



Comprehensive comparative toxicity study on tomato (*Solanum lycopersicum*) and brinjal (*Solanum melongena*) using green labelled insecticides against *Bemisia tabaci*

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ABSTRACT

Bemisia tabaci a serious sucking pest of tomato (*Solanum lycopersicum* L.) and brinjal (*Solanum melongena* L.), is mostly managed using high-dose conventional insecticides, which are harmful for end-users and the environment. Target-insect is also known to exhibit host-dependent variation in insecticidal toxicities; therefore an experiment was conducted during 2020–22 at ICAR-Indian Agricultural Research Institute, New Delhi, to study the host-specific toxicity relationships on tomato and brinjal using market-available safer insecticides with green labels in the Indian context. Bioefficacy evaluated on eggs, nymphs, and adults using leaf-dip bioassay (IRAC) recorded higher LC₅₀ for candidate insecticides (clothianidin, spinetoram and cyantraniliprole), but lower toxicities for azadirachtin and novaluron for test-insects reared on brinjal. The possible role of test-leaf on LC₅₀ was evaluated in a reciprocal way, i.e. test-populations grown in tomato were subjected to brinjal leaf bioassay and *vice versa*. This resulted to no significant change in LC₅₀ and implies no effect of the test leaf. Feeding (honeydew excretion) and oviposition studies showed higher honeydew excretion (76.4 mm²) and egg-laying (43.6 eggs) per 20 adults on brinjal. Detoxification enzymatic activities in control showed the test enzymes, viz. carboxylesterase, glutathione S-transferase and cytochrome P450 monooxygenase, were at higher concentrations in the brinjal population, i.e. 1.75, 1.43 μ mol/min/mg, and 3.10 n mol/min/mg of protein. Results showed that brinjal is the preferred host for *B. tabaci*, on which it develops healthier and resulting in a higher insecticide dose to arrive at the desired lethal effect.

Keywords: Carboxylesterase, Detoxifying enzymes, Glutathione S-transferase, IRAC, Leaf-dip bioassay

Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a serious pest that has a cosmopolitan presence in various agro-ecological ecosystems (Farina *et al.* 2022). It has been reported to feed on more than 600 hosts, and also regarded as a serious invasive organism (De Barro *et al.* 2012). It is also considered as a cryptic species complex which comprises of 44 morphologically indistinguishable species, which can behave contradictorily in feeding behaviour, host range, virus spreading efficiency, and insecticide resistance (Hussain *et al.* 2019). In the world, India ranks second in production of tomato (*Solanum lycopersicum* L.) and brinjal (*Solanum melongena* L.), with an annual production of 20.3 and 12.7 million metric tonnes of tomato and brinjal, respectively (National Horticulture Board 2021–22). In both of these crops, *B. tabaci* damage by both direct and indirect means. In direct damage, it sucks sap from the plant and causes yellowing, curling, and sooty mould growth. Through indirect means, it is a vector for various viral diseases (Pal *et al.* 2021). There are diverse

approaches for management of *B. tabaci*, viz. physical control, biological control (Sani *et al.* 2020), genetic control (Mishra *et al.* 2016), and chemical control (Naveen *et al.* 2017). But among all of these strategies, most popular is the chemical control (Naveen *et al.* 2017) and the main insecticides used at field level are dichlorvos, imidacloprid, clothianidin, sulfoxaflor, and flupyradifurone (Smith *et al.* 2016). Most of these insecticides are conventional insecticides, which are required in high doses, non-specific, and result in serious environmental pollution. These problems can be mitigated by substituting them with green-labelled insecticides. As we hypothesise that substituting safer alternatives for conventional insecticides with other green-labelled insecticides will be more acceptable among farmers than recommending an entirely new management strategy. Thus, in this investigation, we plan to study host-specific comparative toxicity studies against the *B. tabaci* using safer and greener insecticides on tomato and brinjal.

MATERIALS AND METHODS

Host plants, test insect and test insecticides: Tomato and brinjal were chosen as host plants for the study. *B.*

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Table 1 Details of insecticides used in the study

Insecticide	Trade name and formulation	IRAC ^a category	Mode of action ^b (IRAC)	Different safety labels		
				Label ^c colour	WHO ^d class	USEPA ^e category
Clothianidin	Dantotsu® (50% WDG)	4A	nAChR competitive modulators	Green	II	III
Spinetoram	Delegate® (11.7% sc)	5	nAChR allosteric modulators – Site I	Green	U	IV
Cyantraniliprole	Benevia® (10.26% OD)	28	Ryanodine receptor modulators	Green	U	IV
Azadirachtin	Econeem® (1% EC)	UN	Uncertain MoA	Green	U	IV
Novaluron	Rimon® (10% EC)	15	Inhibitors of chitin biosynthesis affecting CHS1	Green	U	IV
Imidacloprid	Confidor® (17.8% SL)	4A	nAChR competitive modulators	Yellow	II	II

^aIRAC Mode of action classification scheme version 10.5, March 2023; ^bIRAC, Insecticide resistance action committee; ^cBased on CIBRC classification; Insecticides Rule 1971, Govt. of India; ^dAccording to World Health Organization (WHO) classification, 2019; Ia, Extremely hazardous; Ib, Highly hazardous; II, Moderately hazardous; III, Slightly hazardous; U, Unlikely to present acute hazard; ^eAccording to United States Environmental Protection Agency (USEPA) classification; nAChR, Nicotinic acetylcholine receptor.

tabaci population was collected from the tomato from research fields in ICAR-Indian Agricultural Research Institute, New Delhi and were reared on tomato for more than a year (2020–22). Thus lab-susceptible population was later transferred to brinjal and multiplied again for 5–6 generations to stabilize. Rearing was done in Insect Proof Climate Control Chamber, at a temperature of 27±1°C, 65–70% RH, and photoperiod 14 L: 10 D. Details of selected test insecticides are mentioned in Table 1.

Insecticide bioassays

Ovicidal screening: Susceptibility of eggs to insecticides was determined using ovicidal screening method (IRAC 2009). Forty adult *B. tabaci* were collected, anaesthetized using CO₂, and released into cylindrical clip cages (diameter: 3 cm, height: 3.5 cm) made of transparent over-head projector (OHP) sheets. These clip cages then attached to the under surface of the respective host plant leaf. After 24 h, adult *B. tabaci* were removed from clip cages. For each leaf, at least 35–40 eggs were selected and removed the remaining. Leaves containing eggs were dipped in desired insecticide concentrations for 20 sec. Plants were kept under controlled conditions until first-instar nymphs emerged. After 7 days of treatment, the number of un-hatched eggs and first-instar nymphs were counted. Treatments were replicated thrice.

Nymphicidal screening: Susceptibility of nymphs to insecticides was determined using nymphicidal screening method (IRAC 2009). Forty adult *B. tabaci* were collected, anaesthetized using CO₂ and released into clip cages. These clip cages were attached to the underside of the respective host plant leaf. All the adult *B. tabaci* were removed from clip cages after 24 h and egg-laying was confirmed. After 8–9 days, when the eggs advanced into 2nd stage instars, the

leaf containing nymphs were immersed in desired insecticide concentration (20 sec). Mortality was evaluated after 7–8 days of treatment; the number of nymphal instars dead and the number of fourth instars were recorded.

Adulticidal screening: The leaf-dip bioassay method was followed for testing the susceptibility of adults to selected insecticides (IRAC 2016). Test-host leaves of similar size were collected, washed and dipped (20 sec) in desired concentration of insecticide solution and shade-dried for 2 h. After that, the petiole was inserted into an agar cube (2%; 1.2 × 1.2 × 1.2 cm³) and placed into a petri plate (90 mm × 15 mm). Then about 30–40 adult *B. tabaci* (pre-starved for 3 h) were collected, anaesthetized using CO₂ and released into each petri plate containing the respective host plant leaf treated with insecticide. All petri plates were placed at 27±1°C, 65–70% relative humidity, and a photoperiod of 14 L: 10 D. Mortality observation was recorded after 72 h (IRAC 2016). Adults that remained incapable of moving were counted as dead.

Median lethal concentration comparison on tomato and brinjal–Reciprocal approach: The effect of test-host leaf in the leaf-dip bioassay was determined by reciprocal leaf exchange. The protocol for the experiment was the same as that of the leaf-dip bioassay mentioned above, but used the opposite test leaf in the leaf-dip bioassay setup, i.e., tomato leaf for the leaf-dip bioassay of the brinjal population and brinjal leaf for tomato population. Mortalities were recorded after a stipulated time period of 72 h (IRAC 2016). Adults that were incapable of moving were counted as dead. Treatments were replicated thrice.

Estimation of feeding potential using honeydew excretion test: The experimental setup contained a single leaf with its petiole inserted into an agar cube (2%; 1.2 × 1.2 × 1.2 cm³) wrapped in aluminium foil, which helps to

avoid unwanted moisture contamination, and placed in a petri plate (90 mm × 15 mm) containing Whatman No. 1 filter paper treated with bromocresol green solution (0.1% solution in alcohol). In this setup, 20 adults were released and after 24 h, the experiment was terminated, and filter papers having white spots with a blue margin were collected. The area of spots (honeydew excretion) were calculated using the graph paper method, exact size of the spots was traced onto standard graph paper and area was calculated in mm² (He *et al.* 2013).

Estimation of oviposition rate: Twenty adult *B. tabaci* were released inside clip cages and attached to the underside of the respective leaves of hosts. The readings were taken after 24 h and eggs on the leaf was counted under a stereo microscope (30x) (He *et al.* 2013).

Detoxification enzyme assays

Carboxylesterase (CarE) activity: CarE activity was studied using 1 mM α -naphthyl acetate as substrate. Fifty live adult *B. tabaci* were collected and homogenised in sodium phosphate buffer (0.1 M, pH 7.5). The homogenate was kept for centrifugation at 10,000 rpm at 4°C for 10 min and supernatant used as enzyme source. By using 1-naphthol standard curve was prepared. The reaction mixture (250 μ l) includes 25 μ l sodium phosphate buffer (0.1 M, pH 6.0), 25 μ l enzyme source, and 200 μ l substrate solution (1 mM). The specific activity of CarE enzyme was measured at 25°C in a BioTek microplate reader for 10 min, giving a 1 min time interval at 450 nm (Feng *et al.* 2010).

Glutathione S-transferase (GST) activity: GST activity was determined using Glutathione reduced (GSH) and 1chloro-2,4-dinitrobenzene (CDNB) as substrates. Fifty live adult *B. tabaci* were collected and homogenised in sodium phosphate buffer (0.2 M, pH 7.5). The homogenate was kept for centrifugation at 10,000 rpm at 4°C for 10 min and supernatant used as enzyme source. The reaction mixture (300 μ l) includes 100 μ l enzyme source, 100 μ l CDNB (0.4 mM), and 100 μ l GSH (4 mM). The GST activity was measured at 25°C in a BioTek microplate reader for 10 min, giving a 1 min interval at 340 nm (Wu and Miyata 2005)

Cytochrome P450 monooxygenase (C-P450) activity: C-P450 activity was assayed using 4-nitroanisole (*p*-NA) as substrate. Fifty live adult *B. tabaci* were collected and homogenised in sodium phosphate buffer (0.1 M, pH 7.5, 1 mM of ethylene diamine tetraacetic acid (EDTA), 1 mM of 1, 4-dithioerythritol (DTT), 1 mM of propylthiouracil (PTU), and 1 mM of PMSF). The homogenate was kept for centrifugation at 10,000 rpm at 4°C for 10 min, and supernatant used as enzyme source. The reaction mixture includes 100 μ l *p*-NA (8 mM), 10 μ l NADPH (6 mM), and 90 μ l enzyme sources. Mixture was then incubated at 34°C in an airy atmosphere for 30 min. The C-P450 activity was measured at 25°C in a BioTek microplate reader for 10 min, giving a 1 min interval at 405 nm (Feng *et al.* 2010).

Statistical analysis: The estimate of lethal concentrations at the 95% confidence interval was determined by log-dose

probit analysis using software PoloPlus 2.0 (LeOra Software, Petluma, CA). The data obtained from the experiments were analyzed using SPSS (version 16.0). Mean values were compared using two way ANOVA and statistical significance at a significance level of P=0.05 was determined using Tukey's HSD test.

RESULTS AND DISCUSSION

Insecticide bioassays

Ovicidal screening: In tomato, cyantraniliprole 10.26% OD exhibited maximum ovicidal activity among test-insecticides (LC₅₀ = 0.599 mg/L), and least effective was novaluron 10% EC (LC₅₀ = 202.485 mg/L). Whereas, in brinjal, azadirachtin 1% EC was the most effective ovicide (LC₅₀ = 1.120 mg/L), and the least effective ovicide was novaluron 10% EC (LC₅₀ = 171.739 mg/L). The comparison of concentration-mortality response among test-hosts revealed that four test-insecticides, viz. clothianidin 50% WDG, spinetoram 11.7% SC, cyantraniliprole 10.26% OD and imidacloprid 17.8% SL, exhibited higher ovicidal effect against *B. tabaci* population reared on tomato than brinjal. Whereas, azadirachtin 1% EC and novaluron 10% EC exhibited better ovicidal activity against brinjal population (Table 2). Similar finding was reported by Chen *et al.* (2018) that cyantraniliprole showed higher ovicidal activity against *B. tabaci* feeding on chilli compared to brinjal.

Nymphicidal screening: In tomato, spinetoram 11.7% SC exhibited best nymphicidal action (LC₅₀ = 0.016 mg/L), and azadirachtin 1% EC, found to be least effective (LC₅₀ = 88.993 mg/L). Whereas, in brinjal spinetoram 11.7% SC showed maximum nymphicidal action (LC₅₀ = 0.026 mg/L) and azadirachtin 1% EC was least effective (LC₅₀ = 62.537 mg/L). The comparison of concentration-mortality response among test-hosts revealed that clothianidin 50% WDG, spinetoram 11.7% SC, cyantraniliprole 10.26% OD, and imidacloprid 17.8% SL were exhibiting maximum toxicity against nymphal stage of *B. tabaci* reared on tomato, whereas azadirachtin 1% EC and novaluron 10% EC showed more toxicity on nymphs of *B. tabaci* reared on brinjal (Table 2). Cyantraniliprole showed higher nymphicidal activity against *B. tabaci* feeding on brinjal compared to chilli (Chen *et al.* 2018).

Adulticidal screening: In tomato, spinetoram 11.7% SC was found to be most effective (LC₅₀ = 0.098 mg/L) and novaluron 10% EC was the least effective (LC₅₀ = 1550.353 mg/L). In brinjal, spinetoram 11.7% SC was most effective (LC₅₀ = 0.166 mg/L), and novaluron 10% EC was least effective (LC₅₀ = 1289.607 mg/L). The comparison of concentration-mortality response among test-hosts revealed that clothianidin 50% WDG, spinetoram 11.7% SC, cyantraniliprole 10.26% OD, and imidacloprid 17.8% SL exhibited maximum toxicity against adults of *B. tabaci* reared on tomato, whereas azadirachtin 1% EC and novaluron 10% EC showed more toxicity against adults of *B. tabaci* reared on brinjal (Table 2). *B. tabaci* population reared on tomato showed higher LC₅₀ values for imidacloprid, and

Table 2 Comprehensive summary of median lethal concentration for test-insecticides against different stages of *Bemisia tabaci*

Test insecticides	Host-stage	n	$\chi^2(df)^a$	Slope \pm SE	LC ₅₀ (mg/L)	Ratio ^b
Clothianidin 50% WDG	Tomato-Egg	790	7.11(5)	0.522 \pm 0.05	4.12	1.512
	Brinjal-Egg	806	5.808(5)	0.498 \pm 0.053	6.235	
	Tomato-Nymph	799	2.491(5)	0.889 \pm 0.088	0.049	2.102
	Brinjal-Nymph	848	3.908(5)	0.843 \pm 0.087	0.103	
	Tomato-Adult	661	5.238(4)	0.640 \pm 0.086	4.907	1.478
	Brinjal-Adult	741	2.976(5)	0.818 \pm 0.082	7.255	
Spinetoram 11.7% SC	Tomato-Egg	790	7.536(5)	1.312 \pm 0.185	2.221	1.559
	Brinjal-Egg	708	6.734(4)	0.533 \pm 0.074	3.463	
	Tomato-Nymph	802	9.786(5)	1.338 \pm 0.143	0.016	1.625
	Brinjal-Nymph	810	9.278(5)	0.788 \pm 0.087	0.026	
	Tomato-Adult	691	2.583(4)	0.558 \pm 0.072	0.098	1.693
	Brinjal-Adult	745	7.110(5)	1.171 \pm 0.118	0.166	
Cyantraniliprole 10.26% OD	Tomato-Egg	793	2.88(5)	0.525 \pm 0.055	0.599	4.178
	Brinjal-Egg	804	8.290(6)	0.428 \pm 0.044	2.503	
	Tomato-Nymph	822	8.793(5)	1.130 \pm 0.119	0.224	2.857
	Brinjal-Nymph	720	4.040(4)	0.663 \pm 0.081	0.640	
	Tomato-Adult	745	6.335(6)	0.652 \pm 0.063	5.075	3.198
	Brinjal-Adult	785	10.84(6)	0.674 \pm 0.067	16.23	
Azadirachtin 1% EC	Tomato-Egg	788	6.842(5)	0.455 \pm 0.047	1.750	0.64
	Brinjal-Egg	785	2.635(5)	0.502 \pm 0.051	1.120	
	Tomato-Nymph	824	10.19(5)	1.657 \pm 0.179	88.993	0.702
	Brinjal-Nymph	811	7.014(5)	0.600 \pm 0.079	62.537	
	Tomato-Adult	729	6.56(5)	2.333 \pm 0.293	255.91	0.571
	Brinjal-Adult	668	7.537(4)	1.021 \pm 0.104	146.21	
Novaluron 10% EC	Tomato-Egg	793	8.980(5)	0.665 \pm 0.08	202.48	0.848
	Brinjal-Egg	792	8.370(5)	1.621 \pm 0.178	171.73	
	Tomato-Nymph	716	6.641(4)	0.629 \pm 0.079	0.694	0.550
	Brinjal-Nymph	812	7.246(5)	0.793 \pm 0.088	0.38	
	Tomato-Adult	689	2.289(4)	6.359 \pm 0.65	1550.3	0.831
	Brinjal-Adult	690	2.369(4)	5.168 \pm 0.554	1289.6	
Imidacloprid 17.8% SL	Tomato-Egg	700	7.99(4)	0.525 \pm 0.055	3.578	1.329
	Brinjal-Egg	797	8.450(5)	0.594 \pm 0.065	4.758	
	Tomato-Nymph	796	4.897(5)	1.029 \pm 0.108	42.875	1.206
	Brinjal-Nymph	811	10.96(5)	0.651 \pm 0.091	51.743	
	Tomato-Adult	730	4.816(5)	1.321 \pm 0.120	107.58	1.190
	Brinjal-Adult	771	5.948(6)	0.985 \pm 0.091	128.05	

^a χ^2 at P=0.05 is of df 4= 9.49; 5=11.07; 6=12.59; ^bRatio= $\frac{LC_{50} \text{ of Brinjal}}{LC_{50} \text{ of Tomato}}$

cyantraniliprole compared to populations reared on sweet pepper, except for azadirachtin (Gravalos *et al.* 2015).

Median lethal concentration comparison on tomato and brinjal-Reciprocal approach: While using brinjal leaf for leaf-dip bioassay of tomato population it was clear that there was no significant difference between it and the results of leaf dip bioassay using tomato leaf with tomato

population (Table 3). Similarly, when tomato leaf used in leaf-dip bioassay of brinjal population indicated same i.e. there were no significant difference in leaf dip bioassay results of brinjal leaf with brinjal population (Table 3). Thus this reciprocal approach exhibited no significant variation in LC₅₀ values for both the situations, thus it is inferred least impact of host leaves.

Table 3 Median lethal concentration comparison on tomato and brinjal-reciprocal approach

Insecticides	Treatment	n	X ² (df)	Slope ± SE	LC ₅₀ (mg/L)
Clothianidin 50% WDG	Tomato population with Tomato leaf	661	5.238(4)	0.640 ± 0.086	4.907 ^f (0.690)
	Tomato population with Brinjal leaf	687	5.472(4)	0.689 ± 0.07	5.21 ^f (0.717)
	Brinjal population with Brinjal leaf	741	2.976(5)	0.818 ± 0.082	7.255 ^e (0.860)
	Brinjal population with Tomato leaf	711	3.421(4)	0.782 ± 0.078	6.73 ^e (0.831)
Novaluron 10% EC	Tomato population with Tomato leaf	689	2.289(4)	6.359 ± 0.65	1550.353 ^a (3.190)
	Tomato population with Brinjal leaf	724	3.214(4)	1.242 ± 0.72	1561.21 ^a (3.193)
	Brinjal population with Brinjal leaf	690	2.369(4)	5.168 ± 0.554	1289.607 ^b (3.110)
	Brinjal population with Tomato leaf	713	4.231(4)	2.782 ± 0.624	1302.53 ^b (3.114)
Imidacloprid 17.8% SL	Tomato population with Tomato leaf	730	4.816(5)	1.321 ± 0.120	107.58 ^d (2.030)
	Tomato population with Brinjal leaf	728	4.242(5)	1.264 ± 0.21	100.86 ^d (2.00)
	Brinjal population with Brinjal leaf	771	5.948(6)	0.985 ± 0.091	128.052 ^c (2.109)
	Brinjal population with Tomato leaf	726	5.423(5)	1.345 ± 0.08	120.58 ^c (2.081)

X² at P=0.05 is of df 4= 9.49; 5=11.07; 6=12.59, values in parentheses are log transformed data, two way ANOVA test was carried out, means within each column followed by the same superscript letters are not significantly different (Tukey's HSD test, P<0.05).

Estimation of feeding potential and oviposition rate on two different hosts: B. tabaci excrete more honeydew on brinjal (76.4 mm² per 20 adults) compared to tomato (65.3 mm² per 20 adults). *B. tabaci* oviposition rate is higher on brinjal (43.6 eggs per 20 adults) compared to tomato (28.7 eggs per 20 adults). The highest number of eggs, nymphs and adults of *B. tabaci* were found on brinjal compared to tomato hence brinjal is the most preferred host for feeding and oviposition (Hossain et al. 2018).

Detoxification enzyme assays: Higher CarE activity was found in *B. tabaci* feeding on brinjal (1.75 μ mol/min/mg of protein) plants compared to that on tomato (1.57 μ mol/min/mg of protein) (Fig 1A). Higher GST activity was recorded in *B. tabaci* feeding on brinjal (1.43 μ mol/min/mg of protein) plants compared to tomato (1.29 μ mol/min/mg of protein) (Fig 1B). More C-P450 activity found in *B. tabaci* feeding on brinjal (3.1 n mol/min/mg of protein) plants compared to tomato (2.2 n mol/min/mg of protein) (Fig 1C). Among the various detoxifying enzymes in *B.*

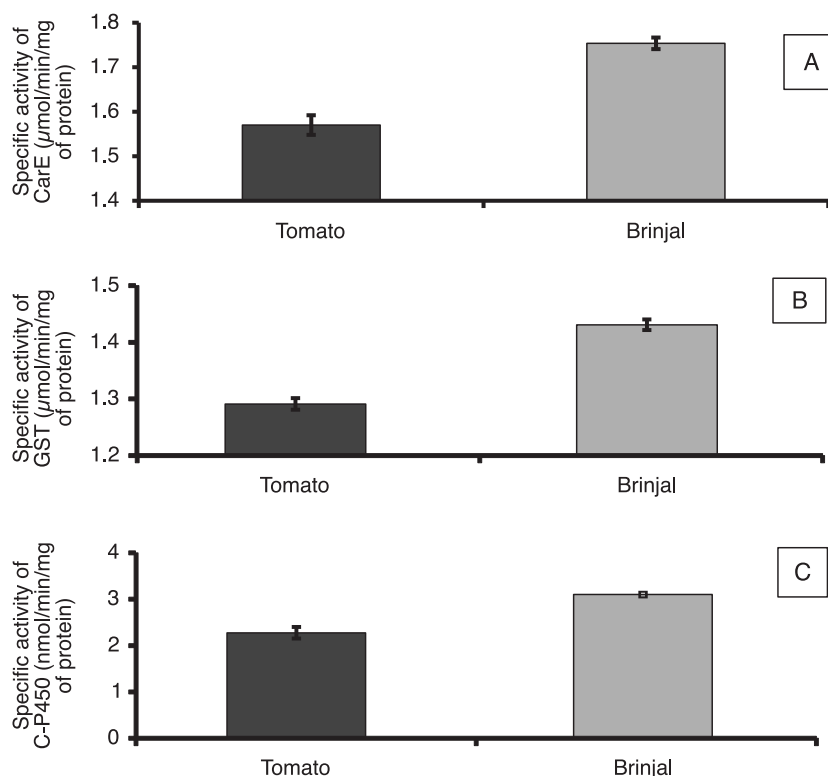


Fig 1 Comparison of specific activity (A) CarE, (B) GST, and (C) C-P450 in *Bemisia tabaci* adult population on tomato and brinjal at control condition.

Bar and error bar indicates Mean + SEM, respectively.

tabaci Q, only GSTs showed significantly higher activity in the resistant strain compared to the susceptible strain (Yang *et al.* 2016).

On comparison of both hosts it was evident that, *B. tabaci* population on brinjal required more dose of insecticide to arrive at desired toxicity (mortality). The insect feeding (as implied from honeydew excretion), oviposition parameters recorded on control populations implied brinjal to be a better and more preferred host than tomato. Comparatively higher detoxification enzymatic activities, viz. CarE, GST and C-P450 on brinjal than those in tomato explain the toxicities assayed. This higher enzyme activity in the brinjal reared pest population can be attributed to the enhanced biological performance, increased fitness, and overall healthier condition of the insect, as compared to those reared on tomato.

It is inferred that green-labelled insecticides are more effective in managing whiteflies and are considered safer due to their higher oral LD₅₀ values against rats. Green-labelled insecticides exhibit superior ovicidal, nymphicidal, and adulticidal properties compared to imidacloprid. Novaluron, classified as an insect growth regulator (IGR), demonstrates better efficacy against nymphal stages while it was very poor in managing remaining two stages. While spinetoram, clothianidin, and novaluron are not currently registered for the management of whiteflies both in tomato and brinjal, but they show promising nymphicidal and/or adulticidal effects. Henceforth, they may be considered for label claim extension with further field evaluations.

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