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RESPONSE OF HELICOVERPA ARMIGERA TO VARIOUS VOLATILES IN HOST PLANTS

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Abstract

Adult H. armigera were tested using a four-choice olfactometer to see how they orientated in response to volatiles from marigold, tomato, chickpea, and pigeonpea plants. Both female and male H. armigera moths were found to be most attracted to the volatiles of marigold plants, with a 35.01 percent attraction to uncut twigs and a 42.32 percent attraction to cut twigs, respectively. The volatile compounds from tomato plants elicited the second highest reaction. Volatiles from the host plant were identified using GC-MS testing. Eleven volatile compounds were isolated from marigolds, ten from tomatoes, seventeen from pigeonpeas, and ten from chickpeas.

Keywords: *Response, Helicoverpa Armigera, Volatiles, Host Plants.*

1. Introduction

Helicoverpaarmigera (Hübner) (Lepidoptera: Noctuidae), with its extensive host range, poses a significant threat to agricultural output across the globe. H. armigera causes damage to a wide variety of commercially important plants, including cotton, tobacco, pigeonpea, chickpea, maize, sunflower, and many more field and horticulture crops. This pest is found all over the world, and it eats plants from the Leguminosae, Solanaceae, Asteraceae, Malvaceae, and Poaceae families, as well as many others.[1]

Damage to plants and the price tag associated with pest control measures add up to a staggering US$ 4 billion annually in losses due to this pest's prevalence on crops across the world. In India, annual crop losses are estimated to be over US$ 927 million for chickpeas and pigeonpeas, and over US$ 530 million for pulses and cotton. Almost US$127.5 million is spent annually on insecticides for control of this pest. H. armigera is responsible for annual quantitative losses in chickpea of 5–70%, tomato of 50–100%, cotton of 30–40%, and safflower of 10–80% throughout the nation.[2]

The incubation time for these eggs is between three and five days, and they are hemispherical in form and a yellowish white colour. Larvae are born a dirty white colour and change to pinkish brown and light green throughout the course of their 23–25 day larval stage (6 instars). The brownish pupae develop in approximately 14 days. Moths lived for just 9-11 days and might be either a greenish-grey male or an orange-brown female. The average insect lives for between 51 and 53 days. Changes in average temperature may affect how long a given species lives.[3-4]

When deciding which plants to employ as hosts, insects mainly depend on allelochemicals to make their decisions. Learning how kairomones impact the preferences of insects for their host plants might lead to the development of more effective techniques for dealing with insect pests. However, further study is necessary to establish how H. armigera reacts to the volatiles generated by its host plants.[5]

Host plant volatiles are chemicals released by plants or secondary plant metabolites that facilitate insect-plant interactions. Some of these organic volatiles molecules have insect-repelling or pest-attracting properties that might be used in integrated pest control strategies. Moths are drawn to many plants, either to eat on the plants' nectar or to use as oviposition sites, and their larvae feed on the plants' fruiting bodies. There is mounting evidence that volatiles and secondary metabolites from plants play a significant role in guiding moths to their hosts. Selecting an appropriate host may include using volatiles from the host plant as ovipositional stimulants, deterrents, or communication bridges.[6]

The ability of pregnant females of H. armigera to locate and oviposit on a wide range of host plants belonging to different families is one factor contributing to the widespread pest status of this species. It has been hypothesised that this phenomenon occurs due to the fact that all host plants have a same set of signals or cues that the insects utilise to direct their behaviour. It's possible that the selection of a host is influenced by chemical or visual cues.[7]

2. Literature review

Ogunwande, I. A., &Olawore, N. O. (2019) Cotton, pigeonpea, sweet corn, mung bean, bean and common sowthistle were all included as host plants in this investigation. When pregnant female moths were provided plants, they were at the flowering stage. Pigeonpea flowers attracted the highest oviposition of any plant and were the preferred food source for first-instar larvae. The pigeonpea plant not only generated the most fertile moths, but also aided in the development of the healthiest larvae and pupae. The oviposition habit and larval performance of Australian H. armigera moths in relation to pigeonpea are therefore similar with those of Indian H. armigera moths. The findings provide credence to the idea that pigeonpea is a potential major host for H. armigera and imply that the insect's host identification and acceptance behaviour is consistent across its range.[8]

Ragesh, P. R. & Singh, A. K. (2018) When determining the food utilisation indices of H. armigera's fifth instar larvae, the impact of several host plants was taken into account. These included carnation, pigeonpea, bathua, chickpea, sorghum, mothbean, tomato, capsule of castor, cotton, sonchus, and cowpea. Therefore, chickpea was declared the most preferred host of H. armigera, followed by tomato and cotton, on the basis of its values for efficiency of conversion of ingested food to body substances (ECI=17.65%), approximate digestibility (AD=87.56%), efficiency of conversion of digested food to body substances (ECD=20.15%), and growth rate (GR=0.388).[9]

Rajapakse & Walter, G. H. (2017) Only seven of the 34 individual compounds examined were considerably appealing to H. armigera, whereas six were highly repellant. However, out of 31 blends examined, 21 were considerably appealing and just 1 was significantly repelling when offered as mixtures of two or more volatiles. Four- to six-component mixtures, including 2-phenylethanol and phenylacetaldehyde and volatiles predominantly found in leaves like green leaf volatiles and terpenoids, were the most appealing.[10]

Walter, G. H., & Cribb, B. W. (2016) In the one and only field trial conducted on cotton, tomato and country bean by a British scientist in India (Guntur, Andhra Pradesh), he found that a blend of phenylacetaldehyde, salicylaldehyde, methyl 2-methoxybenzoate, linalool, and limonene with a ratio of about 50:20:10:10:10 was effective against H. armigera. The orientational reactions of neonate H. armigera to the leaf volatiles of four leguminous crops were bioassayed in the lab. The crops tested were chickpea (Cicer arietinum L.), pigeonpea (Cajanus cajanMillsp. Larvae of the H. armigera species responded well to the leaves of all test plants. H. armigera larvae preferred chickpea, pigeonpea, and blackgram leaves over cowpea leaves.[11]

Mohan, K. S. & Rao, N.G.V. (2015) Laboratory studies using no-choice and two-choice procedures were conducted on the orientation response of neonatal H. armigera to the green leaf volatiles of three leguminous crops: pigeonpea (C. cajan), mungbean (V. radiate), and blackgram (V. mungo). All of the test plants' leaves were effective in guiding the larvae in the right direction. Pigeonpea, on the other hand, was preferred by larvae over blackgram and mungbean. compared the directional responses of H. armigera neonate larvae to odour sources generated from six plant species (Nicotiana tabacum, Capsicum annuum, Solanum esculentum, Gossypium hirsutum, Arachis hypogaea, Zea mays). Larvae of H. armigera showed a more specialised reaction to tobacco than those of other species.[12]

3. Methodology

The "Orientation response of Helicoverpaarmigera (Hübner) to different host plant volatiles" was studied in 2019-20 at Agricultural University, Anand (Gujarat)."

3.1 Insect collection and rearing

The okra field of Anand Agricultural University in Anand, India was sampled for H. armigera larvae. To prevent cannibalism among the field-collected larvae, we housed them in separate plastic vials (Plate 3.1). Lab-reared larvae were fed a semi-synthetic diet based on chickpeas every other day until pupation (Table 3.1). To ensure a parasite- and disease-free culture, unhealthy larvae and pupae were removed from the population before they could spread throughout the raising process. The breeding space was maintained at (25 2) oC, with a photoperiod of 10: 14 (L: D) hours and a relative humidity of 50-60%. Male and female pupae were separated under the microscope (Plate 3.2), and then moved to an incubation chamber to shorten the pupal phase in the lab. Following adult emergence, five male and female insects were placed in an oviposition cage and fed a 10% honey solution.

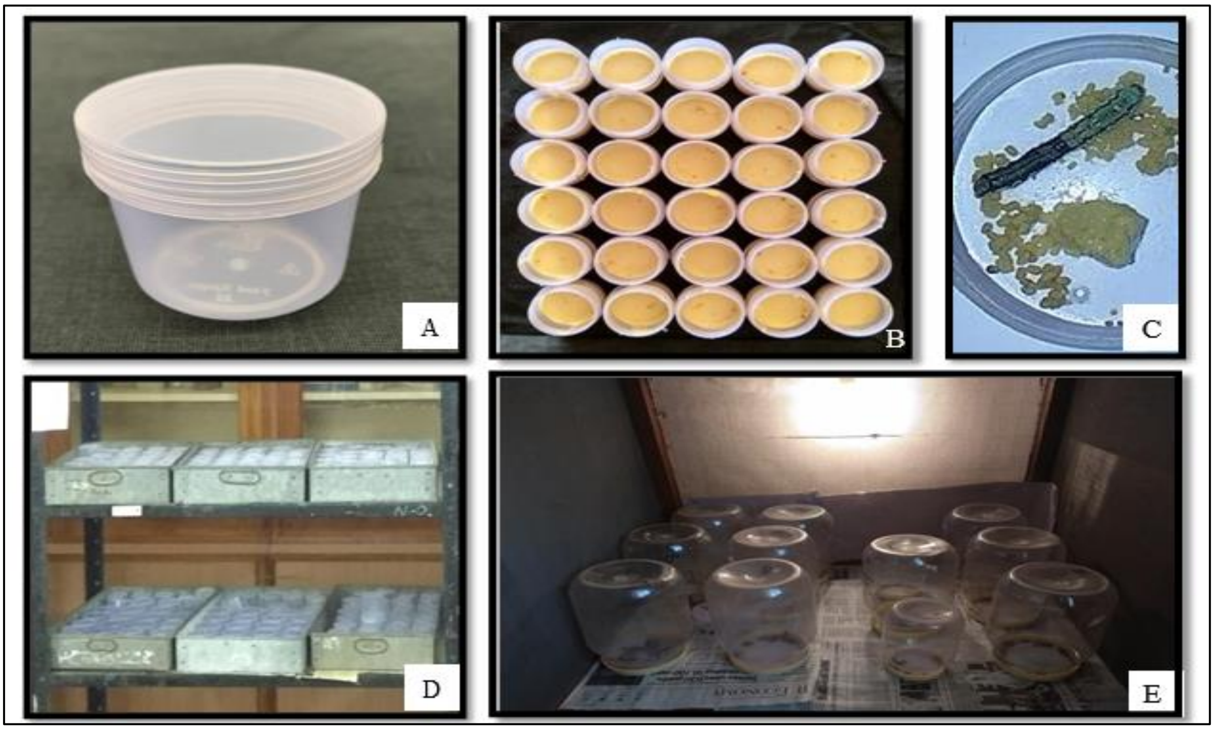
Table 3.1: Semi-synthetic diet components based on chickpeas

Component	Quantity
Chickpeaflour	300 g
Yeast	10 g
Methylparaben	3 g
Sorbicacid	0.84 g
Ascorbicacid	4.68 g
Agar	17.48 g
Multi-vitamintablets	2
Streptomycinsulphate	40 mg
Carbendazim	1 g

Formalin	3 ml
Water	1000 ml

3.2 Raising of host plants

At Regional Research Station, Anand (India) host plants of H. armigera for the current research were grown in containers. These included chickpea, pigeonpea, tomato, and marigold. A conventional potting mix (4:1, soil: vermi-compost combination) was mixed to provide the soil mixture needed for plant cultivation. In order to obtain blooming at some point in all crops for the experiment, regular seeding was done at regular intervals of 10 days. Researchers employed plants with both flowering adult plants and sensitive leaves for larval experiments.



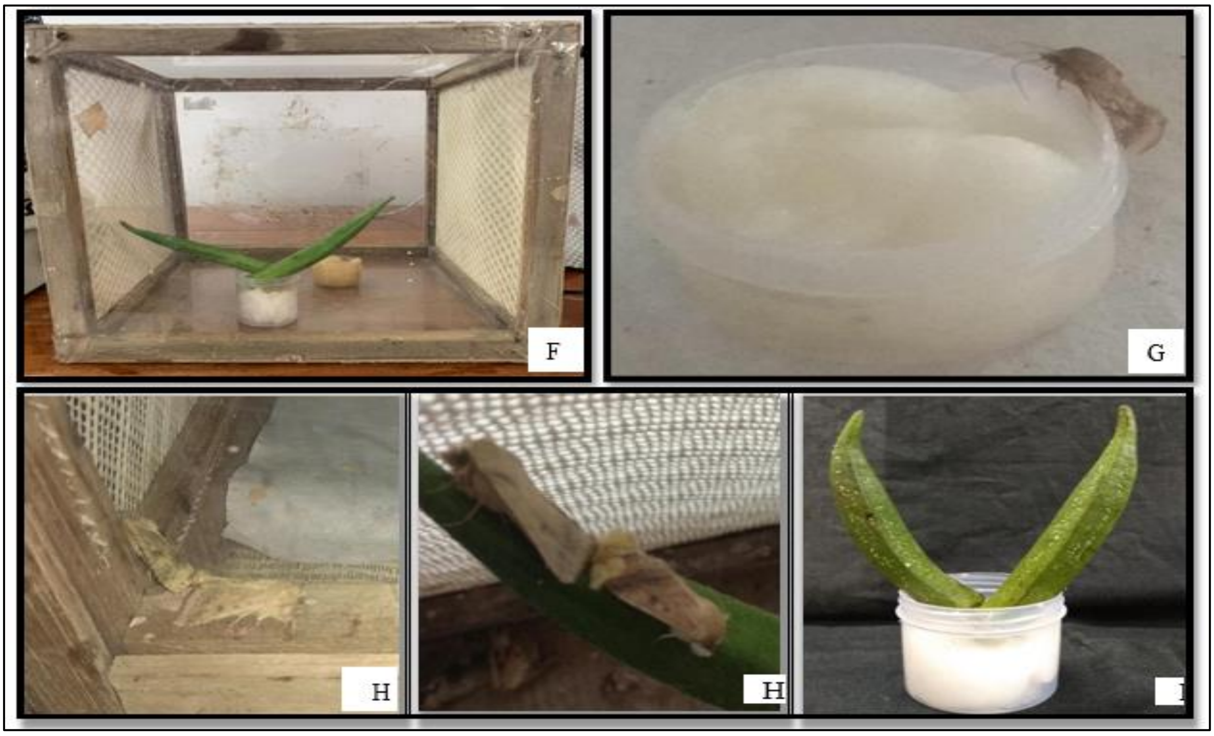
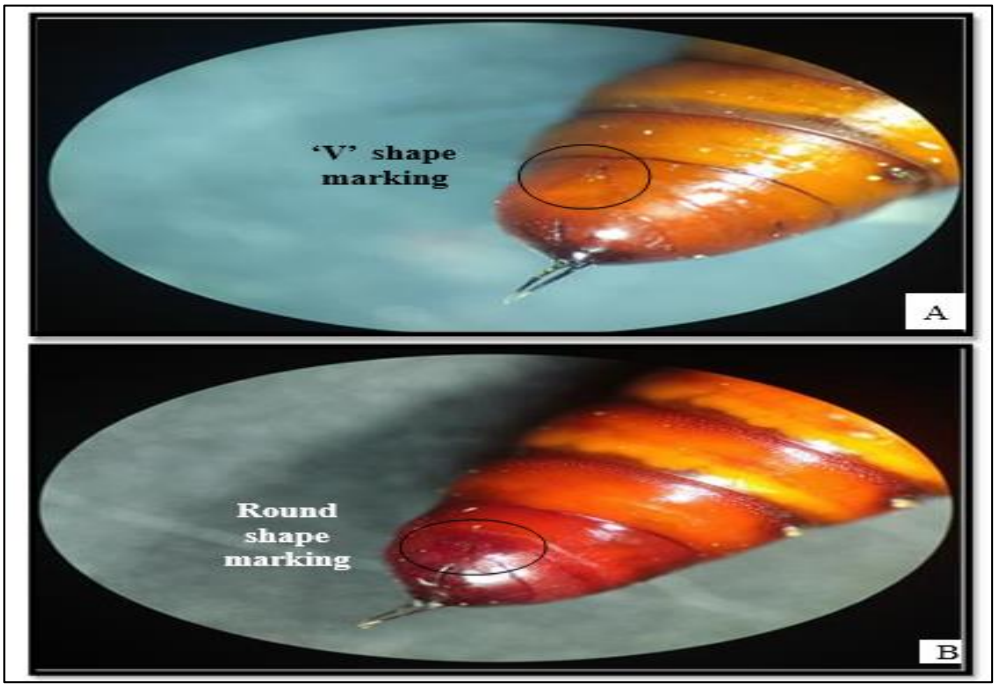


Plate 3.1: H. armigera captive breeding



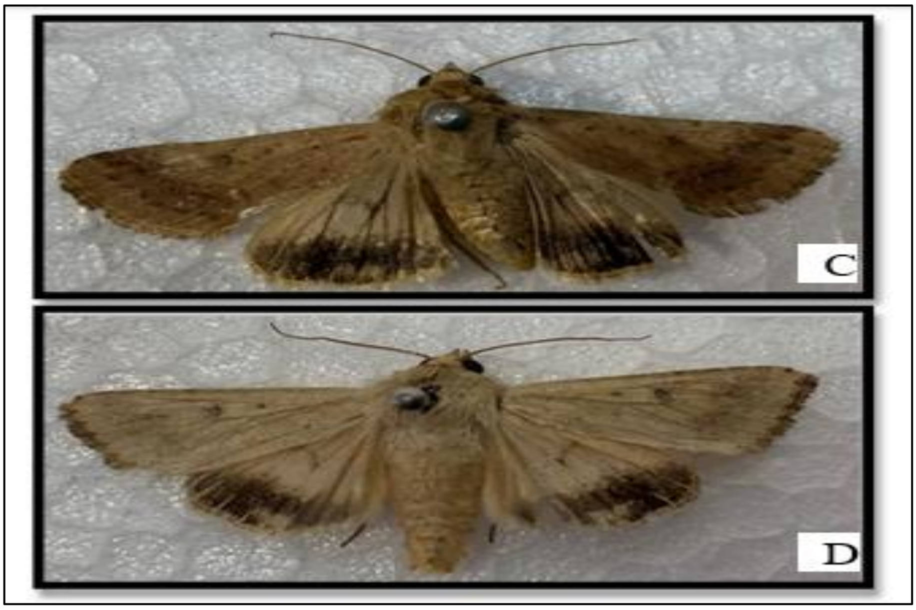


Plate 3.2: Male and female H. armigera taxonomy

3.3. Assessment of the orientation response of adults to volatiles of different host plants

3.3.1 Details of Experiment

Location	AINPVPM:AgriculturalOrnithologyLaboratory,AAU,Anand
Year	2019-20
Design	CompletelyRandomizedDesign
Treatments	4 T_1: Chickpea plantT_2:PigeonpeaplantT_3:Marigol d plant T_4:Tomato plant
Repetitions	5
Observatio n recorded	No.of mothspermothcollection chamber

Standard statistical methods, including a randomized design, arc sine transformation, and the LSD test, were used to analyze the data.

3.4 collection and identification of host plant volatiles from different host plants

3.4.1 Identification of Host Plant Volatiles

For examination by Gas Chromatography - Mass Spectrometry (GC - MS), samples of host plant volatiles were sent to the National Bureau of Agricultural Insect Resources in Bengaluru. Sample volumes were brought down to 100 l using a nitrogen evaporator after volatiles were extracted in 500 l of dichloromethane (DCM).

3.4.1.1 GC-MS methodology

The 2l injected sample of plant volatiles was analyzed using a gas chromatograph (GC7890A; Agilent Technologies USA Ltd.) linked to a 5977B mass selective detector from the same company, operating in electron impact mode at a source temperature of 230 °C and a transfer line temperature of 250 °C. An HP-5 MS phenyl methyl siloxane non polar capillary column (0.25 mm x 30 m x 0.25.) max 350 °C was utilised for the fractionation process (Agilent component No. 190915-433). The mobile phase was purified using the universal trap; it was 99.999 percent pure helium purchased from Bhuruka gas Ltd. There was a split inlet ratio of 50:1, and the input temperature was 260 C.

3.5 Estimation of the orientation response of neonate larvae of h. Armigera to volatiles of different host plant leaf

3.5.1 Details of Experiment

Standard statistical methods, including a randomised design, arc sine transformation, and the LSD test, were used to analyse the data.

Location	AgriculturalOrnithologyLaboratory, Anand, Gujarat
Design	CompletelyRandomizedDesign
Treatments	4 T_1: Chickpea leafT_2:PigeonpealeafT_3:Marigold leaf T_4:Tomatoleaf
Repetitions	5
Observation	No.oflarvaeper arm
Year	2019-20

4. Results

In the results of our investigation into the "Orientation response of Helicoverpaarmigera (Hübner) to different host plant volatiles," broken down into a number of categories and discussed in the context of a review of the literature that is directly or indirectly related to our work.

4.1 Assessment of the orientation response of adults to volatiles of different host plants

In 2019-20, researchers from AINPVPM: Agricultural Ornithology, AAU, Anand, used an olfactometer to perform an experiment. With the goal of identifying the host plant that releases the most enticing plant volatiles, researchers examined the orientation response of adult H. armigera against volatiles of various host plants.

In Table 4.1, you'll find information on how male and female H. armigera, both a day old, react to volatiles from uncut twigs of various host plants, and how this affects their orientation. One-day-old female H. armigera showed a similar orienting reaction to both the marigold plant volatile (35.01%) and the tomato plant (24.59%). The chickpea plant had the lowest percentage response (10.39%), along with the pigeon pea plant (10.69%).

The volatile marigold plant received the highest percentage of the one-day-old male H. armigera's orientation reaction (36.67%), followed by the tomato plant (23.12%). Pigeonpea plant had the lowest percentage of responses (9.97%) compared to the other plants studied (12.63%).

Table 4.1: Adult H. armigera react differently to volatiles from various host plants, based on where the twig was cut from

Tr. No.	Treatments	Mothsattracted (%)	
		Female	Male
T_1	Marigoldplant	36.28a (35.01)	37.30a (36.72)
T_2	Chickpeaplant	18.80b (10.39)	20.82c (12.63)
T_3	Pigeonpeaplant	19.08b (10.69)	18.41c (9.97)
T_4	Tomatoplant	29.73a (24.59)	28.74b (23.12)

S.Em. ±	2.33	1.58
CDat 5%	7.00	4.73
C.V.(%)	20.11	13.42

The orientation response of female and male H. armigera to volatiles from cut twigs of numerous host plants is shown in Table 4.2.

The percentage of moths that were drawn to each host plant was used to illustrate the females' orientation response. The attraction of female moths to marigolds was the strongest (42.32 percent). The next highest percentage (26.23%) was for the tomato plant. The chickpea plant attracted 8.42% as many female moths as the pigeonpea plant did 11.02%.

The marigold plant had the strongest reaction from male moths (39.18%), followed by the tomato plant (25.29%). Pigeonpea plant response (4.92%) was equal to chickpea plant response (14.13%) for lowest percentage of plants responding.

Table 4.2: H. armigera adults' orientation in reaction to volatiles from various host plants (in the instance of a severed twig)

Tr. No.	Treatments	Mothsattracted (%)	
		Female	Male
T_1	Marigoldplant	40.58a (42.32)	38.75a (39.18)
T_2	Chickpeaplant	16.87c (8.42)	14.08d (5.92)
T_3	Pigeonpeaplant	19.39c (11.02)	22.08c (14.13)
T_4	Tomatoplant	30.81b (26.23)	30.19b (25.29)
S.Em. ±		2.42	1.92
CDat 5%		7.27	5.77
C.V.(%)		20.14	16.39

4.2 Collection and identification of host plant volatiles from different host plants

During 2019-20, researchers at the National Bureau of Agricultural Insect Resources in Bengaluru used gas chromatography mass spectrometry to identify the volatiles of host plants that had been gathered using volatile collecting tubes in the laboratory of AINPVPM:

Agricultural Ornithology, AAU, Anand. Unfortunately, during transit to Bengaluru, the fragile glass tubes containing the host plant volatile extracts were broken. As a result, below are the host plant volatiles found in freshly cut plants.

Table 4.3 lists the volatile compounds found in marigold, which include n-Hexadecanoic acid, 2,3-dihydroxypropyl elaidate, octadecanoic acid, gamma-sitosterol, and 17-pentatriacontene, cyclohexane, heptane, toluene, and cyclohexane.

Table 4.3: Specifics about the volatiles found in marigold plants, the host plants

Compoundname	Formula	Chemical Abstracts Service(CAS)Number
Pentane,3-methyl-	C_6H_{14}	96-14-0
2-Pentane,3-methyl-, (Z)-	C_6H_{12}	922-62-3
Pentane,3,3-dimethyl-	C_7H_{16}	562-49-2
Cyclohexane	C_6H_{12}	110-82-7
Heptane	C_7H_{16}	142-82-5
Toluene	C_7H_8	108-88-3
17-Pentatriacontene	$C_{35}H_{70}$	6971-40-0
n-Hexadecanoicacid	$C_{16}H_{32}O_2$	57-10-3
2,3-Dihydroxypropylelaidate	$C_{21}H_{40}O_4$	2716-53-2
Octadecanoicacid	$C_{18}H_{36}O_2$	57-11-4
Gamma-sitosterol	$C_{29}H_{50}O$	83-47-6

Tomato plants were found to contain a variety of volatile chemical compounds, including cyclohexane, toluene, dotriacontane, 1,3-dioxane, and 1,4-dioxane (hexadecyloxy).Table 4.4 lists many compounds, including 2-pentadecyl-, 2,3-dihydroxypropyl elaidate, octadecanoic acid, and gamma-sitosterol.

Table 4.4: Details of host plant volatiles identified from tomato plant

Compoundname	Formula	ChemicalAbstracts Service(CAS)Number
Pentane,2,2,3,4-tetramethyl-	C_9H_{20}	1186-53-4
2-Pentane,3-methyl-, (E)-	C_6H_{12}	616-12-6
Pentane,3,3-dimethyl-	C_7H_{16}	562-49-2
Cyclohexane	C_6H_{12}	110-82-7
Toluene	C_7H_8	108-88-3
Dotriacontane	$C_{32}H_{66}$	544-85-4
1,3-Dioxane,4-(hexadecyloxy)-	$C_{35}H_{70}O_3$	56599-40-7

2-pentadecyl-		
2,3-Dihydroxypropylelaidate	$C_{21}H_{40}O_4$	2716-53-2
Octadecanoicacid	$C_{18}H_{36}O_2$	57-11-4
Gamma-sitosterol	$C_{29}H_{50}O$	83-47-6

Heptacosane, naphthalene, 1-(4-ethylphenyl) ethanone, tetradecanoic acid, n-hexadecanoic acid, 4-(4-(p-phenylene) diisopropylidene) diphenol, oleic acid, octadecanoic acid, phosphoric acid, tris(3-methylphenyl) ester, and Pigeonpea plant volatiles were identified as Nonacosane, 2-methyl-Nonacosane, 3-methyl-Nonacosane, Triacontene, 2-methyl-Tetratriacontene, 3-methyl-Hentriacontene, and Dotriacontene (Table 4.5).

Table 4.5: Pigeonpea plant volatiles: a detailed account

Compound name	**Formula**	**ChemicalAbstracts Service(CAS)Number**
Naphthalene	$C_{10}H_8$	91-20-3
Ethanone,1-(4-ethylphenyl)	$C_{10}H_{12}O$	937-30-4
Tetradecanoicacid	$C_{14}H_{28}O_2$	544-63-8
n-Hexadecanoicacid	$C_{16}H_{32}O_2$	57-10-3
Heptacosane	$C_{27}H_{56}$	593-49-7
4-4’-((p-phenylene)diisopropylidene) diphenol	$C_{24}H_{26}O_2$	2167-51-3
Phosphoricacid, tris(3-methylphenyl) ester	$C_{21}H_{21}O_4P$	563-04-2
Oleicacid	$C_{18}H_{34}O_2$	112-80-1
Octadecanoicacid	$C_{18}H_{36}O_2$	57-11-4
Nonacosane	$C_{29}H_{60}$	630-03-5
Nonacosane,2-methyl-	$C_{30}H_{62}$	1560-75-4
Nonacosane,3-methyl-	$C_{30}H_{62}$	14167-67-0
Triacontene	$C_{30}H_{62}$	638-68-6
2-Methyltriacontene	$C_{31}H_{64}$	1560-72-1
Tetratriacontene	$C_{34}H_{70}$	14167-59-0
Hentriacontene,3-methyl-	$C_{32}H_{66}$	4981-99-1
Dotriacontene	$C_{32}H_{66}$	544-85-4

Chemicals including cyclohexane, toluene, 1,3-dioxane, 5-(hexadecyloxy)-1, and 2-pentene may be extracted from the chickpea plant.Table 4.6 lists the compounds found to be 2-pentadecyl-,trans-1-cyclohexyldimethylsilyloxyoctadecane; 1-cyclohexyldimethylsilyloxyoctadecane; 9-octadecenoic acid (Z)-tetradecyl ester; and octadecanoic acid.

Table 4.6: Specifics of chickpea plant volatiles identified as host plant volatiles

Compoundname	**Formula**	**ChemicalAbstractsService(CAS) Number**
Pentane,2,2,3,4-tetramethyl-	C_9H_{20}	1186-53-4
2-Pentane,3-methyl-, (Z)-	C_6H_{12}	922-62-3
Cyclohexane	C_6H_{12}	110-82-7
Pentane,3,3-dimethyl-	C_7H_{16}	562-49-2
Toluene	C_7H_8	108-88-3
1,3-Dioxane,5-(hexadecyloxy)-2-pentadecyl-,trans-	$C_{35}H_{70}O_3$	34315-34-9
1-Cyclohexyldimethylsilyloxyoctadecane	$C_{26}H_{54}OSi$	1000281-96-8
9-Octadecenoicacid(Z)-,tetradecylester	$C_{32}H_{62}O_2$	22393-85-7
Octadecanoicacid	$C_{18}H_{36}O_2$	57-11-4
1,3-Dioxane,5-(hexadecyloxy)-2-pentadecyl-,trans-	$C_{35}H_{70}O_3$	34315-34-9

4.3Estimation of the orientation response of neonate larvae of h. Armigera to volatiles of different host plant leaf

Studying the orientation response of H. armigera neonate larvae against volatiles of different host plants leaf was performed in the laboratory of AINPVPM: Agricultural Ornithology, AAU, Anand during the academic year 2019-20 with the intention of identifying the host plant leaf that releases the most enticing volatiles.. In Table 4.7, you'll find information on how volatiles from various host plant leaves affect the direction of newly hatched larvae.

The highest percentage of responding neonates (29.46%) were exposed to tomato leaf, followed by those exposed to marigold leaf (15.84%). Chickpea leaf reaction was the lowest at 5.92%, tied with pigeonpea leaf response at 11.02%.

Table 4.7: H. armigera neonate larvae's orienting reaction to volatiles from various host plants' leaves.

Tr. No.	**Treatments**	**Larvalattraction (%)**

T_1	Marigoldleaf	23.45b (15.84)
T_2	Chickpealeaf	14.08c (5.92)
T_3	Pigeonpealeaf	19.39bc (11.02)
T_4	Tomatoleaf	32.87a (29.46)
S.Em. ±		1.80
CDat 5%		5.39
C.V.(%)		17.93

5. Conclusion

In order to determine which host plant releases the most enticing plant volatiles, researchers examined the orientation response of adult H. armigera to volatiles from four distinct host plants (marigold, tomato, chickpea, and pigeonpea) using a four-choice olfactometer. In trials using both whole and broken twigs from the same plant, both sexes reacted most strongly to volatiles from marigold plants, followed by those from tomatoes. In order to determine which of eight synthetic host plant volatiles performed best in a four-choice olfactometer, this experiment was done in a controlled laboratory setting. In the first experiment, participants were drawn to one of four distinct synthetic volatiles (HPV01 to HPV04), with HPV01 receiving the highest percentage of female participants (55.21%) and the lowest percentage of male participants (49.58%).

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