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Pathogenicity of *Beauveria bassiana* and laboratory assessment with selective pesticides

S. M. Bisandre[a], **Y. V. Ingle**[b,*], **G. K. Lande**[c], **M. D. Giri**[d]

[a] Department of Plant Pathology, Dr. PDKV, Akola-444104 (MS) India
[b] AICRP on Fruits, Dr. PDKV, Akola-444104 (MS) India
[c] Department of Agricultural Entomology, Dr. PDKV, Akola-444104 (MS) India
[d] Pulses Research Unit, Dr. PDKV, Akola-444104 (MS) India

*Corresponding author, e-mail: yvingle@pdkv.ac.in

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ABSTRACT: *Beauveria bassiana* is one of the most promising entomopathogenic fungus species utilized for pest control. The pathogenicity of *B. bassiana* was tested by spraying spore suspension directly on third instar larvae of *Helicoverpa armigera*. The highest larval mortality (80.00%) was achieved by application of spore load of 10^{11} conidia/ml. It is needed to determine the effects of pesticides on *B. bassiana* as it is to be included in a pest management program. The compatibility of fungi with selected pesticides commonly used in plant protection was investigated in this study. Nine different insecticides were evaluated at two different doses (recommended dose and half of recommended dose) under *in vitro* condition against *B. bassiana* by poison food technique. Fipronil and spiromesifen significantly supported the maximum growth (4.03 and 3.85 cm, respectively) of *B. bassiana* at half of recommended dose, while the remaining insecticides appeared to be toxic to the tested fungus. Ten different fungicides were also tested *in vitro* against *B. bassiana* at half and recommended doses; potassium phosphonate supported maximum radial mycelial growth of 2.98 cm (at half of recommended dose) and 2.36 cm (at recommended dose), whereas carbendazim + mancozeb and fluxapyroxad + pyraclostrobin appeared harmful by restricting the growth of the *B. bassiana* at both doses. This study demonstrated the varying pesticide effects on fungi; however, their actual effects at the cellular and field levels must be investigated to determine whether the effects are permanent or temporary.

KEYWORDS: *Beauveria bassiana*, compatibility, fungicides, insecticides

INTRODUCTION

India has rich entomopathogen biodiversity, and exploitation of these natural and renewable resources is considered for a successful biocontrol strategy. Cultural production and cryopreservation of blastospores of these fungi have been reported [1]. Entomogenous fungi can be the most adaptable biological control agents due to their diverse host range, resulting in natural epizootics. One appealing feature of these fungi is infectivity by direct contact, and actual action is through penetration [2]. These fungi are highly diverse and have over 100 genera and 750 species reported from various insects [3, 4]. An entomopathogenic fungus, *B. bassiana* (Balsamo) Vuillemin, has the potential to infect many arthropods with an ability to infect more than 200 species of insects and acaridae [5, 6]. *Beauveria* comes under Ascomycota, Order Hypocreales, Family Clavicipitaceae [7].

There are numerous examples where the combination of selective chemical pesticides and fungi provides satisfactory control of many agricultural insect pests. Combined utilization of selective pesticides and entomopathogens can enhance the management efficiency by reducing the pesticide amount and minimizing environmental contamination and risk of pest resistance [8]. The use of entomopathogens with full or reduced doses of chemical pesticides is a promising pest-control option for minimizing adverse chemical effects. Information on compatibility of entomopathogenic fungi with pesticides may aid in the selection of appropriate products for integrated pest management (IPM) programs that include the fungus as an important pest control agent [9].

To understand at what extent certain agrochemicals inhibit the development and reproduction of entomopathogenic fungi, the information on the compatibility of entomopathogenic fungi with agrochemicals is urgently required. Data generated from such studies would certainly be helpful to the farmers in selecting appropriate compounds and plan application schedule of microbial and agrochemical treatments to reap compatible set's benefits. If *B. bassiana* is to be incorporated into a pest management program, it is also necessary to determine the effects of pesticides on it. Many insecticides and fungicides have broad spectrum activities, and hence entomopathogen suppression can be anticipated. Therefore, understanding the adverse effects of different insecticides and fungicides on entomopathogenic fungi is highly essential. The main purpose of this study was to screen some of the broad-spectrum insecticides and fungicides that are commonly used in a variety of field crops to reduce pest and disease incidence.

MATERIALS AND METHODS

B. bassiana isolate (PDKV Bb-1) used in this study had initially been isolated from naturally mycosis larvae of *H. armigera*. The fungus was grown on SDAY (Sabouraud's dextrose with 1% yeast extract agar medium), and conidia produced were used for studies.

Pathogenicity test

The pathogenicity test of *B. bassiana* was carried out using the procedure mentioned earlier [10]. The fresh fungal spores of *B. bassiana* were harvested in 25–30 ml of sterilized distilled water containing 0.05% Tween 20 and vortexed for one min to produce homogenous suspension. The conidial suspension was then diluted with sterile distilled water to make concentrations of 10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{11} conidia/ml by serial dilution and topically applied on third instar larvae (7.5 to 13 mm length with the average of 280 to 286 mg weight) of *H. armigera* (Hubner). Before spraying spore suspension on insect body, all instar larvae were treated with 1% sodium hypochloride solution and rinsed gently twice with distilled water. The excess water was removed with blotting paper. Ten larvae were taken in a plastic Petri dish which was lined by a filter paper at the bottom for absorbing excess moisture. Ten ml of six different concentrations, viz., 10^6, 10^7, 10^8, 10^9, 10^{10}, and 10^{11} conidia/ml were directly sprayed on the larvae using a hand atomizer. The treated larvae were then transferred to each plastic Petri dish containing fresh chickpea seed. These Petri dishes were then kept inside a BOD incubator at 25 ± 1 °C. The larval mortality was recorded at 24 h interval until 10 days of treatment. The percent larval mortality due to mycosis was calculated. Development of symptoms was recorded up to ten days. The isolate was compared to the original culture of *B. bassiana* after re-isolation on SDAY medium.

In vitro compatibility of *B. bassiana* with insecticides and fungicides

Insecticides and fungicides (Tables 1 and 2) were assessed at two levels, i.e. recommended dose (RD) and half of the recommended dose (HD). The 100 ml of Sabouraud dextrose with 1% yeast extract agar medium (SDYA) was transferred into 250 ml conical flask, then autoclaved at 1.04 kg/cm^2 for 15 min. The desired concentrations were obtained by adding a sufficient amount of insecticides to SDYA medium by poison food technique [11] in Petri plates. Following media solidification, each plate was inoculated with a small disc (1 mm deep, 6 mm diameter) of SDYA with *B. bassiana*, which was deposited in the centre of each plate containing the mixture of SDYA and pesticide. Each pesticide concentration in combination with fungus and corresponding control was replicated three times. Non-insecticide and fungicide SDYA medium was inoculated with *B. bassiana* as the control. The plates were sealed with parafilm and incubated at 25 ± 1 °C in an incubator for 10 days. The colony diameter was measured with a ruler (cm) at 10 days after inoculation (DAI). Treatment data were compared to those of the control to determine the percentage value of inhibitory growth. The percent inhibition of mycelial growth was calculated by following the previous formula [12].

Pesticides were further classified into evaluation categories based on a 1–4 scoring index. According to the classification by Hassan [13], they can be categorized as 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50–79%), 3 = moderately harmful (80–90%), and 4 = harmful (>90%) in *in vitro* toxicity tests. A completely randomized design (CRD) was used in all experiments. The data of each character were subjected to ANOVA, and means were compared by critical difference ($p = 0.01$) [14].

RESULTS AND DISCUSSION

Pathogenicity test of *B. bassiana* against *H. armigera*

The pathogenicity of *B. bassiana* was tested against third instar larvae of *H. armigera* by direct spraying of spore suspension on the insect's body at concentrations ranging from 10^6 to 10^{11} spore/ml. The larval mortality was recorded up to 10 days, and cumulative mean percent larval mortality was calculated. The isolate of *B. bassiana* was found pathogenic to the tested insect (Table 3). The isolate of *B. bassiana* showed pathogenicity towards *H. armigera* with varying mortality rates at different concentrations. The highest percentage of larval mortality (80.00%) was achieved by application of spore load of 10^{11} spores/ml, and the lowest (43.33%) was achieved at the lowest concentration of 10^6 spores/ml. No larval mortality was recorded in control. Data indicated that susceptibility of the larvae was positively associated with spore concentration; a decrease in concentration corresponded to decrease in mortality.

From these findings, it can be inferred that *H. armigera* appeared to be susceptible to *B. bassiana*, as significant mortality was found in the tested instar larvae. There are great variations in the doses of application used by the different workers for evaluating pathogenic abilities against different pests under laboratory and field conditions. The topical inoculation method was used to test efficacy of *B. bassiana* against *H. armigera* third instar larvae at various concentrations, including 10^6, 10^7, 10^8, 10^9, 10^{10}, and 10^{11} spores. The highest percent mortality (80.0%) was observed for third instar larvae at 10^{11} spores/ml, indicating that larval mortality increased in accordance with the increase in concentrations of fungal spore suspension.

Results obtained in this investigation are also sup-

Table 1 Insecticides and their doses used in this study.

SN	Chemical name	Trade name	Formulation	Conc. (%)	
				RD	HD
1	Imidacloprid 17.8%	Confidor	Soluble concentrate	0.1	0.05
2	Quinolphos 25% EC	Celquin	Emulsifiable concentrate	0.2	0.1
3	Chlorpyriphos 20% EC	Tricel	Emulsifiable concentrate	0.1	0.05
4	Thiamethoxam 12.6% + Lambda cyhalothrin 9.5 ZC	Alika	A mixed formulation of capsule suspension and suspension concentrate	0.1	0.05
5	Dicofol 18.5% EC	Difol	Emulsifiable concentrate	0.2	0.1
6	Fipronil 5% SC	Regent	Suspension concentrate	0.25	0.125
7	Ethion 50% EC	Fosmite	Emulsifiable concentrate	0.25	0.125
8	Dimethoate 30% EC	Tafgor	Emulsifiable concentrate	0.1	0.05
9	Spiromesifen 22.9% SC	Oberon	Suspension concentrate	0.12	0.06

RD (recommended dose); HD (half of the recommended dose).

Table 2 Fungicides and their doses used in this study.

SN	Chemical name	Trade name	Formulation	Conc. (%)	
				RD	HD
1	Propineb 70%	Antracol	Wettable powder	0.3	0.15
2	Copper oxychloride 50%	Cuprina	Wettable granules	0.3	0.15
3	Fosetyl-Al 80%	Aliette	Wettable powder	0.25	0.125
4	Metalaxyl 8% + mancozeb 64%	JU-Redomil	Wettable powder	0.25	0.125
5	Carbendazim 12% + mancozeb 63%	Nagarjuna combi plus	Wettable powder	0.2	0.1
6	Cymoxanil 8% + mancozeb 64%	Bullet	Wettable powder	0.25	0.125
7	Azoxystrobin 23%	Amistar	Suspension concentrate	0.1	0.05
8	Metalaxyl 3.3% + chlorothalonil 33.1%	Folio gold	Suspension concentrate	0.2	0.1
9	Fluxapyroxad167G/L + pyraclostrobin333G/L	Priaxor	Suspension concentrate	0.2	0.1
10	Potassium phosphonate (potassium salt of phosphonic acid 98%)	K-Phonic	Water soluble powder	0.3	0.15

RD (recommended dose); HD (half of the recommended dose).

Table 3 Pathogenicity of *B. bassiana* against *H. armigera*.

Entomopathogenic fungi	Conc. (conidia/ml)	No. of larva		%Mortality (10 DAI)
		treated	mycosis	
B. bassiana	10^6	30	13	43.33
	10^8	30	15	50.00
	10^9	30	19	63.33
	10^{10}	30	21	70.00
	10^{11}	30	24	80.00
Control	Tween 80 (0.05%)	0.00	0.0	

ported by other studies [10, 15], where *B. bassiana* also appeared pathogenic to all stages of *H. armigera* and caused 60–100% mortality. Topical application of *B. bassiana* also found effective on fourth instar larvae of *H. armigera* (Hubner) when tested with four different concentrations (0.1, 0.125, 0.2, and 0.25 ml $\times 10^8$ conidia/ml) [16], and 76.70% mortality was observed at the highest dose (0.25 ml $\times 10^8$ conidia/ml) in 3 to 6 days of incubation. Similarly, Prabhukarthikeyan et al [17] observed 72.59% mortality of third instar *H. armigera* larvae after 4.59 days at a concentration of 1×10^8 conidia/ml. The reduction in spore concentrations resulted in a significant reduction in percent mortality [18, 19]. The percent mortality levels from different studies are not comparable precisely because of various factors such as different strains and concentrations, diverse ecological situation, and local test insect culture. Differential response of various strains of *B. bassiana* on *H. armigera* is also attributed to differences in level of virulence against different hosts and the source of *B. bassiana* isolates [20, 21]. The previous study [22] confirmed the current investigation by reporting variation in virulence with different hosts among different isolates of *N. rileyi* collected from different geographical areas. Similarly, the Ecuador and Mississippi biotypes of *Nomuraea rileyi* with the different pathogenic abilities were reported [23]. Differences in the level of virulence of isolates may be due to microbe's selection, recombination and mutation as isolates were collected from diversified ecological situation might have influenced their genetic make-up.

In vitro compatibility of *B. bassiana* with different insecticides

Except for the control plate, all insecticides partially or completely inhibited the mycelial growth of *B. bassiana* at both concentrations (Table 4). Fipronil was the least toxic insecticide tested against *B. bassiana*, followed

Table 4 *In vitro* compatibility of *B. bassiana* with different insecticides at 10 DAI.

SN	Insecticide	Half of the recommended dose			Recommended dose		
		Radial mycelial growth (cm)*	% Growth inhibition	Grade†	Radial mycelial growth (cm)*	% Growth inhibition	Grade†
1	Imidacloprid 17.8% SL	3.72[b]	58.66	2	2.27[b]	74.77	2
2	Quinalphos 25% EC	2.28[c]	74.66	2	1.63[c]	81.88	3
3	Chlorpyriphos 20% EC	3.42[b]	62.00	2	1.59[c]	82.33	3
4	Thiamethoxam 12.6% SL + Lambda Cyhalothrine 9.5% ZC	3.37[b]	62.55	2	1.38[c]	84.66	3
5	Dicofol 18.5% EC	2.06[c]	77.11	2	0.00	100	4
6	Fipronil 5% EC	4.03[b]	55.22	2	1.79[c]	80.11	3
7	Ethion 50% EC	2.95[c]	67.22	2	0.00	100	4
8	Dimethoate 30% EC	2.55[c]	71.66	2	0.00	100	4
9	Spiromesifen 22.9% SC	3.85[b]	57.22	2	2.40[b]	73.33	2
10	Control	9.00[a]			9.00[a]		
	SE(m) ±	0.26			0.11		
	CD ($p = 0.01$)	1.07			0.46		

* Average of three replications; DAI, days after inoculation; means followed by the same letter do not differ significantly at the 0.01 probability level.

† Grade: 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50–79%), 3 = moderately harmful (80–90%), 4 = harmful (>90%) in *in vitro* toxicity tests.

by spiromesifen, imidacloprid, chlorpyriphos, and thiamethoxam at HD dose. The maximum radial mycelial growth was observed in fipronil (4.03 cm), and percent growth inhibition was 55.22% at HD dose. Insecticides including spiromesifen, imidacloprid, chlorpyriphos, and thiamethoxam + lambda cyhalothrine showed minimum inhibitory to *B. bassiana* as they exhibited 3.85, 3.72, 3.42, and 3.37 cm colony growth with the inhibition of 57.22, 58.66, 62.55, and 62.00%, respectively, at HD dose. At RD dose, fipronil appeared moderately toxic to the test fungus; however, spiromesifen significantly supported the maximum growth (2.40 cm) of *B. bassiana* over the rest of the insecticides, followed by imidacloprid. Other insecticides were found to be harmful to the entomopathogen as recorded that there was more than 50% inhibition in growth at RD dose. Dicofol, ethion, and dimethoate were harmful at RD dose and showed 100% growth inhibition. Fipronil 5% EC, spiromesifen 22.9% SC, imidacloprid 17.8% SL, chloropyriphos 20% EC, and thiamethoxam 12.6% SL + lambda cyhalothrine 9.5% ZC at HD dose showed the highest radial mycelial growth for *B. bassiana* when compared to the control. Robust study should be undertaken on this line to validate the results reported in our study at field level, and harmful effects of entomopathogen (if any) on beneficial insects also need to be tested.

The results of our experiments were consistent with prior findings, indicating potential effects of insecticides on *B. bassiana* under laboratory. Isaiah et al [24] demonstrated that the increased concentration of pesticides reduced the radial development of *B. bassiana*. We found that spiromesifen (ketoenol insecticide) and imidacloprid (neonicotinoid insecticide) were comparatively more compatible at their tested doses than other chemical insecticides. Effects of imidacloprid at different doses: (0.5 × DF (dose of field), 1 × DF, and 2 × DF) investigated against *B. bassiana* [25] showed that all three doses of imidacloprid had the lowest inhibitory effect (< 22%). In previous studies, *B. bassiana* were found to be highly compatible with the insecticides such as imidacloprid and spinosad with no inhibition of growth, sporulation, and viability [26]. Imidacloprid was neurotoxic to insects but had no adverse effect on *B. bassiana* with ability to metabolize and liberate compounds as secondary nutrients [27]. When used with *B. bassiana*, chemical pesticides such as thiamethoxam, fipronil, and spiromesifen showed satisfactory compatibility [28–31]. In this study, dicofol (organochlorine insecticide), ethion, quinolphos, chloropyriphos, and dimethoate (organophosphorus insecticide) had the most negative effect on *B. bassiana* vegetative growth most likely because it acts both by contact and through ingestion with inhibitory activity towards the fungal enzymes that cause decreased or delayed growth, abnormalities of cellular metabolic pathways, and damage of cellular structures. Insecticides may interfere with the uptake of carbohydrates and nitrogen from exogenous sources (media), which are required for the growth and sporulation of entomogenous fungi [32].

Compatible combination can lower the cost of cultivation by shortening the time required to apply each component separately. The observed variations in the inhibitory potential could be attributed to the inherent variability of chemical insecticides in their interactions with biocontrol agents. The inhibitory potential of these chemicals varies both between and

Table 5 *In vitro* compatibility of *B. bassiana* with different fungicides at 10 DAI.

SN	Fungicide	Half of the recommended dose			Recommended dose		
		Radial mycelial growth (cm)*	% Growth inhibition	Grade†	Radial mycelial growth (cm)*	% Growth inhibition	Grade†
1	Propineb 70% WP	1.20[d]	86.66	3	0.00	100	4
2	Copper oxychloride 50% WG	2.18[c]	75.77	2	0.00	100	4
3	Fosetyl-Al 80% WP	2.70[b]	70.00	2	1.34[c]	85.11	3
4	Metalaxyl 8% + mancozeb 64% WP	1.32[d]	85.33	3	0.00	100	4
5	Carbendazim 12% + mancozeb 63% WP	0.00	100	4	0.00	100	4
6	Cymoxanil 8% + mancozeb 64% WP	1.87[c]	79.22	2	1.17[c]	87.00	3
7	Azoxystrobin 23% SC	1.49[d]	83.44	3	0.73[d]	91.88	4
8	Metalaxyl 3.3% +Chlorothalonil 33.1% SC	1.42[d]	84.22	3	0.00	100	4
9	Fluxapyroxd 167G/L + pyraclostrobin 333G/L SC	0.00	100	4	0.00	100	4
10	Potassium phosphonate	2.98[b]	66.88	2	2.36[b]	73.77	2
11	Control	9.00[a]			9.00[a]		
	SE(m) ±	0.09			0.11		
	CD ($p = 0.01$)	0.39			0.47		

* Average of three replications; DAI, days after inoculation; means followed by the same letter do not differ significantly at the 0.01 probability level.

† Grade: 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50–79%), 3 = moderately harmful (80–90%), 4 = harmful (>90%) in *in vitro* toxicity tests.

within chemical classes [33]. A given insecticide may have different antifungal effects on the fungus at different stages of the pest [34]. Potential inhibitory effects of pesticides on germination and mycelia growth of biocontrol fungi differ between taxa and strains [35]. Moreover, results may differ in the field because fungi are exposed to pesticides at their highest levels *in vitro*, which do not occur in the field. Incompatible pesticides may inhibit the development and reproduction of entomopathogens such as *B. bassiana*, limiting their potential use in IPM strategies.

In vitro compatibility of *B. bassiana* with different fungicides

Radial mycelial growth of *B. bassiana* was significantly affected by all fungicides used. The *B. bassiana* radial mycelial growth was least inhibited by potassium phosphonate (66.88%), followed by fosetyl-Al (70.0%), copper oxychloride (75.77%), and cymoxanil 8% + mancozeb (79.22%) and recognized as slightly harmful as per Hassan classification equation at HD dose. The moderately harmful fungicides included azoxystrobin (83.44%), metalaxyl + chlorothalonil (84.22%), metalaxyl + mancozeb (85.33%), and propineb (86.66%) whereas carbendazim + mancozeb and fluxapyroxd + pyraclostrobin were observed as high inhibitory as recorded of 100% inhibition of the test fungus even at HD dose (Table 5).

At RD dose, maximum mycelial radial growth of 2.36 cm was achieved in potassium phosphonate with 73.77% growth inhibition of *B. bassiana*. Fosetyl-Al and cymoxanil + mancozeb were found to be moderately harmful to *B. bassiana* with mycelial radial growth of 1.34 and 1.17 cm and 85.11% and 87.00% growth inhibition, respectively. However, propineb, copper oxychloride, metalaxyl + mancozeb, carbendazim + mancozeb, metalaxyl + chlorothalonil, and fluxapyroxd + pyraclostrobin were found to be more toxic, exhibiting 100% inhibition at RD dose.

In conclusion, our research revealed significant variation in the sensitivity of the entomopathogenic fungus, *B. bassiana*, to the chemical fungicides tested. The experiment results revealed a significant reduction in case of radial growth of *B. bassiana* treated with fungicides at RD dose, whereas potassium phosphonate showed relatively slight fungal inhibition at both HD and RD doses. Propineb, copper oxychloride, azoxystrobin, metalaxyl + mancozeb, carbendazim + mancozeb, metalaxyl + chlorothalonil, and fluxapyroxd + pyraclostrobin are all broad-spectrum fungicides and have both protective and curative actions that are expected to inhibit *B. bassiana* growth. These fungicides may inhibit fungus development by interfering with spindle formation during mitosis during cell division, preventing germ tube development, appressorium formation, and mycelial growth. In the presence of fungicides, Butters et al [36] observed an abnormal bent appearance of the germination peg of *B. bassiana* with bursting at the tip. Similarly, total inhibition of mycelial growth was observed in at tested concentrations in the current study. The fungicides inhibited the vegetative growth of *B. bassiana* to varying degrees depending on the chemical nature and concentrations of the active compounds. The current findings are strongly supported by the work from Machowicz and Stefaniak [37] reporting that fungicides such as copper oxychloride, carbendazim, thiram, and benomyl were incompatible with *B. bassiana*. Metalaxyl + mancozeb,

chlorothalonil, maneb, thiophanate-methyl, and zineb were found to inhibit the growth of *B. bassiana* [38]. Similarly, Wari et al [39] demonstrated that fungicides such as metalaxyl + mancozeb, kasugamycin + copper oxychloride, azoxystrobin, difenaconazole, and iprodione inhibited mycelial growth of *B. bassiana* (GHA strain).

We have demonstrated that metalaxyl + chlorothalonil and fluxapyroxd + pyraclostrobin were incompatible with *B. bassiana* and inhibited its growth completely or strongly. As per the existing literature search, we have shown for the first time that metalaxyl + chlorothalonil and fluxapyroxd + pyraclostrobin were not safe for *B. bassiana* at both HD and RD doses.

Potassium phosphonate is a mono- and dipotassium salt of phosphonic acid that is mostly used to treat *Pythium* and *Phytopthora* induced diseases and has unique symplastic ambimobility. It primarily acts as a systemic resistance inducer expressing the defensive molecules such as phytoalexins and pathogen related (PR) proteins to block the pathogen directly. When considering their mode of action, it is possible that the tested fungus is not adversely affected in the amended medium. Compatibility between potassium phosphonate and *B. bassiana* may be due to specific action of the active ingredients against the fungi like organisms, oomycetes. This fungicidal effect on *B. bassiana* has not been documented to our knowledge. Our results demonstrated that potassium phosphonate is compatible with *B. bassiana*. The availability of fungicides that are compatible with *B. bassiana* has important agronomic implications as it allows IPM programs to combine the use of the entomogenous fungus and fungicides to control both insects and diseases.

CONCLUSION

We have demonstrated that insecticides such as imidacloprid 17.8% SL and spiromesifen 22.9% SC as well as the fungicides including potassium phosphonate were compatible with *B. bassiana* isolate (PDKV Bb-1) at both HD and RD doses, while the rest of the insecticides and fungicides inhibited its development slightly to strongly. In integrated pest management, these formulations could be used in conjunction with this insect pathogenic fungus.

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