35 days (Padmanaban *et al.,* 2001 and Priyadarshini *et al*., 2014).

The fifth instar larvae before entering into prepupal stage makes the cocoon by winding short pieces of fibrous materials of the leaf sheath around body. The cocoon is dark brown, elongate and cylindrical. The pre - pupal period lasted for 3 to 5 days. The pupa is exarate type, yellowish with setae on the head and base of the rostrum and a tuft of hairs on the anal region. The pupal period lasted for 17 to 20 days (Azam *et al.,* 2010; Thippaiah *et al.,* 2012 and Priyadarshini *et al.,* 2014).

The adult weevil is robust with black to reddish brown. The weevil has a long life span and many adults live for a year. The adults are less active and weak fliers. The thorax is hard, elytra has longitudinal grooves and do not extend upto the posterior end of the abdomen. The mouthparts are of biting and chewing type and are present at the tip of snout. The antenna is elbow type. The adult individuals reared in the laboratory survive for 65 to 80 days (Shukla and Kumar, 1969; Azam *et al*., 2010; Thippaiah *et al.,* 2012 and Priyadarshini *et al.,* 2014).

The biology of the BSW has been studied and there are six overlapping generations in a year (Zhou and Wu, 1986), the 4th and 5th instars are active and caused heavy damage (Luo *et al.*, 1985). The total life cycle is about 42 days with an adult longevity of about 90-120 days (Visalakshi *et al.*, 1989). The developmental rates are highly dependent on climatic factors with the duration of the life stages longer in the winter than in the summer. Under laboratory conditions, the duration from egg to adult stage is 44 days (Padmanabhan and Sathiamoorthy, 2001). There are two population peaks in April-May and September - October (Zhou and Wu, 1986). The activity is high from February - November (Prasad and Singh, 1988). The density is high from late May to June and from late September to mid-October that caused heavy damage (Luo *et al.*, 1985).

Sexual dimorphism

Dutt and Maiti (1972) separated the sex on the basis of rostral characters. They found that rostral punctuations in males are larger than those in the females and each punctuation is placed on raised area. The number of such punctuations per linear unit is more in males than females, giving the rostral surface a more or less rough appearance.

2.4 Sex and Aggregation pheromone

Successful chemical identification of insect pheromones (Butenandt *et al.,* 1959) led to an interest in pheromone research. Pheromones were considered a new generation of pest control agents and rapid progress was made in the identification and isolation of pheromones from a wide range of insect species. The term pheromone is derived from Greek word, "*pherein*" for carrying and "*horman*" for stimulation (Regnier and Law, 1968).

The first sex pheromone was identified in the late 1950's by extraction of half a million glands of female silkworm moths, *Bombyx mori* (L.) (Butenandt *et al*., 1959). Since then, sex pheromone components have been identified in more than 1,500 species of moths (Arn *et al*., 1997).

Since the identification of the first insect pheromone there has been continuing interest in behaviour-modifying chemicals and their potential role in integrated pest management. Many studies in early stages concentrated on pheromones used by social insects. But subsequently attention has turned to non-social insects, especially members of the Lepidoptera and Coleoptera (Hall, 1995). Pheromones can provide a means of monitoring and controlling insects which is non-toxic to animals and plants and specific for the target pest. As such, they are fully compatible with other methods of pest supression and thus ideal for IPM programmes (Cork and Hall, 1998).

Sex and aggregation pheromones are common among weevils. Aggregation pheromones lead to aggregation of members of both sexes resulting in mating and aggregation at a food source (Foster and Harris, 1997). These are often produced by males. Male produced aggregation pheromones have been demonstrated for a number of weevil species such as *Rhynchophorus palmarum* (L.) (Rochat *et al.*, 1991a, 1991b), *R. cruentatus* (Weissling *et al.,* 1993), *R. phoenicis* (Fabricus) (Gries *et al.,* 1993), *Metamasius hemipterus* (L.) (Giblin-Davis *et al.*, 1994), *Cosmopolites sordidus* (Budenberg *et al.*, 1993b), and *Sitophilus* spp. (Walgenbach *et al.*, 1983).

Aggregation pheromones are potential attractants that many weevil and beetle species produce. Aggregation pheromones have been studied in storage weevils and other beetles (Levinson and Levinson, 1995), bark beetles (Borden, 1982), boll and pepper weevil (*Anthonomus* sp.) (Tumlinson *et al.,* 1969 and Eller *et al.,* 1994), and palm weevils (*Rhynchophorus* sp.) (Rochat *et al.,* 1993). Release of aggregation pheromones with frass and/or during plant-feeding is shown for a range of coleopteran species such as the boll weevil (*A. grandis*), palm weevil (*R. palmarum*), banded fruit weevil (*Phlyctinus callosus*), sugarcane rootstalk borer weevil (*Diaprepes abbreviatus*), mountain pine beetle (*Dendroctonus ponderosae*), and the Japanese beetle (*Popillia japonica*) (Tumlinson *et al.,* 1969; Pitman, 1971; Billings *et al.,* 1976; Barnes and Capatos, 1989; Loughrin *et al.,* 1996; Sánchez *et al.,* 1996; Harari and Landolt, 1997). All these examples support the idea that feeding-induced odours may serve as an indicator of conspecifics feeding on a high-quality host.

The potential exists for using pheromones to control coleopteran populations by mass trapping. The advantage is that, with few exceptions, coleopterans use aggregation pheromones, attracting both sexes which negatively affect the reproductive capacity of the population (Trematerra, 1997 and Bartelt *et al*., 1991).

2.5 Calling and mating Behaviour

Literature on calling behaviour of *O. longicollis* females is scanty and the mating Behaviour of this pseudostem weevil is also not well documented.

Research on the use of both biotechnological and biocontrol tools are needed to reduce the damage caused by this weevil, in order to build-up eco-friendly and low-cost control strategies. In this regard, research on the mating Behaviour of insects is the first step toward a full understanding of their evolutionary Behavioural ecology (Kirkendall, 1983).

The mating Behaviour has been studied for a number of terrestrial weevil species. For example, *Anthonomus grandis* Boheman (Mayer and Brazzel, 1963), *Diapprepes abreviatus* (Sirot *et al.,* 2007), *Cosmopolites sordidus* (Viana and Vilela, 1996), *Euscepes postfasciatus* (Sato and Kohama, 2007) and *Hypera postica* Gyllenhal, (LeCato and Pienkowski, 1970). Certain Behaviours are commonly described for weevils, such as the postcopulatory mate guarding (Sato and Kohama, 2007; Polak and Brown, 1995 and Vanderbilt *et al*., 1998), the tapping or stroking mechanism during courtship (Sirot *et al*., 2007 and Vanderbilt *et al*., 1998) and several matings with the same partner (LeCato and Pienkowski, 1970 and Wen *et al*., 2004). Mate recognition is based on visual, olfactory, auditory or tactile cues (Gillot, 2005).

Ravi and Palaniswami (2002) documented the calling Behaviour of females and courtship Behaviour of males of BSW. The calling Behaviour of the female observed included (i) knocking the substrate with rostrum, (ii) vibrant antennal beating on the substrate, (iii) lying close to the ground to rub the entire abdomen on the floor, (iv) raising the abdomen off the substrate with full extent of the hind leg and stretching the abdomen, (v) dilation of body segments with wings kept loosely above the abdomen, (vi) bending the abdomen and partly exposing the concealed ovipositor through the genital opening, (vii) extruding the ovipositor and curving down to the substrate accompanied by rapid protrusion and retraction of the ovipositor, and (viii) pressing the substrate with the extruded ovipositor. The Behavioural responses of males when exposed to the female scent included (i) lifting of the head, (ii) holding the antennae out at right angles to the body across the fume and (iii) proceeding to walk or run into the test chamber having the caged female (iv) moving around the female, (v) antenna-to-antenna contact, (vi) downward bending of the posterior end of the abdomen and (vii) mounting. The males exhibited Behavioural responses to the scent of virgin as well as mated females.

LeCato and Pienkowski (1970) studied the mating Behaviour of the alfalfa weevil, *H. postica* (Gyllenhal) (Coleoptera: Curculionidae) in laboratory. Male *H. postica* in the laboratory were extremely aggressive, mounting females as often as 10 times in 12 hours. Prior to mounting, males displayed no detectable courtship Behaviour. After attaining the copulatory position, the male generally touched the females head and prothorax with antennae and simultaneously extended the aedeagus. The female was usually receptive and copulations lasted up to 12 hours, though most were of much shorter duration.

Polak and Brown (1995) reported that males of tropical weevil *Cleogonus rubetra* search within aggregations of con-specifics for receptive females and upon finding a suitable partner, males mount and perform courtship Behaviour consisting of stroking the eyes and sides of the female's abdomen. Males also stridulate and emit a sequence of short buzzing sounds. While mounting, males actively prevent rival males from mating with their partner. Males defend their mates for a mean duration of 3.7 h.

Courtship and mating Behaviour of *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) was studied by Viana and Vilela (1996). The mating Behaviour was observed in individual pairs and groups. Two different sequences of courtship were described: a complex one when the male tapped the abdomen, antenna and head of the female before the copulation and the simple courtship without tapping on the abdomen. When in a group of males and females, a post-copulatory Behaviour with mate guarding by the males was observed.

Mating Behaviour of *Rhynchophorus cruentatus* (Coleoptera: Curculionidae) was investigated by Vanderbilt *et al.* (1998). Males exhibited rostral rubbing and antennal tapping on the female’s elytra before copulation and guarding females afterwards. Field-collected and virgin males in groups were significantly quicker to initiate mating Behaviour and attempted to mate more often per session than males in sequestered pairs. Increased sexual stimulation in weevil aggregations appears to be semio-chemically mediated.

Walid Kaakeh (1998) recorded the mating Behaviour sequence of 1, 7, 21 and 45 days old red palm weevil including the pre-mounting, mounting and copulation Behavioural activities. The pre-mounting period was significantly longer for 1 and 7 days old weevils than for 21 and 45 days old weevils and ranged from 5.5 to 18.5 min. There was no courtship Behaviour prior to mounting. Mean mounting duration was not significantly different between age groups and ranged from 35.5 to 43.3 s. Mean copulation period varied significantly with weevil age, length of copulation increased with increasing weevil age and ranged from 3.8 to 9.8 min.

Facundo *et al.* (1999) conducted the laboratory studies on female emergence and pheromone release, male emergence and mate-locating and female and male mating Behaviours of oriental beetle, *Exomala orientalis* (Waterhouse) (Coleoptera: Scarabaeidae). Mate acquisition and copulation occurred on the soil surface near the female emergence site, with both sexes engaging in pheromone-mediated Behaviours after having emerged from the soil. A highly stereotyped female pheromone release or calling Behaviour consisting of insertion of the female's head into the soil and elevation of the tip of abdomen into the air. Female *E. orientalis* also exhibited leg raising and stroking, which may aid in the dissemination of the pheromone by generating air turbulence at the release sites or function as an auxiliary dispenser of the pheromone. Males exhibited two Behaviours that were associated with courtship. First, when the female was deep in the thatch, the male vigorously touched females dorsum by using legs and mouthparts. Second, while in copula, males were observed to stroke the anterior part of the female's elytra by using the maxillary and labial palpi, accompanied by head bobbing. Males also showed post-copulatory mate guarding Behaviour in the presence of a second or rival male.

Sato and Kohama (2007) studied the post-copulatory mounting Behaviour of the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae). Males mount the female's back after copulation. The results revealed that the duration of the postcopulatory association was very brief in comparison with the length of the refractory phase all females showed after copulation. When females were prevented from resisting copulations during the postcopulatory mounted phase males copulated again, while under normal conditions, a second copulation was never observed. This indicated the presence of a sexual conflict over mating. They interpreted the results of this study as, males test whether the copulation has successfully reduced female receptivity by attempting to remate, if females resist the mating, males leave.

Sirot and Lapointe (2008) examined mating patterns of individually-marked tropical root weevil, *D. abbreviates* in the field and follow-up experiments conducted in captivity to clarify the consequences of the patterns observed in the field. Matings occurred throughout the day but the percentage of individuals mating tended to peak during the middle of the day. Mating duration ranged from less than 3 h to over 9 h. Most males and females mated repeatedly, with the same partner multiple times or with multiple partners. Both same-sex and extra-pair mountings occurred at low frequencies. In a follow-up study conducted in captivity, found no short-term direct benefits of multiple mating to females but female fertility decreased dramatically by 2 weeks after mating if females were not allowed to re-mate. Thus, female *D. abbreviates* may re-mate, in part, to replenish sperm stores.

Ana Mutis *et al*. (2009) investigated on the use of contact pheromones in mating Behaviour of raspberry weevil, *Aegorhinus superciliosus* (Guerin) (Coleoptera: Curculionidae). Males of raspberry weevil mounted females only after antennal contact with the female cuticle and only 33 % of the males attempted to mate with dead females washed with solvent. When a glass rod was coated with female cuticular extracts, males exhibited Behaviours similar to those observed with females. A preliminary GC-MS analysis of cuticular extracts indicated that males and females share a series of aliphatic hydrocarbons but that the relative abundance of some of these compounds differs between the sexes. These results suggested that cuticular lipids mediate mating Behaviour of the raspberry weevil and provide the first evidence of contact pheromones in curculionids.

Flay *et al.* (2009) studied the influence of male density on the courtship and mating duration of male rice weevils, *Sitophilus oryzae.* Results showed that males spent less time in courting females with antennal contact at density 1 when compared to densities 5 and 10. Courtship duration increased from day 1 to day 3 at male densities of 1 and 10. Mating duration increased with increasing male density. The mechanism behind this phenomenon may be (1) a mate guarding strategy to enhance reproductive success by reducing the chance of sperm displacement by competitors and (2) a strategy to avoid damage to the reproductive organs of their mates by multiple mating.

Studies on the mating procedures of virgin males and females of *Neochetina eichhorniae* were made in the laboratory at a temperature range of 28-30 ^{0}C. Five trials were made, in each trial, a pair of weevils was placed together for one hour. A minimum copulation period of 60 seconds and maximum period of 120 seconds with a mean copulation period of 97.67 seconds was observed. Adult male and female of *N. eichhorniae* mated repeatedly both day and night, in their life-time. Mating frequency was 50% greater at night than during the day (Oke, 2011).

Female calling and male flight orientation and searching Behaviours in *Callisphyris apicicornis* (Coleoptaera: Cerambycidae) studied by Curkovic and Ferrera (2012). The calling Behaviour of females included diagnostic movements of the abdomen, legs, elytra, and wings. A total of 59 successful (ending in male attraction) calling sequences were recorded, lasting on average, 1.6 ± 1.2 min. The orientation and searching Behaviour of males included zigzagging flights, landing on and searching for females in cages. Searching was considered to have ended when the male reached the female. Unmated females keep calling despite approaches from males. A total of 209 sequences of males flying to and approaching calling females were recorded, lasting an average of 3 ± 0.93 min. Females called and males responded mainly between 09.00 AM and 01.00 PM.

The mating Behaviour of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) was observed under laboratory conditions. More than 50 % of the *H. hampei* pairs less than 24 h old and all pairs (100 %) more than 24 h old performed their first mating during the 24-h evaluation period. The beetles started to mate within a few hours of emergence. Repeated mating occurred during the 24-hour period and increased in frequency with age. Multiple matings were more frequent in older pairs than in younger ones. They also observed a number of Behavioural steps during the pre-copulatory phase. In most of the pairs (50 %), the male approached the female from the back. In 80 % of the pairs, the male touched the female's elytra with their antennae and rostrum and in 70 % of the pairs, the male mounted the female from the back. During the copulatory phase, the female remained motionless in 60 % of the pairs. All of the pairs performed the same Behavioural steps during the post-copulatory phase. The copulatory phase was significantly longer than the precopulatory and postcopulatory phases. The durations of the phases were 2.20 ± 0.10, 1.35 ± 0.12, and 0.90 ± 0.10 (mean ± SE) minutes, respectively (Silva *et al.*, 2012).

The mating Behaviour of Rice water weevil, *Oryzophagus oryzae* (Coleoptera: Curculionidae) was recorded by Martins *et al*. (2013) indicating that the rice water weevil pairs had intense sexual activity in the first 3 h of photophase and scotophase. Adults mated during day and night and the main peak mating period occurred during the 2nd hour of photophase when 87 % of the couples mated. Adults mated and re-mated at any time of day in the presence of water. However, in the absence of water, only 10 % of adults mated, indicated that water was important for pairs to find each other. In the sequence of pre-mating Behaviours bioassay observed that females were always attracted to males, seeking them before copulation, after mating males showed guarding activity of variable duration, allowing remating. In the first steps of pre-mating sequence, males attempted to copulate several times before females accepted them as partners and females refused the first mating attempt in 90 % of the pairs. Pairs also reacted against the interest of rival males during or after mating occurred.

A study conducted by Nancy Barreto-Triana *et al.* (2014) to determine the reproductive Behaviour of the sugarcane weevil *Sphenophorus levis* (Coleoptera: Curculionidae). The matings occurred at any hour of the day and multiple mating can occur. Fourteen to thirty five days-old couples showed the highest mating frequencies 76 and 88%, respectively. The mean duration of the first mating was 5.75 ± 4.22 h and the total mating time fluctuated between 1 to 17 h. They concluded that *S. levis* 21 to 35 days-old adults presented better receptivity to mating and reproduction.

Benelli *et al.* (2014) observed *Aclees sp. cf. foveatus* Voss (Coleoptera: Curculionidae) mating Behaviour under field conditions. The mating Behaviour is divided into precopulatory, copulatory and postcopulatory phases. Simultaneous antennal tapping and rostral rubbing acts performed by the male on female's body raised the male's chances to copulate, while males that performed only antennal tapping on the partner's body did not achieved mating success. Copulation attempt phase lasted 37.80 ± 7.65 s. Copulation lasted 1116.00 ± 229.73 s and was followed by a motionless postcopulatory phase (8.40 ± 1.21 s). Same-sex sexual interactions were observed among *A. sp. cf. foveatus* males. Male-male sexual interactions lasted about 140.17 ± 19.45 s and were composed of antennal tapping and rostral rubbing on conspecific males followed by one or more mounting attempts.

Uzakah and Odebiyi (2015) recorded the mating behaviour of the banana weevil, *C. sordidus* Germar. Both sexes of the banana weevil mate repeatedly in their lifetime. No elaborate courtship Behaviour was seen before mating in the laboratory. However, ‘sniffing’ (an activity by which male weevils used their antennae to contact the abdominal tip of the female), often preceded mating. This observation may be an indication of a chemical pheromone involved in the mating behaviour of this insect. Male banana weevils generally displayed aggressive mating Behaviour. Matings occurred under both light and dark conditions, but significantly more in the dark. A direct and positive linear correlation was observed between mating and sniffing under both light and dark conditions. Similar trends or correlations were observed between mating and mating duration, mating and sniffing durations respectively. Repeated matings were observed involving the same or different partners.

2.6 Chemical identification of pheromone of Banana pseudostem weevil

The studies regarding chemical identification of pheromone components of other weevils are given below, as the work regarding the chemical identification of BSW not reported so for.

Two related volatile compounds were identified from each of two species of *Pissodes* bark weevils and implicated as components of their aggregation pheromones. Grandisol (*cis*-2-isopropenyl-1-methylcyclobutaneethanol) and its corresponding aldehyde, grandisal, were isolated from males of both *Pissodes strobi* and *Pissodes approximatus* and were found in the abdomens and hindguts of the respective species. In field tests synthetic grandisol and grandisal together with odors from cut pine acted synergistically in attracting both sexes of *P. approximatus*. This response was similar to that elicited by male *P. approximatus* feeding on cut pine. Males and females of natural populations of *P. strobi* were more responsive to caged males feeding on leaders of white pine than they were to leaders alone. The combination of grandisol, grandisal and leaders was less attractive than males on leaders, but more attractive than leaders alone. From isolation of pheromone components at different times of the year, it was determined that males of both species produced grandisol and grandisal only at times when cohort females were reproductively mature (Booth *et al.*, 1983).

Rochat *et al.* (1991b) reported that male American palm weevils (APWs), *Rhynchophorus palmarum* (L.) produced two sex-specific compounds, which were disclosed by volatile collections on Supelpak-2 and gas chromatography. Comparisons of trapped volatiles from sugarcane, APW males + sugarcane and APW females + sugarcane revealed the presence of two male-specific compounds. Females produced no detectable sex-specific volatiles. Extracts containing male-produced + sugarcane volatiles elicited very strong EAGs in both APW males and females. Extracts containing volatiles from sugarcane alone or from APW females + sugarcane induced EAGs as weak as those to air and solvent. All GC-EAD recordings from both male and female APWs showed high EAG responses to major compound and no or weak response was recorded for minor compound, not always detected. The major male-produced volatile identified as (2E)-6-methyl-2-hepten-4-ol through coupled gas chromatography-mass spectrometry and gas chromatography-Fourier transform infrared spectrometry, proton nuclear magnetic resonance spectrometry and rational synthesis. They proposed the trivial name rhynchophorol for this new molecule.

Eller *et al.* (1994) reported that volatile collections and gas chromatography study revealed the presence of six male-specific compounds in the aggregation pheromone of pepper weevil, *Anthonomus eugenii* Cano. These compounds were identified using chromatographic and spectral techniques as: (Z)-2-(3,3-dimethylcyclohexylidene) ethanol, (E)-2-(3,3-dimethylcyclohexylidene) ethanol, (Z)-(3,3-dimethylcyclohexylidene) acetaldehyde, (E)-(3,3-dimethylcyclohexylidene) acetaldehyde, (E)-3,7-dimethyl-2,6-octadienoic acid (geranic acid), and (E)-3,7-dimethyl-2,6-octadien-l-ol (geraniol). The emission rates of these compounds from feeding males were determined to be about: 7.2, 4.8, 0.45, 0.30, 2.0, and 0.30 µg/male/day, respectively.

Beauhaire *et al.* (1995) achieved the synthesis of diastereo-selective (IS,3R,SR,7S) 2,8-dioxa 1-ethyl 3,5,7-trimethyl bicylo [3,2,1] octane 1d using key-step of the regioselective Baeyer-ViUiger reaction of 2,6-disubstituted cyclohexanone. It confirms the identification of a new male pheromone emitted by the banana weevil, *C. sordidus*.

Aggregation pheromone of the banana weevil *C. sordidus* was investigated by Cabrera *et al*. (1995). The volatile compounds emitted by stimulated insects were trapped on adsorbent polymers Porapak-Q and Supelpack-2. An air stream was drawn 10 minutes per hour during 15 days, into flasks with and without insects. Compounds were desorbed from the polymers with acetone and bioassays were undertaken to confirm biological activity. Extracts were analyzed by Gas Chromatography - Mass Spectrometrj (GC-MS). The compound 8-methyl-5-nonen-3-ol was tentativily identified by GC-MS as one of the components of the aggregation pheromone of the banana weevil *C. sordidus.*

Palm weevils in the subfamily Rhynchophorinae (Curculionidae) (*Rhynchophorus* spp., *Dynamis borassi, Metamasius hemipterus, Rhabdoscelus obscurus* and *Paramasius distortus*) use male-produced aggregation pheromones for intraspecific chemical communication. Pheromones comprise 8, 9 or 10 carbon, methyl-branched, secondary alcohols. (4*S*, 5*S*)-4-Methyl-5-nonanol (ferrugineol) is the major aggregation pheromone for *R. ferrugineus*, *R. vulneratus*, *R. bilineatus*, *M. hemipterus* and *D. borassi* and a minor component for *R. palmarum*. (5*S*, 4*S*)-5-Methyl-4-octanol (cruentol), (3*S*, 4*S*)-3-methyl-4-octanol (phoenicol), and (4*S*, 2*E*)-6-methyl-2-hepten-4-ol (rhynchophorol) are the main aggregation pheromones for *R. cruentatus*, *R. phoenicis* and *R. palmarum*, respectively. Plant kairomones strongly enhance pheromone attractiveness but none of the identified volatiles, such as ethyl acetate, ethyl propionate or ethyl butyrate are as synergistic as fermenting plant (palm or sugarcane) tissue (Giblin-Davis *et al.*, 1996).

Female cowpea weevils *Callosobruchus maculatus,* produce a sex pheromone that elicits orientation and sexual Behaviour in males. Bioassay directed isolation of the sex pheromone compounds in the active fraction were identified and synthesized. The most active GC fraction contained the following five 8-carbon acids identified by GC-MS and comparison with synthetic candidates: 3-methyleneheptanoic acid, (Z)-3-methyl-3-

heptenoic acid, (E)-3- methyl-3-heptenoic acid, [Z)-3-methyl-2-heptenoic acid, and (E-3-methyl-2- heptenoic acid. Each of the synthetic acids active individually for males and combinations of two or more of the acid pheromones had an additive effect. Upwind flight responses to natural and synthetic pheromones were observed in a flight tunnel. (Z)-3-Methyl-2-heptenoic acid was previously identified as the sex pheromone for the related *C. analis*, but this and the other four acid pheromones from *C. maculatus* were inactive for male *C. analis* (Phillips *et al*., 1996).

The male pecan weevil pheromone is a mixture of four components; I as both the *Cis* and *trans* isomers of 2-propenyl-l-methyl-cyclobutaneethanol [also identified as *(1R,2S)*-(+ and -)-grandisol], II [(Z)-3,3-dimethylcyclohexane-$\Delta^{1,ß}$ -ethanol], III [(Z)-3,3-dimethylcyclohexane- $\Delta^{1,\alpha}$-acetaldehyde] and IV [(E)-3,3-dimethylcyclohexane- $\Delta^{1,\alpha}$-acetaldehyde]. These components were synthesized by the male pecan weevil, but not by the female, in the ratio 7:16:3:3 of I, II, III and IV, respectively. The same compounds were earlier identified as the pheromone of the male boll weevil, *A. grandis*, in which they were isolated from frass in the ratio 6:6:1.5:1.5. However, only the (+) isomer of grandisol was synthesized by male boll weevil. In laboratory tests, 80 % of female pecan weevils were attracted to a synthetic formulation based on the ratio found in male pecan weevils, while only 28 % of the females were attracted to a synthetic formulation based on the ratio found in boll weevil frass. The attraction of males to these synthetic formulations was minimal (14, 4, and 2 %, respectively). Live males and their extracts were also attractive to females, but males did not respond to male or females. Preliminary field tests demonstrated that females were attracted to males and the synthetic pecan weevil formulation, but not to the synthetic boll weevil formulation (Hedin *et al*., 1997).

Gunawardena and Dissanayake (2000a) reported that male banana pseudostem weevil produced a pheromone, 2-methyl-4-heptanol which attracted both male and female banana weevils. This aggregation pheromone *i.e.*, 2-methyl-4-heptanol works only in the presence of food bait under field conditions using bucket traps.

Comparison of volatiles produced by field collected, overwintering individuals of each sex of the strawberry blossom weevil, *Anthonomus rubi*, led to identification of three male-specific compounds—(*Z*)- 2-(3,3-dimethylcyclohexylidene) ethanol, (*cis*)- 1-methyl-2-(1-methylethenyl) cyclobutaneethanol and 2-(1-methylethenyl)- 5-methyl-4-hexen-1-ol (lavandulol)—in amounts of 6.1, 1.2 and 0.82 µg/day/male. The first two compounds are components of the aggregation pheromone of the boll weevil, *A. grandis*, grandlure II and grandlure I, respectively. Grandlure I was the (1*R*, 2*S*)-(C) enantiomer and lavandulol was a single enantiomer, although the absolute configuration was not determined. Trace amounts of the other two grandlure components (*Z*)-(3, 3-dimethylcyclohexylidene) acetaldehyde (grandlure III) and (*E*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure IV) were also detected. (*E*, *E*)-1-(1-Methylethyl)-4-methylene- 8-methyl-2,7-cyclo-decadiene (germacrene-D), a known volatile from strawberry plants, *Fragaria ananassa* was collected in increased amounts in the presence of pheromone-producing weevils. In field trials using various combinations of synthetic grandlures I, II, III, and IV and lavandulol, significantly more weevils were caught in traps baited with blends containing grandlure I and II and lavandulol than in those baited with blends without lavandulol or unbaited controls. Addition of grandlure III and IV had no significant effect on attractiveness (Innocenzi *et al.*, 2001).

Gas chromatographic and mass spectral (GC-MS) analysis of hindgut volatiles of the red turpentine beetle *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae) revealed the presence of *trans*-verbenol, *cis*-verbenol, myrtenol, myrtenal and verbenone, which are frequently reported pheromone components of *Dendroctonus* spp. Electroantennogram tests indicated that *D. valens* possess olfactory sensitivity for these three compounds. Additionally, *D. valens* displayed anemotaxis toward all three compounds in a Y-tube olfactometer. In field tests, both *trans*-verbenol and myrtenol signifcantly increased catches of beetles in traps baited with host kairomone [a blend of three host monoterpenes: (+)-α-pinene, (-)-ß-pinene and (+)-3-carene] (Zhang and Sun, 2006).

Zarbin *et al.* (2007) evaluated the Behavioural responses of adult male and female *Pseudopiazurus obesus* (Boheman) (Coleoptera: Curculionidae) to several odour sources in a Y-tube olfactometer. Results indicated that male and female insects were significantly more attracted to a combination of volatiles released by males of the species and host plant, suggesting the existence of a male-produced aggregation pheromone. Comparative analysis of the volatiles released by males and females revealed three male-specific compounds, in a ratio of 77:14:9, providing a chemical support to the Behavioural observations. (1*R*, 2*S*) - (+)-Grandisal and (1*R*,2*S*) - (+)-grandisol were identified as the major and intermediate compounds, respectively, while the chemical structure of the minor compound, that seems to be a new grandisol derivative, still remains to be determined.

Combined gas chromatography electroantennography (GC-EAD) analysis of male volatile extracts of agave weevil, *Scyphophorus acupunctatus* showed that four peaks elicited antennal responses from males and females. The peaks identified by GC-MS as (i) 2-methyl-4-heptanol, (ii) 2-methyl-4-octanol, (iii) 2-methyl-4-heptanone and (iv) 2-methyl-4-octanone. EAG recordings of both sexes to 0.01, -0.1, -1, and -10 μg stimulus load of synthetic compounds showed that the dose of the tested compounds and weevil sex significantly influenced the antennal response of *S. acupunctatus*. Antennae of *S. acupunctatus* were most sensitive to compounds (ii) and (iv), reaching the threshold at a 0.01-μg stimulus load. Weevil antennae were less sensitive to compounds (i) and (iii), and the threshold response to these compounds was 0.1μg. Behavioural evaluation of the synthetic compounds showed them to be attractive to both males and females in a Y-tube olfactometer (Ruiz-Montiel *et al*., 2008).

Volatiles from both sexes of the Brazilian soybean stalk weevil *Sternechus subsignatus* Boehman were collected by aeration and the behavioural response of males and females was evaluated using a Y-olfactometer. The results demonstrated that the communication in *S. subsignatus* is mediated by aggregation pheromone as both sexes were attracted to host plant (HP) volatiles and this attraction was increased by the addition of male volatiles. Five male-specific compounds were detected in the chromatographic analysis, providing chemical support to the Behavioural data. Release of these volatiles is dependent on the presence of the HP as the amount of compounds differs significantly when volatiles are collected from weevils with or without access to food. The release takes place mainly during photophase, showing a peak between 4 and 6 h after its beginning, which is also a peak of the insect activity in the field (Ambrogi and Zarbin, 2008).

Analyses of the headspace volatiles produced by males and females of *Sternechus subsignatus* Boheman (Coleoptera: Curculionidae) revealed seven male-specific compounds. The major component was (E)-2-(3,3-dimethylcyclohexylidene)-ethanol, and the minor components were 1-(2′-hydroxyethyl)-1-methyl-2- isopropenylcyclobutane (grandisol), 7-methyl-3-methyleneoct- 6-en-1-ol, (Z)-2-(3,3-dimethylcyclohexylidene)-ethanol, (Z)- and (E)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde and (E)-2-(3,3-dimethylcyclohexylidene) acetic acid. Only four of the seven identified compounds showed electrophysiological activity. Enantioselective gas chromatography showed that the natural grandisol was the (1R, 2S)-stereoisomer. The major component, (E)-2-(3,3-dimethylcyclohexylidene)-ethanol, attracted *S. subsignatus* in olfactometer bioassays (Ambrogi *et al*., 2012).

ZE Sang-Zi *et al*. (2010) investigated on the screening of attractant semiochemicals of the Armand pine bark weevil, *Pissodes punctatus* (Coleoptera: Curculionidae). Volatiles of adult frass and male hindguts were analysed and tested both in laboratory and in the field. GC-MS analysis confirmed that besides familiar monoterpenes such as α-pinene, ß-pinene and 3-carene, cis-2-isopropenyl-1-methylcyclobutannethanol (grandisol) also exists in adult frass and male hindguts of *P. punctatus*. *P. punctatus* weevils displayed anemotaxis towards low dosage of 3-(+)-carene, grandisol and its corresponding aldehyde, grandisal, in a Y-tube olfactometer. In field tests, both grandisol and grandisal exhibited attraction activity to the bark weevils. The results suggested that grandisol is a component of aggregation pheromone of *P. punctatus.*

Szendrei *et al*. (2011) reported that the cranberry weevil, *Anthonomus musculus* Say were highly attracted to traps baited with the *A. eugenii* Cano aggregation pheromone, indicating that these congeners share common pheromone components. To identify the *A. musculus* aggregation pheromone, headspace volatiles were collected from adults feeding on blueberry or cranberry flower buds and analyzed by gas chromatography-mass spectrometry. Three male-specific compounds were identified: (Z)-2-(3, 3-dimethyl-cyclohexylidene) ethanol (Z grandlure II). (Z)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure III) and (E)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure IV). A fourth component, (E)-3,7-dimethyl-2,6-octadien-1-ol (geraniol) was emitted in similar quantities by males and females. The emission rates of these volatiles were about 2.8, 1.8, 1.3, and 0.9 ng/adult/d, respectively.

Lapointe *et al*. (2012) isolated, identified and synthesized an unsaturated hydroxy-ester pheromone from the headspace and feces of male *Diaprepes abbreviatus*. The pheromone, methyl (E)-3-(2-hydroxyethyl)-4-methyl-2-pentenoate was discovered by gas chromatography-coupled electroantennogram detection (GC-EAD) and identified by gas chromatography– mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR). The synthesis yielded an 86:14 mixture of methyl (E)-3-(2-hydroxyethyl)-4-methyl-2-pentenoate (active) and methyl (Z)-3-(2-hydroxyethyl)-4-methyl-2-pentenoate (inactive), along with a lactone breakdown product. The activity of the synthetic E-isomer was confirmed by GC-EAD, GC-MS, NMR, and bioassays. No antennal response was observed to the Z-isomer or the lactone. In a two-choice olfactometer bioassay, female *D. abbreviates* moved upwind towards the synthetic pheromone or natural pheromone more often compared with clean air. Males showed no clear preference for the synthetic pheromone. This pheromone, alone or in combination with plant volatiles, may play a role in the location of males by female *D. abbreviates*.

Jyothi *et al.* (2014) investigated the olfactory and behavioural responses of groundnut seed-beetle, *Caryedon serratus* Olivier to semiochemicals isolated from conspecific insects as well as from groundnut seed headspace volatiles by coupled Gas Chromatography-Electroantennogram (GC-EAD) and Y-tube olfactometer bioassays. Significant orientation responses of male bruchids to the headspace volatiles from females and orientation responses of both sexes of seed beetles to headspace volatiles from male extracts confirm the existence of both sex and aggregation pheromone systems in *C. serratus*. Coupled GC-EAD analyses of headspace volatiles of female extract revealed seven bioactive peaks with strongest antennal responses of males towards flame ionization (FID) peaks at retention times of 3 min and 26 min, respectively. GC-EAD profiles of male headspace volatile extract indicated the presence of three distinct bioactive peaks elicited by both male and female antennae.

The comparison of gas chromatograms of airborne volatiles produced by males and females of the guava weevil *Conotrachelus psidii* (Coleoptera: Curculionidae: Molytinae) revealed the presence of one male-specific compound. Analysis of the headspace extracts of *C. psidii* males via GC/EAD repeatedly showed this male-specific component to elicit strong responses on the antennae of both male and female weevils. Comparative GC-MS analysis showed the presence of a male-specific electroantennographically active compound identified as (1*R*, 2*S*, 6*R*)-2-hydroxymethyl-2, 6- dimethyl-3-oxabicyclo [4.2.0] octane (papayanol). Release of this compound was dependent on the photoperiod, taking place primarily during the scotophase. Analysis of the extracts obtained during three consecutive photophases and scotophases showed that *C. psidii* male release 85 % of their pheromone during the scotophase ($p < 0.005$), with release rates gradually declining between onset and end of the scotophase. Overall release rates ranged between 198 ± 27 to 496 ± 29 ng of pheromone⁄insect/day, and higher amounts of production were observed between 2 h and 6 h after the onset of the scotophase. The presence of the host plant in the aeration chambers during the headspace extract collection stimulates the pheromone release of male adults. This Behaviour has been documented occurring in some other curculionidae species such as *A. grandis*, *A. musculus, Rynchophorus phoencis, R. palmarum, Pissodes strobe* and *P. approximates* among others (Palacio-Cortés *et al.*, 2015).

2.7 Laboratory and field evaluation using identified pheromone

2.7.1 Laboratory bioassay's with pheromone components of Aggregation pheromone

Male and female banana weevils *C. sordidus* were attracted to freshly cut banana rhizome and pseudostem in a still air olfactometer. Females responded similarly to odours from a comparatively resistant and from a susceptible cultivar of banana, both when presented as freshly cut tissue and as Porapak-trapped volatiles. Females were also attracted to live connnspecific males, to Porapak-trapped volatiles from them and to their dissected hindguts, but not to live females, their hindguts or volatiles from males, but not to those from females. It was suggested that male banana weevils release an aggregation pheromone via the hindgut (Budenberg, 1991).

Coupled gas chromatographic–electroantennographic detection (GC-EAD) analyses and coupled gas chromatographic-mass spectrometry (GC-MS) of volatiles produced by male and female West Indian sugarcane weevils (WISW) *Metamasius hemipterus sericeus* (Oliv.) revealed eight male specific, EAD active compounds: 3-pentanol (1), 2-methyl 4-heptanol (2), 2-methyl 4-octanol (3), 4-methyl 5-nonanol (4), and the corresponding ketones. In field experiments in Florida, alcohols 1–4 in combination with sugarcane were most attractive, whereas addition of the ketones or replacement of alcohols with ketones significantly reduced attraction. In Costa Rica field experiments testing alcohols 1–4 singly and in all binary, ternary and quaternary combinations revealed 4 in combination with 2 was the major aggregation pheromone, equally attracting male and female WISW. Stereoisomeric 4 and (4*S*, 5*S*) 4, the only isomer produced by WISW, were equally attractive. Addition of 4*S*, 4*R* or (±) 2 to (4*S*, 5*S*) 4 significantly enhanced attraction. Sugarcane stalks in combination with 2 plus 4 (ratio of 1:8) were highly synergistic, whereas EAD active sugarcane volatiles ethyl acetate, ethyl propionate, or ethyl butyrate only moderately increased attractiveness of the pheromone lure (Perez *et al.*, 1997).

The relative roles of male and female weevils, frass, food and combinations of these odor sources in aggregation formation were studied by Harari and Landolt (1997). Female and male rootstalk borer, *Diaprepes abbreviates* were attracted by food, males, females and female or male frass. Females were most often attracted by damaged food (broken green beans), whereas males were similarly attracted to damaged food and either female frass, male frass, or heterosexual pairs. No enhancement of attraction by either sex was found when males and male frass were combined with damaged food.

The response of *C. sordidus* to the volatiles of its host banana plant *(Musa paradisiaca* cv. Prata) and conspecific adults was studied by de Mendonça *et al*. (1999). They observed the attraction of males and females of *C. sordidus* to rhizome and pseudotem of the banana, fresh or rotting. The results suggested that the aggregation pheromone was produced by males and that it attracts both sexes and there was another pheromone produced by females which attracts males only. The results also suggested that the females are the first responsible for the aggregation of the species, but the male pheromone mainly responsible for the mass aggregation.

Ravi and Palaniswami (2002) conducted the olfactory assay with *O. longicollis.* They showed evidence for an air-borne sex attractant from the females which attracted the males for courtship. In the olfactometer when females (20 numbers) were placed in the release chamber and a male, either mated or unmated, was introduced into the test chamber, the air current carrying the male odour caused no Behavioural change in the females. The females were neither attracted nor stimulated by the male odour. However, when the males (20 numbers) were kept in the release chamber and a virgin female introduced in the test chamber, the males showed excitement and courtship Behaviour. Similarly the experiment examining the attraction of males to females of various age groups provided evidence for the change in intensity of response. The females of pre-emergence resting stage as well as females of up to four-day-old were non-attractive to the males. This may probably be due to sexual immaturity of the individuals. The female begins calling males only five days after emergence. Twenty-day-old females elicited maximum male responses. A maximum attraction index of 0.938 (± 0.740) was observed in twenty-day-old females and the approach latency was also comparatively shorter. When the females were of more than one month old, the attraction remained more or less constant.

Tinzaara *et al.* (2003) conducted different bioassays for investigating orientation responses of the banana weevil, *C. sordidus* to show additive effects of host plant volatiles and the synthetic pheromone. The results indicated that the weevils responded significantly stronger to the combination of pheromone and fermented pseudostem than to pheromone or pseudostems alone in locomotion compensator. In Double port olfactometer bioassay, *C. sordidus* moved more frequently to the port releasing the synthetic pheromone than to that releasing the odour of fermented pseudostem. The weevil responded significantly more to the combination of pheromone and pseudostem than to the pseudostem alone. The comparison of *C. sordidus* response to the combination of the pheromone and pseudostem with the pheromone alone did not show a significant difference. In Double pitfall olfactometer bioassay, *C. sordidus* did not show a significant difference between the synthetic pheromone and fermented pseudostem odour. There was a significantly higher response of *C. sordidus* to the combination of pheromone and pseudostem than to either the pheromone or volatiles from the pseudostem alone.

Tinzaara *et al.* (2007) conducted laboratory bioassay to determine an additive effect of pseudostem tissue and pheromone. The results revealed that *C. sordidus* was strongly attracted to both host plant volatiles and the synthetic pheromone presented singly or in combination as compared to clean air in a double pitfall olfactometer. Fermented pseudostem tissue was more attractive to *C. sordidus* than fresh tissue. The presence of fresh or fermented pseudostem tissue enhanced attractivity of pheromone lures in comparison to pheromone lures alone. There were no differences in attractivity between pheromone + fermented tissue and pheromone + fresh tissue.

EAG results obtained from stimulating male and female antennae with n-hexane, whole-body extracts of weevils, host plant extract and combinations of weevil-body extracts and leaf sheath extracts, all the stimuli tested elicited significant EAG responses from male and female BSWs in comparison to control (n-hexane) indicating the presence of certain antennally active constituents in these extracts. The highest absolute EAG response recorded with male antenna against male body extract + banana sheath extract was 4.25 mV. EAG responses of females to their own body extracts were found to be low compared to control. Male body volatile extracts elicited stronger EAGs ranging upto 1.45 mV in female antenna. The results of olfactometer bioassays showed that the per cent activity of males (75-98.3 %) in all the tests found to be higher than that of females (35-63.33%). These finding confirm the male based aggregation pheromone system in BSW (Prasuna *et al*., 2008). Volatiles from both sexes of soybean stalk weevil, *Sternechus subsignatus* were collected by aeration. The results of Y-olfactometer on the behavioural responses of males and females showed that the communication in *S. subsignatus* is mediated by aggregation pheromone as both sexes were attracted to host plant volatiles and this attraction increased by the addition of male volatiles (Ambrogi and Zarbin, 2008).

The effect of different dosages of aggregation pheromone at 1, 10, 100 and 1000 ng/1 µl hexane on the diurnal responses of virgin and mated male and female *Rhynchophorus ferrugineus* (Olivier) (Coleoptera Dryophthoridae) was studied under laboratory conditions by Poorjavad *et al.* (2009). Pheromone concentration, as well as sex and mating status, was found to affect the behavioural responses of the weevils. In both sexes the response to ferrugineol increases with mating. In the case of aggregation pheromone at 1000 ng dose elicited a significantly greater response that of 1 ng, while the remaining doses gave intermediate responses. Female weevils responded to the pheromone more than males and mated individuals more than their virgin counterparts.

Olfactometer studies indicated that the aggregation pheromone of red palm weevil and rhinoceros beetle attracted significantly more number of weevils (13.4 females and 7.6 male weevils) and beetles (6.5 male and 12.3 female beetles), respectively than control. Similarly, field studies found that both 750 and 1000 mg pheromone dosage lures of red palm weevil and rhinoceros beetle trapped significantly higher numbers of weevils (695.80 and 789 weevils, respectively) and beetles (98 and 108 beetles, respectively) in traps ($P<0.05$), respectively. On an average 80-85 % red palm weevil and 72-78 % rhinoceros beetle population got trapped (Chakravarthy *et al*., 2014).

Behavioural responses of *C. psidii* males and females in the Y-tube olfactometer to different odor sources showed that the attraction of males and females to the natural headspace extract of males containing the host plant was significant as well as the attraction to the combination of host plant headspace extract with the synthetic pheromone component ((1*R*, 2S, 6R)-Papayanol) to males and females. On the other hand, neither the synthetic compound nor the host plant volatiles alone were attractive to males and females (Palacio-Cortés *et al.,* 2015). Evidences from mating behaviour studies of the banana weevil and from the weevil's responses to trapped volatiles of mature adults clearly suggested that two types of pheromones were produced by the *C. sordidus,* a female produced sex pheromone and a male produced aggregation pheromone. Olfactory responses to trapped adult volatiles revealed that males responded significantly to the trapped adult volatiles of both males and females, however, females responded significantly to only the trapped adult male volatiles and not to the trapped adult female volatiles (Uzakah *et al.,* 2015).

2.7.2 Host Plant volatiles

Insect Behaviour is governed by complex interactions among chemical and physical signals in the environment. For example, host plant volatiles may (i) facilitate orientation of insects to potential feeding sources, (ii) provide a mechanism for insects to avoid non-hosts or (iii) either enhance or disrupt Behavioural responses to pheromones (Dickens *et al.,* 2002).

Herbivores are known to use plant volatiles (kairomones) to locate a food plant (Visser, 1986). When herbivores feed on a plant, cell damage results in a release of volatiles from the wound site (Dicke *et al.,* 1990). The amount of volatiles released by herbivores or by herbivore infested plants may affect herbivore response to the plant. The response to an information source by the herbivore may depend on physiological state, previous experiences and abiotic conditions (Dicke *et al.,* 1998).

Hord and Flippin (1956) reported that fresh pseudostems are more attractive to *C. sordidus* than fermented material. Male and female *C. sordidus* were attracted to freshly cut banana rhizome and pseudostem in a still-air olfactometer. Females responded similarly to odors from a comparatively resistant and from a susceptible cultivar of banana, when presented as either freshly cut tissue or as Porapak-trapped volatiles. Females were also attracted to rotting banana pseudostem and to volatiles collected from it. Males and females gave similar responses to host tissue in both the Behavioural bioassay and to collected volatiles in EAG recordings. Fresh and rotting materials were equally attractive. Weevils did not respond, either Behaviourally or electrophysiologically, to a synthetic mixture of mono- and sesqiterpenes, which made up over 9 % of the volatiles collected from pseudostem (Budenberg *et al.,* 1993).

Gunawardena and Dissanayake (2000b) analyzed the steam distillates of freshly cut banana stem by combined GC-EAD and combined GC-MS. They identified n-hexanol, n-hexanal, n0pentanol and cis-3-hexenol as host attractants for the banana pseudostem borer *O. longicollis*. Female weevils responded slightly more (EAG 1.41 ± 0.01) than male weevils (EAG 1.04 ± 0.03) to the steam distillate. Synthetic equivalents of the above attractants were subjected to EAG assay and n-hexanol elicited the highest EAG response (0.39 ± 0.02mV). A mixture of the above synthetic attractant mixture (SAM) also elicited a poor EAG response (0.2mV) in *O. longicollis*. In field experiments, n-hexanol singly or in combination with the aggregation pheromone of *O. longicollis* failed to attract any weevils into traps. The SAM also was not field attractive while its combination with the aggregation pheromone of *O. longicollis* was attractive with a maximum mean catches of 0.66 ± 0.21 weevils/traps/week. Under the same conditions, the aggregation pheromone + banana stem tissue baited trap, had a maximum mean catch of 29.16 ± 7.02 weevils/trap/week.

Van Tol *et al.* (2002) reported that odors of weevil damaged yew (*Taxus baccata*) and spindle trees (*Euonymus fortunei*) are attractive to the vine weevil *Otiorhynchus* *sulcatus* but Rhododendron and strawberry are not. Undamaged *Euonymus* is attractive to the weevils in spring time but not in late summer. When clean air or undamaged *Euonymus* was the alternative, weevils strongly prefer weevil-damaged *Euonymus* foliage and this preference was retained throughout the year. Hence, plant damage plays a role in attraction of the vine weevil. In contrast to the permanent attractiveness of weevil-damaged *Euonymus*, mechanically damaged plants gradually lose the attractiveness that they have early in the growing season. This suggested that emission of volatiles, produced by the plants in response to weevil damage is important for attraction of the weevils because, the weevils may use these plant odors to find suitable food plants throughout the season. Apart from weevil-damage-related plant volatiles, green leaf volatiles also play significant role, as indicated by the fact that weevils prefer: early season undamaged *Euonymus* over clean air; early season mechanically damaged *Euonymus* over undamaged *Euonymus* and throughout the season, had no preference when mechanically damaged *Euonymus* was tested against weevil-damaged *Euonymus*. They suggested that monitoring can be done by the use of green leaf volatiles and/or herbivore-induced volatiles as attractants in traps.

Van Tol *et al.* (2004) tested the Behavioural responses of adult vine weevils *O. sulcatus* at night to odours from the frass of conspecifics and closely related species *O. salicicola* and to odors from the non-host plants (Ivy, *Hedera helix* and cherry laurel, *Prunus laurocerasus*). Freshly collected frass from *O. sulcatus* and from *O. salicicola* males and females were attractive. Vine weevil was attracted to mechanically damaged leaves of both plant species, whereas undamaged leaves were not attractive. Only undamaged young unfolding leaves of *H. helix* were also attractive. The attraction to odours from mechanically damaged host and non-host plants suggested the involvement of compounds that are commonly found in many plant species. Valde´s-Rodrı´guez *et al.* (2004) reported that *Scyphophorus acupunctatus* Gyllenhal was attracted to extracts of freshly cut henequen leaves in laboratory bioassays.

Sahayaraj and Kombiah (2009) studied the olfactory responses of the pseudostem weevil *O. longicollis* against the decayed pseudostem and its extract under laboratory conditions. Banana pseudostem were allowed to decay for 1, 3, 5 and 7 days and used for the experiment. Results revealed that both 7 days decayed pseudostem (53.35 %) and its extract (75.0 %) has more attractant property compared to other experimental decayed pseudostem and its extracts. The results suggest that decayed pseudostem based attractant can be used as the trapping agent for the management of *O. longicollis.*

Saïd *et al*. (2011) developed a bioassay and a mathematical model to evaluate synergy between aggregation pheromone (P) and host plant odors (kairomone: K) in the American palm weevil, *Rhynchophorus palmarum*, showing enhanced responses to P+K mixtures. Responses to synthetic P and natural K were obtained using a four-arm olfactometer coupled to a controlled volatile delivery system. Results showed that: (1) Response thresholds were ca. 10 and 100 pg/s respectively for P and K. (2) Both stimuli induced similar maximum response. (3) Increasing the dose decreased the response for P to the point of repellence and maintained a maximum response for K. (4) P and K were synergistic over a 100-fold range of doses with experimental responses to P+K mixtures greater than the ones predicted assuming additive effects. Responses close to maximum were associated with the mixture amounts below the response threshold for both P and K.

Palanichamy *et al*. (2011b) prepared a microwave oven assisted extract from banana pseudostem and tested its kairomonal activities on *O. longicollis* using Electroantennogram technique. In addition, the results were tested with two other methods such as Solvent extraction and Air-entrainment extraction for comparison of its activities. The results revealed that the micro oven assisted extract elicited the maximum EAG response in female, whereas, solvent extract produced higher antennal activity in male only and the air-entrainment extract elicited poor response both in male and female *O. longicollis*.

Silva *et al.* (2012) tested the olfactory responses of the guava weevil to odors from green and ripened fruits, leaves and flower buds of *P. guajava*, guava weevils and their feces in a Y-tube olfactometer. Results indicated that flower buds odors were attractive to *C. psidii* males suggesting the presence of host kairomones. Male and female weevils feces attracted both male and female weevils, indicating the existence of aggregation pheromones or allelochemicals (undigested plant compounds) in the feces.

A Study on the Behavioural responses of *O. longicollis* to volatiles from banana pseudostems with different physiological status was conducted by Yin Jiong *et al*. (2012). Solid phase microextraction (SPME) was used to extract volatiles from banana pseudostems in the following four physiological states: fresh, rotten but not fed on by weevils, rotten and fed on by weevils and fresh and fed on by weevils. The extracted volatiles were analyzed by gas chromatographymass spectrometry (GC-MS). A total of 10 components were identified from volatiles extracted from fresh and rotten pseudostems that had been fed on by weevils, whereas a total of 11 components were identified from extracts from rotten pseudostems and fresh pseudostems that had been fed on by weevils. The extracted volatiles included hydrocarbon, esters, ketone and heterocyclic compounds, the relative abundance of which differed with pseudostems physiological state. The species and relative abundance of extracted volatiles were closely related to damage inflicted by the banana pseudostem weevil. There was a corresponding reduction in the relative content of the common component alloocimene after *O. longicollis* infestation. Olfactometer bioassay revealed that both male and female *O. longicollis* were attracted to the volatiles from banana pseudostems in different physiological states. Compared to fresh pseudostems, weevil feeding activity and rottenness enhanced the attractiveness of the extracted volatiles from banana pseudostems to male and female *O. longicollis*.

2.7.3 Field evaluation of aggregation pheromone

Eller *et al*. (1994) reported that sticky traps baited with a synthetic blend of (Z)-2-(3,3-dimethylcyclohexylidene) ethanol, (E)-2-(3,3dimethylcyclohexylidene)ethanol, (Z)-(3,3-dimethylcyclohexylidene) acetaldehyde, (E)-(3,3-dimethylcyclohexylidene) acetaldehyde, (E)-3,7-dimethyl-2,6-octadienoic acid (geranic acid), and (E)-3,7-dimethyl-2,6-octadien-l-ol (geraniol) compounds captured more pepper weevils (both sexes) than did unbaited control traps or pheromone-baited boll weevil traps. Commercial and laboratory formulations of the synthetic pheromone were both attractive. However, the commercial formulation did not release geranic acid properly and geranic acid is necessary for full activity.

Gunawardena and Bandarage (1995) conducted a preliminary field assay with 4-methyl-5-nonanol (ferrigineol), a synthetic component of aggregation pheromone of *Rhynchophorous ferrugineus.* At a release rate of 0.38 ± 0.08 mg synthetic ferrugineol per day from capillaries suspended in bucket traps filled with soap water, significantly more weevils were caught compared toa control trap (0.23 ± 0.04 weevils/trap/day vs. 0.00 weevils/trap/day, respectively) in the field. Significant differences were not observed between male and female trap catches using ferrugineol as bait (0.12 ± 0.02/trap/day and 0.11 ± 0.01 trap/day, respectively).

In field tests in a banana plantation in Costa Rica, compounds exo-ß-sordidin and exo- α -sordidin significantly increased capture rates of standard pseudostem traps. Comparable numbers of adults were attracted to pseudostem traps baited with the major naturally occurring isomers of sordidin, endo-α-sordidin+exo- α -sordidin or exo-ß-sordidin and exo- α -sordidin. Although addition of the minor naturally occurring isomers of sordidin (exo-ß-sordidin and *endo- ß -sordidin*) to pseudostem traps increased capture rates above controls, these compounds did not increase attraction of pseudostem traps to the same extent as exo-ß-sordidin and exo- α –sordidin (Jayaraman *et al*., 1997). Cerda *et al*. (1998) conducted field trials with sordidin, an aggregation pheromone of *C. sordidus*, to evaluate its attractiveness with host plant tissue. They reported that sordidin attracts few insects when it was presented without host plant tissue. However, the attractiveness of host plant tissue increases more than ten fold when it was presented simultaneously with sordidin in field traps.

Field monitoring of *Sitophilus zeamais* and *S. oryzae* (Coleoptera: Curculionidae) using refuge and flight traps baited with synthetic pheromone and cracked wheat was conducted by Likhayo and Hodges (2000). Traps baited with the aggregation pheromone (Sitophilure) and traps baited with pheromone and food bait (cracked wheat) were field tested. Both traps types were effective in capturing *Sitophilus spp.* and gave higher weevil catch when baited with pheromone and cracked wheat combined than with pheromone or cracked wheat alone. The combination of pheromone and cracked wheat had an additive effect on trap catch and not a synergistic effect as reported in earlier laboratory trials.

The comparison of adult trapping methods such as pseudostem traps, pitfall traps containing a pheromone [either Cosmolure (Pheromone A) or Cosmolure$^+$ (Pheromone B)] and unbaited pitfall traps (control) for trapping banana weevil, *C. sordidus*, in field trial showed that Pheromone A proved to be the most effective of the different traps (De Graaf *et al*., 2005).

Experiments were conducted to assess the effect of pheromone blend, trap design and trap height on the capture of potato tuber moths *Phthorimaea operculella* (Zeller) in New Zealand. Water traps caught more moths per day. Sticky traps (delta-shaped) caught more moths than A-trap sticky traps (cylinder-shaped) and funnel traps. The 1:1 and 1:1.5 pheromone blends caught more moths than the 1:4 blend. Trap height had no significant effect on moth catch. It is concluded that the DeSIRe sticky trap is the most suitable for commercial use. The 1:1.5 pheromone blend is recommended for commercial use in New Zealand and overseas (Herman *et al*., 2005).

Tinzaara *et al.* (2007) conducted field trials to determine an additive effect of pseudostem tissue and pheromone. The results showed that in trials 1 and 3 under field conditions, pheromone was significantly more attractive than the fermented tissue and control but equally attractive to the combination of the pheromone and the fermented tissue. However, in trials 2 and 4, the addition of fermented tissue to pheromone-baited traps increased attractivity to *C. sordidus* by 50 % compared to pheromone alone. Fresh pseudostem tissue contributed significantly to the attraction of *C. sordidus* only in trial 4.

Field experiments with synthetic components of male aggregation pheromone of agave weevil, *Scyphophorus acupunctatus* confirmed the laboratory results, showing that all components, singly or in blends, were attractive to the weevils. In general, traps baited with the quaternary blend of compounds (i) 2-methyl-4-heptanol, (ii) 2-methyl-4-octanol, (iii) 2-methyl-4-heptanone and (iv) 2-methyl-4-octanone captured significantly more weevils than traps baited with males. However, compounds (iii) and (iv) were sufficient to obtain captures equivalent to those by the quaternary blend (Ruiz-Montiel *et al*., 2008).

Field experiments in highbush blueberry (New Jersey) and cranberry (Massachusetts) examined the attraction of *A. musculus* to traps baited with the male-produced compounds and geraniol presented alone and combined with (Z)-3-hexenyl acetate and hexyl acetate and to traps baited with the pheromones of *A. eugenii* and *A. grandis*. In both states and crops, traps baited with the *A. musculus* male produced compounds attracted the highest number of adults. Addition of the green leaf volatiles did not affect *A. musculus* attraction to its pheromone but skewed the sex ratio of the captured adults towards females (Szendrei *et al*., 2011)

Palanichamy *et al.* (2011a) conducted field experiment to develop an efficient trapping method to monitor and control the pest. Funnel traps baited with the lure of aggregation pheromone 2- methyl-4-heptanol combined with host plant extract caught significantly more weevils than traps baited with either pheromone or host plant extract alone. A mean capture of 3.222 weevils was recorded in the trap baited with pheromone + host plant extract. During the 2nd week of the experiments, weevil capture was reduced and the maximum mean capture was recorded in the trap baited with pheromone + host plant extract. During the 3rd week of the experiments, there was no significant weevil capture in the traps baited with different semiochemicals indicated the reduction of the weevil population at the experimental site.

A study on effect of dispenser type and trap design for capture of dogwood borer (DWB), *Synanthedon scitula* Harris was conducted in apple orchards in West Virginia and North Carolina. Wing-style sticky traps baited with polyethylene vial captured more male DWB over the first 2 months and later decreased (Zhang *et al*., 2013).

III MATERIAL AND METHODS

Studies were carried out on “Chemical Ecology and Behaviour of Banana pseudostem weevil (BSW), *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae)” at Department of Agricultural Entomology, UAS, GKVK and Bio Control Research Laboratory (BCRL), Sreeramanahalli, Bengaluru during 2014-2016. The material used and methods employed for experiments in laboratory and cage conditions on different aspects related to electrophysiological and Behavioural responses of banana pseudostem weevil, *O longicollis* infesting banana are presented here. The aspects studied are calling and mating Behaviour and chemical identification of Behaviourally active pheromone components of *O. longicollis*. The details of the experiments carried out on above aspects are described in this chapter.

3.1 Behavioural responses of Banana pseudostem weevil related to pheromones

3.1.1 Laboratory culture of the weevil

The weevil, *O. longicollis* pupae and adults were collected from infested banana fields in and around Bangalore rural district (13.18’, 77.55’, 980 m AMSL). The field collected pupae were kept separately in plastic boxes (7 cm dia, 12 cm height) and daily checked for adult emergence. Emerged adults were separated into males and females by rostrum characteristics (Plate 1). The adult weevils collected from the banana fields were separated into males and females based on rostrum characteristics. Males were having short snout with punctuations on the rostrum. This gives the rostrums rough surface-look, while in female snout is longer compared to males having no punctuations on the rostrum giving smooth, shiny, surface-look under stereo zoom microscope. Both male and female weevils were maintained in separate plastic containers (29 cm x 17 cm x 33 cm) in the laboratory under the photoperiod of 12 light (L): 12 dark (D) regime with 25 ± 2 ^{0}C and relative humidity of 70 ± 10 %. Weevils were provided with freshly cut banana pseudostem pieces (approx. 25cm long) as food (Plate 2). The pseudostem pieces were changed once in 5 days. Insect colonies were maintained at the rearing facility of Biocontrol Research laboratories (BCRL), Pest Control (India) Pvt. Ltd. (PCI), Sriramanahalli, Bengaluru.

3.1.2 Periodicities of mating

The study was conducted to determine the peak time of mating activity of *O. longicollis.* This was to facilitate the time of volatile collection and for Behavioural studies. Experiments were conducted during scotophase and photophase of weevils in the BioControl Research Laboratory, Bengaluru to develop an understanding on time of activity of weevils. In this study, number of mating pairs and frequency of matings in each hour were recorded. Five males and females were placed in separate transparent plastic boxes (19 cm L x 15 cm W x 9 cm H) covered by a muslin cloth tied with an elastic ribbon. A fresh piece of banana pseudostem (3 cm x 2cm) was placed inside the boxes. Pairs were analyzed every 5 min for a 12 hour period both during scotophase (6 pm to 6 am) and photophase (6 am to 6 pm). During the scotophase, observations were facilitated using a dim red bulb (5 W). Observations on number of copulation events per pair and mean number of mating pairs were recorded and observations were repeated two times.

3.1.3 Female calling Behaviour

The study was conducted to determine the periodical pattern of female calling Behaviour of *O. longicollis.* Experiments were conducted during photophase of weevils in the BCRL laboratory to develop an understanding on calling Behaviour. During the female calling study, the time of calling, frequency of calling per day, duration and posture of calling were studied. Twenty females were confined in a transparent plastic container (12 cm L x 7 cm W x 11 cm H) individually, covered by a muslin cloth tied with an elastic ribbon. A fresh piece of banana pseudostem (3 cm x 2 cm) was provided as food inside the boxes. The calling Behaviour of weevils was recorded every 10 min. The calling Behaviour was replicated four times. Four groups of females were observed with 5 individuals in one group. Mean number of calling females on each hour was compared using one way ANOVA followed by Tukey's post-hoc test was conducted using IBM-SPSS (version 21). A level of $P<0.05$ was accepted as statistically significant for all analyses.

3.1.4 Mating Behaviour

Laboratory study was conducted to determine the male and female courtship and mating Behaviour. In this study, duration of mounting, mating, patterns of courtship and mating Behavioural activities, the courtship posture, number of copulation events per pair and mean number of mating pairs on each hour were studied. Thirty pairs of active male and female weevils were kept separately (one pair/box) in a transparent plastic container (12 cm L x 7 cm W x 11 cm H) to mate and the containers were covered by a muslin cloth tied with an elastic ribbon. Observations recorded immediately after pairing. Pairs were analyzed every 10 min throughout the photophase for six days. Weevils were provided with a piece of fresh banana pseudostem (3 cm x 2 cm) inside the containers as a food source to the weevils. The average percentage of pairs starting to mate every 15 min was estimated for each hour. The onset time of mating, duration for each mating, courtship Behaviour and mating posture were recorded during photophase (6 am to 6 pm) for a 12 hr period. Courtship Behaviour like male approach to the female, downward bending of posterior end of abdomen and exposing the aedeagus partly or fully were recorded. Six groups of thirty pairs with 5 pairs in each group were observed for six consecutive days. The ethogram was prepared using probability of each event of courtship and mating occuring.

3.2 Chemical identification of pheromone components of Banana pseudostem weevil

3.2.1 Volatile collection

Prior to insect volatile collection, preliminary trials were conducted to determine the peak time of mating activity of male and female BSWs during scotophase and photophase under caged conditions. Based on the observations, the volatile collection was carried out during photophase between 0900 to 1600 h by air entrainment method (Ambrogi and Zarbin, 2008; Ruiz-Montiel *et al*., 2008; Prashant, 2014 and Palacio-Cortés *et al.,* 2015). Groups of 50 male and 50 female weevils were maintained

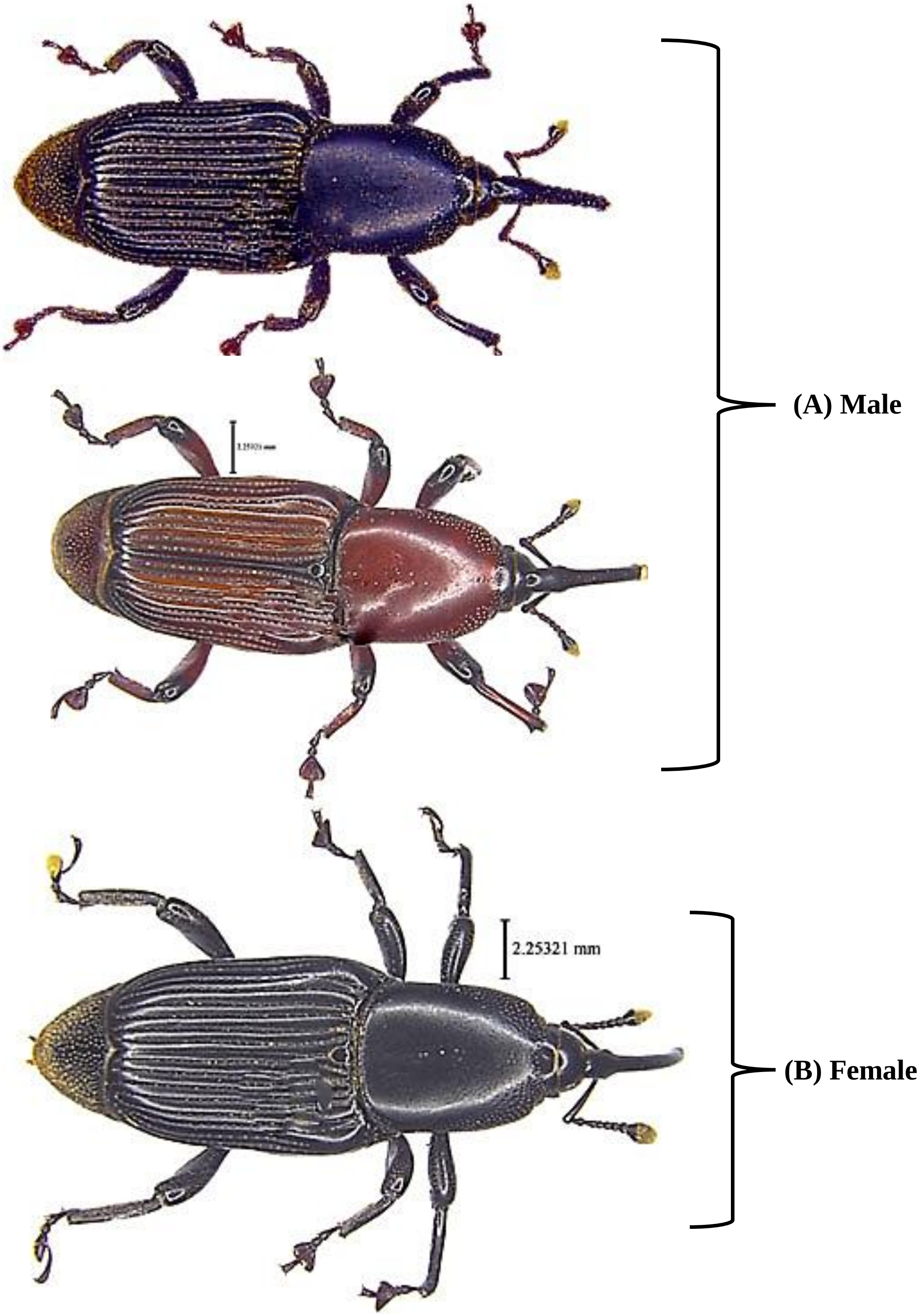


Plate 1. Banana pseudostem weevil, *Odoiporus longicollis*.
(A) Male (B) Female

Plate 2. **Banana pseudostem weevil culture maintained at BCRL, Bangalore**

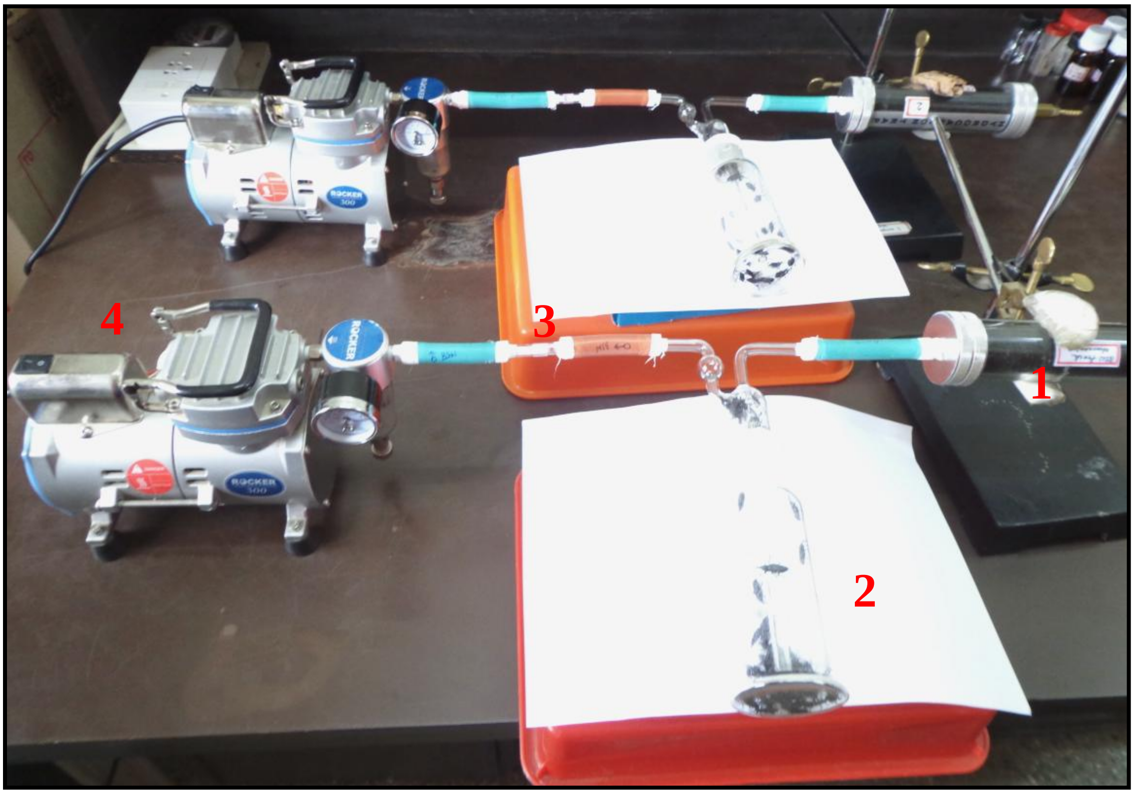


Plate 3. **Insect volatile collection by air entrainment method, 1-Hydrocarbon, 2-Insect release chamber, 3-Poropaq- Q tube and 4 Vaccum suction pump.**

separately in different glass aeration chambers (5 cm dia. x 15 cm ht). Thirty male and 30 female were held separately in wire mesh cages and both cages were kept in a single plastic acrylic container (6.5 cm width x 12 cm ht x 17 cm length) for volatile collection together from both male and female BSWs. Before collecting insect volatiles, blank collection was done with an acrylic box. The weevils were not starved before the collection of the volatiles. A charcoal filtered humidified air flow at a rate of 1.8 L min^{-1} was pushed through the aeration system using a vacuum system. The air was pulled through 1) activated charcoal which removed large hydrocarbon molecules and any oils present in the system 2) a molecular sieve, which removed any smaller organic molecules, 3) Gilmont #l flow meter, which monitored the air flow rate, 4) A glass aeration chambers (5 cm dia. x 15 cm ht) which contained either male/female weevils and finally through the glass column trap filled with a polymer - conditioned Porapak-Q 50/80 adsorbent (Plate 3).

Volatile chemicals which were released from the male and female weevils were collected daily for 2-4 hour and trapped on Porapak-Q adsorbent using the previously mentioned apparatus. The volatile collection was conducted at 25 ± 2 ^{0}C and 70 ± 10 % RH under a 12 L: 12 D regime at Biocontrol Research laboratories, Sriramanahalli, Bengaluru. The volatiles from weevils were collected from the Porapak-Q with 1.5 ml of HPLC grade Dichloromethane (DCM) in 15 min. A dilution of 300 weevils were prepared and concentrated to 1 ml (*i.e.,* 3 weevil volatiles per 10 µl of DCM) under nitrogen stream before analysis, so that the volatiles are in diluted form for use in experiments. Porapak-Q has been demonstrated as an effective adsorbent of insect volatiles and pheromones (Byrne *et al.,* 1975; Guss *et al.,* 1982; Guss *et al*., 1983; Hall *et al*., 1987; Jang *et al*., 1989; Leal *et al*., 1996 and Phillips *et al*., 1996). Volatiles from 30 males and 30 females (Prashant, 2014) were collected for use in the Behavioural assay. The aforementioned procedure as indicated earlier for the volatile collection was employed.

3.2.1.1 Porapak-Q Conditioning

The Porapak-Q adsorbent was conditioned before and after volatile collection. It involved placing the Porapak-Q into a Soxhlet apparatus and refluxing it with dichloromethane from a round bottom flask (50 ml) for 5 hours on a heating mantle at 45 ^{0}C (Bowers *et al*., 1991).

3.2.1.2 Solvent Evaporation

The volatile extracts were concentrated before use for electroantennography or EAG linked GC analysis and Behavioural assay. The solvent was evaporated applying a gentle stream of dry nitrogen under a fume hood to obtain a final volume of 0.5 ml. This concentration was used for the preparation of all stimuli. The stimulus concentration was prepared based on the number of weevils used in the volatile collection. Therefore, the stimulus concentration (in weevil equivalents) represents at most 100 per cent participation in volatile production. Any dilution of volatile extracts that was not used immediately for stimulus preparation was stored in a refrigerator at -20 ^{0}C.

3.2.2 Gas Chromatography (GC)

The gas chromatographic technique is useful in isolating the chemical compounds and in establishing the purity of isolated compounds. Failure to ensure the isolated material in pure form results in thwarted identification of bio-active material. Though 100 % separation of several chemical constituents is not ensured in GC, criteria of analysis of a compound were established that is useful to detect the purity of the synthesized compound. The effective use of GC for the analysis of pheromones and the determination of their purity needs a clear understanding of the chromatographic process. Capillary Gas Chromatography is the most useful method now available for pheromone analysis for which it provides maximum resolution needed for the isolation/separation of complex mixtures and the sensitivity to detect minute amounts of impurities. The contents of the male and female volatiles and standard compounds were analyzed using Agilent 7890A GC system. The GC was fitted with a DB-5 column (both 30 m× 0.25 mm i.d., 0.25 µm film; J&W Scientific, Folsom, CA, USA). The carrier and makeup gas was nitrogen (99.999 %) at the flow rate of 5 ml min^{-1}. The GC oven was programmed from 60 °C for 2 min, 10 °C per min to 220 °C for DB-5, hold time for 28 min.

3.2.3 Coupled Gas Chromatography-Electroantennogram Detection (GC-EAD)

The pheromone extracts of BSW were injected to Agilent 7890A GC system, fitted with the Flame Ionization Detector (FID). Gaschromatography was fitted with a DB-5 column (30 mm length: 0.25 mm inner diameter: 0.25 µm film thickness; J&W Scientific, Folsom, California, USA) for separation of compounds. A flame ionization detector (FID) was ignited using high purity hydrogen gas and reference gas zero air (99.99 %) (White and Chamber, 1989). The carrier gas was nitrogen (99.99 % purity) (0.5 kg cm^{-2}) and injection temperature was 250 ^{0}C in the split mode. The GC oven was programmed from 60 ^{0}C for 2 min, 10 ^{0}C per min to 220 ^{0}C for DB-5 with column flow of 1ml/m, held for 30 min. Solvent extracts (1 µl aliquots) were analyzed in Split less mode. The effluents emerging from the column were split using Agilent splitter with half of the sample going to the FID detector and the other half to the EAD through column (The column effluent was split 1:1 between the FID and EAD apparatus). The portion directed to the EAD was diluted in a humidified air stream that was directed over the antennal preparation that consisted of the excised terminal 4–5 antennal segments of a weevil, placed over the positive and negative electrode of a Combi-probe using a high conducting electrode gel (Signagel, Parker Laboratory Fories. Inc. USA). A single antennal preparation was used for one run. One µl of the sample was injected into the injector and traces were recorded using the Agilent Chemstation. The antenna of the weevil (male and female) was excised and fitted in the micromanipulator assembly of the electroantennogram and the antenna was kept at close proximity to the stimulus delivery tube described earlier so that the traces of effluents from GC are gently blown onto the antennae after elution in the GC (Plate 4). The traces from FID and EAD were plotted and the matching peaks along with their retention times were measured. The compound was considered electroantennographic active when it elicited corresponding antennal responses at least three times.

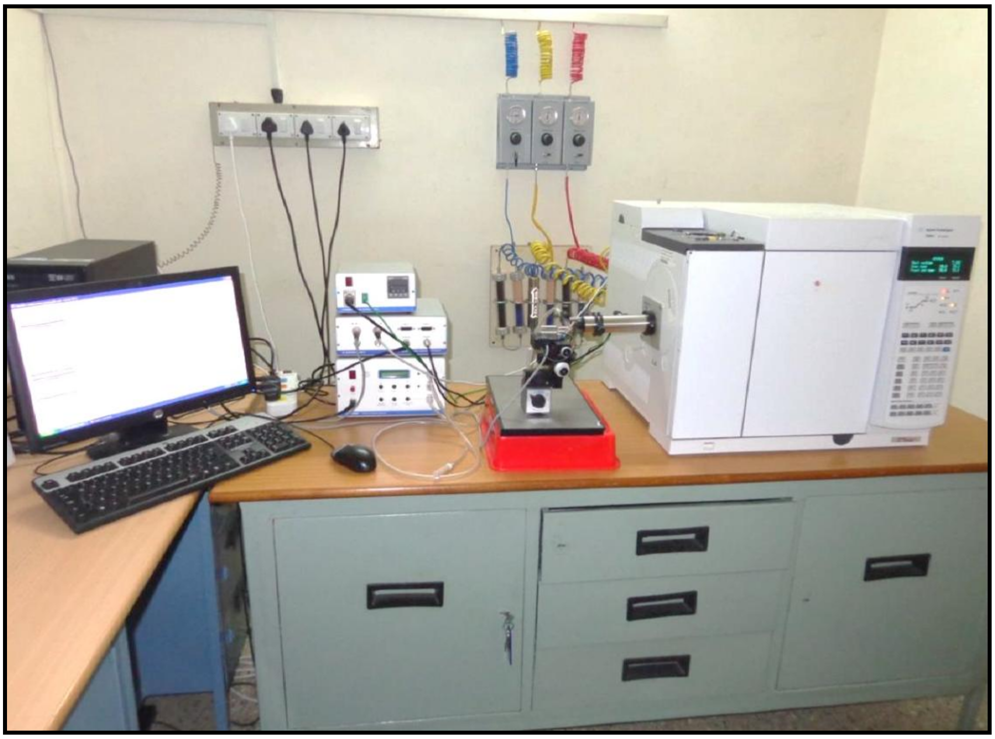


Plate 4. **Coupled gas chromatography-electro antennogram detector (GC-EAD) instrument**

Plate 5. **Coupled gas chromatography-mass spectrometry (GC-MS) instrument**

3.2.4 Gas Chromatography–Mass Spectrometry (GC-MS) Analyses

GC-MS analyses of BSW volatile extracts were carried out with an Agilent 6890 N GC interfaced to a 5790C mass selective detector (MSD) (Agilent, Santa Clara, CA, USA). The GC was fitted with an HP5-MS column (30 mm × 0.25 mm i.d., 0.25 μm film). The carrier and makeup gas was helium. Temperature was programmed from 60 (held for 2 min) to 220 °C at 10 °C (held per 10 min) min^{-1}. Injector temperature was 250 °C and injections were made in the splitless mode. Specific peaks were tentatively identified by matching their mass spectra to the GC/MS database (National Institute of Standards and Technology, Gaithersburg, MD) and confirmed by comparing spectra and retention times with KI authentic standards. KI were calculated for unknowns and standards relative to blends of straight chain hydrocarbons. For increased precision, KI values on the DB-Wax column were obtained using a GC oven program rate of 5 rather than 10 ^{0}C/min (Plate 5). Identifications of peaks were confirmed by coinjections of extracts with authentic standards (BCRL, Bengaluru).

3.2.5 Quantification of volatiles

3.2.5.1 Quantification of identified pheromone compounds from insect volatiles of only males and Male + Female BSW collected during full day

For the quantification of identified pheromone compounds, insect volatiles were collected from 50 males and Male+ Female BSW separately from 11.00 AM to 04.00 PM for 3 consecutive days by following the above mentioned method. Samples were injected to GC along with internal standard (Cue lure for Diphenylamine and 14-Acetate for 1-Hexadecene and Hexadecane). The GC oven was programmed from 60 ^{0}C for 2 min, 10 °C per min to 220 °C for DB-5, hold for 28 min and using the chromatograms, volatiles were quantified using the following formula,

$$\text{Quantity of volatile} = \frac{\text{Concentration of Internal standard} \times \text{Area per cent of volatile}}{\text{Area per cent of internal standard}}$$

3.2.5.2 Quantification of identified pheromone compounds from insect volatiles collected during different time intervals (*i.e.*, morning and afternoon)

For this study volatiles were collected from 50 male BSW from 11.00 AM to 01.00 PM and 02.00 PM to 04.00 PM for 3 consecutive days by following the above mentioned method. Samples were injected to GC along with internal standard with initial temperature and using the chromatograms, these pheromone compounds were quantified following the above formula.

3.2.5.3 Quantification of identified pheromone compounds from insect volatiles collected during night time

This study was conducted to know the diel periodicity of pheromone release and for the quantification of pheromone compounds produced during night. The insect volatiles were collected from 50 male BSW for 3 consecutive nights by following the above mentioned method. Samples were injected to GC along with internal standard with initial temperature and using the chromatograms, these pheromone compounds were quantified using the above formula.

3.2.5.4 Quantification of identified pheromone compounds from insect volatiles collected in the presence and absence of banana pseudostem as food

In order to investigate the effect of food plant availability on the release of pheromones, the volatiles were collected from 50 male BSW with fresh pseudostem pieces (three pieces of 3 cm x 2 cm) provided as food inside the volatile collection glass chambers. The volatiles were collected from 11.00 AM to 04.00 PM for 3 consecutive days, either in the presence of food or without food, following aforementioned method. Samples were injected to GC with internal standard with initial temperature and the chromatograms, these pheromone compounds were quantified using the formula stated earlier.

3.2.5.5 Quantification of identified pheromone compounds from insect volatiles from starved weevils

In order to investigate whether the identified pheromone compounds were derived from the food consumed by the weevils, a study was conducted to quantify these compounds from starved insects and fully fed insects. Two batches of weevils, each batch with 30 male BSW was starved for 24 hr (Batch-I) and 48 hr (Batch-II). The volatiles were collected from two batches of starved weevils and fully fed weevils during day time from 11.00 AM to 04.00 PM following the above mentioned method. Samples were injected into GC along with internal standard with initial temperature and by using the chromatograms, these pheromone compounds were quantified using aforementioned formula.

3.3 Studies on host volatiles

3.3.1 Extraction of plant volatiles

The present study was conducted employing electroantennographic (EAG) technique and Behavioural bioassays to determine the effective host-plant volatile(s) for the attraction of *O. longicollis* adults. Volatiles were collected from healthy banana pseudostem, BSW damaged pseudostem, mechanically damaged pseudostem and decaying pseudostem. Banana pseudostem samples for volatile extraction were obtained from the Elakki variety, BCRL, Bengaluru, Karanataka.

Solvent extraction method was followed for volatile collection. Pseudostem pieces (1 cm) of 5 gm each from healthy banana plant, BSW damaged plant, mechanically damaged plant (3 days after mechanical damage) and 20 days decayed pseudostem were collected and immersed in 10 ml dichloromethane (CH_2Cl_2) (HPLC grade) at room temperature (25 ± 2 ^{0}C) for 3 days (72 h). Three days after soaking, the solvents were decanted into another bottle (25 ml) separately. The 1.5 ml of collected volatile was concentrated to 250 µl under a gentle stream of nitrogen and kept in the deep freezer under -20 ^{0}C until used for bioassays.

3.3.2 Gas Chromatography (GC)

The contents of all the four types of pseudostem extracts were analyzed using Agilent 7890A GC system. The GC was fitted with a DB-5 column (both 30 m x 0.25 mm i.d., 0.25 μm film; J&W Scientific, Folsom, CA, USA). The carrier and makeup gas was nitrogen (99.999 %) at the flow rate of 5 ml min^{-1}. The GC oven was programmed at 60 ^{0}C for 2 min, 10 ^{0}C per min to 220 ^{0}C for DB-5, hold time was 28 min.

3.4 Laboratory and field evaluation using identified pheromone components

3.4.1 Laboratory bioassay of *Odoiporus longicollis* pheromone components and blend combinations

3.4.1.1 Electrophysiology (EAG)

Electroantennography (EAG) bio-assays were performed for quick verification of insect response to crude extracts and to various synthetic compounds and screening of blends because it is a versatile technique for detecting electrophysiological activity of compounds using live insects (Barsi, 1998). EAG is generally the sum of many olfactory receptor potentials recorded more or less simultaneously by an electrode located in the chemosensory organ (antenna) of an insect. This technique is useful in the determination of structural, chemical and electrical aspects of olfactory sensilla of an insect (Barsi, 1998). The biological activity can be detected by EAG. Exposure of an insect antenna to the chemicals detected by receptors on the antenna causes depolarization, which can be recorded by means of a microelectrode at either end of the insect antenna. This technique gives a vital clue for further determination of the bio active pheromone compounds.

Electrophysiological responses of male and female *O. longicollis* antennae to volatile dilutions and to individual as well as blends of synthetic compounds were recorded using commercially available electroantennographic system (Syntech, Hilversum, The Netherlands). The system consisting of a probe having positive and negative electrodes (filled with electrolyte, 0.1 M KCl and 1 % PVP) for antenna fixation, a CS-55 stimulus controller and IDAC-2 (2-channel signal acquisition interface Intelligent Data Acquisition Controller).

The *O. longicollis* antenna was carefully excised from the head using a microscissor in such a way that all the antennal segments and the basal nerve were still attached to the antenna. Antennal depolarization was detected with a high-resistance EAG probe. An excised antenna was fixed onto an antenna holder for the EAG probe in such a way that the tip of the antenna was in contact with -ve electrode and base *i.e.* scape was in contact with +ve electrode. This was accomplished using electroconductivity gel (Sigma-gel, Parker Laboratory Fories. Inc. USA). The DC potential was recorded on a computer using a custom-built amplifier (Hanson B-102) and an IDAC (Intelligent Data Acquisition Controller) A/D converter and software (Auto Spike v. 3.0, Syntech, Hilversum, The Netherlands). Filtered and humidified air was continuously flowing over the mounted antenna (approximately 0.5 m/s).

The samples were subjected to EAG by applying 20 μl dilution of test chemical in a defined amount on to the filter paper strips (6 cm length and 5 mm width; Whatman

No.1) and placed into the Pasteur pipette (Dimensions- 5.75 mm dia; Length- Overall 145 mm; tip length 47.0 mm). This was connected to the stimulus controller (CS- 05, Syntech, Hilversum, The Netherlands) by non-adsorbent, odourless polyesterene rubber tube. After 10 seconds, the solvent was blown out with first puff. Another 60 seconds, the stimulus was puffed on to the antenna by injecting the vapour phase of the chemical stimuli through a polyesterene tube along with the continuous air stream (pulse rate 0.5 s, continuous flow 25 ml s-1, pulse flow 21 ml s-1) to the antenna (Plate 6). The solvents used for the stimuli were tested independently and elicited minimal EAG responses, indistinguishable from responses to air. The different stimulus puffs were given at 30 s intervals. For each sample 5 replicates were performed per sex with fresh antennae and each replicate represented one antenna.

Electrophysiological responses (mV) of various stimuli were statistically analyzed by using a one-way Analysis of Variance (ANOVA) and treatment means were separated by Tukey's post hoc test using IBM-SPSS (version 21).

3.4.1.2 Y-tube Olfactometer assays

The Behavioural responses of *O. longicollis* males and females were tested to a range of volatile extracts from weevils and banana plants both *per se* and in blends. The pheromone compounds and plant volatile extracts were tested in Perspex acrylic Y-tube olfactometer (BCRL, Bengaluru). Dilutions for each compound were made either in dichloromethane or in hexane.

The olfactometer consisted of Y-shaped acrylic tube of 6 cm dia. The main tube (stem) of the olfactometer and the two arms were each 30 cm in length at 90^0. The olfactometer arms were closed with muslin cloth and the samples were placed at the end of each arm. One arm of the Y-tube held the odour source and the other served as a control. The air-delivery unit consists of two small battery operated fans, placed at 5 cm distance from the two arms of the Y-tube (outside) to blow air to pass through the two odor chambers. Airflow through each of the olfactometer arms was maintained at 0.5 L min^{-1}. From each odour chamber, the air passed into the respective arms of the Y-tube and then through the main tube (stem) of the olfactometer. A known number of weevils were released at the end of the olfactometer stem in a small container (7 cm dia, 12 cm height). They were allowed to acclimatize for half an hour and then the fans were switched on and the barrier for the weevils removed. The Behaviour of weevils was observed for 15 min. For each experiment a group of 3 weevils were used and each experiment was replicated 7 times with fresh weevils (the total number of weevils of each sex used per sample and odor sources was 21). When a weevil crossed the choice line 10 cm after the division of the main tube and remained there for at least 20 s, it was recorded as a choice for the odor source in that arm (Plate 7). If the weevils stayed in the main tube or at the junction of the two arms and did not make a choice during this time were considered a non-responding individual and were excluded from the statistical analysis.

The application of odors or their sources to each arm of the olfactometer was reversed between tests to eliminate directional bias. After each test, the olfactometer and components were washed with soap solution, rinsed with acetone followed by distilled

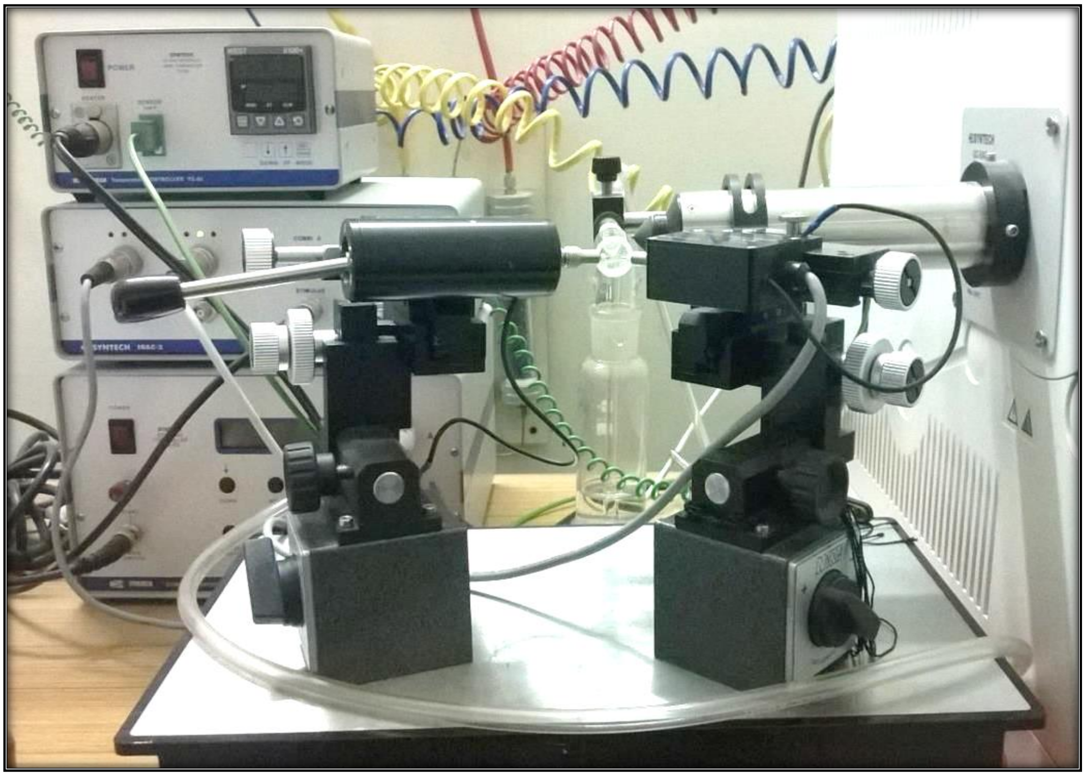


Plate 6. Electroantennography (EAG) system

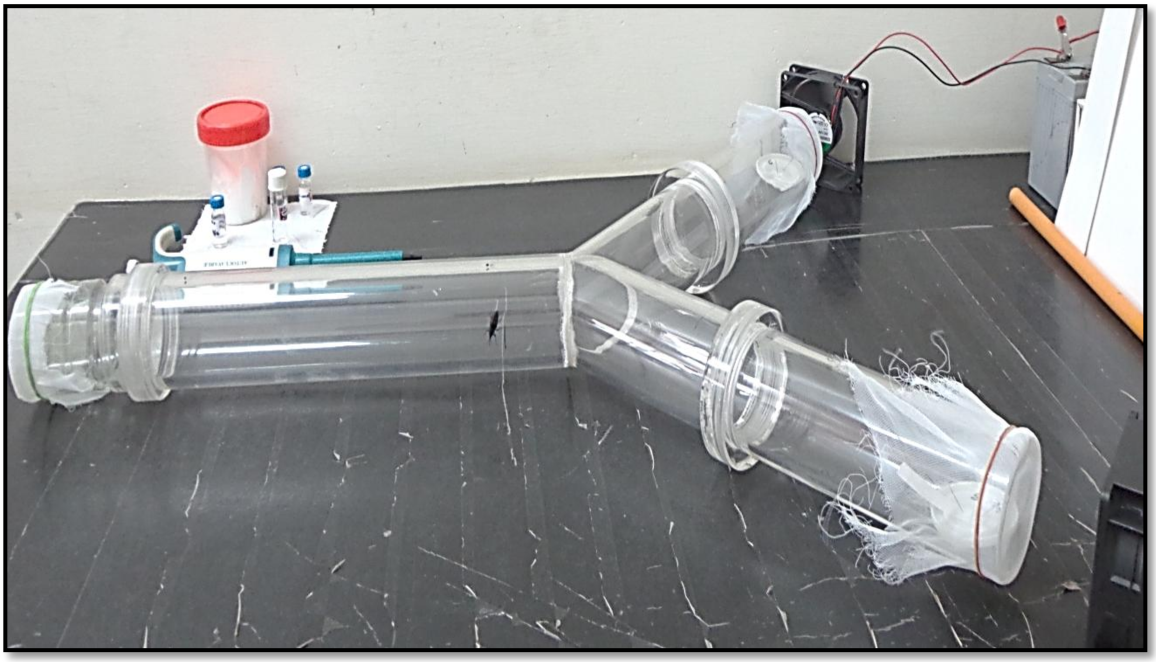


Plate 7. Y-tube olfactometer for bioassay

water and air dried for at least 30 min to remove any volatile contaminants. For each odor source tested, the known amount of dilution were loaded in Whatman filter paper strips of 1 x 5 cm and the solvent was allowed to evaporate for 60 s before placing in to the chamber of odor source. The other chamber served as control providing filter paper strips (1 x 5 cm) loaded with equal volume of HPLC-grade DCM or hexane solvent. New filter papers with the odor source and DCM were used for each trial (for every 3 insects). After being tested, weevils were returned to the containers with food. The same insects were not tested on consecutive days. Separate experiment was conducted with the male and female weevils for each odour source. The trials were carried out between 11:00 to 17:00h during photophase coinciding with the peak time of mating of weevils. The responses of weevils were analyzed with a binomial test (χ^2 test; SPSS 21.0 version software) and means were compared by Tukey HSD test ($P < 0.05$) (Jaramillo *et al.*, 2013).

3.4.1.3 Dose response studies

3.4.1.3.1 Dose response study of Diphenylamine, 1-Hexadecene and hexadecane to male and female BSW

The different doses of synthetic compounds, *viz.*, Diphenylamine, 1-Hexadecene and hexadecane were tested with male and female antenna. Five different doses *viz.*, 0.5 µl, 5 µl, 10 µl, 20 µl and 40 µl (2 % solution *i.e.*, 20 mg/ml) were tested to find out the response of weevils to individual synthetic compounds. Most preparations would last 2-3 hr without degradation, thus allowing a full dose series for one chemical to be tested. Stimulus dilutions were presented in order from the lowest to the highest doses. Five replications were maintained for each sex using antenna of different weevils at each dose.

3.4.1.3.2 Dose response study of best performed blend of DPA, 1-16C and 16C to male and female BSW

Best performed blend (screened by EAG and Y-tube olfactometer bioassay) were further tested at five different doses *viz.*, 10 µl, 20 µl, 40 µl, 80 µl and 100 µl (2 % solution *i.e.*, 20 mg/ml) to find out the dose variation response. Most preparations would last 2-3 hr without degradation, thus allowing a full dose series for one chemical to be tested. Stimulus dilutions were presented in order from the lowest to the highest doses. Five replications were maintained for each sex using antenna of different weevils at each dose.

3.4.2 Bioassay of Plant volatiles

3.4.2.1 Electrophysiology (EAG)

Electroantennography (EAG) bioassay was carried out to assess the olfactory sensitivity of both male and female *O. longicollis* adults to different pseudostem extracts. The procedure followed was same as mentioned in 3.4.1.1. The antennal response was also recorded for the combinations of plant volatiles and male insect volatiles against both male and female antennae.

3.4.2.2 Y-tube Olfactometer Assays

The Y-tube olfactometer studies were conducted using *O. longicollis* males and females after EAG screening to determine Behavioural response to different pseudostem extracts at Insect Behaviour Testing Lab (IBTL), BCRL. The procedure followed was same as mentioned in 3.4.1.2. Also these insects were tested for response to the combination of plant volatiles and male insect volatiles.

3.4.3 Cage studies

3.4.1.1 Pheromone trap evaluation

Pheromone trap evaluation was conducted using pseudostem pieces and live BSW weevils as attractant in different types of traps in nylon mesh cage (10 m x 10 m x10 m) in the field experiment station of BCRL, Bengaluru. Male (10 Nos.) and female (10 Nos.) weevils were separately confined in a wire mesh cage (5 cm L x 3 cm H x 4 cm W). Four types of pheromone traps viz., Bucket trap (5 l capacity; 22 cm H x 23 cm dia. four windows of 7 cm x 2 cm), Wota-T water trap (1.5 l capacity), china funnel trap [(consisting of a funnel attached with a cylinder (20 cm H x 10 cm dia.)] and cross vane trap (white cardboard sheet provided with adhesive surface) [Marketed by PCI (Pvt.) Ltd.] were evaluated for effectiveness in trapping BSW weevils (Plate 8). The weevil confined cages were suspended by a thin wire in the middle of the traps during 17:00 h. Ten milliliter of soap water was added per litre of water in Wota-T water trap and China funnel trap, to prevent the weevils escaping from the trap. The traps were suspended to a support by a wire attached at 1.5 m from the ground level. Two traps of each type were placed in two opposite corners of the cage. Ten male and female weevils from the laboratory culture, were collected from the field and maintained in the laboratory in separate plastic containers, were released in to the cage daily up to six days. Next day, at 10.00 hr the number of weevils captured in the traps was collected, brought to the laboratory, male and female weevils were separated and their numbers were recorded. Weevils not captured in the traps were collected back. For each trial, fresh insects were used in the cage and traps were cleaned. The traps position was changed regularly to avoid the positional effect. The observations on trap catches were subjected to paired t-test analysis. Relative attraction was worked out [(number of weevils on trap A- number of weevils on trap B)/ Total number of weevils on both traps] × 100 (Tasin *et al*., 2011). The trap catches in different traps were compared using one-way ANOVA followed by Tukey post hoc test using IBM SPSS version 21. Details of different traps and attractants used for the trap evaluation are presented in Table 1.

3.4.1.2 Synthetic pheromone blend evaluation

Pheromone blend evaluation was conducted by using cross vane trap with a bucket suspended to it for retaining trapped weevils in a nylon mesh cage (10 m ×10 m × 10 m) in the field experiment station of Bio Control Research Laboratory, Bengaluru (Plate 9). Three blends were tested in the field cage study *viz*., 0.5:10:20 (DPA: 1-16C: 16C), 100:8:5 (DPA: 1-16C: 16C) and 8:5 (1-16C: 16C). Two cross vane traps were placed at opposite corners of the cage. Traps were suspended to a support by a wire attached 1.5 m from the ground level. Ten male and female weevils from the laboratory culture were released into the cage daily up to six days. Next day, at 10.00h the number

Plate 8. **Different types of traps used for evaluation. A-Bucket; B-Wota-T; C-Cross vane, D-Cross vane with bucket;E- China funnel, F-cages with male and female BSW**

of weevils captured in the traps was collected, brought to the laboratory, male and female weevils were separated and their numbers were recorded. Weevils not captured in the traps were collected back. For each trial fresh insects were released and traps were cleaned. The traps position was changed regularly to avoid the positional effect. The observations on trap catches in both source and respective control were subjected to paired t-test analysis. The trap catches in different lures were compared using one-way ANOVA followed by Tukey post hoc test using IBM SPSS version 21.

Table 1. Treatments used in the pheromone trap evaluation

Sl. No.	Treatments
1	Bucket trap + pseudostem pieces v/s Pseudostem trap
2	Wota-T water trap + 10 male BSW in a cage v/s Wota-T water trap + 10 female BSW in a cage
3	China funnel trap + 10 male BSW in a cage v/s China funnel trap+ 10 female BSW in a cage
4	China funnel trap + pseudostem piece v/s China funnel trap no pseudostem piece
5	Cross vane trap + pseudostem piece v/s only pseudostem trap
6	Cross vane trap + 10 male BSW in a cage v/s Cross vane trap + 10 female BSW in a cage
7	Cross vane trap with bucket + pseudostem piece v/s Cross vane trap with bucket + no pseudostem piece

3.4.4 Field studies

3.4.2.1 Field evaluation of synthetic pheromone of BSW using cross vane trap

Field experiments were conducted during 2015 in infested banana field (Variety G-9) at Haniyur village, Hesaraghatta Hobli, Bengaluru, Karnataka. Experiment was conducted using four treatments and each treatment was replicated five times in randomized blocks. Cross vane trap obtained from BCRL, Bengaluru baited with 100 mg of synthetic male pheromone (0.5:10:20) in white plastic septa, suspended in the middle of the trap. Similarly, small pseudostem pieces (8 cm L X 2 cm width) were suspended in the middle of the traps (as shown in Plate 9). The pheromone traps with and without synthetic pheromone lures were suspended at 1.5 m height from the ground. Details of the treatments used in the study are presented below. Observations were recorded at 4 days intervals and dried pseudostem pieces were replaced once in 4 days.

Table 2. Treatments used in the evaluation of synthetic pheromone of BSW

Sl. No.	Treatments
1	Cross vane trap + synthetic male pheromone lure
2	Cross vane trap + pseudostem piece
3	Cross vane trap + pseudostem piece + synthetic male pheromone lure
4	Control (Only cross vane trap)

Plate 9. Field cage to study the synthetic pheromone blend evaluation

IV RESULTS AND DISCUSSION

Investigations on chemical ecology of the banana pseudostem weevil (BSW), *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) were carried out at Department of Agricultural Entomology, UAS, GKVK and Bio Control Research Laboratories (BCRL), Bengaluru during 2014-16. The results of the studies are presented and discussed in this chapter.

4.1 Behavioural responses of Banana pseudostem weevil to pheromone components

4.1.1 Time of mating

Observations on the time of day for mating were recorded during the scotophase and photophase. Observations revealed that adults mated during both day and night (Figure 1). The pairs started mating in the 1st hour of scotophase and photophase. During scotophase, the maximum mating activity was observed in the 1st hour (3.67 ± 1.33) itself, later the number of copulations decreased as the scotophase progressed. The peak mating period occurred during 6th hour (5.33 ± 1.45) and 9th hour (5.67 ± 2.40) of photophase when 100% of the pairs mated. During both scotophase (p=0.255, F=1.358) and photophase (p=0.227, F=1.420) there were no significant differences between individual time periods. There were clear differences in the mean number of copulations between individual time periods in scotophase and photophase. However, when photoperiods were compared, they were significantly different (t=2.524, p=0.028) and distinct pattern was observed with maximum number of matings during day time (Figure 1). Since, mating activity was observed constantly from 4th to 11th hour of photophase with two peak periods at 6th and 9th hours, the volatile collection from adult male and female BSW weevils and laboratory bioassay activities were carried out during this period.

In coleopterans, the mating occurs at any time of the day (Silva *et al*., 2012; Martins *et al*., 2013; Khairmode *et al*., 2015 and Nancy Barreto-Triana *et al*., 2014). *O. longicollis* mate throughout the year at day time and even at night (Khairmode *et al*., 2015). Mating is observed at any time outside the pseudostem or in the space between the outer most and inner sheaths of the pseudostem and frequent matings are observed (Dutt and Maiti, 1972). In rice water weevil, *Oryzophagus oryzae* adults mated during day and night and the main peak mating period occurred during the 2nd hour of photophase when 87 % of the pairs mated (Martins *et al*., 2013). The banana weevil, *Cosmopolites sordidus* Germar mates repeatedly in its lifetime. Matings occur under both light and dark conditions, but significantly more in the dark (Uzakah and Odebiyi, 2015). Sirot and Lapointe (2008) reported that in tropical root weevil, *Diaprepes abbreviates* (Linneaus) matings occurred throughout the day but the percentage of individuals mating tended to peak during the middle-of the-day.

Mating Behaviour in some Cerambycids occurs only under daylight illumination (Linsley, 1959). Mating is regulated by circadian rhythms in *Nadezhdiella cantoni* Cantoni (Coleoptera: Cerambycidae). Thus, light illumination is considered a necessary environmental condition during attempts to collect pheromones from calling females under laboratory conditions (Wang *et al.*, 2002). Both sexes of *Callisphyris apicicornis* Fairmaire and Germain remain motionless during the scotophase and during most of the daylight hours, except during a clear peak of activities in both sexes between 09.00 AM and 01.00 PM. Light acts as a triggering factor for sexual Behaviour in *C. apicicornis* and suggest that this time window would be the optimal time for collecting the pheromone from females and for testing the responses of males to pheromone components (Curkovic, and Ferrera, 2012). Matings in *Hypothenemus hampei* (Ferrari) may occur at any time of the day, possibly because these activities occur only inside the coffee berry. The beetles are not directly exposed to adverse climatic conditions or to natural enemies (Silva *et al*., 2012). In red palm weevil, *Rhynchophorus ferrugineus* Oliver, mating activity occurs during photophase at 02.00-06.00 pm (Walid Kaakeh, 1998). *Neochetina eichhorniae* Waner mate repeatedly, both day and night, in their life-time. However, mating frequency was 50 % greater at night than during the day (Stark and Goyer, 1983; Oke, 2011).

4.1.2 Female calling Behaviour

Observations on the calling Behaviour and peak calling activity of *O. longicollis* females were recorded during photophase and results are presented in Table 3 and Figures 2. The calling activity of *O. longicollis* females was observed on the 2nd to 12th hour of the photophase. The female calling activity started in the 2nd hour of the photophase, showed increasing trend and achieved maximum calling activity in the mid of the day, followed by decreasing calling activity at the end of the photophase. A clear difference was observed in the mean number of calling females between individual time periods but they were not significantly different ($F_{11, 36}$ =1.897, p=0.073). The maximum numbers of calling females were observed during 6th, 8th, 9th and 10th hour photophase (32.00 ± 0.18 and 36.00 ± 0.15), that was considered as the peak time of female calling period. Lower number of calling females was observed on the 2nd, 3rd, 5th and 12th hour of photophase. Fourth hour of photophase recorded the lowest number of calling females (8.00 ± 0.06). In the 1st hour of photophase, no females were in calling posture (Figure 2).

Table 3. Female calling pattern of *O. longicollis* during the day

Female	Mean no. of female callings/day	Mean calling duration /day
F-1	4	2.3025
F-2	2.5	2.03
F-3	8.25	5.845
F-4	3.5	2.33
F-5	8	3.61
Mean	**5.25**	**3.22**
SD	**2.681**	**1.588**

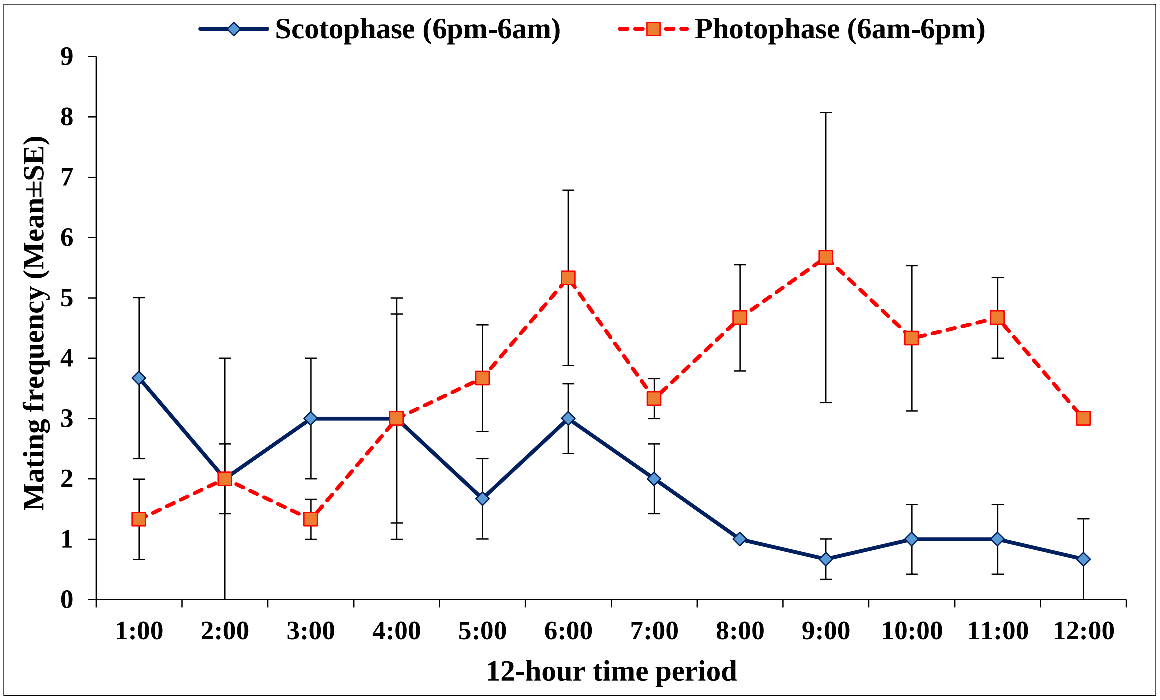


Fig. 1. Frequency of matings (Mean ± SE) estimated for every 1 hour throughout the 1st to 12th hour of photophase and scotophase. (n=15 pairs)

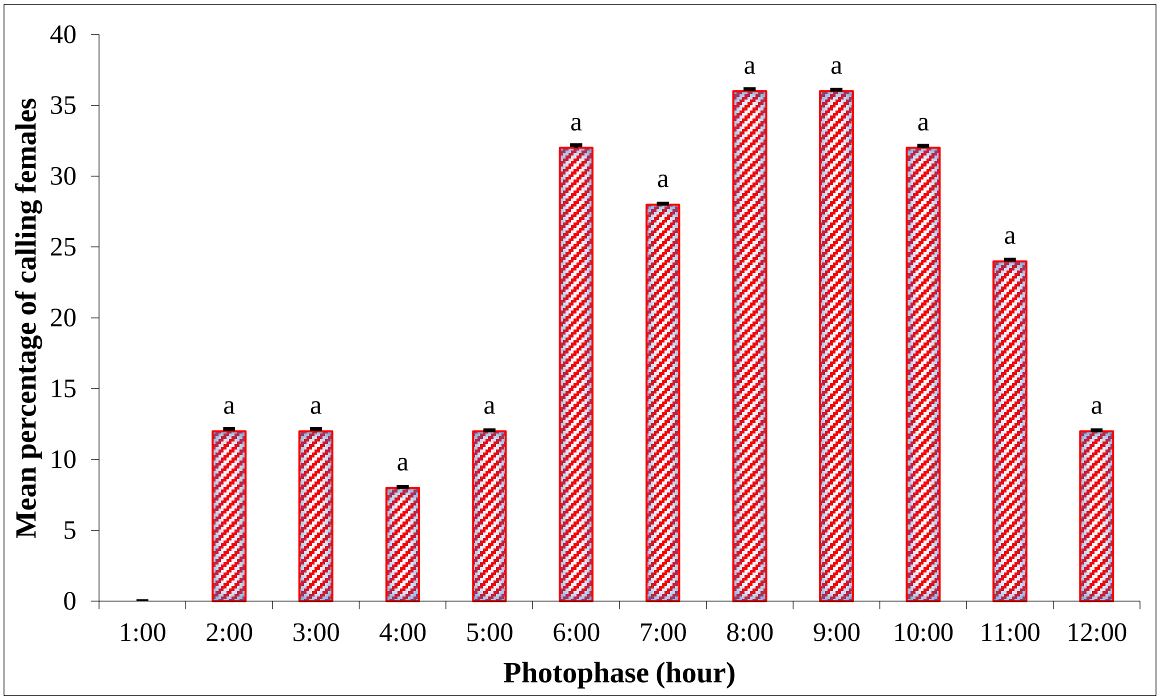


Fig. 2. Female calling patterns during photophase. Mean percentage of calling females in 4 days observations. (n=20, error bars indicate the standard error of mean, the column having same letters are not significantly different at 0.05 % (Tukey post hoc test)

Observations on female calling Behaviour revealed that female became active after 60 min in the photophase. Prior to exhibiting calling posture, females began to move around actively inside the container and showed hovering and fluttering wings (restlessness). During this time, females searched for a suitable place for calling. After locating a suitable place, female began beating the substrate with rostrum and simultaneous vibrant antennal beatings on the substrate. Female positioned her abdomen parallel to the substrate and lying close to the ground to rub the entire abdomen on the floor. The abdomen was raised off the substrate to the full extent of the hind leg and started stretching the abdomen. Dilation of body segments was observed with wings kept loosely above the abdomen. This was followed by bending of abdomen and partly exposing the concealed ovipositor through the genial opening. The ovipositor was fully exposed and curved down to the substrate accompanied by rapid protrusion and retraction of the ovipositor. This was followed by pressing the substrate with the extruded ovipositor to release the pheromones (Plate 10). Then female rest until the next calling, which began with attempts to fly and active movements. Before calling posture, female frequently show flying Behaviour. The number and duration of calling posture per female per day varied from 2.50 – 8.25 and 2.03-5.85 min, respectively. The mean number of female callings per day was 5.25 ± 2.68 and the mean duration of calling per female per day was 3.22 ± 1.59 min (Table 3).

Literature on the calling Behaviour of *O. longicollis* females is scanty. The calling and courtship Behaviours of females and males *O. longicollis* described in the present study resemble previous reports of Ravi and Palalniswami (2002). They reported that beating of the substrate, vibrant antennal beating, bending of abdomen, exposing the concealed ovipositor, rapid protrusion and retraction of the ovipositor and pressing the substrate with the extruded ovipositor were the main characteristic calling Behaviour of *O. longicollis* females. The elevation of tip of the abdomen into the air and leg raising and stroking are the main characteristics of the calling posture of oriental beetle, *Exomala orientalis* (Waterhouse) (Facundo *et al*., 1999). The diagnostic movements of the abdomen, legs, elytra and wings are the characteristic sequences of calling Behaviour as reported in *Callisphyris apicicornis* Fairmaire and Germain females (Curkovic and Ferrera, 2012).

4.1.3 Courtship and mating Behaviour

The temporal distribution pattern and sequence of events in mating of *O. longicollis* was recorded during photophase and results are presented in Figures 3 and 4. The mating activity of *O. longicollis* began in the 1st hour of photophase (72.00 ± 0.10) and continued till the end of the photophase. A clear difference was observed in the mean number of mating pairs between individual time periods and statistically significant differences was recorded between time periods ($F_{11, 60}$ =2.735, p=0.006). The mating activity was recorded throughout the photophase and the maximum numbers of mating pairs were recorded during 6th, 8th and 12th hour of photophase (84.00 ± 0.11 and 92.00 ± 0.03). The main peak period of mating activity and significantly higher number of mating pairs was recorded during the 10th hour of photophase (100.00 ± 0.08) when 100 % of the couples mated. The lowest numbers of mating pairs were observed on the 3rd and 4th hour of photophase (40.00 ± 0.10 and 44.00 ± 0.10, respectively). The mating activity was observed even when females were not calling (Figure 3).

The mating Behaviour sequence observed in *O. longicollis* pairs exhibited three distinct phases *i.e.*, pre-copulatory phase, copulatory phase and post-copulatory phase. In the precopulatory phase, on initiation of the calling by females, the males are active, they initiate searching of the females with their antennal movements. The courtship Behaviour mainly consisted of perception of female calling signals by holding the antennae upright (Figure 4 and Plate 11) male actively moves around, loosening of its wings, bending of abdomen, partly or full exposure of aedeagus, pressing it against the substrate, walk towards receptive female and copulation. Once the male approach the calling female, male moves around or behind the female and male approaches the female towards her posterior, lateral or frontal side. In most of the pairs (50 %), the male approached the female from the posterior side and remaining 30 % and 13.33 % of the pairs approached the female from the lateral and frontal side, respectively. After approaching, the male touched the females elytra, abdominal tip (70 %) and pronotum (30 %) with antennae and rostrum. The male mount the female from any direction. In 80 % of the pairs, male mounted the female from posterior side and 20 % of the pairs from the lateral and frontal sides. When male mounted the female from lateral or frontal side, the male aligns the body with the longitudinal axis of the females (34 %). In the posterior mounting, the male moved his body in a posterior direction (66 %). During mounting, in all the mating pairs (100 %), male holds the female in position with hind legs at the end of the abdomen, middle legs at the middle part of the body and forelegs at the thoracic region and rostrum on the pronotum (Plate 11). After mounting, at the end of precopulatory phase, male bends abdomen ventrally and extrudes aedeagus (Figure 4 and Plate 11).

In the copulatory phase, male positioned its body perpendicular to that of the female and inserted aedeagus into the female genital chamber (100 %). During copulation, both sexes remained motionless in 70 % of the pairs and in the rest 30 % of the pairs, short spurts of walks carried out by the female, before becoming motionless. Occassionally, copulation was briefly disturbed by conspecific males (6.67 %) that performed mounting attempts on the female or mounting pairs. The rival male does not achieve the mating success in most of the cases. The copulatory phase ended with genital disengagement. Male remained in mounting position for short time after mating (Figure 4).

In the post-copulatory phase, the male dismounted slowly after genital disengagement. The partners remained close to each other and motionless for a few seconds and then separated. All the pairs (100 %) performed the same Behavioural sequences during the post-copulatory phase (Figure 4).

Mating occurred throughout the day but the peak was recorded during the middle of the day (Sirot and Lapointe, 2008). The courtship Behaviour is characteristic for several species of weevils (Ravi and Palalniswami, 2002; Sirot *et al.*, 2007 and Vanderbilt *et al.,* 1998). Lifting of head, holding antennae at right angles to the body across the fume, moving around the female and downward bending of posterior end of

1. Actively moving around and Flight

2. Knocking the substrate with the rostrum

3. Vibrant antennal beating on the substrate

4. Lying close to the ground to rub the entire abdomen on the floor

5. Raising the abdomen off the substrate with full extent of the hind leg and stretching the abdomen

6. Dialation of body segments with wings kept loosely above the abdomen

7. Bending the abdomen and partly exposing the concealed ovipositor through the genital opening

8. Full exposure of ovipositor and curving down to the substrate accompanied by rapid protrusion and retraction of the ovipositor

9. Pressing the substrate with the extruded ovipositor

Plate 10. Sequence of female calling behaviour

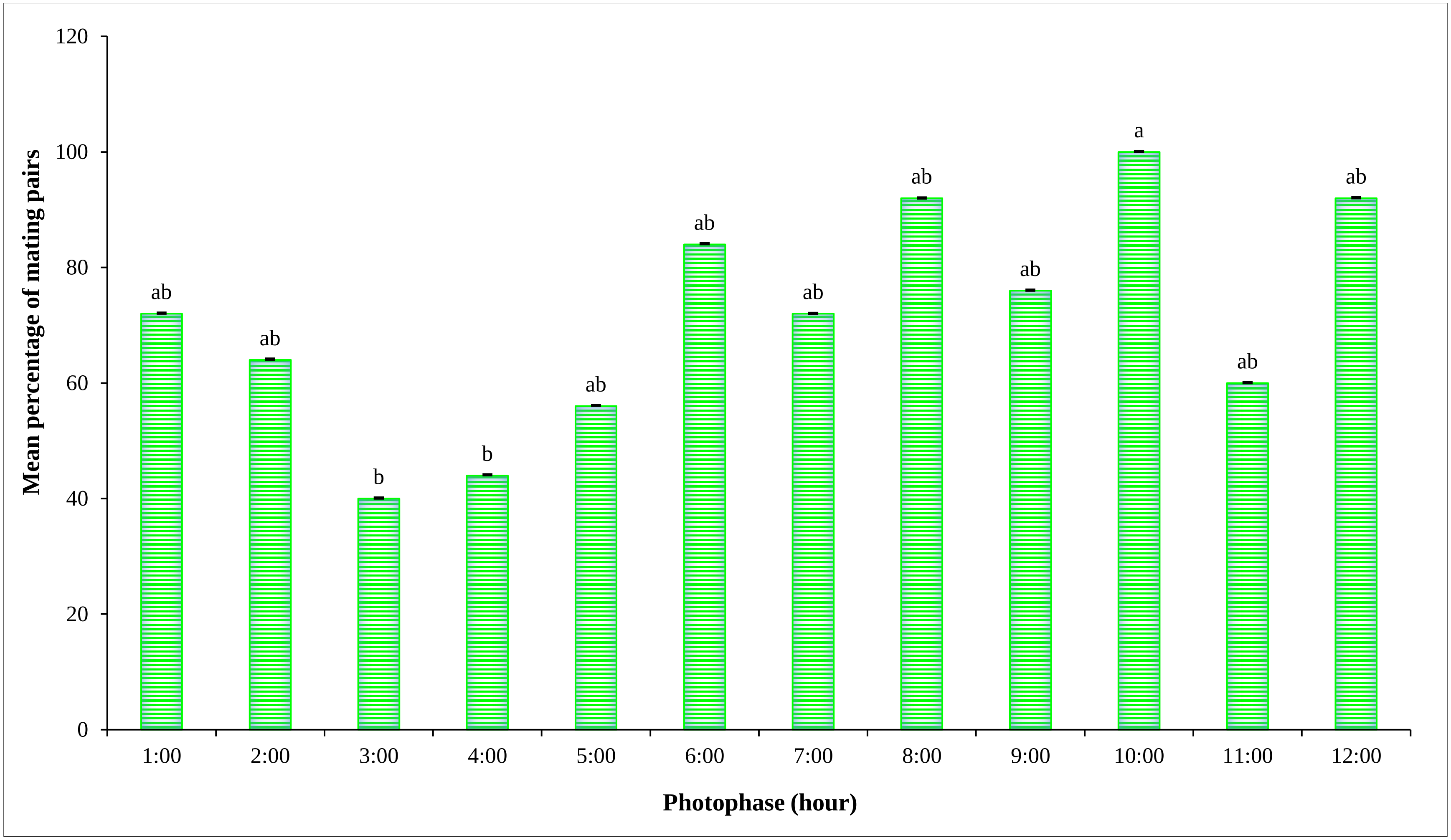


Fig. 3. Temporal mating patterns of *O. longicollis* during photophase. Bars indicated mean number of mating pairs in each hour during 6 days observation, each day consisting 5 pairs (n=30 pairs). Error bars indicate the standard errors of mean, the column having same letters are not significantly different at 0.05 % (Tukey post hoc test)

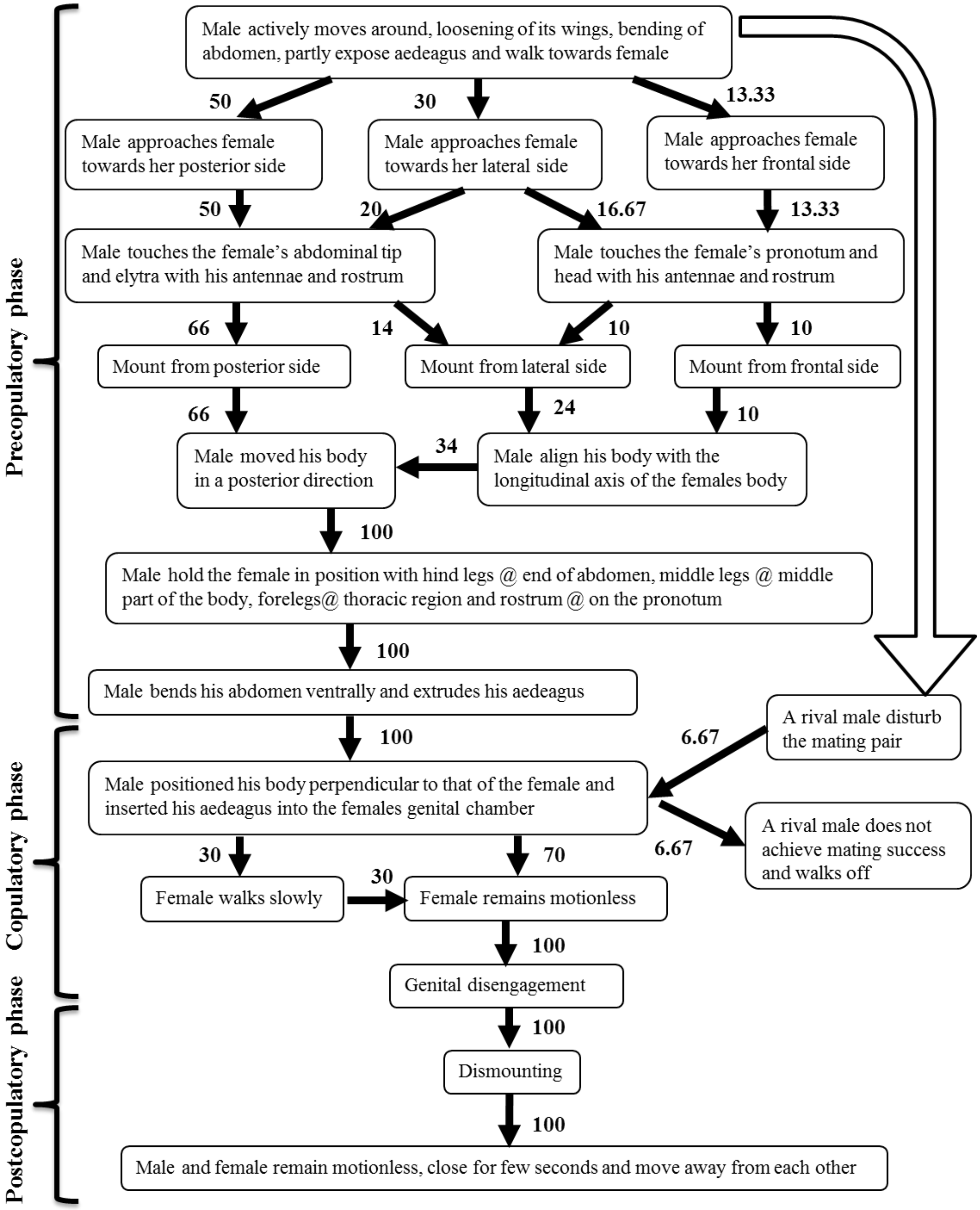


Fig. 4. **Ethogram of *Odoiporus longicollis* mating Behaviour (n=30 pairs). Numbers are percentage of mating pairs that proceeded to the next step**

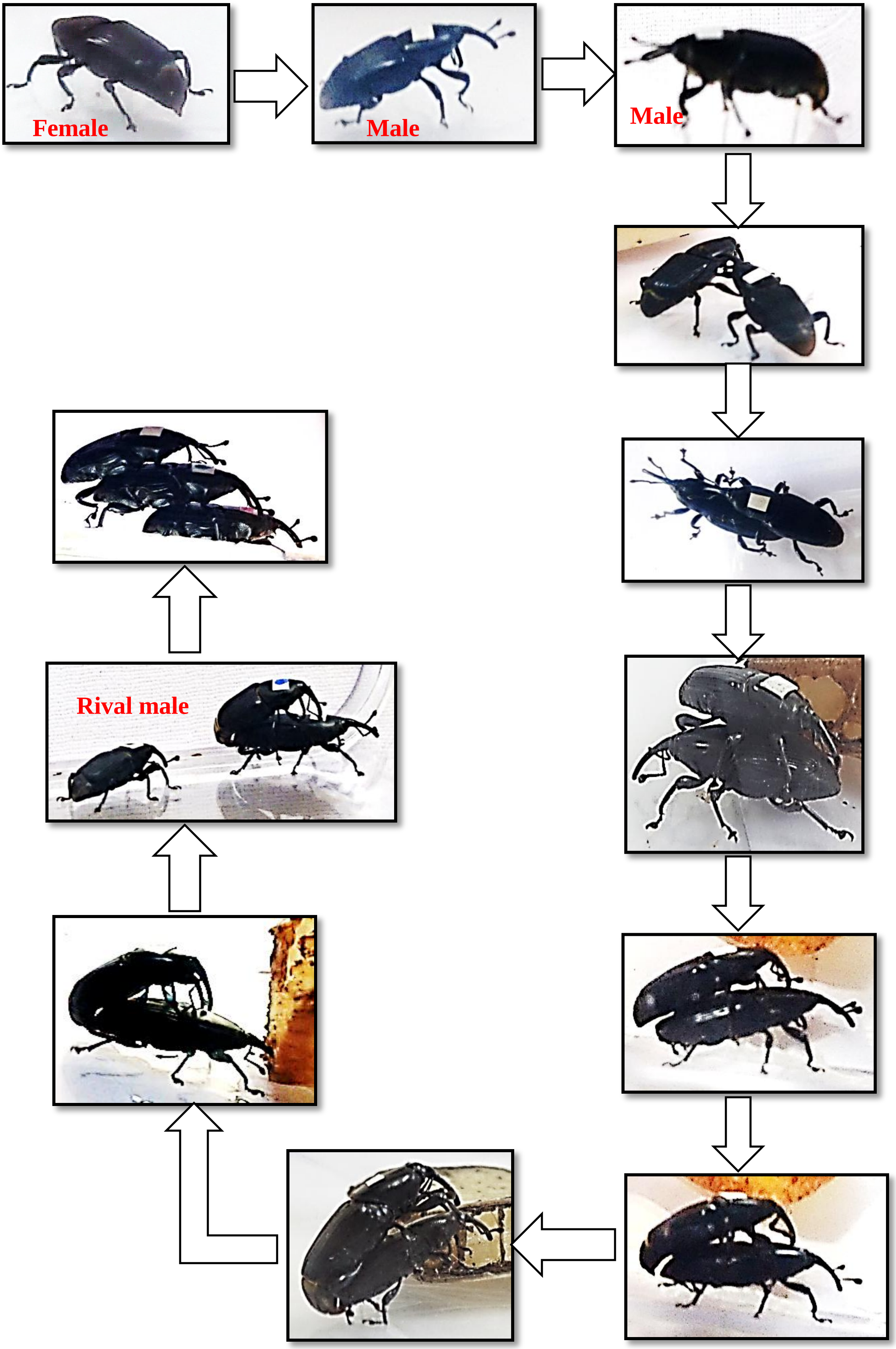


Plate 11. Male mating behaviour sequence

the abdomen are the main characteristics of the courtship Behaviour of many coleopterans (Ravi and Palalniswami, 2002). Tapping of the abdomen, antennae and head of the female as a courtship Behaviour was reported by Viana and Vilela (1996) in *C. sordidus*. Uzakah and Odebiyi (2015) reported that there was no elaborate courtship Behavioural response in *C. sordidus* before mating. However, sniffing often preceded mating. Rostral rubbing and antennal tapping on the female's elytra before copulation are the characteristic courtship Behaviours in *Rhynchophorus cruentatus* (Fabricius) (Vanderbilt *et al.,* 1998) and *Aclees sp. cf. foveatus* Voss (Benelli *et al*., 2014).

The mating Behaviour has been recorded for a number of terrestrial weevil species such as *A. grandis* Boheman (Mayer and Brazzel 1963), *Diapprepes abreviatus* (Linneaus) (Sirot *et al.,* 2007), *C. sordidus* (Viana and Vilela, 1996), *Euscepes postfasciatus* (Fairmaire) (Sato and Kohama, 2007), and *Hypera postica* (Gyllenhal) (LeCato and Pienkowski, 1970). Certain Behaviours are commonly described for weevils, such as the post-copulatory mate guarding, where males mount the females back after copulation to test whether the copulation has successfully reduced the female's receptivity by attempting to mate (Sato and Kohama, 2007) or to prevent the rival males from mating with their partner (Polak and Brown, 1995 and Vanderbilt *et al.,* 1998).

Observations in this study revealed that repeated mating was observed with the same pair for several times. This repeated mating has also been reported in *C. sordidus*, involving the same or different partners. Martins *et al*. (2013) also reported remating in the rice water weevil, *Oryzophagus oryzae* (Costa Lima). The significance of multiple matings could be for increased fecundity. Many species, in Coleoptera, require more than one mating for maximum fecundity and male insects are capable of inseminating females throughout the female's lifetime (Hinton, 1981). The frequent disturbance of mounted pairs and sometimes the mounted mating males in the current study, this Behaviour often led to an aggregation of weevils around or on top of a mating pair (LeCato and Pienkowski, 1970; Vanderbilt *et al.,* 1998; Benelli *et al*., 2014 and Uzakah and Odebiyi, 2015). The pre-copulatory, copulatory and post copulatory Behaviours of *O. longicollis* described in the present study are in agreement with previous reports (Benelli *et al*., 2014; Silva *et al*., 2012 and Martins *et al*., 2013).

4.2 Chemical identification of pheromone components

Volatile samples collected from *O. longicollis* males were extracted with 1.5 ml of dichloromethane (DCM) and concentrated using nitrogen gas.

4.2.1 Gas Chromatography (GC) and Gas Chromatography linked electroantennogram detector (GC-EAD) analysis

The BSW male volatile profile revealed that approximately 25 peaks were detected in the chromatogram (Figure 5). GC analysis of male volatiles (Figure 5) and GC-EAD analysis of male volatiles against male-female excised antennae of *O. longicollis* followed a similar pattern of peaks in chromatogram. The Gas chromatography linked electroantennogram detector analysis of male volatile of *O. longicollis* revealed that three compounds elicited the EAD response in male and female antennae (EAD active compounds) (Figures 6 and 7). The retention time of EAD active peaks were indicated in the representative chromatogram in GC-EAD and also in the chromatogram of male volatile (Figure 5). The retention times indicated in the chromatogram of male volatile (5. 935 RT, 6.005 RT and 6.465 RT) (Figure 5) and GC-EAD chromatogram of male volatiles to male antennae (5.93 RT, 6.00 RT and 6.45 RT, respectively) (Figure 6) and female antennae (5.95 RT, 6.03 RT and 6.46 RT, respectively) (Figure 7) were the same, with slight variation in the seconds. Hence, the same sample was subjected to GC-MS.

4.2.2 Gas Chromatography-Mass spectrometry (GC-MS) analysis

Gas chromatography-Mass spectrometry (GC-MS) analysis of BSW male volatile indicated that same peaks (as in GC-EAD) appeared at the retention times of 19.862, 19.992 and 20.479 (Figure 8). These peaks were identified as 1-Hexadecene (19.862), Hexadecane (19.992) and Diphenylamine (20.479), respectively. The change in retention time was due to different columns used in the GC-EAD and GC-MS. The spectral analysis of 1-Hexadecene, Hexadecane and Diphenylamine are provided in Figures 9, 10 and 11.

The mass spectra of EAD active peak,1-Hexadecene (1-16C) and synthetic compound (authentic) exhibited ions at m/z 55, 69, 83, 97, 111, 125, 154, 182, 196 and a diagnostic molecular ion at m/z 224 [M+]. Based on the pattern of peak, retention time (19.862) and the molecular weight and mass spectra matching with authentic data, the compound was consistent with 1-Hexadecene. The spectral analysis of 1-Hexadecene is depicted in Figure 9. The mass spectra of EAD active peak, Hexadecane (16C) and synthetic compound (authentic) exhibited ions at m/z 43, 57, 71, 85 and a diagnostic molecular ion at 222 [M+]. Based on the pattern of peak, retention time (19.992) and the molecular weight and mass spectra of the EAD active peak in male volatile (222) almost matching with authentic data (226), the compound was consistent with Hexadecane. The spectral analysis of Hexadecane is shown in Figure 10.

Similarly the mass spectra of EAD active peak, Diphenylamine (DPA) and synthetic compound (authentic) exhibited ions at m/z 51, 65, 84, 89, 115, 141, 154 and a diagnostic molecular ion at 169 [M+]. Based on the pattern of peak, retention time (20.479) and the molecular weight and mass spectra of the EAD active peak in male volatile matching with authentic data. Hence, the compound was consistent with Diphenylamine. The spectral analysis of Diphenylamine is depicted in Figure 11. Mass spectral analysis of male volatiles (arrow mark in Figure 8) indicated that 1-Hexadecene (19.862) Hexadecane (19.992) and Diphenylamine (20.479) matched with the reference compounds.

4.2.3 Gas Chromatography (GC) and Gas Chromatography linked electroantennogram detector (GC-EAD) analysis of synthetic compounds

The identified compounds were synthesized at Bio Control Research Laboratory (BCRL). A 2 % dilution of each compound was prepared and tested in GC. The gas chromatogram analysis of synthetic compounds of EAD active peaks were depicted in Figures 12, 13 and 14. The retention time of synthetic 1-Hexadecene at 5.961 (Figure 12) and Hexadecane at 6.031 are matching with natural male volatile peaks (Figure 13).

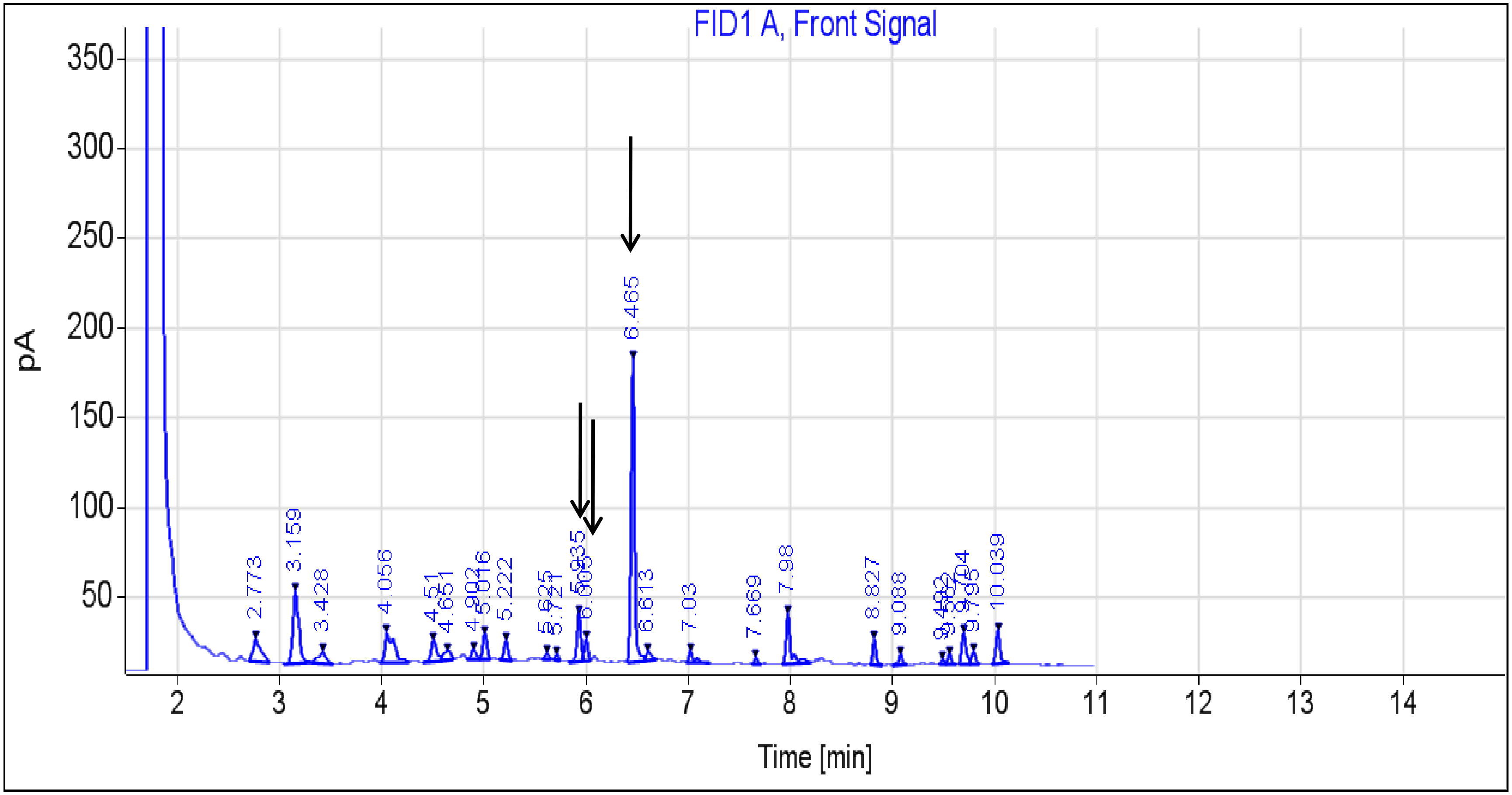


Fig. 5. Gas chromatography analysis of porapak extracts of air entrainment odors from a male *O. longicollis*. The peaks at 5.935 min, 6.005 min and 6.465 min, were identified as 1-Hexadecene, Hexadecane and Diphenylamine, respectively (300 males)

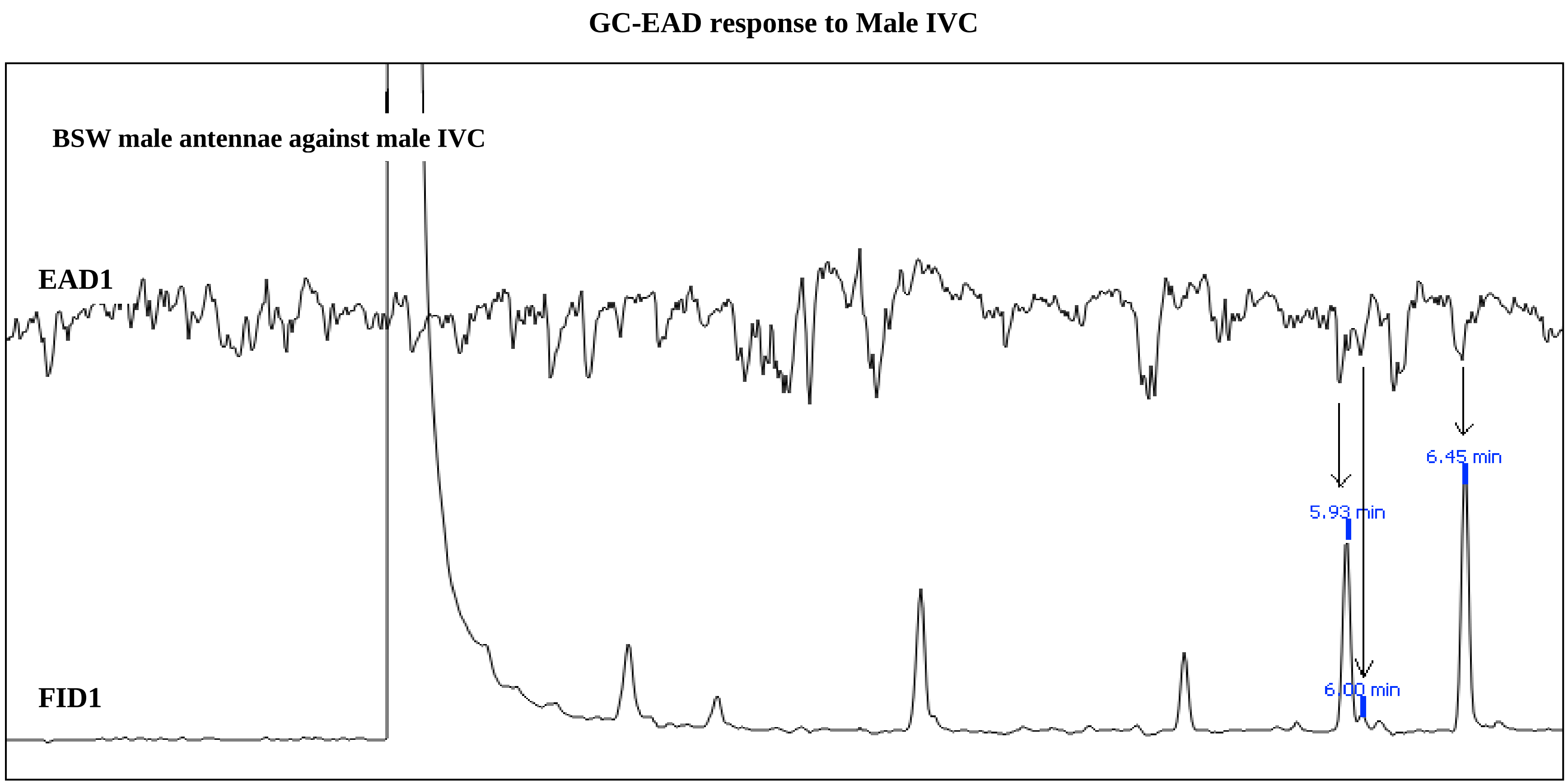

GC-EAD response to Male IVC

Fig. 6. Representative coupled gas chromatography linked –electroantennogram (GC-EAD) analysis of porapak extracts of air entrainment odors from a male *O. longicollis.* Lower trace is the flame ionization detector (FID*)* chromatogram, upper inverted traces are the electroantennogram signal from the antennae of the male *O. longicollis.* The response at 5.93 min, 6.00 min and 6.45 min were elicited by 1-Hexadecene, Hexadecane and Diphenylamine, respectively

BSW female antennae against male IVC

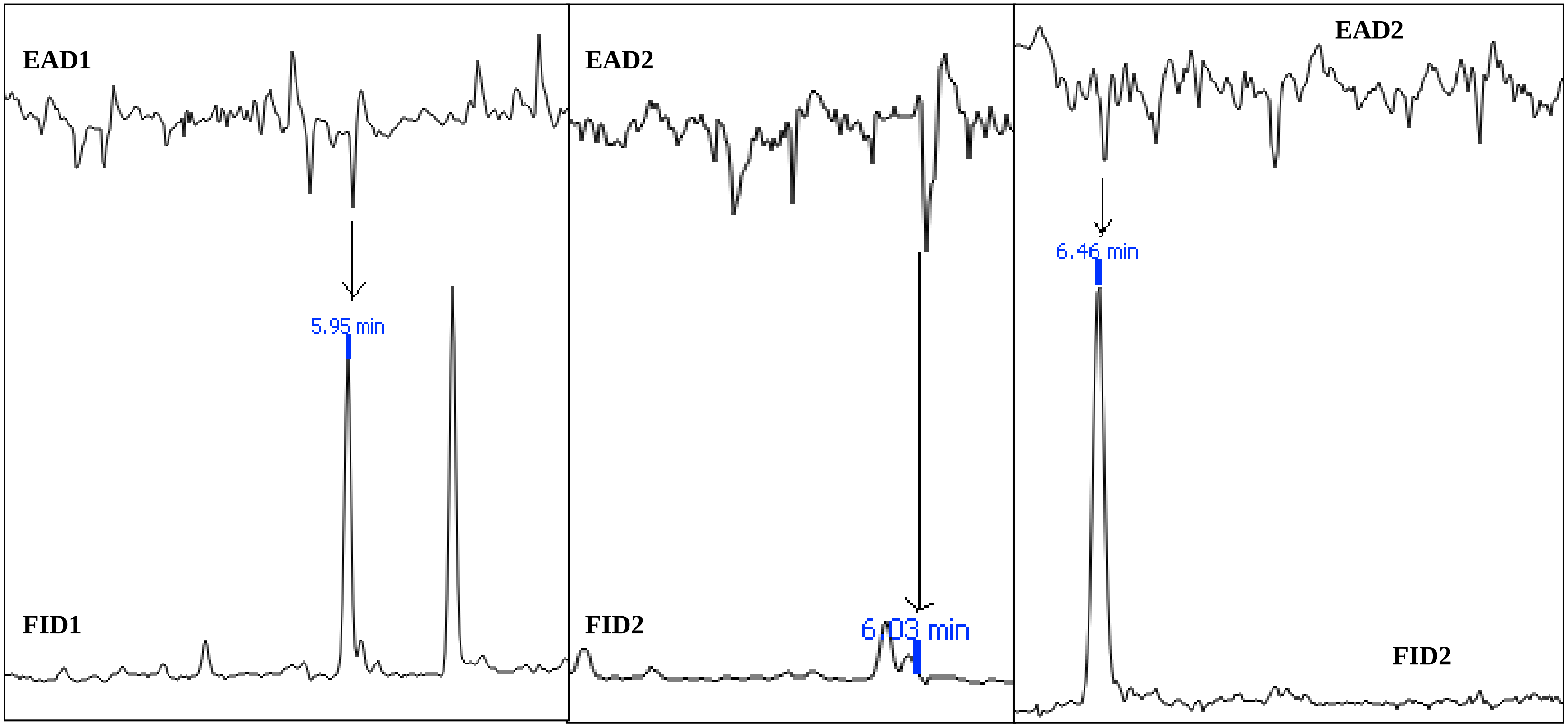


Fig. 7. Representative coupled gas chromatography linked –electroantennogram (GC-EAD) analysis of porapak extracts of air entrainment odors from a male *O. longicollis.* Lower trace is the flame ionization detector (FID*)* chromatogram, upper inverted traces are the electroantennogram signal from the antennae of the female *O. longicollis.* The response at 5.95 min, 6.03 min and 6.46 min were elicited by 1-Hexadecene, Hexadecane and Diphenylamine, respectively

GC-MS of Male IVC

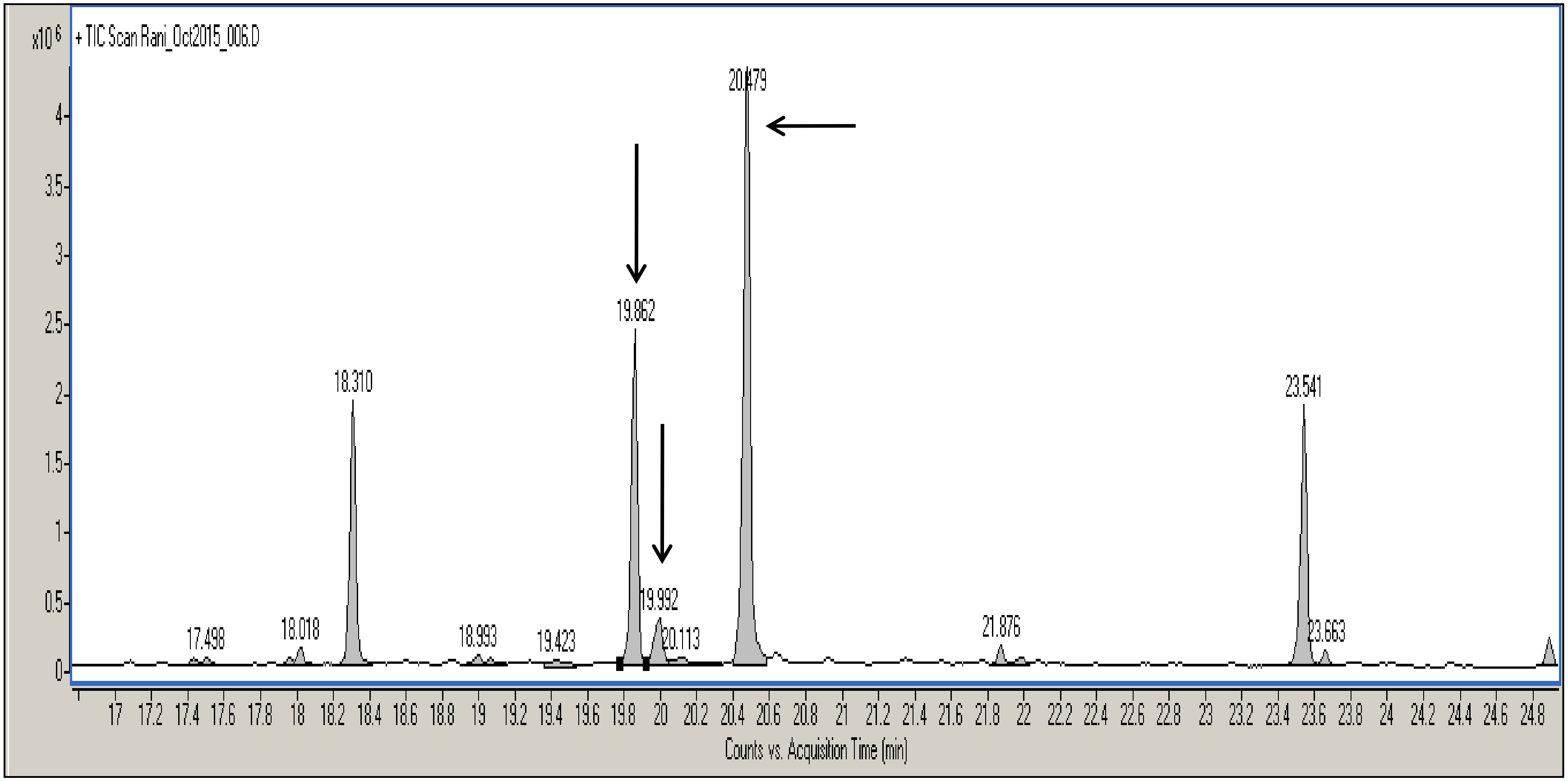


Fig. 8. Coupled gas chromatography linked –mass spectrometry (GC-MS) analysis of porapak extracts of air entrainment odors from a male *O. longicollis*. The peaks at 19.862 min, 19.992 min and 20.479 min were identified as 1-Hexadecene, Hexadecane and Diphenylamine, respectively

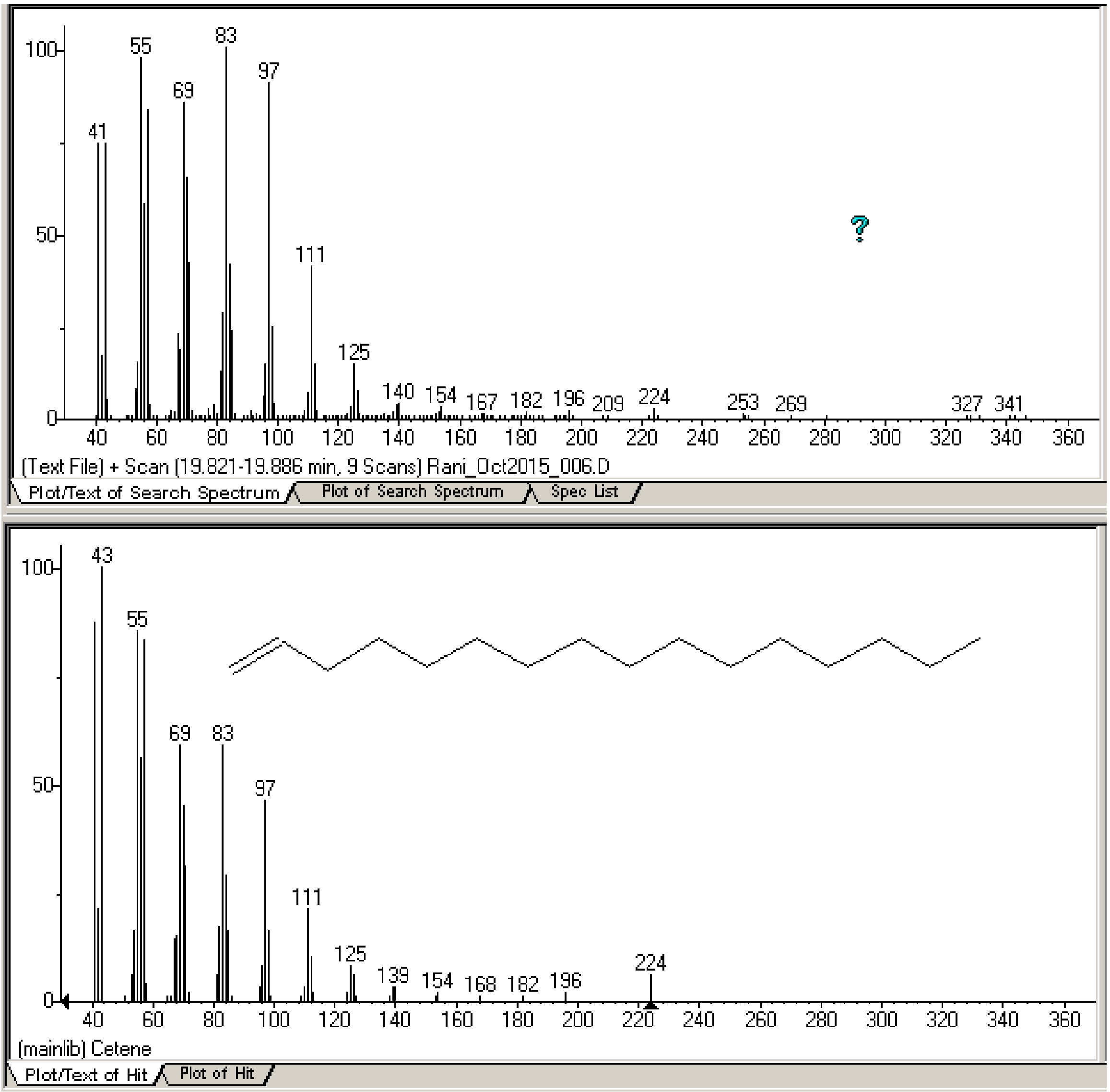


Fig. 9. Electron impact ionization (70-eV) mass spectra of 1-Hexadecene in an air entrainment extract from male *O. longicollis* (top) and an authentic standard (bottom)

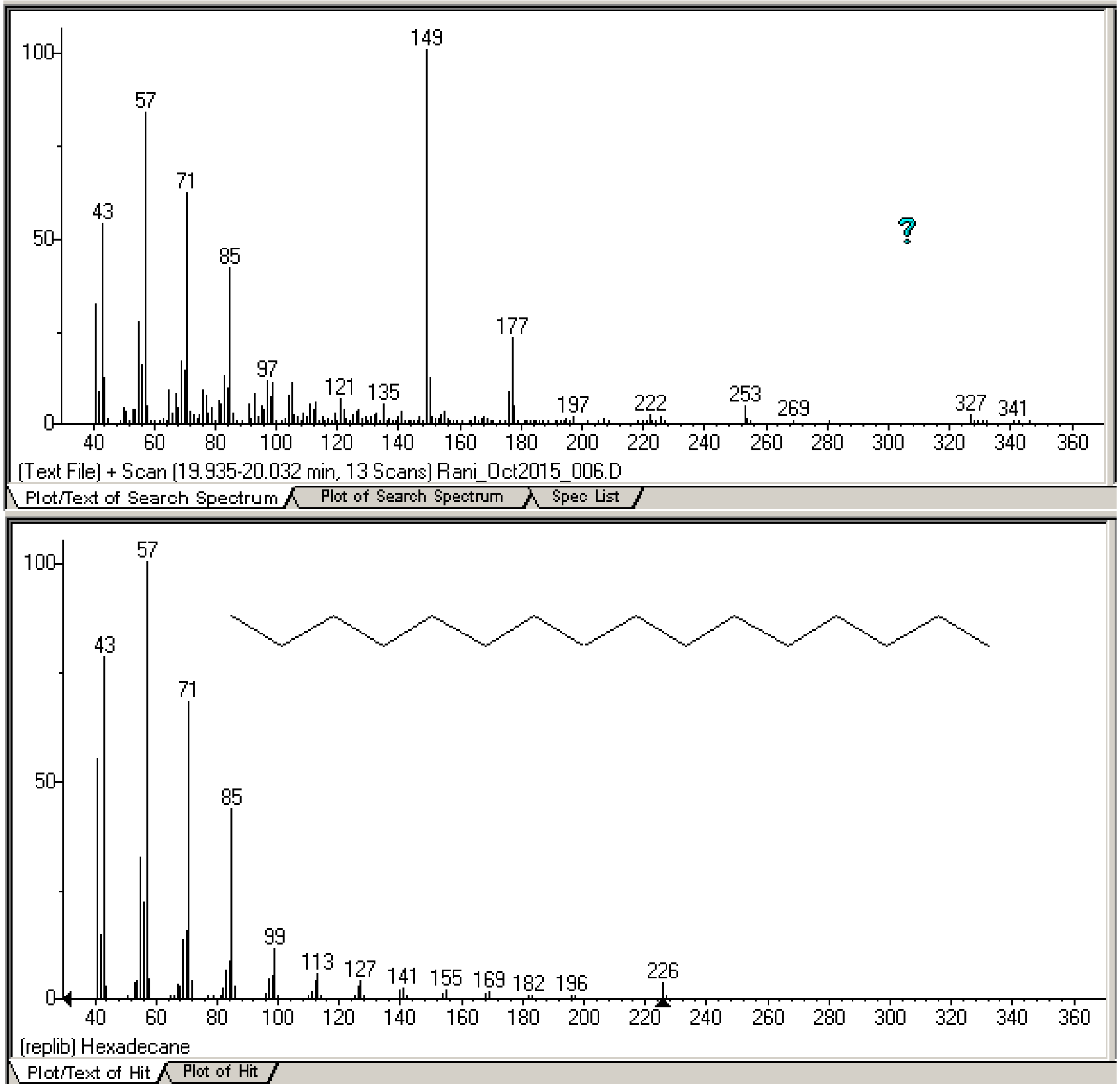


Fig. 10. **Electron impact ionization (70-eV) mass spectra of Hexadecane in an air entrainment extract from male *O. longicollis* (top) and an authentic standard (bottom)**

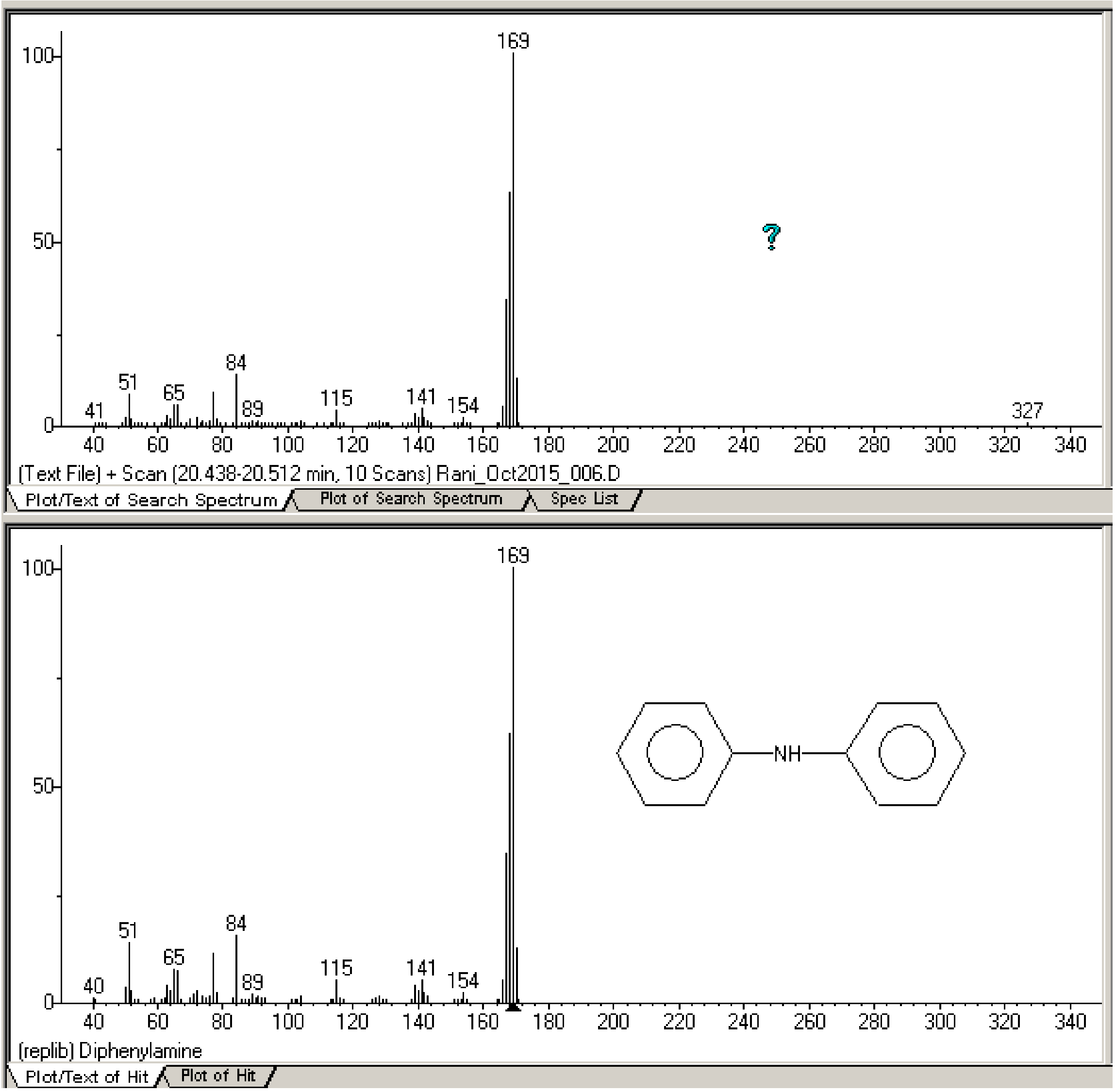


Fig. 11. **Electron impact ionization (70-eV) mass spectra of Diphenylamine in an air entrainment extract from male *O. longicollis* (top) and an authentic standard (bottom)**

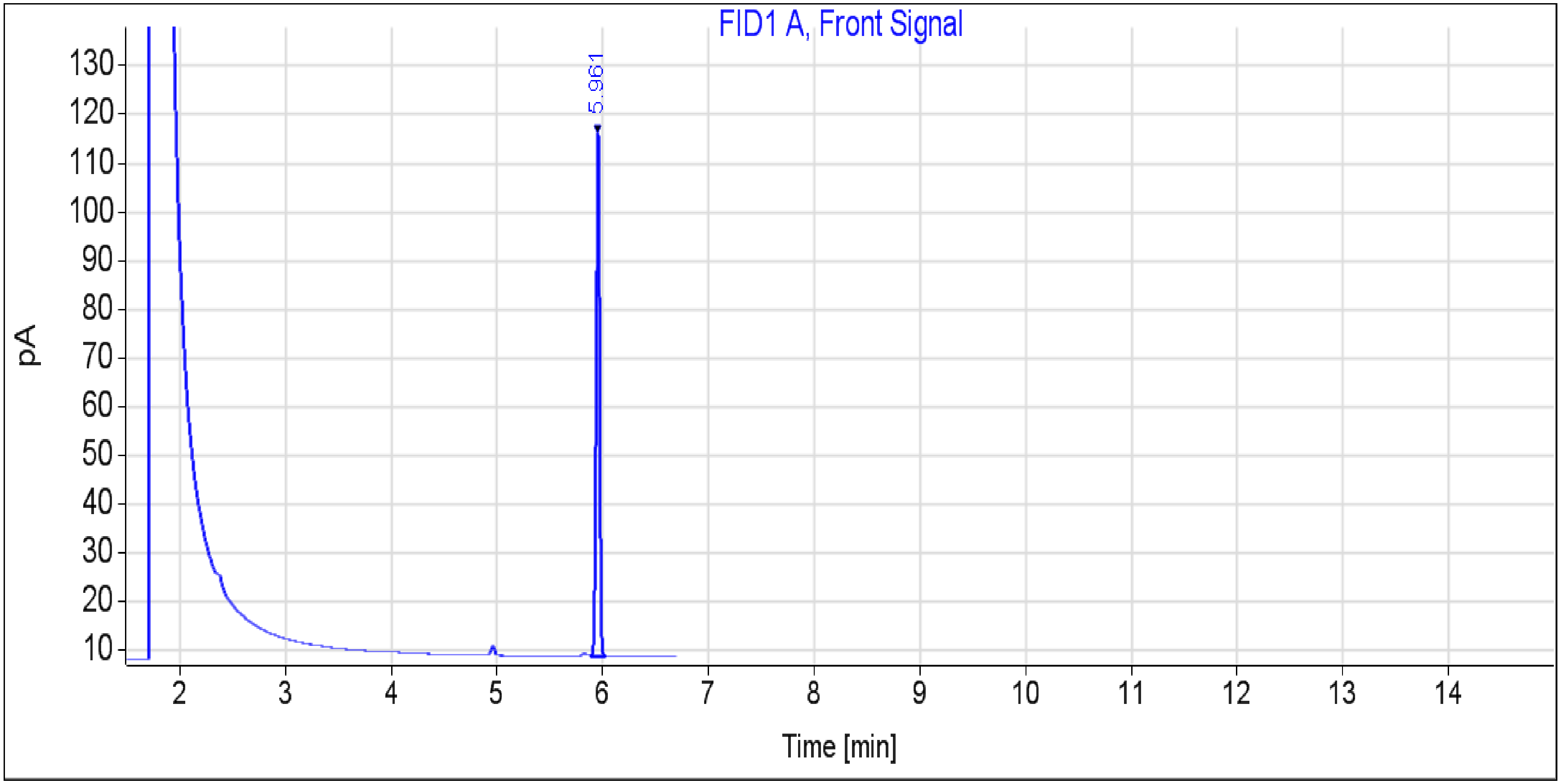


Fig. 12. Gas chromatography analysis of synthetic 1-Hexadecene. The peak at 5.961 min was identified as 1-Hexadecene

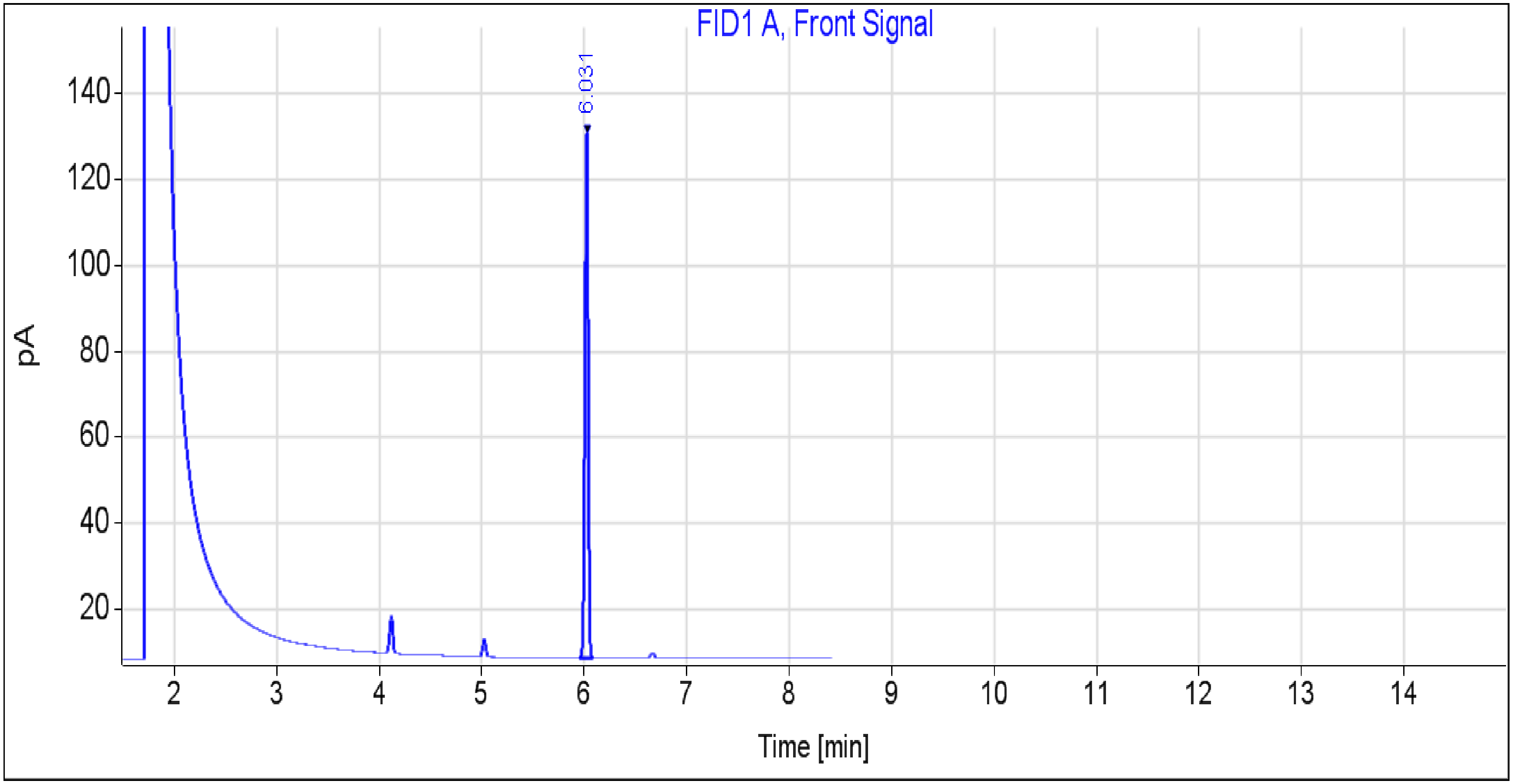


Fig. 13. Gas chromatography analysis of synthetic Hexadecane. The peak at 6.031 min was identified as Hexadecane

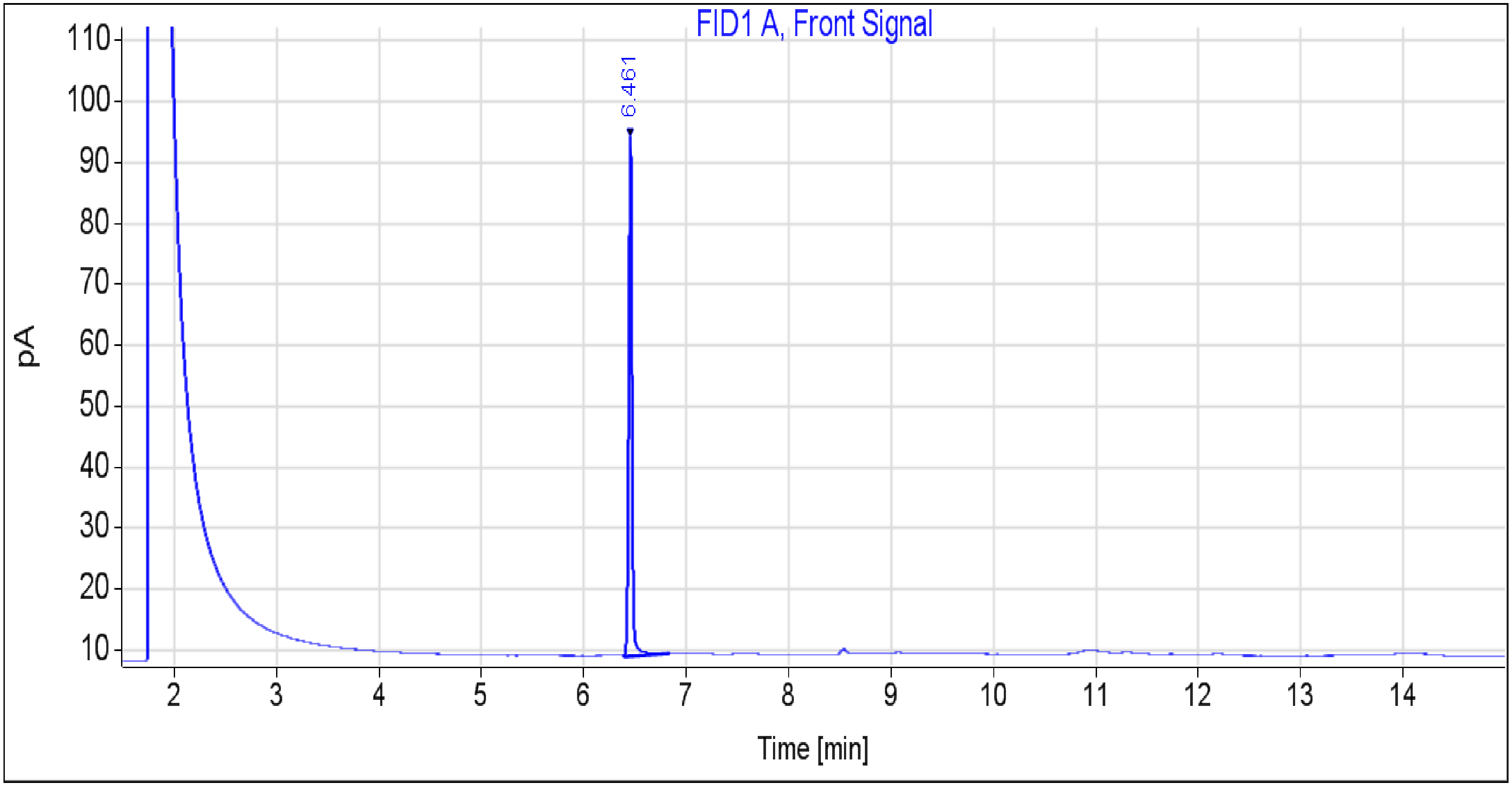


Fig. 14. Gas chromatography analysis of synthetic Diphenylamine. The peak at 6.461 min was identified as Diphenylamine

Similarly, the peak of synthetic Diphenylamine at 6.461 retention time matching with natural one (Figure 14).

Identified synthetic compounds (1-Hexadecene, Hexadecane and Diphenylamine) were subjected to GC-EAD experiments against male and female antennae of BSW (Figures 15, 16 and 17). The synthetic 1-Hexadecene elicited greater response in male and female antennae at the retention time of 5.95 min and 5.96 min, respectively (Figure 15) and the peak matched with the GC. Similarly, synthetic Hexadecane produced greater response at the retention time of 6.02 min in both male and female antennae and the peak matched with the GC (Figure 16). The response of male and female antennae to the synthetic Diphenylamine was greater at the retention time of 6.45 min and 6.48 min, respectively (Figure 17) and the peak matched with the GC.

4.2.4 Quantification of volatiles of banana pseudostem weevil

4.2.4.1 Quantification of DPA, 1-16C and 16C in insect volatiles of male and males+females of BSW collected during full day

The quantity of DPA (0.04 ng ± 0.001), 1-16C (0.02 ng ± 0.006) and 16C (0.003 ng ± 0.0003) was more in volatiles collected from males compared to volatiles collected together from males + females (Figure 18). Within the male volatile, higher quantity of DPA was recovered compared to 1-16C and 16C. In Male + female volatile the quantity of 1-16C was higher (0.007 ng ± 0.003) compared to DPA (0.004 ng ±0.001) and 16C (0.002 ng ± 0.0006) (Figure 18).

4.2.4.2 Quantification of DPA, 1-16C and 16C in insect volatiles of male BSW collected at different time intervals in a day

The quantity of DPA and 1-Hexadecene was more (0.015 ng ± 0.003 and 0.032 ng ± 0.006, respectively) in male insect volatiles collected during 02.00 PM-04.00 PM compared to volatiles collected during 11.00 AM-01.00 PM (0.012ng ± 0.005 and 0.022 ng ± 0.002, respectively) (Figure 19). The quantity of 1-16C was more than the quantity of DPA in volatiles collected during both morning and afternoon hours. The Hexadecane was unquantifiable due to negligible amount in the volatiles collected during both morning and afternoon (Figure 19).

4.2.4.3 Quantification of DPA, 1-16C and 16C in insect volatiles of male BSW collected in the presence and absence of banana pseudostem as food

The quantity of DPA, 1-16C and 16C was more in the volatiles collected from males in the presence of banana pseudostem (0.193 ng ± 0.057, 0.434 ng ± 0.080 and 0.032 ng ± 0.009, respectively) compared to volatiles collected in the absence of banana pseudostem (0.038 ng ± 0.009, 0.235 ng ± 0.142 and 0.018 ng ± 0.007, respectively). The quantity of 1-Hexadecene was more than the DPA in the volatiles collected with or without pseudostem (Figure 20).

4.2.4.4 Quantification of DPA, 1-16C and 16C in insect volatiles of male BSW collected during scotophase and photophase

The quantity of DPA remains almost same in volatiles collected both during scotophase (0.05 ng ± 0.014) and photophase (0.04 ng ± 0.009). The quantity of 1-16C was more in volatiles collected during photophase (0.235ng ± 0.142) compared to scotophase (0.019 ng ± 0.005). Where as the quantity of 16C was more in volatiles collected during photophase (0.018 ng ± 0.007) and it was unquantifiable in volatiles collected during scotophase (Figure 21).

4.2.4.5 Quantification of DPA, 1-16C and 16C in insect volatiles of male BSW collected from 24 hour and 48 hour starved BSW male insects

The quantity of DPA was more in volatiles collected from 24 hour starved BSW males (0.281 ng ± 8.427) compared to volatiles collected from 48 hour starved BSW males (0.195 ng ± 5.838). The quantity of 1-16C and 16C remains almost same in volatiles collected both from 24 hour (0.022 ng ± 0.663 and 0.023 ng ± 0.69) and 48 hour starved BSW male insects (0.020 ng ± 0.588 and 0.022 ng ± 0.66, respectively) (Figure 22).

Males of many coleopteran species were reported to produce aggregation pheromones (Prasuna *et al*., 2008). Within the superfamily Curculionoidea, the majority of known pheromones are long-range, male produced aggregation pheromones (Seybold and Vanderwel, 2003). The chemical identification of aggregation pheromones has been reported in at least nine families of Coleoptera and in many species of the family Curculionidae (Booth *et al.,* 1983; Eller *et al*., 1994 and Beauhaire *et al*., 1995) and they have been elucidated and successfully applied in the field for several curculionidae species (Tumlinson *et al*., 1969; Rochat *et al*., 1991b; Budenberg *et al*., 1993; Ruiz-Montiel *et al*., 2008 and Tinzaara *et al*., 2000). Gunawardena and Dissanayake (2000a) reported that male banana pseudostem weevil produced a pheromone, 2-methyl-4-heptanol which attracted both male and female banana weevils. They also reported that this aggregation pheromone *i.e.,* 2-methyl-4-heptanol works only in the presence of food bait under field conditions. Prasuna *et al.* (2008) showed that BSW males produce a volatile aggregation pheromone.

In the present study, the volatiles produced by male *O. longicollis* showed the presence of three electroantennographically active compounds, *viz.,* Diphenylamine (DPA), 1-Hexadecene (1-16C) and Hexadecane (16C). All the three compounds elicited antennal responses in both male and female weevils. Male and female antennae of *O. longicollis* responded strongly to two major compounds (DPA and 1-16C). Weaker but consistent responses were also found to the minor compound (16C). This was confirmed by the EAG, Y-tube olfactometer bioassay, GC-EAD and field cage trials with traps. This is the first report of chemical identification of aggregation pheromone in banana pseudostem weevil, *O. longicollis*.

Booth *et al*. (1983) identified two related volatile compounds (Grandisol and Grandisal) from each of two species of *Pissodes* male bark weevils and implicated as components of their aggregation pheromones. The males of *Sternechus subsignatus*

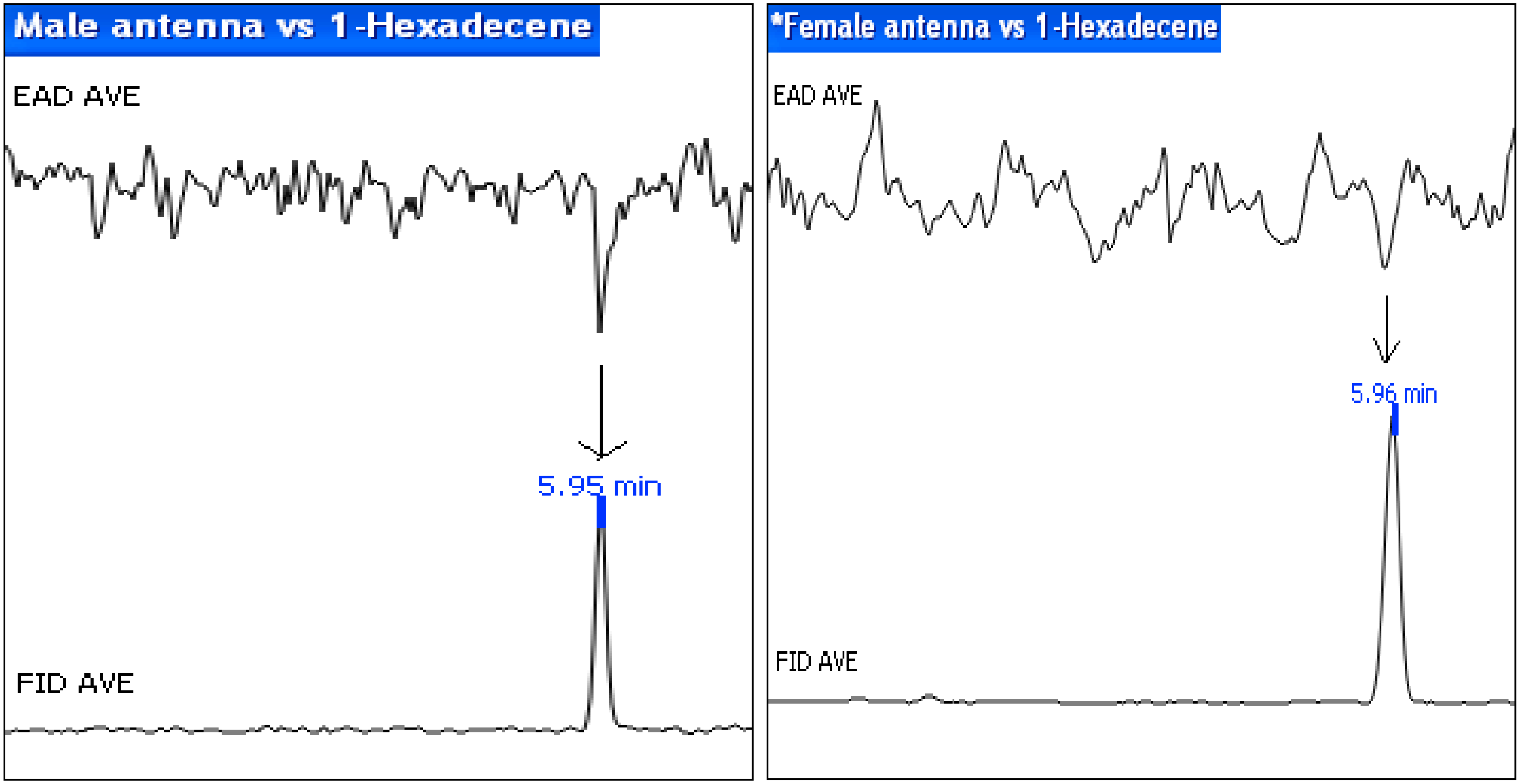


Fig. 15. **Representative coupled gas chromatography linked –electroantennogram (GC-EAD) analysis of synthetic 1-Hexadecene. Lower trace is the flame ionization detector (FID) chromatogram, upper inverted trace is the electroantennogram signal from the antennae of male and female *O. longicollis.* The response at 5.95 min or 5.96 min (RT) was elicited by 1-Hexadecene**

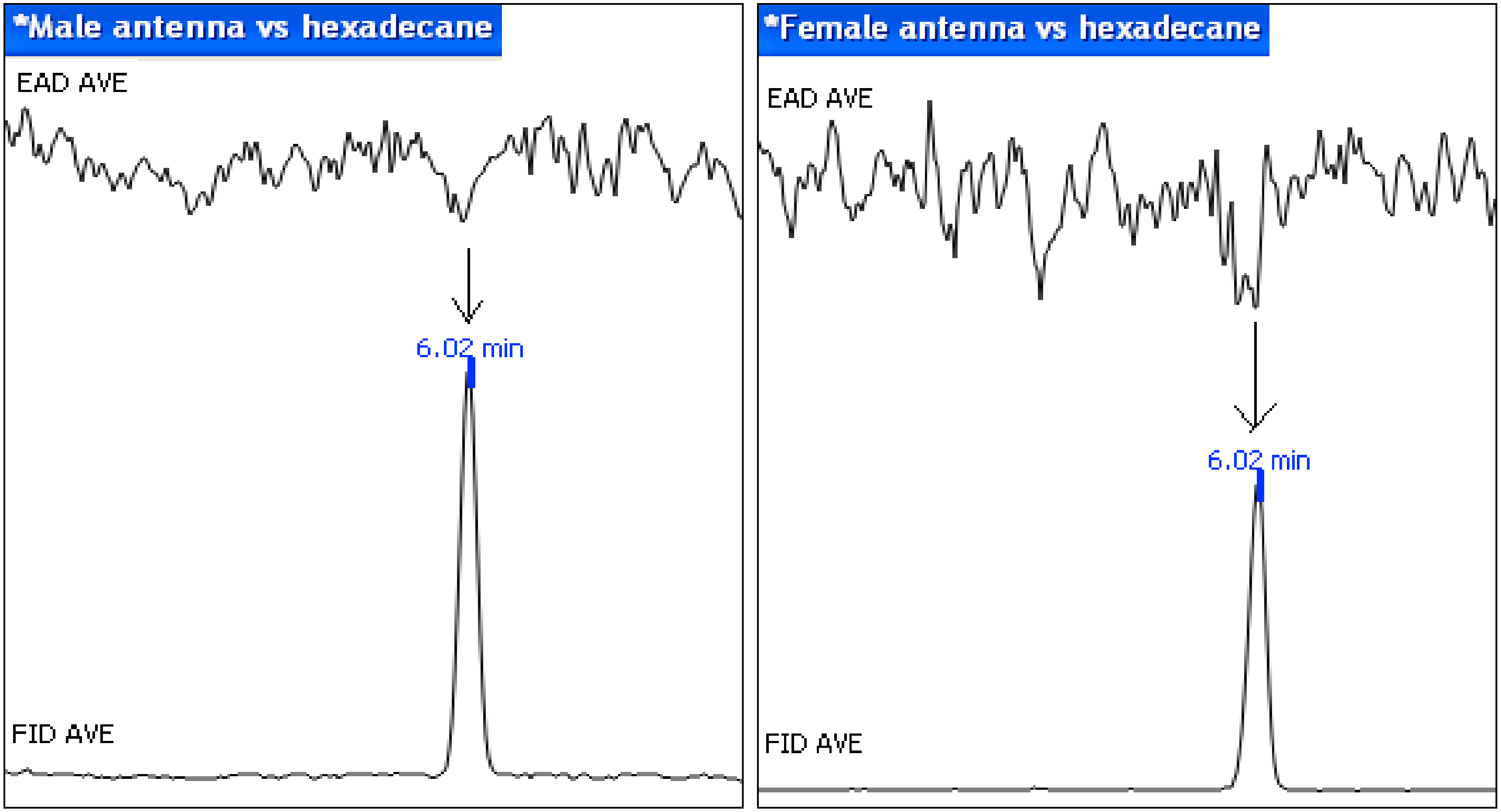


Fig. 16. Representative coupled gas chromatography linked –electroantennogram (GC-EAD) analysis of synthetic Hexadecane. Lower trace is the flame ionization detector (FID) chromatogram, upper inverted trace is the electroantennogram signal from the antennae of male and female *O. longicollis.* The response at 6.02 min (RT) was elicited by Hexadecane

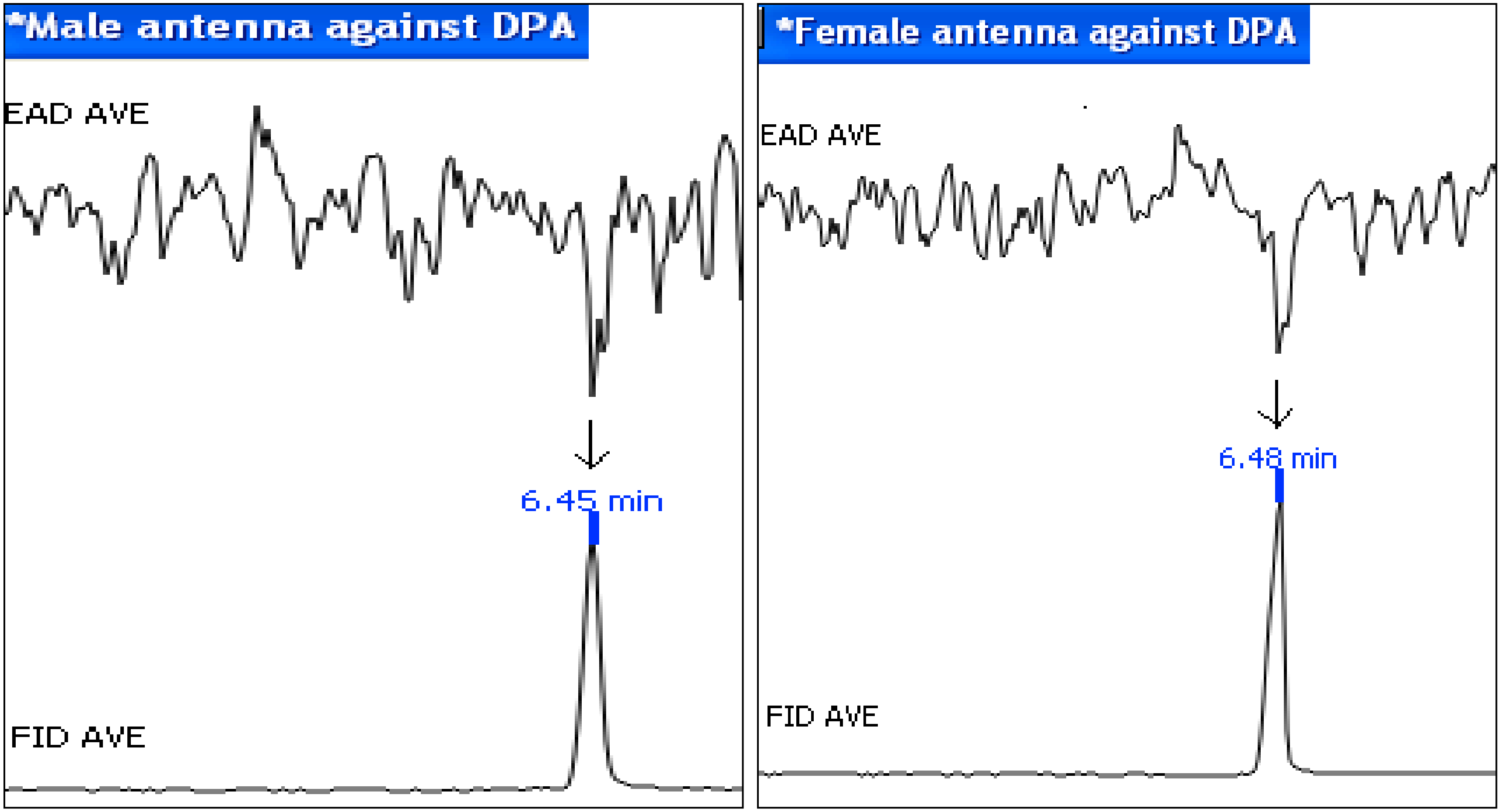


Fig. 17. Representative coupled gas chromatography linked –electroantennogram (GC-EAD) analysis of synthetic Diphenylamine. Lower trace is the flame ionization detector (FID) chromatogram, upper inverted trace is the electroantennogram signal from the antennae of male and female *O. longicollis.* The response at 6.45 min or 6.48 min (RT) was elicited by Diphenylamine

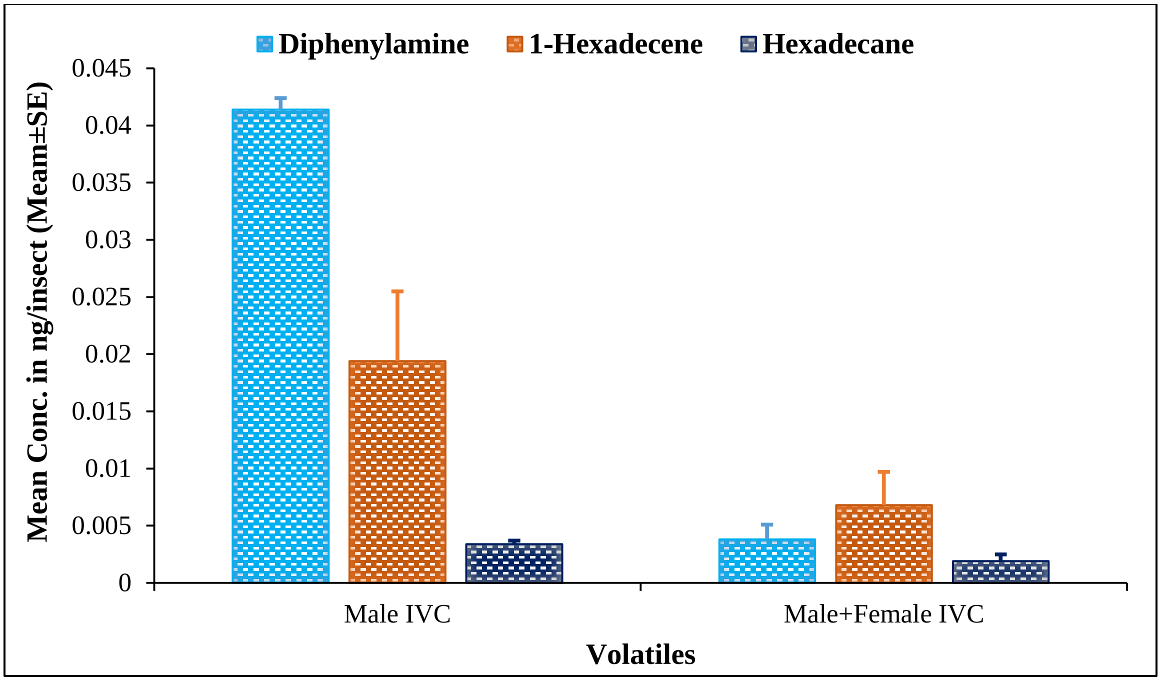


Fig. 18. **Mean concentration (ng/insect) of Diphenylamine, 1-Hexadecene and Hexadecane in porapak extracts of air entrainment odors from male and male + female volatiles of BSW collected during full day from 11 am-4 pm (n=4 batch, 300 insects in each batch)**

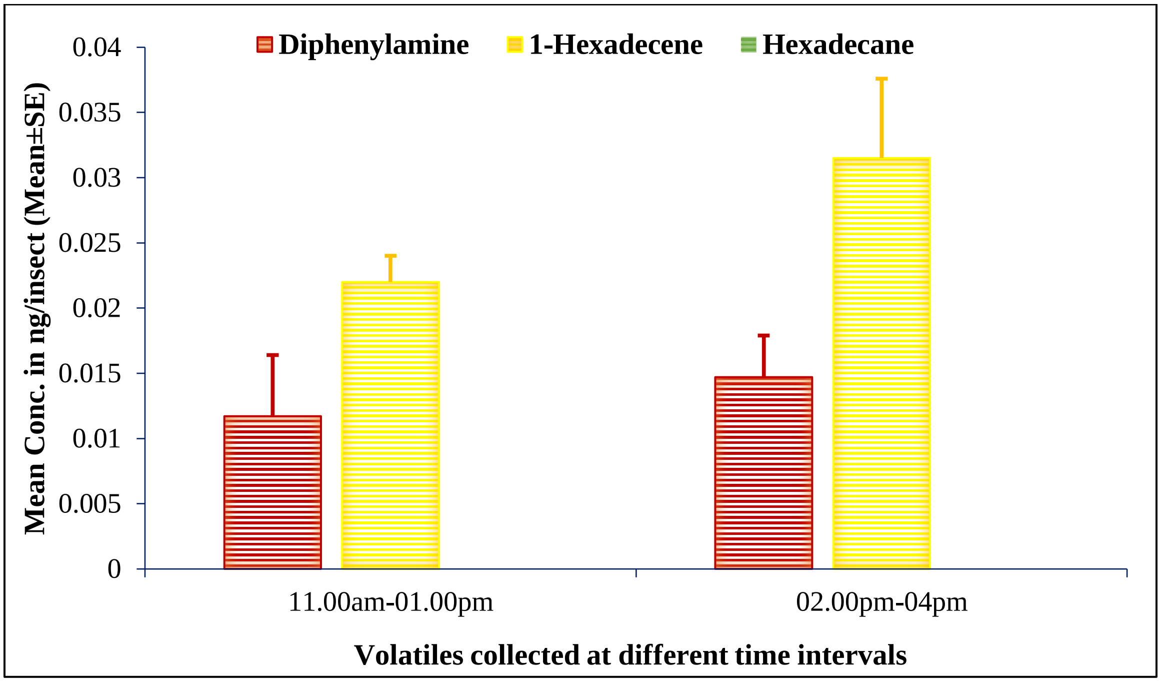


Fig. 19. **Mean concentration (ng/insect) of Diphenylamine, 1-Hexadecene and Hexadecane in porapak extracts of air entrainment odors from male and male + female volatiles of BSW collected at different time intervals of the day (n=3 batch, 50 insects in each batch)**

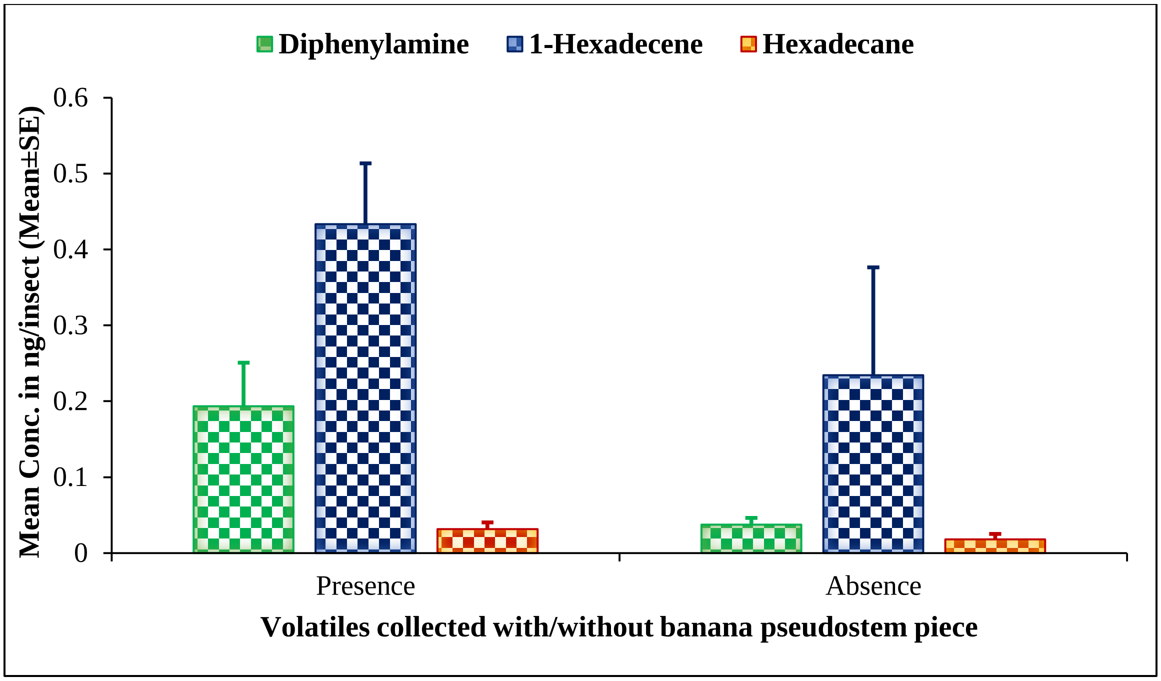


Fig. 20. **Mean concentration (ng/insect) of Diphenylamine, 1-Hexadecene and Hexadecane in porapak extracts of air entrainment odors from male volatiles of BSW in the presence and absence of banana pseudostem (n=3 batch, 50 insects in each batch)**

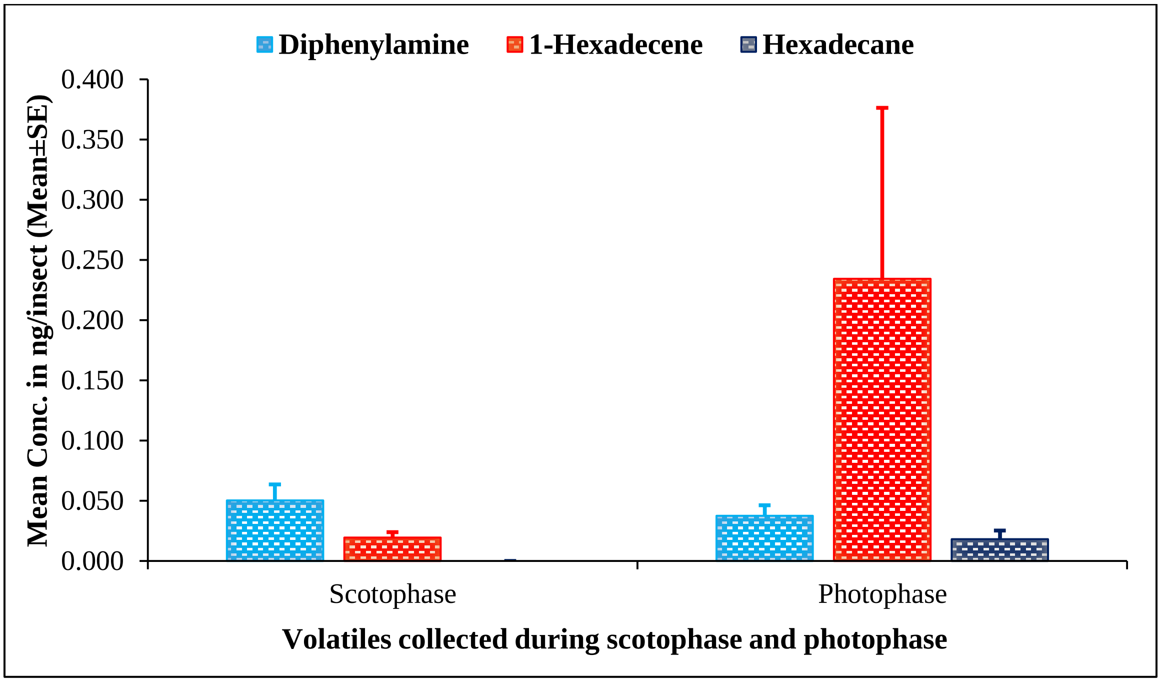


Fig. 21. **Mean concentration (ng/insect) of Diphenylamine, 1-Hexadecene and Hexadecane in porapak extracts of air entrainment odors from male volatiles of BSW collected during scotophase and photophase (n=3 batch, 50 insects in each batch)**

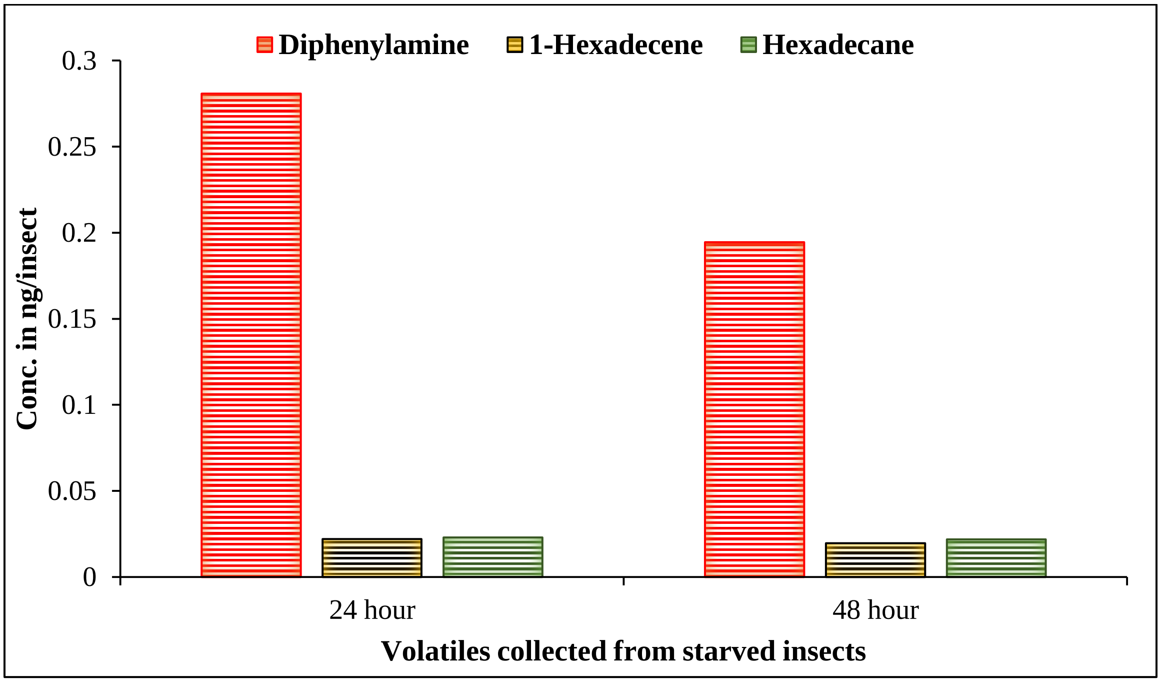


Fig. 22. Mean concentration (ng/insect) of Diphenylamine, 1-Hexadecene and Hexadecane in porapak extracts of air entrainment odors from male volatiles of BSW collected from 24 hour and 48 hour starved BSW male insects (n=30 insects)

Boheman produced seven sex-specific compounds, but only four of these seven showed electrophysiological activity (Ambrogi *et al.,* 2012). The Comparative GC-EAD and GC-MS analysis of airborne volatiles produced by males and females of the guava weevil *Conotrachelus psidii* Marshall showed the presence of a male-specific electroantennographically active compound identified as (1*R*,2*S*,6*R*)-2-hydroxymethyl-2,6- dimethyl-3-oxabicyclo[4.2.0]octane (papayanol) (Palacio-Cortes *et al*., 2015). The male American palm weevils (APW), *Rynchophorus palmarum* (Linnaeus) produced two sex specific compounds, one was minor compound, not always detected and the other was major compound identified as (2E)-6-methyl-2-hepten-4-ol which proved to be the essential component of the APW aggregation pheromone by EAG, GC-EAD and Behavioural bioassays (Rochat *et al.,* 1991b). The comparative analysis of the volatiles released by males and females of *Pseudopiazurus obesus* (Boheman) revealed three male-specific compounds in 77:14:9 (Zarbin *et al.*, 2007).

The quantification of all the three pheromone compounds in male volatiles revealed that Diphenylamine and 1-Hexadecene were produced in abundance than Hexadecane. Hence, the first two compounds were suggested as major aggregation pheromone compounds of *O. longicollis.* Because, in the previous reports on other weevils, the compounds released in the greatest quantities by males constituted major aggregation pheromone (Perez *et al.,* 1997 and Giblin-Davis *et al*., 2000). Eller *et al.* (1994) reported the presence of six male-specific compounds in the aggregation pheromone of pepper weevil, *Anthonomus eugenii Cano*. The emission rates of these compounds from feeding males were determined to be about: 7.2, 4.8, 0.45, 0.30, 2.0, and 0.30 µg/male/day, respectively.

The production of pheromone components depended on the presence of the host plant, as insects feeding on pseudostem released more volatiles than weevils alone (Ambrogi and Zarbin, 2008 and Palacio-Cortes *et al*., 2015). Food has been shown to stimulate pheromone release in other beetles (Ruiz-Montiel *et al*., 2008). This Behaviour has been documented in other curculionid species such as *Anthonomus grandis* Boheman (Dickens, 1989), *Anthonomus musculus* Say (Szendrei *et al*., 2011) *Rynchophorus phoencis* (F.) (Jaffe *et al*., 1993), *Rynchophorus palmarum* ((Linnaeus) (Oehlschlager *et al.*, 1992), *Pissodes strobe* (Peck) and *Pissodes approximates* Hopkins (Booth *et al*., 1983).

Observations in the current study revealed that pheromone release takes place mainly during photophase with a maximum production recorded between 02.00 PM-04.00 PM. Release of pheromone in *O. longicollis* depended on the photoperiod. The present observations are in agreement with the previous reports (Ambrogi and Zarbin, 2008 and Palacio-Cortes *et al*., 2015). The high pheromone release during photophase suggested that the activity of BSW weevils is predominant during daytime. This is in accordance with field observations, in which the adult's activity was observed mainly during daytime. The diel rhythm of pheromone release was influenced by the photoperiod and this is previously reported in the *A. grandis* (Gueldner and Wiygul, 1978) and in select species of scarab beetles (Leal *et al*., 1993).

4.2.5 Gas chromatography of plant volatiles

Four different types of volatiles extracted from banana pseudostem of different physiological states i.e., healthy, BSW damaged, decaying and mechanically damaged pseudostem in 10 ml of dichloromethane (DCM) and further concentrated using nitrogen gas. The chromatograms of these volatiles showed that the number of volatile peaks present in the healthy pseudostem extract was more than that of BSW damaged, decaying and mechanically damaged pseudostem extracts (Figures 23 and 24). The volatiles collected from four different types of banana pseudostem were used in EAG studies and Y-tube olfactometer bioassays.

In the GC chromatograms of healthy pseudostem volatiles, approximately 46 volatile peaks were detected. Among the detected peaks, 20 were major peaks with their area percentage varying from 1.0118 to 23.1770 (Figure 23A). BSW damaged pseudostem volatile chromatogram indicated 23 volatile peaks, among them 18 were major peaks with their area percentage ranging from1.0876 to 15.6114 (Figure 23B).

The GC chromatogram of decaying pseudostem volatile profile revealed that, approximately 28 volatile peaks were detected among them, 22 were major peaks with the area percentage ranging from 1.0217 to 14.7788 (Figure 24A). In the mechanically damaged pseudostem volatile chromatogram, about 24 volatile peaks were detected among the detected peaks 14 were major peaks with the area percentage varying from 1.1255 to 35.0893 (Figure 24B).

Herbivores are known to use plant volatiles (kairomones) to locate a food plant (Visser, 1986). When herbivores feed on a plant, cell damage results in the release of volatiles from the wound site (Dicke *et al.*, 1990). The amount of volatiles released by herbivores or by herbivore infested plants affects the herbivore response to the plant. The response to an information source by the herbivore depends on physiological state, previous experiences and abiotic conditions (Dicke *et al.*, 1998).

A study on the Behavioural responses of *O. longicollis* to volatiles from banana pseudostems with different physiological status was conducted by Yin Jiong *et al*. (2012). Solid phase microextraction (SPME) was used to extract volatiles from banana pseudostems in the following four physiological states: fresh, rotten but not fed on by beetles, rotten and fed on by beetles, and fresh and fed on by beetles. The extracted volatiles were analyzed by gas chromatography mass spectrometry (GC-MS). A total of 10 components were identified from volatiles extracted from fresh and rotten pseudostems that had been fed on by beetles, where as a total of 11 components were identified from extracts from rotten pseudostems and fresh pseudostems that had been fed on by beetles. The relative abundance of compounds in the extracted volatiles differed with pseudostems' physiological state. The species and relative abundance of extracted volatiles were closely related to damage inflicted by the banana pseudostem weevil.

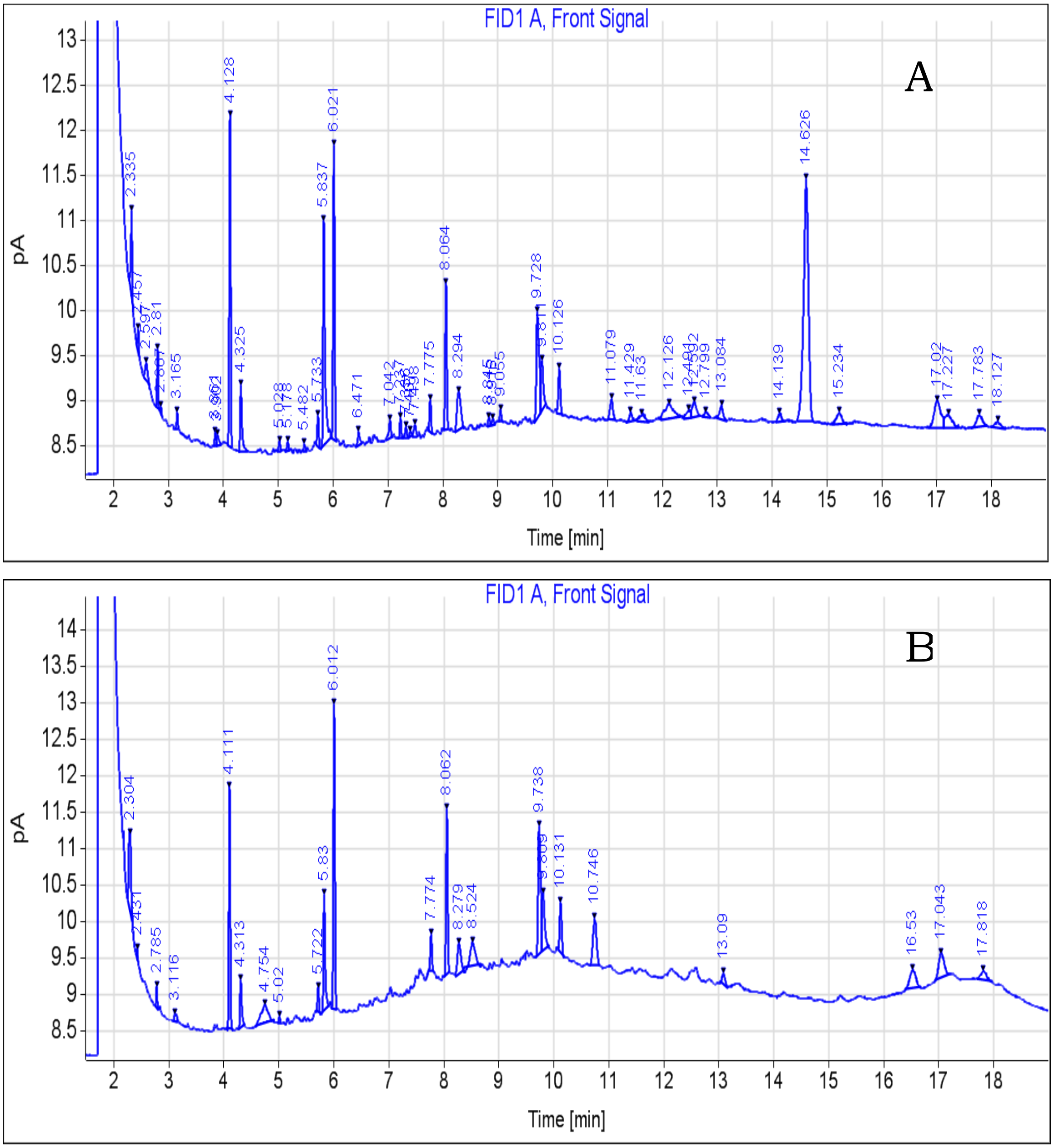


Fig. 23. Gas chromatography analysis of pseudostem extracts of Healthy banana plant (A) and BSW damaged banana plant (B)

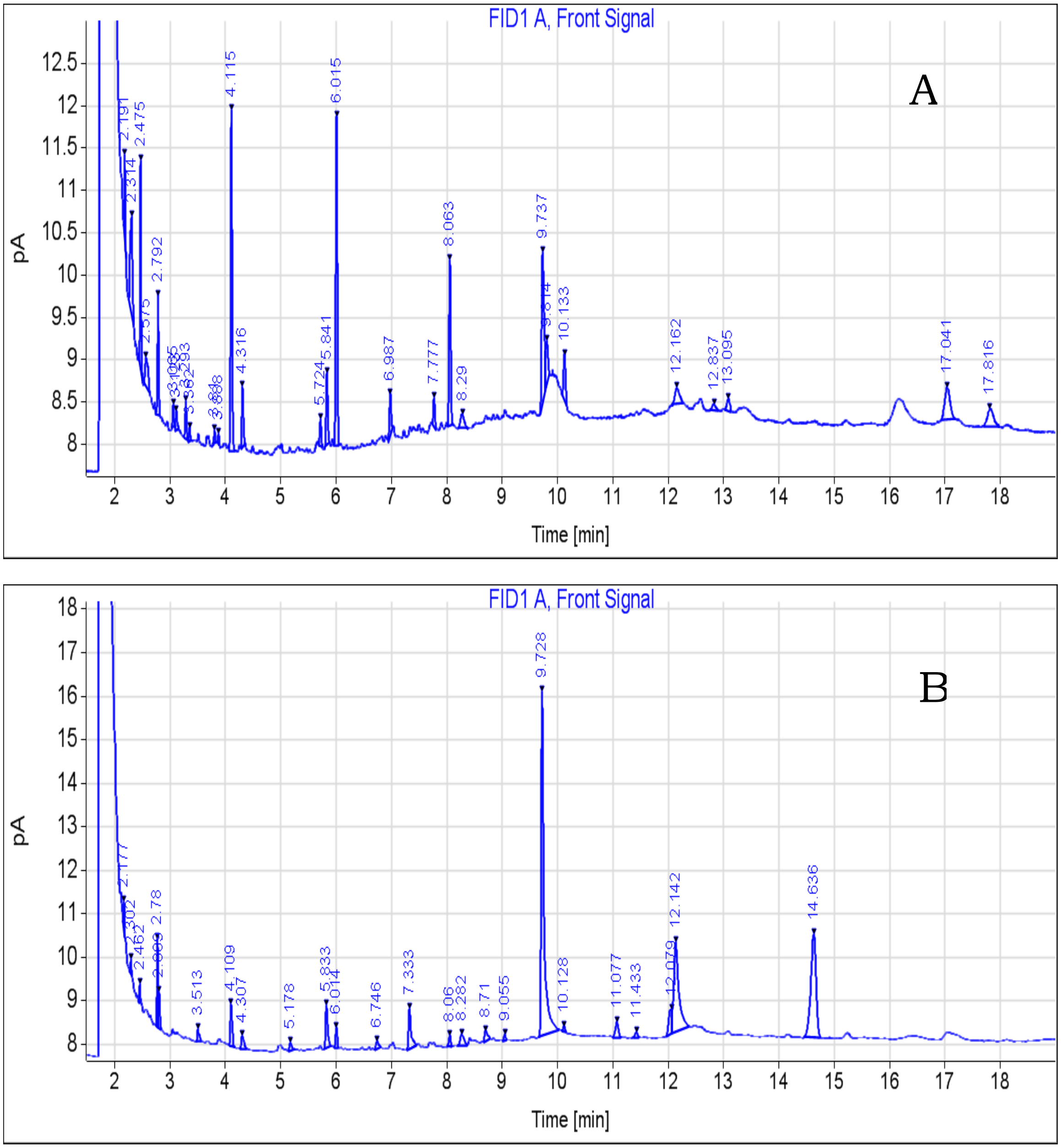


Fig. 24. **Gas chromatography analysis of pseudostem extracts of Decaying banana plant (A) and mechanically damaged banana plant (B)**

4.3 Laboratory and Field evaluation of identified pheromone compounds

4.3.1 Laboratory bioassay of *O. longicollis* pheromone components and blends

Bioassays were carried out for identified *O. longicollis* aggregation pheromone components involving Diphenylamine, 1-Hexadecene and Hexadecane and different blend combinations of these compounds for evaluating biological activity to male and female BSW. The data are presented and discussed here under. The bioassay study was also conducted with 2-methyl 4-heptanol reported as aggregation pheromone of *O. longicollis* by Gunawardena and Dissanayake (2000a), to determine the Behavioural responses of weevils to this pheromone.

4.3.1.1 Electroantenography (EAG)

Results of electroantennography (EAG) responses of male and female antennae of *O. longicollis* for different volatiles of BSW, synthetic pheromone compounds and their different blend combinations were conducted at BCRL, Bengaluru and are presented in Tables 4 to 14 and Figures 25 to 35.

Mean EAG responses of BSW male antennae to different insect volatiles revealed that there was a significant difference among the tested volatiles ($F_{3, 16} = 4.288$; $p = 0.021$). The antennae of male BSW produced significantly higher EAG response to male volatile compared to any other stimulus source tested. The highest EAG response recorded with male antenna against male volatile was 1.378 ± 0.350 mV (Table 4 and Figure 25). Male + Female volatiles and Female volatile evoked second higher antennal response and these two were statistically on par (0.801 ± 0.204 and 0.756 ± 0.133 mV, respectively). Control evoked the lowest response (0.287 ± 0.065) (Table 4 and Figure 25).

Female antennal responses to male volatile, female volatile and male + female volatile were assayed by EAG. The results revealed that the responses were significantly different ($F_{3, 16} = 6.686$; $p = 0.004$). The female BSW antennae produced significantly higher EAG responses to male and female volatiles (1.704 ± 0.246 and 1.368 ± 0.239, mV respectively) and these two were statistically on par followed by male + female volatile (1.250 ± 0.209 mV) and DCM (0.452 ± 0.080) (control) (Table 4 and Figure 25).

The EAG responses of *O. longicollis* male and female to 2-methyl 4-heptanol, BSW male volatile and hexane were conducted. The results were presented in Table 5 and Figure 26. The mean male antennal responses to the tested volatile compounds were significantly different ($F_{2, 12} = 18.085$; $p = 0.0001$). The male antennal response to 2-methyl 4-heptanol and BSW male volatile were significantly higher and they were statistically on par (1.809 ± 0.198 and 1.428 ± 0.159 mV, respectively). Hexane (control) recorded the lowest response (0.523 ± 0.090 mV) (Table 5 and Figure 26).

Mean EAG responses of *O. longicollis* female to 2-methyl 4-heptanol, BSW male volatile and hexane revealed that the responses were significantly different ($F_{2, 12} = 18.246$; $p = 0.0001$). The EAG response of female antennae was significantly higher for

2-methyl 4-heptanol (1.888 ± 0.204 mV) followed by male volatile (1.297 ± 0.134 mV) and the lowest response for the control (0.619 ± 0.083 mV) (Table 5 and Figure 26).

Table 4. EAG response (Mean ± SEm) of BSW male and female antennae against different volatiles from BSW weevils

Sl. No.	Treatments	EAG Response (mV) (Mean ± SEm)	
		Male	Female
T1	Male volatile	1.378±0.350^{a}	1.704±0.246^{a}
T2	Female volatile	0.756±0.133^{ab}	1.368±0.239^{a}
T3	Male + Female volatile	0.801±0.204^{ab}	1.250±0.209^{ab}
T4	Control (DCM)	0.287±0.065^{b}	0.452±0.080^{b}
DF		3, 16	3, 16
F test		4.288	6.686
P value		0.021*	0.004**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), * Significant difference at p<0.05, ** Significant difference at p<0.01, N=5.*

Table 5. Antennal response (Mean ± SEm) of *O. longicollis* male and female to 2-methyl 4-heptanol and BSW male volatile

Sl. No.	Treatments	EAG Response (mV) (Mean ± SEm)	
		Male	Female
1	2-methyl 4-heptanol	1.809±0.198^{a}	1.888±0.204^{a}
2	BSW Male IVC	1.428±0.159^{a}	1.297±0.134^{b}
3	Hexane	0.523±0.090^{b}	0.619±0.083^{c}
DF		2, 12	2, 12
F test		18.085	18.246
P value		0.0001**	0.0001**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), ** Significant difference at p<0.01, N=5.*

The EAG responses of *O. longicollis* male and female to synthetic pheromone compounds *viz.*, Diphenylamine, 1-Hexadecene and Hexadecane with male volatile and hexane was conducted. The results revealed that there was a significant difference among the tested compounds ($F_{4, 20}$ =9.694; p = 0.0001). Male volatile evoked significantly higher response in male antennae (1.428 ± 0.159 mV) and it was followed by 1-

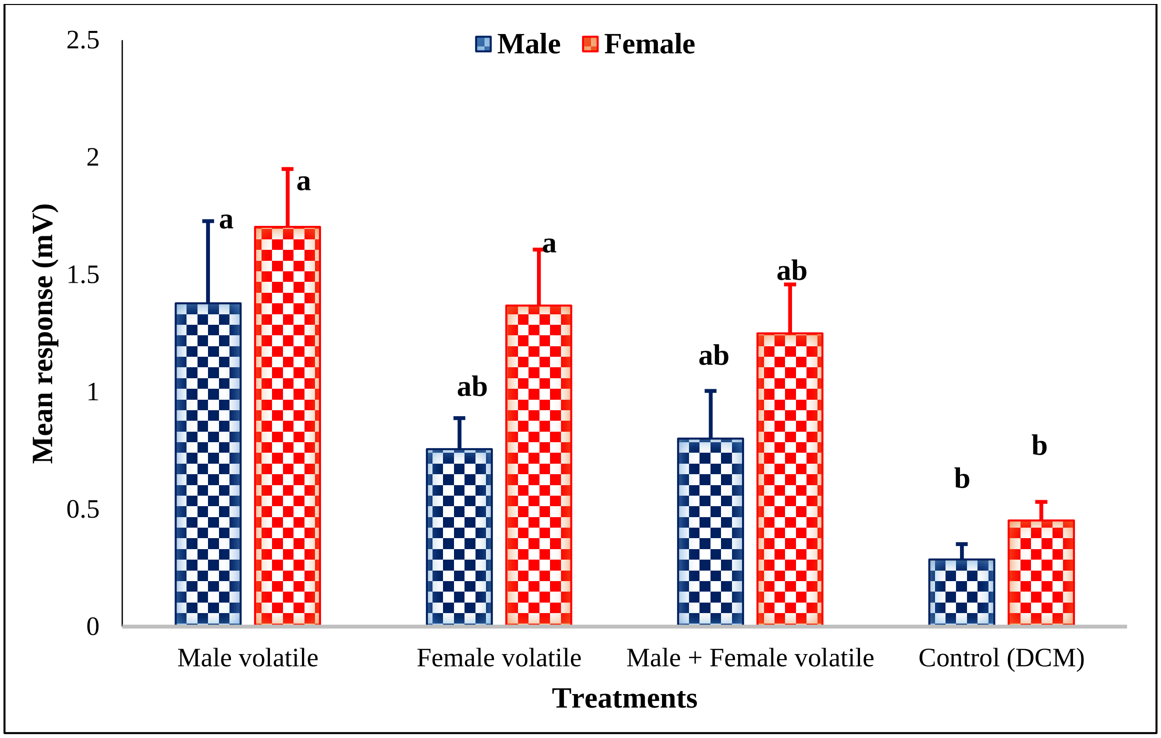


Fig. 25. **EAG response (Mean ± SEm) of BSW male and female antennae against different volatiles of BSW weevils. N=5, Error bars indicate ± SE**

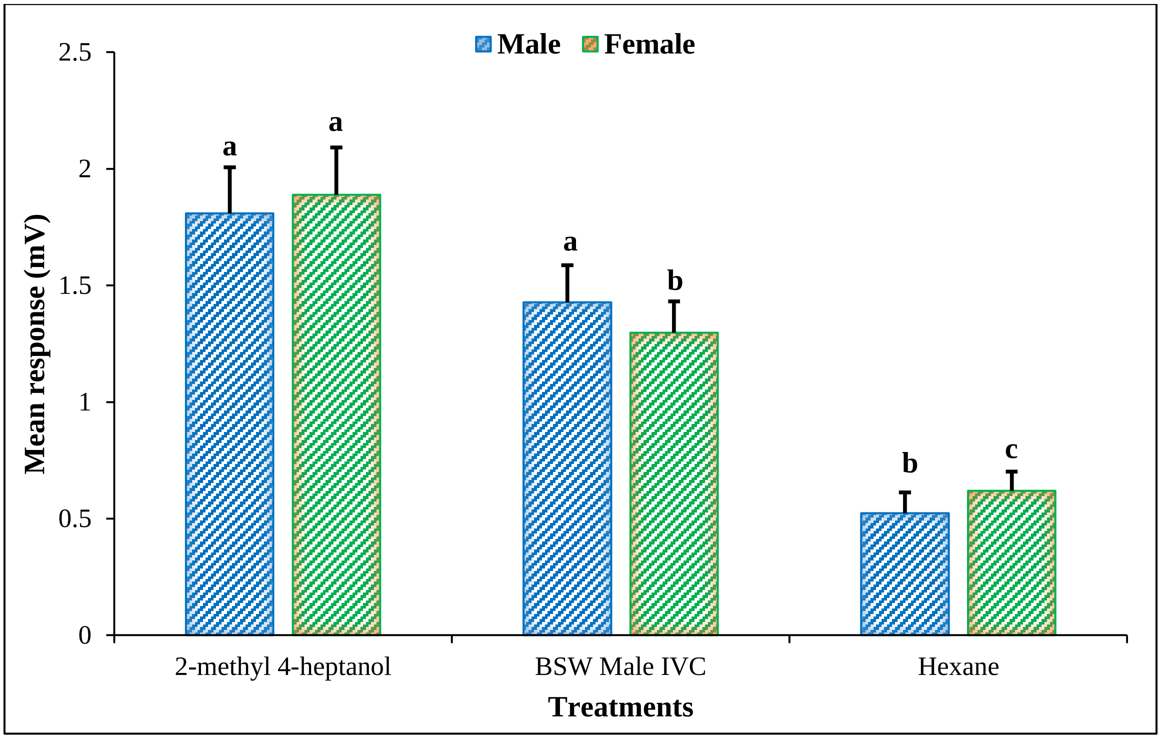


Fig. 26. **EAG response (Mean ± SEm) of BSW male and female antennae against 2-methyl 4-heptanol and BSW male volatile. N=5, Error bars indicate ± SE**

Hexadecene evoked the second higher response (1.243 ± 0.119 mV). Diphenylamine and Hexadecane elicited lower antennal response and these two were statistically on par (1.094 ± 0.094 and 1.033 ± 0.056, respectively) (Table 6 and Figure 27).

Mean EAG responses of female to synthetic pheromone compounds *viz.*, Diphenylamine, 1-Hexadecene and Hexadecane with male volatile and hexane revealed that the responses were significantly different ($F_{4, 20} = 7.121$; $p = 0.001$). Further, the female antennal response to male volatile (1.297 ± 0.134) was significantly higher than rest of the treatments. Hexadecane, 1-Hexadecene and Diphenylamine evoked the second higher response (0.846 ± 0.067, 0.774 ± 0.060 and 0.767 ± 0.117, respectively) and these were statistically on par with the control (0.619 ± 0.083) (Table 6 and Figure 27).

Results of the present study revealed that male volatile elicited significantly higher EAG responses in male and female antennae and there was a significant difference in EAG responses of males and females to different treatments. Females produced higher EAG deflections than male weevils in all the tests except for the control. These results are in agreement with the Prasuna *et al*. (2008). These results suggest that male and female *O. longicollis* antennae are equally sensitive in perceiving the chemicals. Both male and female weevils showed antennal response to synthetic pheromone compounds and elicited higher EAG response in male antennae than in female antennae.

Table 6. Antennal response (Mean ± SEm) of *O. longicollis* male and female to synthetic components of male pheromone

Sl. No.	Treatments	EAG Response (mV) (Mean ± SEm)	
		Male	Female
1	Diphenylamine (DPA)	1.094 ± 0.094^{b}	0.767 ± 0.117^{b}
2	1-Hexadecene (1-16C)	1.243 ± 0.119^{ab}	0.774 ± 0.060^{b}
3	Hexadecane (16C)	1.033 ± 0.056^{b}	0.846 ± 0.067^{b}
4	Male IVC	1.428 ± 0.159^{a}	1.297 ± 0.134^{a}
5	Hexane	0.523 ± 0.090^{c}	0.619 ± 0.083^{b}
DF		4, 20	4, 20
F test		9.694	7.121
P value		0.0001**	0.001**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), ** Significant difference at $p<0.01$, N=5*

4.3.1.1.1 Dose response studies of synthetic pheromone compounds

The synthetic pheromone compounds *viz.*, Diphenylamine, 1-Hexadecene and Hexadecane were tested at five different doses *viz.*, 0.5, 5, 10, 20 and 40 µl separately when dispensed from filter paper to find out the dose variation responses with increasing doses. The results are shown in Tables 7, 8, 9 and Figures 28, 29, 30.

Antennal responses of males and females to different doses of Diphenylamine revealed that all doses elicited EAG response. In case of male antennae dose-dependent increase in EAG activity was observed with increase in dose up to 5µl and then decreased with further increase in dose and similar kind of response was recorded with the female antennae (Table 7 and Figure 28). The EAG response of male to different doses of 1-Hexadecene increased with increase in dose up to 10µl and then decreased with further increase in dose. Likewise female weevil antennae increased up to 20 µl and decreased with further increase in dose (Table 8 and Figure 29).

The mean antennal response of male to different doses of Hexadecane increased with increase in dose up to 20 µl and then decreased with further increase in dose while that of female antennae increased with increase in dose (Table 9 and Figure 30). The results of dose response studies of synthetic pheromone compounds indicated that Diphenylamine at 5 µl elicited higher EAG response and the responses decreased with increase in doses in both male and female antennae. While 1-Hexadecene and hexadecane produced increased antennal response in both sexes with increased doses.

Table 7. Antennal response (Mean ± SEm) of *O. longicollis* male and female to doses of Diphenylamine (2 %)

Sl. No.	**Treatments**	**Dose (µl)**	**EAG Response (mV) (Mean ± SEm)**	
			Male	**Female**
1	Diphenylamine (10µg)	0.5	0.676±0.089 ^{a}	0.829±0.045^{ab}
2	Diphenylamine (100µg)	5	0.890±0.032 ^{a}	0.933±0.053^{a}
3	Diphenylamine (200µg)	10	0.788±0.103 ^{a}	0.820±0.059^{ab}
4	Diphenylamine (400µg)	20	0.618±0.125 ^{a}	0.802±0.067^{ab}
5	Diphenylamine (800µg)	40	0.585±0.104 ^{a}	0.670±0.068^{b}
DF			4, 20	4, 20
F test			1.729	2.534
P value			0.183^{NS}	0.072 ^{NS}

Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), NS=Non-significant, N=5

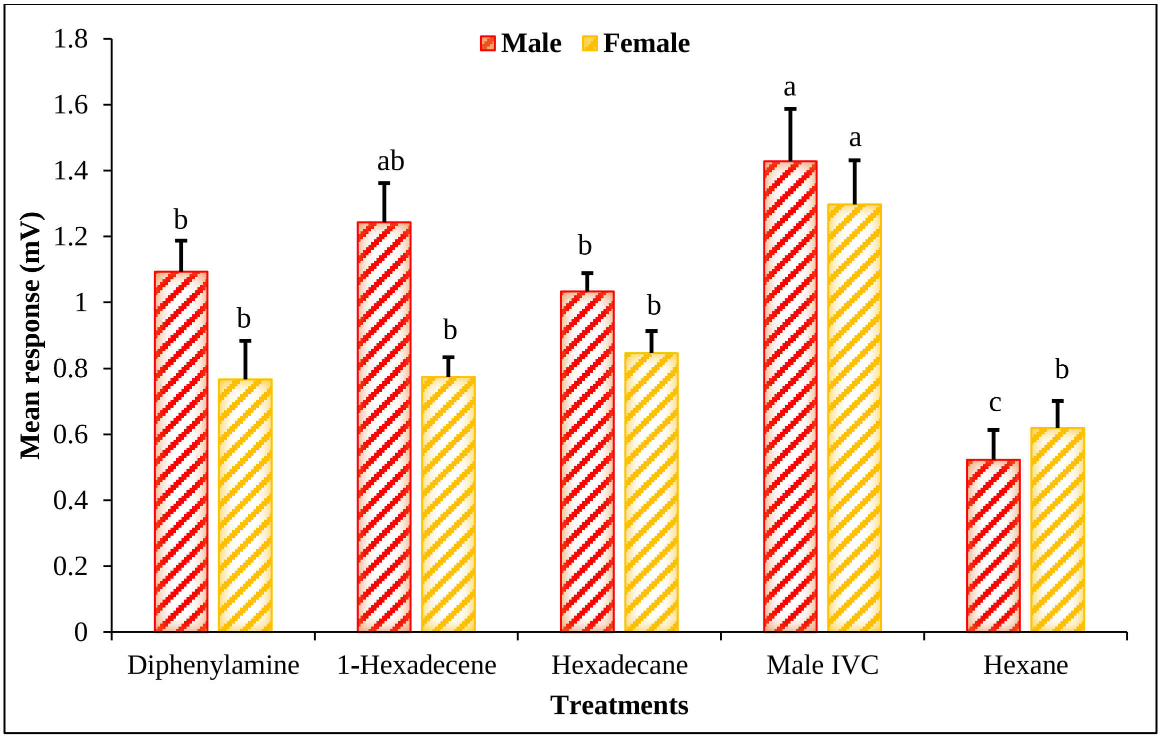


Fig. 27. **EAG response (Mean ± SEm) of BSW male and female antennae to synthetic components of male pheromone. N=5, Error bars indicate ± SE**

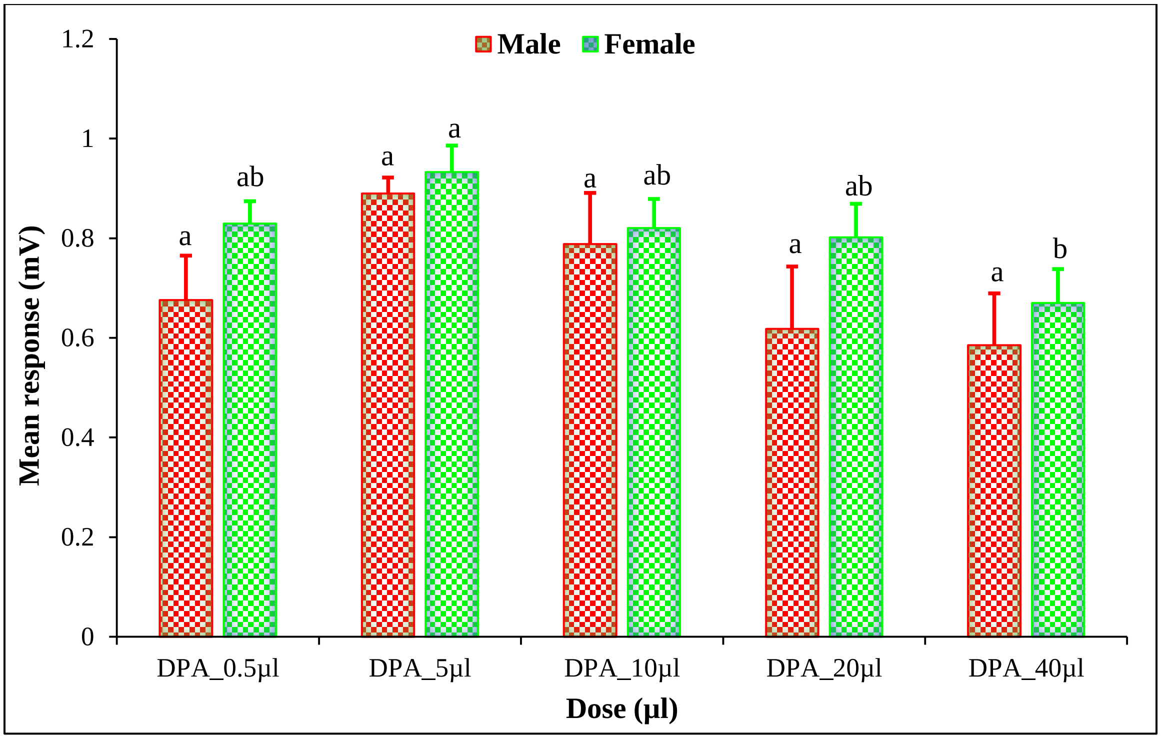


Fig. 28. **EAG response (Mean ± SEm) of BSW male and female antennae to doses of Diphenylamine (2 %). N = 5, Error bars indicate ± SE**

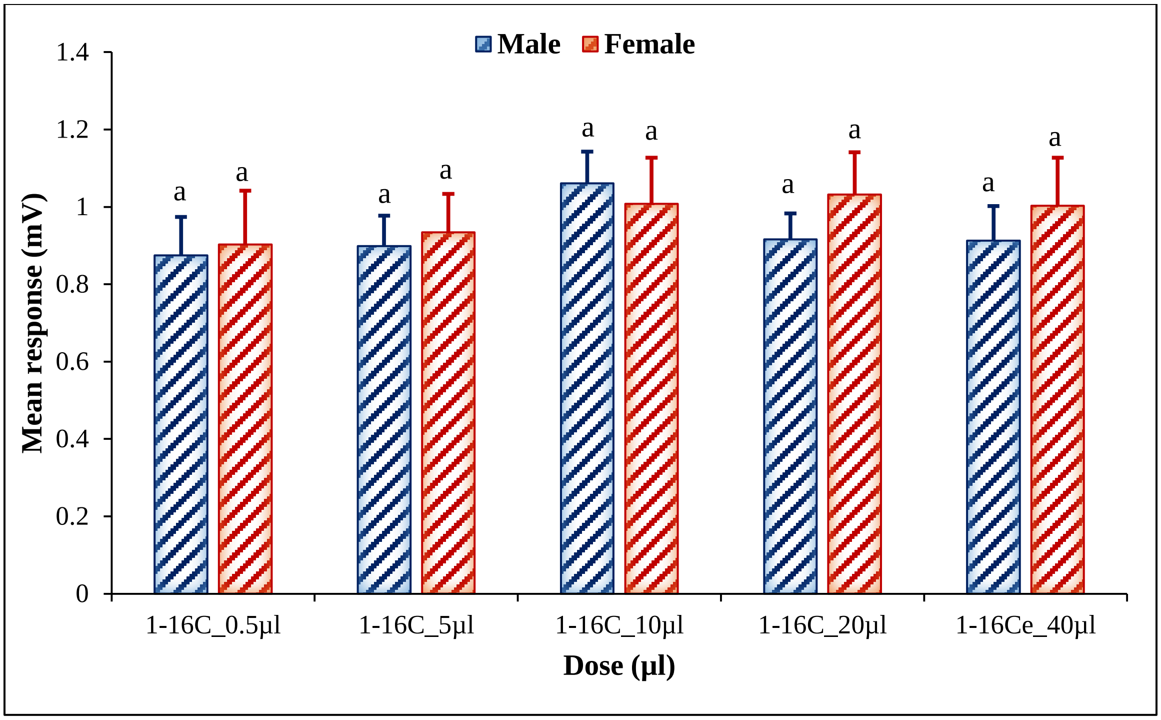


Fig. 29. EAG response (Mean ± SEm) of BSW male and female antennae to doses of synthetic 1-Hexadecene (2 %). N=5, Error bars indicate ± SE

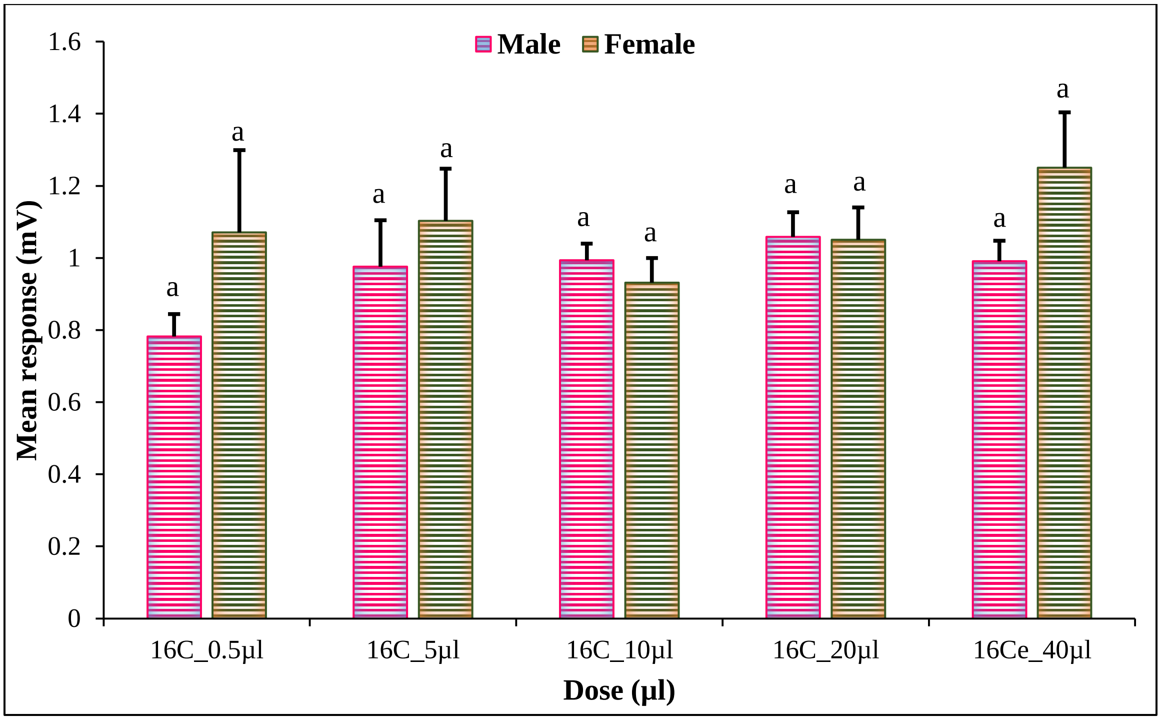


Fig. 30. EAG response (Mean ± SEm) of BSW male and female antennae to increasing doses of synthetic Hexadecane (2 %). N=5, Error bars indicate ± SE

Table 8. Antennal response (Mean ± SEm) of *O. longicollis* male and female to doses of 1-Hexadecene (2 %)

Sl. No.	Treatments	Dose (µl)	EAG Response (mV) (Mean±SEm)	
			Male	Female
1	1-Hexadecene (10µg)	0.5	0.875±0.099 ^{a}	0.903±0.139 ^{a}
2	1-Hexadecene (100µg)	5	0.899±0.078 ^{a}	0.934±0.100 ^{a}
3	1-Hexadecene (200µg)	10	1.061±0.082 ^{a}	1.008±0.119 ^{a}
4	1-Hexadecene (400µg)	20	0.916±0.067 ^{a}	1.032±0.109 ^{a}
5	1-Hexadecene (800µg)	40	0.913±0.089 ^{a}	1.003±0.124 ^{a}
DF			4, 20	4, 20
F test			0.774	0.211
P value			0.555 ^{NS}	0.930 ^{NS}

Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), NS=Non-significant, N=5

Table 9. Antennal response (Mean ± SEm) of *O. longicollis* male and female to doses of Hexadecane (2 %)

Sl. No.	Treatments	Dose (µl)	EAG Response (mV) (Mean±SEm)	
			Male	Female
1	Hexadecane (10µg)	0.5	0.782±0.062 ^{a}	1.071±0.228 ^{a}
2	Hexadecane (100µg)	5	0.976±0.129 ^{a}	1.103±0.145 ^{a}
3	Hexadecane (200µg)	10	0.994±0.046 ^{a}	0.931±0.069 ^{a}
4	Hexadecane (400µg)	20	1.058±0.069 ^{a}	1.050±0.090 ^{a}
5	Hexadecane (800µg)	40	0.991±0.057 ^{a}	1.250±0.154 ^{a}
DF			4, 20	4, 20
F test			1.764	0.602
P value			0.176 ^{NS}	0.666 ^{NS}

Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), NS=Non-significant, N=5

4.3.1.1.2 EAG studies of Diphenylamine, 1-Hexadecene and Hexadecane in combinations

Two sets of EAG studies were conducted with different combinations of two compounds viz., Diphenylamine and1-Hexadecene. Study-1 was conducted with 100:0, 100:2, 100:4, 0:100, male volatile and hexane against both male and female antennae. For study-2 with 100:0, 100:6, 100:8, 100:10, male volatile and hexane against male antennae and 0:100, 100:6, 100:8, 100:10, male volatile and hexane against female antennae. The final EAG study was conducted with different combinations of three compounds viz., Diphenylamine, 1-Hexadecene and Hexadecane. The study-3 with 100:8:5, 100:8: 100:5, 8:5, 0.5:10:20, male volatile and hexane against both male and female antennae. The results are presented in Tables 10, 11, 12, 13 and Figures 31, 32, 33, 34.

The mean EAG response of male antennae against different blends of Diphenylamine and 1-Hexadecene in study-1 revealed that the Behavioural responses were significantly different ($F_{5, 24}$ = 3.772; p=0.012). Male volatile evoked significantly higher response (1.513 ± 0.292) than rest of the treatments. Diphenylamine (100:0) and 1-Hexadecene (0:100) evoked higher response and these two were on par (0.836 ± 0.172 and 0.775 ± 0.192, respectively). The lowest response was elicited by Diphenylamine: 1-Hexadecene at 100:4 and 100:2 (0.673 ± 0.152 and 0.614±0.149, respectively). These two blends were statistically on par with the control (0.483 ± 0.113) (Table 10 and Figure 31).

Table 10. EAG response (Mean ± SEm) of BSW male and female antennae to blends of Diphenylamine and 1-Hexadecene (Study-I)

Sl. No.	Treatments	EAG Response (mV) (Mean±SEm)	
		Male	Female
1	Diphenylamine	0.836 ± 0.172^{ab}	0.615 ± 0.053^{bc}
2	Diphenylamine: 1-Hexadecene (100:2)	0.614 ± 0.149^{b}	0.706 ± 0.036^{bc}
3	Diphenylamine: 1-Hexadecene (100:4)	0.673 ± 0.152^{b}	0.640 ± 0.059^{bc}
4	1-Hexadecene	0.775 ± 0.192^{ab}	0.730 ± 0.086^{b}
5	Male IVC	1.513 ± 0.292^{a}	1.023 ± 0.139^{a}
6	Hexane	0.483 ± 0.113^{b}	0.488 ± 0.077^{c}
DF		5, 24	5, 24
F test		3.772	4.788
P value		0.012*	0.004**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), * Significant difference at p<0.05, ** Significant difference at p<0.01, N=5*

The antennal response of female antennae to different blends in study-1 revealed that the responses were significantly different ($F_{5, 24}$ = 4.788; p=0.004). Mean EAG response of female antennae was significantly higher in male volatile (1.023 ± 0.139) followed by 1-Hexadecene (0:100) (0.730 ± 0.086). The blends of Diphenylamine: 1-Hexadecene at 100:2, 100:4 and 100:0 evoked lower response and are statistically on par (0.706 ± 0.036, 0.640 ± 0.059 and 0.615 ± 0.053, respectively). The lowest response was elicited by hexane (Table 10 and Figure 31).

The mean EAG response of male antennae against different blends of Diphenylamine and 1-Hexadecene in study-2 revealed that the Behavioural responses were significantly different ($F_{5, 24}$ = 22.072; p=0.0001). The male antennal response was significantly higher in male volatile (1.405 ± 0.123) than rest of the treatments. The blends of Diphenylamine: 1-Hexadecene at 100:6, 100:8 and 100:0 evoked higher response (0.677 ± 0.103, 0.669 ± 0.035 and 0.603 ± 0.027, respectively), significantly higher than hexane (control) and are statistically on par. The lowest response was elicited by Diphenylamine: 1-Hexadecene at 100:10 (Table 11 and Figure 32).

Table 11. EAG response (Mean ± SEm) of BSW male antennae to blends of Diphenylamine and 1-Hexadecene (Study-II)

Sl. No.	Treatments	EAG Response (mV) (Mean±SEm)
		Male
1	Diphenylamine	0.603 ± 0.027^{b}
2	Diphenylamine: 1-Hexadecene (100:6)	0.677 ± 0.103^{b}
3	Diphenylamine: 1-Hexadecene (100:8)	0.669 ± 0.035^{b}
4	Diphenylamine: 1-Hexadecene (100:10)	0.546 ± 0.042^{bc}
5	Male IVC	1.405 ± 0.123^{a}
6	Hexane	0.366 ± 0.075^{c}
DF		5, 24
F test		22.072
P value		0.0001**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), ** Significant difference at p<0.01, N=5*

Mean EAG response of female antennae to different blends in study-2 revealed that the responses were significantly different ($F_{5, 24}$ = 3.995; p=0.009). The mean EAG response of female antennae was significantly higher for male volatile (1.239 ± 0.213). The higher antennal response was elicited by Diphenylamine: 1-Hexadecene at 100:6 and 0:100 (0.863 ± 0.109 and 0.818 ± 0.091, respectively), that were significantly higher than hexane and are statistically on par. Further, Diphenylamine: 1-Hexadecene at 100:8 and 100:10 evoked lower response (0.792 ± 0.126 and 0.761 ± 0.099, respectively) and these two were on par (Table 12 and Figure 33).

Table 12. EAG response (Mean ± SEm) of BSW female antennae to blends of Diphenylamine and 1-Hexadecene (Study-II)

Sl. No.	Treatments	EAG Response (mV) (Mean±SEm)
		Female
1	1-Hexadecene	0.818±0.091 ^{b}
2	Diphenylamine: 1-Hexadecene (100:6)	0.863±0.109 ^{b}
3	Diphenylamine: 1-Hexadecene (100:8)	0.792±0.126 ^{bc}
4	Diphenylamine: 1-Hexadecene (100:10)	0.761±0.099 ^{bc}
5	Male IVC	1.239±0.213 ^{a}
6	Hexane	0.452±0.065 ^{c}
DF		5, 24
F test		3.995
P value		0.009**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), ** Significant difference at p<0.01, N=5*

The mean EAG response of male antennae against different blends of Diphenylamine, 1-Hexadecene and Hexadecane in study-3 revealed that all the blends tested elicited EAG activity. Mean EAG response of male antennae was higher in male volatile (1.548 ± 0.404) followed by Diphenylamine: 1-Hexadecene: Hexadecane (0.5:10:20) (0.992 ± 0.287), 1-Hexadecene: Hexadecane (8:5) (0.936 ± 0.273), Diphenylamine: 1-Hexadecene: Hexadecane (100:8:5) (0.852 ± 0.156), Diphenylamine: 1-Hexadecene (100:8) (0.832 ± 0.227), Diphenylamine: Hexadecane (100:5) (0.799 ± 0.209) and the lowest response showed to hexane (control) (0.796 ± 0.215) (Table 13 and Figure 34).

The mean EAG response of female antennae against different blends of Diphenylamine, 1-Hexadecene and Hexadecane in study-3 revealed that the responses were significantly different ($F_{6, 28} = 3.126$; p=0.018). The female antennal response was significantly higher in male volatile (1.138 ± 0.228) than rest of the treatments. The second higher antennal response was elicited by 1-Hexadecene: Hexadecane (8:5) (0.761 ± 0.116) followed by Diphenylamine: 1-Hexadecene: Hexadecane (0.5:10:20) (0.725 ± 0.126), Diphenylamine: Hexadecene (100:5) (0.635 ± 0.113), Diphenylamine: 1-Hexadecene: Hexadecane (100:8:5) (0.627 ± 0.086), Diphenylamine: 1-Hexadecene (100:8) (0.586 ± 0.080) and the lowest response showed to hexane (control) (0.371 ± 0.119) (Table 13 and Figure 34).

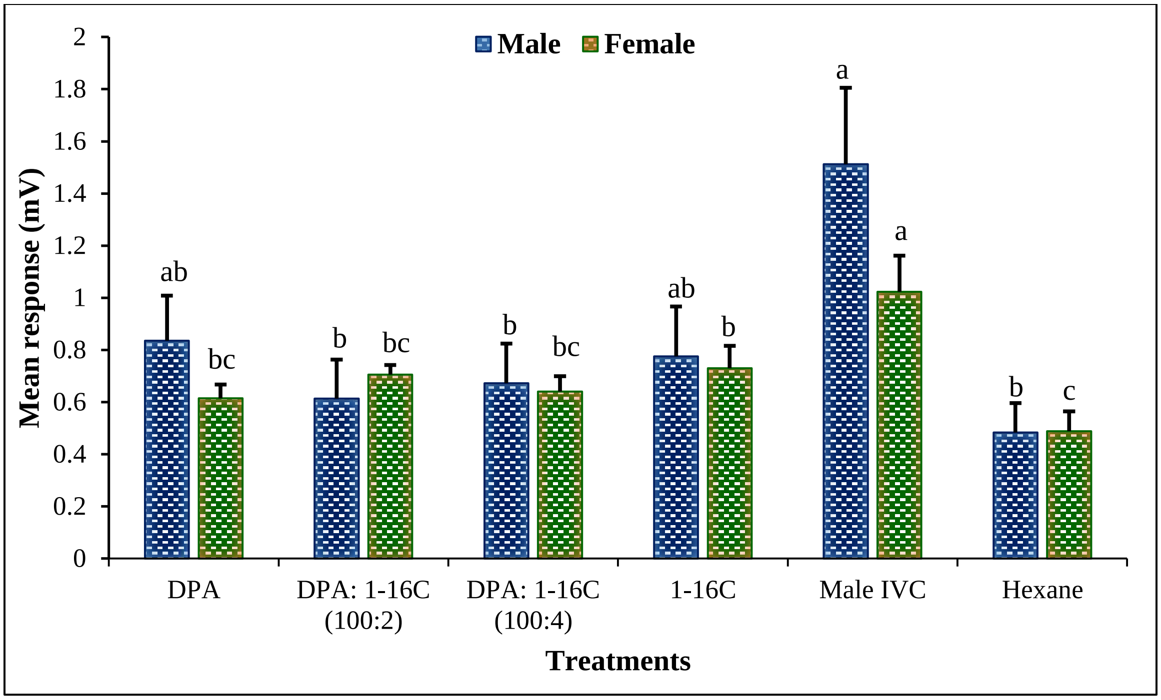


Fig. 31. **EAG response (Mean ± SEm) of BSW male and female antennae against different blends of Diphenylamine and 1-Hexadecene (Study-I). N=5, Error bars indicate ± SE**

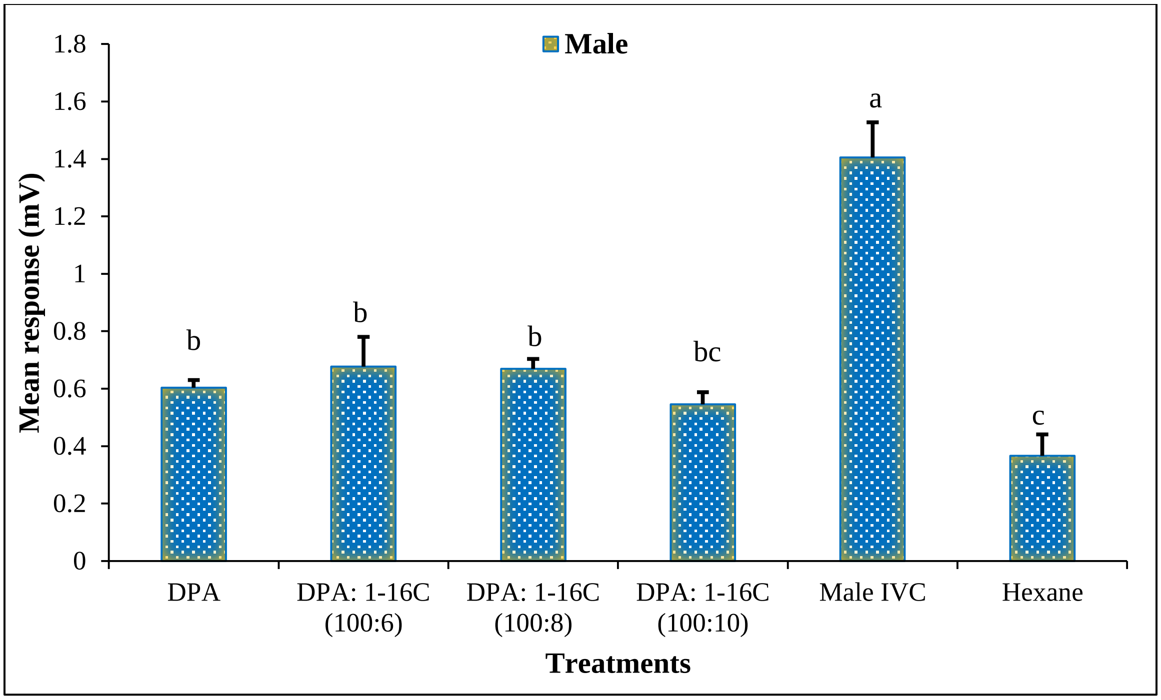


Fig. 32. **EAG response (Mean ± SEm) of BSW male antennae against different blends of Diphenylamine and 1-Hexadecene (Study-II). N=5, Error bars indicate ± SE**

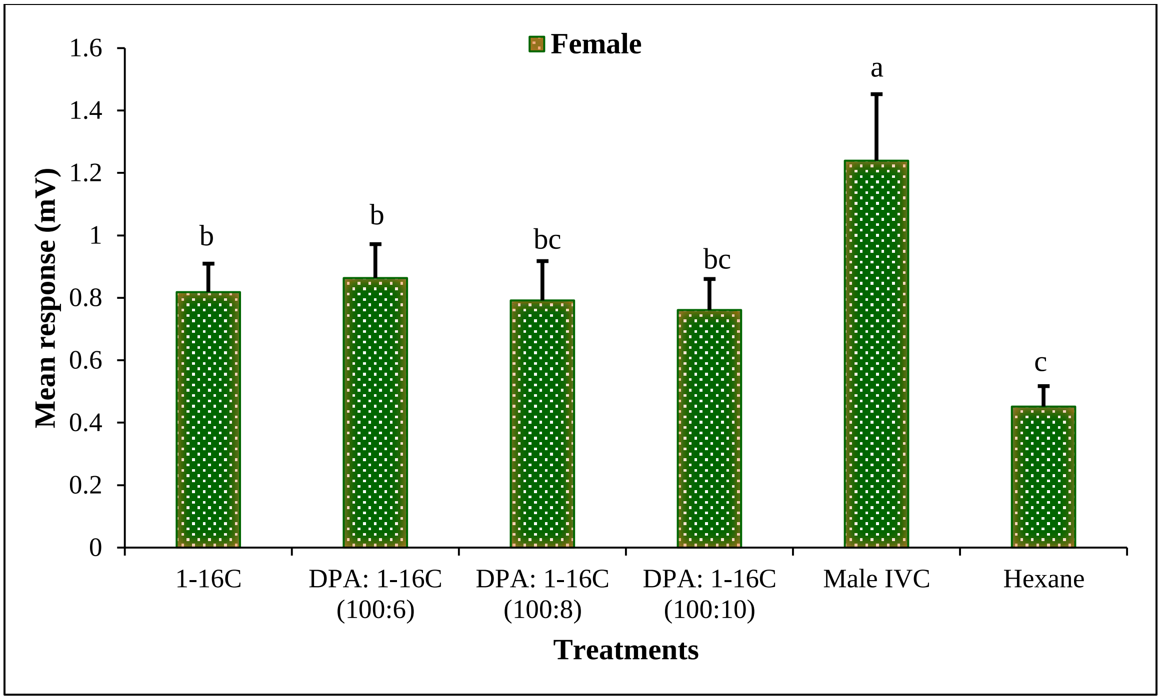
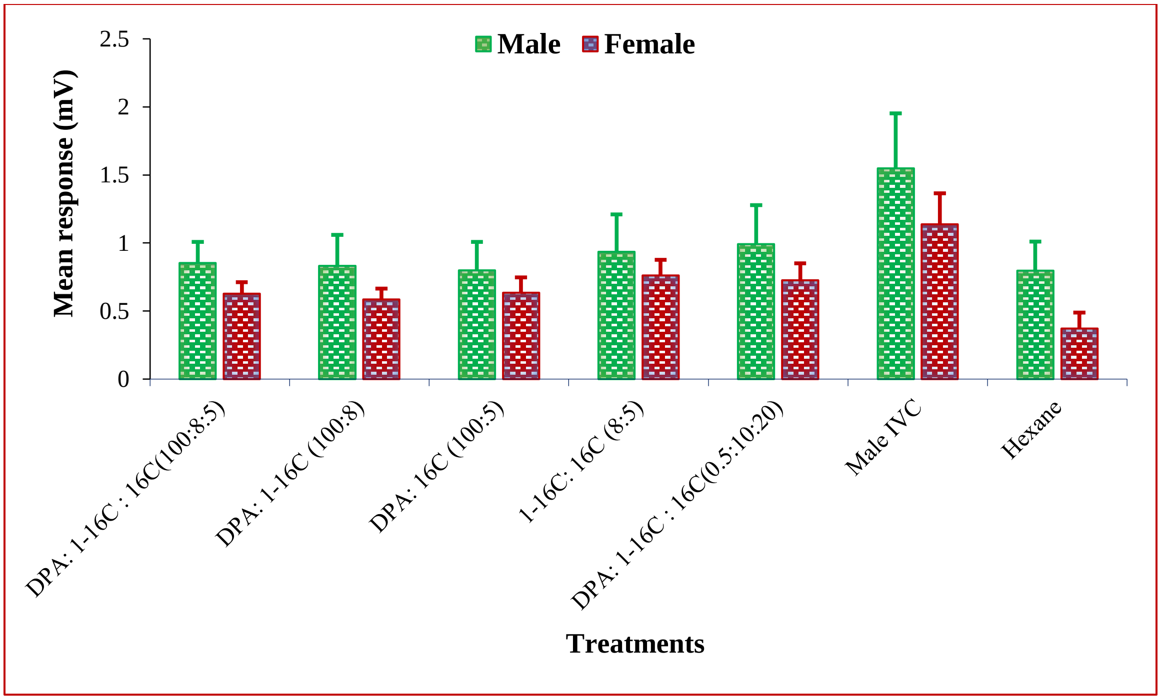


Fig. 33. EAG response (Mean ± SEm) of BSW female antennae against different blends of Diphenylamine and 1-Hexadecene (Study-II). N=5, Error bars indicate ± SE



Fig, 34. EAG response (Mean ± SEm) of BSW male and female antennae against different blends of Diphenylamine, 1-Hexadecene and Hexadecane (Study-III). N=5, Error bars indicate ± SE

Table 13. EAG response (Mean ± SEm) of BSW male and female antennae to blends of Diphenylamine, 1-Hexadecene and Hexadecane (Study-III)

Sl. No.	Treatments	EAG Response (mV) (Mean±SEm)	
		Male	Female
1	Diphenylamine:1-Hexadecene:Hexadecane (100:8:5)	0.852±0.156	0.627±0.086 ^{ab}
2	Diphenylamine: 1-Hexadecene (100:8)	0.832±0.227	0.586±0.080 ^{ab}
3	Diphenylamine: Hexadecane (100:5)	0.799±0.209	0.635±0.113 ^{ab}
4	1-Hexadecene: Hexadecane (8:5)	0.936±0.273	0.761±0.116 ^{ab}
5	Diphenylamine:1-Hexadecene:Hexadecane (0.5:10:20)	0.992±0.287	0.725±0.126 ^{ab}
6	Male IVC	1.548±0.404	1.138±0.228^{a}
7	Hexane	0.796±0.215	0.371±0.119^{b}
DF		6, 28	6, 28
F test		1.028	3.126
P value		0.428^{NS}	0.018*

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), * Significant difference at p<0.05, NS= Non-significant, N=5*

4.3.1.1.3 Dose variation response of best performed blend

Best performed blend from the bioassay seriates i.e., Diphenylamine: 1-Hexadecene: Hexadecane at 0.5:10:20 was tested at five different doses viz., 10, 20, 40, 80 and 100 µl when dispensed from filter paper to find out the dose variation response.

Antennal response of males to different doses of DPA: 1-16C: 16C at 0.5:10:20 blends revealed that all doses elicited EAG response. Dose dependent increase in EAG activity was observed with increase in dose of synthetic pheromone blend. Stimuli elicited higher response at 100 µl (1.123 ± 0.105) and 10 µl had the lowest response (0.821 ± 0.103) (Table 14 and Figure 35). Mean EAG response of females to 0.5:10:20 blend of DPA: 1-16C: 16C revealed that, the response was increased with increase in dose. The higher response was elicited at 100 µl (0.752 ± 0.075) and the lowest response was recorded at 10 µl (0.683 ± 0.144) (Table 14 and Figure 35).

The results of the different blend combinations of synthetic pheromone compounds indicated that the blend of DPA: 1-16C:16C at 0.5:10:20 and 100:8 elicited higher EAG response in both male and female antennae. The increased doses of the best performing blend (0.5:10:20) produced increased antennal response in both the sexes. An increased antennal response was recorded at the dose of 100 µl.

Table 14. EAG response (Mean ± SEm) of BSW male and female antennae to doses of best performing synthetic blend of Diphenylamine, 1-Hexadecene and Hexadecane

Sl. No.	Treatments	Dose (µl)	EAG Response (mV) (Mean±SEm)	
			Male	Female
1	Diphenylamine 1-Hexadecene Hexadecane (0.5:10:20)	10	0.821±0.103	0.683±0.144
2	Diphenylamine 1-Hexadecene Hexadecane (0.5:10:20)	20	0.953±0.139	0.657±0.159
3	Diphenylamine 1-Hexadecene Hexadecane (0.5:10:20)	40	0.986±0.189	0.670±0.147
4	Diphenylamine 1-Hexadecene Hexadecane (0.5:10:20)	80	1.018±0.136	0.711±0.126
5	Diphenylamine 1-Hexadecene Hexadecane (0.5:10:20)	100	1.123±0.105	0.752±0.075
DF			4, 20	4, 20
F test			0.630	0.079
P value			0.647^{NS}	0.988^{NS}

NS= Non-significant, N=5

4.3.1.2 Y-tube olfactometer studies

Dual choice Y-tube olfactometer studies were conducted to determine the Behavioural responses of *O. longicollis* males and females against volatiles of banana pseudostem weevil, synthetic pheromone compounds, their blend combinations, different doses of single pheromone compounds and blends.

The Behavioural responses of *O. longicollis* males to different volatiles of BSW weevils are presented in Table 15 and the percentage responding males are presented in Figure 36. The results indicated that significantly higher number of males were attracted towards the male volatile (χ^2=11.842, df=1, p=0.001, 17/2), male + female volatile (χ^2=7.143, df=1, p=0.008, 12/2) and female volatile (χ^2=5.333, df=1, p=0.021, 10/2) compared to control (Table 15 and Figure 36).

The responses of *O. longicollis* females towards different volatiles of BSW weevils are presented in Table 16 and the percentage responding females are presented in Figure 37. The results indicated that, significantly higher number of females was attracted towards the male volatile and female volatile compared to control. The female attraction towards the male + female volatile was not significantly different (p<0.05) from the control (Table 16 and Figure 37).

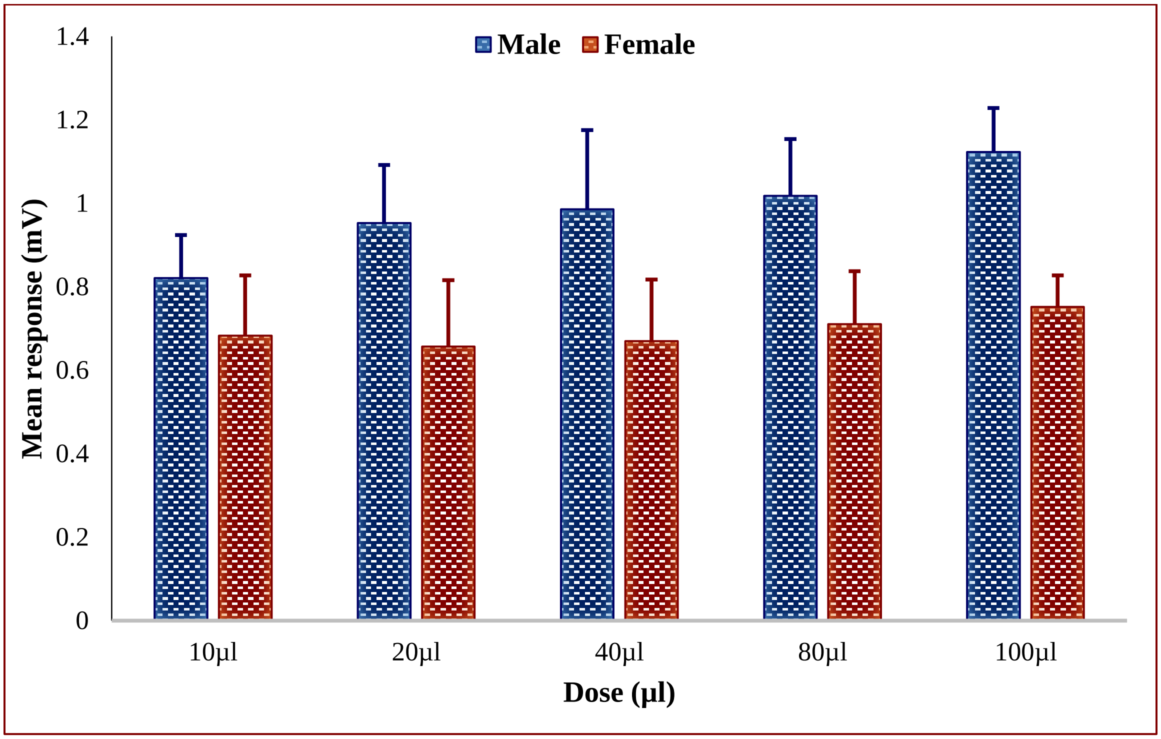


Fig. 35. **EAG response (Mean ± SEm) of BSW male and female antennae to increasing doses of best performing synthetic blend of Diphenylamine, 1-Hexadecene and Hexadecane. N=5, Error bars indicate ± SE**

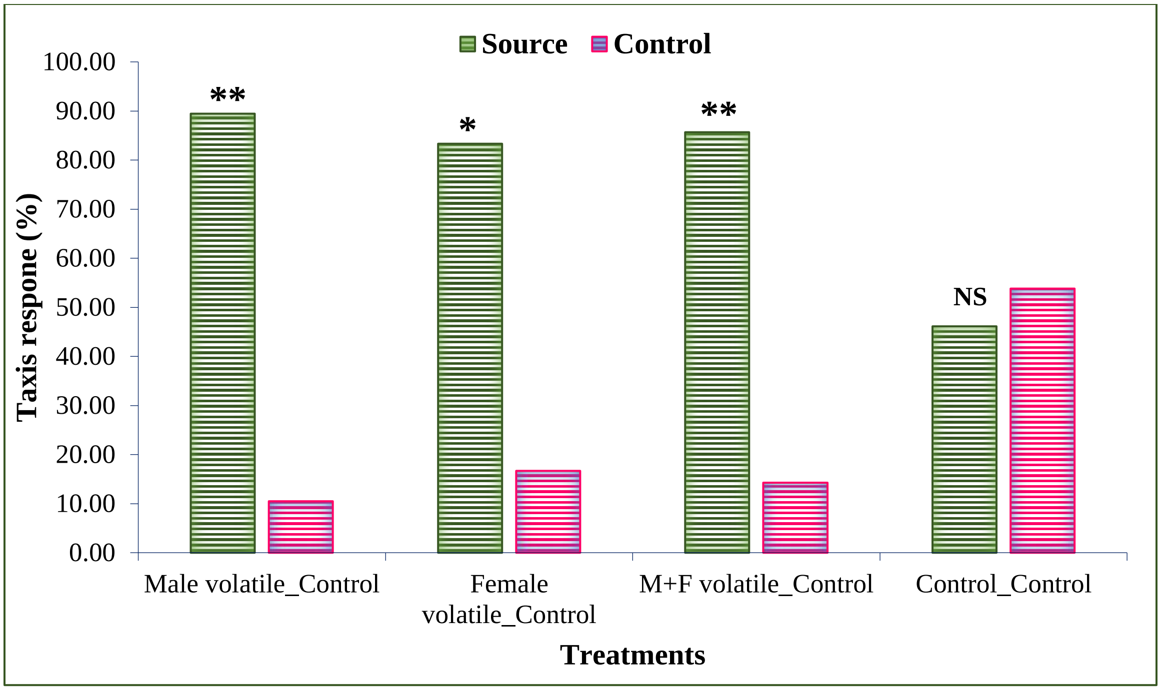


Fig. 36. Taxis response of *O. longicollis* male to different volatiles of BSW weevils in Y tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$ and ** at $p<0.01$, NS= non-significant different

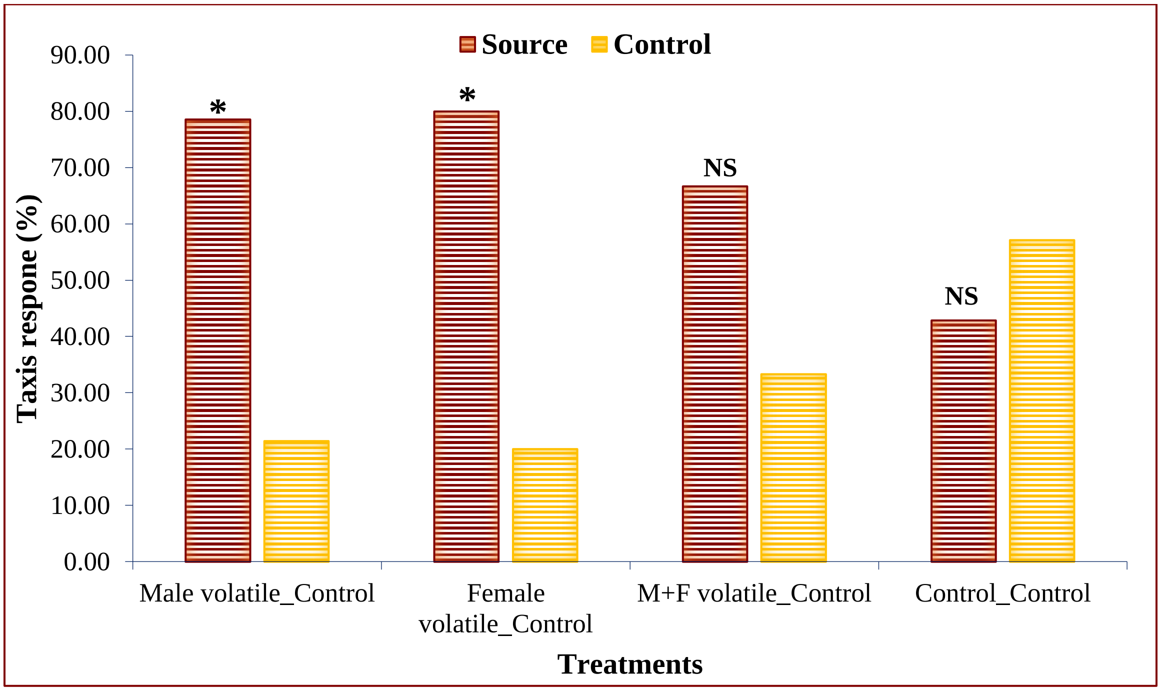


Fig. 37. Taxis response of *O. longicollis* female to different volatiles of BSW weevils in Y tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$, NS= non-significant different

Table 15. Behavioural responses of *O. longicollis* males to different volatiles of BSW weevils

Treatments	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Male volatile vs. Control	17	2	11.842	19	0.001	**
Female volatile vs. Control	10	2	5.333	12	0.021	*
M+F volatile vs. Control	12	2	7.143	14	0.008	**
Control vs. Control	6	7	0.077	13	0.782	NS

** Response between source and control is significantly different at p<0.05, ** at p<0.01, NS= non-significant different. ‡ n= 21, df=1*

Table 16. Behavioural responses of *O. longicollis* females to different volatiles of BSW weevils

Treatments	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Male volatile vs. Control	11	3	4.571	14	0.033	*
Female volatile vs. Control	12	3	5.400	15	0.020	*
M+F volatile vs. Control	12	6	2.000	18	0.157	NS
Control vs. Control	6	8	0.286	14	0.593	NS

**Response between source and control is significantly different at p<0.05, NS= non-significant different. ‡ n= 21, df=1*

The responses of *O. longicollis* males to synthetic pheromone compounds and the percentage responding males are presented in Table 17 and Figure 38. The results indicated that male weevils were highly attracted to the male volatile (χ^2=11.842, df=1, p=0.001, 17/2) than the control. The responses of males towards the synthetic Diphenylamine (χ^2=2.882, df=1, p=0.090,12/5), 1-Hexadecene (χ^2=1.471, df=1, p=0.225,11/6), Hexadecane (χ^2=2.250, df=1, p=0.134,11/5) and 2-methyl 4-heptanol (χ^2=0.600, df=1, p=0.439,9/6) were not significantly different over respective controls (Table 17 and Figure 38).

The responses of *O. longicollis* females to synthetic pheromone compounds and the percentage responding females are presented in Table 18 and Figure 39. Significantly higher number of females were attracted to male volatiles compared to control. The responses of females towards the synthetic Diphenylamine (χ^2=1.667, df=1, p=0.197,10/5), 1-Hexadecene (χ^2=2.571, df=1, p=0.109,10/4), Hexadecane (χ^2=2.000, df=1, p=0.157,12/6) and 2-methyl 4-heptanol (χ^2=0.286, df=1, p=0.593,6/8) were not statistically different over respective controls (Table 18 and Figure 39).

Table 17. Behavioural responses of *O. longicollis* males to synthetic components of male pheromone

Treatments	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Diphenylamine (DPA) vs. Control	12	5	2.882	17	0.090	NS
1-Hexadecene (1-16C) vs. Control	11	6	1.471	17	0.225	NS
Hexadecane (16C) vs. Control	11	5	2.250	16	0.134	NS
2-methyl 4-heptanol vs. Control	9	6	0.600	15	0.439	NS
Male IVC vs. Control	17	2	11.842	19	0.001	*

** Response between source and control is significantly different at $p<0.05$, NS- non-significant different. ‡n= 21, df=1*

Table 18. Behavioural responses of *O. longicollis* females to synthetic components of male pheromone

Treatments	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Diphenylamine (DPA) vs. Control	10	5	1.667	15	0.197	NS
1-Hexadecene (1-16C) vs. Control	10	4	2.571	14	0.109	NS
Hexadecane (16C) vs. Control	12	6	2.000	18	0.157	NS
2-methyl 4-heptanol vs. Control	6	8	0.286	14	0.593	NS
Male IVC vs. Control	11	3	4.571	14	0.033	*

**Response between source and control is significantly different at $p<0.05$, NS- non-significant different. ‡n= 21, df=1*

4.3.1.2.1 Dose-response studies of synthetic pheromone compounds

The responses of *O. longicollis* males to different doses of synthetic Diphenylamine and the percentage responding males are presented in Table 19 and

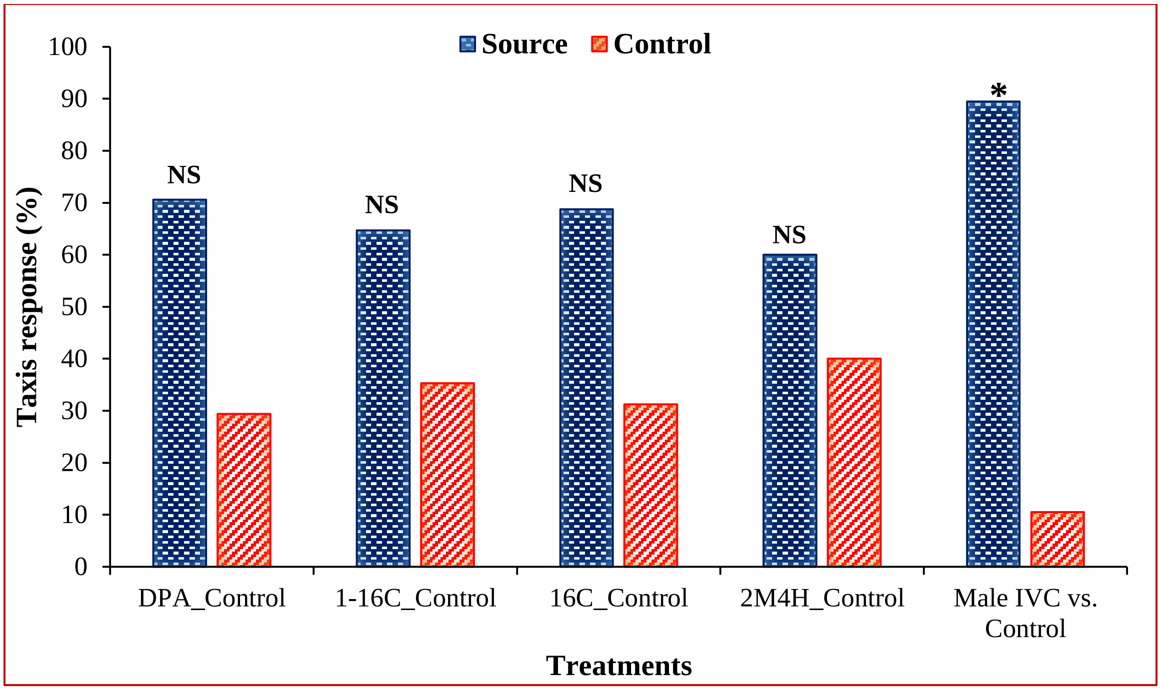


Fig. 38. Taxis response of *O. longicollis* males to synthetic components of male pheromone in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at p<0.05, NS= non-significant different

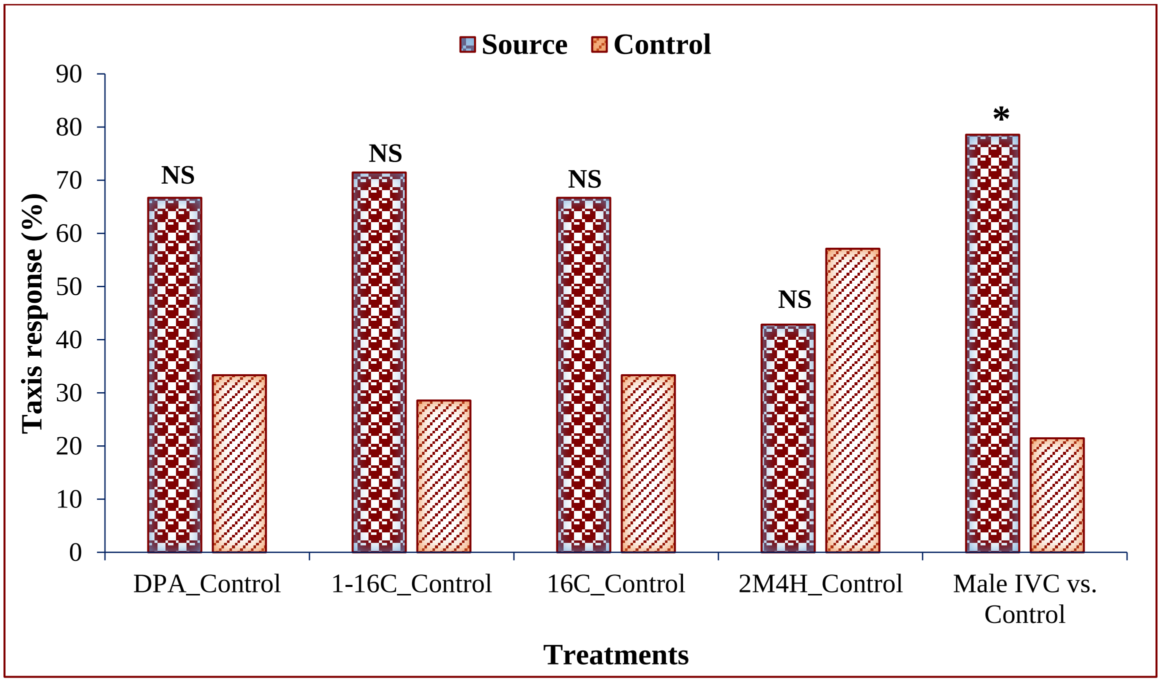


Fig. 39. Taxis response of *O. longicollis* females to synthetic components of male pheromone in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at p<0.05, NS= non-significant different

Figure 40. The results indicated that significantly higher number of males responded to 0.5µl of Diphenylamine (χ^2=5.400, df=1, p=0.020, 12/3) over the respective control. The number of responding males decreased with increased doses of Diphenylamine. The responses of males to 5µl (χ^2=2.000, df=1, p=0.157, 12/6), 10µl (χ^2=2.882, df=1, p=0.090,12/5) and 20µl of Diphenylamine (χ^2=2.273, df=1, p=0.132,8/3) were not significantly different over respective control (Table 19 and Figure 40).

Table 19. Behavioural responses of *O. longicollis* males to doses of Diphenylamine

Doses (µl)	**Number of response**		**Chi square value**	**‡Responded individual (N)**	**P**	
	Source	**Control**				
DPA_0.5 µl vs. Control	12	3	5.400	15	0.020	*
DPA_5 µl vs. Control	12	6	2.000	18	0.157	NS
DPA_10 µl vs. Control	12	5	2.882	17	0.090	NS
DPA_20 µl vs. Control	8	3	2.273	11	0.132	NS

**Response between source and control is significantly different at p<0.05, NS- non-significant different, ‡n= 21, df=1*

The responses of *O. longicollis* females to different doses of synthetic Diphenylamine and the percentage responding females are presented in Table 20 and Figure 41. The number of responding females decreased with increased doses of Diphenylamine. The responses of females to 0.5 µl (χ^2=3.267, df=1, p=0.071,11/4), 5 µl (χ^2=2.882, df=1, p=0.090,12/5), 10 µl (χ^2=1.667, df=1, p=0.197,10/5) and 20 µl of Diphenylamine (χ^2=1.667, df=1, p=0.197,10/5) were not significantly different over their respective control (Table 20). The females showed higher percent response to 0.5 µl and 5 µl followed by 10 µl and 20 µl (Figure 41).

Table 20. Behavioural responses of *O. longicollis* females to doses of Diphenylamine

Doses (µl)	**Number of response**		**Chi square value**	**‡Responded individual (N)**	**P**	
	Source	**Control**				
DPA_0.5 µl vs. Control	11	4	3.267	15	0.071	NS
DPA_5 µl vs. Control	12	5	2.882	17	0.090	NS
DPA_10 µl vs. Control	10	5	1.667	15	0.197	NS
DPA_20 µl vs. Control	10	5	1.667	15	0.197	NS

NS= Non-significant different, ‡ n= 21, df=1

The responses of *O. longicollis* males to different doses of synthetic 1-Hexadecene are presented in Table 21 and the percentage responding males are presented in Figure 42. The responses of males to 10 µl (χ^2=3.000, df=1, p=0.083,9/3), 20 µl (χ^2=1.471, df=1, p=0.225,11/6), and 40 µl (χ^2=1.667, df=1, p=0.197,10/5), were not statistically different from the control (Table 21). The number of responding males decreased with increased doses of 1-Hexadecene. The males showed higher percentage of response to 10 µl of 1-Hexadecene and relatively lower response to 40 µl and 20 µl (Figure 42).

Table 21. Behavioural responses of *O. longicollis* males to doses of 1-Hexadecene

Doses (µl)	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
1-Hexadecene _10 µl vs. Control	9	3	3.000	12	0.083	NS
1-Hexadecene _20 µl vs. Control	11	6	1.471	17	0.225	NS
1-Hexadecene _40 µl vs. Control	10	5	1.667	15	0.197	NS

NS= Non-significant different, ‡ n= 21, df=1

The percentage of responding females to different doses of synthetic 1-Hexadecene are presented in Table 22 and Figure 43. The results revealed that, significantly higher number of females responded to 10 µl of 1-Hexadecene (χ^2=4.571, df=1, p=0.033, 11/3) over the respective control. The responses of females to 20 µl (χ^2=2.571, df=1, p=0.109, 10/4) and 40 µl of 1-Hexadecene (χ^2=0.600, df=1, p=0.439,9/6) were not statistically different from the control (Table 22). The number of responding females decreased with increased doses of 1-Hexadecene. Female weevils showed higher response to the 10 µl followed by 20 µl and 40 µl (Figure 43).

Table 22. Behavioural responses of *O. longicollis* females to doses of 1-Hexadecene

Doses (µl)	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
1-Hexadecene _10 µl vs. Control	11	3	4.571	14	0.033	*
1-Hexadecene _20 µl vs. Control	10	4	2.571	14	0.109	NS
1-Hexadecene _40 µl vs. Control	9	6	0.600	15	0.439	NS

**Response between source and control is significantly different at p<0.05, NS= Non-significant different, ‡n= 21, df=1*

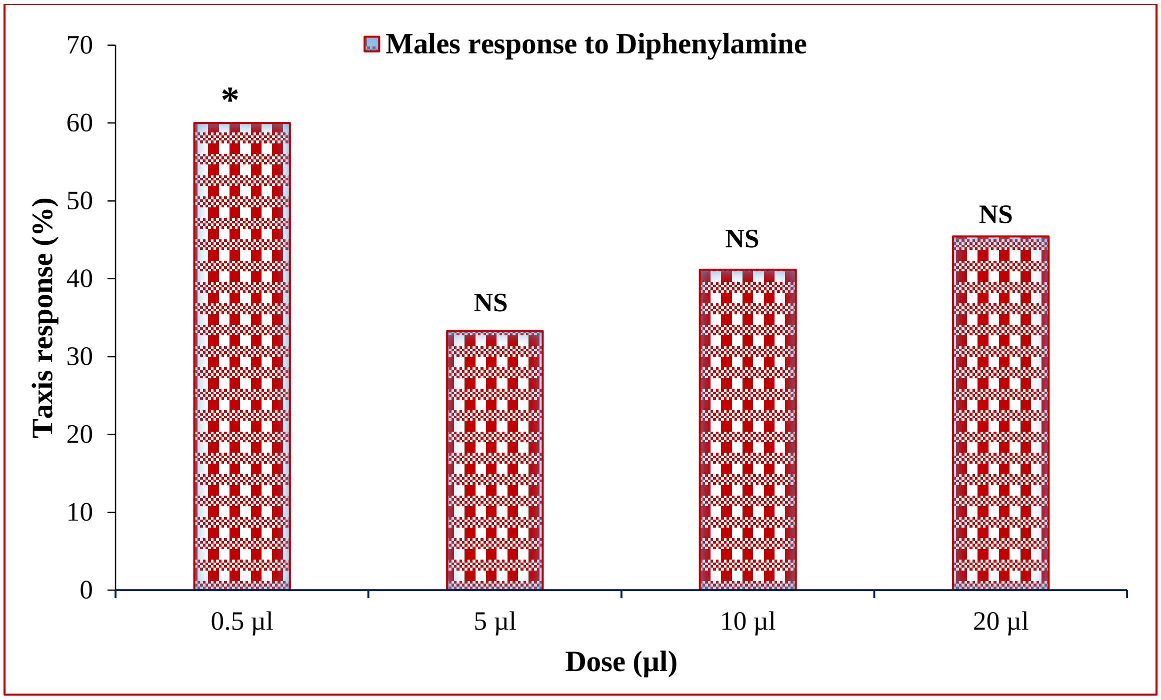


Fig. 40. Taxis response of *O. longicollis* males to doses of Diphenylamine in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at p<0.05, NS= non-significant different

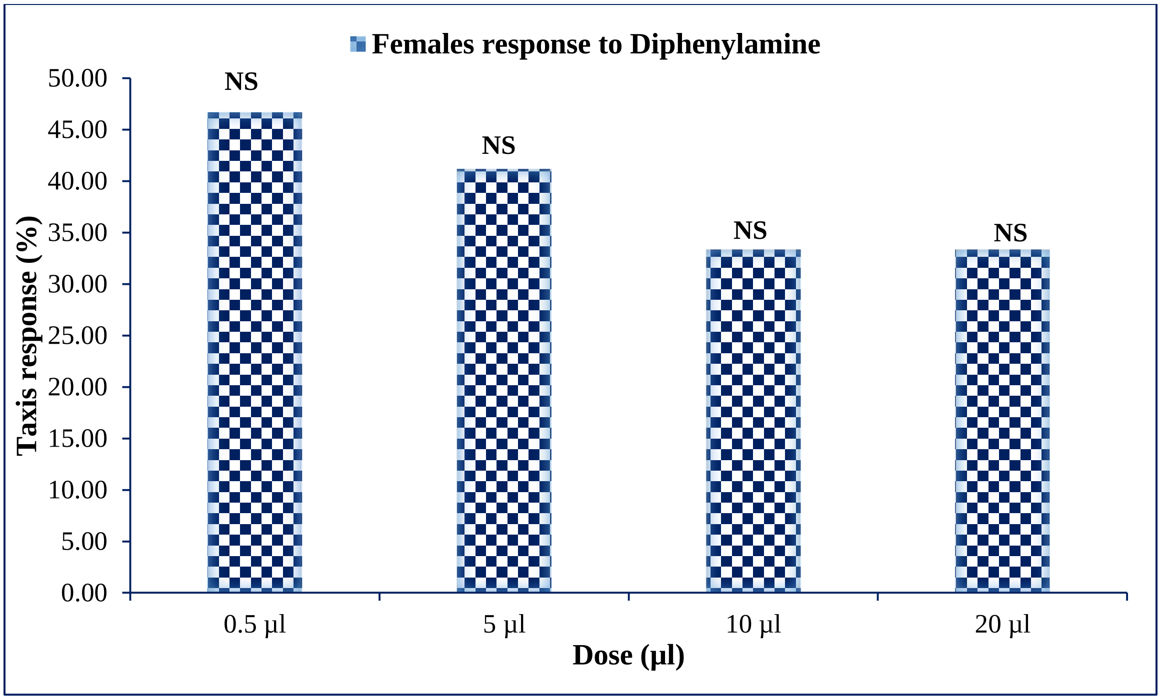


Fig. 41. Taxis response of *O. longicollis* females to doses of Diphenylamine in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at p<0.05, NS= non-significant different

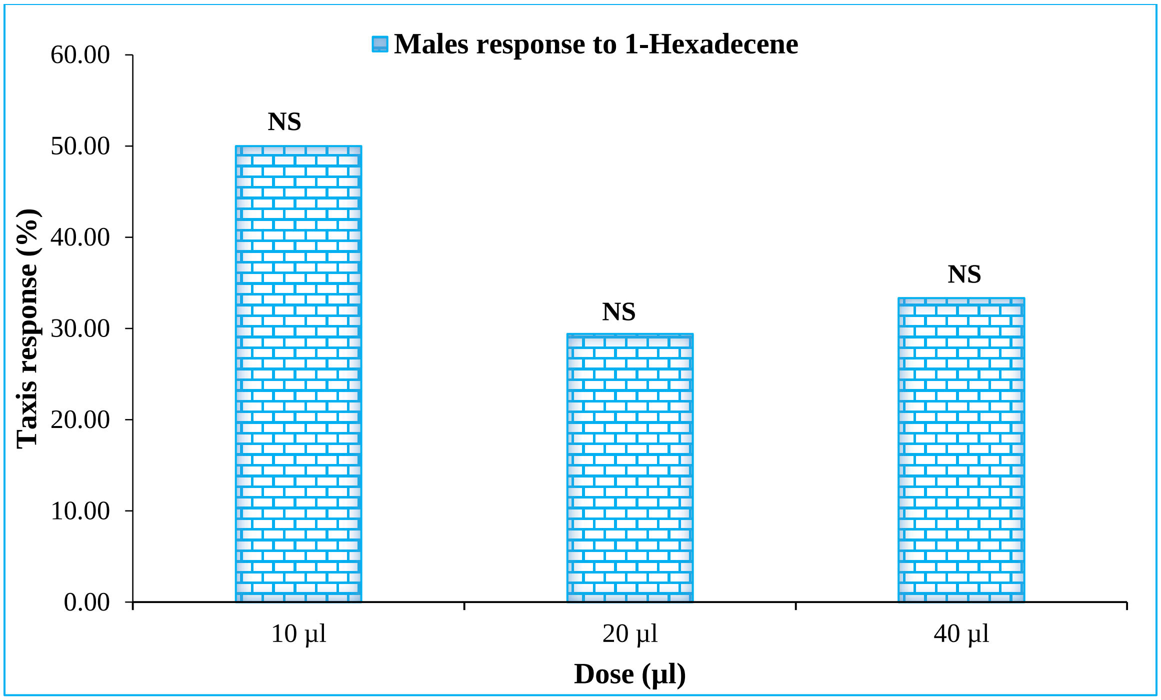


Fig. 42. Taxis response of *O. longicollis* males to doses of 1-Hexadecene in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at p<0.05, NS= non-significant different

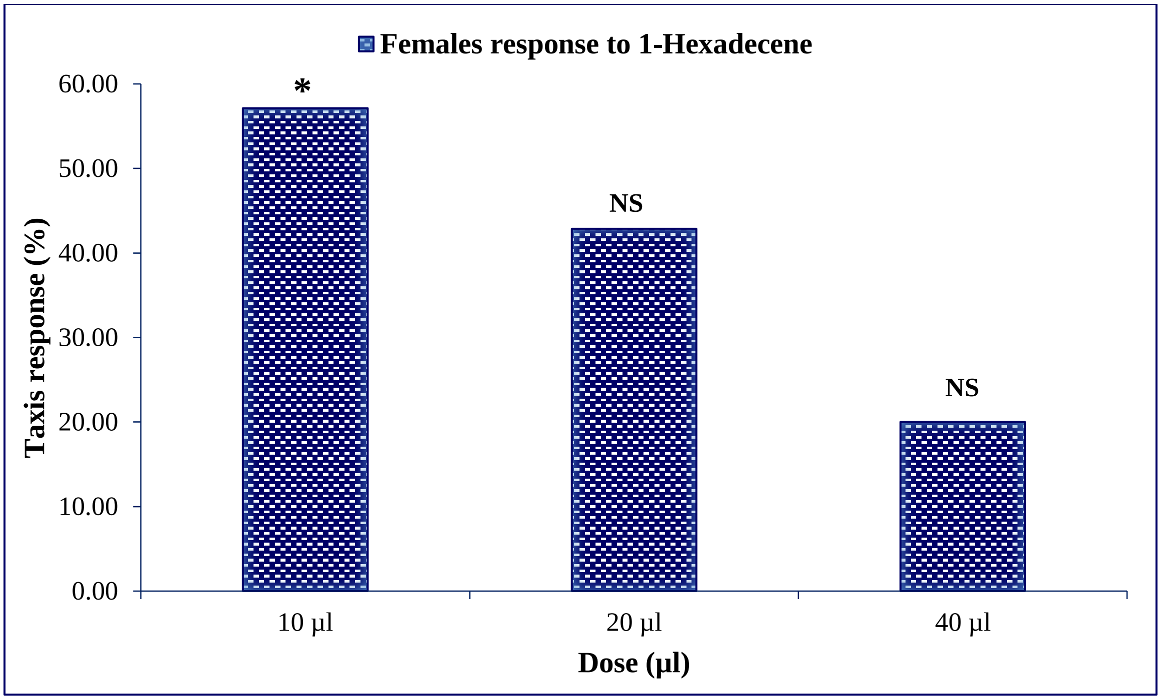


Fig. 43. Taxis response of *O. longicollis* females to doses of 1-Hexadecene in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at p<0.05, NS= non-significant different

The responses of *O. longicollis* males to different doses of synthetic Hexadecane are presented in Table 23 and the percentage responding males are presented in Figure 44. The responses of males to 10 µl (χ^2=0.286, df=1, p=0.593,8/6), 20 µl (χ^2=2.250, df=1, p=0.134,11/5), and 40 µl (χ^2=1.143, df=1, p=0.285,9/5) were not statistically different from the control (Table 23). The number of responding males increased with increased doses and then decreased with increased dose of Hexadecane. Males showed more response to the 20 µl of Hexadecane followed by 40 µl and 20 µl (Figure 44).

Table 23. Behavioural responses of *O. longicollis* males to doses of Hexadecane

Doses (µl)	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Hexadecane _10 µl vs. Control	8	6	0.286	14	0.593	NS
Hexadecane _20 µl vs. Control	11	5	2.250	16	0.134	NS
Hexadecane_40 µl vs. Control	9	5	1.143	14	0.285	NS

NS= Non-significant different, ‡ n= 21, df=1

The responses of *O. longicollis* females to different doses of synthetic Hexadecane and the percentage responding males are presented in Table 24 and Figure 45. The responses of females to 10 µl (χ^2=0.600, df=1, p=0.439,9/6), 20 µl (χ^2=2.000, df=1, p=0.157,12/6), and 40 µl (χ^2=1.667, df=1, p=0.197,10/5) were not significantly different from the control (Table 24). The number of responding males increased with increased doses and then decreased with increased dose of Hexadecane. Females showed more response to the 20 µl of Hexadecane followed by 40 µl and 20 µl (Figure 45).

Table 24. Behavioural responses of *O. longicollis* females to doses of Hexadecane

Doses (µl)	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Hexadecane _10 µl vs. Control	9	6	0.600	15	0.439	NS
Hexadecane _20 µl vs. Control	12	6	2.000	18	0.157	NS
Hexadecane_40 µl vs. Control	10	5	1.667	15	0.197	NS

NS= Non-significant different, ‡ n= 21, df=1

4.3.1.2.2 Olfactometer studies of Diphenylamine, 1-Hexadecene and Hexadecane in combinations

The Behavioural responses of *O. longicollis* males to different blends of synthetic pheromone compounds are presented in Table 25 and the percentage responding males are presented in Figure 46. The results revealed that significantly higher number of males were attracted to the blend of DPA: 1-16C:16C at 0.5:10:20 (χ^2=4.263, df=1, p=0.039, 14/5) compared to control. The male responses to other blend combinations were not significantly different over respective controls (Table 25). The blend combination of DPA: 1-16C:16C and DPA: 1-16C at 100:8:5 (χ^2=2.571, df=1, p=0.109, 10/4) and 100:8 (χ^2=2.000, df=1, p=0.157,12/6) attracted more number of males followed by 1-16C:16C (8:5) and DPA: 16C (100:5) and are not statistically different (Figure 46).

Table 25. Behavioural responses of *O. longicollis* males to blends of synthetic pheromone

Blend components	Ratio of components	Number of response		Chi square value	‡Responded individual (N)	P	
		Source	Control				
Diphenylamine: 1-Hexadecene: Hexadecane	100:8:5	10	4	2.571	14	0.109	NS
Diphenylamine: 1-Hexadecene	100:8	12	6	2.000	18	0.157	NS
Diphenylamine: Hexadecane	100:5	10	9	0.053	19	0.819	NS
1-Hexadecene: Hexadecane	8:5	10	8	0.222	18	0.637	NS
Diphenylamine: 1-Hexadecene: Hexadecane	0.5:10:20	14	5	4.263	19	0.039	*

**Response between source and control is significantly different at p<0.05, NS= Non-significant different, ‡ n= 21, df=1*

The responses of *O. longicollis* females to different blends of synthetic pheromone compounds and the percentage responding females are presented in Table 26 and Figure 47. The results revealed that significantly higher number of females were attracted to the 0.5:10:20 (DPA: 1-16C:16C) (χ^2=4.000, df=1, p=0.046,12/4) and 100:8 (DPA: 1-16C) (χ^2=4.571, df=1, p=0.033,11/3) blends compared to control followed by 100:8:5 (DPA: 1-16C:16C) (χ^2=3.769, df=1, p=0.052,10/3) blend. The responses of females to 8:5 (1-16C:16C) and 100:5 (DPA: 16C) blends were not significantly different from respective controls (Table 26 and Figure 47).

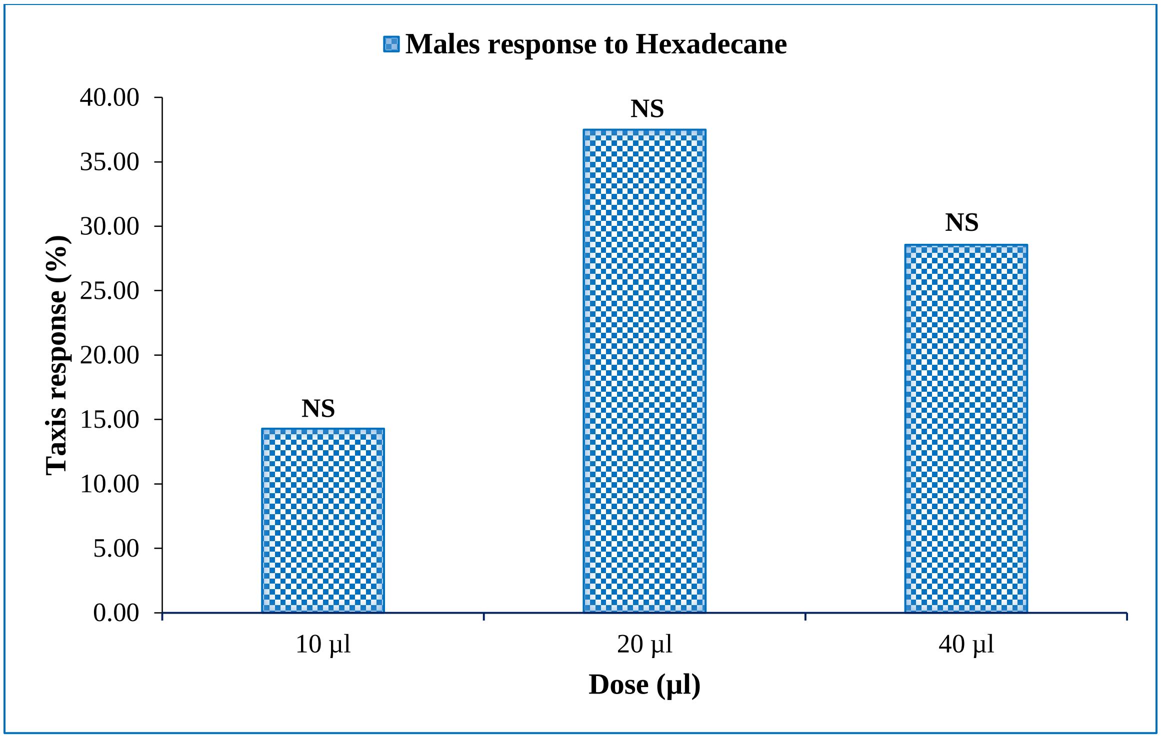


Fig. 44. Taxis response of *O. longicollis* males to doses of Hexadecane in a Y-tube olfactometer. N=21, NS= non-significant different

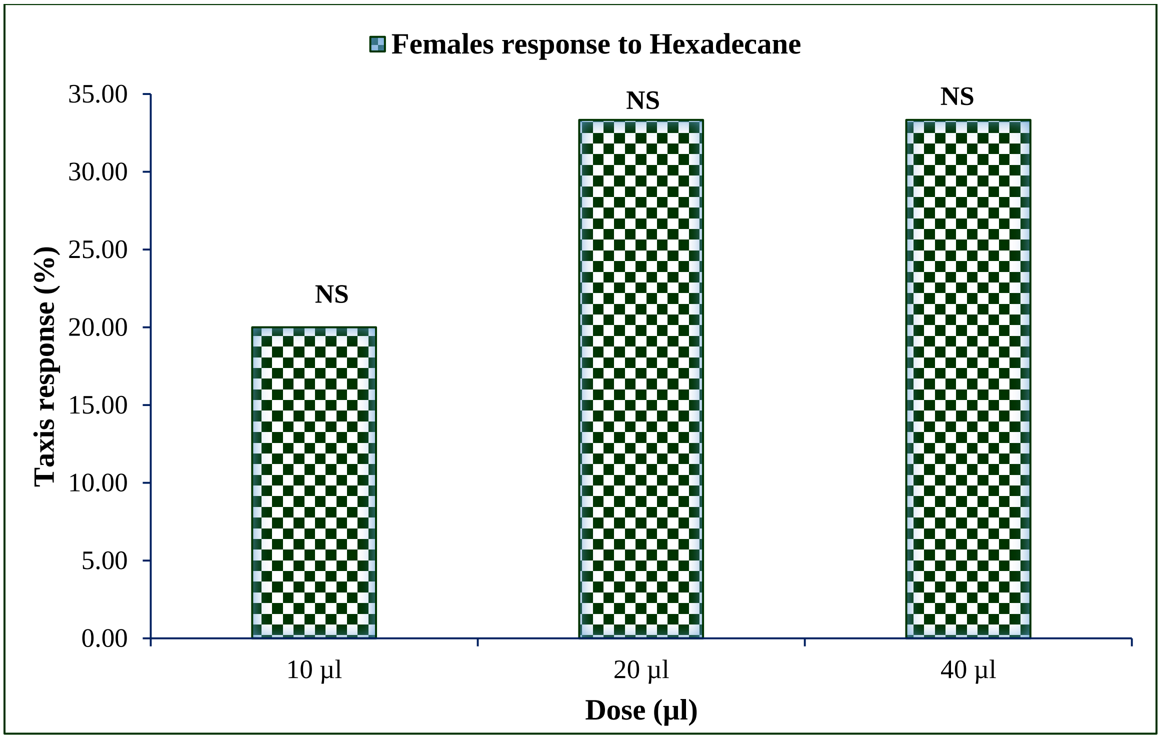


Fig. 45. Taxis response of *O. longicollis* females to doses of Hexadecane in a Y-tube olfactometer. N=21, NS= non-significant different

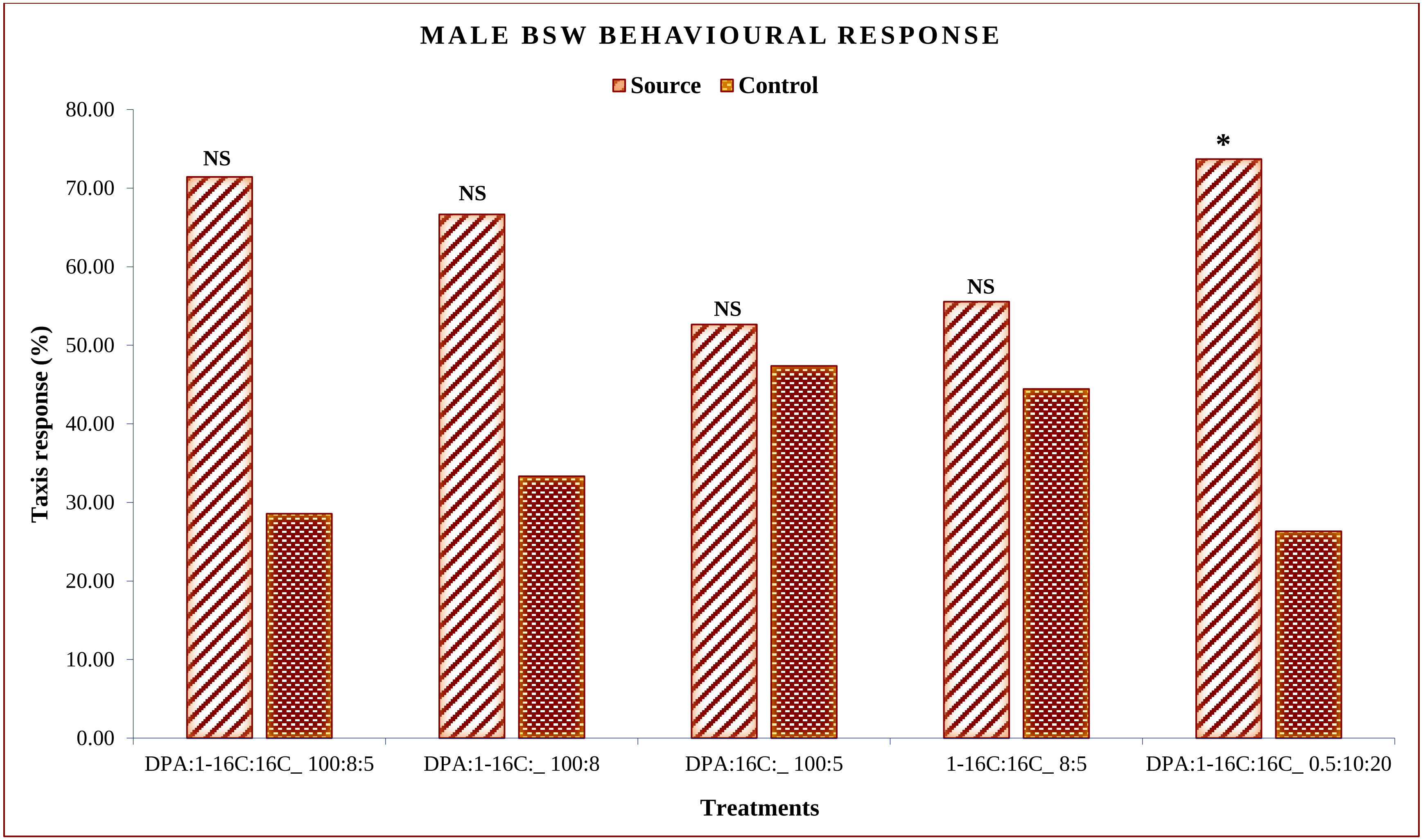


Fig. 46. Taxis response of *O. longicollis* males to blends of synthetic components of male pheromone in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$, NS= non-significant different

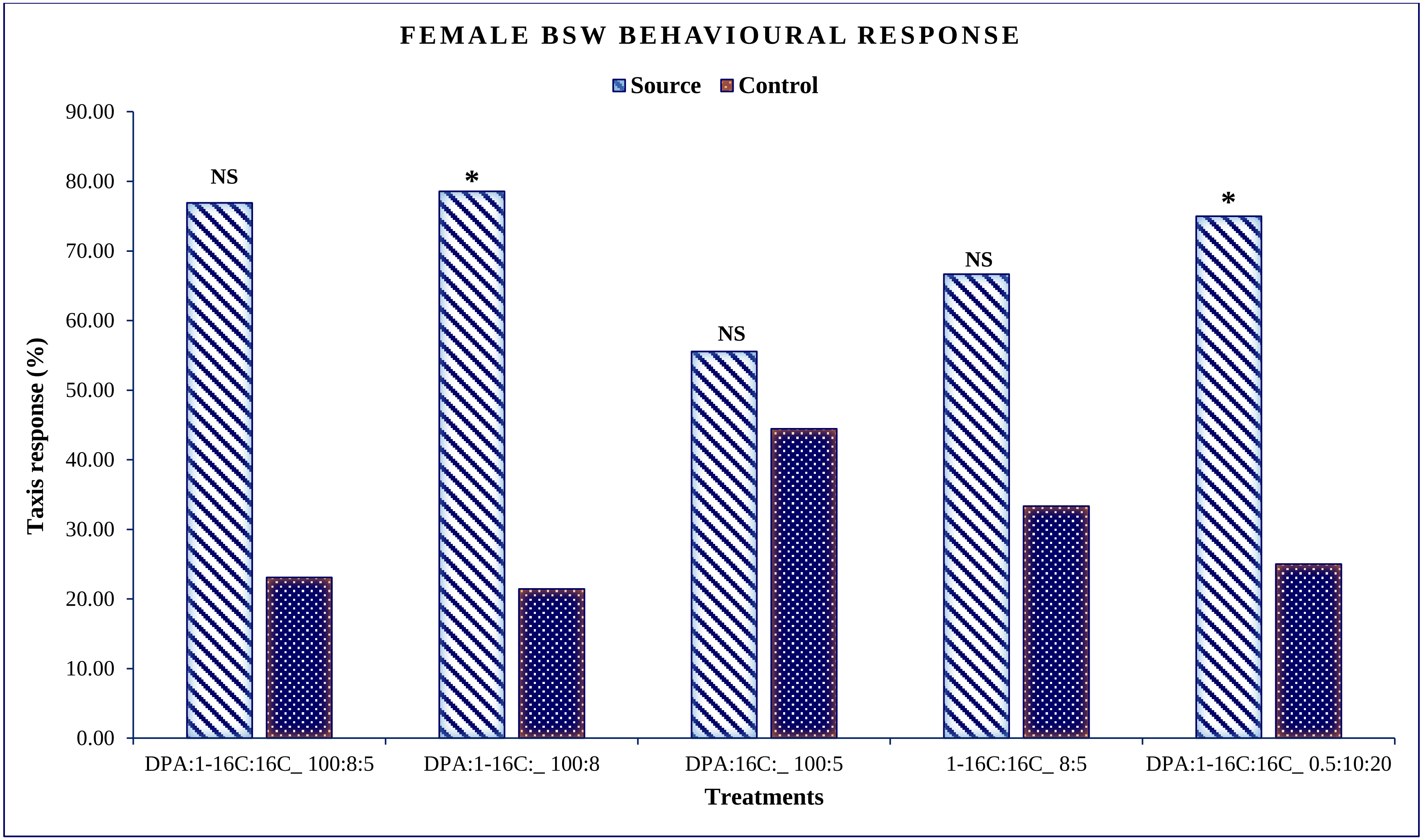


Fig. 47. Taxis response of *O. longicollis* females to blends of synthetic components of male pheromone in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$, NS= non-significant different

Table 26. Behavioural responses of *O. longicollis* females to blends of synthetic pheromone

Blend components	Ratio of components	Number of response		Chi square value	‡Responded individual (N)	P	
		Source	Control				
Diphenylamine: 1-Hexadecene: Hexadecane	100:8:5	10	3	3.769	13	0.052	NS
Diphenylamine: 1-Hexadecene	100:8	11	3	4.571	14	0.033	*
Diphenylamine: Hexadecane	100:5	10	8	0.222	18	0.637	NS
1-Hexadecene: Hexadecane	8:5	10	5	1.667	15	0.197	NS
Diphenylamine: 1-Hexadecene: Hexadecane	0.5:10:20	12	4	4.000	16	0.046	*

**Response between source and control is significantly different at p<0.05, NS= Non-significant different, ‡ n= 21, df=1*

4.3.1.2.3 Dose variation response of best performed blend

The Behavioural responses of *O. longicollis* males to different doses of best performing blend of synthetic pheromone compounds and the percentage responding males are presented in Table 27 and Figure 48. The number of responding males increased with increased doses of the synthetic blend. The results revealed that significantly higher number of males were attracted to 100 µl (χ^2=9.000, df=1, p=0.003, 14/2) and 80 µl (χ^2=6.250, df=1, p=0.012, 13/3) of the blend over the respective control. The responses of males to 40 µl (χ^2=3.267, df=1, p=0.071, 11/4), 20 µl (χ^2=1.923, df=1, p=0.166, 9/4) and 10 µl (χ^2=1.667, df=1, p=0.197, 10/5) were not significantly different from control (Table 27 and Figure 48).

The responses of *O. longicollis* females to different doses of best performing blend of synthetic pheromone compounds are presented in Table 28 and the percentage responding males in Figure 49. The number of responding males increased with increased doses of the synthetic blend. The results revealed that significantly higher number of males were attracted to 100 µl (χ^2=4.765, df=1, p=0.029, 13/4) and 80 µl (χ^2=4.000, df=1, p=0.046, 12/4) of the blend over the respective control. The responses of males to 40 µl (χ^2=3.267, df=1, p=0.071, 11/4), 20 µl (χ^2=1.000, df=1, p=0.317, 10/6) and 10 µl (χ^2=0.889, df=1, p=0.346, 11/7) were not significantly different from the control (Table 28 and Figure 49).

Table 27. Behavioural responses of *O. longicollis* males to doses of best performing blend of synthetic pheromone

Blend components	Doses (µl)	Number of response		Chi square value	‡Responded individual (N)	P	
		Source	Control				
DPA:1-16C:16C (0.5:10:20)	10	10	5	1.667	15	0.197	NS
DPA:1-16C:16C (0.5:10:20)	20	9	4	1.923	13	0.166	NS
DPA:1-16C:16C (0.5:10:20)	40	11	4	3.267	15	0.071	NS
DPA:1-16C:16C (0.5:10:20)	80	13	3	6.250	16	0.012	*
DPA:1-16C:16C (0.5:10:20)	100	14	2	9.000	16	0.003	**

**Response between source and control is significantly different at p<0.05 and ** at p<0.01, NS= Non-significant different, ‡ n= 21, df=1*

Table 28. Behavioural responses of *O. longicollis* females to doses of best performing blend of synthetic pheromone

Blend components	Doses (µl)	Number of response		Chi square value	‡Responded individual (N)	P	
		Source	Control				
DPA:1-16C:16C (0.5:10:20)	10	11	7	0.889	18	0.346	NS
DPA:1-16C:16C (0.5:10:20)	20	10	6	1.000	16	0.317	NS
DPA:1-16C:16C (0.5:10:20)	40	11	4	3.267	15	0.071	NS
DPA:1-16C:16C (0.5:10:20)	80	12	4	4.000	16	0.046	*
DPA:1-16C:16C (0.5:10:20)	100	13	4	4.765	17	0.029	*

**Response between source and control is significantly different at p<0.05, NS= Non-significant different, ‡n= 21, df=1*

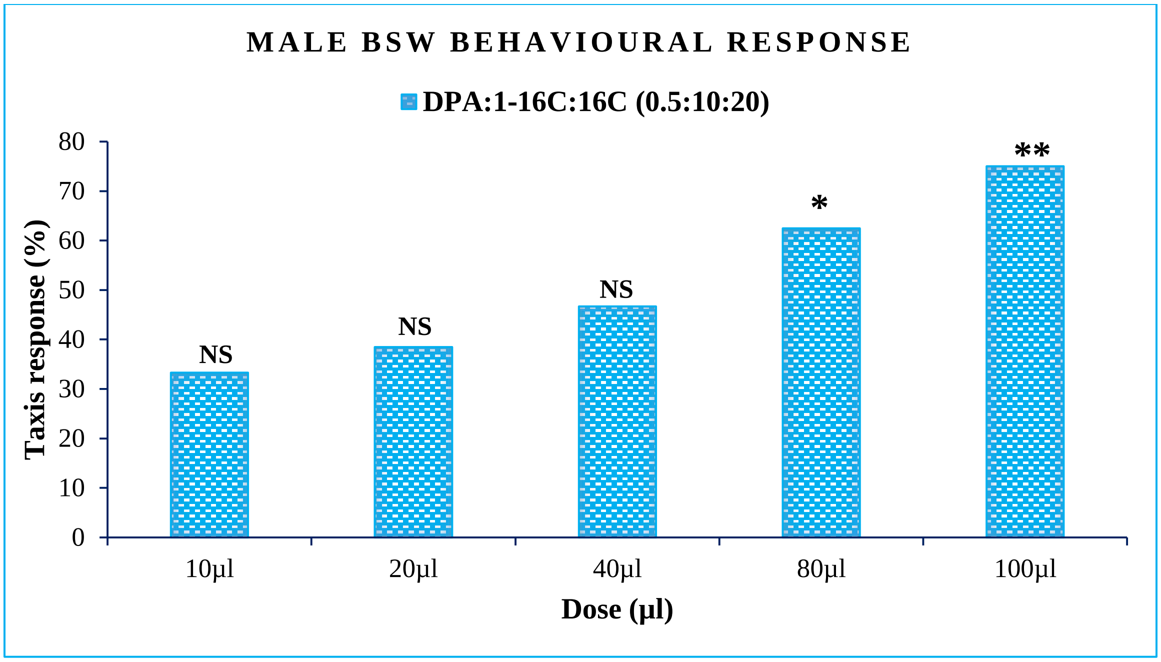


Fig. 48. **Taxis response of *O. longicollis* males to increasing doses of best performing blend of synthetic male pheromone components in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$ and **$p<0.01$, NS= non-significant different**

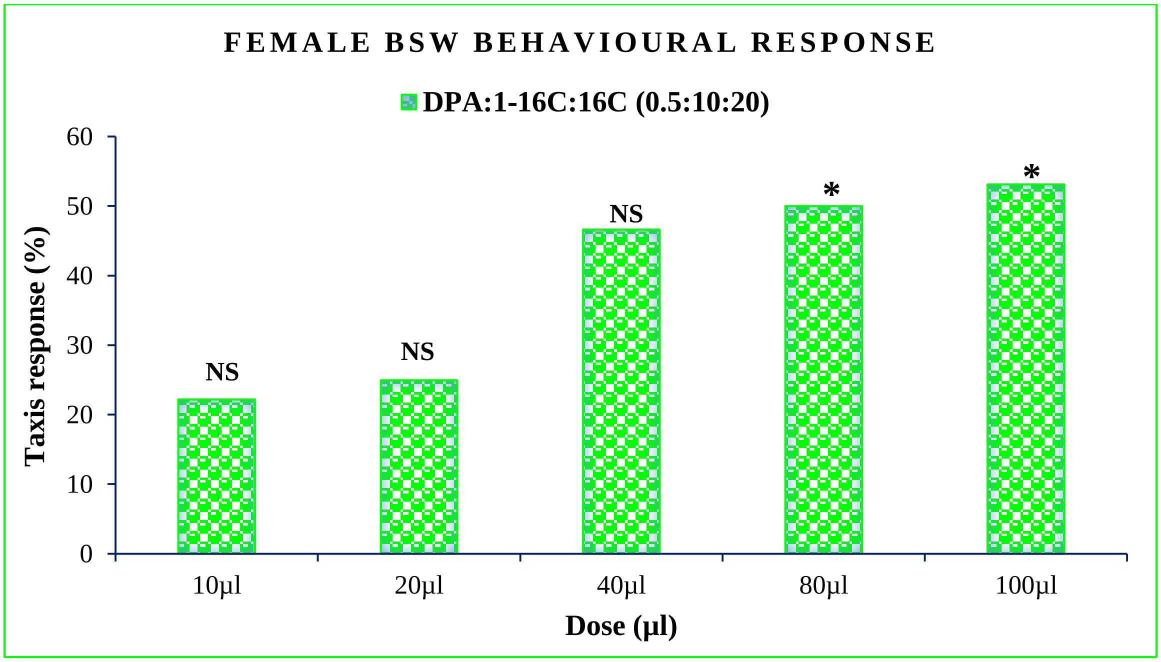


Fig. 49. **Taxis response of *O. longicollis* females to increasing doses of best performing blend of synthetic male pheromone components in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$ NS= non-significant different**

The results of Y-tube olfactometer study indicated that the attraction of both sexes to male volatile and male weevil's attraction to M+F volatile indicated the presence of male produced aggregation pheromone in the species. Males were more attracted to the aggregation pheromone compared to female, suggesting that these compounds could be used as chemical cues primarily by males of the species to find an appropriate site for mating. The results of this study are in consistent with Prasuna *et al*., 2008, Ambrogi and Zarbin, 2008 and Palacio-Cortes *et al*., 2015. Parallel results were reported by Uzakah *et al*. (2015). They reported that, olfactory responses of *C. sordidus* to trapped adult volatiles revealed that males responded significantly to the trapped volatiles of both males and females. However, females responded significantly to only the trapped male volatiles and not to the trapped female volatiles.

The electrophysiological and Behavioural evaluation of the three synthetic compounds in the laboratory showed that they are biologically active. The synthetic compounds when presented individually are not attractive to both male and female BSW. Individual synthetic compounds when tested at different doses, DPA at 0.5 µl, 1-Hexadecene at 10 µl and Hexadecane at 20 µl elicited attraction Behaviour in both sexes. The results also showed that, the combination of all the three pheromone compounds are necessary for eliciting Behavioural response in both sexes. The blend of DPA: 1-16C :16C at 0.5:10:20 are highly attractive to both male and female BSW. Both EAG and Y-tube olfactometer studies confirmed that 0.5:10:20 (DPA:1-16C :16C) is the best performing blend and this blend evoked increased Behavioural responses with increased doses in both the sexes and they showed higher response at 100µl of synthetic blend. These results are in agreement with Poorjavad *et al.* (2009).

They tested different dosages of aggregation pheromone at 1, 10, 100 and 1000 ng/1 µl hexane on the diurnal responses of *R. ferrugineus*. The results showed that aggregation pheromone at 1000 ng elicited a significantly greater response of 1 ng, while the remaining doses yielded intermediate responses. Perez *et al.,* (1997) reported that in Costa Rica field experiments testing alcohols 3-pentanol (1), 2-methyl 4-heptanol (2), 2-methyl 4-octanol (3) and 4-methyl 5-nonanol (4) singly and in all binary, ternary, and quaternary combinations revealed 4 in combination with 2 was the major aggregation pheromone, equally attracting male and female West Indian sugarcane weevils (WISW), *Metamasius hemipterus sericeus* (Oliv.).

4.3.2 Bioassays of plant volatiles

Bioassay was carried out for screening plant volatiles to determine the effective host-plant volatile(s) for the attraction of *O. longicollis* adults and the results are presented in Tables 29, 30 and Figures 50, 51.

4.3.2.1 Electroantenography (EAG)

All the pseudostem volatiles elicited antennal response in both male and female antennae. Antennal responses of male *O. longicollis* to different pseudostem volatiles revealed that the responses were significantly different ($F_{4,\,20}$=4.652; P=0.008) (Table 29 and Figure 50). Among different extracts, healthy and BSW damaged pseudostem volatiles (1.187 ± 0.102 and 1.274 ± 0.102, respectively) elicited significantly higher response than control (0.730 ± 0.056) and are statistically on par. While volatiles of decaying and mechanically damaged pseudostem evoked lower antennal response (1.001 ± 0.092 and 1.058 ± 0.119, respectively) and these two were on par (Table 29 and Figure 50).

Table 29. EAG response (Mean ± SEm) of BSW male and female antennae against banana pseudostem extracts

Sl. No.	Treatments	EAG Response (mV) (Mean±SEm)	
		Male	Female
1	Healthy pseudostem extract	1.187 ± 0.102^{a}	0.578 ± 0.070^{abc}
2	BSW damaged pseudostem extract	1.274 ± 0.102^{a}	0.867 ± 0.080^{ab}
3	Decaying pseudostem extract	1.001 ± 0.092^{ab}	0.528 ± 0.096^{bc}
4	Mechanically damaged pseudostem extract	1.058 ± 0.119^{ab}	0.942 ± 0.116^{a}
5	Control (DCM)	0.730 ± 0.056^{b}	0.377 ± 0.081^{c}
DF		4, 20	4, 20
F test		4.652	6.984
P value		0.008**	0.001**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), ** Significant difference at p<0.01, N=5*

Mean EAG response of female *O. longicollis* to different pseudostem volatiles revealed that, all the volatiles elicited significantly higher antennal response ($F_{4, 20}$=6.984; P=0.001) than control (Table 29 and Figure 50). Among different pseudostem volatiles, mechanically damaged pseudostem volatiles (0.942 ± 0.116) elicited significantly higher EAG response. BSW damaged pseudostem volatiles (0.867 ± 0.080) elicited second higher response followed by healthy and decaying pseudostem volatiles (0.578 ± 0.070 and 0.528 ± 0.096, respectively). The lowest response was recorded for DCM (control) (Table 29 and Figure 50).

The EAG responses of *O. longicollis* male and female to different odor stimuli such as male volatile, healthy and mechanically damaged pseudostem volatiles and their combinations was conducted. The results were presented in Table 30 and Figure 51. The mean male antennal responses to the volatile compounds were significantly different ($F_{4, 24}$=5.285; P=0.002) than control. Male volatile and male volatile + mechanically damaged pseudostem volatile evoked significantly higher response (2.231 ± 0.468 and 2.231 ± 0.479, respectively) and these two were on par. Nevertheless, male volatile + healthy pseudostem volatile, healthy and mechanically damaged pseudostem volatiles were found on par with each other (Table 30 and Figure 51).

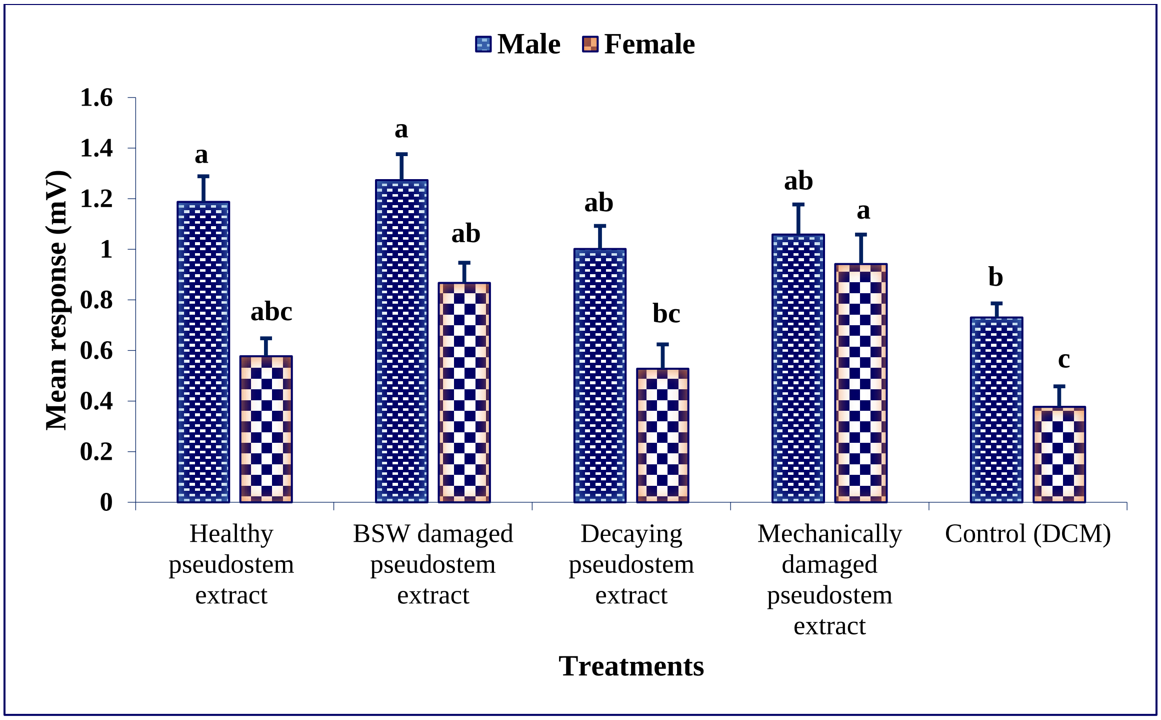


Fig. 50. EAG response (Mean ± SEm) of BSW male and female antennae against banana pseudostem extracts. N=5, Error bars indicate ± SE

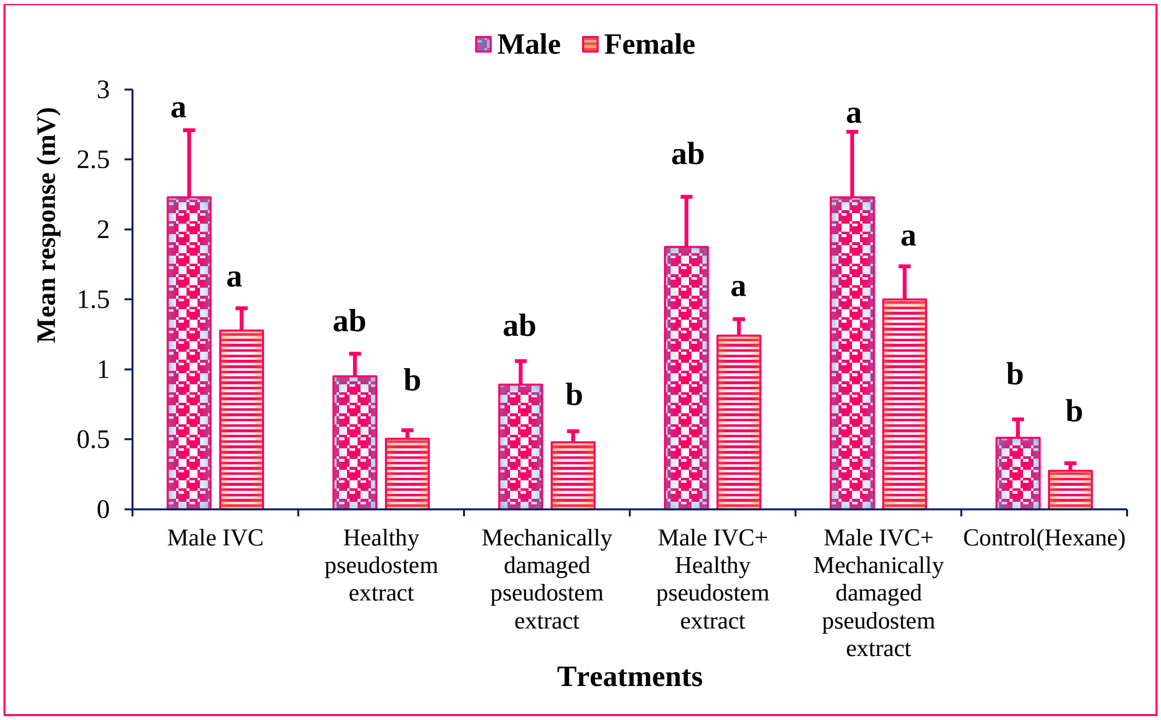


Fig. 51. EAG response (Mean ± SEm) of BSW male and female antennae against different odor stimuli from BSW male weevils and banana pseudostem. N=5, Error bars indicate ± SE

Table 30. EAG response (Mean ± SEm) of BSW male and female antennae against different odour stimuli

Sl. No.	Treatments	EAG Response (mV) (Mean±SEm)	
		Male	Female
1	Male IVC	2.231±0.479^{a}	1.279±0.157^{a}
2	Healthy pseudostem extract	0.952±0.159^{ab}	0.506±0.058^{b}
3	Mechanically damaged pseudostem extract	0.893±0.165^{ab}	0.481±0.077^{b}
4	Male IVC+ Healthy pseudostem extract	1.877±0.357^{ab}	1.242±0.118^{a}
5	Male IVC+ Mechanically damaged pseudostem extract	2.231±0.468^{a}	1.502±0.234^{a}
6	Control(Hexane)	0.511±0.131^{b}	0.277±0.051^{b}
DF		5, 24	5, 24
F test		5.285	15.251
P value		0.002**	0.0001**

*Values followed by same letters in each column are not significantly different (Tukey HSD post –hoc test), ** Significant difference at p<0.01, N=5*

Mean EAG response of female *O. longicollis* to different odor stimuli, revealed that there was a significant differences among the tested volatiles ($F_{4,\ 24}$=15.251; P=0.0001). Female antennal responses to male volatile, male volatile + mechanically damaged pseudostem and male volatile + healthy pseudostem volatiles were significantly higher than others (1.279 ± 0.157, 1.502 ± 0.234 and 1.242 ± 0.118, respectively) and they were found statistically on par with each other. The lower antennal response was elicited by healthy and mechanically damages pseudostem volatiles (0.506 ± 0.058 and 0.481 ± 0.077, respectively) and are found on par with control (Table 30 and Figure 51).

The EAG results of both male and female weevils showed that both sexes produced higher antennal response to male volatile and combination of male volatile with the plant extracts compared to plant extracts alone. These results are in agreement with Prasuna *et al*. (2008). Palanichamy *et al*. (2011a) reported that, all the odorous stimuli of banana pseudostem, extracted by different methods elicited higher EAGs in male compared to female, indicating that male perceived pheromones/kairomones may be involved in mate/host plant selection. The study also revealed that males showed more antennal activity than females.

4.3.2.2 Y-tube olfactometer studies

Dual choice Y-tube olfactometer studies were conducted to determine the Behavioural responses of male and female *O. longicollis* adults to different fractions of banana pseudostem extracts and combination of male volatile with pseudostem extracts.

The Behavioural responses of *O. longicollis* males to different fractions of banana pseudostem extracts and the percentage of responding males are presented in Table 31 and Figure 52. The results indicated that significantly higher number of males were attracted towards the healthy (χ2=7.118, df=1, p=0.008, 14/3) and mechanically damaged pseudostem exracts (χ2=8.067, df=1, p=0.005, 13/2) compared to control. The male responses towards decaying (χ2=3.267, df=1, p=0.071, 11/4) and BSW damaged pseudostem extracts (χ2=0.333, df=1, p=0.564, 7/5) were not statistically different from the respective control (Table 31 and Figure 52).

Table 31. Behavioural responses of *O. longicollis* males to different types of banana pseudostem volatiles

Treatments	**Number of response**		**Chi square value**	**‡Responded individual (N)**	**P**	
	Source	**Control**				
Healthy vs Blank	14	3	7.118	17	0.008	*
BSW damaged vs Blank	7	5	0.333	12	0.564	NS
Mechanically damaged vs blank	13	2	8.067	15	0.005	*
Decaying vs Blank	11	4	3.267	15	0.071	NS
Blank vs Blank	6	7	0.077	13	0.782	NS

** Response between source and control is significantly different at p<0.05, NS= Non-significant different.*
‡n= 21, df=1

Similarly, the responses of females to different volatiles of banana pseudostem extracts are presented in the percentage of responding females are presented in. The results indicated that, significantly more number of females were attracted towards the BSW damaged (χ2=7.143, df=1, p=0.008, 12/2), healthy (χ2=6.231, df=1, p=0.013, 11/2) and decaying pseudostem extracts (χ2=5.400, df=1, p=0.020, 12/3) over respective control (Table 32 and Figure 53). Responses of females to mechanically damaged pseudostem extract (χ2=0.600, df=1, p=0.439, 9/6) was not statistically different from the control (Table 32 and Figure 53).

The responses of male *O. longicollis* to volatiles of male pseudostem weevils and combination of male volatile with healthy and mechanically damaged pseudostem extracts are presented in Table 33 and the percentage responding males are presented in Figure 54. The attraction of male *O. longicollis* to the male volatile (χ^2=15.211, df=1, p=0.0001, 18/1), healthy pseudostem extracts (χ^2=7.118, df=1, p=0.008, 14/3) and to the combination of male volatile with mechanically damaged pseudostem extract (χ^2=8.000, df=1, p=0.005, 15/3) was found highly significant over respective control. Similarly, the extracts of mechanically damaged pseudostem alone (χ^2=5.400, df=1, p=0.020, 12/3) and the combination of male volatile with healthy pseudostem extract (χ^2=4.765, df=1, p=0.029, 13/4) was also more attractive to male weevils compared to control (Table 33 and Figure 54).

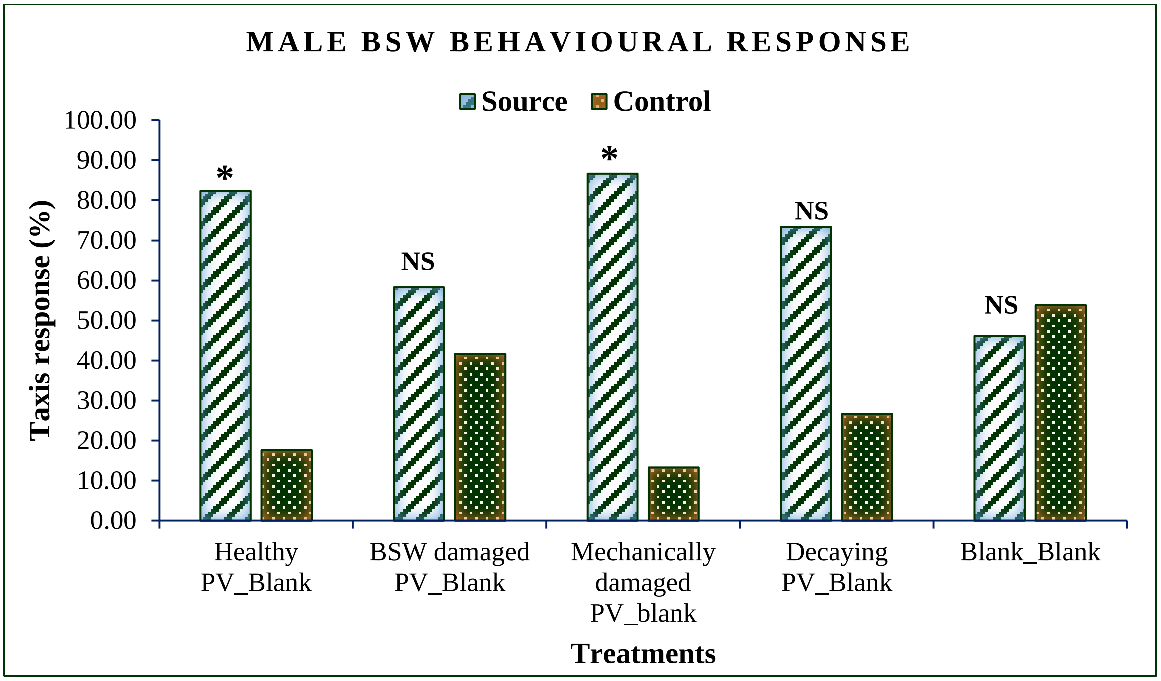


Fig. 52. **Taxis response of *O. longicollis* males to different types of banana pseudostem volatiles in Y tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$, NS= non-significant different**

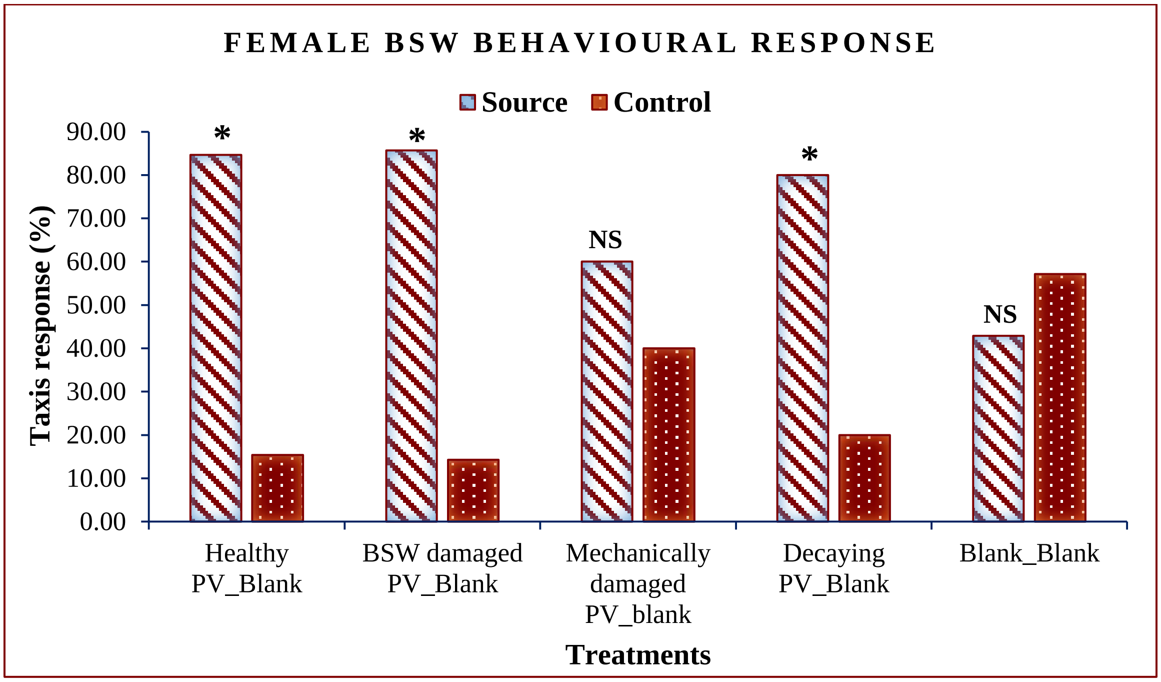


Fig. 53. **Taxis response of *O. longicollis* females to different types of banana pseudostem volatiles in Y tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$, NS= non-significant different**

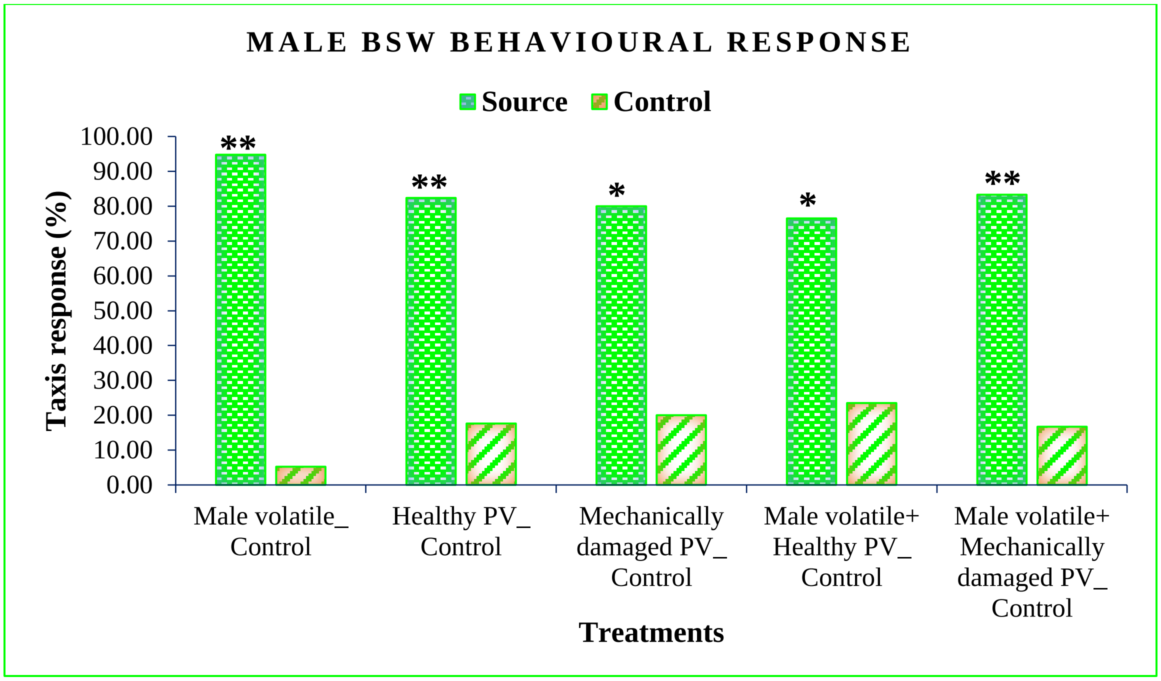


Fig. 54. Taxis response of *O. longicollis* males to volatiles from male weevils and banana pseudostem extract in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$ and **$p<0.01$, NS= non-significant different

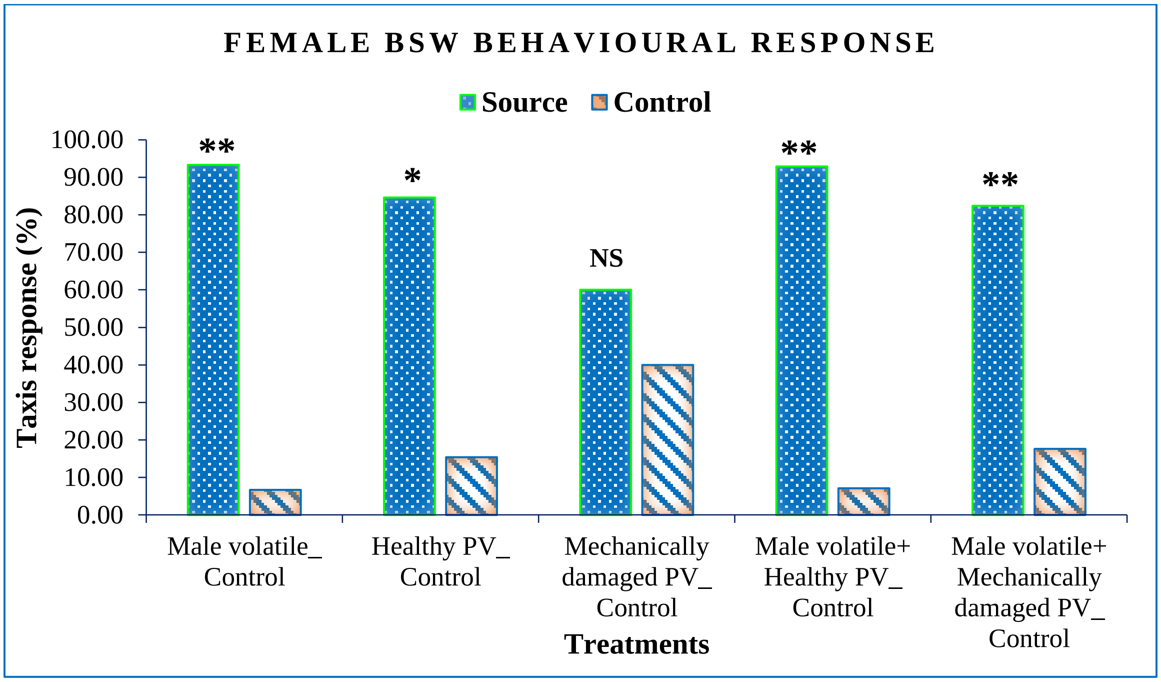


Fig. 55. Taxis response of *O. longicollis* females to volatiles from male weevils and banana pseudostem extract in a Y-tube olfactometer. N=21, *Percentage response between source and control is significantly different at $p<0.05$ and **$p<0.01$, NS= non-significant different

Table 32. Behavioural responses of *O. longicollis* females to different types of banana pseudostem volatiles

Treatments	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Healthy vs Blank	11	2	6.231	13	0.013	*
BSW damaged vs Blank	12	2	7.143	14	0.008	*
Mechanically damaged vs blank	9	6	0.600	15	0.439	NS
Decaying vs Blank	12	3	5.400	15	0.020	*
Blank vs Blank	6	8	0.286	14	0.593	NS

** Response between source and control is significantly different at p<0.05, NS= Non-significant different. ‡n= 21, df=1*

Table 33. Behavioural responses of *O. longicollis* males to volatiles from male weevils and banana pseudostem extract in a Y-tube olfactometer

Treatments	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Male IVC vs. Control	18	1	15.211	19	0.0001	**
Healthy plant extract vs. Control	14	3	7.118	17	0.008	**
Mechanically damaged plant extract vs. Control	12	3	5.400	15	0.020	*
Male IVC+ Healthy plant extract vs. Control	13	4	4.765	17	0.029	*
Male IVC+ Mechanically damaged plant extract vs. Control	15	3	8.000	18	0.005	**

** Response between source and control is significantly different at p<0.05, ** at p<0.01, NS= Non-significant different. ‡ n= 21, df=1*

The responses of *O. longicollis* females to male volatile and combination of male volatile with healthy and mechanically damaged pseudostem extracts and the percentage responding females are presented in Table 34 and Figure 55. The results revealed that, significantly higher number of females were attracted towards male volatile (χ^2=11.267, df=1, p=0.001, 14/1) and the combination of male volatile with healthy (χ^2=10.286, df=1, p=0.001, 13/1) and mechanically damaged pseudostem extracts (χ^2=7.118, df=1, p=0.008, 14/3) compared to respective control. Healthy pseudostem extract alone (χ^2=6.231, df=1, p=0.013, 11/2) attracted significantly more number of females than control, while the attraction of females to the mechanically damaged pseudostem extract (χ^2=0.600, df=1, p=0.439, 9/6) was statistically not different from the control (Table 34 and Figure 55).

Table 34. Behavioural responses of *O. longicollis* females to volatiles from male weevils and banana pseudostem extract in a Y-tube olfactometer

Treatments	Number of response		Chi square value	‡Responded individual (N)	P	
	Source	Control				
Male IVC vs. Control	14	1	11.267	15	0.001	**
Healthy plant extract vs. Control	11	2	6.231	13	0.013	*
Mechanically damaged plant extract vs. Control	9	6	0.600	15	0.439	NS
Male IVC+ Healthy plant extract vs. Control	13	1	10.286	14	0.001	**
Male IVC+ Mechanically damaged plant extract vs. Control	14	3	7.118	17	0.008	**

** Response between source and control is significantly different at p<0.05, ** at p<0.01, NS= Non-significant different. ‡ n= 21, df=1*

From the EAG and Y-tube olfactometer studies, it is clear that, all the pseudostem extracts elicited differential Behavioural response in both male and female weevils. Healthy pseudostem extracts were attractive to both male and female weevils. BSW damaged and decaying pseudostem extracts were attractive to females but not to the males, whereas mechanically damaged pseudostem extracts were attractive to males but not to the females. Fresh and rotting materials equally attractive to weevils (Budenberg *et al.*, 1993 and Hord and Flippin, 1956). Emission of volatiles, produced by the plants in response to weevil damage, is important for attraction of the weevils because the weevils may use these plant odors to find suitable food plants. Hence, the plant damage also plays a role in attraction of weevils. Apart from weevil-damage-related plant volatiles, green leaf volatiles also play significant role (Van Tol *et al.*, 2002). Van Tol *et al.* (2004) reported that vine weevil was attracted to mechanically damaged leaves only.

Similarly, the present study also showed that the combination of male volatiles and plant volatiles are more attractive compared to only plant volatiles. The results suggested that, *O. longicollis* uses plant volatiles in addition to male pheromone to identify and locate the hosts for feeding, mating and for oviposition by both male and female. These results are in agreement with Gunawardena and Dissanayake (2000b), Prasuna *et al.* (2008), Ambrogi and Zarbin (2008), Sahyaraj and Kombiah (2009), Palanichamy *et al*. (2011b) and Palacio-Cortes *et al*. (2015). The Behavioural responses of *O. longicollis* to volatiles from banana pseudostems with different physiological status, results revealed that both male and female *O. longicollis* were attracted to the volatiles from banana pseudostems in different physiological states. Compared to fresh pseudostems, beetle feeding activity and rottenness enhanced the attractiveness of the extracted volatiles from banana pseudostems to male and female *O. longicollis* (Yin Jiong *et al.,* 2012).

Ambrogi and Zarbin (2008) reported that both sexes of soybean stalk weevil, *Sternechus subsignatus* were attracted to host plant volatiles and this attraction increased by the addition of male volatiles. Harari and Landolt (1997) reported that rootstalk borer, *D. abbreviates* females were most often attracted by damaged food. While males were attracted to damaged food. Tinzaara *et al.* (2003) reported that banana weevil, *Cosmopolites sordidus* responded significantly more to the combination of pheromone and pseudostem than to the pseudostem alone. *C. sordidus* showed stronger attraction towards both host plant volatiles and the synthetic pheromone when presented singly or in combination. Fermented pseudostem tissue was more attractive to *C. sordidus* than fresh tissue (Tinzaara *et al.,* 2003).

4.3.3 Cage studies

Before the blends are tested in the field, different traps were evaluated for suitable trapping of BSW weevils. Synthetic pheromone blends were tested in the cages by using a superior trap.

4.3.3.1 Pheromone trap evaluation using live BSW weevils and Pseudostem pieces

Efficiency of traps was assessed by releasing known number of weevils (10-15 male and 10-15 female weevils/day for 6 days) into the field cage made up of nylon mesh. Mean number of male BSW weevils caught in different types of traps presented in Table 35 and the Relative Attraction Index (RAI) of males per day are presented in Figure 56. Among the four different tested traps, cross vane trap with a bucket + pseudostem piece (t=11.023, df=5, p=0.0001) and only cross vane trap + pseudostem piece (t=4.609, df=5, p=0.006) significantly outperformed the bucket trap, Wota-T water trap and china funnel trap in capturing and retaining *O. longicollis* male weevils compared to the respective control (Table 35). Significantly higher relative attraction index of male weevils per day was found in the cross vane trap with a bucket + pseudostem piece (69.23 %) than others (Figure 56).

Similarly, mean number of female BSW weevil catches are presented in Table 36 and the Relative Attraction Index (RAI) of females per day in different types of traps are presented in Figure 57. Significantly higher number of female weevils were caught and retained in the cross vane trap with a bucket + pseudostem piece (t=11.129, df=5, p=0.0001) and only cross vane trap + pseudostem piece (t=7.460, df=5, p=0.0006) compared to other types traps tested (Table 36). Significantly higher RAI of female weevils per day was recorded in cross vane trap with a bucket + pseudostem piece (71.83 %) than other tested traps (Figure 57). The results of the cage studies for evaluation of pheromone traps confirmed that for trapping *O. longicollis* weevils, cross vane trap with

Table 35. Mean catches of *O. longicollis* males among different types of pheromone traps baited with live BSW weevils and pseudostem pieces as bait in field cage

Code No.	Trap type	*Mean number weevils caught/day (Mean ± SE)		Paired t-test		ANOVA	
		Treatment	Control	t-Stat	P-value	F-value	P-value
A	Bucket trap + pseudostem pieces v/s Pseudostem trap	2.50±0.43	3.33±0.67	-0.916	0.402	1.106	0.318
B	Wota-T water trap + 10 male BSW in a cage v/s Wota-T water trap + 10 female BSW in a cage	1.33±0.42	1.17±0.31	0.237	0.822044	0.102	0.756
C	China funnel trap+ 10 male BSW in a cage v/s China funnel trap+ 10 female BSW in a cage	2.50±0.76	2.0±0.45	0.473	0.656	0.319	0.585
D	China funnel trap+ pseudostem piece v/s China funnel trap no pseudostem piece	2.67±0.49	1.00±0.36	2.331	2.571	7.353	0.022
E	Cross vane trap + pseudostem piece v/s only pseudostem trap	9.33±0.76	3.67±0.49	4.609	0.006	39.054	0.0001
F	Cross vane trap + 10 male BSW in a cage v/s Cross vane trap + 10 female BSW in a cage	8.17±0.60	4.50±0.43	5.129	0.004	24.694	0.001
G	Cross vane trap with a bucket + pseudostem piece v/s Cross vane trap with bucket + no pseudostem piece	11.00±0.58	2.00±0.37	11.023	0.0001	173.57	0.0001

** 10 to 15 weevils were released per day (N=6 days)*

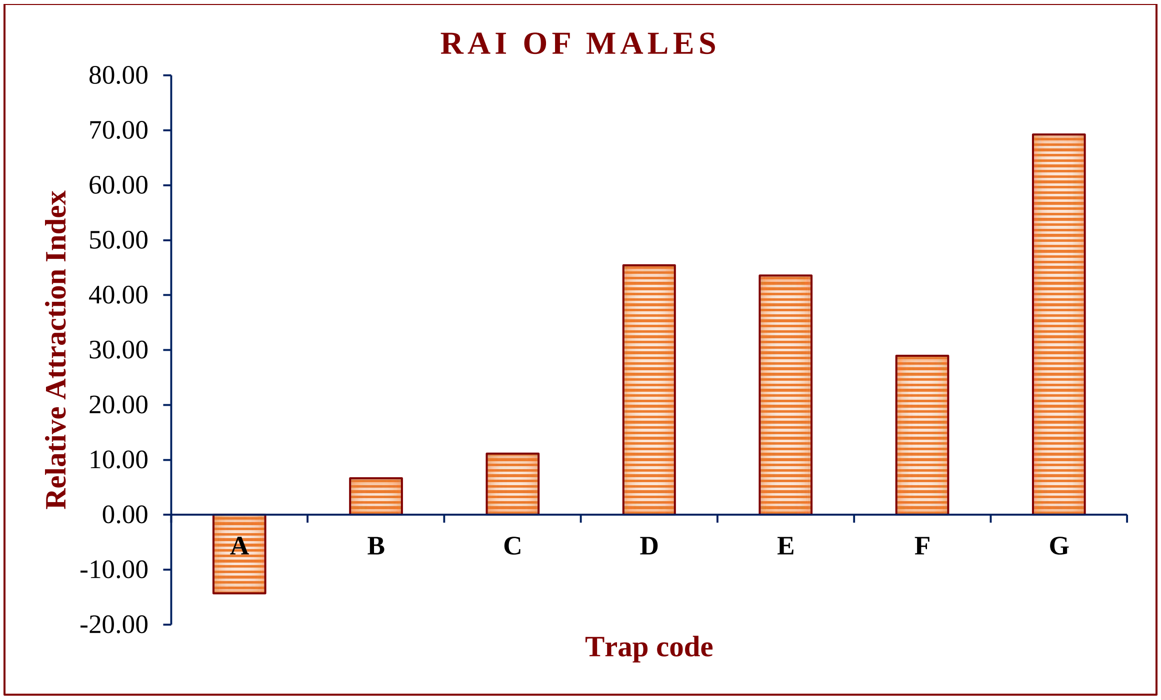


Fig. 56. **Relative Attraction Index (RAI) of male *O. longicollis* among different types of pheromone traps baited with live BSW weevils and pseudostem pieces as bait in field cage**

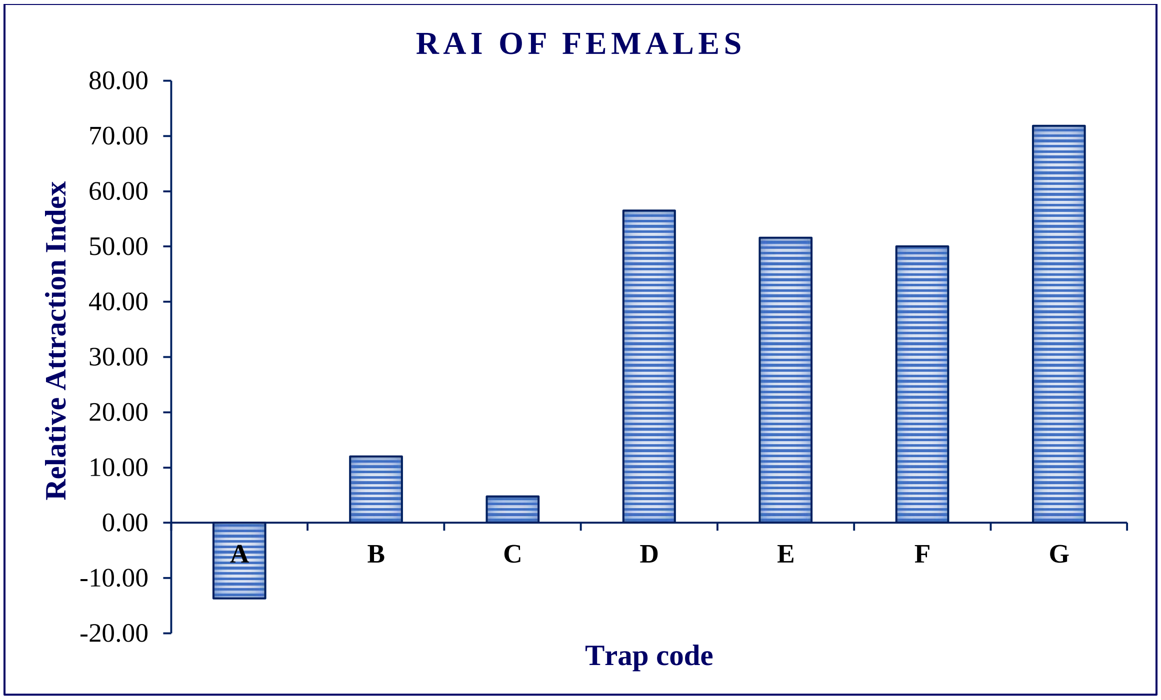


Fig. 57. **Relative Attraction Index (RAI) of female *O. longicollis* among different types of pheromone traps baited with live BSW weevils and pseudostem pieces as bait in field cage**

Table 36. Mean catches of *O. longicollis* females among different types of pheromone traps baited with live BSW weevils and pseudostem pieces as bait in field cage

Code No.	Trap type	*Mean number weevils caught/day (Mean ± SE)		Paired t-test		ANOVA	
		Treatment	Control	t-Stat	P-value	F-value	P-value
A	Bucket trap + pseudostem pieces v/s Pseudostem trap	3.67±0.71	4.83±0.65	-0.863	0.428	1.450	0.256
B	Wota-T water trap + 10 male BSW in a cage v/s Wota-T water trap + 10 female BSW in a cage	2.33±0.71	1.83±0.60	0.522	0.624	0.287	0.604
C	China funnel trap+ 10 male BSW in a cage v/s China funnel trap+ 10 female BSW in a cage	1.83±0.48	1.67±0.67	0.210	0.842	0.041	0.843
D	China funnel trap+ pseudostem piece v/s China funnel trap no pseudostem piece	3.00±0.58	0.83±0.31	3.993	0.010	10.974	0.008
E	Cross vane trap + pseudostem piece v/s only pseudostem trap	7.83±0.60	2.5±0.43	7.460	0.0006	52.245	0.0001
F	Cross vane trap + 10 male BSW in a cage v/s Cross vane trap + 10 female BSW in a cage	7.50±0.76	2.50±0.43	4.841	0.005	32.609	0.0001
G	Cross vane trap with bucket + pseudostem piece v/s Cross vane trap with bucket + no pseudostem piece	10.17±0.48	1.67±0.33	11.129	0.0001	213.197	0.0001

** 10 to 15 weevils were released per day (N=6 days)* a bucket as a container to prevent the escape of trapped insects proved more effective than other types of traps.

It was found that only cross vane traps or with a bucket was better than remaining traps which is in part agreement with previous study by Chandrashekaraiah (2013). Studies on trap type indicated that the cross vane trap attracting higher number of *Opisian arenosella* moths than other types. A study on effect of dispenser type and trap design for capture of dogwood borer (DWB), Synanthedon scitula Harris was conducted in apple orchards in West Virginia and North Carolina. Wing-style sticky traps baited with polyethylene vial captured more male DWB over the first 2 months and later decreased (Zhang et al., 2013). Herman et al. (2005) reported that water traps caught more potato tuber moths, (*Phthorimaea operculella* (Zeller)) per day. Sticky traps (delta-shaped) caught more moths than A-trap sticky traps (cylinder-shaped) and funnel traps. The 1:1 and 1:1.5 pheromone blends caught more moths than the 1:4 blend. It is concluded that the DeSIRe sticky trap is the most suitable for commercial use. The 1:1.5 pheromone blend is recommended for commercial use in New Zealand and overseas (Herman *et al.*, 2005).

4.3.3.2 Pheromone blends evaluation

Cage studies were conducted to determine *O. longicollis* male and female weevil's attraction to the selected blends. The lures were prepared using a white color plastic vial dispensers and the cross vane trap with a bucket (proved superior in the previous study) were used. The results of the cage studies are presented in Tables 37 and 38. The cage study results indicated that, males were significantly attracted to all the tested blends. Significantly more males were attracted to 100:8:5 (DPA: 1-16C:16C) blend when compared with 8:5 blend (1-16C:16C) (t=7.746, df=5, p=0.0006). When, three combination blends were compared, 0.5:10:20 (DPA: 1-16C:16C) blend attracted the significantly higher number of males than 100:8:5 (t=5.000, df=5, p=0.004) (Table 37). Similarly, more number of females were attracted to 100:8:5 (DPA: 1-16C:16C) comapred to 8:5 (1-16C:16C) blend (t=2.291, df=5, p=0.071) and 0.5:10:20 (DPA: 1-16C:16C) blend attracted the higher number of females than 100:8:5 (t=1.754, df=5, p=0.139). The attraction of females to all the tested blends was not statistically different (Table 38).

Table 37. Mean catches of *O. longicollis* males to different blends of BSW synthetic pheromone lures

Trap type	*Mean number weevils caught/day (Mean ± SE)		Paired t-test		ANOVA	
	Treatment	Control	t-Stat	P-value	F-value	P-value
100:8:5 vs. 8:5	5.5±0.43	1.5±0.43	7.746	0.0006	43.636	0.0001
0.5:10:20 vs. 100:8:5	6.17±0.48	2.00±0.45	5.000	0.004	40.584	0.0001

** 10 male weevils were released per day (N=6 days)*

Table 38. Mean catches of *O. longicollis* females to different blends of BSW synthetic pheromone lures

Trap type	*Mean number weevils caught/day (Mean ± SE)		Paired t-test		ANOVA	
	Treatment	Control	t-Stat	P-value	F-value	P-value
100:8:5 vs. 8:5	4.83±0.60	2.67±0.42	2.291	0.071	8.711	0.014
0.5:10:20 vs. 100:8:5	5.00±0.37	3.67±0.56	1.754	0.139	4.000	0.073

** 10 female weevils were released per day (N=6 days)*

The results of this preliminary cage study revealed that the traps baited with the blends containing three compounds captured more weevils than trap baited with only two compounds. The combination of synthetic compounds both at 0.5:10:20 and 100:8:5 blend attracting both sexes and indicated that all the three components are necessary for the attraction of banana pseudostem weevils.

Cage studies confirmed the laboratory bioassays and therefore the identified compounds can be considered as aggregation pheromone compounds of *O. longicollis*. Eller *et al*. (1994) reported that sticky traps baited with a synthetic pheromone blend of pepper weevils captured more pepper weevils (both sexes) than did unbaited control traps or pheromone-baited boll weevil traps. Ruiz-Montiel *et al*. (2008) reported that traps baited with the quaternary blend of compounds (i) 2-methyl-4-heptanol, (ii) 2-methyl-4-octanol, (iii) 2-methyl-4-heptanone and (iv) 2-methyl-4-octanone captured significantly more weevils than traps baited with males.

4.3.3.3 Field evaluation of synthetic pheromone of BSW using cross vane trap

Field study was conducted with synthetic pheromone blend of DPA: 1-16C:16C at 0.5:10:20 ratio using cross vane trap. The traps baited with synthetic pheromone lure, pseudostem piece as well as their combination failed to attract any weevils into traps. This was mainly due to trap design that failed to capture and retain the weevils in the field conditions. Trap and lure standardisation were suggested as a rational approach to reduce the variability in performance of traps (Riedl and Loher, 1980). Tinzaara *et al*. (2005) reported that, insights into the factors affecting trap effectiveness are essential for developing efficient pheromone trapping systems. Hence, there is a need to optimize and establish pheromone trapping plans including the trap design, trap height, trap placement (e.g., trap height, position and density) and weather (e.g., temperature, wind speed and rainfall) on trap catches, effect of pheromone release rate and longevity of synthetic pheromone on attraction of weevils.

Mass trapping is appropriate for weevils because of their long life cycle and adult longevity and reliance on aggregation pheromone and host volatiles. This ultimately reduces the amount of insecticides required for the control and and due to its specificity also increases the efficiency of beneficiary insects in the field. In addition, the pheromone can be used for monitoring the population trends of *O. longicollis* and for evaluating the effects of control measures.

The results of the present study finally open up the possibility that the identified pheromone components can be used in the development of a mass trapping programme as a viable alternative for the management of banana pseudostem weevil, *O. longicollis*. From the practical point of view, ternary blend is sufficiently attractive to be used in the development of the trapping programme. However, before the strong conclusion further studies can be suggested such as large scale cage and field trapping studies.

V SUMMARY

The results of investigations on chemical ecology and Behaviour of Banana pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) conducted at Department of Agricultural Entomology, UAS, GKVK and Bio Control Research Laboratory (BCRL), Sreeramanahalli, Bengaluru rural district during 2014-2016 are summarized in this chapter.

O. longicollis adults- mated during both day and night. Mating started in the 1st hour of scotophase and photophase. The maximum number of matings was observed during photophase. The peak mating period occurred during 6th hour (5.33 ± 1.45) and 9th hour (5.67 ± 2.40) of photophase when 100 % of the pairs mated. There were significant differences between scotophase and photophase with respect to mean number of copulations and distinct pattern was observed recording maximum number of matings during daytime. Since, the constant mating activity was observed from 4th to 11th hour of photophase with two peak periods at 6th and 9th hours, the volatile collection from adult male and female Banana pseudostem weevils and laboratory bioassays activities were carried out during this period.

The calling activity of *O. longicollis* females was observed on the 2nd to 12th hour of the photophase. The female calling activity started in the 2nd hour of the photophase and achieved maximum calling activity in the middle of the day, followed by a decreasing calling activity observed at the end of the photophase. The maximum numbers of calling females were observed during 6th, 8th, 9th and 10th hour photophase (32.00 ± 0.18 and 36.00 ± 0.15), that was considered as the peak time of female calling period. Before calling, female showed restlessness Behaviour with frequent flying. Subsequently, female stayed at one place and began knocking on the substrate with rostrum and simultaneous vibrant antennal beating on the substrate. Dilation of body segments was observed with wings held loosely above the abdomen. This was followed by bending of abdomen and partly exposing the concealed ovipositor through the genital opening. The ovipositor was fully exposed and curved down to the substrate which was accompanied by rapid protrusion and retraction of the ovipositor. This was followed by pressing the substrate with the extruded ovipositor to release the pheromones. The number and duration of calling posture per female per day varied from 2.5 – 8.25 and 2.03-5.85 min, respectively. Mean number of callings per female per day was 5.25 ± 2.68 and the mean duration of calling per female in a day was 3.22 ± 1.59 min.

The mating activity of *O. longicollis* began in the 1st hour of photophase (72.00 ± 0.10) and continued till the end of the photophase. A clear difference was observed in the mean number of mating pairs between individual time periods and statistically significant difference was recorded between time periods. The mating activity was observed throughout the photophase and the maximum numbers of mating pairs were recorded during 6th, 8th and 12th hour of photophase (84.00 ± 0.11 and 92.00 ± 0.03 per cents). The peak period of mating activity and significantly higher number of mating pairs was recorded during the 10th hour of photophase (100.00 ± 0.08) when 100 % of the pairs mated. The mating Behaviour sequence observed in *O. longicollis* pairs exhibited three distinct phases *i.e.*, pre-copulatory phase, copulatory phase and post-copulatory phase. In the pre-copulatory phase, the males were active and they initiated searching of the females with their antennal movements. Males showed distinct courtship Behaviour by bending of abdomen, partial or full exposure of aedeagus pressing against the substrate. In the copulatory phase, 100 % of the pairs exhibited genital contact. The mating occurred throughout the day but the peak mating was recorded during the middle of the day.

The Gas chromatography linked electroantennogram detector analysis of male volatile of *O. longicollis* revealed that three compounds elicited the EAD response in male and female antennae (EAD active compounds). The retention times in the chromatogram of male volatile (5. 935 RT, 6.005 RT and 6.465 RT) and GC-EAD chromatogram of male volatiles to male antennae (5.93RT, 6.00RT and 6.45RT, respectively) and female antennae (5.95 RT, 6.03 RT and 6.46 RT, respectively) were found the same. Gas chromatography-Mass spectrometry (GC-MS) analysis of BSW male volatile indicated that same peaks (as in case of GC-EAD) appeared at the retention times of 19.862, 19.992 and 20.479. These peaks were identified as 1-Hexadecene (19.862), Hexadecane (19.992) and Diphenylamine (20.479), respectively.

The quantity of Diphenylamine (0.04 ng ± 0.001), 1-Hexadecene (0.02 ng ± 0.006) and Hexadecane (0.003 ng ± 0.0003) was more in volatiles collected from males compared to volatiles collected from males + females. Pheromone release took place mainly during photophase with its maximum production recorded between 02.00 PM-04.00 PM compared to volatiles collected during 11.00 AM-01.00 PM. The production of pheromone components depend on the presence of host plant and insects feeding on pseudostem released more volatiles than in the absence of food. Male volatile extract elicited significantly higher antennal response (1.378 ± 0.350 mV and 1.704 ± 0.246 mV, respectively) and Behavioural responses (80.95 % and 60 %, respectively) in both male and female weevils.

Synthetic pheromone compounds elicited higher EAG response in male antennae than in the female antennae. The results of dose-response studies of synthetic pheromone compounds indicated that Diphenylamine at 5 µl elicited higher EAG response and the responses decreased with increased doses in both male and female antennae. Where as 1-Hexadecene and Hexadecane produced increased antennal response in both sexes with increased doses. The results of the different blend combinations of synthetic pheromone compounds indicated that the blend of DPA: 1-16C:16C at 0.5:10:20 elicited higher EAG response in both male and female antennae. The increased doses of this best performing blend (0.5:10:20) produced higher antennal response in both the sexes.

The synthetic compounds when presented individually are not attractive to both male and female BSW, while the combination of all the three pheromone compounds are necessary for eliciting Behavioural response in both sexes. The blend of DPA: 1-16C:16C at 0.5:10:20 are highly attractive to both male and female BSW. EAG and Y-tube olfactometer studies confirmed that 0.5:10:20 (DPA:1-16C :16C) is the best performing blend and this blend evoked increased Behavioural responses with increased doses in both the sexes and they showed higher response at 100 µl of the synthetic blend.

Volatiles from healthy and mechanically damaged pseudostem extract elicited significantly higher antennal response in male (1.187 ± 0.102 mV) and female (0.942 ± 0.116 mV), respectively. Both male and female produced significantly higher antennal response to male volatile (2.231 ± 0.479 and 1.279 ± 0.157 mV) and its combination with healthy (1.877 ± 0.357 and 1.242 ± 0.118 mV) and mechanically damaged pseudostem extract (2.231 ± 0.468 and 1.502 ± 0.234 mV).

All the pseudostem extracts elicited differential Behavioural responses in both male and female weevils. Significantly more males were attracted towards the healthy (66.67 %) and mechanically damaged pseudostem extract (61.90 %), while females showed more attraction towards BSW damaged (57.14 %)) and decaying pseudostem extract (57.14 %) in addition to healthy plant volatile (52.38 %). The combination of male volatiles and plant volatiles are more attractive compared to plant volatiles alone.

Different traps evaluated using live BSW weevils and pseudostem piece as attractants for suitable tapping of males and females indicated that cross vane trap with a bucket + pseudostem piece and only cross vane trap + pseudostem piece significantly out-performed the bucket trap, Wota-T water trap and china funnel trap in capturing and retaining *O. longicollis* male and female weevils compared to the respective controls. The results of preliminary cage study revealed that the combination of synthetic compounds at 0.5:10:20 and 100:8:5 blend attracted both sexes and indicated that all the three components are necessary for the attraction of banana pseudostem weevils.

Finally, the results of present study open up the possibility that the three identified pheromone components can be used in the development of a mass trapping programme as a viable pest management alternative for *O. longicollis*. From a practical point of view, ternary blend is sufficiently attractive in the development of the trapping programme. However, it is necessary to conduct large scale cage and field trapping studies to arrive at strong conclusion.

Future line of work

- Identification and location of sensillae in BSW.
- Pheromone attractiveness and trap efficiency need to be enhanced.
- Several components of mass trapping system need to be optimized *viz.*, trap type, trap placement (e.g., trap height, position and density) and influence of weather conditions on trap catches.
- The effect of release rate and longevity of synthetic pheromone blends on attraction of *O. longicollis* should be investigated under laboratory and field conditions.
- Mass trapping should be conducted for evaluation of the efficacy of new lures.
- Identification and evaluation of plant volatiles from Healthy and mechanically damaged pseudostem.
- Laboratory and field studies on synergistic or additive effects of plant volatile with synthetic pheromone compounds are warranted.

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Behavioral and Electrophysiological Responses of the Banana Pseudostem Weevil *Odoiporous longicollis* Olivier to Host Plant Volatiles

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Abstract

Electroantennography (EAG) and behavioral bioassays were conducted to determine the effective host-plant volatile(s) for the attraction of *Odoiporus longicollis* adults. Volatiles from healthy and mechanically damaged pseudostem extract elicited significantly higher antennal response in male (1.187±0.102mV) and female weevils (0.942±0.116mV), respectively. The Y tube olfactometer study revealed that significantly more males were attracted towards the healthy (66.67%) and mechanically damaged pseudostem extract (61.90%), while females showed more attraction towards BSW damaged (57.14%)) and decaying pseudostem extract (57.14%) in addition to healthy plant volatile (52.38%). Hence, these plant volatiles may be used as attractants for the management of the pest.

Banana (*Musa* spp., Musaceae) is one of the most important fruit crops of South East Asian region. India is the largest producer of banana in the world with 822 thousand ha and 29,221 thousand tons production (NHB, 2014). Banana is infested with nineteen insect pests from planting to harvesting in India (Padmanaban *et al*., 2001). Of these, the banana pseudostem weevil (BSW) *Odoiporus longicollis* Olivier (Coleoptera: Curculionidae) is gaining importance as a serious pest causing heavy losses to the growers. Now-a-days, this weevil poses a serious threat to the banana cultivation in the banana growing belts of India (Ravi and Palaniswami, 2002).

Banana pseudostem weevil is a monophagous pest. Adult females of *O. longicollis* lay eggs in the outermost leaf sheath of banana. Larvae hatched from the eggs bore into the living tissue, producing frass-filled tunnels that weaken the affected parts of the host plant and permit invasion of fungal and bacterial pathogens. Mature larvae pupate in cocoons made from plant fibers close to the exit holes. The severity of the loss is greater when infestation occurs at the early vegetative stage (5 months old). It is estimated that banana pseudostem weevil incurs 10-90 per cent yield loss depending on the crop growth stage and management practice (Padmanaban and Sathiamoorthy, 2001).

At present, banana pseudostem trapping is the only method available to monitor this pest. The endophytic behavior of the larvae and long life span of adults complicates the management of this pest as the insecticides were ineffective. Hence, there is an urgent need to explore an alternative ecofriendly practical approach. Use of kairomones is a recent trend in developing semiochemical-based pest monitoring and management, which is one of the viable technologies to combat this pest. Owing to its restricted feeding habit and monophagy, *O. longicollis* may use specific host plant volatiles to find its host for feeding and oviposition. Exploiting these behaviorally active plant volatiles as a potential tool for monitoring and mass trapping purposes offers an ecofriendly management option. In the present study, electroantennographic (EAG) technique was employed to screen different extracts from the banana pseudostem for detecting volatiles with possible semiochemical property.

O. longicollis larvae were collected from damaged banana plants and reared on banana pseudostem pieces under laboratory conditions at 12L: 12D at 25 ± 2^0 C, 70 ± 10 per cent RH at Biocontrol Research laboratories (BCRL), Pest Control (India) Pvt. Ltd. (PCI), Bengaluru. Emerged adults were separated into males and females by rostrum characteristics. They were maintained in separate plastic containers (29cm x 17cm x 33cm). Weevils were provided with freshly cut pseudostem pieces as food, replaced every 5 days.

Solvent extraction method was followed for volatile collection. Pseudostem pieces of 5gm each from healthy plant, BSW damaged plant, mechanically damaged plant and decaying plant were collected and immersed in 10 ml dichloromethane (CH_2Cl_2) (HPLC grade) at 25 ± 2^0 C for 3 days. The solvents were filtered and were concentrated under a gentle stream of nitrogen. On condensing, the sample was stored at -20^0 C until used for bioassay.

Electroantennography (EAG) (Syntech, The Netherlands) bioassay was carried out at BCRL to assess the olfactory sensitivity of both male and female *O. longicollis* adults to different pseudostem extracts. Ten µl of aliquot placed on a filter paper strip (60mm long, 5mm wide Whatman No. 1) inside a glass Pasteur pipette (Dimensions – 5.75, Length – Overall 145.0 mm; tip-47.0 mm) was used for stimulus delivery. This was connected to the stimulus controller by silicone rubber tubing. After 10 seconds, the solvent was blown out with first puff. Another 60 seconds later, the stimulus was puffed on to the excised antenna by injecting the vapour phase of the chemical stimuli through a polysterene tube along with a continuous air stream (pulse rate 0.5 s, continuous flow 25 ml s^{-1}, pulse flow 21 ml s^{-1}) to the antenna. Five samples were used, for each sample 5 replicates were performed per sex and each replicate represented one antenna.

The behavioral responses of *O. longicollis* males and females to different pseudostem extracts after EAG screening were further tested in a dual choice Y-tube olfactometer at Insect Behavior Testing Lab (IBTL) of BCRL. The olfactometer consisted of Y-shaped acrylic tube of 6 cm dia. The main tube (stem) of the olfactometer and the two arms were each 30 cm in length at 90^0. The air-delivery unit (model) was connected to the two arms of the Y-tube to draw purified air to pass through the odor sources in the Y-tube. Airflow through each of the olfactometer arms was maintained at 0.5 L min^{-1}. The olfactometer study was carried out in a room separated from *O. longicollis* culture. The bioassay was carried out between 11:00 and 1600 h during photophase, which corresponded with the peak mating behavior of *O. longicollis*. Fixed number of pseudostem extracts (40 il) were loaded in Whatman filter paper strips of 1 x 3 cm size and were placed in one of the Y-tube chambers and the other chamber served as control (equal volume of HPLC-grade DCM). New filter papers with the extracts and DCM were used for each trial (for every 3 weevils). The position of treatments was alternated after each trial, to avoid directional bias. A group of 3 BSWs were introduced into the base tube of the olfactometer, and the behavior was observed for 15 m. when a weevil crossed the choice line 10 cm after the division of the base tube and remained there for at least 20 s, it was recorded as a choice for the odor source in that arm. If the weevils stayed in the common tube or at the junction of the two arms and did not make a choice during this time were considered a non-responding individual and were excluded from the statistical analysis. Age and mating status of the weevils were not controlled during the bioassays because of the difficulty of rearing these insects under laboratory conditions.

All the extracts elicited antennal response in both male and female BSW. Among different extracts, healthy pseudostem and BSW damaged pseudostem extract elicited significantly higher response ($F_{4, 20}$=4.652; P=0.008) in male antennae than control. While extracts of decaying pseudostem and mechanically damaged pseudostem were found on par. When female antenna was exposed to above mentioned extracts, mechanically damaged pseudostem extract elicited significantly higher response ($F_{4, 20}$= 6.256; P=0.001) than other extracts and control (Table I).

Dual choice Y-tube olfactometer studies were conducted to determine the behavioral responses of male and female *O. longicollis* adults to different fractions of banana pseudostem extracts. The results indicated that, significantly higher number of males were attracted towards the healthy pseudostem (÷2=7.118, p=0.008) and mechanically damaged pseudostem exracts (÷2=8.067, p=0.005) compared to control. The male responses towards BSW damaged (÷2=0.333, p=0.564) and decaying pseudostem extracts (÷2=3.267, p=0.071) were not statistically different from respective control (Table 2). Similarly, male weevil attraction towards healthy (÷2=6.231, p=0.013), BSW damaged (÷2=7.143, p=0.008) and decaying pseudostem extracts (÷2=5.400, p=0.020) were significantly higher than the control. Response of males toward mechanically damaged pseudostem extract

TABLE I

EAG response (Mean (±SEM)) of BSW male and female antennae against banana pseudostem extracts

Treatments	EAG Response (mV) (Mean±SEM)	
	Male	Female
Healthy pseudostem extract	1.187±0.102^{a}	0.578±0.070^{abc}
BSW damaged pseudostem extract	1.274±0.102^{a}	0.867±0.080^{ab}
Decaying pseudostem extract	1.001±0.092^{ab}	0.528±0.096^{bc}
Mechanically damaged pseudostem extract	1.058±0.119^{ab}	0.942±0.116^{a}
Control (DCM)	0.730±0.056^{b}	0.377±0.081^{c}
DF	4, 20	4, 20
F test	4.652	6.256
P value	0.008**	0.001**

Note : Figures within a column followed by a common letter are not significantly different by Tukey post hoc test ($p<0.05$)

TABLE II

Behavioral responses of O. longicollis males to plant volatiles

Treatments	Number of response		Chi square value	‡Responded individual (N)	p	
	Male	Female				
Healthy vs Blank	14	3	7.118a	17	0.008	*
BSW damaged vs Blank	7	5	0.333b	12	0.564	NS
Mechanically damaged vs blank	13	2	8.067c	15	0.005	*
Decaying vs Blank	11	4	3.267c	15	0.071	NS
Blank vs Blank	6	7	0.077d	13	0.782	NS

‡ Response between source and control is significantly different at $p<0.05$, NS- non-significant different. ‡n= 21, df=1

TABLE III

Behavioral responses of O. longicollis females to plant volatiles

Treatments	Number of response		Chi square value	‡Responded individual (N)	p	
	Male	Female				
Healthy vs Blank	11	2	6.231a	13	0.013	*
BSW damaged vs Blank	12	2	7.143b	14	0.008	*
Mechanically damaged vs blank	9	6	0.600c	15	0.439	NS
Decaying vs Blank	12	3	5.400c	15	0.020	*
Blank vs Blank	8	6	0.286b	14	0.593	NS

‡ Response between source and control is significantly different at $p<0.05$, NS- non-significant different. ‡n= 21, df=1

(÷2=0.600, p=0.439) was not statistically different compared to control (Table III).

In this study, both male and female weevils responded to all pseudostem extracts, which may be used to identify and locate the hosts for feeding, mating and for oviposition by both male and female, respectively. Gunawardena and Dissanayake (2000) suggested that the host volatiles have additional function as an oviposition stimulant for females in addition to other function. Both male and female *O. longicollis* weevils use volatile chemicals of banana for locating their mates (Prasuna *et al.,* 2008) and for oviposition (Abera, 1997 and Gold *et al.,* 2001). Sahayaraj and Kombiah (2009) suggested that *O. longicollis* use decayed pseudostem as cues for food location. Palanichamy *et al.* (2011) suggested that microwave assisted pseudostem extract can be used as kairomones for attracting weevils particularly in the fields where, female population preponderates male. Hence, major volatiles released by the host plants can be used to develop semiochemical-based-management practices for *O. longicollis*.

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Behavioral Responses of Banana Pseudostem Weevil, *Odoiporus longicollis* Ilivier (Coleoptera: Curculionidae) to Insect and Plant Volatiles

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Abstract *Odoiporus longicollis* (Coleoptera: Curculionidae) is the most important monophagous pest of banana, suggesting specific volatiles from banana pseudostem may be attractive to this pest. A study was conducted to determine the begavioral response of *O. longicollis* adults to male produced chemicals and plant volutiles. lectroantennography (EAG) and behavioral bioassay was conducted using different odor sources collected from male weevils and the host plant. The EAG result showed that, both male and female produced significantly higher antennal response to male volatile (2.231±0.479 and 1.279±0.157 mV) and its combination with healthy (1.877±0.357 and 1.242±0.118 mV) and mechanically damaged pseudostem extract (2.232±0.468 and 1.502±0.234 mV). In the olfactometer study, per cent response of males (ranging from 57.14 to 85.71%) was found to be higher than that of females (ranging from 42.86 to 66.67%) in all the tests. These findings hint a possible role of male produced chemicals along with host plant volatiles in attraction of weevils. Studies are underway to chemically identify the constituents responsible for the attraction of weevils.

Keywords Behavioral bioassay, Electroantennography, *Odoiporous londicollis*, Olfactometer, Semiochemicals.

Entroduction

Banana (*Musa* spp.) is an important druit commonly grown in tropical and subtropical parts of the world. India is the largest producer of banana in the world and occupies top position among total fruit production in India. It is cultivated in India in 822 thousand ha with 29.221 thousand tons production [1]. Banana is attacked by different insect pests among which, banana pseudostem weevil (BSW) *Odoiporus longicollis* Oliver (Coleoptera: Cureculionidae) is a key pest [2]. BSW is a serious and devastating pest of nanana in many parts of the world. Both adults and larvae cause severe damage to banana plants [3]. Currently in India, this weevil is posing a serious threat

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to the banana cultivation in Andaman Islands, Uttar Pradesh, Bihar, West Bengal, Assam, Lerala, Tamil Nadu and Karnataka [4]. BSW distribution and severity of infestation in South Karnataka was not related to geographical location but influenced by the varieties cultivated [5].

Adult BSW are attracted by the volatiles released by the banana plants. Infestation of the weevil normally starts in five-month-old plants [2]. The female weevil lays eggs inside the air chamber of the outer sheath of the pseudostem through holes made by its rostum. Emerging grubs make extensive tunnels in the pseudostem for feeding and pupate inside the pseudostem to become adults. Early symptoms of the infestation are the presence of small pin-head-sized holes on the stem, exudation of a gummy substance from such holes and fibrous extrusions from bases of leaf petioles. Extensive tunnelling both in the leaf sheath and pseudostem occurs at the advanced stages of infestation. Rotting occurs due to secondary infection of pathogens and a foul odour is emitted. When the true stem and peduncle are tunnelled after flowering, the fruits do not develop properly, presenting a dehydrated condition with premature ripening of the bunch itself. It is estimated that banana pseudostem borer causes 10–90% yield loss depending on the growth stage of the crop and management efficiency [6]. The problem is generally noticed only when the damage is in the advanced stage and grubs are fully grown [7]. The pest status of the banana weevil varies depending on agro-ecological condition [8].

Table 1. EAG responses of *O. longicollis* males to different odorous stimuli. Figures within a column followed by a common letter are not significantly different by Tukey post hoc test Pd”0.05).

Treatments	EAG Response (mV) (Mean±SEM)
Male IVC	2.231 ± 0.479^{a}
Healthy pseudostem extract	0.952 ± 0.159^{ab}
Mechanically damaged pseudostem extract	0.893 ± 0.165^{ab}
Male IVC+ Healthy pseudostem extract	1.877 ± 0.357^{ab}
Male IVC+ Mechanically damaged pseudostem extract	2.231 ± 0.468^{a}
Control (DCM)	0.511± 0.131^{b}
DF	5, 24
F test	5.285
P value	0.002

Farmers adopt several control measures, but all have not been effective or validated or integrated with other practices. The endophytic behavior of the larvae and long life span of adults complicates the management of this pest and insecticides failed to reach them. At present, banana pseudostem trappling is used to monitor this pest, but it is difficult to maintain the pseudostem traps for long periods. The banana pseudostem tissue used in conventional trapping becomes less efficient due to rapid dehydration lead to decrease in the attractiveness after few days [9]. Use of semiochemical (pheromone and kairomone) based pest management is a recent trend in horticultural and plantation cropping systems. Several attempts have been made to optimize and develop semiochemical based methods for the management of *O. longicollis* but these were not successful. Most of the insects locate host plants using the plant volatiles. Host attractants as kairomones can be used as attractant for insects either male or female/ both [10]. Both male and female *O. longicollis* weebils use volatile chemicals emitted by the host plant as cues for their location [11]. In many coleopterans, males initiate host location and produce semiochemicals (pheromones) to enhance the attraction of conspecifics towards the host for feeding and mating [12]. Therefore, an attempt was made to determine the behavioral response of *O. longicollis* adults to male produced chemicals and plant volatiles. In this study volatiles collected from the male weevils and plant extracts of healthy and mechanically damaged pseudostems of banana plants were used, since the weevils were observed to get attracted to both healthy banana plants as well as to the cut pseudostem traps in field conditions but the chemicals responsible for attraction were not identified. An air entrainment method was followed for the collection of insect volatiles [13—15] rather than solvent extraction of insects. So that specific volatiles released by the male

Table 2. EAG responses of *O. longicollis* males to different odorous stimuli. Figures within a column followed by a common letter are not significantly different by Tukey post hoc test Pd"0.05).

Treatments	EAG Response (mV) (Mean±SEM)
Male IVC	2.279 ± 0.157^{a}
Healthy pseudostem extract	0.506 ± 0.058^{b}
Mechanically damaged pseudostem extract	0.481 ± 0.077^{b}
Male IVC+ Healthy pseudostem extract	1.242 ± 0.118^{a}
Male IVC+ Mechanically damaged pseudostem extract	1.502 ± 0.234^{a}
Control (DCM)	0.277 ± 0.051^{b}
DF	5, 24
F test	15.251
P value	0.0001

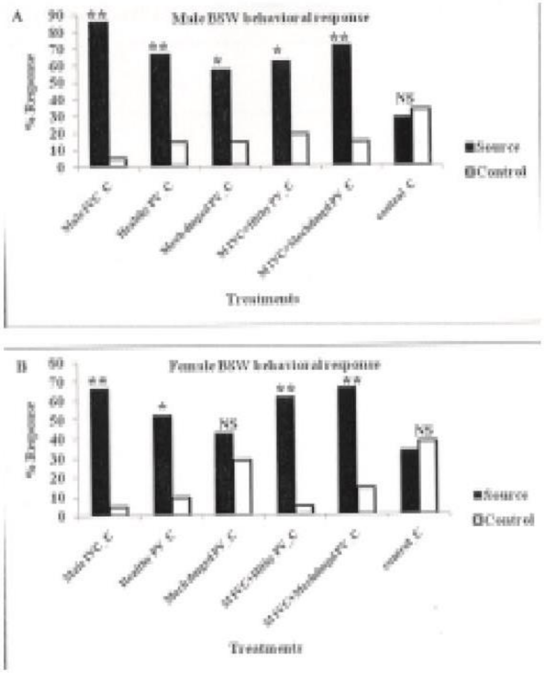

Fig. 1. Behavioral response (%) of *Odoiporus longicollis* (A) Males and (B) Females to volatiles from BSW and Banana plant in a Y-tube olfactometer. Differences between paired bars (χ^2 test) indicated by $*P<0.05$, $**P<0.01$, NS=Non-significant. M IVC: Male Insect volatile Collection; PV: Plant volatile; C: Control.

weevils attractive to conspecifics can be identified.

Materials and Methods

Insects

The pupae of banana pseudostem weevils were collected from infested banana plants, maintained in separate plastic containers (7 cm × 5 cm) and daily checked for adult emergence. Emerging adults were sexed based on rostrum characteristics. Male and female weevils were maintained in separate containers (29 cm × 17 cm) in the laboratory under the photoperiod of 12 light (L): 12 dark (D) with 25 ± 2°C and 70 ± 10% RH until use at Biocontrol Research laboratories (BCRL), Sriramanahalli, Bengaluru (13.18´, 77.55´, 980AMSL). Weevils were provided with freshly cut pseudostem pieces as food, replaced every 5 days.

Collection of insect volatiles

Groups of 50 male weevils were maintained separately in 6 cm dia. × 19 cm long glass aeration chambers. A charcoal filtered humidified airstream was pushed through the aeration system at a flow rate of 1.8 L min^{-1} was achieved using a vaccum system. Emitted volatiles were collected daily and trapped in glass tubes with a 30 mg of polymer-conditioned Porapak-Q adsorbent. The weevil volatiles were collected from the Porapak-Q with 1.5 ml of HPLC grade Dichloromethane (DCM) in 15 min. A dilution of 200 weevils were prepared by mixing 4 days volatile collection and were concentrated to 1 ml (20 weevil volatiles per 100 µl of DCM) under nitrogen stream before analysis, so that the volatiles are in diluted form for direct use in different studies. The volatile collection was conducted at 25 ± 2°C temperature and 70 ± 10% RH at Biocontrol Research laboratiries (BCRL), Sriramanahalli, Bengaluru. Extracts that were not used immediately were stored at -20°C in a refrigerator. Solvent extraction method was followed for collection of banana pseudostem extract. To prepare the banana pseudostem extract, 5 g each of fresh pesudostem and mechanically damaged pseudostem were cut into small pieces and soaked in 10 ml dichloromethane (CH_2Cl_2) (HPLC grade) separately in a glass vial at 25 ± 2°C for 65 h. The solvent extract from both the samples were carefully transferred to separate glass vials. The extracts were concentrated under a gentle stream of nitrogen. On condensing, the sample was stored at -20°C until used for bioassay. The antennal and behavioral responses of weevils to male volatiles, plant volatiles and their combinations were conducted using Electroantennography (EAG) and Y-olfactometer at Insect Behavior Testing Laboratory (IBTL), BCRL, Bengaluru.

Electroantennography (EAG)

Electrophysiological responses of male and female *O. longicollis* antennae to different volatile extracts collected from weevils and plants were recorded using Syntech EAG system. Ten µl of aliquot placed on a filter paper strip (60 mm long, 5 mm wide Whatman No. 1) inside a glass Pasteur pipette (5.75, Length – Overall 145.0 mm; tip-0.47 mm) was used for stimulus delivery. This was connected to the stimulus controller by silicone rubber tubing. After 10 seconds, the solvent was blown out with first puff. Another 60 seconds later, the stimulus was puffed on to the excised antenna by injecting the vapour phase of the chemical stimuli through a polysterene tube along with a continuous air stream (pulse rate 0.5 s, continuous flow 25 ml s^{-1}, pulse flow 21 ml s^{-1}) to the antenna. For each sample dilution, 5 replicates were performed per sex and each replicate represented one antenna.

Laboratory bioassay

The test volatile extracts were further subjected to a dual choice Y-tube olfactometer to determine the behavioral responses of *O. longicollis* males and females. The oriented movements of weevils to different volatile samples were recorded. The olfactometer consisted Y-shaped acrylic of 6 cm dia. The main tube (stem) of the olfactometer and the two arms were each 30 cm in length at 90°. The air-delivery unit was connected to the two arms of the Y-tube to draw purified air to pass through the odor sources in the Y-tube. Airflow through each of the olfactometer arms was maintained at 0.5 L min^{-1}. 40 µl of volatile extracts in DCM were loaded in Whatman filter paper strips (1 × 3 cm) and were placed in one of the Y-tube chambers and the other chamber served as control (equal volume of HPLC-grade DCM). New filter parers with the extracts in DCM were used for each trial (3 weevils). The position of treatments was alternated after each trial, to avoid directional bias. A group of 3 BSWs were introduced into the base tube of the olfactometer and the behavior was observed for 15 min. Totally 21 weevils were used for each treatment in 7 batches, each batch with 3 weevils. When a weevil crossed the choice line 10 cm after the division of the base tube and remained there for at least 20s, it was recorded as choice for the odor source in that arm. If the weevils stayed in the common tube or at the junction of the two arms and did not make a choice during this time were considered a non-responding individual and were excluded from the statistical analysis.

Statistical Analysis

Electroantennogram responses were compared using a one-way analysis of variance (ANOVA) followed by a honestly significant different (HSD) Tukey test (SPSS 16.0 version software). Results of all olfactometer bioassays were analyzed with a binomial test (χ^2 test; SPSS 16.0 version software) and means were compared by Tukey HSD test ($p<0.05$).

Results and Discussion

All the stimuli tested elicited significant EAG responses from both male and female antennae in comparison to control indicating the presence of certain antennally active constituents in the extracts. Tested males produced significantly higher EAG deflections than female weevils in all the tests except for control (Tables 1 and 2).

Statistical analysis of results with Tukey's post hoc test revealed significant differences in EAG responses of males to different treatments. Male volatile alone and its combination with mechanically damaged pseudostem extract elicited significant antennal

Table 3. Responses of male and female BSW adults to select semiochemicals in Y-tube olfactometer. Control: DCM, Statistically significant differences using binomial test at $*p<0.05$; $**p<0.01$ and NS=Non-significant.

	Frequencies	
Odor sources	Male	Female
Male IVC vs. Control		
Male IVC	18**	
14**Control	01	01
Not decided	02	06
Total	21	21
Healthy plant extract vs. Control		
Healthy plant extract	14**	11*
Control	03	02
Not decided	04	08
Total	21	21
Mechanically damaged extract vs. Control		
Mechanically damaged plant extract	12*	09^{NS}
Control	03	06
Not decided	06	06
Total	21	21
Male IVC+Healthy plant extract vs. Control		
Male IVC+ Healthy plant extract	13*	13**
Control	04	01
Not decided	04	07
Total	21	21
Male IVC+ Mechanically damaged plant extract vs. Control		
Male IVC + Mechanically damaged plant extract	15**	14**
Control	03	03
Not decided	03	04
Total	21	21
Control vs. Control		
Control	06^{NS}	06^{NS}
Control	07	08
Not decided	08	07
Total	21	21

responses in males than any other stimulus and these two were statistically on par (2.231±0.489 mV and 2.231±0.468 mV, respectively). The EAG responses of male BSW antennae to the combination of male volatile with heatthy pseudostem extract (1.877±0.357 mV) was higher than the response to healthy pseudostem extract (0.952±0.269 mV) and mechanically damaged pseudostem extract when tested alone (0.893±0.165 mV) (Table 1).

Analysis of results with Tukey's post hoc test revealed significant differences among the treatments in antennal responses of female BSWs. The female antennae produced significantly higher EAG response to male volatile extract (1.279±0.157 mV) and combination of male volatile extract with mechanically damaged and healthy pseudostem extract (1.502±0.234 mV and 1.242±0.118 mV, respectively), were statistically at par. The healthy and mechanically damaged pseudostem extracts when given individually without male volatile extract elicited significantly lower EAG response in female antennae and they were statistically on par (Table 2).

The EAG result of both male and female weevils showed that, both sexes produced higher antennal response to male volatile and combination of male volatile with the plant extracts compared to plant extracts alone. Others also reported that body extracts of male weevils in combination with nanana sheath extract produced larger EAGs in both males and female *O. longicollis* than banana sheath extracts alone [11]. All the odorous stimuli of banana pseudostem extracted by different methods elicited higher EAGs in male compared to female, indicating that male perceived pheromones/kairomones may be involved in the process of mate/host plant sciection [10]. The study also shows that males showed more antennal activity than females.

The responses of *O. longicollis* males and females in Y-tube olfactometer to different odor sources are shown in Table 3. The attraction of *O. longicollis* male to the male weevils volatile extract (P=0.0001, χ^2=15.211), healthy pseudostem extracts (p=0.008, χ^2=7.118) and to the combination of male volatile extract with mechanically damaged pseudostem extract (p=0.005, χ^2=8.000) was found highly significant than control. Similarly, the extracts of mechanically damaged pseudostem alone (p=0.020, χ^2=5.400) and combination of male volatile with healthy pseudostem extract (p=0.029, χ^2=4.765) was also more attractive to male weevils compared to DCM solvent as control (Table 3). On the other band, male volatile (p=0.001, χ^2=11.267) and the combination of male volatile with healthy and mechanically damaged pseudostem extracts (p=0.001, χ^2=10.286 and p=0.008, χ^2=7.118, respectively) were highly attractive to female weevils than healthy pseudostem extract (p=0.013, χ^2=6.231). The attraction of females to the mechanically damaged pseudostem extract (p=0.439, χ^2=0.600) was statistically non-significant (Table 3). Ambrogi and Zarbin reported that combination of male extract and soybean stem was more attractive to males and female of *Sternechus subsugnatus* Boehman, weevils than soybean stem alone [13]. The attraction of the guava weevil, *Conotrachelus psidii* Marshall, males and females to the natural headspace extract of males was significant as well as the attraction to the combination of host plant headspace extract with the synthetic pheromone component. On the other hand, neither the synthetic compound nor the host plant volatiles alone were attractive to males and females [15]. Others have also observed that in the laboratory, volatiles from pseudostem tissue had an additive effect on attraction of *Cosmopolites sordidus* Germar, weevils to the pheromone but the effect was not significant in the field [9].

The per cent response of male and female weevils to different odor sources in each experiment was calculated due to significant differences was observed in the movement of weevils in the olfactometer bioassay. In all the tests, per cent response of males (ranging from 57.14 to 85.71%) was found to be higher than that of females (ranging from 42.86 to 66.67%). On the other hand, both seces showed similar type of response to all the odor sources except mechanically damaged pseudostem extract was more attractive to males but not to the females (Fig. 1). These results were in confirmation with other workers [11]. The percent activity of both male and female was more towareds the conbination of male body extract and banana sheath extract than to the banana sheath extracts per se. These findings lead to the conclusion that, male volatiles along with plant volatile as a possible role in the host plant as well as male location.

The electrophysiological and olfactometer studies revealed that the plant volatiles alone are not effective for attracting the *O. longicollisa* weevils but their combination with male produced volatiles is found to have additive effect in attracting the weevils under the laboratory conditions. Male volatile and its combination with plant extracts produced considerable EAGs and behavioral responses in male and female BSWs. However, male weevils showed higher EAGs and olfactory responses to all the odor stimuli than females. Therefore, male weevils are more responsive to odors and male produced chemicals with plant volatiles play a role in the attraction of both sexes. Since, analysis of the extracts of *O. longicollis* male via GC-EAD showed that, two peaks of male extracts consistently activated the antennae of male and female weevils. Studies are underway to chemically identify the constituents responsible for the attraction of weevils.

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