Host Plant Volatiles Released by *Bracharaira brizantha* and *Desmodium* spp. and Their Effects on the Behaviour of Fall Armyworm

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Abstract- *Desmodium spp. and Napier-like grasses are widely used as push-pull systems for mange fall armyworm (FAW) in Africa. However, the responsible cues for the olfactory responses of the FAW was not been identified yet. Hence, this study was conducted to identify volatile compounds release by Desmodium spp. and Bachiraria brizantha plants and determine their role on behaviour modification of FAW adults and the larvae. Four different plant species i.e., B. brizantha, Desmodium heterophullum, D. triflorum and D. interim were collected, and volatiles were extracted using dynamic head space and solvent extraction methods. The chemical compounds were identified using GC-MS. Behavioural studies were conducted for selected volatiles based on availability. This study identified 29 volatile compounds released by Desmodium spp. where 30 compounds were identified from B. brizantha. Behaviour study results indicated that females were attracted to n-hexane (72.73%) and Limonene (-) (66.67%), where naphthalene (70%) showed a repellent behavior. Males were significantly repelled by 3-Hexen-1-ol, Limonene (-), and Limonene (+). Neonates significantly attract to Limonene (-) (69.23%) and repel by 1-Octen-3-ol. Therefore, it can be concluded that this behavior modified volatile compounds can be used to develop semiochemical based green pest management strategies.*

Keywords: Pest management strategy, Host plant volatiles, Desmodium, Push-pull system

I. INTRODUCTION

Fall armyworm (FAW) *Spodoptera frugiperda* Smith *(*Lepidoptera, Noctuidae*)*, is a native pest of America, widely spread throughout the tropical and subtropical areas and it has been recognized as an economical pest of maize for more than decades (Groote *et al*., 2020). The polyphagous nature of the larvae feeds on a variety of crop species, other than maize *i.e.* sorghum, millet, and cotton, sugarcane, and vegetable crops. The damage beyond the American and African continent appeared on the Indian subcontinent in Asia in May 2018, and it has since spread across India, Nepal, Bangladesh and Sri Lanka (Lamsal *et al*., 2020) and presently become a major concern in food security in these countries. In 2017, Centre for Agriculture and Bioscience International (CABI) estimated that, due to unavailability of effective control methods, FAW causes annual maize yield losses of 8.3 to 20.6 million tons (that equal to feed 40.8 to 101 million people) which estimated 2.5-6.2 USD billion in 12 African maize-producing countries (Day *et al*., 2017). To control the pest, farmers have been tried several methods including the application of chemical pesticides, biopesticides, genetically improved plants containing Bt genes. Even though access is easy to chemical pesticides, it may cause development of resistant populations, reduction of natural enemies moreover lack of awareness on proper use, consistency of use, low purchasing power and the limited choice of pesticide products (Midega *et al*.2017) becoming the chemical control less effective. Hence Integrated Pest Management packages with minimizing pesticide application and use of natural enemies becoming popular among farmers in Africa and identified as a suitable and cost-effective method is known as the push-pull system (Day *et al*.,2017) The "Push-Pull" also known as stimulus-deterrent diversionary strategy technique to control the pest through using both strategies at the same time as employing attracting and repellent from the crops (USAID, 2021). In push-pull strategy, they used Napier grass as the border crop with silver leaf desmodium (*Desmodium uncinatum*) as the intercrop with maize. In push-pull strategy, they used Napier grass as the border crop with silver leaf desmodium (*Desmodium uncinatum*) as the intercrop with maize. The desmodium plant emits volatile compounds that are disgusting to behave female moths hence acting as a 'push' whilst a grass such as Napier emits attractive chemical compounds that ‘pull’ and attract the moths towards itself. Volatiles, (E)-ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene emitted by the desmodium repel (push) the stemborer pests while chemicals produced by Napier and Sudan grasses attract (pull) moths to lay eggs in the grass instead of maize. Further, hexane, (E)-2, hexenal (Z),-3, Hexen-1-ol have been shown in Napier grass than maize or sorghum in host plants to produce higher levels of attraction for Stem borers(Khan *et al*., 2011). According to Ayurvedic Plants of Sri Lanka: Plants List in Sri Lanka listed several spp of desmodium viz. *Desmodium heterophyllum* (Maha udupiyaliya), *D. gangeticum* (Shaliparni), *D.triflorum* (Heen udupiyaliya) and *D. uncinaium* (Jayawardana, 1985) present in Sri Lanka and similar volatiles can be found from them. As same *Brachiaria brizantha* (Signal grass) is one of the major pasture grasses largely grown in the country (Fernando *et al*.,1958) may having similar volatiles to the napier grass due to one of the host plant of FAW may be having the potential to use as push pull systems with using identified volatile cues. Therefore, this study was conducted to identify volatile compounds released by *Desmodium* spp and *B. brizantha* plants and effect of their behavior modification of FAW neonates and moths.

II. MATERIALS AND METHODS

A. *Plant materials and insect culture*

The plant materials of *B. brizantha*, *D. heterophullum, D. triflorum* and *D. intortum* were collected from different locations of Sri Lanka from September 2020 to February 2021. Healthy leaf samples which were well exposed to light and free from disease and insects, weather or mechanical injury were used for volatile extraction.

FAW larvae were collected from maize fields at Agrotec park, Malwatte and reared on natural diets as a method given by Du Plessis *et al*., (2020) in particular conditions in the laboratory as 12 L:12D photoperiods under 30°C temperature and 70-75% RH condition until moth emerge. Then the moths were placed in the 2.5 L plastic bottles while providing the 10 % honey solution. The lid of the bottle was covered with muslin cloth to facilitate the ventilation and substrate for egg laying. After laying egg, egg masses were kept separately in the new bottles as same above and were monitored daily until hatching, as soon as the first instars emerged, they were provided fresh leaves from maize seedlings until third instar. To avoid the cannibalism third instar larvae were placed individually in ventilated small plastic jars (150 ml capacity) while providing fresh maize leaves. The pupae were separated male and female under the dissecting microscope following the method described by Sharanabasappa *et al*., (2018). Adult males and females were mixed in oviposition cages by providing 10 % honey solution and freshly mated females were obtained for behavior studies after two days of emergence.

B. *Volatile collection*

The solvent extraction and dynamic headspace collection methods were used to extract volatiles. Insolvent extraction method 50g of each sample was measured using an electronic balance (KERN $_{\mathrm{PFB}}$) and cut into small pieces (<1 cm). Then samples were transferred to the reagent bottles and 50 ml 99.9% purity dichloromethane (DCM) HPLC grade was added and kept overnight at room temperature for the extraction completion. Extractions were filtered through a silica gel column to remove impurities. In the headspace collection method, leaf samples were placed in a desiccator and a gently filtered airstream was allowed to pass through the sample. The odour captured air was trapped to porapak Q absorbent (Supelco) 30 mg (50-80 mesh size) with glass wool by aiding the laboratory suction pump. The collection was made 12 hours and trapped volatiles in the absorbent was eluted to 2 ml DCM HPLC grade. The extractions were concentrated using gent nitrogen flow and stored in chromatography vials at -20 ℃ until bioassay.

C. *GC-MS analysis*

The chemical analysis of extracts was carried out using the Agilent Gas Chromatography (GC 8890) coupled Mass Spectrometry (5978B MSD) system at chemical ecology laboratory. The carrier gas Helium (99.999%) was used with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2µl of eluted sample was injected into the column with the injector temperature at 280ºC. GC oven temperature-programmed initially at 50 ºC and hold for 2 min and it was raised to 180 ºC at the rate of 10 ºC/min, and hold 5 min. Then raised to 250 ºC at the rate of 7 ºC/min and hold for 5 min and increase up to 280 ºC and hold for 9 min with a program rate of 5 ºC/min. The injector and detector temperatures were set at 280ºC and 280ºC respectively. The mass data of samples was gained through electron ionization at 70 eV process and the mass detector was operated in scan mode up to 450 amu. The total running time was 50 min. The obtained masses were identified by comparing NIST libraries using chem station software.

D. Chemicals and behavioural studies

The selected pure synthetic (99.99%) chemicals identified from the GCMS analysis purchased from sigma Aldrich were used for the olfactometer studies, it was carried out to check moth behavior against the volatiles using dual choice olfactometer (Figure 1). Selected chemicals with 1% concentration were made dissolving in a solvent (hexane, HPLC grade). The prepared test chemical 100µl was placed one arm of the olfactometer was served as treatment and the other arm was as control. Five insects were placed in the middle of the chamber and the movement of insects was observed and recorded in their active period at night. The bioassay was replicated ten times for each volatile for males and females separately. The end of the bioassay olfactometer was turned 180º from the position to eliminate side effects and at the end of each bioassay, olfactometer was cleaned using 90% ethanol (v/v).

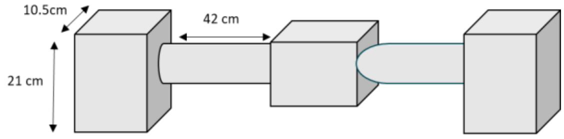

Figure 1: Dual choice olfactometer made up using transparent OHP sheets

E. Olfactometer studies for neonates

The same pure volatile compounds used for the above experiment were used for this study. A dual choice olfactometer test was carried out. The 100 µl of the volatile was sprayed on maize leaf sample and kept on one side of the end of the arm and unsprayed same type of maize leaves were kept on the other side as the control. Ten Neonates were released into the middle of each cylinder and movement of the neonates after 24 hours were recorded. The bioassay was replicated 6 times per each selected volatile (Sisay *et al*., 2019).

F. Data analysis

The Percentage responses of moths to the behavioural studies were compared using Chi-square test as assuming the equal probability (50:50) for both test and control arms using IBM SPSS version 25.

III. RESULTS AND DISCUSSION

The replicated GC-MS analysis results indicated that, 30 volatile compounds from *B. Brizantha* and 29 compounds of all types of *Desmodium spp*. These volatiles consist the alcohols, aldehydes, ketones and esters. Caryophyllene, 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, Hentriacontane, Pentadecane, 1,4-Benzenedicarboxylic acid, dimethyl ester, Oxalic acid, 2-Ethylhexyl tetradecyl ester, Benzoic acid, 4-ethoxy-, ethyl ester, Dodecane, Oxalic acid, allyl hexadecyl ester, Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester were the major component in *B. brizantha* (Table 1 and Figure 3). The volatile compounds *i.e.* Pentadecane, 1,4-Benzenedicarboxylic acid, dimethyl ester, Oxalic acid, 2-Ethylhexyl tetradecyl ester, Cyclooctane, 1,4-dimethyl-, cis-, Benzoic acid, 4-ethoxy-, ethyl ester, 2-methylhexacosane, Oxalic acid, allyl pentadactyl ester, 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate, Heptadecane released by the desmodium spp (Table 2 and Figure 2). According to Khan, *et al* (2007) Africa, molasses grass and *Desmodium uncinatum* (Jacq), releases repellent volatiles to reduce pest populations in maize crops. Forage grasses showed a greater diversity of volatile compounds after damage, including menthone, eucalyptol and camphor while (E)-ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene, released by the desmodium (Silva *et al*., 2019). However, *plant induces leaf volatiles* such as (E)-4, 8-Dimethyl-1, 3, 7-nonatriene, Decanal, (E)-Caryophyllene, Linalool, linalool (plus Nananal), E-β-farnesene, Methyl salicylate and (3E, 7E)-4, 8, 12-trimethyl-1, 3, 7, 11-tri-decatetraene were released by the *B. brizantha* plants when intercropping with *B. brizantha* (Magara *et al*., 2013).

Table 1: Volatile compounds present in *B. Brizantha* identified by GC-MS analysis

Volatile compound	RT	Area
Ethylene oxide	1.3	0.09
Methane, bromo-	1.4	0.17
beta.-Myrcene	10.2	0.21
Octanal	10.5	0.40
3-Hexen-1-ol, acetate, (E)-	10.7	1.58
Eucalyptol	11.4	2.75
D-Limonene	11.2	0.09
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	11.9	0.27
1,6-Octadien-3-ol, 3,7-dimethyl-	13.4	0.22
Nonanal	13.5	0.75
(E)-2-Butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester	13.8	1.36
Levomenthol	15.3	1.13
Naphthalene	15.6	0.29
Decanal	16.1	0.46
Pentadecane	19.0	0.24
Caryophyllene	21.2	0.25
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	21.9	0.17
Hentriacontane	22.7	0.16
Pentadecane	22.7	0.30
1,4-Benzenedicarboxylic acid, dimethyl ester	22.8	0.21
Oxalic acid, 2-Ethylhexyl tetradecyl ester	22.9	0.19
Benzoic acid, 4-ethoxy-, ethyl ester	23.3	1.03
Dodecane	23.6	0.32
Oxalic acid, allyl hexadecyl ester	23.8	0.30
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	24.7	2.53
Heptadecane	26.8	0.45
n-Hexane	1.8	22.08
Ethyl Chloride	1.9	0.72
Toluene	3.4	0.42
3-Hexen-1-ol, acetate, (E)-	10.7	0.16

Table 2: Volatile compounds present in *Desmodium spp* identified by GC-MS analysis

Volatile compound	RT	Area
2-Hexenal, (E)-	5.6146	0.45
1-Octen-3-ol	9.7736	2.50
3-Octanone	10.0169	0.84
Cyclotetrasiloxane, octamethyl-	10.5001	0.63
Eucalyptol	11.3337	1.51
2(3H)-Furanone, 5-ethyldihydro-	12.0266	0.36
Nonanal	13.475	0.49
Cyclohexanol, 5-methyl-2-(1-methylethyl)	15.296	0.75
Naphthalene	15.5372	0.98
Decanal	16.1354	0.25
Eicosane	17.9348	0.29
2-Decene, 7-methyl-, (Z)-	18.5433	0.40
Ketone, methyl 2,2,3-trimethylcyclopentyl	18.7397	0.28
Pentane, 3-methylene-	18.9321	0.26
2-Bromo dodecane	18.999	0.61
Decane, 3,7-dimethyl-	19.4273	0.30
Benzeneacetaldehyde, .alpha.,2,5-trimethyl-	19.7759	0.42
Hentriacontane	22.6558	0.36
Pentadecane	22.7111	0.53
1,4-Benzenedicarboxylic acid, dimethyl ester	22.7613	0.40
Oxalic acid, 2-Ethylhexyl tetradecyl ester	22.8801	0.41
Cyclooctane, 1,4-dimethyl-, cis-	23.2281	0.26
Benzoic acid, 4-ethoxy-, ethyl ester	23.2826	0.91
2-methylhexacosane	23.5571	0.68
Oxalic acid, allyl pentadecyl ester	23.7676	0.59
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	24.7208	3.68
Heptadecane	26.8146	0.49
Butane, 2-chloro-2-methyl-	2.192	0.36
Propanoic acid, 2-methyl	24.7287	1.25

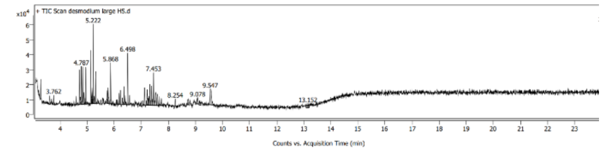

Figure 2: Total ion chromatogram (TIC) obtained by coupled GC-MS analysis of *Desmodium heterophyllum* volatiles

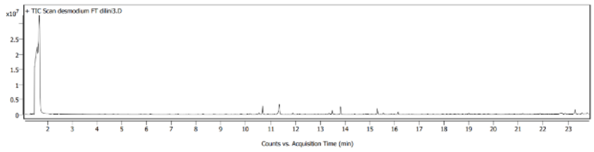

Figure 3: Total ion chromatogram (TIC) obtained by coupled GC-MS analysis of *Brachiaria brizantha* volatiles

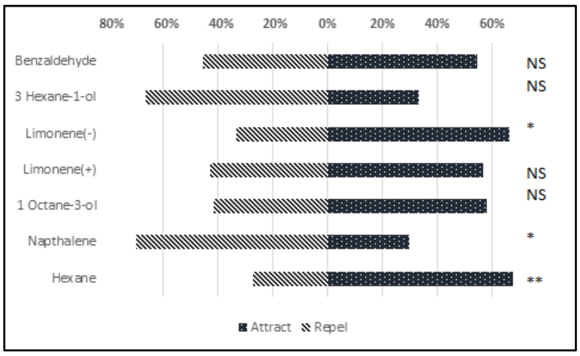

Figure 4: Behavioural response of FAW virgin females to plant volatiles; bars indicate the mean percentage moths found on each sides. * Percentage response between source and control is significantly different at p<0.05 and ** at p<0.01 and NS = non-significant.

Behavioural responses of female for selected volatiles observed a significantly different (p<0.05) with compared to the control. Among them significant proportion of females were attracts towards the hexane (72.73%) (χ2 = 6.81, df = 1, p = 0.009) and Limonene(-) (66.67%)(χ2 = 4.00, df = 1, p = 0.046) at the same time naphthalene (χ2 = 4.80, df = 1, p = 0.028) showed a repellent behaviour by repelling 70% of the female moths form the volatile. However, tested other volatiles showing a neutral effect (Figure. 4).

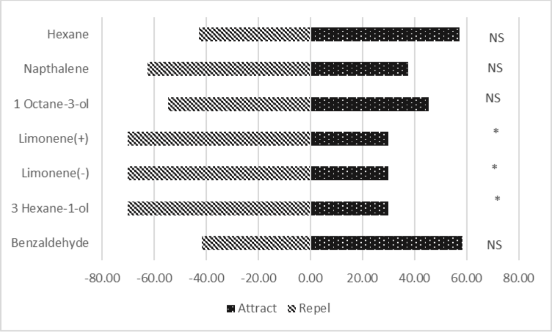

Figure 5: Behavioural response of FAW virgin males to plant volatiles; bars indicated the mean percentage moths found on each side. * Percentage response between source and control is significantly different at p<0.05 and ** at p<0.01 and NS = non-significant.

The behavioural responses of male moths revealed a significantly different repulsive behaviour for selected volatiles i.e. 3-Hexen-1-ol, Limonene (-) and Limonene (+) (χ2 = 4.80, df = 1, p = 0.028) where, others showed a neutral behavior (Figure 5).

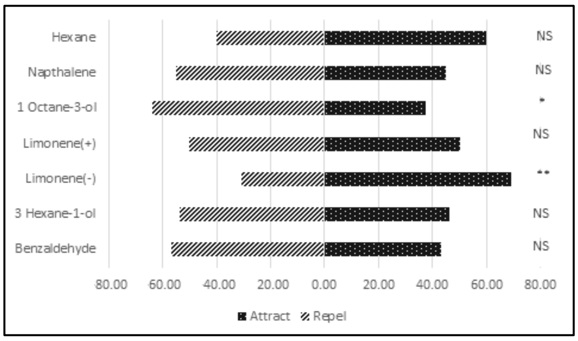

Figure 6: Behavioural response of FAW neonate larvae to plant volatiles; bars indicted the mean percentage moths found on each side. * the bars indicating percentage response between source and control is significantly different at p<0.05 and ** at p<0.01 and NS = non-significant.

The behaviour studies for FAW neonates showed a non-significant neutral effect for the Benzaldehyde, 3 Hexen-1-ol, Limonene (+), Naphthalene and n-Hexane however, higher attraction (69.23%) was reported towards Limonene (-) (χ2 = 11.538, df =1, p = 0.001). Meanwhile significant amount of neonates (63.75%) was repel from the 1-Octen-3-ol (χ2 = 5.44, df = 1, p = 0.02) (Figure 6).

Generally, lepidopteran larvae including FAW are considered as less mobile stages and inability to long-distance movements, so mothers host selection behavior where female moth is mainly responsible for host selection for their progeny survival (Rojas *et al*., 2018). The host finding for the egg-laying and feeding may be correlated thus we use both adults and larvae for the study. Early instar larvae were used due to later stages of FAW larvae known to be less mortality and confined their selected niche where neonatal choices regarding host finding must be important. According to neonate larvae are believed to disperse randomly from plants because they engage in ballooning (Zalucki *et al*., 2002). But available studies had not addressed whether neonate larvae disperse randomly, nor whether larvae that initially colonize an unsuitable host will disperse further, either directionally or randomly. But our study was found that they use chemical cues for host selection. Further, we got the idea for females to use chemical cues for host selection and we found that some chemicals use to attract the host and some are showing repulsive behavior. To confirm the results further studies is needed and have to use more volatile chemicals and different blends due to we selected only a few chemicals and plants not release volatiles as individual compounds and releases at a various rate of combinations and ratios (McCormick *et al*., 2012).

IV. CONCLUSION

Our study can be concluded that n-hexane is a significant attraction for females while male neonates showing an attractant movement which is the major component in *B. brizantha*. Moreover, limonene (+) and limonene (-) both showing a repellent property for males which is the major volatile component in *Desmodium spp*. In the case of neonates 1-octane 3-ol showing significant repellent properties. These identified volatile compounds can be used to develop behavioural modifying pest management strategies.

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