Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 440-445

Received: 10-08-2020
Accepted: 18-09-2020

P Arunkumar
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

D Rajabaskar
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

JS Kennedy
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Muthukumar
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

R Anandham
Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
JS Kennedy
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Influence of volatiles emitted from watermelon bud necrosis virus (WBNV) infected watermelon plants over healthy plants

P Arunkumar, D Rajabaskar, JS Kennedy, M Muthukumar and R Anandham

Abstract
Melon thrips, *Thrips palmi* Karny (Family: Thripidae; Order: Thysanoptera) is a polyphagous pest and mainly infest on Solanaceae, Cucurbitaceae and Leguminosae plants in tropical countries. An exploratory study was conducted to comprehend the changes in volatile profile of healthy and Watermelon Bud Necrosis Virus (WBNV) infected watermelon plants responsible for thrips attraction. Headspace VOCs emitted from healthy and WBNV infected plants were collected with volatile collection chamber and analyzed by Gas chromatography coupled to mass spectrometry. Host volatiles recorded with higher area percent influence strong induction of volatiles among other volatiles. The results showed Nonadecane recorded from healthy plants and Piperazine & 2-Pentene, 4,4-dimethyl from WBNV infected plants conveys higher area percent over other volatiles. Majority of host volatiles recorded from both healthy and infected plants belongs to hydrocarbon group of VOCs.

Keywords: Thrips**,** vector-virus-host interactions, WBNV, *Watermelon bud necrosis virus*, VOCs, volatile organic compounds

Introduction

Watermelon (*Citrullus lanatus,* Family: Cucurbitaceae), a vine like flowering plant originated from West Africa. It is an economically important fruit crop besides good source of lycopene and citrulline. Lycopene is a carotenoid, has antioxidant properties [1] and citrulline is a non-essential amino acid found rich in rind of water melon it helps the athletic ability and strengthening of immune system in human being [20].

Melon thrips, *Thrips palmi* was been recorded on tobacco plants in Sumatra during 1925 and later it spread to all the tropical countries as invasive pest [18]. It acts as direct as well as indirect pest. As direct pest, both adult and nymphs feeds on the lower surface of the leaves that leads to silvering of leaves along the midrib, stunted growth and severe cases plants show bronzed appearance [2].

Plants infected with pathogen, regulates the release of Volatile Organic Compounds (VOCs) which attracts the vector of pathogen and enhance the transmission and spread of virus [4]. Plant virus can induce changes in host plants via, changes in nutrition or VOC, that causes the vector to prefer more on virus infected plants compared to non-infected plants [7, 25, 3, 16, 17, 22]. The preference of thrips toward infected plants was influenced due to release of VOCs [14]. Insect vector favors more towards virus infected plants than on healthy plants [8, 11, 12, 13, 21]. *Myzus persicae* were attracted to volatiles produced from Potato leaf roll virus (PLRV) infected plants [7].

Our overall objective was to identify the host volatiles changes associated with Watermelon Bud Necrosis Virus (WBNV) infected and non-infected watermelon plants which will be responsible for attraction of vectors.

Materials and Methods

Test plants for volatile extraction

Healthy plants: Healthy watermelon plants were grown in small pots and maintained in 4” plastic pots containing mixture of (Sand: compost: Loamy soil) in the ratio 1:2:1 enclosed by insect proof bugdorm at growth chamber at 25 ± 1^{0}C. Two weeks year old watermelon plants are chosen for volatile collection.

Infected plants

The infected watermelon, *Citrullus lanatus* showing typical symptoms of WBNV like stunted growth, chlorosis, bud necrosis, stem pitting, stem necrosis, leaf mottling, bronzing and drying of leaves [10, 24] were collected from field and used as the source of virus inoculum. Samples were collected in re-sealable plastic bags and mechanical inoculation [9] was done for virus maintenance in two leaf stage healthy watermelon plants. One to two seeds were sown in 4" plastic pots containing mixture of (Sand: compost: Loamy soil) in the ratio 1:2:1 and seedlings were maintained in insect proof bugdorm at screenhouse conditions of 28-30^{0}C and 70-80% RH. One gram of WBNV infected leaf samples were macerated using 0.1M phosphate buffer adjusted to pH 7 under refrigerated condition. Buffer was prepared by stirring (6.15 ml+3.85ml) of KH_2 PO_4 and K_2HPO_4 and make upto 100ml. Then, 0.3g of Sodium sulphate (Na_2SO_4) and 0.01M 2-mercaptoethanol was added to it. Mechanical inoculation was done by swabbing the virus inoculum on two leaf stage watermelon plant during the early morning or evening hours. Before swabbing the inoculum to the leaves, injury was caused with small amount of abrasives namely, 1% celite 545 and Carborundum 320 grit (Fisher scientific, USA). 2-3 minutes after swabbing the inoculum to the leaves, washed with distilled water. During swabbing care was taken to avoid injury to the leaves and then inoculated plants were kept inside the insect proof bugdorm with label for providing ideal condition for establishment of virus. 7-10 after inoculation plants will express the symptom of virus infection. 2-week-old virus inoculated plants were selected for volatile collection.

VOC Analysis and quantification

HPLC grade Dichloromethane was used as solvent for extraction of VOCs. Before collection, the volatile collection chamber was wiped with DCM. Plant pot containing soil was covered with aluminium foil sheet to prevent any deviation from volatiles. Then the watermelon plant was kept inside the volatile collection chamber which was connected to push and pull motor using tubes on left and right sides of chamber with light source at the top (Figure 1). During volatile collection, the compressed air filtered with activated carbon was passed through the inert tube at flow rate of 300 cm^3 / min to the chamber. The existing air was trapped inside the Porapak Q sorbent tube that was connected to the outlet of chamber. Then the entrapped air was eluted from porapak using HPLC grade DCM at 500µl. Eluted samples were analysed using GCMS instrument for volatile profile analysis [23].

Gas chromatography-Mass spectrometry (Agilent technologies- 7890B GC interfaced with Agilent 5977B mass selective detector) was used for injection of sample, separation and detection of VOCs using autosampler. About 500µl of eluent (plant volatile extracts) were injected using the autosampler into the GC inlet port. GC column was a 30m length DB-5MS fused silica capillary with 0.25 mm internal diameter. The carrier gas used was Helium (99.999% purity) maintained at a flow rate of 1 mL/min with 12.445 psi column head pressure. Sample was injected at 250 ºC temperature with split mode of 5:1 at a volume of 500 µl with 280 ºC transfer line temperature, 230°C ion source temperature and 150 ºC quadrapole temperature. Total run time for each sample was 42 min per sample. The oven temperature was initially 60°C for 2 min, then increased to 230°C at 5°C/min and then finally raised to 280°C at 20°C/min which was held for 21 min. Peaks of plant extract constituents were identified based on the retention data and comparison with spectral matches in the National Institute of Standards and Technology mas spectra (NIST) library database and published kovats indices.

Results

Headspace VOCs emitted from healthy and WBNV infected watermelon plants

The GC-MS analysis of changes in the volatile emission of Healthy and WBNV infected Watermelon plants (Fig 2) were studied. Total headspace volatile compounds emitted from both the plants were trapped and documented (Table 1). Totally six classes of VOCs *viz;* Aldehyde, Hydrocarbon, Ketone, Amine, Acyclic olefins, and Alcohol were documented from both Healthy and WBNV infected Watermelon plants (Figure 3 & 4). Among them there was a strong induction of class Hydrocarbon from both Healthy (Hydrocarbon- 58%) and WBNV infected (Hydrocarbon- 46%) Watermelon plants.

VOCs emitted from Healthy watermelon plants documented were Nonanal (0.09), Decanal (0.08), 2-Undecenal (0.15), Dodecanal (0.14), Piperazine (0.01), Nonadecane (0.90), Pentadecane (0.22), Dodecane (0.12), Undecane (0.12), 2-Dodecanone (0.10), 2-Undecanone (0.12), 1,6-Dioxacyclododecane-7,12-dione (0.31).

VOCs from WBNV infected plants documented were Pyrazinamine (0.02), Benzenamine 2-methyl-3,5-dinitro- (0.03), Decanal (0.12), Dodecanal (0.10), 13-Methyltetradecanal (0.17), Piperazine (0.76), Nonadecane (0.08), Pentadecane (0.26), Dodecane (0.10), Undecane (0.20), 2,4-Hexadiyne (0.13), Tricosane (0.15), 1,6-Dioxacyclododecane-7,12-dione (0.23), 2-Pentene, 4,4-dimethyl- (0.80), 5-Isoxazolol (0.09), 2-Coumaranone (0.08), 2-Pentanamine (0.10), 2-Decen-1-ol, (E)- (0.17), Norpseudoephedrine (0.02).

PCA analysis of 24 compounds from healthy and WBNV infected watermelon plants reveals the strong induction of compounds among them (Figure 6). Total 24 compounds were numbered and plotted. It shows that the compound number 9 (Nonadecane) from healthy watermelon and compound numbers 8 (Piperazine) and 18 (2-Pentene, 4,4-dimethyl-) from WBNV infected watermelon plant have strong induction of VOCs emitted compared to other compounds.

Heat map analysis (Figure 5) of area percent of volatiles detected from healthy and infected watermelon shows three compounds have strong induction of volatiles compared to other compounds. Nonadecane from healthy and Piperazine & 2-Pentene, 4,4-dimethyl**-** from infected plants reveals strong induction of volatiles through colour chart.

Discussion

The intact headspace analysis of WBNV infected and healthy watermelon plants showed differences in their VOC emissions (Figures 2, 5, & 6). This may be due to the altered physiology of the plant after virus infection and those cues from VOCs would be used by the herbivore for host selection process**.** These volatiles are very specific in function and varies with age, phenotype and genotype of the plant [6].

After the infection of maize plants with MCMV induces changes in volatile profiles of infected plants [19]. There was a strong induction of volatile organic compounds Nonadecane on healthy plants and Piperazine & 2-Pentene, 4,4-dimethyl on watermelon plants inoculated with WBNV. Brassicaceae plants infected with Turnip yellows virus (TuYV) shows significant increase in volatile emissions [5]. In this study volatile profile of infected plants changes due to virus infection and results are on par with earlier reports by [19, 16] where the aphid behaviour was influenced by virus induced volatiles in *M. persicae*-PLRV pathosystem. However, the VOCs emission was very dynamics in which the amount and composition of VOCs production differs with infection status and disease progression [2, 26, 23].

Higher amounts of volatiles released by infected plants were responsible for vector attraction than the specific metabolites [5]. VOCs emitted from virus infected plants differ from healthy plants that are important mediators for the attraction and decision making for the insect vectors during selecting of a host plant [16].

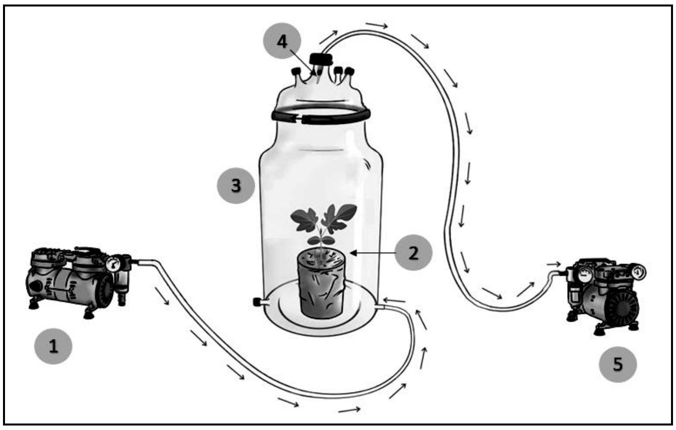


Fig 1: Setup for collection of VOCs from Volatile collection chamber

Filtered air is pumped by Push pump (1) into air tight volatile collection chamber (3) containing watermelon plant (2) in which soil was enclosed by aluminium foil. Headspace VOCs trapped with the help of Porapak Q sorbent tube (4) which was connected to pull pump (5).

Table 1: List of total VOCs trapped from headspace of healthy and WBNV infected watermelon plants

S. No	Name of the compound	Class
1.	Pyrazinamine	Amine
2.	Benzenamine, 2-methyl-3,5-dinitro-	Amine
3.	Nonanal	Aldehyde
4.	Decanal	Aldehyde
5.	2-Undecenal	Aldehyde
6.	Dodecanal	Aldehyde
7.	13-Methyltetradecanal	Aldehyde
8.	Piperazine	Hydrocarbon
9.	Nonadecane	Hydrocarbon
10.	Pentadecane	Hydrocarbon
11	Dodecane	Hydrocarbon
12.	Undecane	Hydrocarbon
13.	2,4-Hexadiyne	Hydrocarbon
14.	Tricosane	Hydrocarbon
15.	2-Dodecanone	Ketone
16.	2-Undecanone	Ketone
17.	1,6-Dioxacyclododecane-7,12-dione	Ketone
18.	2-Pentene, 4,4-dimethyl-	Acyclic olefins
19.	5-Isoxazolol	Alcohol
20.	2-Coumaranone	Ketone
21.	Indole-2-one	Ketone
22.	2-Pentanamine	Monoalkylamine
23.	2-Decen-1-ol, (E)-	Alcohol
24.	Norpseudoephedrine	Phenethylamine

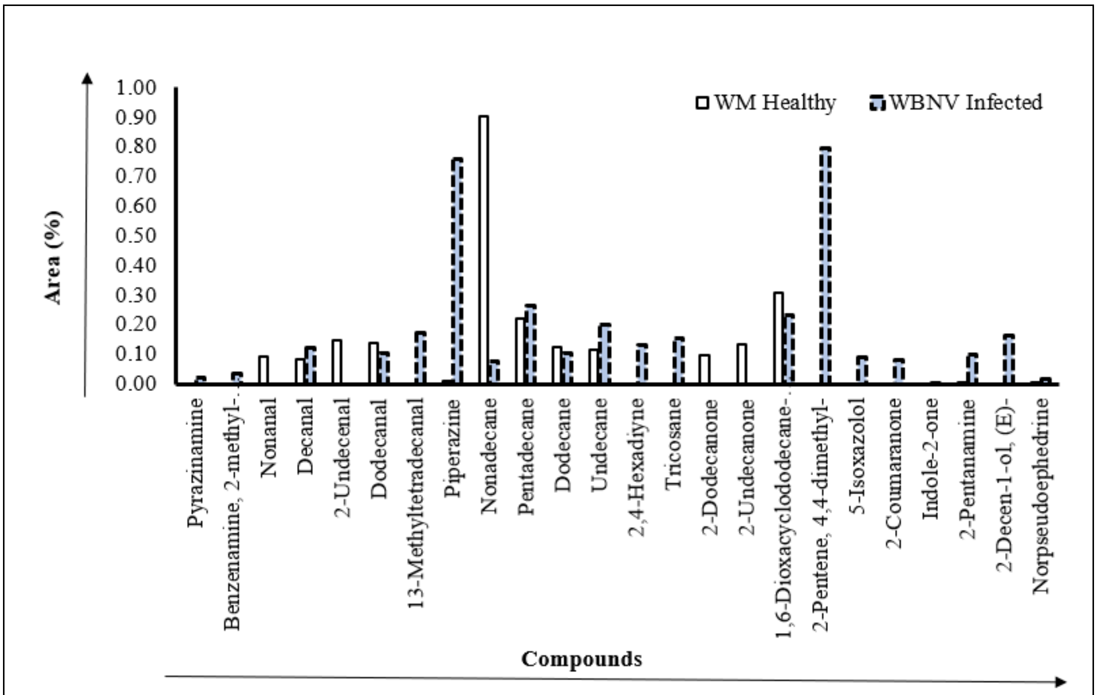


Fig 2: VOCs emitted from Healthy and WBNV infected watermelon based on area percentage Among the compounds documented from both healthy and infected plants, Nonadecane was recorded highest in Healthy watermelon plants and Piperzine, 2-pentene, 4,4-dimethyl- was recorded highest in WBNV infected watermelon plants.

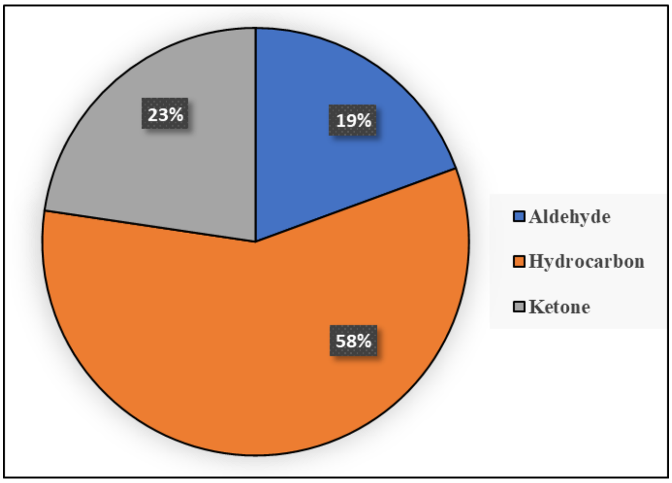


Fig 3: Proportion of major classes of Volatile organic compounds collected from Healthy watermelon plants

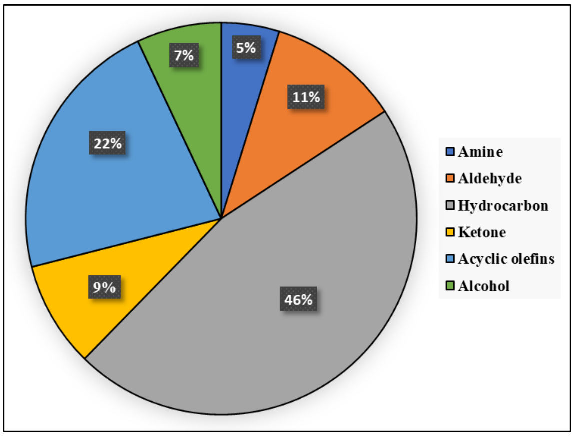


Fig 4: Proportion of major classes of Volatile organic compounds collected from WBNV infected watermelon plants Hydrocarbon class of VOCs were recorded highest from both healthy and WBNV infected watermelon plants.

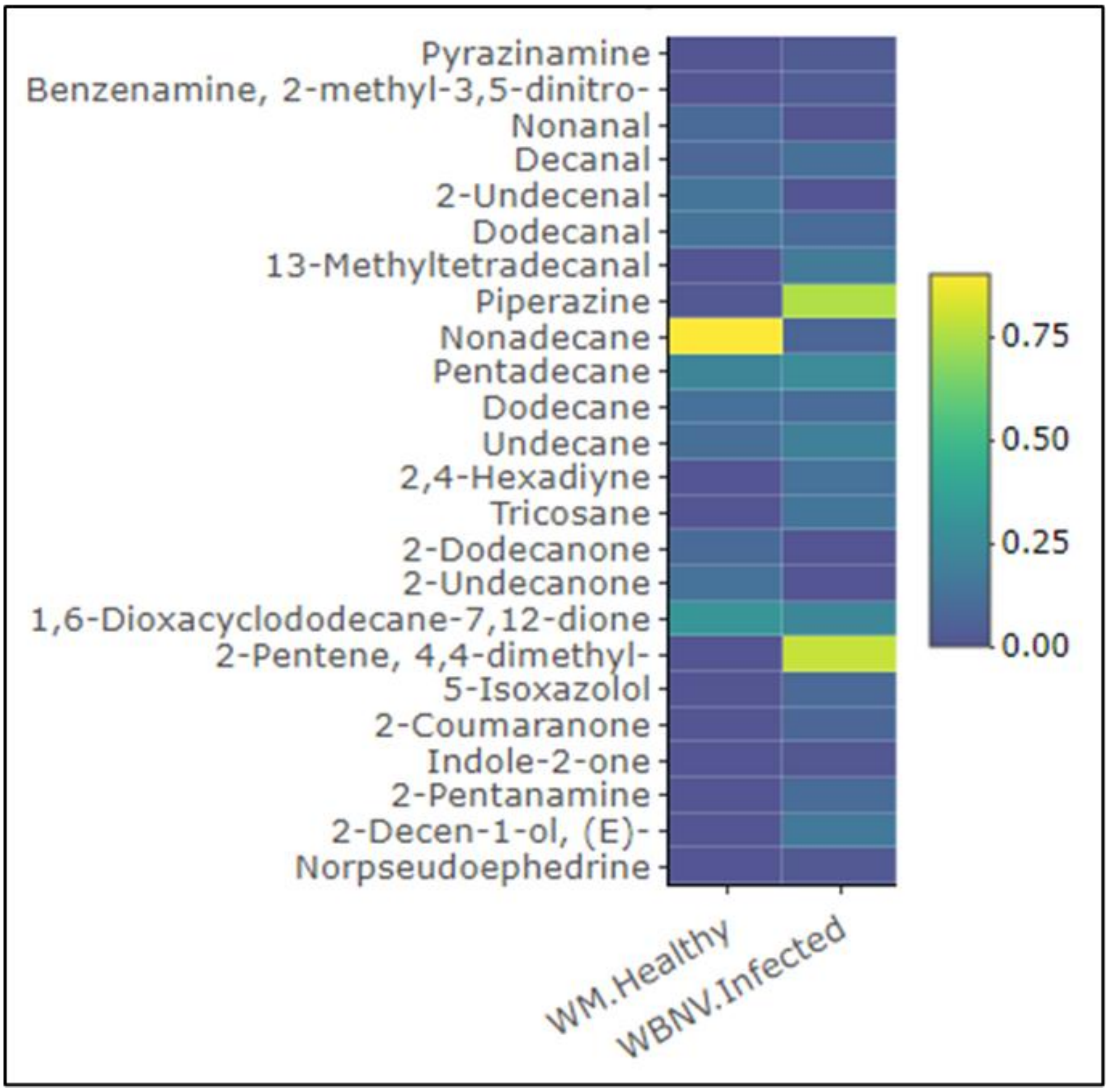
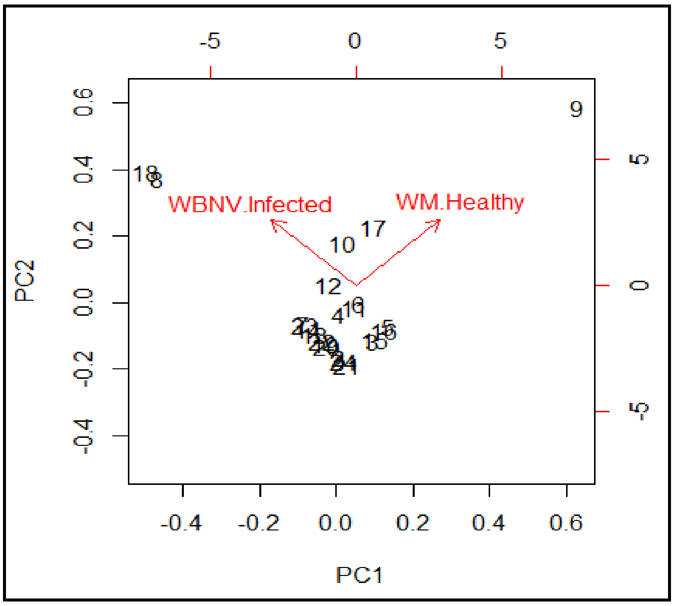


Fig 5: HEAT MAP: Analysed by R software version 3.6.1 Comparison of the volatiles in healthy and infected watermelon in terms of area percent. Colour represents area percent of VOCs emitted. Nonadecane in yellow colour indicates it was recorded highest in Healthy watermelon plants and Piperazine, 2-pentene, 4,4-dimethyl- in light green colour indicates it was recorded highest in WBNV infected watermelon plants.



1.	**Pyrazinamine**
2.	Benzenamine, 2-methyl-3,5-dinitro-
3.	Nonanal
4.	Decanal
5.	2-Undecenal
6.	Dodecanal
7.	13-Methyltetradecanal
8.	Piperazine
9.	Nonadecane
10.	Pentadecane
11.	Dodecane
12.	Undecane
13.	2,4-Hexadiyne
14.	Tricosane
15.	2-Dodecanone
16.	2-Undecanone
17.	1,6-Dioxacyclododecane-7,12-dione
18.	2-Pentene, 4,4-dimethyl-
19.	5-Isoxazolol
20.	2-Coumaranone
21.	Indole-2-one
22.	2-Pentanamine
23.	2-Decen-1-ol, (E)-
24.	Norpseudoephedrine

Fig 6: Principle Component Analysis (PCA): Analysed by R software version 3.6.1 Principle Component Analysis (PCA) for volatiles emitted from healthy and infected plants reveals that the 9th compound recorded from healthy watermelon plants and 8th, 18th compound recorded from infected watermelon plants influence more than the other compounds.

Conclusion

The experiments have been conducted with VOCs changes in healthy and circulative persistent Tospovirus, WBNV infected plants. It shows that host volatiles may influence the behaviour of vector for virus spread and transmission in which such host volatiles were identified. This host volatiles may be used as synthetic blend for attraction of thrips in future work.

Acknowledgement

This study was conducted for M.Sc. research in Department of Entomology, Pheromone laboratory, Tamil Nadu Agricultural University. Fund was provided by DBT-GOI, New Delhi was great fully acknowledged.

References

1. Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. Canadian medical association journal 2000;163(6):739-744.
2. Aishwarya P, Karthikeyan G, Balakrishnan N, Kennedy JS, Rajabaskar D. Seasonal incidence of melon thrips (*Thrips palmi* Karny) and watermelon bud necrosis virus (WBNV) in watermelon (Citrullus lanatus). Journal of entomology and zoology studies 2019;7(3):1470-1474.
3. Alvarez AE, Garzo E, Verbeek M, Vosman B, Dicke M, Tjallingii WF. Infection of potato plants with potato leafroll virus changes attraction and feeding behaviour of Myzus persicae. Entomologia Experimentalis et Applicata 2007;125(2):135-144.
4. Belliure B, Janssen A, Maris PC, Peters D, Sabelis MW. Herbivore arthropods benefit from vectoring plant viruses. Ecology Letters 2005;8(1):70-79.
5. Claudel P, Chesnais Q, Fouché Q, Krieger C, Halter D, Bogaert F *et al*. The aphid-transmitted turnip yellows virus differentially affects volatiles emission and subsequent vector behavior in two brassicaceae plants. International journal of molecular sciences 2018;19(8):2316.
6. Dudareva N, Pichersky E. Metabolic engineering of plant volatiles. Current opinion in biotechnology. 2008;19(2):181-189.
7. Eigenbrode SD, Ding H, Shiel P, Berger PH. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, Myzus persicae (Homoptera: Aphididae). Proceedings of the Royal Society of London. Series B: Biological Sciences 2002;269(1490):455-460.
8. Fereres A, Lister RM, Araya JE, Foster JE. Development and reproduction of the English grain aphid (Homoptera: Aphididae) on wheat cultivars infected with barley yellow dwarf virus. Environmental Entomology. 1989;18(3):388-393.
9. Holkar SK, Basavaraj YB, Mandal B, Jain RK. Optimization of a more efficient protocol for mechanical inoculation for watermelon bud necrosis orthotospovirus and its validation with different watermelon genotypes. Crop Protection 2018;108:110-119.
10. Holkar SK, Mandal B, Reddy MK, Jain RK. Watermelon bud necrosis orthotospovirus-An emerging constraint in the Indian subcontinent: An overview. Crop Protection 2019;117:52-62.
11. Jiménez-Martínez ES, Bosque-Pérez NA, Berger PH, Zemetra RS, Ding H, Eigenbrode SD. Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to Barley yellow dwarf virus–infected transgenic and untransformed wheat. Environmental Entomology 2004;33(5):1207-1216.
12. Kennedy JS, Booth CO. Host alternation in Aphis fabae Scop. I. Feeding preferences and fecundity in relation to the age and kind of leaves. Annals of Applied Biology 1951;38(1):25-64.
13. Laurema S, Markkula M, Raatikainen M. The effect of virus diseases transmitted by the leafhopper Javesella pelucida (F.) on the concentration of free amino acids in oats and on the reproduction of aphids. Ann. Agric. Fenn 1966;5:94-99.
14. Maris PC, Joosten NN, Goldbach RW, Peters D. Decreased preference and reproduction, and increased mortality of *Frankliniella occidentalis* on thrips-resistant

pepper plants. Entomologia experimentalis et applicate 2004;113(3):149-155.
15. Mauck KE, Chesnais Q, Shapiro LR. Evolutionary determinants of host and vector manipulation by plant viruses. In Advances in virus research 2018;101:189-250.
16. Mauck KE, De Moraes CM, Mescher MC. Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proceedings of the National Academy of Sciences 2010;107(8):3600-3605.
17. McMenemy LS, Hartley SE, MacFarlane SA, Karley AJ, Shepherd T, Johnson SN. Raspberry viruses manipulate the behaviour of their insect vectors. Entomologia Experimentalis et Applicata 2012;144(1):56-68.
18. Monteiro RC, Zucchi RA, Mound LA. Record of *Thrips palmi* Karny, 1925 (Thysanoptera, Thripidae) in the state of SÃO PAULO, Brazil. Brazilian journal of Agriculture-Revista de Agricultura 1995;70(1):53-55.
19. Mwando NL, Tamiru A, Nyasani JO, Obonyo MA, Caulfield JC, Bruce TJ *et al*. Maize chlorotic mottle virus induces changes in host plant volatiles that attract vector thrips species. Journal of chemical ecology 2018; 44(7-8):681-689.
20. Perkins-Veazie P, Collins JK, Pair SD, Roberts W. Lycopene content differs among red-fleshed watermelon cultivars. Journal of the Science of Food and Agriculture. 2001; 81(10):983-987.
21. Quiroz C, Lister RM, Araya JE, Foster JE. Effect of symptom variants derived from the NY-MAV isolate of barley yellow dwarf virus on the life cycle of the English grain aphid (Homoptera: Aphididae) and on yield components in wheat and oats. Journal of economic entomology 1991;84(6):1920-1925.
22. Rajabaskar D, Bosque-Pérez NA, Eigenbrode SD. Preference by a virus vector for infected plants is reversed after virus acquisition. Virus research 2014;186:32-37.
23. Rajabaskar D, Ding H, Wu Y, Eigenbrode SD. Different reactions of potato varieties to infection by Potato leafroll virus, and associated responses by its vector, Myzus persicae (Sulzer). Journal of chemical ecology 2013; 39(7):1027-1035.
24. Rajabaskar D, Rabeena I, Aishwarya P, Karthikeyan G, Usharani TR, Kennedy JS. Melon thrips Thrips palmi karny association with bud necrosis disease in water melon. Indian Journal of Entomology 2020;82(1):80-84.
25. Srinivasan R, Alvarez JM, Eigenbrode SD, Bosque-pérez, NA. Influence of hairy nightshade Solanum sarrachoides (Sendtner) and Potato leafroll virus (Luteoviridae: Polerovirus) on the host preference of Myzus persicae (Sulzer) (Homoptera: Aphididae). Environmental Entomology 2006;35(2):546-553.
26. Werner BJ, Mowry TM, Bosque-Pérez NA, Ding H, Eigenbrode SD. Changes in green peach aphid responses to Potato leafroll virus–induced volatiles emitted during disease progression. Environmental entomology 2009;38(5):1429-1438.