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Comparative Efficacy of Different Insecticides against *Bemisia tabaci* (Gennadius) under Controlled Conditions

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MA and SF conceived and designed the research. MA and MR conducted experiments and collected data. SF supervised the experiments. MA, JA, MR and SF analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Key words
White fly, Insecticides, Biochemical characterization, Resistance, IGR, Enzymes

ABSTRACT

Bemisia tabaci is the critical polyphagous pest of different crops and insecticides belonging to different groups are being used for its control. The present investigation was performed to estimate the efficacy of conventional and new synthetic insecticides along with their biochemical analysis on *B. tabaci*. The data affirmed that utmost nymphal mortality 89.80% was detected in the buprofezin and 81.46% mortality in thiamethoxam at highest concentration. Results of adult bioassay revealed maximum mortality i.e., 84.33% in the bifenthrin followed by 66.66%, 42.34%, 36.84% mortality in chlorfenapyr, profenofos and imidacloprid, respectively. Detoxification enzyme analysis showed maximum activities of acetylcholinesterase, esterases, glutathione S-transferase, acid and alkaline phosphatases in the highest concentration of insecticides and minimum activities were observed in the control groups. Findings of the current research showed that the rotational use of insecticides may be highly effective for *B. tabaci* and can also minimize risk of resistance development.

INTRODUCTION

Bemisia tabaci (Gennadius) is most significant pest of ornamentals and vegetables (Perring *et al*., 2018). Its host range varies from ornamentals to solanaceous crops including tobacco, brinjal, tomato, cotton, potato and chilies (Kanakala and Ghanim, 2019; Smith and Krey, 2019; Kunjwal and Srivastava, 2018). It damages the plants by sucking cell sap from leaves (Wang *et al*., 2017; Eslamizadeh *et al*., 2015; Mascarin *et al*., 2013) and also excretes honeydew which causes the formation of mold and it inhibits the photosynthesis (Gao *et al*., 2017; Xie *et al*., 2014). In addition, nymphs inject enzymes that alter the plant physiology resulting in an unequal ripening of fruits and underdeveloped color (Legg *et al*., 2014). It also acts as vector of pathogens including torradoviruses, begomoviruses, ipomoviruses, carlaviruses and criniviruses (Cuthbertson, 2013; Stansly and Natwick, 2010; Kumarasinghe *et al*., 2009). Out of these begomoviruses are well known for causing the losses in many crops (Gangwar and Charu, 2018).

In Pakistan, *B. tabaci* has developed resistance against carbamates, pyrethroids and organophosphates due to the excessive usage (Ahmad *et al*., 2002, 2010; Ali, 2011). Neonicotinoids have novel mode as related to the conventional insecticides as they specifically focus on the central nervous system of insects (Stenersen, 2004). In the present cropping systems, neonicotinoids are being used against sucking (plant and leaf hoppers, aphid and whiteflies) and certain chewing (coleopterans and lepidopterans) pests (Jeschke *et al*., 2011).

Alternatively, insect growth regulators (IGR's) have shown the potential for controlling *B. tabaci* under green house and field crops (Ishaaya *et al*., 1988). IGR's act by interrupting the metamorphosis and development of insects and also disrupt their expression of activity, that's why these insecticides act in slow pace than other insecticides (Sial and Brunner, 2010; Horowitz *et al*., 2005). Synthetic pyrethroids act on the voltage-gated calcium, sodium and chloride ducts and GABA-gated chloride channels

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(Forshaw and Ray, 1990; Song and Narahashi, 1996). Bifenthrin being a 3rd generation pyrethroid is effective for managing the pests of cotton (Balakrishnan *et al.*, 2009), fruits (Reddy and Rao, 2002), rice (Veeravel and Ravivarman, 2010) and vegetables (Gupta *et al*., 2009).

One of the causes of insecticide resistance is the physiological changes leading to an increased level of enzymes involved in the detoxification process (Heckel, 2012; Vontas *et al*., 2002). Furthermore, the detoxification process can lead to cross-resistance to certain other insecticides (Zhang *et al*., 2016; Mitchell *et al*., 2012; Qian *et al*., 2008). Considering the importance of *B. tabaci* as a potential economic pest, this study was planned to evaluate the effectiveness of insecticides on *B. tabaci* and the involvement of detoxification enzymes in the resistance development.

MATERIALS AND METHODS

Bemisia tabaci

Bemisia tabaci was collected with the help of manual aspirator from cotton fields of Bahauddin Zakariya University. Adults were reared on cotton plants in glasshouse without any exposure of insecticides under optimum conditions (T= 25 ± 2°C and RH = 65 ± 5%) until 5 generations. Plants were monitored for egg laying after 48-72 h, and plants containing eggs were transferred into rearing cages to attain uniform generation.

Insecticides

Commercial formulations of imidacloprid 20 SL Confidor®, Syngenta, chlorfenapyr 360 SC Squadron® FMC, profenofos50 EC Curacron®, Syngenta and bifenthrin 10EC Talstar®, FMC were used against adults, while thiamethoxam 25 WG Actara®, Syngenta, buprofezin 25 WP Buprofezin®, Syngenta were used against nymphs. All concentrations were made by serial dilution method.

Insecticides bioassay for adults

For bioassay, leaf dip method as reported by Luo *et al*. (2010) was used with slight variations to assess the effect of lethal and sublethal concentration (LC_{50} and LC_{30}). Briefly 2 mL out of 1.5% agar solution was taken into petri dishes of 1 ×3.5 cm and placed at a cool place. Petri dishes were modified by making a round hole in the cover lid with a cut of 2.8 cm in diameter for infusion of air into the petri dishes, mesh size 100 was used to make insect proof cover for the opening. To avoid large leaf veins, hole of 3.5 cm circumference was made. Leaves discs were carefully submerged in the dilutions while ddH_2O water was used to treat control insects. Leaves were left for drying with their abaxial sides facing up and then put in petri dishes to get attached with agar having no space between them.

Bemisia tabaci adults were placed in the petri dishes by making the adults immobilized by placing them at -20 °C for 10 s. 15 individuals per petri dishes were used for experiment. Bioassay was performed under completely randomized design (CRD) design having 5 treatments and 3 replications. Data was recorded by counting the dead insects and those not moving normally were also counted as dead.

Nymphal bioassay

Methodology of Zheng *et al*. (2017) was followed for nymphal bioassay. Cotton plants at two true leaves stage were grown in the glasshouse and then transferred into cages. Fifty *B. tabaci,* adults were shifted in the cages for egg-laying. After 24 h, egg-laying was observed under microscope and adults were removed. Number of nymphs were counted using magnifying lens when they reached up to 2nd nymphal instar. Infested leaves were dipped into the dilutions of the insecticides for 20 s and mortality was assessed after seven days when nymphs developed into pupae. Those nymphs were considered as dead that could not transform into pupae. Experiment contained five treatments including one control and three replicates.

Determination enzymatic activity

Samples for biochemical analysis of *B. tabaci* were collected by following Malik *et al*. (2019). Five treated adults were taken into Eppendorf tubes (1.5 mL) and 100 µL of 0.15 M NaCl was added for crushing and volume was made 500µL. Samples were spun for 10 min at 10,000 rpm and supernatant was used for determination of enzymatic activity glutathione (GST), esterases (EST), acetylcholinesterase (AChE), alkaline phosphatases (ALP) and acid phosphatase (ACP).

Habig *et al*. (1974) was followed for estimation of GST activity. Methodology of Damayanthi and Karunaratne (2005) was used for determining the EST activity. For determining the AChE activity, methodology of Ellman *et al*. (1961) was followed. The procedure of Otto *et al*. (1946) was followed to check the activities of ALP and ACP. Tris-HCl buffer 0.05M was used with 0.23mM disodium 4-nitrophenyl phosphate (PNPP), while for ACP 0.05M citrate buffer was used. Samples were incubated for 2 h at room temperature. After incubation, 425µL 0.05M NaOH was mixed in the samples for coloration. Activities were observed at λ 410 nm.

Data analysis

The software POLO-PC (LeOra, 2003) was

used for establishing both lethal (LC_{50}) and sub-lethal concentrations (LC_{30}). Mortality data was then analyzed using the software Statistix 8.1, and the Tukey's test was used to differentiate between the means of different treatment. Graphs describing enzymatic activities were created using Microsoft Excel 2010 and Prism Graph Pad (version 9.0.0). Mean values with standard errors were determined using the bootstrap methods (Meyer *et al*., 1986). All experiment were repeated twice.

RESULTS

Mortality of B. tabaci *after exposure to insecticides*

Toxicity of insecticides was evaluated using leaf dip bioassay. Mortality data for insecticides efficacy on adults of *B. tabaci* was observed 2nd day post treatment which depicted highest mortality in bifenthrin i.e., 84.33 % followed by chlorfenapyr, profenofos and imidacloprid 66.66, 42.34 and 36.84%, respectively compared to the untreated groups (Table I). The data depicted varying LC_{50}, LC_{30} and slopes values for different insecticides used against adult *B. tabaci* (Table II).

Mortality data for insecticides efficacy on *B. tabaci* nymphs was observed 7th day post treatment until nymphs changed to pupae. Results showed that highest mortality of nymphs was caused by buprofezin (89.80 %). Thiamethoxam also demonstrated notable mortality of 81.46% as compared to the untreated groups (Table 1). Similarly, buprofezin and thiamethoxam treatments on *B. tabaci* nymphs showed LC_{50} and slope 1.039, LC_{50}, LC_{30} and slope 0.796 values, respectively (Table II).

Enzymatic activity of B. tabaci *after insecticides exposure*

In bifenthrin treatment, highest AChE activity 23.53 µmol/min mg protein was observed in the maximum concentration treatment followed by GST, EST, ALP activities 18.53,15.53, 7.17 µmol/min mg protein, while least activity of ACP i.e., 5.39 was recorded in the similar treatment (Fig. 1). In chlorfenapyr treatment highest activity of GST i.e., 29.20 was found in the maximum concentration followed by EST 28.53, AChE 22.54, ALP 6.05 µmol/min mg protein and minimum activity of ACP 5.19 was found in highest concentration (Fig. 2).

Table I. Percent mortality of different stages of *B. tabaci* after exposure to insecticides.

Concentration (ppm)	Adult				Concentration (ppm)	Nymph	
	Bifenthrin (n=270)	Chlorfenapyr (n=270)	Profenofos (n=270)	Imidacloprid (n=270)		Buprofezin (n=270)	Thiamethoxam (n=270)
100	84.33 ± 0.64^{a}	66.66 ± 1.70^{a}	42.34 ± 1.11^{a}	36.84 ± 0.69^{a}	20	89.80 ± 1.82^{a}	81.46 ± 1.11^{a}
75	46.66 ± 0.74^{ab}	56.66 ± 0.74^{ab}	35.66 ± 1.29^{ab}	27.33 ± 0.74^{a}	10	74.87 ± 1.02^{a}	70.00 ± 1.29^{ab}
50	33.33 ± 0.74^{b}	40.00 ± 1.82^{bc}	25.32 ± 1.29^{bc}	20.00 ± 1.58^{ab}	5	69.33 ± 0.74^{ab}	60.00 ± 1.29^{ab}
25	20.00 ± 1.82^{c}	30.00 ± 1.82^{c}	20.00 ± 1.58^{ca}	16.66 ± 1.05^{b}	1	53.33 ± 0.91^{bc}	50.00 ± 1.58^{bc}
12.50	13.33 ± 1.29^{cd}	23.33 ± 1.29^{cd}	13.33 ± 1.05^{a}	10.00 ± 1.58^{b}	0.5	36.66 ± 1.05^{cd}	33.33 ± 1.05^{c}
Control	6.66 ± 1.82^{d}	3.33 ± 1.82^{d}	6.66 ± 1.82^{e}	3.33 ± 1.82^{c}	Control	7.66 ± 1.82^{d}	6.66 ± 0.82^{d}

Table II. LC_{50} and LC_{30} of different insecticides treated against laboratory population of *B. tabaci*.

Stage	Insecticides	LC_{50}	LC_{30}	Slope ± SE	χ^2	df	P
Adult	Profenofos	0.742-3.186	0.039-1.004	0.681 ± 0.179	2.344	3	0.781
	Bifenthrin	1.636-9.009	0.843-1.554	0.546 ± 0.175	0.171	3	0.057
	Imidacloprid	1.559-3.893	0.302-1.258	0.973 ± 0.186	0.655	3	0.218
	Chlorfenapyr	3.113-14.159	3.622-4.202	0.636 ± 0.178	1.418	3	0.473
Nymph	Buprofezin	1.076-2.688	0.234-1.214	1.039 ± 0.190	5.385	3	0.185
	Thiamethoxam	1.047-3.387	0.112-1.203	0.796 ± 0.181	0.253	3	0.084

* S.E, Standard Error; χ^2, Chi-square value; df, Degree of freedom; *P*, p-value.

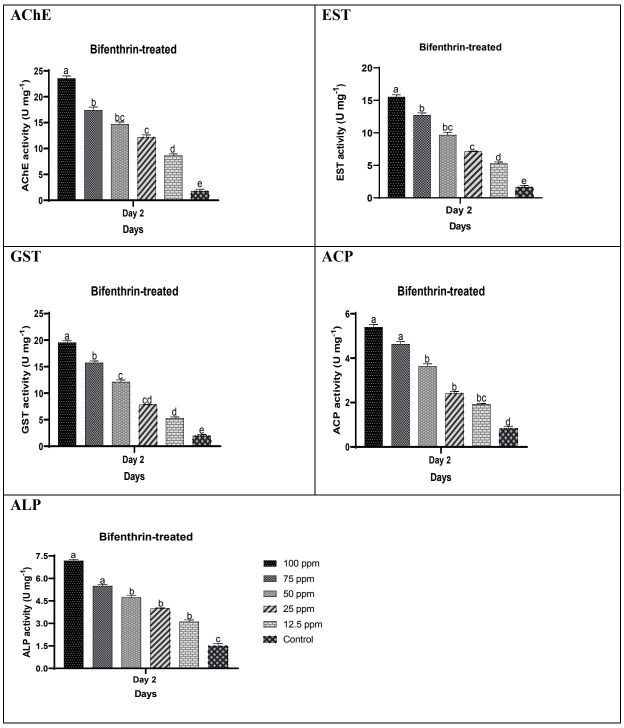

Fig. 1. Activities of AChE, EST, GST, ALP and ACP in *B. tabaci* adults treated with bifenthrin. Bars with same letters represent non-significant difference (P>0.05).

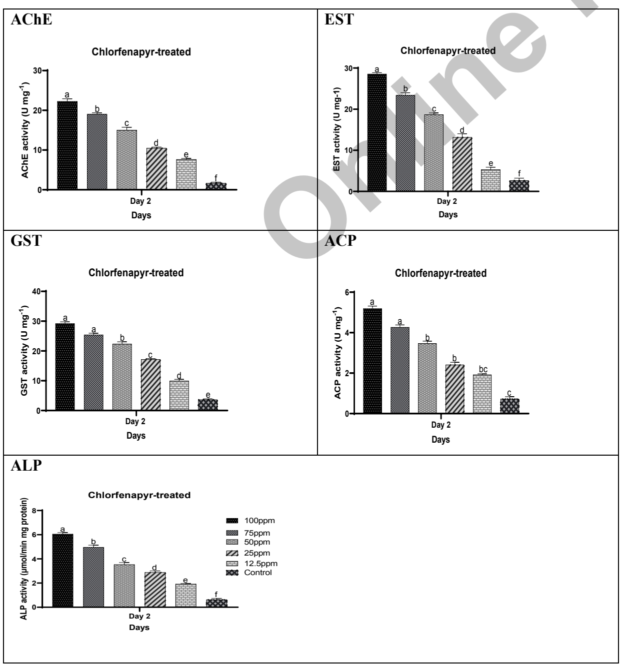

Fig. 2. Activities of AChE, EST, GST, ALP and ACP in *B. tabaci* adults treated with chlorfenapyr. Bars with same letters represent non-significant difference (P > 0.05).

In the imidacloprid treatment maximum activity of EST 31.20 was recorded in highest concentration treatment followed by 25.53 in GST, 15.53 in AChE, 4.41 in ACP while minimum activity of ALP 3.97 was observed in highest concentration treatment (Fig. 3). In the profenofos treatment, maximum activity of GST 29.25 was found in the highest concentration treatment followed by 26.75, 20.15, 5.17 µmol/min mg protein of EST, AChE, ALP while minimum activity was observed in the ACP (Fig. 4).

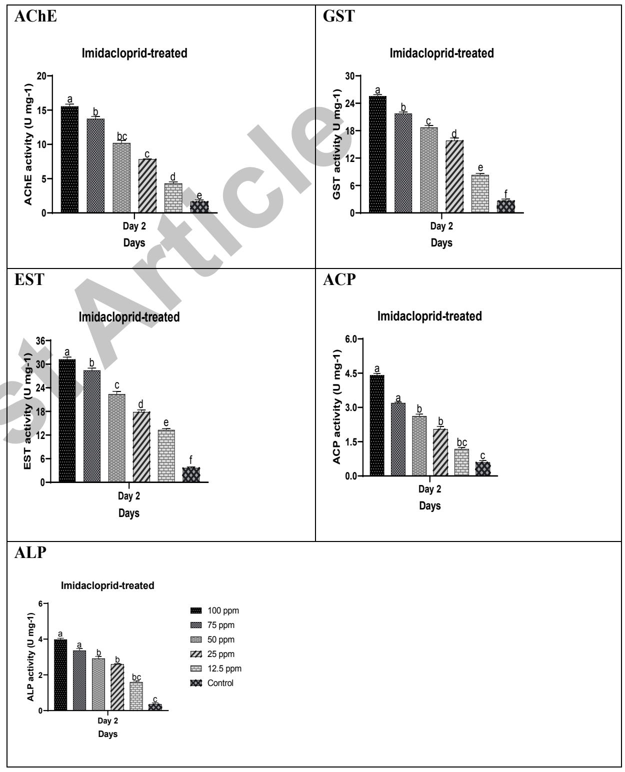

Fig. 3. Activities of AChE, EST, GST, ALP and ACP in *B. tabaci* adults treated with imidacloprid. Bars with same letters represent non-significant difference (P > 0.05).

Enzymatic activity in nymphs of B. tabaci

In buprofezin treatment maximum activity of GST i.e., 25.53 µmol/min mg protein followed by AChE 23.20, EST 18.87, ALP 9.72 µmol/min mg protein, and minimum level of ACP 9.05 was recorded in highest concentration treatment (Fig. 5). Similarly, in thiamethoxam treatment, maximum activity of AChE i.e., 25.47 was found in highest concentration treatment followed by 20.20, 18.20, 5.73 in GST, EST and ACP while minimum activity of ALP 4.17 was at similar concentration, respectively (Fig. 6).

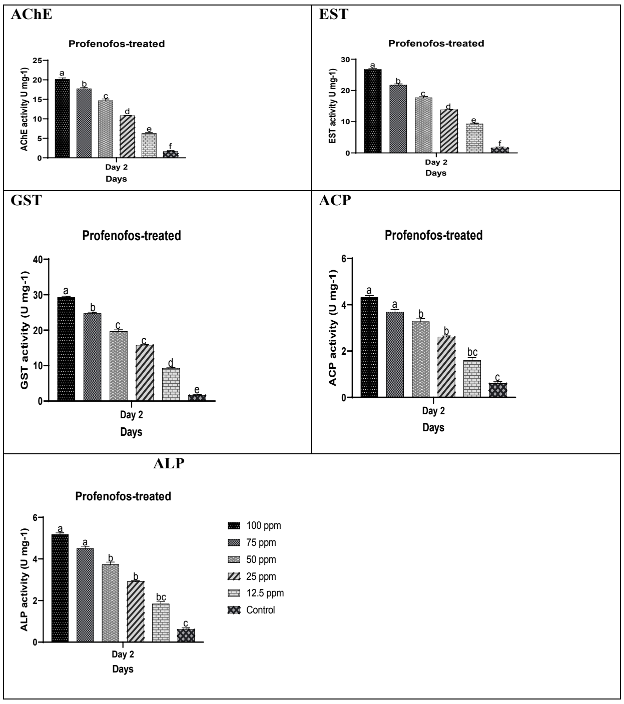

Fig. 4. Activities of AChE, EST, GST, ALP and ACP in *B. tabaci* adults treated with profenofos. Bars with same letters represent non-significant difference (P>0.05).

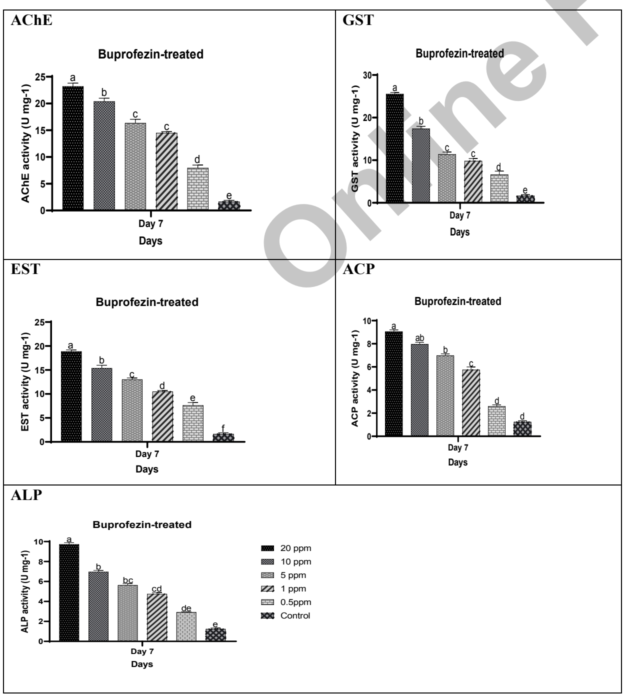

Fig. 5. Activities of AChE, EST, GST, ALP and ACP in *B. tabaci* nymphs treated with buprofezin. Bars with same letters represent non-significant difference (P>0.05).

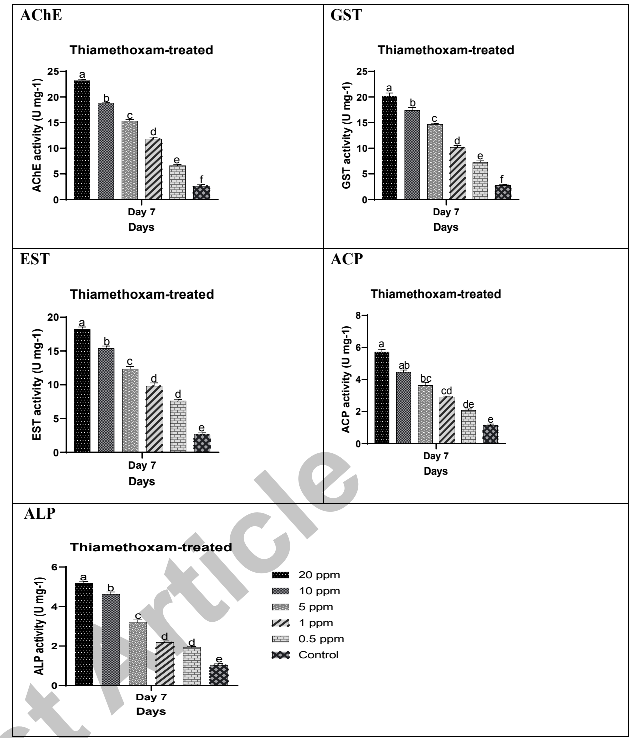

Fig. 6. Activities of AChE, EST, GST, ALP and ACP in *B. tabaci* nymphs treated with thiamethoxam. Bars with same letters represent non-significant difference (P > 0.05).

DISCUSSION

Bemisia tabaci is a versatile pest found in plains of Pakistan all over the year. During summer, cotton, okra, brinjal, and tomatoes flourish in a hotter and drier climate. Small farmers commonly sow these crops adjacently, and whiteflies continue to migrate between them. The occurrence of *B. tabaci* resistance relies on the regularity of pesticide uses instead of whitefly populations on specific crops. Cotton, brinjal and okra are treated with insecticide four times, while tomato is sprayed only two times. In Pakistan, it is common to control the infestation of sucking pests on vegetables and ornamental plants by using new chemicals (Basit *et al*., 2013). In the 1990s, Pakistan permitted the use of novel insecticides in response to a notable rise in resistance to insecticides in the country (Ahmad *et al*., 2002, 2010). For *B. tabaci*, pesticides with novel modes of action proved to be highly effective and were extensively used. In the year 2000, the vulnerability of *B. tabaci* to common insecticides was regained due to increased use of novel chemical insecticides and the decreased reliance on conventional insecticides (Ahmad and Khan, 2017). This study presents the insecticidal effectiveness of seven different insecticides classified as organophosphates, pyrethroids, neonicotinoids, and IGRs. Maximum mortality was observed by buprofezin 89.80%, which is in agreement with Gogi *et al*. (2021) who deliberated the efficacy of insecticides against whitefly and *Encarsia formosa* and stated buprofezin to cause 84.4% nymphal mortality on Bt-cotton. Neonicotinoids are the major insecticides used for the management of whiteflies (Yang *et al*., 2016). Findings of this study indicated that thiamethoxam showed 81.46% mortality of nymphs which is in line with Chen *et al*. (2018) who reported toxicity of thiamethoxm to *B. tabaci* nymphs.

Imidacloprid provides effective control of adults of *B. tabaci* and in the current research 84.33% mortality was recorded which is partially consistent with the outcomes of Babar *et al*. (2013) and Ghosh (2020) who reported 76.0 and 80.1% mortalities of adult *B. tabaci*. Whiteflies in cotton have been difficult to eradicate using standard pesticides instead of pyrethroids (Dennehy and Williams III, 1997). The tolerance of whitefly may be due to the decreased insecticide permeation, the high insecticide dissolution, and lack of sensitivity of the insecticides' target location (Guedes, 1999; Yu, 1988). Low mortalities 36.84 and 42.34%, were caused by bifenthrin and profenofos treatment which agree with the findings of Bacci *et al*. (2007) and Kady and Devine (2003) who reported inefficiency of these insecticides for controlling *B. tabaci*.

AChE, ALP, ACP, EST, and GST enzymes are responsible for neutralizing toxic metabolites in the insect body. These enzymes are essential for maintaining the normal functioning of insect physiology (Chen *et al*., 2017; Zibaee *et al*., 2009). The metabolism of insects depends on enzymes that neutralize the impact of insecticides. The resistance in insect pests to these pesticides depends on the amplified activities of such enzymes (Gong *et al*., 2021).

The study tested the impact of pesticide application on the activities of detoxification enzymes in *B. tabaci*. Increased GST and AChE activities were noted following pesticide treatment on *B. tabaci*, which aligns with the outcomes of Yang *et al*. (2016) and Bilal *et al*. (2017, 2018a, b) who detected a similar rise in AChE activity following exposure to pesticides. Similarly, elevated levels of EST, ALP, and ACP were detected in insects treated with insecticides, which correlates with Wang *et al*. (2002) and Jia *et al*. (2016) who described amplified enzymatic activities in Aphis gossypii and *Locusta migratoria*.

CONCLUSION

This study exposes the difference in vulnerability of *B. tabaci* to different insecticides under laboratory conditions and its biochemical characterization. Results propose that appropriate use of insecticides with rotation can help to reduce *B. tabaci* population and reduce the risk of insecticides resistance.

DECLARATIONS

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Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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