Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(6): 1304-1310

Received: 18-09-2018
Accepted: 21-10-2018

Archana Anokhe
Division of Entomology, Indian Agricultural Research Institute, New Delhi, India

Bikash Mandal
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India

Raju Kumar
ICAR- Indian Agricultural Statistics Research Institute, New Delhi, India

Kirti Sharma
Division of Entomology, Indian Agricultural Research Institute, New Delhi, India

Ranjit Ranjan
Division of Biochemistry, Indian Agricultural Research Institute, New Delhi, India

Sabtharishi Subramanian
Division of Entomology, Indian Agricultural Research Institute, New Delhi, India

Correspondence
Sabtharishi Subramanian
Division of entomology, Indian Agricultural Research Institute, New Delhi, India

Biochemical charecterisation of lipase in host specific *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) population collected from different agro ecological zone

Archana Anokhe, Bikash Mandal, Raju Kumar, Kirti Sharma, Ranjit Ranjan and Sabtharishi Subramanian

Abstract
Whitefly, *Bemisia tabaci* (Genn.) is polyphagus pest causes severe damage to crops, by sucking the sap from plants and as vector of several viral diseases. *B. tabaci* is considered as a species complex of 35 genetic groups recorded so far. Key digestive enzyme lipase was investigations under this study in host specific male and female populations of *B. tabaci* collected from different agro-ecological zone. From the study it was found that Sri Ganganagar population has shown significantly higher lipase activity than Delhi and Coimbatore. Turkey grouping of crop mean comparison revealed that lipase activity was significantly higher in female population reared on host tomato (4.86A µmol/ml/mg) than cotton (3.52B µmol/ml/mg) and brinjal reared population (2.07C µmol/ml/mg). Tomato reared population exposed to *Tomato Leaf Curl New Delhi virus* and discriminating dose (781mg/ml) of insecticide Imidachlorprid (99%) to see the effect of virus and insecticide in dynamics of lipase activity. From result it was found that lipase activity is high in virus acquired population as compare to survival of insecticide exposed and control populations, which indicate that lipase play important role in virus vector relationship. Biochemical characterisation of metabolic enzymes would improve our understanding on the metabolic capabilities of host specific differential lipase activities *vis-a-vis* host plant adaptations and management of *B. tabaci* species complex in crops.

Keywords: Lipase, *B. tabaci*, detoxification, host, viruliferous

1. Introduction
Whitefly, *B. tabaci* (G), a tiny sap sucking insect is a pest of diverse agricultural and horticultural crops. It has become a pest of economic importance owing to its polyphagous pest status with a host range of over 700 different plant species. Due to its ability to transmit more than 200 viruses specifically belonging to the genus *Begomovirus* [3] and large number of genetic group, it has become prevalent globally and difficult to control as well. As per our current understanding, it is regarded as a species complex comprising of at least 35 genetic groups commonly referred as biotypes [1]. These biotypes vary with respect to geography, fecundity, dispersal behaviour, insecticide resistance, natural enemy complex, invasiveness and plant virus transmission. Asia has the greatest diversity of *B. tabaci* species complex [8] with the presence of 16 of the 35 genetic groups described so far. However, our knowledge on indigenous members of *B. tabaci* complex in Asia is very limited, compared to the two invading species of *B. tabaci*, *viz*., Middle East-Asia Minor 1 (B biotype) and Mediterranean (Q biotype).

Lipase enzymes are defined as triacylglycerol hydrolases (EC 3.1.1.3) which break carboxyl ester bonds in diacylglycerols, galactolipids, phospholipids and hydrolyse triacylglycerols to di and monoacylglycerols with free fatty acids as co-products thus playing a key role in controlling the absorption, transport and utilization of lipids [13] [7] [6]. Various lipids are hydrolysed by lipases in the midgut lumen and the products of the hydrolysis are then absorbed into the midgut mucosal cells. The lipid components are re-synthesised to form diacylglycerol (DAG), the main lipid storage form within cells are phospholipids (PL) and triacylglycerol (TAG). The latter two lipids are transported into the haemolymph for transport throughout the insect body via lipophorin and help in metabolic pathways. Digestive lipases have been studied in a few insects like *Rhodinus prolixus* [5],

Naranga aenescens [18] *Andrallus spinidens* [19] which explains that in insects, these enzymes have key roles in utilizing, storing and transporting lipids and also they play important role in basic physiological processes of reproduction, development, and defend against pathogens, oxidative stress, and pheromone signalling [7]. Compared to lipase of higher animal, microorganism and plants lipases, there is limited work on insect lipases has been done [14].

The present investigation was carried out under controlled conditions at 25+2ºC with 60+5% RH (Relative Humidity) during 2015-2018 in the Division of Entomology, Indian Agricultural Research Institute, New Delhi. The details of the rearing equipment and chemicals used, the methodology adopted for recording observations on various aspects under study and statistical analysis used are discussed briefly in materials and methods.

2 Materials and Methods

2.1 Maintenance of Whitefly Populations

The *B. tabaci* populations evaluated in the study were originally collected from Sri Ganganagar (29.9038° N, 73.8772° E), Delhi (28° 38' 4.790" N, 77° 10' 1.590" E), and Coimbatore (11.0168° N, 76.9558° E) from the host brinjal (*Solanum lycopersicum*, L), tomato (*Lycopersicum esculantum*) and cotton (*Gossypium hirsutum*). The insects were maintained at 26+2°C & 70+5% RH in the Insect Proof Climate Control Chambers (IPCC) at Division of Entomology, IARI, New Delhi, India.

2.2 Development of isofemale lines of *B. tabaci* population

Homogenous populations of *B. tabaci* were raised from a single isofemale line by using clip cages on different host plant *i.e* – tomato, cotton and brinjal to maintain the host specific population from each agro ecological zone. One adult pair of whitefly was transferred into a clip cage soon after emergence and allowed to proliferate for further generation. About 40 clip cages were used for raising homogenous populations of *B. tabaci* from Delhi, Sri Ganganagar and Coimbatore and they were maintained in isolated chambers. Homogeneity and purity of the genetic group status was ascertained by periodical *mtCO1* analysis of random samples from these populations.

2.3 Species and genetic group status authentication of *B. tabaci* populations

Species authentication of *B. tabaci* was done by using distinct taxonomic characters [12] and partial sequencing of mitochondrial cytochrome oxidase I *(mtCOI)* gene was done to ascertain the genetic group status of *B. tabaci* populations used in this study [4]. DNA was extracted from single adult female using DNeasy blood and tissue kit (*Qiagen GmbH*, Hilden, Germany). After the PCR amplification, the final product was subjected to sequencing by outsourcing with SciGenom Labs (Cochin, Kerala, India). The *mtCO1*gene sequence obtained from each population was subjected to homology search using *Basic Local Alignment Search Tool* (nBLAST) algorithm at NCBI (http://www.ncbi.nlm.nih.gov). Primer sequence used are CI-J (10 µM) (5'→TTGATT TTTTGGTCATCCAGAAGT→3') and TL2 (10µM), (5'→TCCAATGCACTAATCTGCCATATTA→3')

2.4 Estimation of lipase activity in host specific male and female population of *B. tabaci* collected from different agro-ecological zone

Lipase activity was estimated by microplate assay using *para*-Nitrophenyl Phosphate (pNPP) as substrate. The adults of *B. tabaci* drawn from host specific male and female population collected from different agro-ecological zone *i.e* Delhi, Sri Ganganagar and Coimbatore populations which were reared on respective host from which they have been collected. Male and female of each population reared on tomato, cotton and brinjal were collected and homogenized in 50µl of ice cold 0.05 Mm sodium phosphate buffer using hand held homogenizer. Samples were centrifuged at 13000rpm at 4ºC for 15 min and supernatant was taken for enzyme assay.

Lipase activity was measured by adding 50 µl of enzyme source to microtiter plate well containing 50µl sodium phosphate buffer (0.05M, 8pH) and 250 µl substrate solutions (Sodium deoxycolate and gum arabic in sodium phosphate buffer (0.05M, 8pH) and 1mM pNPP). Absorbance was measured 405nm in SPECTRAmax*plus*^{384} absorbance reader (Molecular Device) for 3 min. The enzyme unit (U/mg protein) was defined as the production of 1 µmol of *para*-Nitrophenyl by the reaction between the substrate and the enzyme. The standard curve of *para*-Nitrophenol was prepared using different concentrations in 10mM sodium acetate-magnesium acetate solution (pH-7). The total protein content of the enzyme sources was determined by the *coomassie* brilliant blue method [2] using BSA as standard.

3 Result

3.1 Genetic group status of *B. tabaci* populations

Genomic DNA was extracted from individual insects of *B. tabaci* populations collected from Sri Ganganagar (29.9038° N, 73.8772° E), Delhi (28° 38' 4.790" N, 77° 10' 1.590" E), and Coimbatore (11.0168° N, 76.9558° E). PCR amplification was done using mitochondrial cytochrome oxidase I (mtCOI) primers (Dinsdale *et al.*, 2010) [4]. The *mtCO1* gene sequence obtained from each population was subjected to homology search using *Basic Local Alignment Search Tool* (nBLAST) algorithm at NCBI (http://www.ncbi.nlm.nih.gov). Sequence analysis revealed that Delhi and Sri Ganganagar population belonged to Asia II-1 whereas Coimbatore population shares the sequence identity to Asia I. From the phylogenetic analysis it was found that Asia I and Asia II-1 population has shown genetic divergent from each other's.

3.2 Estimation of lipase activity in host specific male and female population of *B. tabaci* collected from different ecological zone

Biochemical characterization of lipase was done by measuring enzymatic activity of male and female population of Delhi, Sri Ganganagar and Coimbatore reared on host tomato, cotton and brinjal.

3.2.1 Estimation of lipase activity in host specific male populations of *B. tabaci* collected from different ecological zone

From the results it was found that Sri Gananagar population has shown significantly higher enzyme activity then Delhi and Coimbatore with respect to host cotton and brinjal. Delhi has shown 2.44±0.17 µmol/ml/min, 1.22±0.11 µmol/ml/min, in host cotton and brinjal respectively which is significantly higher to Coimbatore *i.e* 1.02±0.11 µmol/ml/min, 0.78±0.23 µmol/ml/min and lower to Sri Ganganagar which has shown 3.42±0.16 µmol/ml/min, 1.88±0.29 µmol/ml/min in the population reared on host cotton and brinjal respectively. In case of host tomato Sri Ganganagar has shown significantly higher lipase activity (4.48±0.77) then Coimbatore

(2.75±0.18) but it is not significantly high to Delhi population (3.46±0.35)

When rt tukey grouping of population mean was compared it was found that Sri Ganganagar population has shown highest lipase activity *i.e* 3.25 µmol/ml/min which is significantly higher to Delhi (2.37 µmol/ml/min) and Coimbatore (1.51µmol/ml/min). Here Delhi and Coimbatore population also significantly differ with each other in lipase activity. Whereas when rt tukey grouping of crop mean was compared it was found that overall enzyme activity of population was significantly higher in tomato (3.56 µmol/ml/min) followed by cotton (2.29 µmol/ml/min) then in brinjal (1.29 µmol/ml/min). Result given in table-1. Graphical repesentaion of data is dispalyed in fig -1

Table 1: Enzyme activity of host specific male population collected from different agro ecological zone

Male	Delhi	Sri Ganganagr	Coimbatore	Treatment (Mean±SD)
Crop	Enzyme Activity (µmol/ml/min) expressed in Mean ± SD			
Tomato	3.46±0.35ab	4.48±0.77ab	2.75±0.18bc	3.56A
Cotton	2.44±0.17b	3.42±0.16a	1.02±0.11c	2.29B
Brinjal	1.22±0.11b	1.88±0.29a	0.78±0.23c	1.29C
Treatment (Mean±SD)	2.37 B	3.25 A	1.51C	

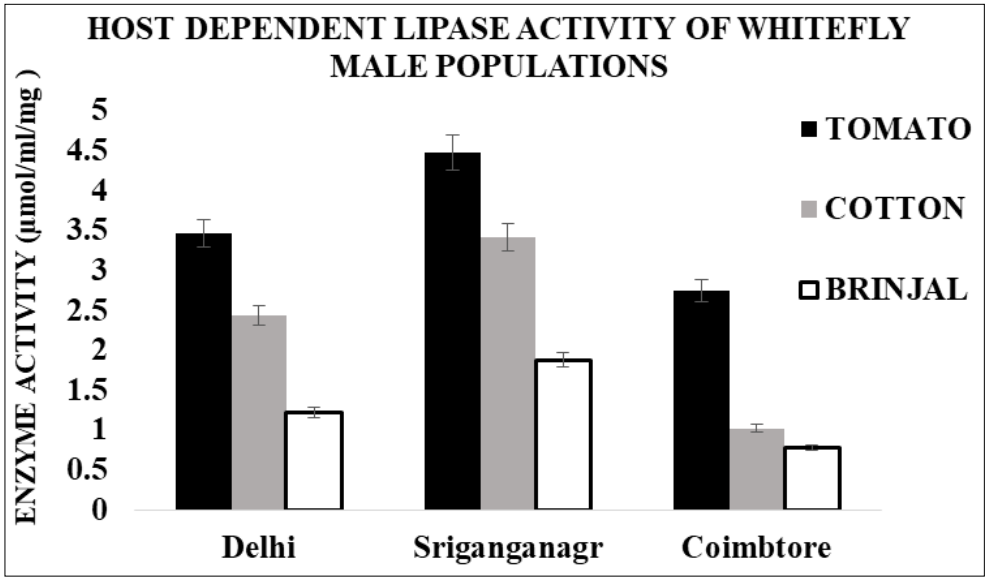
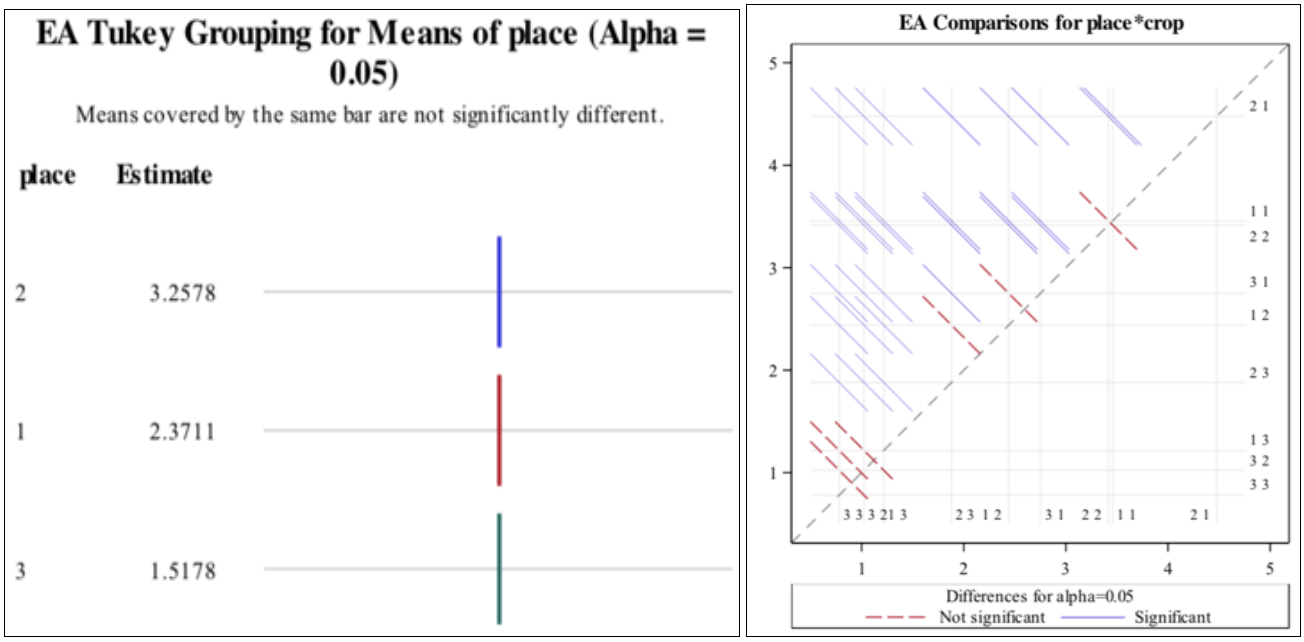


Fig 1: Graphical representation of enzyme activity of host specific male population collected from different agro ecological zone.



2a) **2b)**

Fig 2a: Tukey grouping of population mean of male collected from different agro ecological zone (where 1-Delhi population, 2-Sri Ganganagar and 3- Coimbatore population).

*The lines are not overlapping with each other means the population from places 1, 2 and 3 are significantly different, where population from place 2 has shown higher lipase activity then other remaining populations.

2b) Interaction plot for places and crop represent the behaviour of population with respect to different treatment (Crop).

3.2.2 Estimation of lipase activity in host specific female populations of *B. tabaci* collected from different ecological zone

The lipase activity of female population was also estimated. Result revealed that Sri Ganganagar female has shown significantly higher activity *i.e* 4.94±0.79 µmol/ml/min and 3.13±0.28µmol/ml/min from the population reared on host cotton and brinjal respectively. Delhi population has shown 2.85±0.08µmol/ml/min and 1.94±0.20µmol/ml/min lipase activity which is significantly lower to Sri Ganganagar but not with Coimbatore population, where Coimbatore has shown 2.79±0.13 µmol/ml/min, 1.16±0.38µmol/ml/min enzyme activity in population reared on host cotton and brinjal respectively. In case of population reared on host tomato all the three population has shown significant difference in lipase activity with each other, where Sri Ganganagar has shown highest lipase activity i.e 6.09±1.12 µmol/ml/min followed by Delhi (5.02±0.91 µmol/ml/min) then Coimbatore (3.49±0.41 µmol/ml/min). (Table-2)

When rt tukey grouping of population mean was compared it was found that Sri Ganganagar population has shown highest lipase activity *i.e* 4.71 µmol/ml/min which is significantly higher to Delhi (3.26 µmol/ml/min) and Coimbatore (2.48 µmol/ml/min). Whereas when rt tukey grouping of crop mean was compared it was found that overall enzyme activity of female population was significantly higher in tomato (4.86 µmol/ml/min) followed by cotton (3.52µmol/ml/min) then in brinjal (2.07µmol/ml/min). Result given in table-2. graphical representation of data is displayed in fig -3 and 4 (a & b).

Table 2: Enzyme activity of host specific female population collected from different agro ecological zone

Female	Delhi	Sri Ganganagr	Coimbatore	Treatment(Mean±SD)
Crop	Enzyme Activity (µmol/ml/min) expressed in Mean ± SD			
Tomato	5.02±0.91b	6.09±1.12a	3.49±0.41c	4.86A
Cotton	2.85±0.08bc	4.94±0.79ac	2.79±0.13bc	3.52B
Brinjal	1.94±0.20bc	3.13±0.28ac	1.16±0.38bc	2.07C
Treatment (Mean±SD)	3.26C	4.71B	2.48A	

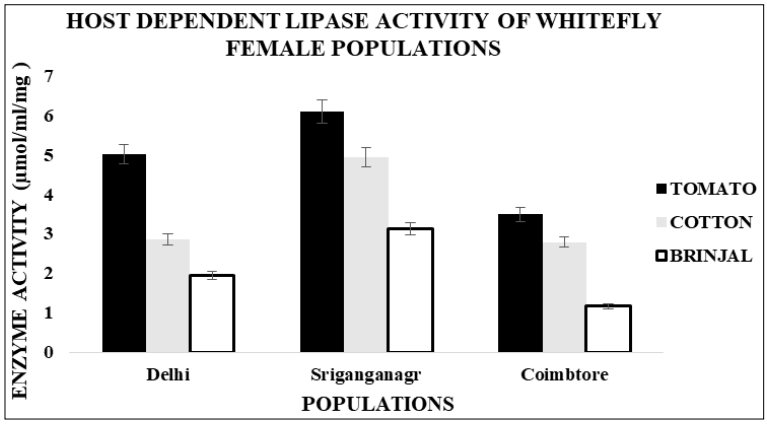
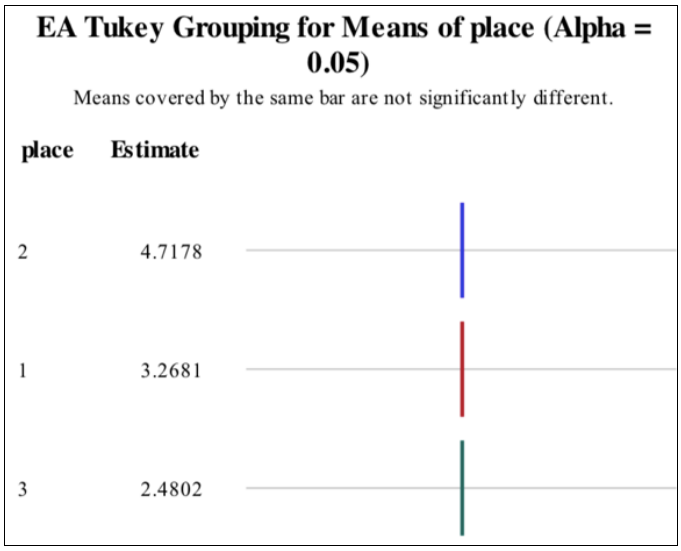
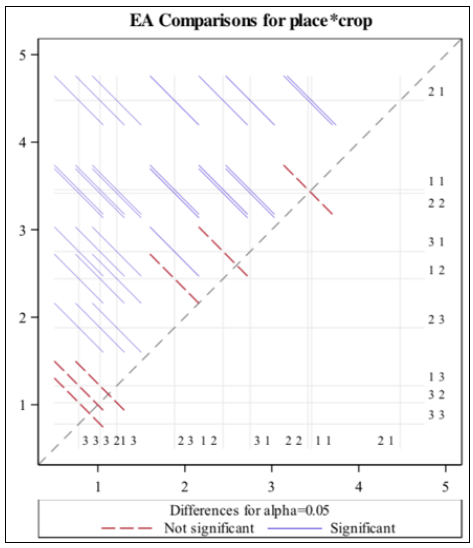


Fig 3: Graphical representation of lipase activity of host specific female population collected from different agro ecological zone.





4a) **4b)**

Fig 4a: Tukey grouping of population mean of female collected from different agro ecological zone (where 1-Delhi population, 2-Sri Ganganagar and 3- Coimbatore population).

*The lines are not overlapping with each other means the population from places 1, 2 and 3 are significantly different, where population from place 2 has shown higher lipase activity then other remaining populations.

4b) Interaction plot for places and crop represent the behaviour of population with respect to different treatment (Crop)

3.2.3 Comparison of lipase activity in host specific male and female populations of *B. tabaci* with respect to host crop- When rt grouping of male and female with respect to host was compared it was found that female population has shown significantly higher lipase activity then male across the host (Table-3)

Table 3: Comparison of lipase activity in host specific male and female populations of *B. tabaci* with respect to host crop

	Female	Male
Crop	Ezyme activity (µmol/ml/mg)	
Tomato (T)	4.86aA*	3.56aB
Cotton (C)	3.52bA	2.29bB
Brinjal (B)	2.07cA	1.29cA

*a, b, c are meant for the comparision between teartment (Crop) and * A, B are meant for the comparision between the male and female, if digit follows the same letter they do not show the significance difference among themselves

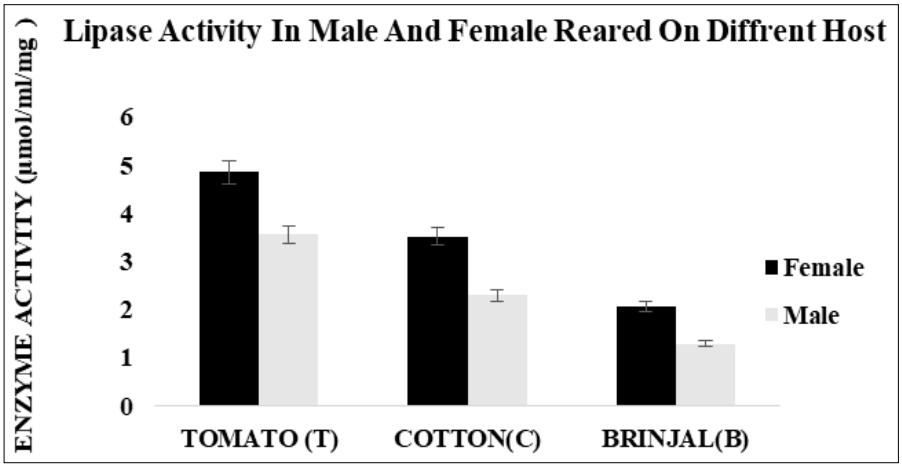


Fig 5: Comparison of lipase activity in male and female population of *B. tabaci* across the host

3.2.4 Comparison of lipase activity in host specific male and female populations of *B. tabaci* collected from different ecological zone

When rt grouping of male and female with respect to population of Delhi, Sri Ganganarag and Coimbatore was compared it was found that female population has shown significantly higher enzyme activity then male across the population (Table-4).

Table 4: Comparison of lipase activity in host specific male and female populations of *B. tabaci* collected from different ecological zone.

Population	Female	Male
	Ezyme activity (µmol/ml/mg)	
Delhi	3.26aA*	2.37aB
Sri Ganganagar	4.71bA	3.25 bB
Coimbatore	2.48cA	1.51cB

*a, b, c are meant for the comparision between teartment (Crop) and * A, B are meant for the comparision between the male and female, if digit follows the same letter they do not show the significance difference among themselves.

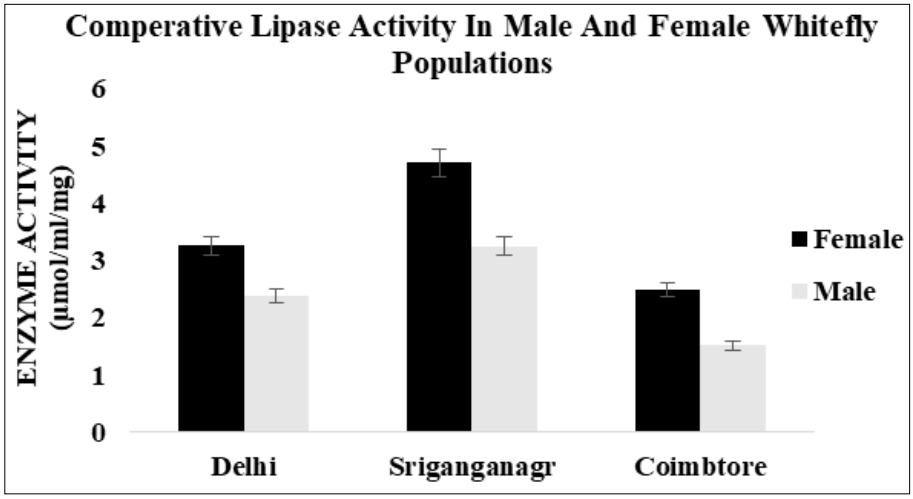


Fig 6: Comparison of lipase activity in male and female population of *B. tabaci* across the population collected from different agro ecological zone.

3.3 Dynamics of lipase activity in viruliferous and pesticide exposed population

When tomato reared female population of Sri Ganganagr, Delhi and Coimbatore is exposed to discriminating dose (781mg/ml- which is determined by using susceptible check of leucania population designated as Pusa population) and *Tomato Leaf Curl New Delhi virus* for 24 hour of acquisition access periods. The result revealed that viruliferous population of Delhi and Coimbatore has shown enhanced level of lipase activity, which is significantly higher to survival population of discriminating dose exposed population and control population. The insecticide population has not shown any significant increase in lipase activity. Sri Ganganagar population population has not shown significant fluctuation in lipase activity in viruiliferous, insecticide exposed and control condition but male and female of this population has shown highest activity with respect to all crop, which state that they have wider and quicker host plant plasticity as well as it overcome the deleterious effect like pesticide exposer or hostile environment created by virus with in the body. This result gives clear indication that lipase has more important role in virus- vector relationship rather then xenobiotic detoxification.

Table 5: Dynamics of lipase activity in control-viruliferous-pesticide exposed population.

	Female		
	Delhi	**Sri Ganganagar**	**Coimbatore**
Viruliferous	6.10±0.75a	6.94±0.72a	5.75±0.75a
Insecticide	5.05±1.09b	6.26±1.30a	3.94±0.80b
Control (B)	5.02±0.91b	6.09±1.12a	3.49±0.41b

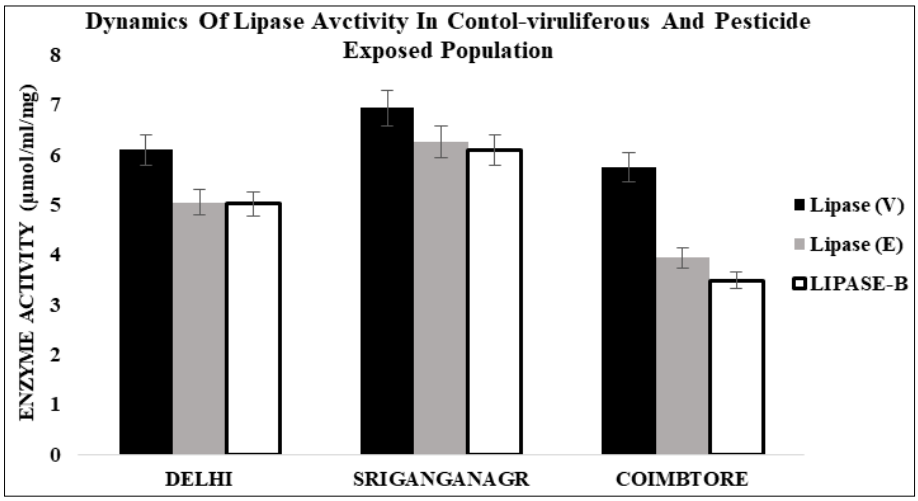


Fig 7: Dynamics of lipase activity in control-viruliferous-pesticide exposed population.

4 Discussion

Triacylglyceride lipases play a crucial role in digestion and absorption of lipids, reproduction and energy supplement [11] [9, 17]. The role of lipases in mediating host defence responses was demonstrated in *Schistocerca gregaria* with the lipases in its oral secretions were associated with the mediation of host defense response in Arabidopsis plants [15]. The salivary lipases of aphids are purported to be involved in digestion of epiculticular wax layer of plants to facilitate phloem-sap sucking process occurred by aphid [10]. Although digestive lipases have been studied in a few insects such as *Rhodnius prolixus* [5] *Naranga aenescens* [18] and *Andrallus spinidens* [19], there is no comparable literature available on enzyme kinetics of lipases in *B. tabaci*

[16] Talaee has studied Triaminoglyceroides (TAG), amylase and alkaline phosphatase activity of the *Chhrysodeixis chalcites* larvae fed on the three host plants including lemon balm (*Melissa officinalis* L.); corn (*Zea mays* L.) and dill (*Anethum graveolens* L.). The activity of Tri amino glyceroides, amylase significantly increased on dill compared with other host plants. Since TAG and amylage play great role in metabolism and dil plant is considered as good inducer of this enzyme in insect body. So these results revealed that dill (*A. graveolens*) is the most appropriate host plant for larvae of *C. chalcites* as evidenced by the highest digestive enzyme activities and intermediary metabolism. From our study tomato is considered as good host due to induction of high lipase activity among the population collected from different agro ecological zone. From our study it was found that all the three population has shown significantly higher lipase activity in tomato as compare to cotton and brinjal so the tomato can be considering as good host which enhances lipase activity in turn help in xenobiotic detoxification.

5. Conclusion

Female has shown higher activity then male which show that female is more efficient in metabolic utilisation of substrate. Among host, in tomato lipase activity is high as compare to cotton and brinjal, it shows that tomato serve as good host because it induces more lipase in body which can catalyse the food substrate in large amount and can make the *B. tabaci* more efficient then other host. In other case *Tomato Leaf Curl New Delhi virus* acquired population has shown significantly higher enzyme activity then survival of insecticide exposed and control population. This result revealed that lipase enzyme has important role in virus- vector relationship rather then xenobiotic detoxification. From the previous study it was revealed that elevated levels of lipase were associated with early metabolism of host.

6. Acknowledgment

The authors acknowledge with thanks the support from Indian Agricultural Research Institute, New Delhi as this work forms part of the Post Graduate thesis submitted by the first author. The financial grant from ICAR-National Fund is also greatly acknowledged. The help and support rendered by Dr. Chitra Srivastava, Dr. Kirti Sharma, Rahul Chandel, Sakshi Gandotra, Ramesh K B are duly acknowledged with thanks.

7. References

1. Ahmed MZ, De Barro PJ, Olleka A, Ren SX, Mandour NS. Use of consensus sequences to identify members of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) cryptic species complex in Egypt and Syria. Journal of Applied Entomology. 2012; 136:510-519.
2. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976; 72:248-254.
3. Brown JK. Molecular markers for the identification and global tracking of whitefly vector-begomovirus complexes. Virus Research. 2001; 71:233-260.
4. Dinsdale A, Cook L, Riginos C, Buckley YM, Barro PD. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Annals of the Entomological Society of America. 2010; 103:196-208.
5. Grillo LA, Majerowicz D, Gondim C. Lipid metabolism in *Rhodnius prolixus* (Hemiptera: Reduviidae): role of a midgut triacylglycerol-lipase. Insect Biochemistry and Molecular Biology. 2007; 37:579-588.
6. Hasan F, Ali Shah A, Hameed A. Methods for detection and characterization of lipases. Biotechnology Advances. 2009; 27:782-798.
7. Horne I, Haritos VS, Oakeshott JG. Comparative and functional genomics of lipases in holometabolous insects. Insect Biochemistry and Molecular Biology. 2009; 39:547-567.
8. Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Archives of Insect Biochemistry and Physiology. 2005; 58(4):216-225.
9. Kawooya JT, Law JH. Role of lipophorin in lipid transport to the insect eggournal of Biological Chemistry. 1998; 263:8748-8753.
10. Khattab H. The Defense Mechanism of Cabbage Plant Against Phloem-Sucking Aphid (*Brevicoryne brassicae* L.). Australian Journal of Basic and Applied Sciences. 2007; 1(1):56-62.
11. Majumder UK, Sengupta A. Triglyceride composition of chrysalis oil, an insect lipid. Journal of the American Oil Chemists' Society. 1979; 56:620-623.
12. Martin JH, Mound LA. An annotated check list of the world's whiteflies (Insecta: Hemiptera: Aleyrodidae). Zootaxa. 2007; 1492:1-84.
13. Mrdakovic M, Lazarevic J, Peric-Mataruga V, Ilijin L, Vlahovic M. Partial characterization of a lipase from gypsy moth (*Lymantria dispar* L.) larval midgut. Folia Biologica (Krakow). 2008; 56(12):103-110.
14. Orscelik O, Akpinar MA, Gorgun S. Partial purification of total body lipase from *Gryllus campestris* L. (Orthoptera: Gryllidae). Fen Bilimleri Dergisi. 2007; 28(2):1-10.
15. Schafer M, Fischer C, Meldau S, Seebald E, Oelmuller R, Baldwin IT. Lipase activity in insect oral secretions mediates defense responses in Arabidopsis. Journal of Plant Physiology. 2011; 156(3):1520-1534.
16. Talaee MM, Rahimi V, Zibaee A. Effects of host plants on digestive enzymatic activities and some components involved in intermediary metabolism of *Chrysodeixis Chalcites* (Lepidoptera: Noctuidae). Journal of entomological and acarological research. 2014. DOI: 10.4081/jear.2014.3224
17. Zang LS, Chen WQ, Liu SS. Comparison of performance on different host plants between the B biotype and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. Entomologia experimentalis et aplicata. 2006; 121:221-227.
18. Zibaee A, Fazeli-dinan M. Purification and characterization of a digestive lipase in *Naranga aenescens* Moore (Lepidoptera: Noctuidae). Scientific Open Access Journals. 2012; 1:38-54.
19. Zibaee A, Hoda H, Fazeli-Dinan MA. TAG-Lipase activity in the salivary secretions of a predaceous bug, *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae). Trends in Entomology. 2012; 9:48-57.
20. Ziegler R. Changes in lipid and carbohydrate metabolism during starvation in adult *Manduca sexta.* Journal of Comparative Physiology. 1991; 161:125-131.