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ABSTRACT

In the present study maize plant volatiles were collected by solvent assisted extraction (SAE) using hexane and dichloromethane (DCM) solvents. The evaluation of volatile extracts against male individuals of *Chilo partellus* through Gas Chromatography-Electroantennogram Detector (GC-EAD) revealed the presence bioactive compounds. The identification of these bioactive compounds through Gas Chromatography-Mass Spectroscopy (GC-MS) revealed that male individuals of *C. partellus* elicited response to 20 maize volatile compounds, out of which benzyl alcohol and myristic acid were detected in both the solvent extracts. These bioactive volatile compounds can be further explored for developing lures either of plant volatiles or in combination with pheromone.

Key words: Solvent assisted extraction, hexane, dichloromethane, *Chilo partellus*, GC-EAD, GC-MS, benzyl alcohol, myristic acid, solvent extracts, bioactive, plant volatiles, pheromone

Plants in addition to non-organic volatiles (CO₂, O₂) emit volatile organic compounds (VOCs) which plays very important role of communication signals between them and organisms in their surroundings (Delory et al., 2016; Johnson et al., 2016). In context of insect-plant interaction these VOCs act as an important signal for herbivores, parasitoids, predators, and neighbouring plants (Aartsma et al., 2017; Bruce et al., 2005). Herbivore insects use VOCs not only to find their host plants but also to find mating partners by means of species-specific pheromones. Though maize plant VOCs and their effect on insect pests have been reported earlier (Birkett et al., 2006; Chamberlain et al., 2006), natural enemies of different pests (Mutyambai et al., 2015; Mutyambai et al., 2016; Suby et al., 2018; Tamiru et al., 2011) but information on the response of male Chilo partellus to maize VOCs is wanting. Therefore, the present study evaluating the electrophysiologically active solvent extracted maize VOCs against male C. partellus.

MATERIALS AND METHODS

The larvae of *C. partellus* were collected from sorghum fields situated at Hapur, Uttar Pradesh, India (28.7306° N, 77.7759° E) and laboratory mass

multiplication was carried out on natural diet (baby corn) in a controlled condition $(27\pm1^{\circ}C, 65\pm5\% \text{ RH},$ L16: D8). To obtain unmated males for the experiment, pupa once formed were isolated through sexing and kept in separate glass jars (15 cm dia and 20 cm height till adult emergence). One to two days old unmated male moths were used for the experiment. Maize plants (variety HQPM-1) were grown in groups of 4-5 plants in a pot (27 cm diameter and 25 cm height) filled with compost and soil in glasshouse. Volatiles were collected from healthy, 15-20 days old plants using DCM (HPLC grade, CDH®) and n-hexane (HPLC grade, CDH®), separately. 100 g of freshly cut leaves were dipped in chilled solvent (300ml) for 5 hrs with intermittent shaking. The extract was filtered using Whatman No. 1 filter paper and passed through anhydrous sodium sulphate to remove moisture. The filtrate was concentrated using vacuum evaporator and stored at -80 °C till further use. Gas Chromatography coupled Electroantennography Detector (GC-EAD) and Gas chromatography coupled mass spectroscopy (GC-MS) was used to identify insect (unmated one day old male moth) response to the volatile compounds contained in extracts. Peaks eluting from the GC column were considered to be active if they elicited EAG

activity in three or more of the fine-coupled run. The traces from Flame Ionization Detector (FID) and EAD were plotted and the matching peaks along with their retention times were determined. Data were analyzed using Chemstation software (version 2.7c, Syntech, Germany).

RESULTS AND DISCUSSION

GC-EAD analysis of DCM extracts revealed eleven bioactive compounds viz., 2-methyl-2-butanol, 2-methyl-2-chlorobutane, cis-4-hexen-1-ol,1-methylcis-4-isopropylcyclohexane, 1-dodecene, benzyl alcohol, 2,6-dimethyldecane, naphthalene, myristic acid, 2-(2,5-dimethoxy-phenyl)-propionaldehyde and 2-hexadecen-1-ol, 3,7,11,15-tetramethy (Fig. 1a). Similarly, eleven bioactive compounds viz., 2-butyltetrofuran, 3-penten-2-one, methyl, 4-hydroxy-4-methyl-2-pentanone, 4-methyl-4-methoxy-2pentanone, benzyl alcohol, 2-methoxy phenol, n-pentadecanal, n-tetradecanoic acid, octadecane, (9E,12E,15E)- octadecatrin-1-ol and hexadecanoic acid were identified from n-hexane extract Fig. 1b. Benzyl alcohol and myristic acid (tetradecanoic acid) was the common bioactive compound in both extracts. VOCs such as myristic acid (tetradecanoic acid), 3,7,11,15-tetramethyl-2-hexadecen-1-ol and hexadecanoic acid were earlier detected in the solvent extracts from maize leaves and stems wherein hexadecanoic acid was reported to be having a role in defence system as it is released in higher concentration from uninfected maize plant (Kasim et al., 2018). Konstantopoulou et al. (2004) reported the presence 3,7,11,15-tetramethyl-2-hexadecen-1-ol and n-pentadecanal from steam distillate of maize leaves. 4-hydroxy-4methyl-2-pentanone was also one

of the bioactive maize volatiles detected in headspace sampling (Guleria et al., 2021). Earlier literature also suggests that the bioactive compounds identified in present study have a role in attraction of different insects. Benzyl alcohol was found eliciting attraction in light brown apple moth Epiphyas postvittana (El-Sayed et al., 2016); gram caterpillar, Helicoverpa armigera (Bruce et al., 2002); dried bean beetle, Acanthoscelides obtectus (Vuts et al., 2021); alfalfa looper moth, Autographa californica (Landolt et al., 2001); Bihar hairy caterpillar, Spilosoma obliqua (Mobarak et al., 2022). Other than insect pests, Proffit et al. (2020) reported benzyl alcohol in the complex mixture of VOCs emitted by receptive fig (*Ficus carica*) as one of electrophysiologically (GC-EAD) active compound to Blastophaga psenes. Naphthalene was reported as an electrophysiologically active VOC from maize against European corn borer Ostrinia nubilalis (Molnar et al., 2015). The bioactive compounds identified in present study from maize plants especially benzyl alcohol and 4-methyl-4-methoxy-2pentenone can be further explored for their application in development of novel lures for attraction of C. partellus males and its behaviour manipulation.

ACKNOWLEDGEMENTS

ICAR- Indian Agricultural Research Institute, ICAR- Indian Institute of Horticultural Research and ICAR- Indian Institute of Maize Research are acknowledged for providing facilities.

AUTHOR CONTRIBUTION STATEMENT

NG and SMN conceived and designed the research problem. NG and PSK conducted the experiment. PDKJ and SMN provide the lab facilities. NG and SMN analysed the data. NG, SBS and SMN wrote



Fig. 1. GC-EAD response of unmated male *Chilo partellus* to constituents of maize (a) Solvent extracts of DCM and (b) Solvent extract of Hexane

the manuscript. All authors read and approved the manuscript

CONFLICT OF INTEREST

No conflict of interest.

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(Manuscript Received: September, 2023; Revised: December, 2023; Accepted: January, 2024; Online Published: February, 2024) Online First in www.entosocindia.org and indianentomology.org Ref. No. e24626