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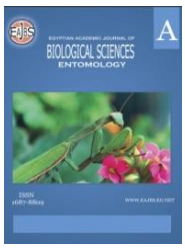

Compatibility of Entomopathogenic Fungi, *Beauvaria bassiana* (Bals. -Criv.) Vuill. and *Metarhizium anisopliae* (Metchn) Sorokin Isolates with Different Agrochemicals Commonly Used in Vineyards.

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ABSTRACT

Compatibility studies of biological control agents with different agrochemicals have paramount importance to provide proper guidelines for their integrated use and time of application of these agents. The effect of twenty-one different agrochemical formulations commonly used in vineyards including 10 fungicides, 5 insecticides, 2 acaricides and four plant growth regulators on conidial germination and vegetative growth of entomopathogenic fungi; *B. bassiana* MN710408 and *M. anisopliae* MN710409 was tested under laboratory conditions**.** The results of this study showed that among tested fungicides, copper hydroxide formulation was highly compatible with both fungal isolates, it even stimulated conidial germination at all tested concentrations. Also, Copper- sulphate® showed a high stimulant effect with *B. bassiana* isolate and to a lesser extent with *M. anisopliae* isolate. The neonicotinoids were compatible with both fungal isolates at certain concentrations. Lufenuron® showed a stimulation effect to both fungal isolates. Regarding plant growth regulators, the Dormex® formulation completely inhibited conidial germination and vegetative growth of both fungal isolates at field recommended concentration, while other types of these agrochemicals showed various degrees of compatibility depending on the fungal isolate.

INTRODUCTION

Compatibility between entomopathogenic fungi (EPF) with different agrochemical is considered essential for IPM. One of the most important factors affecting the efficacy and stability of (EPF) is the side effect of various agrochemicals used in different agro-system. (Mietkiewski *et al*., 1997; Klingen and Haukeland, 2006; Mochi *et al*., 2006). Toxicity of such agrochemicals to EPF may vary with fungus species or strain, chemical nature of the active ingredient, mode of action and recommended labeling rate (Alves and Lecuona, 1998).

Fungicides were among the first of these agrochemicals which attracted attention since they can affect negatively EPF in several ways. The adverse effects of fungicides

depend on fungi species, the type of fungicide the dosage of the active ingredient and the temperature. The inhibitory effect was much greater against the entomophthorales than against the Hyphomycetes. The dithiocarbamate derivations zineb + copper oxychloride, and mancozeb completely inhibited germination *B. bassiana*, *M. anisopliae.* Also, the fungistatic effect was more pronounced at 15°C than at 25°C (Majchrowicz and Poprawski, 1993).

Moorhouse *et al*. (1992) mentioned that the fungicides chlorothalonil and zineb prevented germination of *M. anisopliae* conidia at the commercial concentration; moreover, the carbendazim, totally inhibited growth at 0.1 times the recommended rate. Also, vegetative growth was completely prevented by the fungicides zineb. Kouassi *et al*. (2003) demonstrated that copper-oxide, metalaxyl, and mancozeb at recommended field rate inhibited the radial growth of *B. bassiana* (MK2001 isolate) 8 days post-treatment on the solid media.

Concerning insecticide formulations, Urs *et al*. (1967) demonstrated that BHC ((Lindane) 50% WP and malathion completely inhibited vegetative growth of *Beauvaria bassiana* and *Metarhizium anisopliae* in all tested concentrations. Conversely, Dimecron stimulates the growth of both fungi at all concentrations. *In vitro*, chlorpyrifos completely inhibited the germination of *B. bassiana* (CG 425strain), and contrariwise, abamictin and pyrethroid formulations were more compatible with *B. bassiana* (De Oliveira *et al*., 2003; De oliveira and Neves, 2004). Fiedler and Sosnowska (2017) mentioned that the imidacloprid (Confidor 200 SL) had low toxicity to the *B. bassiana* fungus. Gamma and lambda-cyhalothrin had the lowest adverse effect on conidial germination of the different isolates of *B. bassiana* and *M. anisopliae*, while Significant differences were obtained in the vegetative growth of different fungal isolates (Pelizzaa *et al*., 2018).

With regard to plant growth regulators (PGR), Storey and Gardner (1986) stated that mefluidide was compatible with *B. bassiana* since it caused no significant inhibition of germination and growth of the fungus, but the mortality of fall armyworm, *Spodoptera frugiperda* (Smith) resulting from *B. bassiana* treatment was significantly reduced when larvae were exposed to conidia plus soil treated with paclobutrazol.

This work aims to evaluate the effect of some agrochemicals commonly used in vineyards on conidial germination and vegetative growth of entomopathogenic fungi; *B. bassiana* 3873 PSA and *M. anisopliae* 5130.

MATERIALS AND METHODS

Fungal Isolates:

Experiments were carried out under laboratory conditions. Two fungal isolates of entomopathogenic fungi; *B. bassiana* 3873 PSA and *M. anisopliae* 5130 PSA were obtained from the collection of Assuit University Mycology Center (AUMC). The identity of these isolates was confirmed using molecular techniques. Both fungal isolates were inoculated on sterilized potato dextrose agar medium (PDA) and incubated at 27°C for one week. Then the aerial mycelium was scraped from the culture surface using a sterile scalpel blade. Total DNA was extracted from fungal cultures by using a commercial plant DNA extraction kit (DNeasy Plant Mini Kit, Qiagen, Germany) following the manufacturer's protocol. The experiment was carried out at a plant quarantine pathogens laboratory; Agriculture Center Research (PQPL). The extraction of isolated DNA was done according to a commercial animal and fungi DNA preparation kit protocol (Jena Bioscience, Germany). Genomic DNA was used as a template for PCR amplification of ITS region, using universal primers ITS1 (5′TCC GTA GGT GAA CCT GCG G 3′), and the reverse primer ITS4 (5′TCC TCC GCT TAT TGA TAT GC 3)

(White *et al*., 1990). The sequencing was performed at Macrogen Company, Korea, and submitted to GenBank (accession numbers MN710408 for *B. bassiana* and MN710409 for *M. anisopliae*).

Preparation of Media with Agrochemical Formulations:

Twenty-one of Different agrochemical formulations including 10 fungicides, 5 insecticides, 2 acaricides and four plant growth regulators were evaluated for compatibility with *B. bassiana* and *M. anisopliae* the same as described by Kos and Celar (2013).

Firstly, PDA medium was prepared and sterilized in an autoclave at 120°C for 15min., and cooled to 50-60°C. Then four concentrations FR, 0.5× FR, 0.25× FR and 0.125× FR from each of fungicides, insecticides, acaricides and only one concentration (FR) of plant growth regulators were prepared by serial dilution and added to PDA media. PDA media with the tested agrochemicals, in addition, to control treatment without additive agrochemicals were poured in three replicates of Petri dishes for each concentration. The Trade names, active ingredients, recommended rates, of the evaluated agrochemicals in this study were listed in Table (1).

Table 1: Characteristics of different examined fungicides, insecticides and plant growth regulators.

Type	Trade name	Active ingredient	FR	Mode of action
Fungicides	Zoom®	Copper hydroxide (Fixed copper) 38.37WP.	250g/100L	Contact
	Golden-Blue pentahydrate Copper sulphate®	Copper sulphate (Complex copper) 98.5 % SG.	250g/100L	Contact
	Copperarikh®	Copper oxychloride (Fixed copper) 50%WP	300g/100L	Contact
	Zenga®	(Copper 10%, Mancozeb 30%, Metalaxyl 5%) 45%WP.	125g/100L	Systemic & Contact
	Micronized Soreil KZ®	Sulphur 70%WP.	250g/100L	Contact
	Ridomil® MZ	(Metalaxyl 8% and Mancozeb 64%) 72%WP.	250g/100L	Systemic & Contact
	Tilt®	Propiconazole 25% EC	15 cm^3/100L	Systemic
	Topsin® M	Thiophanate-methyl 70%WP.	80g/100L	Systemic
	Nasr Zim®	Carbendazim 50%WP.	60g/100L	Systemic
	Rizolex-T®	(Tolclofos-Methyl 20%, Thiram 30%) 50 %WP.	250g/100L	Contact
Insecticides	Sunclopride®	Imidachloprid 35%SC.	125 cm^3/100L	Systemic
	Telfast®	Acetampride 20%SP.	25g/100L	Systemic
	Tockthion®	Prothiofos 50%EC.	200cm^3/100L	Contact
	Ictafos®	Chloropyrofos 48%EC.	1L/100L	Contact
	Lenoflag®	Lufenuron 5%EC.	40cm^3/100L	Contact
Acaricides	Artox	Fenpyroximate 5% SC.	50cm^3/100L	Contact
	Arrow®	Abamectin 1.8%EC.	40cm^3/100L	Contact
Plant growth regulators	Dormex®	Hydrogen cyanamide	5cm^3/100L	-
	Gibbest®	Gibberellic acid	150ml/100L	-
	Ethrel®	Ethephon 48%SL	150cm^3/100L	-
	Magictone®	*Naphthaleneacetic acid* (NAA)0.45% and Naphthaleneacetamide (NAAm) 1.25%	60g/100L	-

Effect of Agrochemicals on Conidial Germination:

Conidial suspensions of each fungal isolate were prepared by flooding 20-day cultures with sterile distilled water. The resulting conidial suspensions were filtered through layers of muslin to remove mycelial mats, and then the suspensions were centrifuged at 5,000 rpm for 15 min. the supernatant was decanted and the conidial mass was re-suspended in aqueous solution of 0.01% Tween 20. The stock suspensions of each fungal isolate were subjected to serial dilutions. According to Anderson and Roberts (1983), the dilution giving above 200 colonies of both fungal isolates per plate was used in all further tests.

The Petri dishes containing PDA medium with tested agrochemicals at different concentrations were inoculated with 10µl of the diluted fungal suspension. Petri dishes were held at 25°C, and the colony-forming units (CFU) were counted after 48h by a microscope at 40× magnification. Treatments were repeated three times. The means of CFU (± S.D.) for each treatment were compared with those for the control treatment. The germination inhibition percentage was calculated as follow:

$$\text{Germination inhibition/increase percentage} = \frac{\text{CFU in the control treatment - CFU in treatment}}{\text{CFU in the control treatment}} \times 100$$

Effect of Agrochemicals on Vegetative Radial Growth:

Agar plugs (0.8 cm diameter) with *B. bassiana* and *M. anisopliae* isolates placed in the middle of Petri dishes (9 cm diam.) containing PDA medium with the tested agrochemicals at the selected concentrations, in addition to the control treatment without additive agrochemicals. Then the Petri dishes were incubated at 25°C. The colonies growth was examined daily for 15 days of culturing to measure the colony diameter. Measurements were taken with a mill metric ruler along the same pre-marked radial axis. The replicated values recorded at each reading were averaged (cm ± S.D.). Each treatment was performed in three replicates (Hokkanen and Kotiluoto, 1992). The inhibition/increase percentage of radial growth of each fungus was estimated as follow:

$$\text{Radial growth inhibition /increase percentage=} \frac{\text{Colony diameter in the control - Colony diameter in treatment}}{\text{Colony diameter in the control}} \times 100$$

Statistical Analysis:

The data obtained from various treatments were subjected to a log (X + n) transformation to stabilize the variance and avoid zero values according to **(**Draper and Smith 1981). The transformed data were carried out in replicated randomized complete block design (RCBD) and submitted to analysis of variance ANOVA, general linear model (GLM), and treatment means were post-hoc compared by the Tukey HSD test with the level of significance $p < 0.05$. Statistical analysis was conducted by using SPSS version 16.

RESULTS

The compatibility of *B. bassiana* (MN710408) and *M. anisopliae* (MN710409) with 21 agrochemical formulations commonly used in vineyards were studied *in vitro*. The agrochemical formulation included 10 fungicides, 6 insecticides, 2 acaricides and 4 plant growth, regulators. The compatibility was measured by two parameters, conidial germination and mycelial growth.

Effects of Fungicide Formulations:

The results of general linear model analysis (GLM) showed that a significant difference of germination of *B. bassiana* MN710408 in relation to the examined fungicides, F (10, 108) = 266.033, p<0.0001and its concentration F (3, 108) = 7.780, p<0.0001 compared to control. The conidial germination of *M. anisopliae* MN710409 isolate also affected significantly by fungicide formulation, F (10, 108) =81.6555, p<0.001 and its concentration, F (3, 108) = 22.937, p< 0.0001.

As shown in Table (2), the *in vitro*, the viability of the fungal conidia in the presence of copper fungicides showed that Zoom® based on copper-hydroxide had a stimulating effect on conidial germination of both fungal isolates at all tested concentrations. The stimulation was relatively higher in *B. bassiana* isolate than the isolate of *M. anisopliae.* The highest percentage of stimulating in conidial germination over control was 74% in *B. bassiana* isolate and 29.3% in *M. anisopliae* at 0.25×FR and 0.5×FR concentrations, respectively. Also, the Copper sulphate® formulation had also a positive effect on conidial germination of *B. bassiana* isolate since the stimulatory effect increased linearly by serial dilution of the formulation. The highest percentage of increase in conidial germination over the control (+34.3%) was recorded at the lowest tested concentration. The same formulation had an inhibitory effect on conidial germination of *M. anisopliae* estimated by -34.7% at FR concentration, and mitigated to -10.3% by diluting the concentration to 0.5×FR. However, it showed a stimulant effect being estimated at 14.6% and 24% at the lower concentrations of 0.25×FR and 0.125×FR, respectively.

Adversely, the copper-oxychloride formulation, Copperarikh®, had the largest inhibitory effect among the Copper fungicides on conidial germination of both examined fungi. The formulation completely inhibited the conidial germination of *B. bassiana* isolates at all tested concentrations, except the lowest one which caused 93% inhibition of conidial germination. Also, the Copperarikh® had an adverse effect on conidial germination of *M. anisopliae* isolate, but the effect was lower compared with that of *B. bassiana* isolate. The inhibitory effect on conidial germination increased gradually with doubling the concentration to record the highest percentage (100%) at FR concentration. Micronized Soreil KZ®, sulfur-based formulation, completely inhibited the conidial germination of *B. bassiana* isolate independent of concentration, while the inhibition percentage of *M. anisopliae* conidial germination associated with fungicide concentrations. The inhibitory effect was (100%) at FR concentration, while greatly reduced to -12.7% at 0.125×FR concentration and recorded the highest percentage of inhibition

The treatments contain the fungicide Zenga® had an inhibitory effect on conidial germination of both fungal isolates in a dose-dependant manner, but the inhibition percentage of *B. bassiana* isolate was higher than that of *M. anisopliae* isolate. The conidial germination of both *B. bassiana* and *M. anisopliae* isolate was inhibited by 56.4 and 95.9 over the control treatment, respectively when exposed to FR concentration. The inhibitory effect of this fungicide was mitigated gradually by reducing the concentration reaching 30.3 and 13.6% at the lowest concentration for *B. bassiana* and *M. anisopliae*, respectively. On the other hand, the conidial germination of both fungal isolates was completely inhibited by exposure to the fungicides; Ridomil® MZ, Tilt®, Topsin® M, Nasr Zim® and Rizolex-T®. The fungicidal effect of these formulations was independent of their concentration since the lowest (0.125×FR) concentration of the aforementioned formulations showed the same effect.

With regard to the second parameter, vegetative radial growth, the statistical analysis showed that a significant difference in vegetative radial growth of *B. bassiana* MN710408 in relation to the examined fungicides, F (10, 108) = 380.800, p<0.0001and its concentration F (3, 108) = 25.114, p<0.0001 compared to the control. The radial growth of *M. anisopliae* MN710409 isolate also affected significantly by fungicide formulation, F (10, 108) =126.331, p<0.001 and its concentration, F (3, 108) = 11.867, p< 0.0001.

Table 2: Compatibility of fungicides with *B. bassiana* and *M. anisopliae* isolates based on conidial germination and mycelia growth.

Treatments	Concentration	*B. bassiana* MN710408				*M. anisopliae* MN710409			
		Effect on conidial germination		Effect on vegetative radial growth		Effect on conidial germination		Effect on vegetative radial growth	
		Mean (CFUs)±S.D.	%of inhibition/increase over control	Mean radial growth (cm)	%of inhibition/increase	Mean (CFUs)±S.D.	%of inhibition/increase over control	Mean radial growth (cm)	%of inhibition/increase
Control	-	256.3±24.80 ^{d}	-	6.7±0.04 ^{j}	-	225.6±6.60^{f}	-	6.9±0.30 ^{klmno}	-
Zoom®	FR	270.7±0.80 ^{d}	+5.6	5.4±0.41^{efg}	-19.4	253.6±7.80^{g}	+12.4	7.0±0.45 ^{mno}	+1.4
	0.5×FR	308.3±16.00 ^{de}	+20.3	6.3±0.15 ^{ij}	-5.9	291.7±10.10^{i}	+29.3	7.4±0.40 ^{no}	+7.2
	0.25×FR	445.9±18.70 ^{f}	+74.0	6.7±0.15 ^{j}	-	286.1±8.50^{i}	+26.8	7.6±0.28 ^{o}	+10.1
	0.125×FR	357.6±25.70 ^{e}	+39.5	6.7±0.10 ^{j}	-	261.5±6.00^{gh}	+15.9	7.2±0.3 ^{lmno}	+4.3
Copper sulphate®	FR	278.1±12.70 ^{d}	+8.5	5.4±0.07 ^{ef}	-19.4	147.3±14.10^{c}	-34.7	4.1±0.60 ^{de}	-40.6
	0.5×FR	307.3±18.40 ^{de}	+19.9	5.6±0.15 ^{efg}	-16.4	202.4±14.0^{e}	-10.3	4.7±0.60 ^{ef}	-31.9
	0.25×FR	340.5±13.00 ^{e}	+32.8	5.7±0.28 ^{efg}	-14.9	258.5±47.60^{g}	+14.6	5.6±0.06 ^{ghij}	-18.8
	0.125×FR	344.5±38.70 ^{e}	+34.3	6.8±0.26 ^{j}	+1.5	281.1±18.80^{hi}	+24.6	6.7±0.05 ^{ijklm}	-2.9
Copperarikh®	FR	0 ^{a}	-100	5.2±0.40 ^{e}	-22.4	0^{a}	-100	4.9±0.20 ^{f}	-28.9
	0.5×FR	0 ^{a}	-100	5.5±0.40 ^{efg}	-17.9	103.1±2.80^{b}	-54.3	5.2±0.20 ^{fghi}	-24.6
	0.25×FR	0 ^{a}	-100	5.8±0.30 ^{fgh}	-13.4	164.6±0.50^{d}	-27.1	6.1±0.05 ^{hijk}	-11.6
	0.125×FR	15.9±1.50 ^{a}	-93.8	5.9±0.20 ^{fgh}	-11.9	212.7±1.50^{ef}	-5.7	6.3±0.06 ^{ijk}	-8.7
Micronized Soreil KZ®	FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.5×FR	0 ^{a}	-100	0 ^{a}	-100	89.6±15.60^{b}	-60.3	5.3±0.35 ^{fg}	-23.8
	0.25×FR	0 ^{a}	-100	0 ^{a}	-100	193.6±8.70^{e}	-14.2	6.6±0.06 ^{ijkl}	-4.3
	0.125×FR	0 ^{a}	-100	1.4±0.35 ^{b}	-79.1	197.0±15.60^{ef}	-12.7	6.7±0.10 ^{jklmn}	-2.9
Zenga®	FR	110.5±7.40 ^{b}	-56.9	5.4±0.20 ^{efg}	-19.4	9.2±1.40 ^{a}	-95.9	6.7±0.20 ^{jklmn}	-2.9
	0.5×FR	170.5±35.50 ^{c}	33.5-	5.8±0.20 ^{fgh}	-13.4	135.8±18.30 ^{c}	-39.8	6.8±0.20 ^{jklmn}	-1.4
	0.25×FR	184.4±34.80 ^{c}	-31.0	6.0±0.00 ^{hi}	-5.9	147.1±7.90 ^{cd}	-34.8	6.8±0.05 ^{jklmn}	-1.4
	0.125×FR	186.3±15.70 ^{c}	-30.3	6.4±0.30 ^{ij}	-4.4	194.9±22.80 ^{e}	-13.6	6.8±0.10 ^{jklmn}	-1.4
Ridomil® MZ	FR	0 ^{a}	-100	0.0^{a}	-100	0 ^{a}	-100	1.3±0.13^{b}	-81.2
	0.5×FR	0 ^{a}	-100	2.1±0.10 ^{c}	-68.5	0 ^{a}	-100	2.2±0.10^{c}	-68.1
	0.25×FR	0 ^{a}	-100	3.5±0.80 ^{d}	-47.8	0 ^{a}	-100	2.3±0.47^{c}	-66.7
	0.125×FR	0 ^{a}	-100	3.6±0.34^{d}	-46.3	0 ^{a}	-100	3.6±0.36^{d}	-47.8
Tilt®	FR	0 ^{a}	-100	0.0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.5×FR	0 ^{a}	-100	1.1±0.01^{b}	-84.3	0 ^{a}	-100	0 ^{a}	-100
	0.25×FR	0 ^{a}	-100	1.3±0.01^{b}	-80.6	0 ^{a}	-100	0 ^{a}	-100
	0.125×FR	0 ^{a}	-100	1.5±0.05^{b}	-77.6	0 ^{a}	-100	0 ^{a}	-100
Topsin® M	FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.5×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.25×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.125×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
Nasr Zim®	FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.5×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.25×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.125×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
Rizolex-T®	FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.5×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.25×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
	0.125×FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100

Mean number ± standard deviation calculated from three replications, means followed by the same letter in each column are not significantly different (p < 0.01, Tukey test).FR=field recommended concentrations, 0.5 ×FR= half field recommended concentrations, 0.25× =one-fourth FR field recommended concentrations and 0.125× one-eighth field recommended concentrations**.**

Data obtained in Table (2) showed that the inhibition effect of copper hydroxide formulation (Zoom®) on *B. bassiana* isolate was significant at FR concentration, in comparison to control, while no effect was found at the three other concentrations. On the other hand, this formulation has no adverse effect on the mycelial growth of *M. anisopliae* isolate at FR concentration, but it showed a stimulatory effect at all other tested concentrations compared with control. Both copper-sulphate® and copper-oxychloride (Copperarrikh®) formulations showed inhibition activity to the mycelial growth of both fungal isolates. The variation in mycelial growth inhibition depends on the fungal isolate, the fungicide formulation and the tested concentrations. The copper-sulfate had an inhibitory effect on colony diameter of both fungal isolates, except with the lowest concentration where the mean colony diameter ranged from 5.4- 6.8 and 4.1-6.7cm for *B. bassiana* and *M. anisopliae* isolates, respectively. Zenga® formulation

showed an inhibitory effect on *B. bassiana* isolate and stimulatory effect on *M. anisopliae* isolate where the mean radial growth of mycelium ranged from 5.4-6.4 and 6.7-6.8cm for *B. bassiana* and *M. anisopliae* isolates, respectively. Also, Micronized Soreil KZ® and Ridomil®MZ caused an inhibitory effect on mycelia growth of both fungal isolates, and complete inhibition has occurred at FR concentrations.

Concerning the Topsin®, Nasr Zim®, Rizolex® and Tilt formulations, it was found that the mycelial growth of both *B. bassiana* and *M. anisopliae* isolates was completely inhibited at all tested concentrations of Topsin®, Nasr Zim® and Rizolex®. Tilt® formulation completely inhibited mycelial growth of *M. anisopliae* isolate at all tested concentrations, while the mycelium of *B. bassiana* isolate showed insignificant response by gradual dilution of concentration in comparison with control.

Effects of Insecticides and Acaricides Formulations:

The statistical analysis showed that a significant difference in germination of *B. bassiana* MN710408 in relation to the examined insecticides and acaricides, F (7, 76) = 94.850, p<0.0001and its concentration F (3, 76) = 14.1999, p<0.0001 compared to control. The conidial germination of *M. anisopliae* MN710409 isolate also affected significantly by insecticides and acaricides formulation, F (7, 76) =64.497, p<0.001 and its concentration, F (3, 76) = 19.486, p< 0.0001.

Table (3): Compatibility of insecticides and acaricides with *B. bassiana* and *M. anisopliae* isolates based on conidial germination and mycelial growth.

Treatments	Dose	*B. bassiana* MN710408				*M. anisopliae* MN710409			
		Effect on conidial germination		Effect on vegetative radial growth		Effect on conidial germination		Effect on vegetative radial growth	
		Mean (CFUs)±S.D.	%of inhibition/increase over control	Mean radial growth (cm)	%of inhibition /increase over control	Mean (CFUs)±S.D.	%of inhibition/increase over control	Mean radial growth (cm)	%of inhibition/increase over control
Control	-	256.3±24.80 ^{cdef}	-	6.7±0.04 ^{jklm}	-	225.6±6.60 ^{fgh}	-	6.9±0.30 ^{jklm}	-
Sunclopride®	FR	156.5±7.00 ^{b}	-38.9	5.6±0.10 ^{fgh}	-16.4	201.7±2.80 ^{def}	-10.6	5.1±0.36 ^{ef}	-26.0
	0.5×FR	181.9±10.00 ^{bc}	-29	5.8±0.70 ^{fghij}	-13.4	240.5±10.70 ^{ghi}	+6.6	6.6±0.04 ^{efg}	-4.3
	0.25×FR	214.8±4.00 ^{bcd}	-16.2	5.9±0.70 ^{fghij}	-31.3	256.9±16.60 ^{hij}	+13.9	7.0±0.14 ^{n}	+1.4
	0.125×FR	449.4±3.50 ^{l}	+80.0	5.9±0.50 ^{fghijk}	-14.9	304.1±31.70 ^{k}	+34.8	6.9±0.40 ^{gh}	-
Telfast®	FR	155.3±12.60 ^{b}	-39.4	5.9±0.30 ^{fghijk}	-11.9	203.9±0.50 ^{def}	-9.6	7.0±0.01 ^{jklm}	+1.4
	0.5×FR	246.6±26.80 ^{cde}	-3.8	6.4±0.60 ^{ghijklm}	-4.5	241.4±7.50 ^{ghi}	+7.0	7.3±0.20 ^{lm}	+5.8
	0.25×FR	274.2±9.00 ^{defg}	+7.0	6.9±0.01 ^{klm}	+2.9	247.7±4.50 ^{hi}	+9.8	7.3±0.20 ^{lm}	+5.8
	0.125×FR	304.9±8.30 ^{efghi}	+19.0	6.9±0.10 ^{klm}	+2.9	254.3±1.00 ^{hi}	+12.7	7.5±0.06 ^{mn}	+8.7
Tockthion®	FR	0 ^{a}	-100	1.2±0.17 ^{a}	-82.1	0 ^{a}	-100	0.0 ^{a}	-100
	0.5×FR	0 ^{a}	-100	3.0±0.15 ^{bc}	-55.6	0 ^{a}	-100	2.6±0.20 ^{bc}	-62.3
	0.25×FR	0 ^{a}	-100	3.5±0.40 ^{cd}	-47.38	111.9±0.60 ^{c}	-50.4	3.4±0.10 ^{d}	-50.7
	0.125×FR	0 ^{a}	-100	4.1±0.70 ^{de}	-38.8	131.7±1.20 ^{c}	-41.6	3.5±0.20 ^{d}	-49.2
Ictafos®	FR	0^{a}	-100	2.3±0.20^{b}	-65.7	0 ^{a}	-100	2.0±0.20 ^{b}	-71.0
	0.5×FR	11.3±0.70 ^{a}	-95.6	2.6±0.05^{b}	-61.2	0 ^{a}	-100	2.5±0.40 ^{bc}	-63.8
	0.25×FR	15.3±2.60 ^{a}	-94.0	2.6±0.10^{b}	-61.2	0 ^{a}	-100	2.7±0.20 ^{c}	-60.9
	0.125×FR	23.6±4.70 ^{a}	-90.8	4.2±0.40 ^{cd}	-37.3	211.6±1.50 ^{efg}	-6.2	3.8±0.20 ^{d}	-44.9
Lenoflag®	FR	349.3±17.20 ^{ghijk}	+36.3	6.4±0.11 ^{hijklm}	-4.4	265.6±9.60 ^{hi}	+17.7	6.7±0.10 ^{ijkl}	-2.9
	0.5×FR	358.5±8.60 ^{hijk}	+39.9	6.6±0.11 ^{ijklm}	-1.4	266.0±7.00 ^{hi}	+17.9	6.7±0.06 ^{ijkl}	-2.9
	0.25×FR	360.1±32.10^{hijk}	+40.5	7.0±0.30 ^{lm}	+4.4	267.0±5.80 ^{hij}	+18.3	6.8±0.20 ^{jkl}	-1.4
	0.125×FR	343.6±18.30 ^{ghijk}	+34.1	6.7±0.05 ^{ijklm}	-	290.3±9.60 ^{jk}	+28.7	7.1±0.30 ^{klm}	+2.9
Arrow®	FR	329.3±25.70^{fghij}	+28.5	5.1±0.60 ^{ef}	-23.8	57.8±2.80 ^{b}	-74.4	6.1±0.30 ^{ghi}	-11.6
	0.5×FR	378.3±13.00^{ijkl}	+47.6	5.5±0.05 ^{fgh}	-17.9	132.2±3.00 ^{c}	-41.4	6.4±0.10 ^{hij}	-7.2
	0.25×FR	395.9±7.50^{jkl}	+54.5	6.6±0.21 ^{hijklm}	-1.4	170.3±7.70 ^{d}	-24.5	6.5±0.15 ^{hijk}	-5.8
	0.125×FR	417.5±21.50^{kl}	+62.9	7.1±0.47 ^{m}	+5.9	186.3±10.00 ^{de}	-17.4	7.0±0.26 ^{jklm}	+1.4
Artox®	FR	210.9±8.00^{bcd}	-17.7	5.7±0.49 ^{fghi}	-14.9	300.5±6.50 ^{k}	+33.2	4.9±0.10 ^{e}	-28.9
	0.5×FR	286.3±16.8^{defgh}	+11.7	5.7±0.25 ^{fghi}	-14.9	273.6±25.30 ^{ijk}	+21.3	4.9±0.10 ^{e}	-28.9
	0.25×FR	344.0±11.00^{ghijk}	+34.4	6.0±0.25 ^{fg}	-10.4	263±26.20 ^{ijk}	+16.5	5.3±0.08 ^{ef}	-23.2
	0.125×FR	403.2±6.60^{jkl}	+57.3	6.2±0.30 ^{fghijkl}	-7.4	259±15.30 ^{hij}	+14.8	5.6±0.06 ^{fg}	-18.8

Mean number ± standard deviation calculated from three replications, means followed by the same letter in each column are not significantly different ($p < 0.01$, Tukey test).FR=field recommended concentrations, 0.5 ×FR= half field recommended concentrations, 0.25× =one-fourth FR field recommended concentrations and 0.125× one-eighth field recommended concentrations**.**

Data obtained in Table (3) indicated that the neonicotinoid Sunclopride® was a germination potent stimulant to conidia of *B. bassiana* isolate at 0.125×FR concentration since the germination increased by 80% over the control treatment. It relatively inhibited the conidial germination at all other concentrations. The highest percentage of inhibition was 38.9% over the control treatment at FR concentration. On the other hand, the same

formulation affected positively the conidial germination of *M. anisopliae* isolate, except at the highest concentration which inhibited the germination with 10.6% over the control. The highest percentage of conidial germination stimulation in *M. anisopliae* isolate over the control was +34.8% at the lowest concentration of Sunclopride®.

The other neonicotinoid formulation, Telphast®, showed an inhibitory effect on conidial germination of both fungal isolates at the higher concentrations. The inhibitory effect of Telphast® on *B. bassiana* isolate was higher than that of *M. anisopliae* isolate, where the inhibition percentages were 39.4 and 9.6% over the control at FR concentrations for *B. bassiana* and *M. anisopliae*, respectively. Contrary, this formulation had a stimulating effect on conidial germination of *B. bassiana* and *M. anisopliae* isolates at the lower concentrations. The highest stimulating effect occurred at the lowest concentration (0.125×FR) with percentages of 19.9 and 12.7% on *B. bassiana* and *M. anisopliae*, respectively.

Concerning the organophosphate based formulations, Tockthion® and Ictafos® had a negative effect on conidial germination of *B. bassiana* isolate**.** The inhibitory effect of Tockthion® was independent of concentration, while the Ictafos® formulation strongly inhibited the conidial germination at all concentrations, except at the lowest concentration. The conidial germination of *M. anisopliae* was highly affected by Tockthion® and Ictafos®. The conidial germination of *M. anisopliae* isolate was completely inhibited by Tockthion® at FR and 0.5×FR concentrations, and this effect decreased at the other concentrations to record the lowest percentage of inhibition (41.6%) at 0.125×FR concentration. On the other hand, Ictafos® formulation had a highly negative effect on *M. anisopliae* conidial germination, where it was completely inhibited at all tested concentrations, except at the lowest one since the inhibition of conidial germination was estimated by 6.2% which was on par with the control treatment. Contrary, both fungal isolates showed a positive response to Lenoflag® treatment at all selected concentrations. The highest stimulatory effect was recorded on conidial germination of *B. bassiana* isolate by 40.5% at 0.25×FR, while the germination of *M. anisopliae* conidia recorded a lower stimulating percentage being estimated by 28.7% at the lowest concentration (0.125×FR) of the formulation.

Concerning the acaricide formulations, Arrow® had also a positive effect on conidial germination of *B. bassiana* isolate even at FR concentration. The highest percentage of increase of conidial germination was 62.9% over the control at 0.125×FR concentration. The stimulatory effect of this formulation decreased gradually by reducing the concentration to record the lowest percentage of stimulation (28.5%) at FR concentration. On the other direction, *M. anisopliae* isolate showed high sensitivity toward this acaricide formulation as it had an inhibitory effect in all tested concentrations. The highest percentage of conidial germination inhibition was -74.4% at FR concentration and mitigated gradually by diluting the concentration. The other acaricide formulation Artox® showed a high stimulation effect (+57.3%) on conidial germination of *B. bassiana* isolate at the lowest tested concentration. At 0.5×FR and FR concentrations, the formulation showed a reverse effect where it inhibited the conidial germination by -17.7% at FR concentration. Also, the high stimulating effect of Artox® formulation on conidial germination of *M. Anisopliae* isolate was recorded at FR concentration and estimated by +33.2% increase over control, while this effect decreased gradually by reducing the concentration to record the lowest percentage of stimulation (14.8%) at 0.125×FR concentration.

As shown in Table (3), The Tockthion®, Ictafos®, Sunclopride® and Artox® formulations inhibited the mycelial growth of both fungal isolates by varied percentages according to the fungal isolate, the insecticide or acaricide formulation and the tested

concentrations. The highest inhibition percentages were obtained at FR concentration of Tockthion®, followed by Ictafos® being estimated by (82, 65.7 and 71.0, 100%) for *B. bassiana* and *M. anisopliae* isolates, respectively. Linoflag® and Arrow® formulations showed low inhibition at high tested concentrations and stimulatory effect at the lowest tested concentrations (0.125×FR) on both fungal isolates. Conversely, The Telphast® treatment resulted in stimulation of mycelial growth of both fungal isolates at all tested concentrations, except at FR and 0.5×FR concentrations with *B. bassiana* isolate being estimated by 11.9 and 4.5% inhibition for both concentrations, respectively.

Effects of Plant Growth Regulators:

The statistical analysis showed that a significant difference in the germination of *B. bassiana* MN710408 in relation to the examined plant growth regulators, F (4, 10) = 108.635, p<0.0001 compared to control. The conidial germination of *M. anisopliae* MN710409 isolate also affected significantly by plant growth regulators formulation, F (4, 10) =959.428, p<0.001 and its concentration.

As shown in Table (4), the plant growth regulators (PGRs) formulations were tested at FR concentration. The results showed that the Dormex® formulation had fungicidal effect on both fungal isolates since the germination was completely inhibited. The gibberellic acid-based formulation Gibbest® stimulate the conidial germination by +27% and +3% for *B. bassiana* and *M. anisopliae* isolates respectively. The Ethrel® formulation had significant stimulatory effect being estimated by 41.7% on conidial germination of *B. bassiana* isolate, while the same formulation inhibited conidial germination of *M. anisopliae* isolate by -24.7% over the control. The Magictone® formulation caused inhibition in conidial germination of *B. bassiana* isolate by -45.7% over the control at FR concentration. Conversely, the same formulation stimulated the conidial germination of *M. anisopliae* isolate significantly by +22.9% over the control.

The statistical analysis showed that a significant difference in vegetative radial growth of *B. bassiana* MN710408 in relation to the formulation of plant growth regulators, F (4, 10) = 390.125, p<0.0001. The radial growth of *M. anisopliae* MN710409 isolate also affected significantly by plant growth regulators, F (4, 10) =423.109, p<0.001.

Table 4: Compatibility of plant growth regulators with *B. bassiana* and *M. anisopliae* isolates based on conidial germination and mycelia growth.

Treatments	Dose	*B. bassiana* MN710408				*M. anisopliae* MN710409			
		Effect on conidial germination		Effect on vegetative radial growth		Effect on conidial germination		Effect on vegetative radial growth	
		Mean (CFUs)±S.D.	%of inhibition/increase over control	Mean radial growth (cm)	%of inhibition/increase over control	Mean (CFUs)±S.D.	%of inhibition/increase over control	Mean radial growth (cm)	%of inhibition/increase over control
Control	-	256.3±24.80 ^{c}	-	6.7±0.04^{b}	-	225.6±6.60^{c}	-	6.9±0.30 ^{d}	-
Dormex®	FR	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100	0 ^{a}	-100
Gibbest®	FR	325.5±26.8 ^{d}	+27.0	6.3±0.60 ^{b}	-5.9	233.9±12 ^{c}	+3.7	7.2±0.4 ^{d}	+4.3
Ethrel®	FR	363.1±16.9 ^{d}	+41.7	7.3±0.06 ^{c}	+8.9	169.8±10.9 ^{b}	-24.7	6.1±0.07 ^{c}	-11.5
Majictone®	FR	139.2±10.6 ^{b}	-45.7	7.3±0.35 ^{c}	+8.9	277.3±17.6 ^{d}	+22.9	5.0±0.1 ^{b}	-27.5

Mean number ± standard deviation calculated from three replications, means followed by the same letter in each column are not significantly different ($p < 0.01$, Tukey test).FR=field recommended concentrations.

Data obtained in Table (4) showed that all tested plant growth regulators had an inhibitory effect on mycelial growth of *M. anisopliae* isolate. The highest inhibitory effect was caused by the Dormex® formulation (100% inhibition), followed by Magictone treatment (27.5% inhibition). On the other hand, the Dormex® and the Gibbest® formulations caused inhibition on mycelial growth of *B. bassiana* isolate being estimated by 100 and 5.9% for both formulations, respectively. Conversely, the Ethrel® and Majictone® showed a stimulatory effect on mycelia growth of *B. bassiana* isolate being estimated by 8.9% stimulation.

DISCUSSION

The effect of agrochemical formulations on conidial germination/viability of EPF was considered one of the most important aspects in field compatibility evaluation (Anderson and Roberts, 1983; Malo, 1993; Neves *et al*., 2001). Moreover, fungal conidia is responsible for the preservation of the fungal inoculums in field (Alves and Leucona, 1998).

In the present study, the inhibitory or stimulatory effect of agrochemicals currently used in vineyards on conidial germination and vegetative growth of *B. bassiana* (MN710408) and *M. anisopliae* (MN710409) was dependent on fungus species, formulation of agrochemicals and the concentration in the culture medium which agrees with (Vanninen and Hokkanen, 1988; Anderson *et al*., 1989; Hedimbi *et al.*, 2008; Tkaczuk *et al*., 2016; Tkaczuk and Majchrowska-Safaryan, 2020). The evaluation of currently used copper formulations revealed that copper- hydroxide (Zoom®) was highly compatible with both fungal isolates where it was a potent stimulant of conidial germination of both fungal isolates especially *B. bassiana* isolate, also Copper- sulphate® showed a high stimulant effect with *B. bassiana* isolate and to a lesser extent to *M. anisopliae* isolate. Conversely, copper-oxychloride (Copperarikh®) strongly inhibited the conidial germination of *B. bassiana.*

The germination stimulatory effect of Copper-hydroxide and copper-sulphate was proceeding in a non-linear manner. A number of authors mentioned that copper-hydroxide had a little inhibitory effect on *B. bassiana* compared to Copper-oxychloride (Olmert and Kenneth 1974; Jaros-Su *et al*., 1999; Majchrowicz and Poprawski 1993; Tamai *et al*., 2002; Martins *et al*.,2012). Similarly, Sosa-Gomez (1991), and Sosa-Gomez *et al*. (1987) found that copper- oxychloride completely suppressed conidial production of *H. thompsonii*, while mycelial growth was only reduced. They also mentioned that Copper-sulphate had a fungicidal effect since conidial germination of the fungus was completely inhibited. By contrast with the present results, Challa and Sanivada (2014) demonstrated that Copper- oxychloride promoted the mycelia growth in many isolates of *B. bassiana*. Celar and Kos (2020) reported that Copper-hydroxide 50% WP caused 79.9% inhibition in vegetative growth and 99.1% inhibition in conidial germination of the commercial strain of *B. bassiana* ATCC 74040.

The sulphur formulation Micronized Soreil KZ® completely inhibited the germination of *B. bassiana* isolate even the lowest concentration while conidia and mycelium of *M. anisopliae* isolate showed some tolerance to this formulation by diluting of concentrations. This result agrees with Shaha *et al*. (2009) who mentioned that sulphur inhibited *B. bassiana* and *L. longisporum*, but had a mild inhibitory effect on *M. anisopliae*. On the contrary, Usha *et al*. (2014) demonstrated that sulphur displayed high compatibility to all isolates of *B. bassiana* even at high concentrations. Similary, Tkaczuk *et al*. (2016) reported that the Sulphur-based fungicide; Siarkol Extra 80 WP slightly limited the growth of fungal colonies of *Isaria fumosorosea* and *B. bassiana* and at lower concentrations showed even a stimulating effect which disagrees with the present findings.

The Ridomil® formulation is a mixture of the acylalanine (metalaxyl) and the dithiocarbamate; mancozeb (maneb+zineb) completely inhibited the germination of both fungal isolates even at one-eighth of FR concentration. Although the mycelium of both isolates showed some tolerance to this formulation, the inhibitory effect was significant compared to the control. These findings were consistent with previously published by

Machowicz-Stefaniak (1980) since they reported that maneb (0.5%) was harmful to *B. bassiana* and *V. lecanii* (over 99% reduction in the beneficial capacity of the fungi). Majchrowicz and Poprawski (1993) mentioned that dithiocarbamates completely suppressed *B. bassiana* and *M. anisopliae.* Wie *et al*. (2004) elucidated that mancozeb completely inhibited the germination of the entomophthorelean fungus *Panadora nouryi* even at the manufacturer's lowest recommended concentration for field operation. In addition, Khan *et al*. (2012) demonstrated that metalaxyl (0.1%) and mancozeb (0.2%) were not compatible with *B. bassiana* and *M. anisopliae* since they caused complete inhibition of vegetative growth and spore germination. The same effect of mancozeb on *B. bassiana* was reported by Challa and Sanivada, 2014; Ondráčková *et al.,* 2019). The results showed that the fungicide Zenga® had a lower adverse effect on conidia and mycelium of both fungal isolates than Ridomil® formulation which might be due to the presence of copper in the formulation.

The results showed that the fungicides formulations based on propiconazole triazole derivative (Tilt®), thiophanate-methyl (Topsin®M), carbendazim (Nasr Zim®) and tolclofos-Methyl 20%+ Thiram 30% (Rizolex-T®) had the highest detrimental effect on conidial germination and mycelium growth of both fungal isolates even at high dilution rate which revealed a potential incompatibility of these fungicides with the tested fungal isolates. Earlier, Samuels *et al*. (1989) pointed to the compatibility of Tilt® fungicide with certain isolates of *M. anisopliae* and they suggested that selection of tolerant strains could overcome the problem of incompatibility in-field application. Hassan *et al.* (1991) showed that bitertanol, a triazole-derived fungicide, was highly toxic to *V. lecanii*, but less to *B. bassiana.* Majchrowicz and Poprawski (1993) reported that triazole derivative, triadimefon completely inhibited *B. bassiana* and *M. anisopliae* at field recommended concentration. Similarly, Khan *et al. (*2012) mentioned that members of triazole group including tebuconazole hexaconazole, propiconazole and difenoconazole were not compatible with *B. bassiana* and *M. anisopliae* and caused complete or strong vegetative growth inhibition and spore germination. Silva *et al*. (2013) mentioned that the triazoles difenoconazole and propiconazole reduced conidial germination, mycelial growth and conidiation and should not be applied together with *Metarhizium anisopliae*. A more recent study by Joshi *et al*. (2018), showed that propiconazole 25% EC completely inhibits germination of *B. bassiana* and *M. anisopliae* in its action at all concentrations. Olmert and Kenneth (1974) showed that *B. bassiana* was highly sensitive to Carbendazium which agrees with the present findings. Usha *et al*. (2014) evaluated Bavistin®; carbendizam based formulation, where they demonstrated that the conidial germination of all tested fungal isolates of *B. bassiana* was completely inhibited at field recommended concentration. Fabrice *et al*. (2013) showed that thiophanate-methyl Topsin® was able to inhibit the germination of *M. anisopliae* in the presence of 200 µg/ml of thiophanate-methyl.

The heterocyclic nitromethylenes or neonicotonoids Sunclopride® and Telphast® were highly compatible with tested isolate of *M. anisopliae* and to a lesser extent with the fungal isolate of *B. bassiana*. These results are supported by Neves *et al*. (2001) since they elucidated that acetamiprid (Saurus 200 SP), imidacloprid (Confidor 700 WDGr) and thiamethoxam (Actara 250 WG) had no effect on conidia germination of *B. bassiana*, *M. anisopliae* and *Paecilomyces* sp except under the highest concentration of acetamiprid (1.3×field recommended concentration "FR") in which significant inhibition of *M. anisopliae* occurred.Cuthbertson *et al*. (2005) mentioned that acetaimprid formulation had a significant inhibitory effect on vegetative growth of *B. bassiana* and *M. anisopliae* even at the lowest concentration (0.7×FR). The conidial germination of *L. muscarium* was reduced by 73.5%. Alizadeh *et al*. (2007) reported that the inhibition caused by

imidaclopride formulation was lower than 27% compared to the control treatment. Khan *et al*. (2012) stated that acetameprid (0.004%), thiomethoxam (0.005%), imidachloprid (0.005%) were highly safe and most compatible *B. bassiana* and *M. anisopliae*. Also, the imidachloprid based "Confidor 200SL" showed low toxicity to *B. bassiana* even at 1.5 times the recommended field concentration (Fiedler and Sosnowska, 2017).

The present findings emphasized the adverse effect of organophosphate formulations Tockthion® and Ictafos® on conidial germination and vegetative growth of both fungal isolates suggesting that combined application with these formulations is best avoided. These results agree with those obtained by Urs *et al*. (1967) who mentioned that malathion completely inhibited germination of *B. bassiana* and to *M. anisopliae* while it permitted limited vegetative growth at low concentrations. The Dichlorvos and chloropyrifos formulations at recommended dosages had a strong inhibitory effect on the growth of *Verticilium* spp. (Olmert and Kenneth, 1974). The organophosphate compounds directly interfere with cell wall formation due to inhibition of the enzyme that converts phosphatydilethanalamine into chitin. The same mechanism of inhibition was probably responsible for the drastic reduction in *B. bassiana* conidia germination, vegetative growth and sporulation (Ghini and Kimati, 2000). The conidial germination of *B. bassiana* (CG 425strain) was completely inhibited by triazophos, chlorpyrifos formulations. With regard to the concentration of the formulation, Amutha *et al*. (2010) rated chlorpyrifos 20EC as less toxic to *B. bassiana* while profenophos 50%EC was highly toxic. Usha *et al*. (2014) mentioned that chloropyrofos was extremely toxic to different isolates of *B. bassiana.*

The Lufenuron" benzoylureas" based formulation Lenoflag® acts as IGR by inhibiting synthesis of chitin (Ben-Ziony and Arzi, 2001). The present outcomes indicated that Lenoflag® was one of the compatible and germination stimulating formulations to both tested fungal isolates. Similar results were obtained by Scotty *et al*. (2005) who indicated that lufenuron had no effect on the vegetative growth of *Aspergillus* spp. and *Fusarium* spp. Hector *et al.* (2005) stated that lufenuron had no antifungal properties against *Coccidioides immitis* G.W. Stilies and *Aspergillus fumigates* Fresenius. Alves *et al*. (2011), mentioned that the compatibility of lufenuron with *M. anisopliae* was dose dependant since no interfered with conidia germination of *M. anisopliae* when used in a concentration of 1 mg/ml and increased it in a concentration of 700 µg/ml. Adversely, diflubenzuron showed high inhibition percentage to *L. lecanii* (Hall, 1981). Flufenoxuron inhibited the germination of *B. bassiana* by 96% compared to control (Alizadeh *et al.*, 2007), and hexaflumuron completely inhibited the germination of *M. anisopliae* (Rashid *et al*., 2010).

Concerning acaricide, (Arrow®), abamectine based ormulation was a potent stimulant of *B. bassiana* isolate, while mycelium of the same isolate was significantly inhibited at FR and half of FR concentrations. On the other direction, the same formulation significantly inhibited the conidial germination of *M. anisopliae* isolate, while mycelium was more tolerant to this formulation even at the FR concentration. De oliveira and Neves (2004) mentioned that fenpyroximate and abamectin caused a less detrimental effect on conidial germinationof *B. bassiana* than fenpyroximate. Conversely, abamectin had a more inhibitory effect on vegetative growth than fenpyroximate.

The Dormex®, hydrogen cyanide (HC), completely inhibited germination and vegetative growth of both tested isolates suggesting simultaneous use is best avoided. This formulation is frequently used to break dormancy in grapevine floral buds and various deciduous fruits and commonly used as a nitrogen fertilizer with herbicidal and fungicidal effects (Amberger, 2013). No literature mentioned the effect of this formulation on entomopathogenic fungi, however, Spence *et al*. (2014) found that

Magnaporthe oryzae (Hebert) M.E. Barr infecting rice was negatively impacted by hydrogen cyanide. The gibberillic acid formulation was compatible with tested isolates of *B. bassiana* and *M. anisopliae*. The Ethrel® formulation was compatible with *B. bassiana* isolate, and was a perceptible stimulant of its germination, while it reduced conidial germination of *M. anisopliae*. Conversely, the Magictone® had a significant inhibitory effect on conidial germination of *B. bassiana* isolate, and had a stimulatory effect on conidial germination of *M. anisopliae* isolate. Results partially agree with those obtained by Khan *et al*. (2012) who mentioned that, *In vitro*, naphthalene acetic acid, Ethrel® were compatible with *B.bassiana* and *M. anisopliae*. The vegetative growth of *B. bassiana* and *M. anisopliae* was enhanced by the plant growth regulator Ethrel, IAA (Indol acetic acid, and NAA (Naphthalene acetic acid).

Based on the previous studies, the stimulatory effect of certain formulations to conidial germination or vegetative growth of fungal isolate may be due to utilizing the released compounds from agrochemical metabolism or substances present in the agrochemical formulation and can be used directly as a nutrient by the fungus. Also, the fungus could be making a reproductive effort, thus increasing conidial production (Moino and Alves, 1998). On the other hand, Boucuas *et al.* (1998) indicated that either ionic or molecular formulations may neutralize the electrostatic charge of the surface and/or remove the mucous layer covering conidia, thus affecting the substrate recognition process and the transduction of the signal that initiates germination.

The contrasting themes between present results and previous researches outcomes may due to specific differences between tested fungal isolates, the type of formulation besides the concentration of active indigents in each formulation, 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). Adjutants 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*.

REFERENCES

Alizadeh, A.; Mohamad-Amin, S.; Masood, K.h. and Saberi-Riseh, R. (2007). Compatibility of *Beauveria bassiana* (Bals.) Vuill.with several pesticides**.** *International Journal of Agriculture and Biology,* 9 (1): 31–34.

Alves M. M.; Orlandelli, R. C.; Lourenço, D. A. L. and Pamphile, J. A. (2011). Toxicity of the insect growth regulator lufenuron on the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin assessed by conidia germination and speed parameter. *African Journal of Biotechnology,* 10(47): 9661-9667.

Alves, S. B. and Lecuona, R. E. (1998). Epizootiologia aplicadaao control emicrobiano, Applied epizootiology to microbial control. p. 97–157. In: Alves, S.B., ed. Controlemicrobiano de insetos - Microbial control of insects. Fealq, Piracicaba, SP, Brazil.

Amberger, A. (2013). Cyanamide in plant metabolism**.** *International Journal of Plant Physiology and Biochemistry***,** 5(1):1–10.

Amutha, M.; Banu, J. G.; 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(1):143 – 146.

Anderson, T. E. and Roberts, D. W. (1983). Compatibility of *Beauvaria bassiana*

isolates with insecticides formulations used in Colorado beetles (Coleoptera: chrysomelidae) control. *Journal of Economic Entomology,* 76: 1437-1441.

Anderson, T. E.; Hajek, A. E.; Roberts, D. W.; Preisler, K. and Robertson, J. L. (1989).Colorado potato beetle (Coleoptera: Chrysomelidae): Effects of combinations of *Beauveria bassiana* with insecticides. *Journal of Economic Entomology,* 82(1): 83-89.

Ben-Ziony, Y. and Arzi, B. (2000).Use of lufenuron for treating fungal infections of dogs and cats: 297 cases (1997-1999). *Journal of the American Veterinary Medical Association,* 217 (10): 1510–1513.

Celar, F. A. and Kos, K. (2020). *In vitro* compatibility of *Beauveria bassiana* strain ATCC 74040 with copper fungicides. *Journal of Applied Entomology,* 144: 388–395.

Challa, M. M. and Sanivada, S. K. (2014). Compatibility of *Beauveria bassiana*(Bals.) Vuill.isolates with selected insecticides and fungicides at agriculture spray tank dose. *Innovare Journal of Agricultural Science,* 2(3): 7–10.

Cuthbertson, A. G. S.; Walters, K. F. A. and Deppe, D. (2005).Compatibility of the entomopathogenic fungus *Lecanicillium muscarium* and insecticides for eradication of sweetpotato whitefly, *Bemisia tabaci*. *Mycopathologia,* 160: 35–41.

Da Silva, R. A.; Quintela, E. D.; Mascarin, G. M.; Barrigossi, J. A. F.and Luciano Moraes Lião, L. M. (2013). Compatibility of conventional agrochemicals used in rice crops with the entomopathogenic fungus *Metarhizium anisopliae* . *Scientia Agricola,* 70 (3):152-160.

De oliveira, R. C. and Neves, P. M. O. J. (2004). Compatibility of *Beauveria bassiana* with acaricides. *Neotropical Entomology,* 33(3):353-358.

De Oliveira, C. N.; Neves, P. M.and Lídio Sueki Kawazoe, L. S. (2003).Compatipility between the entomopathogenic fungus *Beauveria bassiana* and insecticides used in coffee plantations. *Scientia Agricola,* 60 (4): 663-667.

Draper, N. R., and Smith, H. (1981).Applied regression analysis, 2nd ed. John Wiley and Sons, Inc., N.Y. 709 pp.

Fabrice, C. E. S.; Tonussi, R. L.; Orlandelli, R. C.; Lourenço, D. A. L. and Pamphile, J. A. (2013). Compatibility of entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin with fungicide thiophanate-methyl assessed by germination speed parameter. *Journal of Agriculture and Environment,* 11 (1): 368 - 372.

Fiedler, Z. and Sosnowska, D. (2017). Side effects of fungicides and insecticides on entomopathogenic fungi in vitro. *Journal of Plant Protection Research,* 57 (4):355-360.

Hall, R. A. (1981). Laboratory studies on the effects of fungicides, acaricides and insecticides on the entomopathogenic fungus, *verticillium lecanii*. *Entomologia Experimentalis et Applicata,* 29: 39-48.

Hassan, S. A.; Bigler, F.; Bogenschütz, H.; Boller, E.; Brun, J.; Calis, J.N.M.; Chiverton, P.; Coremans-Pelseneer, J.; Duso, C.; Lewis, G. B.; Mansour, F.; Moreth, L.; Oomen, P. A.; Overmeer, W. P. J.; Polgar, L.; Rieckmann, W.; Samsoe-Petersen, L.; Stãubli, A.; Sterk, G.; Tavares, K.; Tuset, J. J. and Viggiani, G. (1991). Results of the fifth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. *Entomophaga,* 36:55-67.

Hector, R. F.; Davidson, A. P.; Johnson, S. M. (2005). Comparison of susceptibility of fungal isolates to lufenuron and nikkomycin Z alone or in combination with itraconazole. *American Journal of Veterinary Researc,* 66(6): 1090-1093.

Hedimbi, M.; Kaaya, G. P.; Singh, S.; Chimwamurombe, P. M.; Gindin, G.; Glazer, I. and Samish, M. (2008). Protection of *Metarhizium anisopliae* conidia from Ultra-violet radiation and their pathogenicity to *Rhipicephalus evertsi* ticks. *Experimental and Applied Acarology,* 146: 149-156.

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. *International Organization for Biological Control /WPRS Bulletin,* XI (3):148-151.

Jaros-Su, J.; Groden, E. and Zhang, J. (1999). Effects of selected fungicides and timing of fungicide application on *Beauveria bassiana* induced mortality of the colorado potato beetle (Coleoptera: Chrysomelidae). *Biological Control,* 15:259–269.

Joshi, M; Gaur, N. and Pandey, R. (2018). Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with selective pesticides. *Journal of Entomology and Zoology Studies,* 6(4): 867-872.

Khan, S.; Bagwan, N. B.; Fatima, S. and Iqbal, M. S. (2012). *In vitro* compatibility of two entomopathogenic fungi with selected insecticides, fungicides and plant growth regulators. *Libyan Agriculture Research Center Journal International,* 3 (1): 36-41.

Klingen, I., and Haukeland, S. (2006). The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes. An ecological and societal approach to biological control, eds.Eilenberg, J. and Hokkanen, H.M.T. Dordrecht, The Netherlands: Springer, pp. 145-211.

Kos, K. and Celar, F. A. (2013). Sensitivity of the entomopathogenic fungus Beauveria bassiana (Bals. -Criv.) Vuill. to selected herbicides. *Pest Management Science,* 69: 717–721.

Kouassi, M.; Coderre, D. and Todorova, S. I. (2003). Effects of the timing of applications on the incompatibility of three fungicides and one isolate of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina). *Journal of Applied Entomology,* 127: 421–426.

Majchrowicza, I. and Poprawski, T. J. (1993). Effects in vitro of nine fungicides on growth of entomopathogenic fungi. *Biocontrol Science and Technology,* 3: 321-336.

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

Martins, F.; Soares, M. E.; Oliveira, I.; Pereira, J. A.; Bastos, M. L. and Baptista, P. (2012). Tolerance and bioaccumulation of copper by the entomopathogen *Beauveria bassiana* (bals) vuill.exposed to various copper-based fungicides. *Bulletin of Environmental Contamination and Toxicology,* 89:53–60.

Mietkiewski, R. T.; Pell, J. K. and Clark, S. J. (1997). Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: Field and Laboratory Comparisons. *Biocontrol Science and Technology,* 7: 565-575.

Mochi, D. A.; Monteiro, A. C.; De Bortoli, S. A.; Doria, H. O. S. and Barbosa, J. C. (2006). Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.)

(Diptera: Tephritidae) in soil with different pesticides', *Neotropical Entomology*, 35:382-389.

Moino, J. R.; A.; Alves, A. S. B. (1998). Efeito de imicacloprid e fipronil sobre *Beauveria bassiana* (Bals.)Vuill.e *Metarhizium anisopliae* (Metsch.) Sorok.e no comportamento de limpeza de Heterotermes tenuis (Hagen). *Anais da Sociedade Entomológica do Brasil,* 27, 611-620.

Moorhouse, E. R.; Gillespie, A. T.; Sellers, E. K. and Charnley, A. K. (1992).Influence of fungicides and insecticides on the entomogenous fungus *Metarhizium anisopliaea* pathogen of the vine weevil, *Otiorhynchu ssulcatus*. *Biocontrol Science and Technolog*, 1 (2):49-58.

Olmert, I. and Kenneth, R. G. (1974). Sensitivity of the entomopathogenic fungi, *Beauveria bassiana*, *Verticillium lecanii* and *Verticillium* sp. to fungicides and insecticides. *Environmental Entomology,* 31: 33-38.

Ondráčková, E.; Seidenglanz, M. and Šafář, J. (2019). Effect of seventeen pesticides on mycelial growth of *Akanthomyces*, *Beauveria*, *Cordyceps* and *Purpureocillium* strains. *Czech Mycology*, 71(2): 123–135.

Pelizzaa, S. A.; Schalamukc, S.; Simónd, M. R.; Stengleine, S. A.; Pacheco-Marinof, S. G. and Scorsettib, A. C. (2018). Compatibility of chemical insecticides and entomopathogenic fungi for control of soybean defoliating pest, *Rachiplusia* nu. *Revista Argentina de Microbiología,* 50(2):189-201.

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.

Samuels, K.D.Z.; Pinnock, D. E. and Allsopp, P.G. (1989).The Potential of *Metarhizium anisopliae* (Metchnikoff) Sorokin (Deuteromycotina Hyphomycetes) as a Biological Control Agent of *Inopus rubriceps* (Macquart) (Diptera:Stratiomyidae *Journal of the Australian Entomological Society,* 28: 69-74.

Scotty, N. C.; Evans, T. J.; Giuliano, E.; Johnson, P. J.; Rottinghaus, G. E.; Fotheirgill, A. N. and Cutler, T. J. (2005). *In vitro* efficacy of lufenuron against filamentous fungi and blood concentrations after PO administration in horses. *Journal of Veterinary Internal Medicine,* 19: 878-882.

Shaha, F. A; Ansaria, M. A.; Watkinsa, J.; Phelpsa, Z.; Crossb, J. and Butta, T.M. (2009). Influence of commercial fungicides on the germination, growth and virulence of four species of entomopathogenic fungi. *Biocontrol Science and Technology,* 19, (7):743-753.

Gomez, S. D.R. (1991). Production of three *Hirsutella thompsonii* varieties on semisolid media and different effects of two fungicides. – *Anais da Sociedade Entomológica do Brasil* , 120: 155-163.

Gomez, S. D. R., Manzur, J., Nasca, A. J. (1987). Influence of some pesticides on the three varieties of *Hirsutella thompsonii* Fisher (Hyphomycetes: Moniliales). – *Anais da Sociedade Entomológica do Brasil,* 16: 399-408.

Spence, C.; Alff, E.; Johnson, C.; Ramos, C.; Donofrio, N.; Sundaresan, V. and Bais, H. (2014). Natural rice rhizospheric microbes suppress rice blast infections. *BMC Plant Biology*, 14: 1-17.

Storey, G. K. and Gardner, W. A. (1986). Sensitivity of the entomogenous fungus beauveria bassiana to selected plant growth regulators and spray additives. Appl. Environm. Microbiol., 52(1):1-3.

Tamai, M. A.; Alves, S. B.; Lopes, R. B., Faion, M. and Padulla, L. F. L. (2002). Toxicidade de produtos fitossanita´rios para Beauveria bassiana (Bals.).Vuilli. *Arquivos do Instituto Biologico,* 69(3) :89–96.

Tkaczuk, C. and Safaryan, M. A. (2020). Effect of selected fungicides on the growth of acaropathogenic fungi from the genus *Hirsutella*. *Applied Ecology and Environmental Research,* 18(3):3897-3905.

Tkaczuk, C.; Safaryan, M. A. and Głuszczak, B. (2016). The effect of selected pesticides approved for use in organic farming on the growth of entomopathogenic fungi [Wpływ wybranych środków ochrony roślin dopuszczonych do stosowania w uprawach ekologicznych na wzrost grzybów owadobójczych]. *Progress in Plant Protection,* 56 (3) 2016: 265-271.

Urs, N. V. R.; Govindu, H. C. and Shastry, K. S. S. (1967).The Effect of certain insecticides on the entomogenous fungi *Beauveria bassiana* and *Metarhzium anisopliae. Journal of Invertebrate Pathology,* 9: 398:403.

Usha, J.; Babu, M. N. and Padmaja, V. (2014). Detection of compatibility of entomopathopgenic funus *Beauveria bassiana* (Bals.) Vuill.with pesticides, fungicides and botanicals. *International Journal of plant, Animal Environmmental Sciences*, 4 (2): 612-624.

Vanninen, I. and Hokkanen, H. (1988). Effects of pesticides on four species of entomopathogenic fungi. *Annales Agricultural Fenniae,* 27: 345-353.

Wei, L.; Fang, W. X. and Cheng, F. S. (2004). Impact of sixteen chemical pesticides on conidial germination of two entomophthoralean fungi: *Conidiobolus thromboides* and *Pandora nouryi*. *Biocontrol Science and Technology*, 14 (7): 737-741.

White, T. j.; Bruns, T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In book: PCR - Protocols and Applications - A Laboratory Manual (pp.315-322). Publisher: Academic Press