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The toxic effect and biochemical changes of some insecticides and its mixtures on Peach Fly, *Bactrocera zonata* (Diptera: Tephritidae)

Rokaya S. Hassanein[1], A. A. Romeh[1*], H. M. El-Sharkawi[1], H.M El-Nagar[2]

[1]Faculty of Technology and Development, Zagazig University, Zagazig, Egypt

[2]Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt

Abstract

The peach fruit fly, *Bactrocera zonata*, is one of the most dangerous pests that cause great damage to a wide range of fruits in Egypt. The toxicity of spinosad, alpha cypermethrin, indoxacarb, chlorfenapyr and its mixtures was studied against adult males and females of *B. zonata* under laboratory conditions. Bioassay results indicated that spinosad was the most toxic among the tested insecticides followed by alpha cypermethrin, and indoxacarb, respectively, while chlorfenapyr did not show any toxic effect even with recommended doses or higher than them. The LC_{50} values of spinosad, alpha cypermethrin and indoxacarb for adult males and females of *B. zonata* were reached 0.19 mg/L, 0.71 mg/L, and 126.88 mg/L through 72-h exposure respectively. The results showed that mixing spinosad at the LC_{25} with alpha cypermethrin at the LC_{25} increased the toxic effect to 86% through 72h exposure with a Co-toxicity factors value of 72, indicating the potentiation effect. It is worth mentioning that mixing indoxacarb at the LC_{25} with chlorfenapyr at the recommended dose increased the toxic effect to 79% through 72h exposure with a Co-toxicity factors value of 58, indicating the potentiation effect. The results observed that treatment of adult males and females of *B. zonata* with spinosad, alpha cypermethrin, indoxacarb, chlorfenapyr and its mixtures at LC_{25} caused decreased the activities of total protein, carbohydrate hydrolyzing enzymes (amylase, invertase and trehalase), acetyl choline esterase, protease (except alpha cypermethrin), and chitinase (except alpha cypermethrin) after 24 h of treatment compared to untreated adults. While phenol oxidase was increased (except indoxacarb) after 24 h of treatment compared to untreated adults. These results identified the potential of mixing spinosad with alpha cypermethrin at the LC_{25} and indoxacarb at the LC_{25} with chlorfenapyr at the recommended dose on the population of *B. zonata*, which can be integrated for its management strategy.

Keywords: *Bactrocera zonata*, insecticides, mixtures, toxicity, Biochemical.

Full length article **Corresponding Author*, e-mail: ahmedromeh2006@yahoo.com

1. Introduction

The peach fruit fly, *Bactrocera zonata* (Saunders, Diptera: Tephritidae), is considered severe and polyphagous insect pests that cause great damage to a wide range of fruits in Egypt, including mango, guava, apricot, peach, apple, and fig, in Egypt [1,2]. According to reports, more than 70 species of the genus Bactrocera are thought to be the main crop pests in the entire world [3]. Because of the strong attack of these pests, the economic value of fruits may ultimately decrease. These pests adapt to the various climatic environments found around the world. These are mostly present in tropical and subtropical areas of the world, causing significant economic damage and posing a growing threat of establishment into new areas [4]. Due to its severe damage to fruits and vegetables, *B. zonata* is regarded as a highly significant economic pest in Egypt [5,6]. Fruit fly control has been a major challenge for farmers. They are unable to control fruit flies' defense mechanisms [7]. Farmers rely largely on mulching sprays to control fruit flies and chemical control has been the most important measure against fruit flies in the northern Mediterranean region [8]. Insecticides have been regarded as a key tool for effectively controlling *B. dorsalis*, although their advantages are frequently diminished because of the emergence of pesticide resistance [9]. In addition, because fruit flies lay their eggs inside the fruits and the maggots are still protected in the host tissues, pesticides are ineffective in this situation because they do not reach the targeted area [10,6]. According to reports from tests on fruit flies' susceptibility, resistance to fruit flies has grown around the world, making it difficult to manage them [11]. *B. zonata* has previously been found to be resistant to a variety of organophosphate and pyrethroid pesticides in several regions of Pakistan's Punjab province [7]. The level of susceptibility of insecticides for *B. zonata and B. dorsalis* in Punjab and Pakistan observed that *B. zonata and B. dorsalis* were

susceptible to spinosad whereas resistant to trichlorfon (10-19-fold), lambda-cyhalothrin (4-9-fold), bifenthrin (8-11-fold) and malathion (3-6-fold) [12]. In Egypt, the use of large amounts of synthetic insecticides to overcome production losses resulting from damage by fruit flies, including *B. zonata*, has led to increased development of resistance and disturbance of ecosystems. Furthermore, increasing public awareness about the harmful effects of conventional insecticides on the environment and public health has encouraged the development of safer and environmentally friendly insect control strategies [13]. According to [14,15], the molecular mechanism of resistance is either based on an increase in detoxifying enzyme levels or is connected to a decrease in target-site sensitivity. Finding alternative control measures, such as mixes and rotating programs of insecticides with distinct modes of action, has been prioritized to prevent the development of pesticide resistance in the insects [16,15]. Therefore, the trend is to use new effective methods, such as the use of mixtures of pesticides that increase effectiveness, reduce resistance events, and reduce the doses used. Therefore, the current study's objectives are to assess the effectiveness of various insecticides and mixtures from various groups that have different modes of action against *B. zonata* as well as to examine the impact of sublethal concentrations of the tested insecticides on specific biochemical elements of adult males and females of B. zonata.

2. Material and methods

2.1. Rearing techniques of B. zonata's

Adults of *B. zonata* were reared using the Plant Protection Research Institute Laboratory's Zagazig branch – Sharqia, Egypt's established standard operating procedures. The relative humidity (RH) was kept at 65.5% ± $3C^0$ while the temperature of the peach fruit fly colonies was kept constant at 25.2%± $3C^0$. In Petri plates, a 1:3 mixtures of sugar and protein hydrolysate was given to adults. I filled a little plastic bottle with water. Plastic fruits with several tiny pores were placed within the cage to serve as oviposition receptacles. Eggs were collected twice a week and placed on a diet for larvae. After that, to allow the jumping larvae to pupate, the diet trays were put in a sizable wooden box with sand at the bottom [17].

2.2. The Efficiency of different insecticides and their combinations against the adult B. zonata's

Insecticides belonging to several chemical groups and its combination groups were tested for their toxicity against the adult of *B. zonata* in laboratory settings. Spinosad (Courasd 48%SC), was purchased from Consoukra Commercial Agencies, alpha cypermethrin7% + acetamiprid 3% (Alpha-1, EC), was purchased from AS Agro for the prevention and development of agricultural projects, indoxacarb (Easo 30%WG), was purchased from Shoura Chemicals, chlorfenapyr, (Challenger super 24% suspension concentrate, SC) as conventional formulation was purchased from BASF Agricultural Solutions Small glass jars were used for the toxicity trials, which each contained ten adults of male and ten adults of female flies kept apart, and they were not given any food. Cotton pieces were submerged in a series of five concentrations of each tested pesticide, with three replications for each concentration, and three untreated replications were also put up as a control. After 24 hours, 48 hours, and 72 hours, the little jars were examined, and the dead flies were counted and noted. Individuals' mortality rates were noted in relation to each concentration, and the data were then subjected to a probit analysis [18] to determine the LC_{50} and confidence limits values. The mortality percentages were corrected according to the mortality of control using Abbotts, formula [19]. Toxicity index (T.I) and relative potency (R.P) were estimated according to [20,21] as follows:

Toxicity index = [LC_{50} or LC_{90} of the highest efficient compound / LC_{50} or LC_{90} of the other compound] ×100.

Relative potency (R.P.) = [LC_{50} or LC_{90} of the other compound / LC_{50} or LC_{90} of the highest efficient compound].

The joint toxic effect of the various insecticides on the adult of *B. zonata* was evaluated according to [22]. The expected LC_{25} value, which was obtained from the regression line, was tested for all insecticides except for chlorfenapyr at the recommended dose in the combination, spinosad+alpha cypermethrin, indoxacarb+ chlorfenapyr, indoxacarb+ alpha cypermethrin, chlorfenapyr+alpha cypermethrin, spinosad+indoxacarb, spinosad+chlorfenapyr, alpha cypermethrin+ indoxacarb+ chlorfenapyr and spinosad+indoxacarb+ chlorfenapyr. Blends were evaluated against *B. zonata* adults in the manner indicated above. Following treatment, the observed mortality was noted 24 h, 48 h, and 72 h later. The following equation was used to compute the co-toxicity factor: Co - toxicity factor = (OM - EM)/EM× 100

where EM stands for expected mortality (%) and OM for observed mortality (%). According to [22], a factor between 20 and +20 indicates an additional effect. A factor of -20 or less indicates antagonism. A factor of +20 or more indicates strengthening or potentiation.

2.3. Biochemical studies

2.3.1. Preparing samples for a biochemical test

The purpose of the current study was to evaluate the toxicological effects of pesticide combinations against *B. zonata* adults using the feeding method. This experiment was conducted to examine the effects of sublethal doses (LC_{25}) of the insecticides, spinosad, alpha cypermethrin, indoxacarb, chlorfenapyr, and their mixtures on certain enzyme activities such as total protein, carbohydrate hydrolyzing enzymes (amylase, invertase and trehalase), acetyl choline esterase, protease, and chitinase against adult of *B. zonata* after 24 hours exposure. Using a cold glass Teflon homogenizer, samples of treated and untreated adults of both sexes (males and females) were homogenized in 5 ml 0.1M phosphate buffer (pH 7.4) at reate (50 mg/ml) and replicated four times. Centrifuged homogenates at 5000 rpm for 20 min. at 5 ^oC in a refrigerated centrifuge. The supernatants were stored in a deep freezer at -20 ^oC until they were required for the biochemical experiments, and the deposits were discarded. According to the procedures outlined by [23], total soluble protein in supernatants of homogenate treated and untreated adults of *B. zonata* was determined. The procedure employed to measure the rate of digestion of trehalose, starch, and sucrose by the enzymes trehalase (EC 3.2.1.28), amylase (EC 3.2.1.1), and invertase (EC 3.2.1.26) was like that described by [24]. Using the [25] approach, the reducing end group N-acetyl-glucoseamin

generated from colloidal chitin was measured to estimate the chitinase activity. Acetylcholine bromide (AchBr) was used as the substrate to test AchE (acetylcholinesterase) activity in accordance with the procedure outlined by [26]. Poly Phenol oxidase activity was determined according to [27]. Protease assay was determined with β-casein as substrate according to [28].

2.4. Data analysis

Data were evaluated statistically by one-way ANOVA, and comparison of mean values (mean ± SD) was done by using Tukey's honestly significant difference test at $p \leq 0.05$. The CoStat 6.311 CoHort Statistical Software was used for the analysis [29].

3. Results

3.1. Toxicity of examined insecticides toward adult of B. zonata

The toxicities of spinosad, alpha cypermethrin, indoxacarb, and chlorfenapyr against the adult of *Bactrocera zonata* were noted after 24-h, 48 -h, and 72 -h (Table 1). Data in Table 1 demonstrate that spinosad toxicity was at its peak at the LC50 level following 24-, 48-, and 72-hour exposure. Spinosad was able to induce the highest control efficiency against the adult of *B. zonata* compared with alpha cypermethrin, indoxacarb, and chlorfenapyr. Bioassay results indicated that treating spinosad has high toxicity to adults of *B. zonata* with an LC_{50} of 0.56 mg/L and 0.19 mg/L through 48h and 72h after exposure, respectively, compared with 1.22 mg/L and 0.71 mg/L for alpha cypermethrin**,** 319.89 mg/L and 126.88 mg/L for indoxacarb after 48-h and 72-h of treatment (Table 1). At LC_{50}, the relative potency of spinosad was 166.0 and 289.0**,** indicating 166.0 -fold and 289.0-fold insecticidal activity relative to that of indoxacarb through 48h and 72h after spraying, whereas the relative potency of alpha cypermethrin was 102.35 and 56.94**,** indicating 102.35 -fold and 56.94-fold insecticidal activity relative to that of indoxacarb through 48h and 72h exposure, respectively. It is noted that the toxicity of indoxacarb towards of *B. zonata* adult did not appear within 24 hr of exposure, but it produced its toxic effect within 48h and 72h post treatment. This may be due to the behavior of the endoxacarb towards the insect, where it acts as a strong inhibition of the feeding process, even in sub-lethal doses, which leads to delayed growth, slow development, and very little feeding. Chlorfenapyr did not cause any toxic effect even with the concentrations higher than the recommended concentrations. Within 24 hours, the slope values of the mortality regression lines were less than 1 for spinosad and alpha cypermethrin, indicating a flat line and a heterogeneous population. However, after 48 hours, the slope values increased above 1.0 and reached 1.25 and 1.43 for spinosad and alpha cypermethrin, and reached 2.62 for indoxacarb, indicating a steep, high slope ($b > 2$), indicating the population is relatively homogeneous in the response being measured.

3.2. Combined action studies of tested insecticides against B. zonata adults

The combination between spinosad+alpha cypermethrin, indoxacarb+ chlorfenapyr, indoxacarb+ alpha cypermethrin, chlorfenapyr+alpha cypermethrin, spinosad+indoxacarb, spinosad+chlorfenapyr, alpha cypermethrin+ indoxacarb+ chlorfenapyr and spinosad+indoxacarb+ chlorfenapyr was evaluated at LC_{25} for all insecticides except chlorfenapyr at the recommended dose against *B. zonata* adult through 24h, 48h and 96h exposure. The results showed that the LC_{25} values of spinosad or indoxacarb in the presence of alpha-cypermethrin, also indoxacarb or alpha-cypermethrin in the presence of chlorfenapyr against adults of *B. zonata* through 72h exposure were reduced when compared to the LC_{25} values for each compound separately (Tables 1 and 2). As shown in Table (2) clear that spinosad +alpha-cypermethrin and indoxacarb+ alpha cypermethrin caused 86.0% and 72% mortality in adults of both sexes of *B. zonata* through 72h exposure, which indicates that the Co-toxicity factor produced a potentiation of 72 and 44, respectively. It is noted that spinosad susceptibility in adults of *B. zonata* was increased in the current investigation (Table 1), this might be because of spinosad recent inclusion in tephritid fly management programs. Therefore, mixing pesticides reduces the possibility of resistance occurring, increases the toxic effect, and reduces the recommended doses for control, which reduces environmental pollution. It is worth noting that chlorfenapyr did not cause any toxic effect, even with the recommended, but when mixing it with indoxycarb at LC_{25} increased the toxic effect to 79%, which indicates that the mixture of chlorfenapyr with indoxycarb caused joint action of 58 against adults of *B. zonata* through 72h exposure. Furthermore, Data in Table (2) show that mixing alpha cypermethrin +indoxacarb+ chlorfenapyr increased the toxicity of indoxacarb+ chlorfenapyr from 79% to 100.0% mortality in adults of both sexes of *B. zonata* through 72h exposure, which indicates that the Co-toxicity factor produced a potentiation of 100. From the previous results, it appears that chlorfenapyr increase the toxicity of indoxacarb, as well as increase the toxicity of indoxacarb + alpha cypermethrin. Perhaps the reason for this is that indoxacarb is a pro-insecticide that quickly breaks down into its matching N-decarbomethoxylated metabolite (DCJW), which could effectively block sodium channels in insects and cause their death by flaccid paralysis. While chlorfenapyr interferes with the proton gradient across mitochondrial membranes and therefore hinders mitochondria's capacity to make ATP, which ultimately causes the death of damaged cells and the organism and the result both of compound increased the toxic effect through block sodium channels from indoxacarb + impairs ATP production from chlorfenapyr. In case of three mixtures (alpha cypermethrin +indoxacarb+ chlorfenapyr) increased the toxic effect to 100% which each compound has a toxic effect that differs from the other.

3.3. Biochemical responses of B. zonata to the tested insecticides

Data in Table 3 demonstrated that the tested insecticides (LC_{25}) and their mixtures at LC_{25} for all insecticides except chlorfenapyr at the recommended dose against adult of *B. zonata* through 24-hrs exposure decrease the most level of total protein, carbohydrate hydrolyzing enzymes (amylase, invertase and trehalase), acetyl choline esterase, protease, and chitinase relative to the untreated control.

3.4. Total soluble protein content

After 24 hours of exposure against adult *B. zonata*, data in Table (3) showed the most substantial reduction in the level of total soluble protein in all the tested insecticides and its combinations, except for alpha cypermethrin and Indoxacarb+ Spinosad, which was higher than the control. In the tested insecticides and its mixtures, indoxacarb recorded the highest decreased in the level of total soluble protein (1.87 mg/ g.bw) followed by chlorfenapyr (2.41 mg/ g.bw), indoxacarb+ chlorfenapyr (2.61 mg/ g.bw), spinosad+indoxacarb+ chlorfenapyr (3.0 mg/ g.bw), spinosad+alpha cypermethrin (3.38 mg/ g.bw), indoxacarb+ alpha cypermethrin (3.52 mg/ g.bw), alpha cypermethrin+ indoxacarb+ chlorfenapyr (4.08 mg/ g.bw) and spinosad (8.85 mg/ g.bw), respectively compared with the untreated control (23.65 mg/ g.bw). The corresponding reduction percentages of protein level in indoxacarb, chlorfenapyr, and spinosad were 92.1%, 89.82%, 88.92%, 87.31%, 85.73%, 85.11%, 82.77% and 62.57%. indoxacarb+spinosad recorded the highest increased in the level of total soluble protein (36.00 mg/ g.bw) followed by alpha cypermethrin (23.94 mg/ g.bw), representing an increase of 52.21% and 1.20%. It is noted that indoxacarb and chlorfenapyr and their mixture caused a significant decrease in the level of total soluble protein.

3.5. Carbohydrate hydrolyzing enzymes

Results in Table (3) recorded a changes in the activity of the carbohydrate hydrolyzing enzymes (Invertase, trehalase and amylase) activity, which hydrolyzing sucrose, trehalose, and starch, respectively. Data in Table (3) showed a significantly decrease in the level of carbohydrate hydrolyzing enzymes (invertase, trehalase and amylase) in all the tested insecticides and its combinations, except for indoxacarb+ Spinosad for three enzymes and alpha cypermethrin for invertase, which were higher than the control. The highest reduction percentages in trehalase enzymes were found in case of indoxacarb, spinosad+ alpha cypermethrin and indoxacarb+ chlorfenapyr, recording 90.11%, 89.01% and 87.91%, respectively. Indoxacarb, spinosad+ alpha cypermethrin, and indoxacarb+ chlorfenapyr showed the highest reduction percentages in trehalase enzyme, recording 90.11%, 89.0%, and 87.9%, respectively. While chlorfenapyr, indoxacarb and alpha cypermethrin, showed the highest reduction percentages in amylase enzyme, recording 99.76%, 99.62%, and 96.63%, respectively. In the case of invertase, the highest reduction percentages showed in indoxacarb+ chlorfenapyr, spinosad+alpha cypermethrin and chlorfenapyr, representing 99.71%, 95.61%, and 93.57%, respectively. It's noticed that indoxacarb reduced the activity of trehalase and amylase enzymes, indoxacarb+ chlorfenapyr reduced the activity of trehalase and invertase enzymes, while spinosad+ alpha cypermethrin reduced the activity of trehalase and invertase enzymes. It has been observed that indoxacarb, chlorfenapyr, and their combination significantly reduced the levels of the carbohydrate hydrolyzing enzymes (invertase, trehalase, and amylase).

3.6. Acetylcholinesterase activity

Data in Fig (1a) showed significant decrease in the level of acetylcholinesterase activity in all the tested insecticides and its combinations between 19.09 to 90.24. The highest reduction percentages in acetylcholinesterase activity were found in case of indoxacarb+ alpha cypermethrin+ chlorfenapyr, Spinosad and spinosad+ alpha cypermethrin, recording 90.24%, 90.18% and 89.43%, respectively.

3.7. Protease, chitinase and phenole oxidase

Data in Fig (1, b-c) showed significant reduction in the level of protease and chitinase activity in all the tested insecticides and its combinations, except alpha cypermethrin, spinosad+indoxacarb, which were higher than the control. The highest reduction percentages in protease activity were found in case of chlorfenapyr and indoxacarb, recording 96.11% and 95.42%, respectively. While the highest reduction percentages in kitinase activity were found in case of spinosad, indoxacarb+ alpha cypermethrin, recording 84.76.11% and 78.20%, respectively. As described above, its noticed that endoxacarb, chlorfenapyr and their mixtures recorded higher reduction in the total soluble protein, protease, carbohydrate hydrolyzing enzymes (Invertase, trehalase and amylase) activities, which led to inhibition of chitinase enzyme activity.

4. Discussions

According to these findings, spinosad has a superior relative potency that increases the toxicity effect against *B. zonata*. According to studies by [30,31,32] on beneficial insects, spinosad has a minimal toxicity toward mammals and a high effect on tephritids. Furthermore, [9] suggested that Absence or minor resistance to spinosad and lack of cross-resistance to trichlorfon, suggest that spinosad could be a potential candidate for managing *B. dorsalis*. According to [12], lambda-cyhalothrine was superior to deltamethrin because it effectively reduced the number of melon fruit fly pupae that emerged. Resistance ratio among the 14 populations of *B. zonata* that were evaluated revealed heterogeneity in their resistance factors, ranging from susceptible to low resistance to spinosad by 1.20-fold to 9.95-fold [7]. Three oxygenated monoterpenes, (R)-carvone, (R)-camphor, and (1R, 2S, 5R)-menthol, all had a significant harmful effect on *B. zonata* adults. Nevertheless, (-)-carvone outperformed (1R, 2S, 5R)-menthol and (R)-camphor in terms of insecticidal activity [13]. With LC80, LC90, and LC99 values of 12.28, 17.67, and 33.62 ppm, respectively, spinosad was highly toxic to *B. zonata*. This finding shows that *B. zonata* could be successfully controlled with a spinosad-based strategy [33]. According to the findings by [34], basil oils, onion, peppermint, ginger, garlic, water crass, clove, castor, and mustard had the highest levels of toxicity against *B. zonata* pupa, with LC50 values of 39.704, 50.459, 69.205,78.418, 83.172, 98.0, 101.293, 107.662, and 238.99ml/L, respectively. Following the time intervals of 6, 12, 24, and 48 hours against the peach fruit fly, Bactrocera zonata on guava fruit, the order of toxicity was Coragen > Steward > Flufenoxuron > Acetamiprid > Niten pyramid > Imidacloprid > Neem seed oil > Kor-tuma fruit extract [35]. According to research by [36], spinosad had LC25 values of 1.15 and 1.29, respectively, and lambda-cyhalothrin was the most poisonous to both male and female *B. zonata* organisms, with LC25 values of 0.017 and 0.04. It is known that a unique type of joint response called synergism occurs when one of the chemicals in a mixture can make the other one

more potent, and the combined effect is greater than utilizing each molecule alone [30]. In the past, field strains of *B. zonata* from a few locations in Punjab were also noted to be spinosad-sensitive [7]. However, the widespread use of spinosad in baits or cover sprays has been associated to spinosad resistance in *Bactrocera spp*., according to reports from other nations [37,38]. Identifying pesticide resistance is crucial for creating a successful management plan for insect pests [39]. To reduce the amount of the primary insecticide used in the control program and reduce the chance of rapid development of resistance, numerous different types of efforts have been made. These include limiting the percentage used in terms of the spray areas, using mixtures of insecticides with different modes of action, using the right insecticide formulation [40]. There are numerous instances of successful applications of pesticide combinations in the control of resistance. For example, studies by [7] observed that lambda-cyhalothrin and spinosad were showed susceptible to low resistance of *B. zonata* populations (1.00-fold to 9.57-fold and 1.20 -fold to 9.95-fold). Also, [7] showed that moderate level of λ-cyhalothrin resistance against populations of *B. zonata*. A higher percentage of fatalities was seen when LC_{25} of spinosad was combined with LC_{25} of lemongrass and sesame oil than when each ingredient was used alone [32]. The following are the justifications for using combinations of insecticides made of different chemical types in agriculture: Insects that are resistant to one or more insecticides may be sensitive to a combination of toxicants, and a mixture may provide the best control of a complex of pests with various susceptibilities to the individual components of the mixture. Additionally, combinants may exhibit synergism [41,39].

It is noted that indoxacarb and chlorfenapyr and their mixture caused a significant decrease in the level of total soluble protein, perhaps the reason for this is the cessation of the feeding process caused by indoxacarb or the cessation of the breathing process caused by chlorfenapir, or both together [42]. Another study [43] showed increasing in total soluble protein under the effect of the chlorpyrifos, chlorfluazuron, emamectin benzoate, pyrethrins and its combinations. Also, [44] showed insignificant differences in protein content for chlorpyrifos- methyl resistant strain compared to the susceptible strain. A previous study [45] shown that when insecticides from several chemical groups, including Beticol, Biosad, Elsan, Lufox, Mani, and Match, are used against B. zonata pupae, the amount of total proteins tends to drop at a faster rate. According to [46] study, adult B. zonata flies exposed to spinosad and malathion had lower protein levels than controls. The haemolymph's protein pool serves as a backup supply of the protein synthesis required for the growth and development of the adult stage during the pupal stage [47,48]. It has been observed that indoxacarb, chlorfenapyr, and their combination significantly reduced the levels of the carbohydrate hydrolyzing enzymes (invertase, trehalase, and amylase); this may have been due to the cessation of the feeding process caused by indoxacarb, the cessation of the breathing process caused by chlorfenapyr, or both together [42]. Insect development, including metabolism, metamorphosis, the growth of flight muscles, reproduction, and embryonic development, is significantly influenced by carbohydrates [48]. The suppression of carbohydrate hydrolyzing enzymes may impair the molting process and as previously demonstrated in the toxicological trials, may explain why mortality occurred in adult *B. zonata* [43]. Trehalase activity disruption may make it more difficult to get the glucose needed for chitin synthesis [49]. Previous study [50] who treated 5th instar cotton leafworm larvae of *S. littoralis* with sub-lethal concentrations of thuringeinsin (beta-exotoxin of *B. thuringiensis*) noticed considerable reduction in the carbohydrate hydrolyzing enzymes especially amylase and invertase [51] found that treatment with acetamiprid, chlorpyrifos methyl, and pyriproxyfen inhibited the activity of the carbohydrate hydrolyzing enzymes (amylase, trehalase, and invertase) in the adult whiteflies, *B.tabaci*. According to [41], chemicals such chlorosan, cygron, engeo, and kingbo decreased amylase activity by between -1.05% and -25.26% in comparison to the control. Due to qualitative effects on the acetylcholinesterase enzyme, field-collected *B. zonata* in Egypt were significantly more resistant to malathion (resistance ratio >30) than laboratory susceptible insects [52]. Bio-fly exhibited the highest inhibition of AChE activity for Bactrocera zonata, whereas Chitosans gave the highest inhibition of ATPase activity [53]. Another study [45] demonstrate that Beticol and Radiant, among the tested insecticides, generally boosted the activity of the AChE enzyme in the 2-day pupae of B. zonata. Elsan was the one in charge of other insecticides and control. According to [1], Beticol and Radiant typically boosted the activity of the AChE enzyme in the 2-day pupae of B. zonata compared to other insecticides and control. The in vitro inhibitory effects of the technical compounds abamectin, chlorfenapyr, and pyridaben on AChE isolated from adult female *T urticae* were demonstrated by [54]. There is evidence that additional kinds of pesticides are also involved in lowering AChE activity, in addition to just organophosphorus and carbamate pesticides [55]. According to research done by [41], the chemicals chlorosan, feroban, cygron, engeo, and kingbo significantly altered the acetyl cholinesteras levels in S. littoralis larvae. Its noticed that endoxacarb, chlorfenapyr and their mixtures recorded higher reduction in the total soluble protein, protease, carbohydrate hydrolyzing enzymes (Invertase, trehalase and amylase) activities, which led to inhibition of chitinase enzyme activity, this may be due to the cessation of the feeding process caused by indoxacarb, the cessation of the breathing process caused by chlorfenapyr, or both together [42]. According to [56], B. zonata's total proteins and total carbohydrates are both adversely affected by gamma irradiation. The following are the justifications for using combinations of insecticides made of different chemical types in agriculture: Insects that are resistant to one or more insecticides may be sensitive to a combination of toxicants, and a mixture may provide the best control of a complex of pests with various susceptibilities to the individual components of the mixture. Additionally, combinants may exhibit synergism [41,39]. Previous study [57] discovered a decrease in chitinase activity in S. littoralis larvae treated with chlorpyrifos in their fourth instar. *B. zonata* was exposed to harmful effects and biochemical modifications from the two bioinsecticides Biomctin and Tracer [58] data in Fig (1, d) showed increase in the level of phenol oxidase activity in *B. zonata* treated with the tested insecticides and its combinations, except indoxacarb, however, this increase is not significant.

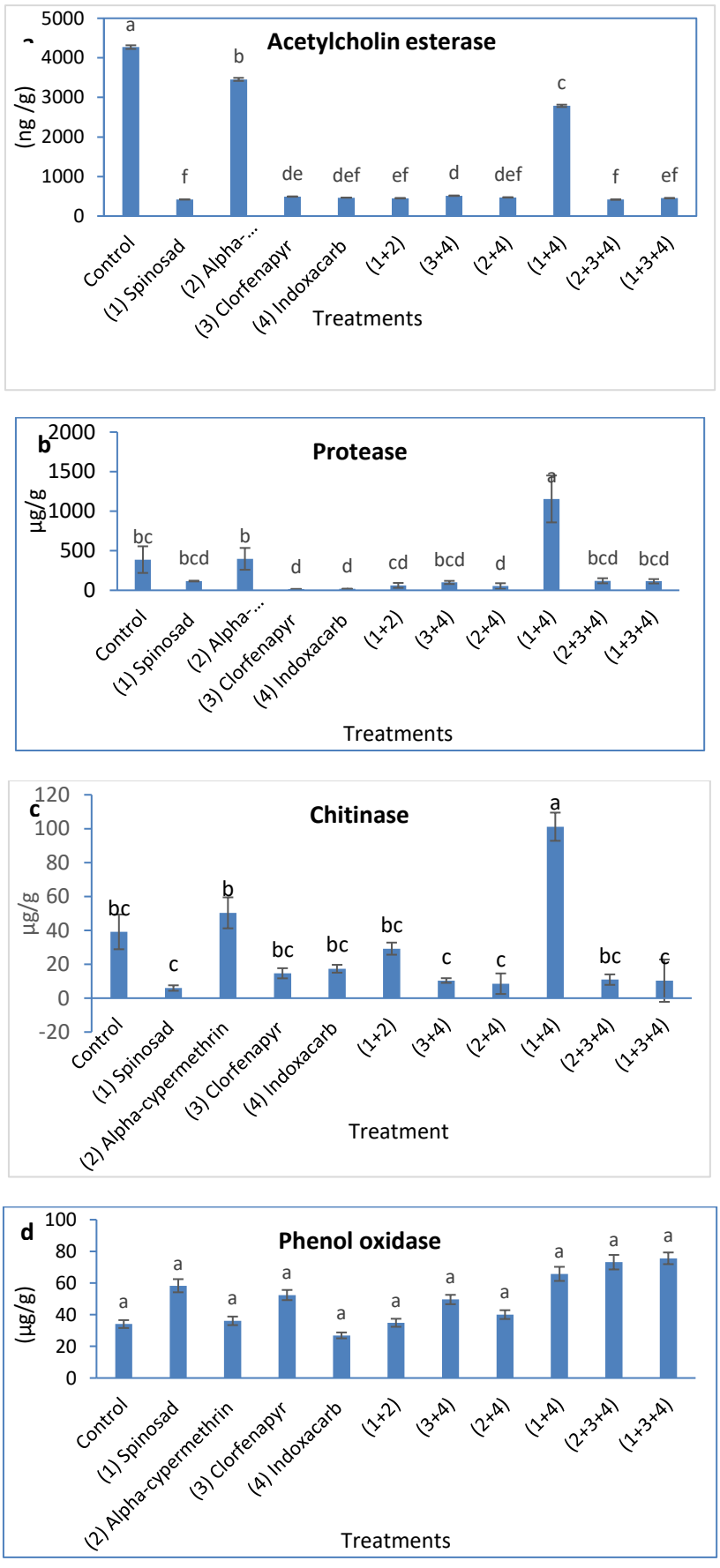

Figure 1: Effect of the tested insecticides and its mixtures on the acetyl choline esterase, protease, chitinase and phenol oxidase of adult *Bactrocera zonata zonata* through 24 hrs exposure. (1+2) refer to Spinosad+ Alpha-cypermethrin, (3+4) refer to Clorfenapyr

+ Indoxacarb, (2+4) refer to Alpha-cypermethrin+ Indoxacarb, (1+4) refer to Spinosad+ Indoxacarb, (2+3+4) refer to Alpha-cypermethrin+ Clorfenapyr+ Indoxacarb and (1+3+4) refer to Spinosad + Clorfenapyr+ Indoxacar

Table 1: Response of laboratory strains of *Bactrocera zonata* to the tested insecticides through 24, 48 and 72hours exposure

Insecticides	LC_{50} (ppm)	Confidence limits		Toxicity index	Relative potency	LC_{90} (ppm)	Confidence limits		Toxicity index	Relative potency	Slope ± S. E
		Lower (ppm)	Upper (ppm)				Lower (ppm)	Upper (ppm)			
24hr											
Spinosad	29.90	—	—	39	1	1543.63	—	—	45	1	0.75 ± 0.25
Alpha -cypermethrin	11.66	6.95	27.1	100	2.56	690.61	169.99	10852.08	100	2.24	0.72 ±0.12
Indoxacarb	—	—	—	—	—	—	—	—	—	—	—
48hr											
Spinosad	0.56	0.41	0.84	100	571.23	5.92	2.95	18.94	100	166.50	1.25 ±0.17
Alpha -cypermethrin	1.22	0.93	1.58	45.90	262.20	9.63	6.74	15.82	61.47	102.35	1.43 ±0.15
Indoxacarb	319.89	—	—	0.175	1	985.66	—	—	0.6	1	2.62 ±0.45
72hr											
Spinosad	0.19	0.15	0.25	100	667.79	1.27	0.86	2.25	100	289.64	1.56 ±0.17
Alpha -cypermethrin	0.71	0.50	0.96	26.76	178.70	6.46	4.61	10.21	19.66	56.94	1.34 ±1.44
Indoxacarb	126.88	106.33	145.59	0.149	1	367.84	284.48	597.04	0.345	1	2.77 ±0.47

Table 2: Combined action studies of tested insecticides against *B. zonata* adults

Compound		LC_{25} (ppm)		Observed percent mortality of mixture A B 1 : 1			Co-toxicity factors		
A	B	A	B	24hr	48hr	72hr	24hr	48hr	72hr
Spinosad	Alpha - cypermethrin	0.071	0.226	15	56	86	-70	12	72 Potentiation
Indoxacarb	Chlorfenapyr	72.46	144	23	54	79	-54	8	58 Potentiation
Indoxacarb	Alpha - cypermethrin	72.46	0.226	15	45	72	-70	-10	44 Potentiation
Chlorfenapyr	Alpha - cypermethrin	144	0.226	17	33	56	-66	-34	12 additive
Spinosad	Indoxacarb	0.071	72.46	8	20	51	-84	-60	2 additive
Spinosad	Chlorfenapyr	0.071	144	0	3	19	-100	-94	-62 antagonistic

Co-toxicity factor >20 means potentiation effect.
Co-toxicity factor <-20 means antagonistic effect.
Co-toxicity factor ranged between -20:20 means additive effect.

Table 3: Effect of the tested insecticides and its mixtures on the total protein contents and Carbohydrate hydrolyzing enzymes (trehalase, amylase and invertase) against adult *Bactrocera zonata* through 24 hrs exposure

Treatments	Total soluble protein (mg/ g.bw)	Change %	Carbohydrate hydrolyzing enzymes (µg glucose/ g body weight)					
			Trehalase	Change %	Amylase	Change %	Invertase	Change %
Control	23.65 ±3.02^{a}	0	0.91 ±0.19^{b}	0	2.08 ±0.38^{b}	0	3.42 ±0.13^{b}	0
Spinosad	8.85 ±5.76^{c}	62.57	0.23 ±0.014^{c}	74.73	0.83 ±0.14^{c}	60.1	0.39 ±0.03^{de}	88.6
Alpha-cypermethrin	23.95 ±1.80^{b}	1.21	1.33 ±0.06^{a}	46.15	0.07 ±0.05^{d}	96.63	1.48 ±0.15^{c}	56.73
Clorfenapyr	2.41± 0.18^{d}	89.82	0.14 ±0.04^{c}	84.62	0.005 ±0.01^{d}	99.76	0.22 ±0.02^{ef}	93.57
Indoxacarb	1.87 ±0.18^{d}	92.10	0.09 ±0.01^{c}	90.11	0.008 ±0.03^{d}	99.62	0.45 ±0.02^{d}	86.84
Spinosad+ Alpha-cypermethrin	3.38 ±0.40^{cd}	85.73	0.10 ±0.01^{c}	89.01	0.54 ±0.09^{cd}	74.04	0.15 ±0.01^{fg}	95.61
Indoxacarb+ Clorfenapyr	2.62 ±0.39^{cd}	88.93	0.11 ±0.01^{c}	87.91	0.42 ±0.12^{cd}	79.81	0.01 ±0.01^{g}	99.71
Indoxacarb+ Alpha-cypermethrin	3.523 ±0.05^{cd}	85.11	0.15 ±0.010^{c}	83.52	0.06 ±0.11^{d}	97.12	0.44 ±0.01^{d}	87.13
Indoxacarb+ Spinosad	36.00 ±2.71^{a}	52.21	1.37 0±.51^{a}	50.55	3.92 ±0.50^{a}	88.46	4.13 ±0.12^{a}	20.76
Indoxacarb+ Alpha-cypermethrin+ Clorfenapyr	4.08 ±0.10^{cd}	82.77	0.22 ±0.03^{c}	75.82	0.40 ±0.07^{cd}	80.77	0.53 ±0.02^{d}	84.5
Indoxacarb+ Spinosad + Clorfenapyr	3.00 ±0.47^{cd}	87.31	0.11 ±0.01^{c}	87.91	0.03 ±0.06^{d}	98.56	0.02 ±0.02^{fg}	99.42

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