Plant Archives Vol. 20, No. 1, 2020 pp. 929-938 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

INTERACTION OF THIAMETHOXAM, SPINOSAD AND *BEAUVERIA BASSIANA* ISOLATES FOR CONTROL WHITEFLY *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE) ON CUCUMBER

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Abstract

This study was carried out to evaluate the interaction of Thiamethoxam, Spinosad and *Beauveria bassiana*, Bb1and Bb4 isolates for control whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) on cucumber. Treatment of eggs with different concentrations of spore's suspension of local *B. bassiana*, Bb1 and Bb 4 isolates appeared very weak reduction in hatching rates compared with control treatment. The mortality rate of *B. tabaci* nymphs exposed to the spores' suspension of local isolates depended on concentrations and isolate. The highest biological index (68.7) was for half field dose of Thiamethoxam that confirm its compatibility with *B. bassiana* and was moderated effect at field dose. Spinosad was recorded toxic effect and incompatible with fungus at field dose, biological index was 27.6, in half field dose, biological index was indicated to moderate effect (42.13). The highest efficiency in controlling this pest after five days was recorded to the combination of SP+Bb1 (89.35%) and it was not significantly different except from isolate Bb4 (68.7%), the superiority of this combination was continued after ten days with a significant differences from all treatments except SP pesticide, the two treatments achieved 82% relative efficiency. The results also showed up there were no synergy and significant increase in the effectiveness when combining both agents and their combination was not exceeding from additive status. According to these results can be combined these pesticides at half field dose with fungus isolates to use it in IPM programs with preference to isolate Bb1.

Key words : Biocontrol, Beauveria bassiana,, Bemisia tabaci, Thiamethoxam, Spinosad.

Introduction

Entomopathogenic fungi are ideal for IPM programs because they are relatively safe to use and have a narrower spectrum of activity than chemical insecticides (Lacey and Goettel, 1995). The entomopathogenic fungus *Beauveria bassiana* (Hypocreales: Cordycipitaceae) (Balsamo) Vuillemin is capable alternative control agents against important agricultural pests, including whiteflies, aphids, thrips, psyllids, weevils, and mealybugs (Shah and Goettel, 1999; Bugti, *et al.*, 2018). The integration of microbial pesticides with chemical practices requires detailed compatibility studies. In some cases, compatible products may be associated with entomopathogenic fungi, increasing the control efficiency, decreasing the amount of insecticides required and minimizing the risks of environmental contamination and pest's resistance expression (Quintela and McCoy, 1998; Purwar and Sachan, 2006). By contrast, use of incompatible insecticides may inhibit growth and reproduction of the pathogens and adversely affect integrated pest management (Duarte *et al.*, 1992; Malo, 1993) hence, an understanding of effects of synthetic insecticides on germination and vegetative growth of fungal biocontrol agents is essential for their application in IPM programs. (Alialzadeh *et al.*, 2007; Rachappa *et al.*, 2007). The objective of this study was to evaluate the compatibility of selected insecticides Thiomethoxam, Spinosad with two isolates of entomopathogenic fungus *B.bassiana*, for using in integrated pest management programmes of sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), that pose a high pest status on a wide range of host plants and the damages it currently inflicts globally are incalculable. Crop losses, noticeably in greenhouse, as well as crops grown in open fields, are caused through direct feeding damage or through transmission of decimating plant viruses

Materials and Methods

Bemisia tabaci

The whitefly, *Bemisia tabaci* which was used in study, was obtained from infected cucumber plants that planted in the greenhouse of the spring season 2019. The greenhouse was prepared and the seeds of cucumber Mansor f1 were planted and all recommended agricultural operations to serve the crop were applied. Plants were left to be infected with insect pests, including white flies, without pesticide control.

Fungal isolates

Two local isolates of *Beauveria bassiana* (Bb1 and Bb4) that were stored in the directorate of Agricultural Research / Ministry of Science and Technology, they previously isolated from Iraqi soil gardens and farms using Galleria bait method (*Galleria mellonella* L. larvae).

Preparation of conidial suspension

The fungal pathogen used in present study were cultured on potato dextrose agar medium (PDA) autoclaved at 121°C (15 Psi) for 15-20 minutes and poured into sterilized Petri plates. The Petri plates containing PDA medium were incubated at 27 ± 1°C, 80 ± 5% relative humidity and photoperiod of 12 hours. The conidia were harvested gently by scraping the surface of 15-days old culture with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween-80. The mixture was stirred on a magnetic shaker for 10 minutes. The hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using haemocytometer. Suspension concentrations (1×10^5, 1×10^7 and 1×10^9 conidia ml^{-1}) was prepared and used in bioassay.

Bioassay

Effect of *Beauveria bassiana* on eggs and nymphs of whitefly

The pathogenicity of local isolates Bb1 and Bb4 was measured at concentrations 10^5, 10^7 and 10^9 spores / ml on eggs and nymphs of the white fly by spraying three plants with each concentration (treatment) and three plants were sprayed with water + Tween 80 at 0.01% as control. The rate of hatching eggs after 5 days and the mortality rate of nymphs after 3, 5 and 10 days were calculated.

Effect of pesticides on nymphs and adults of the white fly

The efficacy of Spinosad and Thiamethoxam was measured at field dose (0.5 ml / l) and half field dose (0.25 ml / l) on nymphs and adults of whitefly in cucumber by spraying three plants per concentration (treatment) with three plants were sprayed with water only as a control. The relative efficiency of pesticides in reducing the number of nymphs and adults was calculated after one day and seven days after spraying by examining three leaves per plant.

Determine the compatibility of insecticides with entomopathogenic fungus *B. basiana*

The tests for fungal vegetative growth, conidia production and conidia germination were done. The fungus were cultured on sterile PDA medium on laminar air flow and incubated for ten days. Insecticides used in this study were Thiamethoxam and spinosad, with two doses each, *i.e.*, at field dose (0.5 ml / l) and half field dose (0.25 ml / l). Vegetative growth was measured by poisoned food technique (Moorhouse *et al.*, 1992). A total of 10 ml warm sterile PDA medium (+ 45 °C) was poured into a Petri dish, then the given dose of insecticide was added aseptically under laminar air flow. The mixture was stirred and poured into a Petri dish (diameter 9 cm), allowed to solidify under laminar air flow. The 5-mm diameter of 10-day-old *B. basiana* isolates were transferred to insecticide-PDA medium. Non-insecticide PDA medium was inoculated with *B. basiana* as a control. The plates were sealed with parafilm and incubated at room temperature. This treatment was replicated four times. The colony diameter was measured with a ruler (cm) at day 10 after inoculation. Treatment data were compared to those of the control to determine the percentage value of inhibitory growth.

To test the conidia production, fungal mycelium from the vegetative growth was taken with needle. The mycelium was placed into a test tube containing ten ml distilled water plus 0.01% Tween 80. The suspension was homogenized in a centrifuge for 10 minutes. The part of that containing the mycelium debris was discarded, while the supernatant containing the conidia was taken. A total of 1 ml conidial suspension was taken and placed on a hemocytometer. Conidia were counted directly at five medium squares on Hemocytometer, and then the average value was calculated. It was replicated four times. The data obtained were compared to the control data.

Conidia germination was tested with modified methods of Alizadeh *et al.*, (2007). Each dose of insecticide was poured into a test tube containing 10 ml of warm sterile PDA (45 °C). Conidial suspension (1 ml) of *B. basiana* (the standard concentration of 10^6 conidia.ml^{-1}) and 0.01% Tween 80 were added into the tube. The medium without insecticides was inoculated with the fungal conidia suspension as the control. Each medium was incubated at room temperature (24–30 °C) for 24 hours. Conidial germination was observed in 5 medium-sized Hemocytometers under a light microscope, and then taken on average. Each treatment was replicated four times. The germinated conidia were characterized by a germ tube. Treatment data were compared to those of the control to obtain the percentage of conidia germination for *B. basiana*. Calculation of Insecticidal Compatibility used the formula below:

BI = 10 (GR) + 47 (VG) + 43 (SPR) 100

Notes: BI = Biological index (level of insecticide toxicity against entomopathogenic fungi in vitro), GR = Comparison of conidia germination treatment with control, VG = Comparison of vegetative growth treatment with control, SPR = Comparison of conidia numbers of treatment with control. Insecticidal toxicity level followed Alves *et al.*, (2007) formula which was based on BI factor calculated by comparing germination data of conidia (GR), vegetative growth (VG) and sporulation (SPR) with control data (%). BI classification was < 42 = toxic, 42–60 = moderately toxic, and > 60 = compatible.

Integrated effect of pesticides and fungus isolates on white fly nymphs on cucumber leaves

The experiment was carried out on cucumber crop in the greenhouse and designed according to Randomized complete block design (RCBD), the house was divided into three block in each was the following treatments:

- Control (untreated)
- Spinosad (0.25 ml / l)
- Thiamethoxam (0.25 ml / l)
- *B.bassiana* Bb1(10^9 spores/ml.)
- *B.bassiana* Bb4 (10^9 spores/ml.)
- Spinosad (0.25 ml / l) + Bb1 (10^9 spores/ml.)
- Spinosad (0.25 ml / l) + Bb4 (10^9 spores/ml.)
- Thiamethoxam (0.25 ml / l) + Bb1(10^9 spores/ml.)
- Thiamethoxam (0.25 ml / l) + Bb4(10^9 spores/ml.)

Weekly samples were collected from three levels for each plant (upper, middle and lower) and were examined in vitro under light microscope.

Synergistic effect

The synergistic between *B.bassiana* with Spinosad or Thiamethoxam was performed. The data corrected by using Abbott (1925) equation. The expected mortality of the treatment of the combination of both agents was obtained using the formula: $E = O_a + O_b (1\text{-}O_a \backslash 100)$ where E represents the expected mortality and O_a represents the observed mortality caused by the neem alone and $O_b$is observed mortality caused by of *B. thuringiensis* alone (Salama, 1984).

Chi squared test X^2 was performed by calculating the X^2v alue using the formula:

$$X^2 = (O_{c\,\_}\,E)^2/ E$$

Where O_c represents observed mortality for the treatment of combination, and then compared to the table value for df 1 (Ã3.84).if the calculated X^2 value exceeds the tabulated value, it indicates a non- additive effect (either synergistic or antagonistic) of the two control agents. A significant interaction of *B.bassiana* and insecticides combination was determined through the difference of ($O_{c\,\_}$ E), where positive = synergistic and negative= antagonistic. If the tabulate value exceeds the calculated X2 value; it represents an additive effect at PdH0.05.

Statistical analysis

Statistical analysis was conducted using completely randomized design CRD, randomized complete Block design RCBD and Duncan test. Probit analysis was used to obtain the median lethal concentrations (LC_{50}) and median lethal time (LT_{50}), and T test within SPSS system, version 20.

Results and Discussion

Effect of *Beauveria bassiana* on whiteflies eggs

Exposure of whiteflies eggs to different concentrations of conidial suspension of local *B.bassiana* isolates, Bb1 and Bb4 appeared too little reduction in hatching rates compared with control treatment. It was 19.2% in case of isolate Bb1 and 15 % for isolate Bb4 at concentration of 10^9 spores / ml (Table 1). The lowest reduction rate for hatching eggs was at the concentration of 10^5 spore / ml, 13.9% in case of isolate Bb1 and 10.8% for isolate B4. These results are consistent with the results of Mohammad (2008) that indicated to high tolerance and greater resistance of eggs and variation in the intensity of the effect of *B. bassiana* on different stages of whitefly. The incidence of eggs was 4.49% despite the use of a high concentration of 6×10^{6}. Spore / ml compared to 42.045% on the second nymph.

Al-Deghairi (2008) in a study of evaluating the effect of *Beauveria bassaina* on eggs and nymphs of the whitefly showed that the percentage of mortality of eggs increases with increasing concentration, it was 1.2, 4.27

and 8% at 2×10^6, 4×10^6 and 6×10^6 spore / ml. He also indicated that the percentage of mortality of eggs was weak compared to the mortality of the nymph of *Bemisia tabaci* when treated with *Beauveria bassaina*. Many studies have confirmed this scientific fact of the whiteflies species against pathogenic fungi, explaining the phenomenon by constructing the chorion layer, which acts as a difficult barrier against the invasion of spore suspension which is an obstacle to germination tube, which requires a long time for germination and penetration of egg shell compared with the speed of embryonic development.

This is due to the presence of antifungal compounds that prevent germination (Meeks *et al.,* 2002 and Abdel-Baky *et al.,* 1998). In another study carried out by Zafar *et al.,* (2016) to evaluate the effect of *Beauveria bassaina* on *Bemisia tabaci* on four different crops it was observed that the fungus was effective on both eggs and other stages and that the effect rate varied depending on the concentration used and the plant host.

Effect of *Beauveria bassiana* on whitefly nymphs

The results of this study table 2 showed the mortality of *B.tabaci* nymphs exposed to the spores suspension of local isolates Bb1 and Bb4 of *B. bassiana* was depended on concentrations and isolate. After 5 days of treatment with Bb1 the concentrations 10^7 and 10^9 were outperformed concentration 10^5 spores / ml in mortality rate with 59, 63 and 48.7% respectively. The differences of effect of the concentration of 10^9 spores / ml after 10 days was significant compared with the other concentrations 10^7 and 10^5 spores / ml, 89, 64 and 54%, respectively. Mortality rate caused by Bb4 isolate was associated with the concentration; it was 47, 51 and 80% after 10 days for the concentrations 10^5, 10^7 and 10^9 Spores / ml, respectively, with a significant difference for the highest concentration. Comparison of pathogenicity of fungal isolates based on LC_{50} concentration showed that Bb1 isolate was highly efficacy with LC50 value 8.9×10^4 spores / ml. after 10 days compared with 1.9×10^5 spores/ml. for Bb4 isolate.

The virulence of the isolates represented by the time required to 50% mortality of the treated insect population (LT50). This time correlated with the concentration. The concentration 10^9 spores/ml. gave the shortest time to achieve this result (3.45 Days). The variance between isolates of the same species is attributed to genetic variance. This discrepancy between the isolates of *Beauveria bassiana* and *Metarhizium anisopliae* has been recorded in many studies (De La Rosa *et al.,* 2002; Garcia *et al.,* 1984). Pathogenesis is the most important indicator to the effectiveness of fungi against pests and the basis for laboratory biological tests. Fungal isolates are selected as successful biological control agents due to their high pathogencity, specialization and ease of mass production and adapt to environmental conditions (Ptlamul and Parasertan, 2012). Sain *et al.,* (2019) in a study to search for the best isolate of entomopathogenic fungi against the whitefly *Bemisia tabaci* indicated that the highest mortality rate of nymphs was achieved at the concentration of 10^7 spores / ml and in the case of isolate *Beauveria bassiana* (Bb) -4511, 95.1% followed by isolate *Beauveria bassiana* -4565 (89.9%) and *Metarhizium anisopliae1299* (86.7%).

In the test of 25 isolates of *B.bassiana* and commercial bio pesticides of the same fungus against the nymph of the whitefly *B.tabaci* at a concentration of 10^7 spores / ml all isolates showed pathogenicity and the mortality rate ranged from 3-85%. In the second series of biological tests on 10 selective isolates at the concentration of 10^5–10^8 spores / ml, the Lc50 of the four most virulent isolates ranged from 1.1×10^5 to 6.2×10^6 spores / ml (Quesada *et*

Table 1: Effect of spore suspension of local isolates Bb1 and Bb4 of *Beauveria bassiana* on eggs of white flies *Bemisia tabaci.*

Treatment	Concentrations (spores / ml)					
	10^5		10^7		10^9	
	Hatching percentage	Reduction percentage	Hatching percentage	Reduction percentage	Hatching percentage	Reduction percentage
Bb1	82	13.9	79	17.1	77	19.2
Bb4	85	10.8	83	12.9	81	15
P value	0.33	0.22	0.1	0.14	0.43	0.29

Table 2: Mortality rates , LC_{50} and LT_{50} of *B.tabaci* nymph exposed to different concentrations of *Beauveria bassiana* isolates.

Isolates	Concentrations Spores/ml.	% Mortality of nymph after 3 days	5 Days	10 days	LT_{50}
Bb1	10^5	44.7a	48.7a	54 a	5.8
	10^7	46.8a	59.3b	64 a	3.4
	10^9	53.4a	63b	89 b	3
LC_{50}		1.4×10^8	10^5	8.9×10^4	
Bb4	10^5	37.3a	41.7 a	47 a	10
	10^7	44.2 ab	47 ab	51 a	6.48
	10^9	47.7 b	59 b	80 b	3.45
LC_{50}		1.6×10^9	9.9×10^6	1.9×10^5	

Similar letters in the same column indicated to none significant differences.

al., 2006). Several studies have confirmed the effectiveness of *B.bassiana* in the control of white flies *B.tabaci* and has achieved good results and the screening process of fungal isolates to determine the characteristics of virulence is vital to the success of strategies to control white flies and other insects (lacy *et al.*, 2008, Faria and Wraight *et al.*, 2001, Cabanillas and Jones, 2009)

Effect of pesticides on nymphs and adult of whitefly

The results of this study table 3 showed that the pesticides Spinosad and Thiomethoxam had a high toxicity against nymphs and adults of white flies on cucumber plant. Spinosad showed high efficiency in reducing the population density of nymphs (74 and 81%) after one and seven days for half field dose and 76.7 and 86% for field dose, efficiency of reducing adult population was 65.4% and 76.2% after one and seven days respectively at half field dose. Thiomethoxam was achieved Relative efficacy 81.9% and 81% in nymph control at half dose after one and seven days respectively, and reduction in adult numbers was 85.5 and 69%. The proposals in the studies related to spinosad were differed, especially on the insect pests, including white flies. Ghosh (2014) found that spinosad (*Saccharopolyspora spinosa*) was the most effective in reducing population density of whiteflies. Atef *et al.*, (2017) scored in a study to assess the effect of spinosad, azadirachtin, *Beauveria bassiana* and *Metarhizium anisopliae* on the population density of the white fly *Bemisia tabaci*. High efficiency of spinosad in the control of whiteflies population on tomato compared to other biological factors. Reducing of whitefly population ranging from 50% to 94.61%. An evaluation of both Spinosad and Azadirachtin on eggs, nymphs and adult whitefly *Bemisia tabaci* in the laboratory and greenhouses showed that the pesticides had a significant effect in reducing fertility and hatching of eggs and the emergence of adults, in addition to large mortality in nymphs and adults, and Azadirachtin Outperforms Spinosad (Hail, *et al.*, 2018). Ochou and Martin (2003) suggested that spinosad could be used in management programs alone or combination with other pesticides. Prabhat and Poehling (2007) reported that spinosad caused heavy mortality in the three nymph stages of the whitefly and the first one was the most sensitive, and Neupane *et al.*, (2016) reported the use of Spinosad, (Tracer 45% SP) reduced the population of white flies by 72.96 %. In a study evaluating the effect of a group of biopesticides, including Spinosad on the whitefly (B and Q Bio-Types of B. tabaci), Kim *et al.*, (2011) found that the most effective pesticides in the whitefly of both types are Spinosad. Yin *et al.*, (2011) confirmed in a similar study to evaluate the effect of eight biopesticides on the whitefly *Bemisia tabaci* that the most effective pesticides in the whitefly are spinosad and Fipronil. The superiority of Spinosad over *Beauveria bassiana* BSA3 was recorded in the reduction of the population density of the white fly, where the proportion of reduction in the numbers of adults 50.61% and 38.55%, respectively (Anbaki, 2016). On the other hand, the speed of the decline of the activity of this pesticide under field conditions and the decrease in the mortality of immature stages and the lack its effectiveness in the recommended doses against white flies (Aslam *et al.*, 2003), as recorded by Ulaganthan and Gupta (2004) spinosad failure in the control of sucking insects on cotton. Ramesh and Ukey (2006) recorded scored the

Table 3: Relative efficiency of Spinosad and Thiamethoxam against adults and nymphs of *B.tabaci*.

The pesticides	The dose	Relative Efficiency of reducing nymphs population		Relative Efficiency of reducing adults population	
		One day later	7 days later	One day later	7 days later
Thiamethoxam	0.25 ml / liter	81.9ab	81a	85.5ab	69 a
	0.5 ml / liter	89.7 a	83.3a	92.3a	71 a
Spinosad	0.25 ml / liter	74b	81 a	76.2 b	65.4 a
	0.5 ml. / l	76.7 b	86 a	84.6 ab	68.3 a

Similar letters in the column indicate no significant difference.

Table 4: Compatibility of Insecticides Spinosad and Thiomethoxam with *B. basiana* based on equation of Alves *et al.* (2007).

Treatments	Dose	Vegetative Growth (cm)	Reduction (%)	Conidial Production x10^7	Reduction (%)	Conidial Germi-nation	Reduction (%)	Biological Index	Classification
TH	0.25 ml / liter	3.7	55.4	6.5	7.14	79	19	68.7	Compatible
	0.5 ml / liter	3.3	60.24	3.5	50	74	24	47.9	Moderately toxic
SP	0. 25ml / liter	2.7	67.46	3	57	83	14	42.13	Moderately toxic
	0.5 ml / liter	2.4	71.1	1	85.7	78	19	27.75	Toxic
Control	-	8.3	-	7	-	96	-		

Less than 42: toxic, 42-60: Moderately toxic, greater than 60: compatible.

effectiveness of the pesticide against white flies on tomato.

Regarding to thiamethoxam pesticide Wafaa 2011 recorded excellence and superiority over acetamiprid, imidacloprid in the control of white flies on cucumbers in summer and autumn in Saudi Arabia, it was achieved reduction in the number of adults and nymphs in the summer, 87.5 and 82.4% respectively for spring season after three days of treatment and 84.7 And 82.1% for autumn agriculture. Kumar *et al.*, (2017) appeared that thiamethoxam 25 WG was the most effective in reducing the population density of white flies on eggplant followed by imidacloprid 17.8 SL (thiamethoxam> imidacloprid> acephate> fipronil> thiacloprid> dimethoate) and therefore there was increased in crop yield. This excellence in the effectiveness of thiamethoxam has been recorded in several studies Bharati *et al.*, 2015; Ghosal and Chatterjee, 2013; Shaikh and Patel, 2012 and Yadav and Kumawat, 2014).

Effect of pesticides on the growth of *Beauveria bassiana*

The effect of Spinosad and Thiomethoxam at field and half field dose on some biological properties of *B. bassiana* was explained in table 4. In the field dose, Spinosad recorded 2.4 cm growth diameter compared with 8.3 cm in control, reduction rate was 71.1% decreased to 67.4% in half field dose. The half field dose of Thiomethoxam caused 55.4% reduction in the fungal growth.

Effect of pesticides on the spore's production was achieved by the recommended field dose of Spinosad 85.7% (10^7 spores / ml) and decreased to 57% at half the dose (3×1^7 spores / ml). The pesticide Thiomethoxam gave reduction in the spore's production by 7.14% (6.57×10^7 spore / ml) at half field dose and increased to 50% in the field dose compared with control (7×10^7 spores / ml). When measuring the germination percentage, the study showed that the greatest reduction was in the field dose of the pesticide Thiomethoxam 24% and less reduction in half field dose for both pesticides 19%. The highest biological index (68.7) was for half field dose of Thiomethoxam that confirm its compatibility with fungus and was moderated effect at field dose. Spinosad was recorded toxic effect and Incompatible with fungus at field dose biological index (27.6). When dose is halved, biological index was indicated to moderate effect (42.13).

Knowledge of the effect of compatibility between pathogenic fungi and pesticides used in crop protection is essential in integrated pest management programs (Todorova *et al.*, 1998). In his evaluation of pesticides imidaclopride, flufenoxuron, teflubenzuron + phuzalon, endosulfane amitraz Alizadeh *et al.*, (2007) found compatibility of imidaclopride with *B. bassiana* (isolate DEBI008) and recommended to use it in integrated control programs. Another study was conducted to determine the compatibility of some pesticides, including Thiamethoxam with some insect pathogens, including *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces farinosus*, *Verticillium lecanii* and *Bacillus thuringiensis*. It was compatible with most entomopathogens and did not affect *Beauveria bassiana* and *Metarhizium anisopliae* (Antonio, *et al.*, 2001). De Oliveira *et al.*, (2003) indicated in a study to evaluate the effect of a group of pesticides Thiamethoxam, Cyfluthrin, Deltamethrin, Cypermethrin, Triazophos, Chlorpyrifos, Fenpropathrin and Endosulfan on the growth of *Beauveria bassiana* fungus (CG 425 strain) and production of spores indicated that Thiamethoxam and Cyfluthrin had the least effect on spore production. Thiamethoxam did not affect the growth of the fungus in low concentrations indicated that it is compatible with the fungus and can be used with the fungus to control pests. In a similar study, De Oliveira and Neves (2004) evaluated the compatibility of 12 miticides with the fungus *B. bassiana*. Amutha *et al.*, (2010) reported in a study to evaluate the effect of a group of pesticides on the vegetative growth of the fungus *Beauveria bassiana* that profenofos, indoxacarb and methyldemeton were highly toxic to the fungus while acetamprid and spinosad showed little toxic effect on the fungus and could be used with fungus in pest control.

Integrated effect of pesticides and fungi isolates on nymphs of white flies on cucumber

The results of using various combinations of pesticides and fungi table 5 indicated that the highest efficiency was recorded after five days of the combination SP+Bb (89.35%) and it was not significantly different except from isolate Bb4 (68.7%) and after ten days superiority of the above combination was continued With a significant difference from all treatments except SP pesticide where the two treatments achieved a relative efficiency of 82%. Whiteflies have a high resistance to pesticides, showing resistance to more than 40 active pesticide substances, and one of the alternative tests for effective control of insect pests known for resistance to pesticides is the use of synergistic mechanism or strengthening between pesticides. The combination of pesticides, low doses and entomopathogens can act synergistically, increasing insect mortality.

This combination is always beneficial because it reduces the doses of pesticides used and thus reduces environmental pollution as well as reduces the resistance of insect pests (Akbar *et al*., 2012)). Several experiments were conducted to investigate the effect of insecticide on entomopathogenic fungi. Karnatak, (2007) tested the effect of insecticides on fungal growth and the results showed that Imidacloprid gave the lowest inhibitory effect (11.1%) followed by deltamethrin (36.7%). In the United Kingdom, fungi and nematodes were used with some chemical pesticides to control white fly *Bemisia tabaci* (Cuthbertson *et al.,* 2007, Cuthbertson and Walters, 2005). Labbe *et al.,* (2009) indicated the compatibility between *Beauveria bassiana*, *Encarsia formosa* and *Dicyphus hesperus* on whiteflies in a study in greenhouses and that the combination of fungus, predator and parsitoid increases the reduction in the population density of the white fly and the fungus did not adversely affect On the parasitoid efficiency but a kind of synergy and increased its efficiency by parasitizing the fly, while no such synergies between the fungus and the predator. Mascarin *et al.,* (2014) in a study to assess the effect of the combination of Trisiloxane with the sporadic suspension of *Beauveria bassiana* (Balsamo) Vuillemin (strain CG1229) and *Isaria fumosorosea* Wise (strain CG1228) on *Bemisia tabaci* (Gennadi). It achieved a synergistic effect with both fungi on the whitefly and achieved the highest mortality rate early in the first nymph stage and lately in the second and third stages. Alizadeh *et al*., (2007) tested the effect of doses of imdocloprid, which is from the same group of Thiamethoxam, half field dose, field dose and double field dose were used to determine its effect on *Beauvria*. The results showed that the lower dose gave a weak inhibition less than 5%.

James and Elzen (2001) noted the compatibility between *Beauveria bassiana* and Imidacloprid in the control of the whitefly *Bemisia argentifolii* and stated that the combination of the fungus and the pesticide has enhanced the efficiency of the fungus in the control of the fly.

Table 5: Relative efficiency of spinosad, thiamethoxam, *B. bassiana* and their combinations against the white fly nymphs on cucumber.

Treatments	Relative efficiency after		
	5 days	10 days	15 days
SP	84.8 a	82 a	67 b
TH	83a	49.5 c	46 c
Bb1	78.4 ab	60.2 b	51.5 c
Bb4	68.7b	60 b	33 d
SP + Bb1	89.35 a	82 a	68 b
SP + Bb4	88.12 a	62.4 b	51.5 c
TH + Bb1	88a	68 b	77 a
TH + Bb4	82.4a	66 b	60 b

Similar letters in the column indicate no significant difference.

Table 6: Synergy bioassay of spinosad and thiomethoxam with *B. bassiana* Bb1 against whitefly nymphs.

X^2	The percentage of mortality				Treatment and concentration	
	observed	expected	SP	Bb1	SP	Bb1
0.54	89.35	96.6	84.8	78.4	0.25 ml./l	10^9
	observed	expected	TH	Bb1	TH	Bb1
0.72	88	96.3	83	78.4	0.25 ml./l	10^9

The value of the Chi square Which is greater than 3.83 at a degree of freedom 1, mean that the factors are synergistic.

Table 7: Synergy bioassay of spinosad and thiomethoxam with *B. bassiana* Bb4 against whitefly nymphs.

X^2	The percentage of death				Treatment and concentration	
	observed	expected	SP	Bb4	SP	Bb4
0.57	88.12	95.35	84.8	68.7		
	observed	expected	TH	Bb4	TH	Bb4
1.6	82.4	94.7	83	68.7	0.25 ml./l	10^9

The value of the Chi square which is greater than 3.83 at a degree of freedom 1 mean that the factors are synergistic.

Synergistic effect

The synergistic between pesticides and isolates of the pathogenic fungus *B.bassiana* (Table 6 and 7) showed that there was no synergy and significant increase in the effectiveness when combining both agents and that their mixing does not exceed the additive status. As an alternative option to manage resistant adult *Listronotus maculicollis*, combinations of the pyrethroid insecticide bifenthrin was done with an emulsifiable oil formulation of the entomopathogenic fungus *Beauveria bassiana* strain GHA (Bb ES). Combinations synergistically enhanced mortality in both insecticide-susceptible and insecticide-resistant L. *maculicollis* adults in the laboratory when bifenthrin was used at LC50s for each population (Wu and Koppenhöfer. 2017). When one-tenth the label dosage of *B. bassiana* and inert carrier oil was combined with neonicotinoids applied in 1 ml water, there were significant increases in *Listronotus maculicollis* mortality over the neonicotinoids alone 1 d after treatment. There was a significant increase (38%) with clothianidin in weevil mortality over clothianidin alone 1 d after treatment *(Clavet et al.,* 2013).

Dayakar *et al.,* (2000) have found that the combination of insecticides with *B. bassiana* and *M. anisopliae* showed 1.05–1.24 and 1.19–1.42 fold increase in virulence over the sole treatment, respectively. Quintela and McCoy (1997) have found that *B. bassiana* and *M. anisopliae* combined with sublethal doses of imidacloprid as a contact or oral treatment increased the mortality synergistically in *Diaprepes abbreviatus*., Thus, the combination of insecticide and entomogenous fungi was more deleterious to the insect than application of insecticides or entomogenous fungi alone.

Evaluate the efficiency of combining *Beauveria bassiana* with certain insecticides (emamectin benzoate, methomyl and *Bacillus thuringiensis*) in tomato field revealed that mixing increases the efficiency of their insecticides. When treated larvae with mixture of fungi (*B. bassiana*) and insecticides percentages mortalities of second and fourth larval instar of emamectin benzoate + *B. bassiana* and methomyl + *B. bassiana* had the highest values after 24hrs. then decreasing gradually, but the highest values of percentage mortality of second and fourth larval instar when mixtures of *Bacillus thuringiensis* + *B. bassiana* were after fifth days at concentrate of insecticide 1/2RC and after third day at concentrate of insecticide 1/4RC. Abdel Aziz *et al.*, (2018).

References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18:** 265–267.

Abdel Aziz, M.F., A.A. Ebrahim and A.R. El-Gabaly (2018). Combined Effect of Entomopathogenic Fungi, *Beauveria bassiana* and Certain Insecticides for the Control of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under Semi-Field Conditions. *J. Plant Prot. and Path*., Mansoura Univ., **9(9):** 573 - 576.

Abdel-Baky, N.F., S. Arafat, Nehal and A.H. Abdel-Salam (1998) Three *Cladosporium* spp. as promising biological control candidates for controlling whiteflies (*Bemisia* spp.) in Egypt. *Pak. J. Biological Sci*., **1:** 188-195.

Akbar, S., S. Freed, A. Hameed, H.T. Gul, M. Akmal, M.N. Malik, M. Naeem and M.B. Khan (2012). Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. *African Journal of Microbiology Research*, **6(17):** 3956-3962.

Al-anbaki Hussain, A.M. (2016). Evaluate relative mortality some pesticides and bio-chemicals to reduce the population density of whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) in beans and cucumber. *Diyala Journal of Agricultural Sciences*, **8(2):** 41-49.

Al-Deghairi, M.A. (2008). Bioassay evaluation of the entomopathogenic fungi, *Beauveria bassaina* Vuellemin against eggs and nymphs of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). *Pak. J. Biol. Sci.*, **11(12):**1551-60.

Alizadeh, A., M.A. Samih, M. Khezri and R.S. Riseh (2007). Compatibility of *Beauveria bassiana* (Bals.) Vuill. Eith several insecticides. *International Journal of Agriculture & Biology*, **9(1):** 31-34.

Alves, S.B., M.L. Haddad, M. Faion, G.C. de Baptista and L.S. Rossi-Zalaf (2007). Novo ýndice biologico para classificacao da toxicidade de agrotoxicos para fungos entomopatogenicos. Brasýlia: Anais do X Siconbiol

Alves, S.B., A. Jr. Monino and J.E.M. Almeida (1998). Produtosfitossanitários eentomopatogênicos. Sâo Paulo: Fealq.

Amutha, M., G.G. Gulsar Banu, T. Surulivelu and N. Gopalakrishnan (2010). Effect of commonly used insecticides on the growth of white Muscardine fungus, *Beauveria bassiana* under laboratory Conditions. *Journal of Biopesticides*, **3 :** 143-146.

Antonio B.F., E.M.A. José and L. Clóvis (2001). Effect of Thiamethoxam on Entomopathogenic *Microorganisms. Neotrop. Entomol.*, **30(3)**.

Atef, S.A., M.A. Nesreen, D. Khaled and M. Abdelhameed (2017). An evaluation of some eco-friendly biopesticides against *Bemisia tabaci* on two greenhouse tomato varieties in Egypt. *Journal of Plant Protection Research*, **57 (1):** 9-17.

Bharati, M.S., S.S. Shetgar and C.G. Sawant (2015). Bio-efficacy of different insecticides against brinjal jassid (*Amrasca biguttula*) and whitefly (*Bemisia tabaci*). *Journal of the Entomological Research*, **39(4):** 369-372.

Bugti, G.A., W. Bin, C. Na and L.H. Feng (2018). Pathogenicity of *Beauveria bassiana* strain 202 against sap sucking insect pests. *Plant Protect. Sci*., **54:** 111–117.

Cabanillas, H.E. and A.J. Walker (2009). Pathogenicity of *Isaria* sp. (Hypocreales: Clavicipitaceae) against the sweet potato whitefly B biotype, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Crop Protection*, **28:** 333–337.

Clavet, C., E. Hampton, M. Requintina and S.R. Alm (2013). Laboratory assessment of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) strain GHA for control of *Listronotus maculicollis* (Coleoptera: Curculionidae) adults. *J. Econ. Entomol.*, **106(6):** 2322-6.

Cuthbertson, A.G.S. and K.F.A. Walters (2005). Pathogenicity of the entomopathogenic fungus, *Lecanicillium muscarium*, against the sweet potato whitefly *Bemisia tabaci*, under laboratory and glasshouse conditions. *Mycopathologia*, **160(4):** 315-319.

Cuthbertson, A.G.S., J.J. Mathers, P. Northing, W.Q. Luo and K.F.A. Walters (2007). The susceptibility of immature stages of *Bemisia tabaci* to infection by the entomopathogenic nematode Steinernema carpocapsae. *Russian Journal of Nematology*, **15(2):**153-156.

Dayakar, S., K.R. Kanaujia and R.R.S. Rathore (2000). Copmatibility of entomogenous fungi with commonly used insecticides for management of *Spodoptera litura* (Fab.). In: Microbials in Insect Pest Management. (Eds.):

S. Ignacimuthu and A. Sen. Oxford and IBH Publishing Co. Pvt. Ltd, M. Delhi, Kolkata, 47-52.

Dayakar, K.R., Kanaujia and R.R.S. Rathore (2000). Compatibility of entomogenous fungi with commonly used insecticides for the management of *Spodoptera litura* (Fab.) S. Ignacimuthu, A. Sen (Eds.), Microbials in Insect Pest Management, Oxford and IBH Publishing Co. Pvt. Ltd, M. Delhi, Kolkata, 47-52.

De la Rosa, W., F.L. Lopez and P. Liedo (2002). Beauveria bassiana as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. *Jour. E. Entomology*, **95:** 36-43.

De Oliveira, R.C. and P.M.O.J. Neves (2004). Biological control compatibility of *Beauveria bassiana* with acaricides. *Neotropical Entomol.*, **33:** 353–8.

Duarte, A., J.M. Menendez and N. Trigueiro (1992). Estudio preliminar sobre la compatibilidad de *Metarhizium anisopliae* com algunos plaguicidas quimicos. *Revista Baracoa*, **22:** 31-39.

Faria, M. and S.P. Wraight (2001). Biological control of *Bemisia tabaci* with fungi. *Crop Prot.*, **20:** 767–778.

Garcia, A.S., C.L. Messias, H.M.L. De Souza and A.E. Piedramuena (1984). Patogenicidade de *Metarhizium anisopliae* var. anisopliae *Ceratitis capitata* (Wied) (Diptera, Tephritidae). *Revi. Bra. D. Entomologia*, **28:** 421-424.

Ghosal, A. and M.L. Chatterjee (2013). Bioefficacy of imidacloprid 17.8 SL against whitefly, *Bemisia tabaci* (Gennadius) in brinjal. *The Journal of Plant Protection Science*, **5(1):** 37-41.

Ghosh, S.K. (2014). Incidence of whitefly (*Bemisia tabaci* Gennadious) and their sustainable management by using pesticides. In: G. Rahmann and U. Aksoy (eds.), Proceedings of the Fourth ISOFAR Scientific Conferences. Building Organic Bridges, the Organic World Congress. pp. 623626. (e print ID 23235). 13-15.

Gupta, P., M.S. Paul and S.N. Sharma (1999). Studies on compatibility of white muscardine fungus *Beauveria bassinia* with neem products. *Indian Phytopathol.*, **52(3):** 278-280.

Hail, K.S., S.A. Malak and L.C. John (2018). Responses of the Whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) to Biologically Based Insecticides. *American Journal of Entomology*, **2(3):** 28-35.

James, R. and G.W. Elzen (2001). Antagonism between *Beauveria bassiana* and Imidacloprid when combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) control. *J. Econ. Entomol.*, **94(2):** 57- 61.

Karnataka (2007). Effect of Agrochemicals on Growth and Sporulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin. *J. Agric. Sci.*, **20(2):** 410-413.

Kim, S.I., S.H. Chae, H.S. Youn, S.H. Yeon and Y.J. Ahn (2011). Contact and fumigant toxicity of plant essential oils and efficacy of spray formulations containing the oils against B-and Q-biotypes of *Bemisia tabaci*. *Pest Management Science*, **67(9):** 1093–1099.

Kumar, V., G.K. Grewal and A.K. Dhawan (2017). Relative toxicity of newly introduced insecticides against sucking insect pests of transgenic Bt cotton. *Pesict. Res. J.*, **28:** 108-12.

Labbe, R.M., D.R. Gillespie, C. Cloutier and J. Brodeur (2009). Compatibility of an entomopathogenic fungus with a predator and a parasitoid in the biological control of greenhouse whitefly. *Biocontrol Sci. Technol.*, **19:** 429–446.

Lacey, L.A., S.P. Wright and A.A. Kirk (2008). Entomopathogenic fungi for control of *Bemisia tabaci* biotype B: foreign exploration, research and implementation. In: J. Gould, K. Hoelmer and J. Goolsby (Eds.), Classical Biological Control of *Bemisia tabaci* in the United States-A Review of Interagency Research and Implementation. *Progress in Biological Control*, **4:** 33–69.

Lacey, L.A. and M.S. Goettel (1995). Current development in microbial control of insect and prospects for the early 21st century. *Entomophaga*, **40(1):** 3-27.

Malo, A.R. (1993). Estudio sobre la compatibilidad del hongo Beauveria bassaina (Bals.) Vuill. con formulaciones comerciales de fungicidas e insecticidas. *Revista Colombiana de Entomologia*, **19:** 151-158.

Mascarin, G.M., N.N. Kobori, E.D. Quintela, S.P. Arthurs and I.D. Ju´nior (2014). Toxicity of non-ionic surfactants and interactions with fungal entomopathogens toward *Bemisia tabaci* biotype. *B. BioControl*, **59:**111–123.

Meeks, E.T.M., S. van Voorst, N.N. Joosten, J.J. Fransen and J.C. van Lenteren (2002). Persistence of the fungal whitefly pathogen, *Aschersonia aleyrodes*, on three different plant species. *Mycol. Res.*, **104:** 1234-1240.

Moorhouse, E.R., A.K. Charnley and A.T. Gillespie (1992). Review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). *Ann. Appl. Biol.*, **121(2):** 431-454.

Neupane, S., S. Subedi, R.B. Thapa, Yubak, G.C. Dhoj, S. Pokhrel, P.R. Regmi and J. Shrestha (2016). Efficacy of Biorational Compounds against Whitefly (*Bemisia tabaci* Genn.) on Blackgram (*Vigna mungo* L.). *Journal of AgriSearch*, **3(1):** 34-39.

Ochou, O.G. and T. Martin (2003). Activity spectrum of spinosad and indoxacarb: rationale for innovative pyrethroid resistance management strategy in west Africa. *Resistant Pest Management Newsletter*, **12(2):** 75- 81.

Petlamul, W. and P. Prasertsan (2012). Evaluation of strains of Metarhizium Anisopliae and Beauveria bassiana against Spodoptera Litura on the basis of their virulence, germination rate, conidia production, radial growth and enzyme activity. *Mycobiology*, **40:** 111-116.

Prabhat, K. and H.M. Poehling (2007). "Effects of Azadirachtin, Abamectin, and Spinosad on Sweetpotato Whitefly

(Homoptera: Aleyrodidae) on Tomato Plants Under Laboratory and Greenhouse Conditions in the Humid Tropics". *Journal of Economic Entomology,* **100(2):** 411 – 420.

Purwar, J.P. and G.C. Sachan (2006). Synergistic effect of entomogenous fungi on some insecticides against Bihar hairy caterpillar *Spilarctia oblique* (Lepidoptera: Arctiidae) *Microbiol. Res*., **161(1):** 38-42.

Quintela, E.D. and C.W. McCoy (1998). Synergistic effect of imidacloprid and two entomogenous fungi on behaviour and survival of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in soil. *J. Econ. Entomol*., **91(1):** 110-122.

Quintela, E.D. and C.W. McCoy (1997). Pathogenicity enhancement of *M. anisopliae* and *B. bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid. *Environ. Entomol.,* **26:** 1173–1182.

Rachappa, V., S. Lingappa and R.K. Patil (2007). Effect of agrochemicals on growth and sporulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin. *Karnataka J. Agri. Sci*., **20(2):** 410-413.

Ramesh, R. and S.P. Ukey (2006). Bio-efficacy of ecofriendly insecticides against tomato jassids and whitefly. *J. Plant Protec. Environ*., **3(1):** 122-126.

Sain, S.K., D. Monga, R. Kumar, D.T. Nagrale, S. Kranthi and K.R. Kranthi (2019). Comparative effectiveness of bioassay methods in identifying the most virulent entomopathogenic fungal strains to control *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Egyptian Journal of Biological Pest Control,* **29:** 31.

Salama, H.S., M.S. Foda, F.N. Zaki, and S. Moawad (1984). Potency of combinations of *Bacillus thuringiensis* and chemical insecticides on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J. Econ. Entomol*., **77:** 885-890.

Shah, P.A. and M.S. Goettel (1999). Directory of microbial control products and services. Division on Microbial control, society for invertebrate pathology, Ginesville, USA.

Shaikh, AA. and J.J. Patel (2012). Bio-efficacy of insecticides against sucking pests in brinjal. AGRES-*An International eJournal*., **1(4):** 423-434.

Todorove, S.I., D. Coderre, R.M. Duchesne and J.C. Côté (1998). Compatibility of *Beauveria bassiana* with selected fungicide and herbicides. *Environ. Entomol*., **27:** 427–33.

Ulaganathan and G.P. Gupta (2004). Effect of Insecticidal Spray Schedules on Sucking Pests of American Cotton, *Gossypium hirsutum* L. *Agnnals of Plant Protection Sciences*, **12:** 279-282.

Wafaa, A. and AL-Kherb (2011). Field Efficacy of Some Neonicotinoid Insecticides on Whitefly *Bemicia tabaci* (Homoptera: Aleyrodidae) and its Natural Enemies in Cucumber and Tomato Plants in Al-qassim Region, KSA. *Journal of Entomology*, **8(5):** 429-439.

Wu, S., O.S. Kostromytska and A.M. Koppenhöfer (2017). Synergistic Combinations of a Pyrethroid Insecticide and an Emulsifiable Oil Formulation of *Beauveria bassiana* to Overcome Insecticide Resistance in *Listronotus maculicollis* (Coleoptera: Curculionidae). *J. Econ. Entomol*., **1;110(4):**1794-1802.

Yadav, S.R. and K.C. Kumawat (2014). Efficacy of chemical and bioinsecticides against major insect pests of brinjal Solanum melongena Linn. *Pesticide Research Journal*, **6(2):**128-135.

Yin, F., X. Feng, D. Zhang, Q. Lin, Z. Hu, Z. Li and H. Chen (2011). Toxicities of 8 insecticides to Bemisia tabaci (Gennadius). *Guangdong Agricultural Sciences*, **18:** 025.

Zafar, J., S. Freed, B.A. Khan and M. Farooq (2016). Effectiveness of *Beauveria bassiana* Against Cotton Whitefly, *Bemisia tabaci* (Gennadius) (Aleyrodidae: Homoptera) on Different Host Plants. *Pakistan J. Zool*., **48(1):** 91-99.