Indian Journal of Entomology 86(1): 44-49 (2024) DoI. No.: 10.55446/IJE.2023.959

ELECTROPHYSIOLOGICAL AND BEHAVIOURAL RESPONSES OF *ANOMALA DIMIDIATA* TO THREE HOST PLANT'S VOLATILES

Nending Muni[1], Yallappa Rajashekar[2] and Jharna Chakravorty[1]*

[1]Department of Zoology, Rajiv Gandhi University, Rono Hills, Itanagar 791112, Arunachal Pradesh, India
[2]Insect Bioresource Laboratory, Animal Bioresources Programme, Institute of Bioresources and Sustainable Development, Department of Biotechnology, Govt. of India, Takyelpat, Imphal 795001, Manipur, India
*Email: jharna.chakraborty@rgu.ac.in (corresponding author): ORCID ID 0000-0003-3705-8970

ABSTRACT

The electrophysiological and behavioural responses of *Anomala dimidiata* (Coleoptera: Rutelinae) were studied for its host kiwi plant's (*Actinidia deliciosa*) three volatiles, (Z)-3-Hexen-1-ol, Linalool, and (-)-α-Pinene. This experiment comprises electroantennographic assay using EAG and field testing in the Kiwi Horticulture field using synthetic compounds. In EAG assays, all three test compounds elicit significant responses with 55.66± 3.41% for (Z)-3-Hexen-1-ol, 44.97± 1.08% for Linalool, and 53.75± 0.33% for (-)-α-Pinene in male beetles and 54.82± 3.52% for (Z)-3-Hexen-1-ol, 44.97± 0.30% for Linalool and 53.75± 4.69% for (-)-α-Pinene in females beetles as compare to reference compound. In the field testing, (Z)-3-Hexen-1-ol baited traps caught maximum beetles while (-)-α-Pinene-baited traps had significantly less number of beetles attracted to them. Linalool-baited traps caught 60.1% fewer beetles than (Z)-3-Hexen-1-ol baited traps but 62.7% more beetles than (-)-α-Pinene- baited traps. This study concludes that (Z)-3-Hexen-1-ol is a potential kairomone for *A. dimidiata*.

Key words: Allelochemical, electroantennogram, leaf-chafer, kairomone, kiwi fruit, plantation, phytophagous, Rutelinae, white grub, baited trap, attraction

Actinidia deliciosa (Kiwi) has recently become an important cash crop in Arunachal Pradesh, India (Singh et al., 2008). The leaf chafer beetles of the subfamily Rutelinae (Coleoptera: Scarabaeidae) consist of phytophagous beetle and is commonly included in the white grub pest family (Sushil et al., 2006). One of these white grub species, *Anomala dimidiata* (Hope), is observed to be infesting the leaves of the kiwi tree. This species is economically significant, with the adults mostly defoliating the leaves of different fruit trees and the larvae feeding on various ground crops (Sharma et al., 2021). They are widely distributed throughout the Indian subcontinent, including the Northeastern state of Arunachal Pradesh (Chandra and Gupta, 2012; Devanda et al., 2022). *Anomala* species are highly diversified generalist pests, and their adults are known to feed on the foliage of various trees and shrubs (Sushil et al., 2006; Rahman et al., 2017). For any generalist insect herbivore, locating its host plants successfully in a complex environment with different arrays of volatiles in the air is crucial yet complex for feeding or oviposition. Unlike specialist species, the polyphagous species utilize hundreds of plant species across multiple plant families for the same (Bernays & Chapman, 1994; Forister et al., 2015). Insect herbivores possess the olfactory mechanisms necessary to detect their host-plant volatile cues, as demonstrated by the mechanisms by which all generalist and specialist herbivores can optimize their foraging for suitable hosts. Previous experiences also enhance host location finding with olfactory and visual cues.

Due to an insect's limited neural capacity to process multiple sensory inputs, it is usually believed that generalist herbivores may find it challenging to choose among alternative host plants (Agrawal, 2001; Carrasco et al., 2015). Thus, competing sensory inputs from the potential host make it slower to decide than the specialist (Bernays and Funk, 1999). Despite this fact, the generalist species like *A. dimidiata* occurs in nature and florist to an enormous extent, even causing substantial economic losses in agriculture fields. However, it is still challenging to ascertain how these herbivores recognize and locate their hosts (Bruce and Pickett, 2011). Many phytophagous chafer species have been explored for sex pheromones and allelochemicals with conformation, and few have been promoted for biomonitoring and pest management in agro-ecosystems (Klein et al., 1981; Leal, 1998; Ruther et al., 2005). Leaf-derived allelochemicals (kairomones) have been identified for several *Anomala* species (Vuts et al., 2014), but none are available in the literature for *A. dimidiata.* This study used synthetic versions of three volatile compounds present in its important host plant (*A. deliciosa*). We tested these compounds for their attractant potential to *A. dimidiata* using Electroantennography and Field application techniques, which can be of huge importance in its management in agroecosystem.

MATERIALS AND METHODS

Adult *Anomala dimidiata* were collected from their infested host plant kiwi trees (*Actinidia deliciosa)* for EAG assay from the field study site in the Old Ziro geographical locality (27°34'19.9" N and 93°48'19.0" E), District Lower Subansiri, Arunachal Pradesh, India during June 2022. For identification purposes, referred specimens to APRC ZSI (Zoological Survey of India), Itanagar, examined by Chandra and Gupta (2012) and pinned specimens were deposited at the same. The electrophysiological assay was done in a 2-channel Signal Aquisition interface IDAC-2, Syntech, Netherlands. Sample preparation for EAG recording was done as suggested by Olsson and Hansson (2013), and Du et al. (2019). It measures the difference in electrical potential between the proximal and distal portions of the antennal flagellum brought on by olfactory stimulation. It counts the total electrical potentials generated by olfactory sensory neurons (OSNs) that are active on an insect's antennae (Roelofs, 1984). Beetles were treated with cold anaesthesia before having an antenna cut off at the base with a dissection scissor. A small amount of electrode gel was used to quickly attach the antenna to both sides of a two-pronged electrode "fork" recording probe (base in the ground position) for EAG recordings. A stimulus air controller (Type CS-55, Syntech, Netherlands) was used to deliver the stimulus. With a supplemental air supply running at 4 m/s connected to the stimulus applicator, the constant air flow was kept at 4-5 m/ s. For the assay, the test compounds (Z)-3-Hexen-1-ol (99%), Linalool (99%), and (−)-α-Pinene (99%) was dissolved in hexane, and aliquots of 10 µl were made, pipetted onto a strip of filter paper (1 cm in diameter), and then put into a fresh, disposable Pasteur pipette for each recording. Hexane alone treated filter strip served as control, and Anethol, a known attractant of *Anomala* spp., was used as a reference. 20 µl (70%) dosage per filter paper strip was chosen as the optimum dose for all recordings after dose-response trials revealed that it elicited a good response. Each recording was carried out independently in 3 replicates, with alternate intervals of 40 seconds to allow for antennal receptor recovery. Signal acquisition and data processing were done using the Syntech IDAC-2 signal acquisition system and EagPro signal acquisition software. For EAG data sorting, the magnitude of depolarization elicited by test compounds was subtracted from the amplitude of the reference response. The % values of the mean reactions to the reference stimuli were converted from millivolt responses for all other stimuli and represented in a graph (Jyothi et al., 2002).

The field study was conducted for three months, from June to August 2022, to evaluate the effect of the volatiles as a potential attractant of *A. dimidiata*. Since the study field is a hilly slope and the plantation is very close to the ground, the baited pitfall trapping method was used with a similar design used for ground beetles (Hoekman et al., 2017) but with few modifications. The plastic cup used in the trap was 20 cm in height and 15cm in diameter. The experiments were done in a kiwi field (≈1.4 acres) at Keliya locality, Old Ziro, Arunachal Pradesh, India (27°34'19.9" N and 93°48'19.0" E). One trap was baited only with the attractant for *Anomala spp.* Anethol (0.1 ml) was considered a positive control; another trap was left unbaited, and the other three traps were baited with test compounds (Z) 3-Hexen-1-ol, Linalool and (-)-α-Pinene (0.1 ml each) respectively. These 5 traps were set out in an experimental field in 3 rows with the alternating position of positive control bait, unbaited and baited with test compounds (all in three replicates each). Traps were spaced 20 m apart in a 3×5 grid within the experimental field. 0.1 ml of 99% test compound in a vial with the pierced cap was taped to the interior wall of a trap to allow evaporation of the chemical. The vials were refilled every week. 5 cm water was added to each trap to preserve captured insects. Insects were collected weekly and identified based on their characteristic morphological tibial features as well as anatomical features of genitalia using a LEICA S8 APO B stereomicroscope (Leica Microsystems CMS GmbH, Wetzlar, Germany). The data were checked for normality using the Kolmogorov-Smirnoff test. EAG responses were analyzed for analysis of variance, followed by Tukey's post hoc test to compare the mean at a significance level of $p < 0.05$. Similarly, the field trapping experiment was analyzed by analysis of variance followed by Dunnett's multiple comparisons test. All these tests were performed using GraphPad Prism 8.4.0. (GraphPad Software Inc., California, USA).

RESULTS AND DISCUSSION

The EAG responses of *A. dimidiata* (Fig. 1), its antennae to all three test compounds are substantial for both male and female beetles (Fig. 2). For every puff of volatile, a typical negative waveform was produced, indicating a rapid depolarization of the antenna shown in negative mV. It was followed by a slower recovery phase, back to the standing potential within a few milliseconds. EAG negative amplitudes were expressed as the mean (±standard error) of the three replicate readings. The two-way analysis of variance showed a significant difference in the mean responses of antennae of *A. dimidiata* to 3 different test compounds as compared to the reference ($F(11,11)=5.549$, $P<0.01$). However, no significant difference was observed in the mean responses of antennae of male and female beetles to 3 different test compounds ($F(1,11)=0.2176$, $P>0.05$). The responses to Linalool are most significant in males and females compared to the reference (Tukey's *post hoc* test; $P<0.05$). The relative responses show a small range of distinction in EAG profiles for 3 test compounds, with overall (-)-α-Pinene having the highest percentage of response intensity and Linalool being the least. The percentage magnitude of depolarization elicited by the test compound compared to the reference amplitude was found to be 55.66± 3.41 for (Z)-3-Hexen-1-ol, 44.97± 1.08 for Linalool and 53.75± 0.33 for (-)-α-Pinene in males; 54.82± 3.52 for (Z)-3-Hexen-1-ol, 44.97± 0.30 for Linalool and 62.00± 4.69 for (-)-α-Pinene in females (Fig. 3). In the field testing, one-way ANOVA showed that a significantly greater number of *A. dimidiata* were caught in (Z)-3-Hexen-1-ol baited traps as compared to Linalool and (-)-α-Pinene baited (male $F(2,114)=66.04$, $P<0.0001$; female $F(2,114)=19.31$, $P<0.0001$). The mean number of beetles also caught significantly differed for three different baited traps in both male and female cases by Dunnett's multiple comparisons test (α= 0.05). The mean number of male beetles caught was found to be 0.179± 0.009 in unbaited traps, 6.59± 0.06 in Anethol baited traps (positive control), 6.85± 0.07 in (Z)-3-Hexen-1-ol baited traps, 2.69± 0.06 in Linalool baited traps and 1.28± 0.04 in (-)-α-Pinene baited traps; and the mean number of female beetles caught was found to be 0.051± 0.006 in unbaited traps, 6.97± 0.06 in Anethol baited traps (positive control), 6.07± 0.08 in (Z)-3-Hexen-1-ol baited traps, 3.79± 0.07 in Linalool baited traps and 2.33± 0.05 in (-)-α-Pinene baited traps during June, July and August 2022 (Fig. 4). The weekly mean number of beetles caught during three months are projected in figure 5 (Fig. 5).

Fig. 1. *Anomala dimidiata* A. Male dorsal view B. lateral view

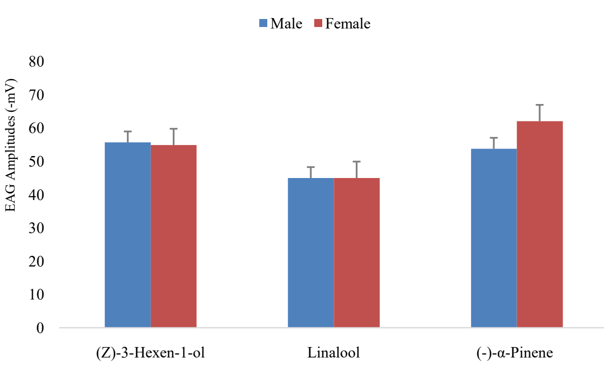

Fig. 2. EAG responses of *A. dimidiata* to three host's volatile compounds. Two-way ANOVA followed by Tukey's Post Hoc Test: **p< 0.05

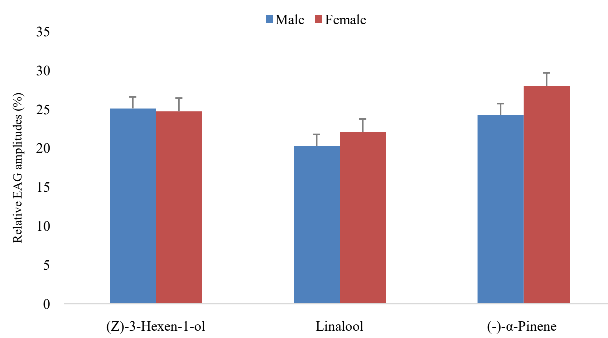

Fig. 3. Relative EAG amplitudes (%) of different test compounds against *A. dimidiata.* Values are the mean (±SEM) of three EAG responses expressed as % responses relative to the reference.

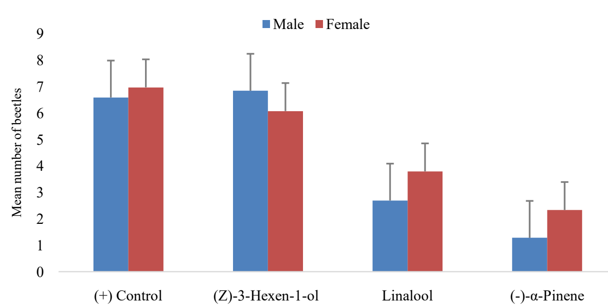

Fig. 4. Mean (±SEM) number of adult *A. dimidiata* caught in different traps from June to August 2022 in Kiwi Field, Ziro, Arunachal Pradesh. Both male and female means differ significantly at $p < 0.05$ (Dunnett's Multiple Comparisons Test).

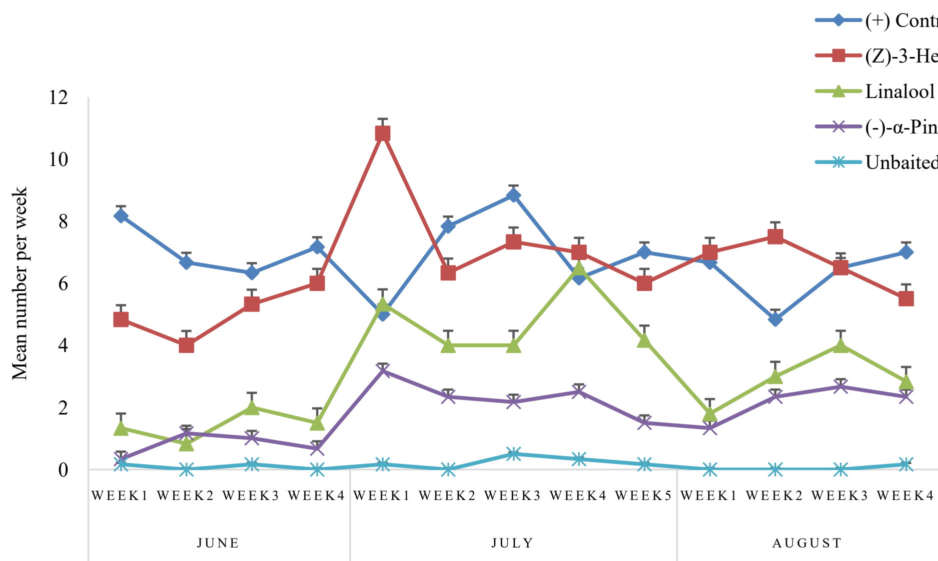

Fig. 5. Weekly Mean (±SEM) number of adult *A. dimidiata* trapped in different baited traps from June to August 2022 in Kiwi Horticulture Field, Ziro, Arunachal Pradesh. (Week1= 1st Sunday of that month)

Our results demonstrated that this economically significant leaf chafer beetle in laboratory experiments responds well to the synthetic version of 3 volatiles present in its host plant, *A. deliciosa*. The GCMS profile of this host plant showed that it contains these three test compounds (Unpublish observation). Our EAG experiments indicate that (-)-α-Pinene elicits a maximum electrophysiological response compared to Linalool and (Z)-3-Hexen-1-ol. Though EAG responses are generally treated as qualitative rather than quantitative indicators of olfactory reception (Olsson and Hansson, 2013), the amplitude elicited by (-)-α-Pinene is equivalent to a reference compound Anethol which is a well-known attractant for many *Anomala spp.* (Leal et al., 1993; Cherry et al., 1996). So far, no literature reports are available conforming (-)-α-Pinene as an attractant to *Anomala* spp. On the other hand, (Z)-3-Hexen-1-ol also has more or less similar attractant potential in EAG as (-)-α-Pinene. Moreover, (Z)-3-Hexen-1-ol is reported as allelochemical with an attraction effect for *Anomala corpulenta* (Motschulsky) and *Anomala exoleta* (Falderman) (Li et al., 2013). Our field observations showed that (Z)-3-Hexen-1-ol baited traps caught the highest number of adult beetles. This common leaf alcohol has been widely explored for its role in the plant-insect interaction of many leaf chafer species. However, no study has been projected in context to *A. dimidiata*. The possible explanation for the attraction of *A. dimidiata* to (Z)-3-Hexen-1-ol may lie in the fact that many related generalists leaf chafer species attract a common allelochemical compound as part of their host detection, which could be the result of host plants overlapping, unlike specialist species. Contrasting to (-)-α-Pinene, monoterpenoid alcohol, Linalool has a few reports of semiochemical roles in *Anomala's* related leaf chafer beetle species, acting as sex pheromone of *Holotrichia parallela* Motschulsky (Leal et al., 1993) which belonging to the subfamily Melolonthinae and as an attractant of *Phyllophaga congrua* (Melolonthinae) (Crocker et al., 1999) and *Popillia japonica* Newman (Rutelinae) (Tumlinson et al., 1977). Nevertheless, Linalool-baited traps caught 60.1% fewer beetles than (Z)-3-Hexen-1-ol baited traps, but it caught 62.7% more beetles than (-)-α-Pinene, which is in contrast to electrophysiological results. Interestingly, the other similar adult leaf chafer species, such as *Phyllophaga* sp., *Holotrichia* sp., *Mimela* sp. and *Lepidiota* sp., were also observed to be sometimes caught in the baited traps along with *A. dimidiata,* which indicates that these test compounds possess attractant potential to those species as well, but further investigation is required.

This study mainly aimed to determine whether any of the three host-leaf volatiles have any field attraction to this important pest species in the kiwi field. Since the attractant activity of (Z)-3-Hexen-1-ol to *A. dimidiata* in the lab experiment and the field test has been demonstrated significantly, it will be prudent to research effective release rates and optimum concentration levels and the impact of using this compound in the agro-ecosystem. (-)-α-Pinene also showed good potential in EAG responses, but the contradicting result in the field study gave an opinion that it is not a major attractant like (Z)-3-Hexen-1-ol to *A. dimidiata*. Linalool, though described as allelochemical with an attraction effect to related leaf chafer species, does not seem to have a major effect but rather a mild attractant to *A. dimidiata.* Exploiting such attractant compounds with potential bioactivity is important for their utilization in field monitoring, mass trapping, and pest management (Murali-Baskaran et al., 2018; Weber et al., 2022).

ACKNOWLEDGEMENTS

The authors wish to thank Director, Institute of Bioresources and Sustainable Development, Manipur, Govt. of India, for kindly permitting the experimental work in his institute and the Head, Department of Zoology, Rajiv Gandhi University, Arunachal Pradesh, for providing the facilities.

FINANCIAL SUPPORT

The authors are thankful to the Department of Biotechnology, Govt. of India (BT/NER/95/SP42584/2021) for the financial support through a project grant to Dr Jharna Chakravorty.

AUTHOR CONTRIBUTION STATEMENT

J C, N M and Y R conceived the idea and designed the study. N M collected the samples, performed the experiments, analyzed the data and provided the first draft. N M, J C, and Y R wrote and edited the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Agrawal A A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294(5541): 321-326.

Bernays E A, Chapman R F. 1994. Host-plant selection by phytophagous insects. London: Chapman & Hall. 312 pp.

Bernays E A, Funk D J. 1999. Specialists make faster decisions than generalists: experiments with aphids. Proceedings of the Royal Society of London. Series B: Biological Sciences 266(1415). pp. 151-156.

Bruce T J A, Pickett J A. 2011. Perception of plant volatile blends by herbivorous insects- finding the right mix. Phytochemistry 72(13): 1605-1611.

Carrasco D, Larsson M C, Anderson P. 2015. Insect host plant selection in complex environments. Current Opinion in Insect Science 8: 1-7.

Chandra K, Gupta D. 2012. Pleurostict scarabs (Coleoptera: Scarabaeidae): new distributional records in Arunachal Pradesh, north-east India. Check List 8(5): 889-893.

Cherry R, Klein M, Leal W. 1996. Attraction of adult *Anomala marginata* (Coleoptera: Scarabaeidae) to anethole. Journal of Agricultural and Urban Entomology 13: 359-364.

Crocker R L, Klein M G, Xu W, Jailon W T. 1999. Attraction of *Phyllophaga congrua*, *Phyllophaga crassissima*, *Phyllophaga crinite*, and *Cyclocephala lurida* (Coleoptera: Scarabaeidae) adults to selected volatile compounds. Southwestern Entomologist 24: 315-319.

Devanda M, Mandyam S, Jayashankar M. 2022. New location reports of *Anomala dimidiata* ssp. *dimidiata* (Hope, 1831) (Coleoptera: Scarabaeidae) in Arunachal Pradesh, India. Insect Environment 25(1).

Du Y, Grodowitz M J, Chen J. 2019. Electrophysiological responses of eighteen species of insects to fire ant alarm pheromone. Insects 10: 403

Forister M L, Novotny V, Panorska A K, Baje L, Basset Y, Butterill P T, Cizek L, Coley P D, Dem F, Diniz I R, Drozd P. 2015. The global distribution of diet breadth in insect herbivores. Proceedings of the National Academy of Sciences 13:112(2): 442-7.

Hoekman D, LeVan K E, Gibson C, Ball G E, Browne R A, Davidson R L, Erwin T L, Knisley C B, LaBonte J R, Lundgren J, Maddison D R. 2017. Design for ground beetle abundance and diversity sampling within the National Ecological Observatory Network. Ecosphere 8(4): e01744.

Jyothi K N, Prasuna A L, Sighamony S, Kumari B K, Prasad A R, Yadav J S. 2002. Electroantennogram responses of *Apanteles obliquae* (Hym., Braconidae) to various infochemicals. Journal of Applied Entomology 126(4): 175-181.

Klein M G, Tumlinson J H, Ladd Jr T L, Doolittle R E. 1981. Japanese beetle (Coleoptera: Scarabaeidae): response to synthetic sex attractant plus phenethyl propionate: eugenol. Journal of Chemical Ecology 7(7).

Leal W S. 1998. Chemical ecology of phytophagous scarab beetles. Annual review of entomology 43(1): 39-61.

Leal W S, Sawada M, Matsuyama S, Kuwahara Y, Hasegawa M. 1993. Unusual periodicity of sex pheromone production in the large black chafer *Holotrichia parallela*. Journal of Chemical Ecology 19(7): 1381-1391.

Li W, Yang L, Shen X, Yuan Y, Yuan G, Luo M, Guo X (2013) Prescription screening and field evaluation of broadspectrum attractants of scarab beetles from *Ricinus communis*. Chinese Journal of Eco-Agriculture 21: 480-486.

Murali-Baskaran R K, Sharma K C, Kaushal P, Kumar J, Parthiban P, Senthil-Nathan S, Mankin R W. 2018. Role of kairomone in biological control of crop pests-A review. Physiological and Molecular Plant Pathology 101: 3-15.

Olsson S B, Hansson B S. 2013. Electroantennogram and single sensillum recording in insect antennae. In Pheromone signaling Humana Press, Totowa, New Jersey. pp. 157-177.

Rahman A, Bathari M, Borah P, Taye R R, Patgiri P. 2017. Diversity of insect species along with their host in Assam Agricultural University, Jorhat. Journal of Entomology and Zoology Studies 5: 2307-2312.

Roelofs W L. 1984. Electroantennogram assays: rapid and convenient screening procedures for pheromones. In Techniques in pheromone research, Springer, New York. pp. 131-159.

Ruther J, Mayer C J. 2005. Response of garden chafer, *Phyllopertha horticola*, to plant volatiles: from screening to application. Entomologia Experimentalis et Applicata 115(1): 51-59.

Sharma P K, Shah M L, Mishra A K. 2021. Biology of white grub *Anomala dimidiata* (Hope)(Coleoptera: Scarabaeidae) in agricultural ecosystem, Doon Valley (UK), India. Environment Conservation Journal 22(1, 2): 147-151.

Singh A, Patel R K, Verma M R. 2008. Popularising kiwifruit cultivation in North East. Envis Bulletin Himalayan Ecology 16(1): 18.

Sushil S N, Mohan M, Selvakumar G, Bhatt J C. 2006. Relative abundance and host preference of white grubs (Coleoptera: Scarabaeidae) in Kumaon hills of Indian Himalayas. Indian Journal of Agricultural Sciences 76: 338-339.

Tumlinson J H, Klein M G, Doolittle R E, Ladd T L, Proveaux A T. 1977. Identification of the female Japanese beetle sex pheromone: inhibition of male response by an enantiomer. Science 197(4305): 789-92.

Vuts J, Imrei Z, Birkett M A, Pickett J A, Woodcock C M, Tóth M. 2014. Semiochemistry of the Scarabaeoidea. Journal of Chemical Ecology 40: 190-210

Weber D C, Konstantinov A S, Khrimian A, Bier A D, Lubenow L A, Knodel J J, Haber A I, Wallingford A K, Mason J A, Kuhar T P. 2022. Trapping of Crucifer-Feeding Flea Beetles (*Phyllotreta* spp.) (Coleoptera: Chrysomelidae) With Pheromones and Plant Kairomones. Journal of Economic Entomology 115(3): 748-56.

(Manuscript Received: December, 2022; Revised: May, 2023;
Accepted: May, 2023; Online Published: May, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23959