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Interaction between some chemical pesticides with the entomopathogenic fungus, *Beauveria bassiana* against immature stages of peach fruitfly, *Bactrocera zonata* (Saunders)

Ahmed Adly Mohamed Ibrahim and Nehad Abdel-Hameed Soliman.

[1] Bio-Insect Plant Protection Institute (PPRI), Agricultural Research Center (ARC), **Egypt**
[2]Horticultural Insect Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), **Egypt**

***Correspondence:** nehadpprie@hotmail.com **Received 19-11-2019, Revised: 20-01-2020, Accepted: 24-01-2020 e-Published: 15-02-2020**

Peach fruitfly, *Bactrocera zonata* (Saunders) is a serious and destructive pest of horticultural fruit in Egypt, tropical and sub-tropical regions in the world. Adding sub-lethal concentrations (LC_{25}) of the tested insecticides, runner, movento and spincer into the SDA showed no inhibition in *Beauveria bassiana* (Bals.) vegetative growth. Combination of sub-lethal concentrations of runner, movento and spincer with *B.bassiana* showed potentiation and additive effects and resulted in better percentages of mortality at tested immature stages and flies.

Keywords: Peach fruitfly, *Beauveria bassiana*, pesticides, potentiation effect.

INTRODUCTION

Peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a destructive polyphagous pest infest all horticultural fruits and some vegetables (Allwood et al., 1999). This pest had been established in Egypt and its presence causes a direct loss to different fresh fruits and vegetables. This pest causes severe damage to the infested fruits when female flies oviposit their eggs inside fruits that hatch into larvae feeding on the fruit flesh causing its destruction, so, they cause high loss in fruit production annually. Larvae pass three larval stages inside fruit, then they pop up to the soil for pupation. In about nine days, new flies emerge to attack fruits again. Dependence on the use of pesticides only as a control method became an obstacle in the way of exporting fresh horticultural products due to pesticides residues. Development of an effective biological control method against *B. zonata* as an alternative to chemical control or as a part of IPM programs is more or less became a necessity. Since most entomopathogenic fungi are natural inhabitants of soil, so they may encounter various insect pests that spend at least one stage of their life cycle in the soil (White and Elson – Harris, 1992). Use of selective pesticides in combination with entomopathogens, may increase the efficacy of control process and reduce the use of insecticides (Oliveira et al., 2003). Insecticides may play an antagonistic or synergistic role on the potentiality of the used entomopathogenic fungus. It is possible to enhance effectiveness through joint action of a pathogen and compatible insecticides. This will not only reduce the costs but also will decrease the harmful effect of insecticides on the environment. This work undertaken to determine the effect of the entomopathogenic fungi, *Beauveria bassiana* (Bals.) alone and in combination with

corresponding doses of five synthetic insecticides against *B.zonata*. In addition, the effects of each fungus-insecticide combination investigated on immature stages and flies. Data obtained from this study might be helpful to recommend the most efficient application of conventional insecticides and bio-pesticides to suppress pest populations of the insect in a way to introduce biological, safe method of insect control and to decrease the excessive use of insecticides that harm human and the environment.

MATERIALS AND METHODS

The experiments were carried out under laboratory conditions (25 ± 2°C, 70 ± 5% R.H.) at the Bio-insecticides Production Unit, Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

Tested insect:

Peach fruit fly, *B.zonata* immature stages and flies obtained from the laboratory colony reared in the Horticulture Insects Department, Plant Protection Research Institute, Dokki, Giza, Egypt. The insect larvae reared using artificial larval rearing medium according to the technique of Tanaka et al., (1969). The flies were provided with food (sugar and enzymatic yeast hydrolysate in ratio 3:1, respectively) El-Sayed, (1979). In present work, full-grown larvae collected immediately after they popped from the artificial larval rearing medium to pupate. Pupation process begun in two to eight hours while pupae need about nine days to complete its duration.

Tested compounds:

Entomopathogenic fungus *B.bassiana*:

Entomopathogenic fungus *B.bassiana* (Bals.) used in the present work was isolated from the white fly, *Bemisia tabaci* in Sharqiya governorate. (Ibrahim, 2006). Cultures of *B.bassiana* was grown at 25 °C, in dark on sabourad dextrose agar (SDA) medium consist of peptone 10 g/L and agar-agar 20 g/L, (constant volume of 15 ml) in standard petri-dishes (90 mm in diameter). To produce inocula for experiments, conidia harvested after two weeks of growth in fungal culture by scraping and suspended in sterile solution of Tween-80 (1ml/L) (polyoxyethylene, sorbitan mono-oleate; 1 ml/L). The resulting suspension wrapped and vortexed for two minutes and agitated for 90 minutes on a flask shaker at room temperature to ensure that conidia are well suspended. Each suspension filtered through sterile muslin to remove mycelia and debris. The concentration of conidia in the filtrate estimated and the suspension held overnight on ice at 4 °C to prevent germination of conidia before use in experiments. Suspension routinely checked before use in bioassays. Only second or third subcultures used to produce inocula for experiments to avoid possible attenuation (loss of virulence) associated with continuous culturing as described by Yeo et al., (2003).

Synthetic compounds and *B.bassiana*:

In the present work, four tested synthetic insecticides and *B.bassiana* used to study their toxicity effect on *B.zonata* immature stages and flies (Table1).

Table 1: Synthetic insecticides used in the bioassay tests

Trade name	Active ingredient and formulation	Mode of action	Applied rate	Manufacturer
Runner®	24% Sc	Methoxyfenozide IGR ecdysone accelerator	25ml/100L	Dow AgroSciences
Movento®	10% Sc	Spirotetramat IGR lipids synthesis inhibitor	75ml/100L	Bayer CropScience
Spincer®	24% Sc	Spinosad acetylcholine disruptor	100ml/100L	Jiangsu Flag Company
Sega Top®	5% EC	Lufenuron Chitin inhibitor	100ml/100L	Jiangsu Flag Company
B.bassiana	4%WP	Microbial insecticide *Beauveria bassiana*	200gm/100L	Insect Pathogen Production Unit, ARC, PPRI,Egypt

Inspecting chemical pesticides as inhibitors for *B.bassiana* growth:

Runner®, movento® spincer® and Sega Top® were tested for their inhibitory action to *B.bassiana* growth by poison food technique CDA in three replicates at the concentrations Recommended (RD), half-recommended (1/2 RD) and quarter recommended (1/4 RD). Each 100 ml portion of the medium was dispensed into a 250 Erlenmeyer conical flask and autoclaved at 121°C for 20 minutes and then cooled to about 45°C. Stock solutions of the pesticides were prepared in sterilized distilled water and incorporated into each flask to provide different levels of concentrates. Each flask was shaked well and poured into sterilized Petri plates (90 mm). A medium without insecticides served as a control. Each plate inoculated with 1×10^{10} condiospores from 12 days old culture of *B. bassiana*. The inoculated plates incubated at 25±1°C. After 15 days of incubation, the growth of *B. bassiana* colony in the Petri plates treated with different insecticides at different concentrates recorded. The produced conidia of each pesticide used harvested by scraping and suspended in sterile solution of Tween-80 (1 ml/L) (polyoxyethylene, sorbitan mono-oleate; 1 ml/L). The resulted suspensions wrapped and vortexed for two minutes and agitated for 90 minutes on a flask shaker at room temperature to ensure that conidia are well suspended. Each suspension filtered through sterile muslin to remove mycelia and debris. The concentration of conidia in the filtrate had estimated.

Effect of some synthetic insecticides and entomopathogenic fungus *B.bassiana* on *B.zonata* soil-borne stages and flies:

The chemical compounds, runner, movento and spincer were tested to evaluate their effect on the immature stages, full-grown larvae, one-day old pupae and one–day old flies. A serial of concentrations for each compound (recommended (RD), half-recommended (1/2 RD) and quarter recommended (1/4 RD) were prepared in distilled water were tested separately and *B.bassiana* concentrations as well. Effect of the tested chemical compounds concentrations and entomopathogenic, fungus, *B.bassiana* on full-grown larvae of peach fruit fly *B. zonata* tested in sterilized glass cylindrical jars of 7 cm diameter containing 75 grams of fine sand soil sieved through 2 mm sieve. The glass jars and the sand sterilized in an oven at 200 ˚C for an hour. Ten milliliters of each prepared dose of each compound added and well distributed in sand using a glass rod. Ten full-grown larvae were collected as they popped out of larval rearing medium at the same time and confined in each jar which containing the treated sandy soil. The same steps proceeded to study the effect on one-day old pupae but by using half quantity of the treated sand in the jars followed by placing ten one-day old pupae gently on the sand surface and then the pupae covered with the rest of the treated sand. The jars covered with pieces of muslin cloth and rubber bands then left under laboratory conditions for ten days until emergence of flies. Five replicates for each concentration were prepared to figure out their effect on the target stage. Other five replicates contained sand and water only accompanied each concentration group as control treatment. The same concentrations of each compound used to test their effect on flies. Sterilized transparent plastic jars measured 9 cm in diameter and 23 cm height were used for this experiment. The jars were sprayed with each concentration separately and left for an hour before provision of flies' diet (three sugar: one protein hydrolysate, in addition of water source) to avoid droplets to affect food. Twenty-five one-day old flies of *B. zonata* introduced gently in each jar using an aspirator allowing them to move on the sprayed dried walls. The jars closed with pieces of muslin for ventilation and rubber bands. Dead flies counted daily for successive five days. *B.bassiana* different concentrations conidia/ml used to test their effect on the previous mentioned target stages. Conidial suspensions sprayed and incorporated into the sand using small sprayer then the sand vigorously mixed with glass rod. Soil moisture measured to reach 10 % using a digital hygrometer (Model HC-520, Gamhouria, Co., Cairo, Egypt). The test on full-grown larvae ran as in insecticides tests. Dead individuals were collected and transferred to sterilized petri dish with moistened piece of cotton and incubated at 25 ˚C until fungal sporulation on the cadavers **(**Ekesi et al., 2002). The same steps repeated to test one-day old pupae as mentioned above. To evaluate toxicity against flies, one-day-old newly emerged flies of *B. zonata* used for the experiment. The same used concentrations of *B.bassiana* were applied separately using the same method applied above. All treatments kept at 25 ± 2 ˚C and 75 %RH cabinets. Dead flies counted daily and mycosis confirmed in sterilized Petri dishes with moistened pieces of cotton. Petri dishes were incubated at 25 ˚C and checked daily

until fungal sporulation appear on the cadavers. Biovar

Interaction of some insecticides and *B.bassiana* and on *B.zonata* immature stages:

B.bassiana LC_{50} concentration with runner, movento and spincer RD, ½ RD, and ¼ RD tested separately against full-grown larvae, one-day old pupae and flies. Full-grown larvae treated as described before using conidial suspension of *B.bassiana* and the used pesticides concentrations prepared separately added to each other in a ratio 1:1. Ten ml of the conidia-pesticide preparations incorporated into the sand using small sprayer (50ml) then well distributed in sand with glass rod. The same steps for conidia-pesticides preparations repeated for testing one-day old pupae. Conidia-pesticides preparations used for testing their effect on flies as mentioned in the previous tests.

Statistical analysis:

Corrected mortality calculated according to Abbott (1925). Toxicity of the synthetic insecticides were calculated as probit analysis (Finney, 1971). Koppenhöffer and Kaya, (1998) formula were used for calculating toxicity of *B.bassiana* and insecticides combinations on *B.zonata* immature stages and flies where expected mortality was calculated as follows: $M_e = M_f + M_i (1 - M_f/100)$, where M_f and M_i were the observed percent mortalities caused by the fungus and the insecticide separately. Positive values of M_{fi}-M_e considered synergistic according to Koppenhöffer and Kaya (1998).

RESULTS AND DISCUSSION

Inspecting some insecticides as inhibitors for *B.bassiana* growth:

The compounds, runner, movento and spincer tested concentrations LC_{25} and LC_{50} impregnated in SDA showed variation in inhibition of *B.bassiana* mycelial growth except sega top that inhibited mycelial growth. Runner, movento and spincer at LC_{25} concentrations did not affect *B.bassiana* mycelial growth. Runner and movento exhibited about 75% mycelial growth at the LC_{50} concentrations while spincer showed about 100% mycelial growth (Table 2). The utilization of appropriately selected insecticides in association with entomopathogenic fungi can increase the efficiency of pest control, allowing a consequent reduction in the amount of the chemical that is applied along with a minimization of environmental contamination and the expression of pest resistance (Oliveira et al. 2003). The sensitivity of different fungal isolates of the same species to a particular insecticide may differ greatly (Olmert and Kenneth 1974). In the present work, we evaluated the interactions between three insecticides, runner®, movento® and spincer® with entomopathogenic fungi, *B.bassiana* under laboratory conditions in order to determine the possible usefulness of combinations of this agent against the peach fruit fly, *B.zonata* full-grown larvae , pupae and flies. The effect of insecticides on an entomopathogenic fungus cannot be generalized. The effect of insecticides on conidial germination should be considered as one of the most important factors affecting success of entomopathogen-insecticide combination. The present study proved that *B. bassiana* was affected by the insecticides runner, movento and spincer lethal doses that partially inhibited the development and reproduction of *B. bassiana* whereas the conidial vegetative growth increased as control at using the sub-lethal doses (quarter-recommended doses). It seems that *B.bassiana* conidial vegetative growth and reproduction are inversely proportional with insecticide dose. These findings are in agreement with Anderson and Roberts (1983) , Alizadeh et al. (2007) and Kumar and Kuttalam, 2010 who proved that fungal germination is an important factor in compatibility evaluation of pesticides with entomopathogenic fungi in pest management because the beginning of epizootics is conditioned by the capacity of conidia to germinate on the host. They stated that expression of low inhibition in the biological properties of *B.bassiana* as observed may be due to the presence of emulsifiers and other additives in the formulated products of insecticides. Generally, wettable powders and flowable formulations cause no inhibition and often increase colony counts, whereas emulsifiable concentrate formulations frequently inhibit *B. bassiana* germination (Anderson et al., 1989). Adjuvants in wettable powders and flowable formulations may act as mild abrasives and break up agglomerations of conidia, which would improve the field performance of *B. bassiana*.

Effect of some synthetic insecticides on immature stages of *B.zonata*:

The toxicity of the tested compounds (runner, movento, spincer and *B.bassiana*) on *B.zonata* full-grown larvae, one-day old pupae and one-day old flies are presented in (Table 3).

Table 2: In Vitro compatibility of *B. bassiana* with used pesticides

Pesticides concentration	Runner®	Movento®	Spincer®	Sega Top®	Control
LC_{25}	1.3×10^7	1.3×10^7	1.3×10^7	-Ve	1.3×10^7
LC_{50}	3.2×10^5	1.3×10^7	3.2×10^5	-Ve	1.3×10^7

Table 3: Efficacy of chemical compounds and *B.bassiana* on *B.zonata* immature stages and flies

Insecticide	Concentration level			χ^2	Slope ± SE
	LC_{25} CL	LC_{50} CL	LC_{90} CL		
		Full-grown larvae			
Runner	102.17 79.71 – 122.12	218.93 180.29 - 295.26	933.13 573.20 - 2346.46	0.220	2.04±0.33
Movento	223.78 151.22 – 281.50	545.40 445.98 -733.15	2963.72 1699.99 - 9260.58	0.164	1.73±0.32
Spincer	19.93 0.104 – 68.56	81.80 69.90 -167.21	1384.47 879.73 - 5641.58	0.034	1.04±0.30
B.bassiana	2.32×10^3 $3.20\times10^2 – 5.04\times10^4$	5.15×10^6 $1.20\times10^5 - 2.52\times10^7$	1.53×10^{10} $1.14\times10^9 - 2.22\times10^{13}$	1.208	0.286±0.073
		One–day old pupae			
Runner	158.21 108.59 – 279.97	463.82 305.14 - 1280.99	307.71 1160.72 - 38735.25	3.490	1.61±0.40
Movento	210.23 135.78 – 268.52	530.74 431.77 - 717.72	3085.78 1725.04 - 10496.79	0.002	1.35±0.32
Spincer	60.83 13.63 – 113.41	160.40 71.45 - 229.99	1009.93 734.24 - 2007.55	0.398	1.60±0.35
B.bassiana	4.70×10^2 $0.033\times10^4 - 2.20\times10^4$	1.45×10^5 $7.32\times10^3 - 1.43\times10^6$	8.03×10^{10} $1.24\times10^9 - 6.15\times10^{11}$	0.801	0.271±0.06
		Flies			
Runner	371.05 286.14 – 602.12	878.53 555.62 - 2204.89	4517.62 1895.40- 26802.95	2.099	1.80±0.32
Movento	1286.13 930.95 – 2514.96	3375.82 1909.91 - 11878.65	21119.22 7248.00 - 234235.46	3.612	1.61±0.32
Spincer	2.83 0.0007 – 20.31	27.25 0.33 - 83.52	2008.63 1086.10 - 22169.47	0.215	0.699±0.22
B.bassiana	2.44×10^4 $2.81\times10^3 – 1.04\times10^5$	1.32×10^6 $3.92\times10^5 - 3.15\times10^6$	2.44×10^9 $9.79\times10^8 - 9.10\times10^9$	0.225	0.391±0.043

CL confidence limits, SE standard error

The most effective tested compound was spincer as showed the lowest LC_{50} value (81.80 ppm) after treating full-grown larvae, followed by runner (218.93 ppm) and movento (545.40 ppm). The bioagents *B.bassiana* recorded LC_{50} value 5.15×10^6 ppm. Spincer showed the lowest LC_{50} value on treating of one-day old pupae (160.40 ppm) followed by runner (463.82 ppm) and movento (530.74 ppm) while *B.bassiana* showed LC_{50} value 1.45×10^5 ppm. On treating flies, spincer was the most effective and recorded 27.25 ppm. Runner and movento showed LC_{50} values 878.53 ppm and 3375.82 ppm, respectively. *B.bassiana* showed 1.32×10^6 ppm. Data in Table (4) showed that mean mortality percentages of *B.zonata* treated individuals were significant at (P<0.05) and significance level at 95%. Mortality percentages were significant after treatment with runner, movento, spincer and showed a concentration dependent pattern. Spincer treatments resulted in the highest mortality percentages of the treated stages. Percentages of larval mortality reflected high significance at LC_{90} (F=782.15, X^2=2.08, P=0.3532), LC_{50} (F=320.28, P<0.0001, X^2=4.77) and LC_{25} (F=515.19, P<0.0001, X^2=1.49,). Pupal mortality percentages revealed high significance at LC_{90} (F=848.54, P<0.0001, X^2=1.11), LC_{50} (F=555.13, P<0.0001, X^2=2.10) and LC_{25} (F=348.83, P<0.0001, X^2=4.30). Flies mortality percentages supported spincer superiority on runner and movento and showed high significant differences at LC_{90} (F=4193.93, P<0.0001,X^2=2.96), LC_{50} (F=465.35, P<0.0001, X^2=13.03) and LC_{25} (F=1821.33, P<0.0001,

X^2=5.30). *B.bassiana* influenced *B.zonata* tested stages significantly more than runner and movento. The resulted percentages of mortality showed concentration pattern dependent (Table 4). The effects of runner, movento and spincer on *B.zonata* full-grown larvae, pupae and flies showed a variety in mortality ranged from low to high percentages. Runner (methoxyfenozide) is an insect regulator from ecdysone agonist group. This compound mimics the natural insect molting hormone by binding competitively to ecdysteroid receptors in insect cells, thus inducing a premature larval molt (Dhadialla et al. 1998). In the present work, runner showed the least effect on *B.zonata* full-grown larvae and one-day old pupae. These results coincides with Fahmy et al. 2013 who tested methoxyfenozide against *B.zonata* full-grown larvae and pupae. Movento contains spirotetramat, an inhibitor of lipids biosynthesis and it is mainly used against sucking insects. In the present work, movento lethal and sub-lethal concentrations reflect a moderate effect on full-gown larvae. Runner (methoxyfenozide) and movento (spirotetramat) reflected a negative effect on *B.zonata* flies and were less toxic than spincer. These findings are supported with Mosleh et al., 2011, who stated that methoxyfenozide had low toxic effect on *B.zonata* flies. At the same point, Bengochea et al. 2013 found that methoxyfenozide did not provoke any negative effect on olive fruitfly, *Bactrocera oleae* Rossi (Diptera: Tephritidae) flies in laboratory and field treatments. On the other hand, Belien et al. (2013) tested movento (spirotetramat) on cherry fruitfly, *Rhagoletis cerasi* (Diptera: Tephritidae) flies and obtained 100% mortality at field treatment in successive two seasons. The spinosyns, a class of macrocyclic lactones produced by the soil actinomycete *Succharopolysporu spinosa*. These products are a complex of several natural metabolites and showed good biological activity against insects of different orders (Adán et al. 1996). Spincer (spinosyns group) caused the highest mortality percentages for tested full-grown larvae, pupae and flies. El-Aw et al. (2008) experimented spinosad and reported that it seem to be acceptable substitutes for organophosphorus and Carbamate insecticides for control tephritid fruit flies such as *B.zonata*. In the present work, spincer reflected significant percentages of mortality on treating *B.zonata* full-grown larvae and one-day old pupae by contact technique in sandy soil in comparison with mortality achieved by methoxyfenozide and spirotetramat using the same technique. These results are supported by Adán et al. (1996) who reported that *C.capitata* full-grown larvae and early age pupae showed positive effect towards spinosad when come in contact at pupation media. Percentages of larval mortality recorded in different studies may be affected by used spinosad doses *i.e.* low doses has weak influence on its target and could not affect acetylcholine upstream. Darriet et al., (2005) tested spinosad by contact solutions on different insecticide-resistant mosquitos and obtained mortality percentages ranged from 0-100% according to the used dose and tested species. Halawa et al. (2013) experimented Radiant® (a second new generation of spinosad used) on *B.zonata* full-grown larvae and one-day old pupae. They revealed that Radiant® was effective against the full- grown larvae of *B. zonata* but pupae were more resistant. It seem that tested stages play an important role in enabling insecticide action as the physiological processes change in each stage. Flies were highly susceptible to spincer more than runner and movento using contact technique. Mostafa et al., (2010) found that spinosad under commercial name “Conserve 0.24% CB” using partial spray could protect the olive fruit crop from infestation of *B.oleae* by 73% and reduced the olive fruit fly population by 77%. According to Khoualdia, (2010) and Thompson et al., 2000, spinosad is an insecticide relatively broad spectrum registered for many cultures. It is deemed effective against Lepidoptera and Diptera. Madiha et al. (2017), controlled *Bactrocera* sp. in guava orchards using spinosad (Tracer® 24% Sc) and reduced fruits infestation by more than 90%. These results are in agreement with those obtained from the present study taking in consideration that commercial spinosad product introduce variation in mortality percentages according to the effective material percentages and the other ingredients. In addition, it seems that there is a relationship not only between the insect and the insecticide but also between the insect different stages and the insecticide.

Table 4: Efficacy of some synthetic insecticides and *B.bassiana* on *B.zonata* immature stages

Compounds											
Runner			**Movento**			**Spicer**			***B.bassiana***		
% of mean larval mortality ±SE											
LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}
14.10±0.98	29.33±0.21	55.61±0.92	28.10±0.02	39.33 ±0.23	58.61±0.29	69.72 ±0.65	78.84±0.84	87.73 ±0.95	59.04±0.51	77.31±0.45	81.53±0.13
Slope±SE=2.042±0.33 χ^2=0.220, P=0.6931			Slope±SE=1.746±0.32 χ^2=0.063,P=0 .8018			Slope±SE=1.043±0.24 χ^2=0.034,P=0.8535			Slope=1.1568±0.23 χ^2=2.965, P=0.0855		
% of mean pupal mortality ±SE											
LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}
13.24±0.32	30.41±0.38	38.90 ±0.20	22.19±0.32	40.11±1.07	59.65±0.63	65.64±0.76	76.53±0.98	92.31±0.54	37.75±0.66	46.59±0.35	55.02±0.63
Slope±SE=1.413 ±0.313 χ^2=0.301, P=0.9598			Slope±SE=1.676 ±0.323 χ^2=0.011, P=0.0083			Slope±SE=1.603±0.353, χ^2=0.398, P=0.5280			Slope±SE=0.6949±0.18 χ^2=0.0296, P=0.8634		
% of mean flies mortality ±SE											
LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}
1.04 ±0.50	8.03±0.66	15.31 ±0.24	1.21±0.13	8.43 ±0.51	13.33 ±0.32	65.24±0.36	79.92±0.05	86.35±0.58	47.08±0.27	60.39±0.23	70.98±0.66
Slope±SE=1.80±0.32 χ^2=2.199, P=0.1483			Slope±SE=1.61 ±0.34 χ^2=3.56, P=0.0591			Slope±SE=0.686±0.22 χ^2=0.215, P=0.6472			Slope±SE=0.9404±0.17 χ^2=0.1690, P=0.6812		

Table (5): Interaction between *B.bassiana* LC_{50} and LC_{25} and LC_{50} of some synthetic insecticides on *B. zonata* immature stages

Treatment	Expected % mortality±SE	Observed % mortality±SE	T-Value	P-Value	M_{fi}-M_e	Co-Toxicity factor	Joint action
Full-grown larvae							
Runner concentrations × *B.bassiana* LC_{50}							
LC_{25}	24.39±0.32	50.49±0.72	19.37	0.0001	26.10	107.01	P
LC_{50}	27.58 ±0.24	57.42±0.51	20.04	0.0001	29.84	108.24	P
Movento concentrations × *B.bassiana* LC_{50}							
LC_{25}	20.65±0.25	50.49±0.81	47.36	0.0001	50.49	144.50	P
LC_{50}	26.31±0.45	58.41±0.52	14.91	0.0001	32.10	122.01	P
Spincer concentrations × *B.bassiana* LC_{50}							
LC_{25}	30.96±0.19	100.00±0.00	09.80	0.0008	68.04	219.87	P
LC_{50}	32.85±0.42	100.00±0.00	14.91	0.0001	66.15	201.49	P
One-day old Pupae							
Runner concentrations × *B.bassiana* LC_{50}							
LC_{25}	19.55±0.24	21.78±0.19	029.59	0.0031	02.23	11.41	AD
LC_{50}	23.69±0.69	50.49±0.27	49.50	0.0001	26.80	113.13	P
Movento concentrations × *B.bassiana* LC_{50}							
LC_{25}	22.68±0.65	29.70 ±0.54	00.53	0.6075	7.02	22.16	AD
LC_{50}	26.31±0.34	58.41±0.56	1.29	0.2323	32.10	122.01	P
Spincer concentrations × *B.bassiana* LC_{50}							
LC_{25}	31.24±0.28	99.00±0.67	09.02	0.0001	67.76	216.90	P
LC_{50}	34.45±0.45	99.00±0.09	10.73	0.0001	64.55	187.43	P
One-day old flies							
Runner concentrations × *B.bassiana* LC_{50}							
LC_{25}	18.27±0.35	72.71±0.06	91.00	0.0001	54.44	297.97	P
LC_{50}	19.91±0.31	52.14±0.37	74.00	0.0001	32.23	161.98	P
Movento concentrations × *B.bassiana* LC_{50}							
LC_{25}	18.31±0.21	89.96±0.31	91.01	0.0001	71.65	391.32	P
LC_{50}	20.01±0.11	83.53±0.25	61.00	0.0001	63.52	317.44	P
Spincer concentrations × *B.bassiana* LC_{50}							
LC_{25}	33.40±0.64	99.13±0.27	12.65	0.0002	65.73	196.86	P
LC_{50}	36.86±0.56	96.87±0.21	14.00	0.0002	60.01	162.81	P

Expected Mortality $M_e = M_f + M_i (1 - M_f/100)$ with M_f and M_i observed mortalities caused by fungus and insecticides alone, respectively. M_{fi} = observed mortality of mixture. Joint action point symbolled means "AD" means additive and "P" means potentiation.

Interaction between *B.bassiana* conidial suspensions and some synthetic insecticides:

Potentiation and additive effects were observed upon application of LC_{25} and LC_{50} of tested insecticides in combination with LC_{50} of *B.bassiana* suspensions after treating *B.zonata* immature stages (Table 5).

Results indicated that larval mortality increased after application of spincer at LC_{25} and LC_{50} in combination with *B.bassiana* at LC_{50} and exhibited the highest potentiation effect, where Co-Toxicity Factor (CTF) values were 219.87 and 201.49, respectively. Movento ranked second and showed potentiation effect, where Co-Toxicity factor (CTF) values were 144.50, 122.01, respectively followed by runner that reflected Co-Toxicity factor values 107.01 and 108.24, respectively. Pupal mortality increased after application of *B.bassiana* combined with Spincer at LC_{25} and LC_{50} and presented high potentiation effect with Co-Toxicity factor values 216.90, 187.43, respectively. Movento and runner increased pupal mortality at LC_{25} as combined with *B.bassiana* and showed additive effect, where the Co-Toxicity factor (CTF) values 22.16 and 11.41, respectively but reflected potentiation at their LC_{50} with Co-Toxicity values 32.10, 26.80, respectively. Spincer at LC_{25} and LC_{50} when combined with *B.bassiana* increased flies mortality with Co-Toxicity factor (CTF) values 196.86 and 162.81, respectively. Flies mortality increased on application of movento LC_{25} and LC_{50} combined with *B.bassiana* showed potentiation with CTF values 391.32, 317.44, respectively followed by runner 297.97, 161.98, respectively. Flies were the most susceptible stage to infection followed by full-grown larvae and pupae. In the present work, we evaluated the interactions between three insecticides, runner®, movento® and spincer® with entomopathogenic

fungi, *B.bassiana* under laboratory conditions in order to determine the possible usefulness of combinations of this agent against the peach fruit fly, *B.zonata* full-grown larvae , pupae and flies. The effect of insecticides on an entomopathogenic fungus can not be generalized. The effect of insecticides on conidial germination should be considered as one of the most important factors affecting success of entomopathogen-insecticide combination. The present study proved that *B. bassiana* was affected by the insecticides runner, movento and spincer LC_{50} concentrations that partially inhibited the development and reproduction of *B. bassiana* whereas the conidial vegetative growth increased as control at using the LC_{25} concentrations. It seems that *B.bassiana* conidial vegetative growth and reproduction are inversely proportional with insecticide concentration. These findings are in agreement with Anderson and Roberts (1983) , Alizadeh et al., (2007) and Kumar and Kuttalam, 2010 who proved that fungal germination is an important factor in compatibility evaluation of pesticides with entomopathogenic fungi in pest management because the beginning of epizootics is conditioned by the capacity of conidia to germinate on the host. They stated that expression of low inhibition in the biological properties of *B.bassiana* as observed may be due to the presence of emulsifiers and other additives in the formulated products of insecticides. Generally, wettable powders and flowable formulations cause no inhibition and often increase colony counts, whereas emulsifiable concentrate formulations frequently inhibit *B. bassiana* germination (Anderson et al., 1989). Adjuvants in wettable powders and flowable formulations may act as mild abrasives and break up agglomerations of conidia, which would improve the field performance of *B. bassiana*. Although the three different insecticides utilized, exhibited variation in their effects (potentiation or additive) with respect to toxicity against *B.zonata* different stages when combined with *B.bassiana* but we never observed an antagonism effect between them. These findings are in agreement with Ericsson et al., 2007 who reported that the combined approaches interact synergistically and result in significantly higher mortality. Based on these observations, the combinations of those insecticides with *B.bassiana* were more toxic to *B.zonata* full-grown larvae, pupae and flies than either of the individual agents used alone. It may indicate that such combined formulations of entomopathogenic fungi and insecticides make less use of active ingredient than what applied separately to achieve the same outcome. Variations in the effect of the entomopathogenic fungus, *B. bassiana* on the insect toxicity of chemical insecticides ranging from antagonistic to additive to synergistic—have been reported (Bitsadze et al., 2013) but antagonism relationship was not detected in the present work. It is not clear how the combination of insecticides and entomopathogenic fungi treatments interact but the physiological effects caused by one agent may increase the effects of the other resulting in higher mortality of the targeted pest. The combinations between *B.bassiana* and spincer LC_{25} and LC_{50} concentrations produced the best results when applied on *B.zonata* full-grown larvae, pupae and flies compared to runner (methoxyfenozide) and movento (spirotetramat). Khorasiya et al., (2018) evaluated the combination between *B.bassiana* with different insecticides and found that spinosad was highly compatible with *B.bassiana*.

CONCLUSION

Combination of *B.bassiana* conidial suspension with synthetic insecticides increased their efficacy against immature stages of *B.zonata* that mostly potentiation and sometimes additive. These obtained results should motivate us to test more groups of synthetic insecticides compatibility with *B.bassiana* and to screen them on orchards level. If the additive or potentiation effect of combinations of synthetic insecticides with *B.bassiana* confirmed in the orchards level, this approach may sparkle a hope and introduce a helpful tool of the integrated pest management of peach fruitfly, *Bactrocera zonata* (Saunders).

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

The authors contributed equally in all parts of this study. The authors read and approved the manuscript.

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