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Olfactory response of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) to the volatiles of healthy and herbivore damaged maize plants and their profiling

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Abstract
Fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is a devastating invasive pest that continues to impede Indian agricultural progress. Olfactory response studies indicated that gravid female moths prefer healthy maize plants over *S. frugiperda* larva damaged maize plants. Among all the treatments, the highest preference of *S. frugiperda* female moths was observed with 86.67 per cent towards the 45-day old healthy maize plant odour. Male moths did not show any specific preference to the different stages of maize *viz*., 15, 30 and 45 days old healthy and damaged plants. Volatile profiling of plants showed significant change in plant volatiles when damaged by *S. frugiperda* larva.

Keywords: Healthy and damaged maize plants, olfactory response, *Spodoptera frugiperda,* volatile profile analysis

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J E Smith), is a destructive invasive pest that was introduced to India in 2018 (Ganiger *et al*., 2018; Sharanabasappa *et al*., 2018) [10, 21] and causes enormous economic loss due to its high dispersal ability, wide host range and high fecundity (Chormule *et al*., 2019) [4]. FAW was not recorded anywhere else until 2015, except in the Americas, where it originated, but it wreaked havoc on African economies after its entrance in 2016. (Goergen *et al*., 2016) [11]. Fall armyworm is a polyphagous pest with a preference for the Poaceae family (Casmuz *et al*., 2010) [3]. It has been shown to feed on 353 different host plants from diverse families (Montezano *et al*., 2018) [16]. Rice, maize, sorghum, sugarcane, cabbage, beet, tomato, potato, onion, cotton, pasture grasses, peanut, soybean, alfalfa, and millets are among the crops severely harmed by FAW (Pogue, 2002; CABI, 2016) [20, 2].

At the outset, we must emphasise the pest's crippling impact on India's maize cultivation. After rice and wheat, maize is India's third most important crop, cultivated on an area of approximately 9.03 million ha with a production of 27.72 million tonnes (FAO STAT, 2019) [9]. Maize has numerous uses as a feed, fodder, and raw material in a variety of industrial applications. According to reports, maize production in India fell from 28.7 million tonnes in 2017 to 27.8 million tonnes in 2019, a 3.2 % decrease due to FAW attacks (Manupriya, 2019) [15].

Farming communities primarily rely on synthetic insecticides to control invasive pests such as *S. frugiperda*, which always poses a significant risk to the environment, consumer health, and negatively affects non-target beneficial insects. Farmers were observed using 2 to 3 sprays of different insecticides without proper knowledge of their efficacy in the year of its introduction (Deshmukh *et al*., 2020) [6].

Fall Armyworm consists of two strains, viz. corn strain ‘C’ which feeds predominantly on maize, sorghum and cotton, and rice strain ‘R’ which prefers rice and turfgrass (Nagoshi and Meagher, 2016) [17]. With reports of R-strain and C-strain in fall armyworm populations (Unbehendit *et al*., 2014) [23], failure of readily available management practises, and a widening host range of fall armyworm in India, it is critical to develop an accurate and precise formulation of sex pheromone lure in combination with plant volatiles for efficient mass trapping and pest management under Indian conditions. Many low-molecular-weight organic compounds, such as alkanes, alkenes, alcohols, ketones, aldehydes, ethers, esters, and carboxylic acids, have been reported to be emitted by plants (Dudareva *et al*., 2004, Niinemets *et al*., 2004) [8, 18].

Study of the olfactory response of both male and female *S. frugiperda* (Smith) (Lepidoptera: Noctuidae) to the volatiles of healthy and damaged maize plants and volatile profiling of healthy and damaged maize plants will provide an insight towards the management of this pest with eco-friendly management strategies.

Materials and methods

Culturing of Insect

Spodoptera frugiperda egg masses were collected from different maize growing research plots at Tamil Nadu Agricultural University (TNAU) campus, Coimbatore and upon hatching the larva were reared in TNAU FAW lablab based artificial diet. After pupation, the pupae were separated and kept in a cage containing sugar solution and *Nerium oleander* twigs as an ovipositional site.

Culturing of host plants

Healthy and *S. frugiperda* infested, 15, 30 and 45 days old maize plants (Variety- COHM-8) raised in pots were used for olfactometer studies and volatile profile analysis. Maize plants without any damage and plants which were inoculated with two, third instar *Spodoptera frugiperda* larvae for one day are taken as healthy and damaged treatments respectively.

Y- tube olfactometer setup

Y- tube olfactometer analysis was done by following the procedure of Signoretti *et al*. (2012) [22] with slight modifications. Experiment was done with 3- 5 days old gravid females and 3-7 days old male moths with no previous experience of host plant volatile exposure. Two choice test with a closed Y-tube olfactometer system was used for analysing the preference of both male and female *S. frugiperda* to the healthy and *S. frugiperda* larva damaged plant volatiles. The Y-tube olfactometer used for the experiment had a 28.5 cm long stem and two 11cm long arms. The internal diameter for the central stem and two arms were 2.6 cm and 2 cm respectively. Air entering into the olfactometer setup was supplied with the aid of an aquarium pump and air pressure was calibrated at 4 L/min using an airflow meter. The air was purified using a charcoal filter and was humidified with a humidifier. Potted 15, 30 and 45 days old healthy and damaged (*S. frugiperda* infested) plants with soil material covered with aluminium foil placed in two 10 L glass chambers served as the odour sources in various treatment combinations. The experiment was conducted at ambient atmospheric conditions of room temperature at 27 ± 1°C and relative humidity at 60–80% at four hours from the start of scotophase.

Moths (30 No's) were individually allowed to enter into the central arm of the Y-tube and were observed for 5 minutes after the initiation of their movement. Moths were considered non-responding when they were unable to move from their entry point even after 5 minutes of their release or when they were not able to enter into one of the arms of the Y-tube or when they were not able to reach the end of the arm. Odour source was renewed after every one hour. Odour source positions were exchanged after the testing of 5 adults to avoid prejudgement by an accidental asymmetry in the experimental setup. After each bioassay the olfactometer setup, glass chambers and connections were wiped with 75% ethanol followed by distilled water and was dried well to avoid odour contamination between consecutive bioassays.

Table 1: Odour source combinations used in Y-tube olfactometer bioassay

15 days old plants	T1	:	Clean air Vs Healthy plants
	T2	:	Clean air Vs Damaged plants
	T3	:	Healthy plants Vs Damaged plants
30 days old plants	T4	:	Clean air Vs Healthy plants
	T5	:	Clean air Vs Damaged plants
	T6	:	Healthy plants Vs Damaged plants
45 days old plants	T7	:	Clean air Vs Healthy plants
	T8	:	Clean air Vs Damaged plants
	T9	:	Healthy plants Vs Damaged plants

Headspace volatile collection from host plants

Plant volatiles were collected from healthy and infested maize plants at different stages of growth, such as 15, 30, and 45 days after sowing, using a push-pull system and volatile collection chamber, as described by Jayanthi *et al*. (2012) [13] with minor modifications (Fig. 1). To trap the volatiles, the entire plant was placed in a 10 L glass container for 12 hours, and carbon filtered air was drawn through the chamber using a mini air compressor (AIHUI: TC-108) with a 21 L/min airflow capacity. Volatiles emitted by the plants were passed through a trap of Porapak-Q adsorbent material and eluted with 750 µl of diethyl ether using a vacuum pump (ROCKER 300). Volatile profiling was carried out in GC-MS (Perkin Elmer Clarus SQ8C with DB-5 MS capillary standard non-polar column and Helium as carrier gas) in the Department of Agricultural Microbiology, TNAU, Coimbatore.

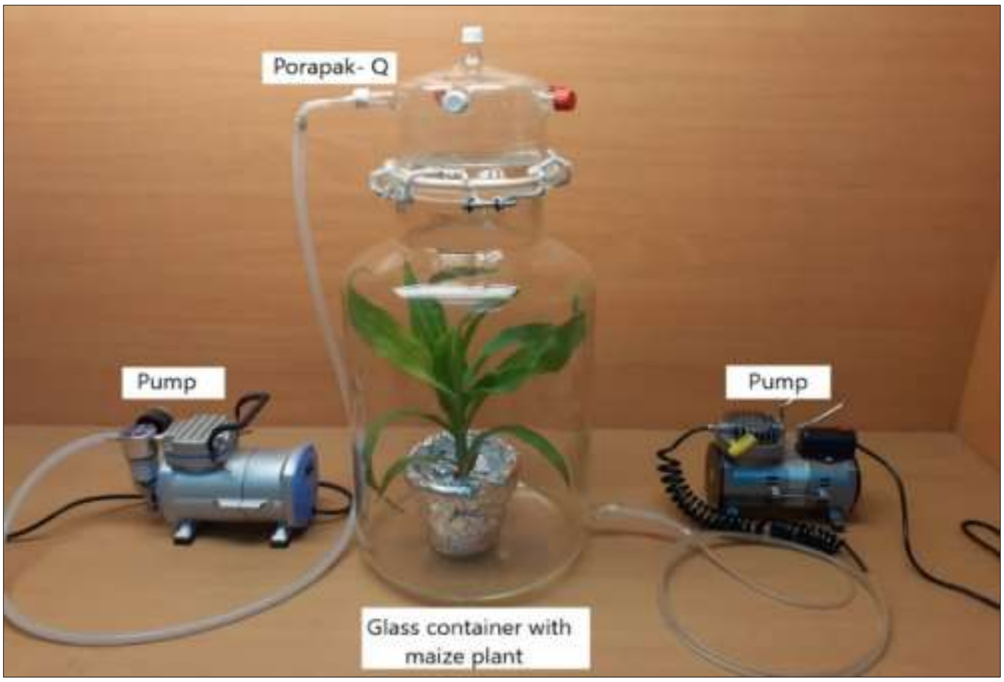

Fig 1: Experimental setup of volatile collection unit used for plant volatile extraction from maize plants

Statistical analysis

Percentage of *S. frugiperda* female and male moths responding to plant volatiles were compared pairwise among experimental treatments using a Chi-square test. Heat maps were prepared online by using Jupyter Notebook, Python.

Results and discussion

Table 2: Preference of female moths to the odour source combinations in a Y-tube olfactometer

Treatments		Mean per cent moths' response ± SE (for respective combinations)		P-value
15 days old plant				
T1	Clean air Vs Healthy plants	23.33	76.66	0.0034
T2	Clean air Vs Damaged plants	63.33	36.66	0.1441
T3	Healthy plants Vs Damaged plants	76.66	23.33	0.0105
30 days old plant				
T4	Clean air Vs Healthy plants	26.66	73.33	0.0105
T5	Clean air Vs Damaged plants	53.33	46.66	0.7150
T6	Healthy plants Vs Damaged plants	80.00	20.00	0.0034
45 days old plant				
T7	Clean air Vs Healthy plants	20.00	80.00	0.0010
T8	Clean air Vs Damaged plants	60.00	40.00	0.2733
T9	Healthy plants Vs Damaged plants	86.66	13.33	0.0002

*Response of 30 moths

Table 3: Preference of male moths to the odour source combinations in a Y-tube olfactometer

Treatments		Mean per cent moths' response ± SE (for respective combinations)		P-value
15 days old plant				
T1	Clean air Vs Healthy plants	36.66	63.33	0.0284
T2	Clean air Vs Damaged plants	56.66	43.33	0.4652
T3	Healthy plants Vs Damaged plants	60.00	40.00	0.0678
30 days old plant				
T4	Clean air Vs Healthy plants	33.33	66.66	0.0678
T5	Clean air Vs Damaged plants	53.33	46.66	0.7150
T6	Healthy plants Vs Damaged plants	63.33	36.66	0.1441
45 days old				
T7	Clean air Vs Healthy plants	33.33	66.66	0.0678
T8	Clean air Vs Damaged plants	56.66	43.33	0.4652
T9	Healthy plants Vs Damaged plants	60.00	40.00	0.2733

*Response of 30 moths

Results indicated that gravid female *S. frugiperda* moths prefer healthy maize plants over *S. frugiperda* larva damaged plants. In fifteen days old maize plants, female moths showed specific preference to healthy plants over clean air (T1: χ^2 =8.53, p=0.003), healthy plants over damaged plants (T3: χ^2 =8.53, p= 0.003) and clean air over damaged plants (T2: χ^2

=2.13, p=0.144). Healthy plants were preferred over damaged plants (T6: χ^2 =10.80, p=0.001) and over clean air (T4: χ^2 =6.53, p=0.011) in thirty days old maize plants. In forty-five days old maize plants, 86.67 per cent of the female moths released showed high preference to healthy plants over damaged plants (T9: χ^2 =16.13, p=0.000) and 80 % of the female moths released preferred healthy plants over clean air (T7: χ^2 =10.80, p=0.001). In male moths, there was no significant difference between the various treatments tested. The present findings are following the results of Signoretti *et al* (2012) [22], who reported that the female moths showed great response to undamaged plant volatiles over herbivore-induced plant volatiles in maize plants. This preference is due to the adaptive strategy of female moths to safeguard its offsprings from natural enemies and competitors. Block *et al*. (2021) [1] found out that *S. frugiperda* females show specific oviposition preference to healthy plants over *S. frugiperda* larva infested plants. Similarly, De Moraes *et al*. (2001) [7] reported that *Heliothis virescens* females are highly repellent to certain herbivore-induced plant volatiles released exclusively during the night due to the feeding of *H. virescens* larva in *Nicotiana tabacum*.

In 15-days old healthy maize plants, a major portion of the plant volatile comprises compounds falling in the group hydrocarbons (50%) followed by compounds under the group of alcohols (15%), esters (15%), ketones (10%) and aldehydes (10%). In 15-days old *S. frugiperda* larva damaged maize plants, there is the presence of amino acid (5%), thiourea (5%), terpene (5%), azane (5%) and cumene (5%) compounds along with hydrocarbon (30%), alcohol (15%), ester (20%) and aldehyde (10%) compounds. Hydroquinone is observed to be present in maximum area per cent in both healthy (7.86%) and damaged (7%) 15-days old maize plants. Benzoic acid, 4-ethoxy-, ethyl ester (0.45%) and octadecane (0.414%) are the compounds present in least area per cent in 15-days old healthy and damaged maize plants respectively (Fig. 2).

Volatile organic compounds of 30-days old maize plants comprise hydrocarbons (30%), alcohols (30%), aldehydes (10%), terpenes (10%), esters (10%), ketones (5%) and amides (5%). In larva damaged 30-days old maize plants, compounds of groups amino acids (5%) and carboxylic acids (5%) are also present along with alcohols (30%), hydrocarbons (25%), aldehydes (10%), ketones (10%) and terpenes (5%). In healthy 30-days old maize plants hydroquinone (7.86%) has the highest area per cent, whereas, in *S. frugiperda* larva damaged maize plants, L-Threonine (8.81%) has the highest value. Glafenin (0.42%) and Methyl tetradecanoate (0.35%) are the compounds with least area per cent in 30-days old healthy and larva damaged maize plants respectively (Fig. 3).

There is the presence of carboxylic acids (5%) and aromatic heterocyclic compounds (5%) in larva damaged 45-days old maize plants in addition to other plant compounds like hydrocarbons (35%), alcohols (35%), esters (10%) and ketones (5%). In 45-days old healthy maize plants, compounds are from the groups of hydrocarbons (45 %), alcohols (25%), aldehydes (10%), esters (10%), terpenes (5%) and ketones (5%). In healthy plants, Nonanal (3.184%) has the highest area per cent and 1-Heptanol, 2-propyl- has the least area per cent, whereas in larva damaged 45 days old maize plants, 2-Norbornanol, 1,2-dimethyl- (2.58%) has the maximum area per cent and Tetradecane, 2,6,10-trimethyl- (0.45%) the least (Fig. 4).

The results on the volatile profile of healthy and damaged maize plants are dissimilar while comparing the volatile organic compounds reported by earlier works on maize plant volatile profile (Malo *et al.*, 2004; D'Alessandro *et al*. 2006; Pinto-Zevallos *et al*., 2016; Yactayo-Chang *et al*., 2021) [14, 5, 18, 24].

Malo *et al.*, 2004 has reported that the antennae of both the sexes of *S. frugiperda* are very sensitive to alcohol moieties, especially at higher doses and *S. frugiperda* female moths are responsive to linalool, which is one among the major compounds released by undamaged maize plants (D'Alessandro *et al*. 2006) [5]. According to Pinto-Zevallos *et al*., 2016 [19], the green leaf volatile (Z)-3-hexenyl acetate, the terpenoids, b-linalool and TMTT as well as indole induced a consistent response in both the mated and virgin *S. frugiperda* females.

Yactayo-Chang *et al*. (2021) [24] has reported that in *S. frugiperda*, methyl salicylate and (E)-alpha-bergamotene act as oviposition attractants and geranyl acetate serves as oviposition attractant or repellent based on of the status of volatiles emitted by the host plant and (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) works as an oviposition deterrent.

Conclusion

Volatile profiling of damaged and healthy maize plants at different stages of the crop helps in understanding the difference in volatile organic compounds with respect to the age of the plant, healthy and herbivore damaged plants. This forms the basis of understanding the role of plant volatiles emanated from host plants in response to the *S. frugiperda.*

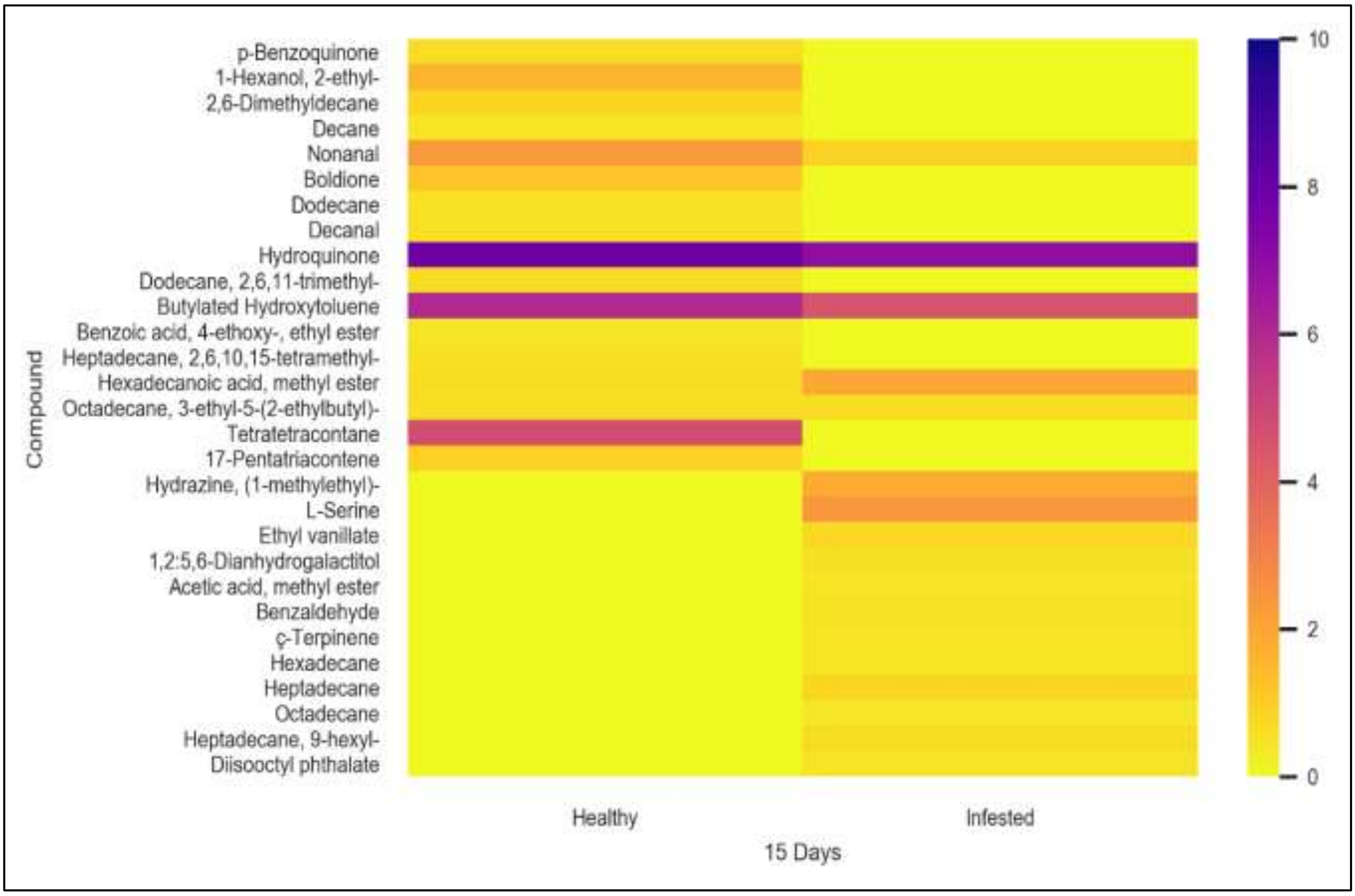

Fig 2: Heat map comparing the plant volatiles of 15 days old healthy and *Spodoptera frugiperda* larva infested maize plants

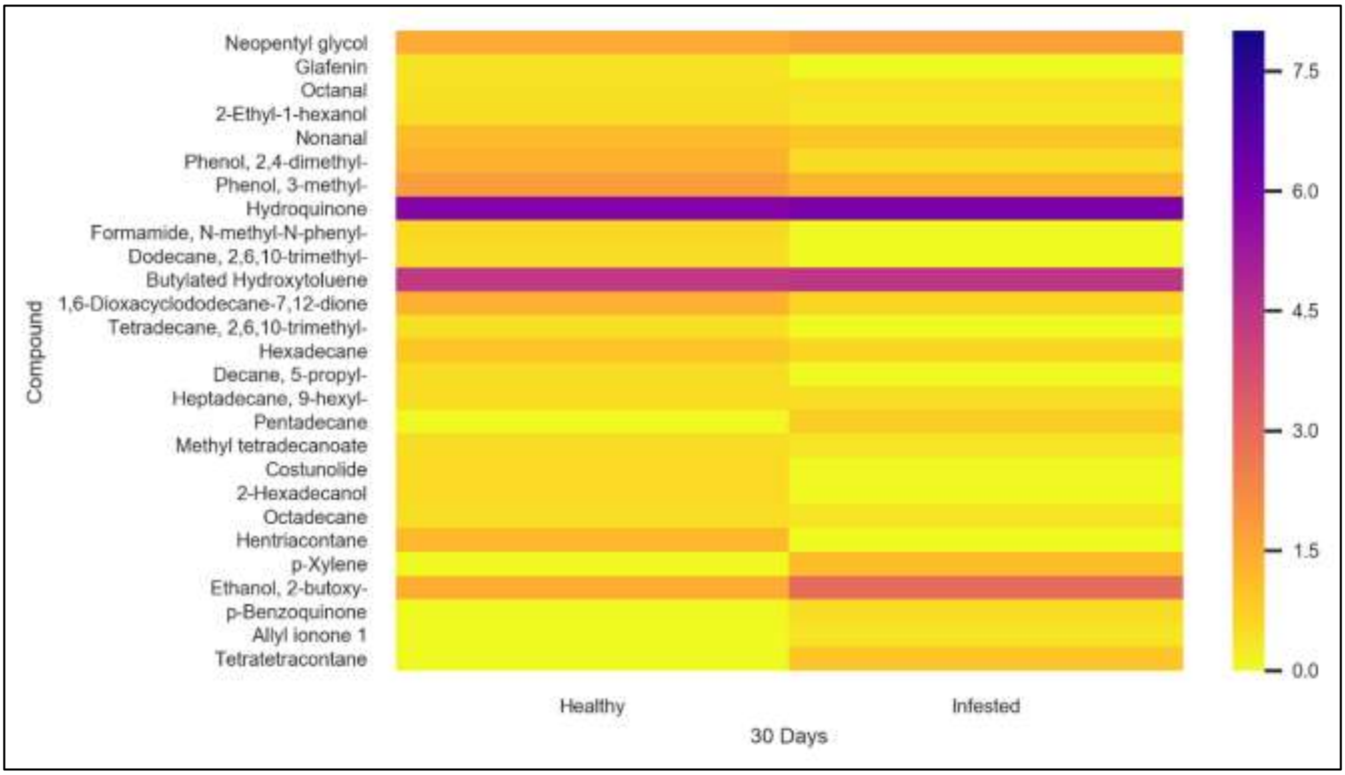

Fig 3: Heat map comparing the plant volatiles of 30 days old healthy and *Spodoptera frugiperda* larva infested maize plants

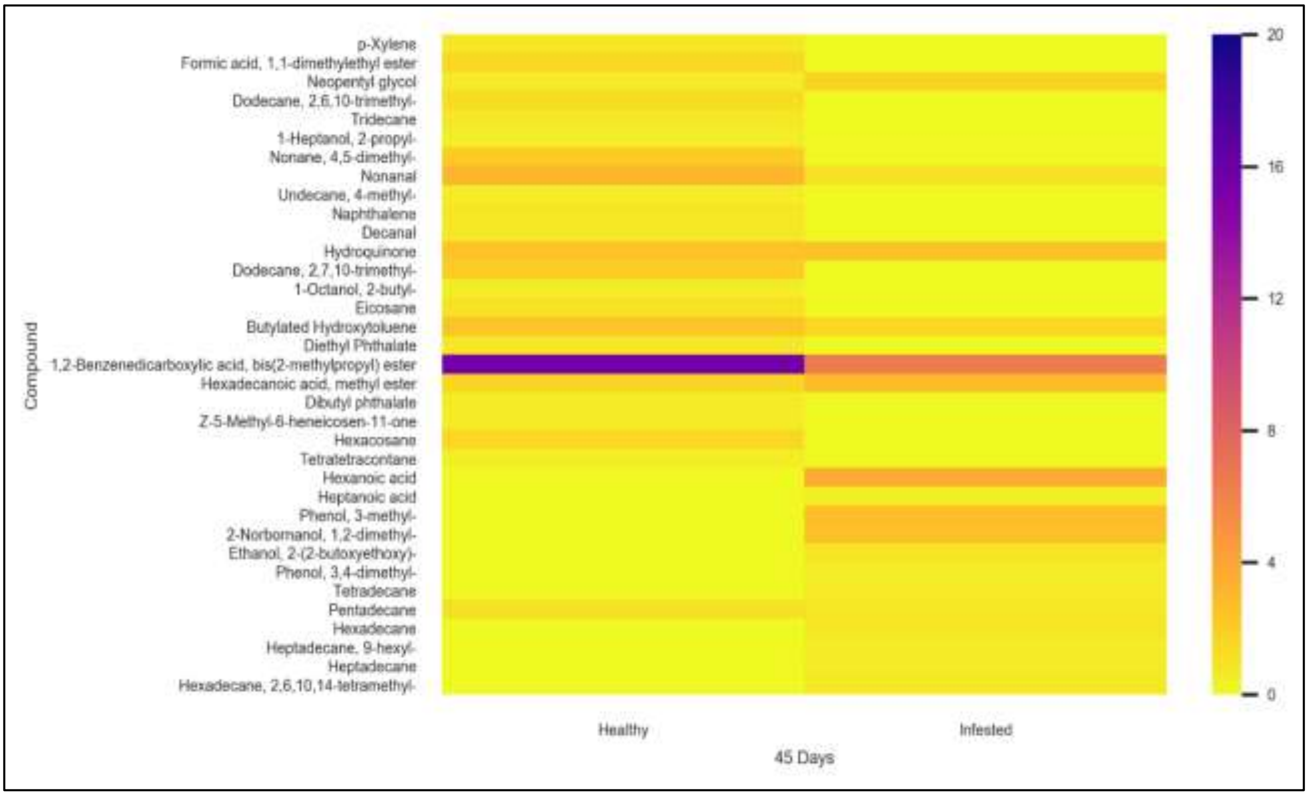

Fig 4: Heat map comparing the plant volatiles of 45 days old healthy and *Spodoptera frugiperda* larva infested maize plants

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