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Bio-pesticides alternative diazinon to control peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)



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Abstract

Background: The bio-pesticide abamectin has been used to control a large variety of insects, including Diptera species, attributed to its high toxicity with virtually no residual effects on treated crops. Its low residual effect ensures the survival of natural enemies and other non-target organisms. Imidacloprid is also widely used for insect pest control due to its potency and high insect selectivity in comparison to mammals. On the other hand, diazinon has been applied extensively to control immature fruit fly stages, mature larvae, pre-pupae, and pupae in soil drench application, thus, affecting the whole agroecosystem, including the natural enemies.

Results: The toxic effects of abamectin and imidaclopride proposed as a replacement for diazinone in soil treatment, were studied against a laboratory strain of the peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) under field-caged conditions. Five days old PFF pupae were treated by each pesticide. PFF pupae exhibited different levels of susceptibility to the tested pesticides. Non-significant differences in the pupal mortality rates were obtained between imidacloprid (77.52%), abamectin (77.22%), or diazinon (73.89%). Diazinon and abamectin achieved the highest percentages of total mortality (100%), followed by imidacloprid (98.89%). Real mortality rates were mostly concentration-dependent, while the deformed flies rate depended on the chemical sub-group of insecticide and concentration. Additionally, the biochemical studies revealed different acetylcholinesterase enzyme (AChE) inhibition levels caused by the pesticides on the treated flies sampled at 24, 48, and 72 hours post fly emergence.

Conclusion: The bio-insecticide abamectin is an option to diazinon for the control of PFF pupae. Also, soil treatment might be an alternative for PFF pupae control.

Keywords: Tephritidae, Fruit flies, Pest control, IPM, Insecticides, Bio-pesticide, Enzymes

Background

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a major destructive tephritid species. Recently, this quarantine pest threats commercial fruits' production in Egypt (EPPO 2002). It is widespread almost in all Governorates of Egypt (Draz et al. 2002).

Pesticides have been usually used for controlling fruit flies with different application methods, such as bait stations and cover spray. The traditional control measure using chemical insecticides has several disadvantages, such as insect resistance, residual problems (El-Gendy 2018), and insecticides' inability to penetrate infested fruits to kill larvae. In Egypt, PFF control methods are focused on eradicating adults using the male annihilation technique (FAO/IAEA 2010). Also, abiotic control measures against the pupae include soil moisture management (El-Gendy and AbdAllah 2019). Many areawide IPM programs have applied insecticidal soil drenches under host trees targeting the pre-pupae and pupae (Ekesi et al. 2007). Diazinon has been used effectively in Florida as a soil drench underneath host plants

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to control fruit fly larvae or gravid adult females. However, chemical control uses increments residues of pesticides in post-harvested fruits. For these reasons, different international organizations, such as the Codex Alimentarius Commission (CAC), the World Health Organization (WHO), and the European Commission (EC), have established and enforced the detection of maximum residue limits (MRLs) in various foods. These are the highest levels of residues expected to be in the food after a pesticide is used according to authorized agricultural regulations. Besides being banned for outdoor residential use, particularly near waterways due to its effects on aquatic organisms in freshwater ecosystems (Stark et al. 2013), diazinon is still used in California and Florida. Several insecticides have been tested in nurseries for fruit fly eradication programs to replace diazinon as a soil treatment (Stark et al. 2014).

Esterases are the most significant enzymes involved in metabolism of many insecticides' groups in insects (Hsu et al. 2004). These hydrolases catalyze the hydrolysis and degradation of a wide range of aliphatic and aromatic esters, choline esters, and organophosphorus compounds (Dauterman 1985). Biochemical tests are useful for monitoring of esterase levels in pest populations or communities. However, AChE plays an essential role in the cholinergic synapses' neurotransmission by catalyzing the acetylcholine neurotransmitter hydrolysis. Organophosphate insecticides (OPs) bind the AChE active site inhibiting the enzyme; this causes an accumulation of acetylcholine in the post-synaptic membrane, preventing the nerve polarization (Oakeshott et al. 2005). After the intensive use of malathion and other OP insecticides in agricultural pest control, resistance mediated by alterations in the AChE has been selected in many insect species (Oakeshott et al. 2005; Mosleh et al. 2011a).

This study aimed to evaluate the toxicity of abamectin and imidacloprid as replacements for diazinon in soil treatments against the pupal stage of *B. zonata*, under field-caged conditions. Furthermore, the in vivo effects of these compounds were studied on acetylcholinesterase activity to elucidate their role in *B. zonata* control.

Methods

To find an appropriate replacement for diazinon in soil tratments against the PFF immature stages, a semi-controlled experiment was set in the field.

Study's location

The present study was carried out in a 200 m² field plot located at the Directorate of Agriculture, Damanhour city, El-Beheira Governorate, Egypt, in 2017. The field plot hosted 20 *Pisidium guajava* fruiting trees, about 20 years old, with a plant spacing of 3.5×4 m.

Insect

The colony of PFF was obtained from the Eradiation of the Peach Fruit Fly Laboratory at Damanhour, El-Beheira Governorate.

Insecticides used

Three commercial insecticides of different chemical subgroups (IRAC, 2020) were tested against PFF pupae: diazinon (Diazinon 60% EC, organophosphates), imidacloprid (Admire 70% WG, neonicotinoids), and avermectin (Abamectin 1.8%, avermectins).

Procedure

Concentrations of the tested insecticides were diluted in tap water, four concentrations each: 0.25, 1.0, 2.0, and 4.0 ppm for abamectin; 1.5, 3.0, 5.0, and 8.0 ppm for imidacloprid; and 0.07, 0.1, 0.5, and 1.0 ppm for diazinon. Five days old PFF pupae (2 days before adults' emergence) were used. The sample size included 600 healthy pupae for each insecticidal treatment and a control, divided in five replicates, 30 pupae for each. Plastic jars (1000 ml, 12 cm wide × 11 cm height) with holes in the bottom (for drainage water) and lined with muslin cloth were filled with sterilized loamy soil, except for the last 50 mm of height. PFF pupae were transferred to the jars, placed carefully on the soil surface, and covered with 30 mm height of the same soil type. Each jar was rinsed with an insecticidal concentration until soil saturation point, according to Rhoades (1982); control treatment received water only. Jars were covered by muslin cloths for aeration and maintained in the field covered by guava trees' canopy, and buried in the ground about 7 cm deep. Daily observations helped to calculate the number of emerged adults. The real pupae mortality rate was calculated, using the following formula:

Pupal mortality no. = (no.of unemerged adult flies/no.of treated pupae) \times 100

Apparent mortality of adult fly was calculated using the formula:

Adult mortality rate = (no.of dead of adult flies/no.of emerged flies) × 100.

The total observed mortality rate of pupae was corrected by Abbott's formula (Abbott 1925).

Acetylcholinesterase (AChE) activity assay

The assay was conducted at the Laboratory of Pesticides Residue Analysis and Toxicity, Damanhour, El-Beheira, Egypt. AChE activity was measured in the emerged flies of PFF pupae treated with LC_{50} of diazinon, imidacloprid, and abamectin, using the method published by Ellman et al. (1961). AChE activity was determined 24, 48, and 72 h post the emergence of adults. The emerged flies of treated and un-treated pupae were collected and

confined in Eppendorf tubes under freezing conditions. Freezing flies were placed on ice and homogenized in a potassium phosphate buffer, pH 7.0 (PPB), using a glass/ Teflon homogenizer at 4 °C. The homogenates were centrifuged at 5000 rpm for 20 min at 0 °C. A mixture of PPB, the supernatant (crude enzyme), 5,5-dithiol-bis-(2-nitrobenzoic acid) (DTNB), and acetylthiocholine iodide (ATChI) was incubated for 30 min at room temperature (27 \pm 2 °C), followed by optical density (OD) spectrophotometrically measures at 412 nm using the T80 UV/Vis spectrophotometer. All treatments were replicated five times. Results are presented as inhibition in AChE activity percentages.

Statistical analysis

Statistical analysis of the AChE inhibition was set as a completely randomized design. A one-way analysis of variance (ANOVA) was conducted for each insecticide and the AChE's inhibition of each treatment. Also, a two-way analysis of AChE inhibition among treatments was performed. A split-plot design was conducted to compare insecticides and their concentrations. The analysis of variance and means separation with Tukey-Kramer's test (p< 0.05) were performed using the CoStat Software (2008, V.6.4).

Results

Real and apparent mortality of the PFF

Results in Tables 1, 2, 3, and 4 showed significant differences in the real mortality rates of PFF pupae as well as the deformed adult-fly rates treated in the pupal stage with different diazinon concentrations (pupal death: F = 8.38, df = 3; p = 0.007: deformed flies; F = 449.53, df = 3, p = 0.0000); however, non-significant differences were found in mortality rates of adult flies among concentrations. Similarly, significant differences based on

abamectin concentration were obtained in both real pupal mortality (F = 5.91, df = 3, p = 0.02) and deformed adult-fly rates (F = 21.69, df = 3, p = 0.0003), as well as non-significant differences in mortality rates of adult flies. With imidacloprid, the real mortality of pupae, apparent mortality of adult flies, and deformed adult-fly rates were significantly different based on insecticide concentration (pupal mortality: F = 8.38, df = 3, p = 0.0075; deformed flies: F = 5.67, df = 3, p = 0.002, adult-fly mortality: F = 11.76, df = 3, p = 0.003).

No differences were obtained among the tested insecticides in the real pupal mortality. However, the highest mortality rates were achieved by imidacloprid (77.52%) and abamectin (77.22%), both significantly different (F = 16.29, df = 3, p = 0.0001) than diazinon (73.89%). The apparent mortality of adult flies were affected significantly by treated insecticides (F = 13.09, df = 2, p = 0.017) and concentrations (F = 7.25, df = 3, p = 0.002), with an interaction effect between them on the mortality rate of adult flies (F = 3.02, df = 6, p = 0.032). Imidacloprid had 35.51% of deformed adult rates, significantly different (F = 8.94, df = 2, p = 0.033) than abamectin (16.41%) and diazinon (10.12%).

In vivo inhibition of the PFF AChE by various selected insecticides

Inhibition rates of AChE activity (AChE activity on μ moles of ATChI hydrolyzed per mg of protein in the homogenate per min) in emerged adult flies were different at each insecticidal treatment, and with each tested time (insecticide treatments: F=1211.21, df = 2, p=0.0000; time: F=132.34, df = 2, p=0.0000). Also, a highly significant interaction was obtained among insecticidal treatments and tested time on AChE inhibition (F=143.27, df = 4, p=0.0000). Abamectin exhibited a highly significant inhibition of AChE activity, followed

Table 1 The real and apparent mortalities, and deformed flies percentages of the peach fruit fly, *Bactrocera zonata*, treated with diazinon pesticide at pupal stage

| Concentrations (ppm) | RM of pupae % | AM of adults % | Total mortality % | Deformed flies % |
|----------------------|---------------|----------------|-------------------|------------------|
| 0.07 | 43.33 b | 100.0a | 100.0a | 23.84 a |
| 0.10 | 68.88 ab | 97.42 a | 100.0a | 16.16 b |
| 0.50 | 88.88 a | 100.0a | 100.0a | 0.0 с |
| 1.00 | 94.44 a | 100.0a | 100.0a | 0.0 c |
| Df | 3.0 | 3.0 | 3.0 | 3.0 |
| SS | 4817.53 | 15.05 | 0.0 | 1305.31 |
| MS | 1605.84 | 5.005 | 0.0 | 435.103 |
| F | 8.38 | 1.44 | - | 449.530 |
| P value | 0.007 | 0.12 | - | 0.0 |
| L.S.D. | 26.06 | 4.21 | 4.21 | 1.85 |

Values with the same letters are not significantly different, L.S.D. least significant difference; RM real mortality; AM apparent mortality, ppm parts per million

Table 2 The real and apparent mortalities, and deformed flies percentages of the peach fruit fly, *Bactrocera zonata*, treated with abamectin pesticide at pupal stage

| Concentrations (ppm) | RM of pupae% | AM of adults % | Total mortality % | Deformed flies % |
|----------------------|--------------|----------------|-------------------|------------------|
| 0.25 | 52.22 b | 100.0a | 100.0a | 2.22 c |
| 1.00 | 78.88 ab | 100.0a | 100.0a | 36.55 a |
| 2.00 | 87.77 a | 100.0a | 100.0a | 8.33 bc |
| 4.00 | 90.00 a | 100.0a | 100.0a | 18.51 b |
| Df | 3.0 | 3.0 | 3.0 | 3.0 |
| SS | 2707.4 | 0.0 | 0.0 | 2030.3 |
| MS | 902.47 | 0.0 | 0.0 | 676.78 |
| F | 5.91 | | | 21.69 |
| P value | 0.02 | | | 0.0003 |
| L.S.D. | 23.27 | 0.0 | 0.0 | 10.52 |

Values with the same letters are not significantly different, L.S.D. least significant difference; RM real mortality; AM apparent mortality, ppm parts per million

by diazinon and imidacloprid, according to the general mean inhibition obtained along the tested time. Results illustrated in Fig. 1 showed that abamectin had a high and significant inhibition of AChE activity, in relation to the tested time (24 h, 20.46%; 48 h, 21.79%; 72 h, 21.87% inhibition) (F=53.80, df = 2, p=0.0004). Also, AChE activity was increasingly and significantly inhibited by diazinon (F=233.03, df = 2, p=0.0001 at 24 (10.65%), 48 (11.89%), and 72 h (22.83% inhibition) (Fig. 2). However, on the adult flies treated with imidacloprid, AChE was significantly inhibited (F=31.75, df = 2, p=0.0014), but inhibition recorded was as follows: at 24 h, 10.02%; 48 h, 3.04%, and 72 h, 6.46% (Fig. 3).

Discussion

Eliminating the adult tephritid emergence from the soil can work side by side with other control measures such as chemical insecticides, bait sprays, soil moisture management, soil compaction, sterile insect release, and male annihilation in eradication programs (Ndlela et al. 2016; El-Gendy and AbdAllah 2019, 2020). In the present study, the tested insecticides were effective in PFF eradication, with different toxicity against PFF pupae and adult flies. Pupal mortality rates depended on the chemical sub-group of the insecticide, reflecting 90-99% of the variability, according to the determination coefficient. It also depended on the insecticidal concentration. For instance, the highest real mortality was obtained with the highest concentration of imidacloprid (99.0%), followed by diazinon (94.44%), and then abamectin (90.0%). Furthermore, diazinon and abamectin can eliminate adult flies at any of the tested concentrations. However, the highest concentrations of imidacloprid are needed to perform similarly.

Diazinon was the most toxic insecticide for the pupal stage, followed by the two biopesticides abamectin and

Table 3 The real and apparent mortalities, and deformed flies percentages of the peach fruit fly, *Bactrocera zonata*, treated with imidacloprid pesticide in pupal stage

| Concentrations (ppm) | RM of pupae % | AM of adults % | Total mortality % | Deformed flies % |
|----------------------|---------------|----------------|-------------------|------------------|
| 1.5 | 66.64 b | 097.22 ab | 96.66 b | 30.55 a |
| 3.0 | 71.01 b | 093.33 b | 98.88 ab | 40.25 a |
| 5.0 | 73.33 b | 100.0a | 100.0 a | 32.33 ab |
| 8.0 | 99.0 a | 100.0a | 100.0 a | 38.88 ab |
| Df | 3.0 | 3.0 | 3.0 | 3.0 |
| SS | 1915.1 | 89.58 | 22.31 | 205.77 |
| MS | 638.37 | 29.86 | 7.43 | 68.59 |
| F | 8.38 | 11.76 | 8.00 | 5.67 |
| P value | 0.0075 | 0.003 | 0.009 | 0.02 |
| L.S.D. | 16.43 | 3.0 | 1.82 | 6.55 |

Values with the same letters are not significantly different, L.S.D. least significant difference; RM real mortality; AM apparent mortality, ppm parts per million

Table 4 The real and apparent mortalities, and deformed flies percentages of the peach fruit fly, *Bactrocera zonata*, treated with pesticides in pupal stage

| Insecticide | Real mortality of pupae % | Apparent mortality of adults % | Total mortality % | Deformed flies % |
|--------------|---------------------------|--------------------------------|-------------------|------------------|
| Diazinon | 73.89 a | 99.35ab | 100.0a | 10.12 b |
| Abamectin | 77.22 a | 100.0a | 100.0a | 16.41 ab |
| Imidacloprid | 77.52 a | 97.64 b | 98.89 b | 35.51 a |
| Df | 2.0 | 2.0 | 2.0 | 2.0 |
| F | 0.125 | 3.58 | 5.5 | 4.40 |
| P value | 0.88 | 0.039 | 0.009 | 0.02 |
| L.S.D. | 10.17 | 1.85 | 0.79 | 18.13 |

Values with the same letters are not significantly different, L.S.D. least significant difference; RM real mortality; AM apparent mortality

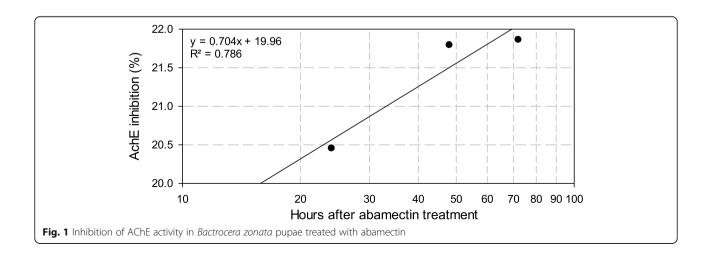
then imidacloprid. These results are in agreement with the results of Stark et al. (2014) who mentioned that diazinon, as a soil treatment, was of the most toxic insecticides against *Ceratitis capitata* pupae, but neither *B*. dorsalis nor B. cucurbitae. In parallel, diazinon was the most toxic insecticide for PFF male and female flies with 0.20 and 0.26 ppm at 24 h post-treatment, followed by malathion, lufenuron, and methoxyfenozide insecticides (Mosleh et al. 2011). Also, Abdullahi et al. (2020) revealed that the diazinon was the most toxicity of the insecticides against B. invadens, followed by chlorpyrifos, cypermethrin + dimethoate, and deltamethrin, respectively. In the same line, Stark et al. (1992) found that puparium formation pesticide concentrationwas dependent, affected by diazinon, not by cyromazine.

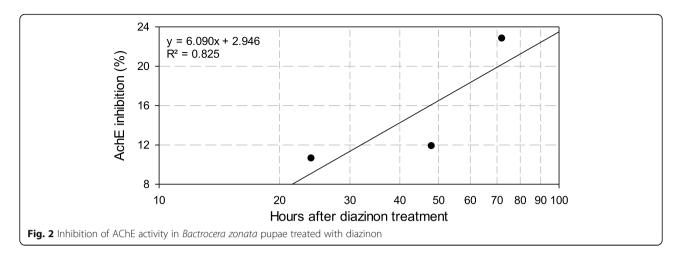
In many fruit fly studies, abamectin 1.8% EC exhibited a high efficiency against various fruit flies stages. According to Halawa et al. (2013), in sandy-soil treatments against 3 days old pupae of PFF and *C. capitata*, abamectin was the most effective treatment, compared to lufenuron or lufenuron + emamectin benzoate. Also, abamectin was of the most effective insecticides against

B. dorsalis adults (Wang et al. 2013). However, imidacloprid obtained the lowest efficiency of the tested insecticides, emamectin benzoate, trichlorfon, and λ -cyhalothrin, against PFF (Khan and Naveed 2016).

PFF response to the tested insecticides varied significantly, which may be due to the insecticide type, mode of action, or residual activity. The present findings are consistent with El-Aw et al. (2008), who reported different toxicity rates of pesticides against PFF adults, where methomyl was the most effective insecticide, followed by thiamethoxam, spinosyn, and malathion. Stark et al. (2014) mentioned that the most effective insecticides as soil treatments for C. capitata, B. cucurbitae, and B. dorsalis pupae were spinosad, lambda-cyhalothrin, permethrin, tefluthrin, and diazinon with no significant difference. However, Raga et al. (2018) mentioned that Anastrepha fraterculus (Wied.) and A. grandis (Macquart) exhibited similar susceptibility to acetamiprid, deltamethrin, flypyradifurone, imidacloprid, phosmet, thiamethoxam, and zeta-cypermethrin.

Recently, the Agriculture Pesticides Committee in Egypt banned diazinon for agricultural uses. However,





residues of diazinon have been found in some Egyptian areas. Diazinon was detected in water samples collected from Lake Qarun and drainage canal in Damietta, Egypt (Abdel-Halim et al. 2006). Its residual effect extended to crop fruits, where fruit samples in oranges and grapes contained 0.01 mg/kg of diazinon (Gad Alla et al. 2015). Hence, it is clear that the importance of finding effective bio-insecticides against immature stages of fruit flies other than diazinon as soil treatments, such as abamectin.

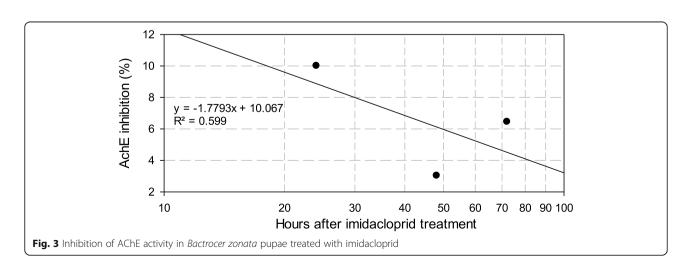
The present study also revealed a decrease in fly AChE activity of previously insecticide-treated pupae. The percentages of AChE activity inhibition were in ascending order for abamectin, diazinon, and imidacloprid. The activity of AChE in treated PFF with malathion, diazinon, methoxyfenozoide, and lufenuron decreased compared to the control (Mosleh et al. 2011), as well as variations among tested insecticides in AChE activities in PFF adult flies treated as pupae were reported (Halawa et al. 2013). The present findings of AChE inhibition by the tested insecticides is

found to be dependent on time after treatment, which was positively correlated to the tested time post-treatment in both abamectin and diazinon insecticides, 24, 48, and 72 h, with a maximum inhibition at 72 h, followed by 48 and 24 h, respectively. As for imidacloprid, AChE inhibition was high after 24 h, followed by 72 and then 48 h.

As a bio-pesticide, abamectin had a high efficacy eliminating immature stages of PFF in the soil, as well as the emerged flies from the treated pupae. Thus, abamectin soil treatment abamectin might be used as an alternative to diazinon for the control program of PFF.

Conclusion

The biopesticide abamectin might be in soil treatments in PFF control, as an alternative to diazinon. The highest percentage of pupal elimination was obtained by imidacloprid, followed by abamectin and diazinon. Diazinon, abamectin, and imidacloprid achieved high percentages of total mortality of PFF.



Abbreviations

PFF: Peach fruit fly; LC_{50} : Lethal insecticide concentrations causing 50% mortality; AChE: Acetylcholinesterase enzyme; ppm: Part per million; IPM: Integrated pest management; IRAC: Insecticide Resistance Action Committee

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Significant statement

This research specifically showed that the bio-pesticide abamectin could be used to control PFF pupae, as an alternative to diazinon in soil treatments, which will make a significant contribution to the protection of Egyptian agro-ecosystems.

Authors' contributions

IR, MI, and JA planned the experiments. IR and MI carried out the experiments. IR analyzed the dada. IR wrote the manuscript with support of MI and JA. IR, MI, and JA discussed the results and contributed the final manuscript. All authors have read and approved the manuscript to publish.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the authors on fair request.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors have declared that no competing interests exists.

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