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The side effect of commonly used chemical pesticides on entomopathogenic *Beauveria bassiana* and *Bacillus thuringiensis* as biopesticides

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Abstract:

Biopesticides are considered to be the best alternative to chemical pesticides that are highly effective, target specific and reduce environmental risks. Compatibility of bio and chemical pesticides is very important for effective pest management. The aim of this work is to study the side effect of used pesticides on the viability of spores (active ingredient) in biopesticides. The tolerance of *Beauveria bassiana* and *Bacillus thuringiensis* to eleven commonly used pesticides as indoxacarb, dimethoate, chlorfenapyre, abamectin, lambda-cyhalothrin, chlorpyrifos, deltamethrin, profenofos, cypermethrin, methomyl and hexaflumuron were tested in the laboratory. The results indicated that chlorfenapyre and abamectin were more compatible with *B. bassiana*. Also, there were not any side effect between them and the fungus. Also, when used the mixtures of *B. bassiana* and chlorfenapyre or abamectin to control the red spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae) were more toxic than use them individually. On the other hand, chlorfenapyre, cypermethrin and methomyl had not any inhibition effect to *B. thuringiensis* compared with other compounds. The obtained results may be useful in the development of Integrated Pest Management strategies using beneficial control agents like microorganisms with chemical pesticides.

Introduction

Biopesticides, in particular when accomplished by entomopathogens, is a method that should be considered as an important factor in Integrated Pest Management (IPM) programs for pest population density reduction (Amutha *et al.*, 2010). The biopesticides, especially *Beauveria bassiana* and *Bacillus thuringiensis* are important natural control agents against many insect pests. Many species of fungi, approximately 1,200 species are described as insect pathogens. From these, seven species are used to control plant pest insects in augmentative biopesticide (Van Lenteren *et al.*, 2017). More than 200 insect species from nine orders of insects, especially Lepidoptera and Coleoptera have been recorded as hosts of the entomopathogenic fungus, *B. bassiana* (De la Rosa *et al*., 2000). *B. bassiana* spores are found naturally in some plants and soils and are regarded as safe biopesticide (Uma Devi *et al*., 2008).

Also, *B. thuringiensis* (*Bt*) is a microbe naturally found in soil. It makes proteins that are toxic to insects. There are many types of Bt. each type targets different group from insects. These insect groups include beetles, mosquitoes, black flies, caterpillars, and moths. There are about one hundred and eighty successful commercial products from Bt in the world (De Maagd *et al*., 2003; Schnepf *et al*., 1998 and Van Franken huyzen, 2009).

The compatibility between microbial pesticides and chemical pesticides requires more studies. The collected data from such studies would enable farmers to select appropriate compounds and schedule microbial and chemical pesticide treatments so that benefits from compatible sets can be accrued and with noncompatible pairs, the deleterious effect of the chemical on the microbe in the biopesticide can be minimized (Butt *et al*., 2001; Inglis *et al*., 2001 and Lacey *et al*., 2001). A microbial pesticide compatible with a commonly used chemical pesticide can be used simultaneously or sequentially with it. To harness the benefits of entomopathogens their compatibility with insecticides becomes decisive for combined use, while the potential inhibitory effects of insecticides on the entomopathogenic fungus or bacteria cannot be ignored. Using incompatible insecticides may suppression the development and reproduction of these pathogens, affecting IPM. IPM programs it is essential to know the influence of compatibility between entomopathogenic fungi or bacteria and pesticides used in crop protection. If *B. bassiana* or *B. thuringiensis* has to be incorporated into a pest management program it is essential to determine the effects of pesticides on it.

It is important to study the side effects of pesticides on beneficial microorganisms for IPM. It is known that pesticides can have different effects on growth, sporulation and pathogenicity of entomopathogens (Tkaczuk, 2001; Rashid *et al*., 2010; Hernandez *et al*., 2012; Tkaczuk *et al*., 2012, 2015 and Pelizza *et al*., 2015). Using biopesticides compatible with classic chemical pesticides is recommended in integrated pest management (IPM) as an effective and environmental sound strategy.

The red spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae) is causing great damage to different agricultural crops. Both chemical and biocontrol must start at low densities for effective control of this pest (Blindeman and Van Labeke ,2003). The main objective of this study is to evaluate the side effects of some common pesticides on the growth and sporulation of entomopathogens, *B. bassiana* and *B. thuringiensis* as well as to the compatibility between them in IPM programs.

Materials and methods

The present study was conducted at Plant Protection Research Institute, Agriculture Research Center, Egypt. Experiments were carried out under laboratory conditions.

1.Tested pesticides:

Eleven commonly used pesticides in Egypt were obtained from Central Laboratory for Pesticides, Agriculture Research Center, Egypt (Table, 1). The side effect of these chemical pesticides was tested on the viability of active ingredient of biopesticides Biovar and Dipel (*B. bassiana* and *B. thuringiensis*, respectively).

2.Tested biopesticides:

B. bassiana and *B. thuringiensis* strains which are used in pesticide formulations Dipel DF and Biovar were used in this study. These strains were obtained from the Plant Protection Research Institute, Agriculture Research Center, Egypt. These strains were refreshed by re-grown on culture media as Potato Dextrose Agar (PDA) for *B. bassiana* and Nutrient Agar (NA) for *B. thuringiensis*.

Table (1): The chemical pesticides which were tested

Trade name	Active ingredient	Field recommended dose
Camfal EC 15%	Indoxacarb	25 ml / 100 L
Dematox EC 40%	Dimethoate	150 ml / 100 L
Macet SC 10%	Abamectin	15 ml / 100 L
Challenger super SC 24%	Chlorfenapyre	60 ml / 100 L
Lamdathrin EC 5%	Lambda-cyhalothrin	20 ml / 100 L
Kafrothrin EC 2.5 %	Deltamethrin	350 ml/fd
Super-methrin EC 25 %	Cypermethrin	600 ml / fd
Helban EC 48%	Chlorpyrifos	1L/fd
Aktacron EC 72%	Profenofos	750ml / fd
Newmel SP 90%	Methomyl	300 gm/ fd
CamEron EC 10%	Hexaflumuron	200ml/ fd

3. Sensitivity of *Beauveria bassiana* to selected pesticides:

The Potato Dextrose Agar medium (PDA) was autoclaved at 121°C for 15 min. When the medium reached 50–60°C precisely measured doses of pesticides were added and thoroughly mixed. The treated medium was poured into Petri dishes and allowed to solidify. An agar disc of 10 days old colony of *B. bassiana* was cut by 6 mm diameter cork borer and transferred into the center of the treated PDA plates. Also, agar disc of *B. bassiana* grown on medium without pesticides as the control. Then Petri dishes were incubated at 25°C. Colony growth was observed every day until 14 days of culturing by measuring the colony diameter. Each treatment was performed in four replications. The results were recorded as the percentage growth inhibition of *B. bassiana* by insecticide-treated PDA (Hokkanen and Kotiluoto, 1992).

$$X = \frac{Y - Z}{Y} \times 100$$

Where: X = Growth inhibition percentage
Y= Growth diameter in control
Z = Growth diameter in treatment

4. Sensitivity of *Bacillus thuringiensis* to selected pesticides:

The sensitivity of *B. thuringiensis* to selected pesticides was measured by plate diffusion method, according to Collins and Lyne (1985) with cultures grown to logarithmic growth phase in nutrient broth medium (NB) for *B. thuringiensis.* Bacterial suspension (2 ml) was mixed carefully with 20 ml of nutrient agar medium in Petri dishes. Wells (8 mm diameter) were punched in the agar using the stainless-steel borer and were filled with 0.1 ml of the selected pesticides concentration. The plates were incubated for 48 hrs at 28 oC and the diameter of resulting clear zones of inhibition was measured using three replicates according to Toda *et al.* (1989).

5. Compatibility test:

The compatibility of the chemical pesticide which has not any side effect on *B. bassiana* and *B. thuringiensis* was tested in vitro against red spider mites (*T. urticae*). Four different treatments were conducted to test their efficacy against the red spider mite, *T. urticae* under laboratory conditions as leaf disk dip bioassay method as following: a) recommended field dose of the chemical pesticide only; b) recommended field dose of chemical pesticide with *B. bassiana* (1×10^8 conidia/ml); **c)** *B. bassiana* (1×10^8 conidia/ml) only; d) control, without any compounds. Mortality was recorded daily until the death of all the tested organisms (Ullah and Lim, 2015 and Pree *et al*., 1989). The percentage of mortalities was corrected using Abbott's (1925) formula.

Results and discussion

Compatibility is the capacity to mix different pesticides without physical or chemical interactions or changes, leading to the enhancement of their biological effects. The sensitivity of *B. bassiana* and *B. thuringiensis* to the tested pesticide in this study showed in Table (2). The growth of *B. bassiana* was inhibited by all pesticides except indoxacarb, abamectin, chlorfenapyre, lambda-cyhalothrin, and methomyl. Abamectin and chlorofenapyr had not any inhibition of colony growth. Indoxacarb proved relatively slightly inhibition (33%), lambda-cyhalothrin and methomyl had moderately inhibition of colony growth were reached 56% and 50% respectively. Other pesticides were highly toxic of *B. bassiana* were reached to 100% inhibition of colony growth. On the other hand, *B. thuringiensis* was more sensitive to all tested pesticides except chlorofenapyr, cypermethrin, and methomyl.

The side effect of chemical pesticides on the entomopathogen (*B. bassiana* and *B. thuringiensis*) have been studied by Tkaczuk *et al*. (2015), Narkhede *et al*. (2017), Amutha *et al.* (2010) and Fiedler and Sosnowska (2017). In the present study abamectin and chlorfenapyre showed no toxic effect to *B. bassiana*. In earlier reports, abamectin was found to be compatible with *B. bassiana* at recommended field dose (Fiedler and Sosnowska, 2017).

Table 2. Sensitivity of *Beauveria bassiana* and *Bacillus thuringiensis* to the tested pesticides.

Pesticides	**Colony growth of *B. bassiana***				**Inhibition zone diameter (cm) of treated *B. thuringiensis* after 3 days**
	Growth diameter (mm)			**Inhibition percentage after 15 days**	
	4 days	**7 days**	**15 days**		
Indoxacarb	0	9	20	33	3.0
Dimethoate	0	0	0	100	2.2
Abamectin	12	17	30	00	1.7
Chlorfenapyre	14	18	30	00	0.0
Lambda-cyhalothrin	0	10	13	56	3.5
Deltamethrin	0	0	0	100	3.5
Cypermethrin	0	0	0	100	0.0
Chlorpyrifos	0	0	0	100	2.4
Profenofos	0	0	0	100	1.8
Methomyl	0	10	15	50	0.0
Hexaflumuron	0	0	0	100	4.0
Control	16	20	30	00	00

In the present study, among the tested pesticides relatively low inhibition of the fungus growth showed based on indoxacarb where the fungus did not grow until four days after treatment and started to grow after five days. But Amutha *et al.* (2010) were found that the indoxacarb was more toxic on the fungus growth until 14 days of treatment. Also, in this study, lambda-cyhalothrin and methomyl have moderately effect on mycelial growth where the growth was slow until 7 days after treatment compared with control but the fungus growth was increased after seven days and reached to about 50% compared with control after 14 days. In previous studies, lambda-cyhalothrin was found to be less toxic to *B. bassiana* (Tkaczuk *et al*., 2015). The compatibility of *B. thuringiensis* with 27 chemical insecticides was studied by Morris (1977). He found that methomyl was the most pesticide compatible with *B. thuringiensis*. This result is agreed with my study.

Compatibility between *B. bassiana* and chlorfenapyre or abamectin and their effect on the response of *T. urticae* when treated with the combination of them was investigated in vitro in this study as shown in Table (3). The combination of the mixture of *B. bassiana* and half recommended the dose of chlorfenapyre or abamectin had a synergistic effect on *T. urticae* resulting 100 % mortality after five days. These results as the same results when used recommended dose from chlorfenapyre or abamectin. The mortality percentage of *T. urticae* reached to 51% and 74% after five and seven days when treated by *B. bassiana*. Also, the half dose from chlorfenapyre and abamectin caused 59% and 60% mortality percentages after seven days of treatment. These results indicated that the application of the mixture of chlorfenapyre or abamectin (half recommended dose) with *B. bassiana* on *T. urticae* showed a higher mortality than when applied individually, illustrating, in this case, an additive effect.

Table (3): Mortality percentage of *Tetranychus urticae* when treated with combinations of *Beauveria bassiana* and chlorfenapyre or abamectin.

Treatments	Mortality percentage after application (Days)			
	1 day	3 days	5 days	7 days
Chlorfenapyr (recommended dose)	81	100	100	100
Chlorofenapyr (half dose)	32	49	56	59
Chlorofenapyr (half dose) + *B. bassiana*	35	78	100	100
Abamectin (recommended dose)	87	100	100	100
Abamectin (half dose)	40	48	51	60
Abamectin (half dose) +*B. bassiana*	43	92	100	100
***B. bassiana* (recommended dose)**	0	34	51	74

Chlorfenapyre and abamectin are acaricides which were evaluated by many authors against *T. urticae* (Abd El-Mageed *et al.*, 2013 and Tawfek and El- Gohary, 2013). They found that chlorfenapyre and abamectin were more toxic against *T. urticae* compared with other compounds and control. Also, many studies investigated the effect of *B. bassiana* on *T. urticae* (Islam *et al*., 2017 and Baruah *et al*., 2008). They reported that mortality percentage increased after 3 days from treatment by *B. bassiana*. More previous studies were used *B. bassiana* as biopesticides against many pests.

Using biopesticides compatible with classic chemical pesticides is recommended in IPM as an effective and environmental sound strategy. The application of the mixture of chlorfenapyre or abamectin with *B. bassiana* on *T. urticae* gave a higher mortality than when applied individually, illustrating, in this study, an additive effect. The compatibility in field trials or semi-field trials of *B. bassiana* with various chemical pesticides was tested by various authors (Shi and Feng, 2009 and Ansari and Sharma, 2005), thus making this fungus an interesting option to control mites in combination with other products. Laboratory studies were developed by Hernández *et al*. (2012) to evaluate the compatibility of flufenoxuron and azadirachtin with Beauveria bassiana against *T. urticae*. They found that the application of flufenoxuron with *B. bassiana* showed a clear synergy while the combination of azadirachtin and *B. bassiana* had an additive effect. Also, they mentioned that these combinations with *B. bassiana* could improve mite control by contributing to reducing the probability of resistance so often described in the literature. Mohan *et al.* (2007) studied the compatibility of azadirachtin A (AZA) and neem oil extract (0.15 % AZA) with thirty different isolates of *B. bassiana*. Of those studied twenty-three combinations were found to be compatible.

It is concluded that the mixture of biopesticides and chemical pesticides may have its implications on the use of a reduced quantity of chemical pesticides, which is a major cause of environmental pollution. In addition, due to the differential mode of action, this combination may also contribute to prolonging the generation of resistance in pest. Significantly, the cumulative strategy may improve the performance of integrated pest management programs.

References

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

Abd El-Mageed, A.E.M.; Tawfek, A.A. and Abohatad, E.E. (2013): Assessment of bioassay techniques and residual effect of certain acaricides against the two spotted spider mite *Tetranychus urticae* Koch and the predatory mite, *Phytioseiulus persimilis* Athias –Henriot. Acarines,7: 63-70.

Amutha, M.; Gulsar Banu, J.; Surulivelu, T. and Gopalakrishnan, N. (2010): Effect of commonly used insecticides on the growth of white muscardine fungus, *Beauveria bassiana* under laboratory conditions. Journal of Biopesticides, 3:143 – 146.

Ansari, M.M. and Sharma, A.N. (2005): Compatibility of *Bacillus thuringiensis* and *Beauveria bassiana* with new insecticides recommended for insect pest control in soybean. Pestology, 29(9):18–20.

Baruah, N.; Deka, A.C.; Kalita, M.C. and Kalita, J.C. (2008): Laboratory evaluation of entomopathogenic fungus, *Beauveria bassiana* on red spider mite (*Oligonychus coffeae* Nietner) a major pest of tea. The Bioscan, 3(3): 277-282.

Blindeman, L. and Van Labeke, M. (2003): Control of the two-spotted spider mite (*Tetranychus urticae* Koch) in glasshouse roses. Communications in agricultural and applied biological sciences, 68(4):249-254 ·

Butt, T. M.; Jackson, C. and Magan, N. (2001) : Fungal biological control agents-Appraisal and recommend dations. In: Butt, T.M.; Jackson, C. and Magan, N., Editors. Fungi as biocontrol agents. Wallingford: CABI Publishing., 377-384.

Collins, C. H. and Lyne, P. M. (1985): Microbiological methods. 5th ed. Butterworths, London, Toronto, 167-181.

De La Rosa, W.; Alatorre, R.; Barrera, J. F. and Toriello, C. (2000): Effect of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycetes) upon the coffee berry borer (Coleoptera: Scolytidae) under field conditions. Journal of Economic Entomology, 93: 1409–1414.

De Maagd, R. A. *et al*. (2003): Structure, diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria. Annu Rev Genet., 37: 409-433.

Fiedler, Ż. and Sosnowska, D. (2017): Side effects of fungicides and insecticides on entomopathogenic fungi in vitro. Journal of Plant Protection Research, 57:(4), 2017, DOI: 10.1515/jppr-2017-0048.

Hernández, M.M.; Martínez-Villar, E.; Peace, C.; Pérez-Moreno, I. and Marco, V. (2012): Compatibility of the entomopathogenic fungus *Beauveria bassiana* with flufenoxuron and azadirachtin against *Tetranychus urticae*. Experimental and Applied Acarology, 58 (4): 395–405. DOI: https://doi.org/10.1007/ s10493-012-9594-1

Hokkanen, H. M. T. and Kotiluoto, R. (1992): Bioassay of the side effects of pesticides on *Beauveria bassiana* and *Metarhizium anisopliae*: standardized sequential testing procedure. IOBC/WPRS Bull., XI (3):148-151.

Inglis, G. D.; Goettel, M. S.; Butt, T. M. and Strasser, H. (2001): Use of hyphomycetous fungi for managing insect pests. In: Butt, T.M.; Jackson, C. and Magan, N., Editors. Fungi as biocontrol agents. Wallingford: CABI Publishing., 23-69.

Islam, T.; Biswas, Md.J. H.; Howlader, M.T.H. and Ullah, M. S. (2017): Laboratory evaluation of *Beauveria bassiana*, some plant oils and insect growth regulators against two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Persian J. Acarol., 6 (3): 203 – 211.

Lacey, L. A.; Frutos, R.; Kaya, H. K. and Vail, P. (2001): Insect pathogens as biological control agents: Do they have a future? Biological Control, 21:230-248.

Mohan, M.C.; Reddy, N.P.; Devi, U.K.; Kongara, R. and Sharma, H.C. (2007): Growth and insect assays of *Beauveria bassiana* with neem to test their compatibility and synergism. Biocontrol Sci Technol, 17(10):1059–1069.

Morris, O. N. (1977): Compatibility of 27 chemical insecticides with *Bacillus thuringiensis* var. Kurstaki. Can. Enr., 109: 855-864.

Narkhede, C.P.; Patil, C.D.; Suryawanshi, R.K.; Koli, S.H.; Mohite, B.V. and Patil, S.V. (2017): Synergistic effect of certain insecticides combined with *Bacillus thuringiensis* on mosquito larvae. Journal of Entomological and Acarological Research, 49:6265, doi:10.4081/jear.2017.6265

Pelizza, S.A.; Scorsetti, A.C.; Fogel, M.N.; Pacheco-Marino, S.G.; Stenglein, S.A.; Cabello, M.N.; and Lange, C.E. (2015): Compatibility between entomopathogenic fungi and biorational insecticides in toxicity against *Ronderosia bergi* under laboratory conditions. BioControl, 60 (1): 81–91.

Pree, D.L.; Cole, K.J. and Fisher, P.A. (1989): Comparison of leaf disc and Petri dish assays for the assessment of dicofol resistance in populations of European red mite (Acari: Tetranychidae) from southern Ontario. Can. Entomol., 12:771-776.

Rashid, M.; Baghdadi, A.; Sheikhi, A.; Pourian, H.R. and Gazavi, M. (2010): Compatibility of *Metarhizium anisopliae* (Ascomycota: Hypocreales) with several insecticides. Journal of Plant Protection Research, 50 (1): 22–27. DOI: https://doi. org/10.2478/v10045-010-0004-6

Schnepf, E. *et al.* (1998): *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev., 62: 775-806.

Shi, W.B. and Feng, M.G. (2009): Effect of fungal infection on reproductive potential and survival time of *Tetranychus urticae* (Acari: Tetranychidae). Exp Appl Acarol., 48:229–237.

Tawfek, A.A. and El-Gohaly, L.R.A. (2013): Effect of some acaricide treatments on some biological aspects of the two spotted spider mite *Tetranychus*

Phytioseiulus persimilis Athias –Henriot. Acarines,7: 47-51.

Tkaczuk, C. (2001): Wpływ wybranych pestycydów stosowanych w ochronie sadów na wzrost grzybów owadobójczych. Biuletyn Naukowy, 12: 375–383.

Tkaczuk, C.; Harasimiuk, M.; Król, A. and Beres, P. K. (2015): The effect of selected pesticides on the growth of entomopathogenic fungi *Hirsutella nodulosa* and *Beauveria bassiana*. Journal of Ecological Engineering, 16: (3)177-183.

Tkaczuk, C.; Krzyczkowski, T.; Głuszczak, B. and Król, A. (2012): The influence of selected pesticides on the colony growth and conidial germination of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. Progress in Plant Protection, 52 (4): 969–974.

Toda, M.; Okuba, S.; Hiy, R. and Shimamura, S. (1989): The bacterial activity of tea and coffee. Lett. Appl. Microbial., 8: 123- 125.

Ullah, M.S. and Lim, U.T. (2015): Laboratory bioassay of Beauveria bassiana against *Tetranychus urticae* (Acari: Tetranychidae) on leaf discs and potted bean plants. Experimental and Applied Acarology, 65: 307–318.

Uma Devi, K.; Padmavathi, J.; Uma Maheswara Rao, C.; Akbar Ali Khan, P. and Murali Mohan, C. (2008): A study of host specificity in the entomopathogenic fungus, *Beauveria bassiana* (Hypocreales: Clavicipitaceae). Biocontrol Science and Technology, 18: 975–989.

Van Franken huyzen, K. (2009): Insecticidal activity of Bacillus thuringiensis crystal proteins. J. Invertebr Pathol., 101: 1-16.

Van Lenteren, J.C.; Bolckmans, K.; Kohl, J.; Ravensberg, J. and Urbaneja, A. (2017): Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl, Doi: 10.1007/s10526-017-9801-4.