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RESEARCH ARTICLE

Compatibility of the Entomopathogenic Fungus *Beauveria bassiana* with Some Fungicides Used in California Strawberry

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Abstract:

Background:

Entomopathogenic fungus *Beauveria bassiana* is pathogenic to several arthropod pests of strawberry in California and an important part of integrated pest management choices. However, fungicides applied for managing foliar and fruit diseases could interfere with the efficacy of *B. bassiana*.

Objective:

To determine the compatibility of some commonly used fungicides in strawberry with *B. bassiana*.

Method:

Laboratory assays were conducted using the larvae of the yellow mealworm, *Tenebrio molitor*. Mortality and infection caused by *B. bassiana* in *T. molitor* was compared in the presence and absence of seven fungicides that have different modes of action.

Result:

Mortality *T. molitor* larvaedue to *B. bassiana* was significantly affected in the presence of fungicides, myclobutanil (Rally) and captan (Captan). A significant reduction in the infection also occurred in the presence of captan, myclobutanil, and sulfur (Microthiol Disperss). Fenhexamid (Elevate) and pyraclostrobin + boscalid (Pristine) were the most compatible fungicides.

Conclusion:

Using certain fungicides with *B. bassiana* can be detrimental to the efficacy of the latter. However, certain fungicides are compatible with *B. bassiana*. These results help identify appropriate fungicides to be used when *B. bassiana* is applied for pest management.

Keywords: *Beauveria bassiana*, Fungicide, Compatibility, Strawberry.

INTRODUCTION

Several arthropod pests infest strawberry crop in California and cause a significant yield loss according to Zalom, Bolda, Dara, and Joseph [1]. Strawberry is an important commercial crop and the annual report from California Department of Pesticide Regulations [2] indicates that large quantities of acaricides, fungicides, herbicides, and insecticides are applied to manage a variety of pests. Chemical acaricides and insecticides are mainly applied to manage the twospotted spider mite, *Tetranychus urticae* Koch and the western tarnished plant bug, *Lygus hesperus* Knight. Dara [3 - 5] demonstrated that entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin, *Isaria fumosorosea* Wize, and *Metarhizium brunneum* Petch. can play an important role in integrated pest management (IPM) and promote sustainable agriculture. However, growers frequently apply fungicides to manage foliar and fruit diseases

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and the potential negative impact of these fungicides on the efficacy of entomopathogenic fungi is a concern for using these beneficial fungi for arthropod pest management. Sulfur and captan are the most widely used fungicides and in general, fungicide use surpasses that of acaricides and insecticides in California strawberries [2]. Earlier studies by Moorhouse, Gillespie, Seller, and Charnley [6], Jaros-Su, Groden, and Zhang [7], Luz, Netto, and Rocha [8], and Brook [9] evaluated the impact of some fungicides on the fungal growth and pest control efficacy of *B. bassiana* or other entomopathogenic fungi through invitro or potted plant studies in multiple crop or pest situations. However, no information specific to the fungicides used in California strawberries is available, especially after the release of some new fungicides in the recent years.

A laboratory study was conducted in 2012 to evaluate the compatibility of *B. bassiana* with seven fungicides that are commonly used in California strawberries. Results are expected to generate information to help growers make appropriate treatment decisions while choosing fungicides for disease management and *B. bassiana* for arthropod pest management in strawberry and other crops where these fungicides are used.

MATERIALS AND METHODS

The larvae of the yellow mealworm, *Tenebrio molitor* L. were used as the host insects to evaluate the pathogenicity of *B. bassiana* alone and in the presence of fungicides. A total of nine treatments including an untreated control were assessed in the study (Table **1**). Medium sized *T. molitor* larvae of about 20 mm long were ordered from a commercial supplier (Fluker Farms, Port Allen, LA, USA) and kept at room temperature (26-27 ^{o}C) for two days prior to using them in the assay. Using the field application rates, 250 ml of each treatment liquid was prepared using deionized water and poured into separate spray bottles.

Table 1. List of treatments with active ingredients, Fungicide Resistance Action Committee (FRAC) mode of action groups, and field application rates.

Treatments/Trade Names		Active Ingredient	FRAC MoA Group	Application Rate/Acre
1*	Untreated control	Untreated	-	-
2	Mycotrol-O	*B. bassiana*	-	1 qt (946.3 ml) in 100 gal (378.4 L)
3	Captan 80 WDG	Captan	Y	3.75 lb (1.7 kg) in 40 gal (151.4 L)
4	Elevate 50 WDG	Fenhexamid	17	1.5 lb (0.68 kg) in 50 gal (189.3 L)
5	Microthiol Disperss	Sulfur	Y	10 lb (4.5 kg) in 60 gal (227.1 L)
6	Pristine	Pyraclostrobin (strobilurin fungicide) + Boscalid (carboximide fungicide)	11	23 oz (652 g) in 100 gal (378.4 L)
			7	
7	Quintec	Quinoxyfen	13	6 fl oz (177.4 ml) in 40 gal (151.4 L)
8	Rally 40 WSP	Myclobutanil	3	5 oz in 100 gal (378.4 L)
9	Switch 62.5 WG	Cyprodinil (aniline-pyrimidines) + Fludioxonil (phenylpyrroles)	9	14 oz (397 g) in 40 gal (151.4 L)
			12	

*Treatments 3-9 have Mycotrol-O along with respective fungicides.

One gallon plastic tubs with screening on the lid were used for treating *T. molitor* larvae. A clean paper towel was placed in the bottom of the tub and sprayed with about 5 ml of the respective fungicides in treatments [3 - 9]. Deionized water was used for the untreated control. Paper towels were allowed to dry before applying 5 ml of *B. bassiana* (Mycotrol-O). A carrot piece, approximately 3 cm long, was cut further into small pieces and placed in one end of the tub. Forty uniformly sized *T. larvae* were placed at the other end of the tub on the treated paper towel allowing them to crawl towards the food source and get more exposure to the treated surface. After a 24-hour exposure to the inoculum, larvae were transferred individually to 5 dram Plexiglas vials with the help of forceps. Each vial had a small piece of carrot and the larva was secured inside by covering the vial with a foam plug. Larvae were incubated at room temperature (26-27 ^{o}C), mortality was recorded daily for seven days, and additional pieces of carrots were placed as needed.

To determine the level of infection, cadavers of the larvae were surface sterilized in 3% sodium hypochlorite solution followed by rinsing in deionized water and incubating on a selective medium (oatmeal agar amended with dodine and crystal violet, according to Chase, Osborne, and Ferguson [10]). Surface sterilization ensures that *B. bassiana* emerging from the cadavers is due to the infection and not saprophytic growth on the surface. Not all insects killed by the fungus show symptoms of infection and the level of infection measured in the study represents fungal sporulation from the cadavers.

Assays were repeated six times over a period of about 3 months. Data were analyzed using one-way analysis of variance. Percent mortality and infection were subjected to arcsine transformation and significant means were separated using Tukey's HSD test.

RESULTS AND DISCUSSION

There was a significant difference ($P < 0.00001$) in the mortality and infection caused by *B. bassiana* (Mycotrol-O) in the presence of various fungicides (Fig. **1**). Effect of the assay on the treatments was not statistically significant ($P > 0.1$). Most of the mortality occurred between 4 and 5 days after the larvae were incubated individually in the vials (Fig. **2**). Captan (Captan) and myclobutanil (Rally) severely affected the mortality in *T. molitor* larvae, which was significantly less than the mortality caused by *B. bassiana* alone. Pyraclostrobin + boscalid (Pristine) and fenhexamid (Elevate) were the most compatible where *B. bassiana* caused 93 and 86% mortality, respectively, which was higher than 70% mortality caused by *B. bassiana* alone. The negative impact of cyprodinil + fludioxonil (Switch) and sulfur (Microthiol Disperss) was moderate compared to others.

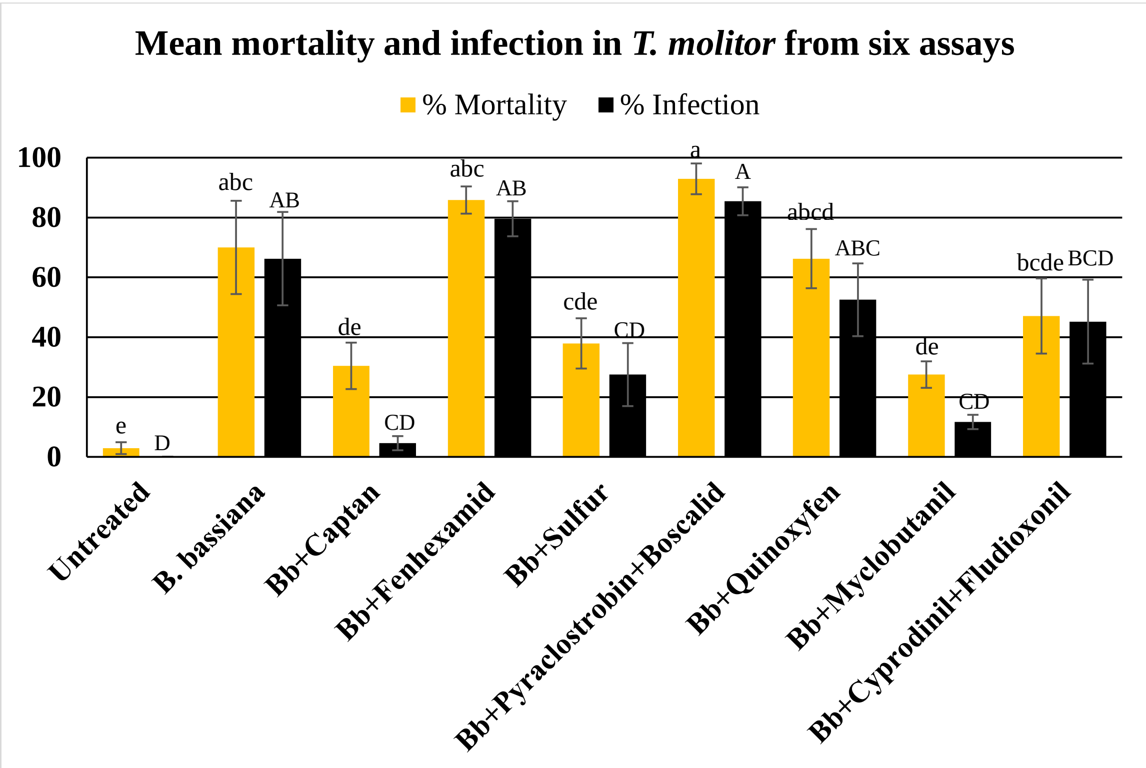

Fig. (1). Mortality and infection caused by *Beauveria bassiana* in *Tenebrio molitor* in the absence and presence of different fungicides. Bars with the same letters (lowercase for mortality and uppercase for infection) are not significantly different using Tukey's HSD test ($P < 0.00001$).

When infection was compared, captan (~5%) and myclobutanil (~12%) had the highest impact followed by sulfur (~28%). The highest level of *B. bassiana* infection occurred in the presence of pyraclostrobin + boscalid (~85%) and fenhexamid (~80%). Infection from *B. bassiana* alone was about 66%. While mortality of the target pest is the primary objective in pest management, sporulation from the infected cadavers could help spread the inoculum in the crop habitat and promote longer control. So, the impact of fungicides on *B. bassiana* infection is also an important aspect to consider.

Jaros-Su, Groden, and Zhang [7] studied the compatibility of *B. bassiana* with some fungicides used for potato disease management. They found out that copper hydroxide fungicide was less harmful to *B. bassiana* than chlorothalonil and mancozeb. These fungicides have multi-site contact activity against fungi, but differed in their compatibility with *B. bassiana*. Interaction between the fungicides and *B. bassiana*, and the resulting mortality of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) varied depending on application rates, intervals, and other factors. An earlier study by Loria, Galaini, and Roberts [11] also reported the detrimental effect of mancozeb and metiram on *B. bassiana*. Chlorothalonil, which has a similar mode of action as mancozeb and metiram, and metalaxyl, which affects RNA polymerase were not harmful to *B. bassiana*.

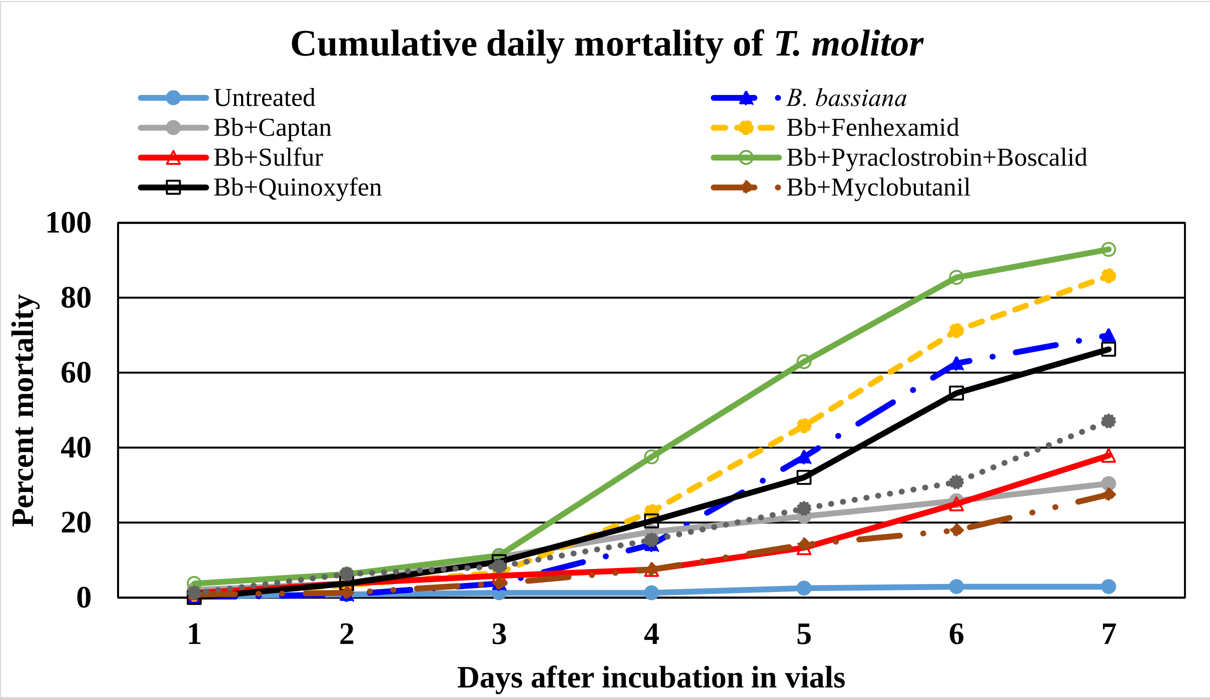

Fig. (2). Mean cumulative daily mortality in *Tenebrio molitor* caused by *Beauveria bassiana* in the absence and presence of difference fungicides.

In a more recent study, Shah, Ansari, Watkins, Phelps, Cross, and Butt [12] evaluated the effect of 15 fungicides on the conidial germination, mycelial growth, and virulence of *B. bassiana*, *I. fumosorosea*, *Lecanicillium longisporum* (Petch) Zare & Gams, and *M. anisopliae* (Metchnikoff) Sorokin. Some of these fungicides are similar to the ones used in the current study. Shah *et al.* infected the larvae of the waxmoth, *Gallaria mellonella* (L.) with commercial formulations of the entomopathogenic fungi and used single spore cultures from the cadavers in the study. Virulence of *B. bassiana* was not affected by any of the 15 fungicides, but conidial germination and mycelial growth were affected to varying levels. Compared to 100% conidial germination in the absence of any fungicides, there was 84, 80, 66, 37, 0, 0, and 0% germination in the presence of the label rates of quinoxyfen, azoxystrobin, fenhexamid, pyrimethanil, sulfur, myclobutanil, and captan, respectively. Mycelial growth was 1.04, 1.11, 1.92, 1.95, 1.96, 1.97, and 2.18 mm in the presence of myclobutanil, azoxystrobin, fenhexamid, captan, sulfur, pyrimethanil, and quinoxyfen, respectively, compared to 2.93 mm in the absence of any fungicide. In the current study, pyraclostrobin, (one of the active ingredients of Pristine) is similar to azoxystrobin, and cyprodinil (one of the active ingredients of Switch) is similar to pyrimethanil, which were used by Shah *et al.*

There are some variations between findings of the current study and those of Shal *et al.*'s. However, the highly negative impact of captan (Captan), myclobutanil (Rally), and sulfur (Microthiol Disperss) and the moderate impact of quinoxyfen (Quintec) in the current study were similar to what Shah *et al.* observed for conidial germination and mycelial growth. While fenhexamid moderately affected *B. bassiana* in Shah *et al.*'s study, it (Elevate) had no such impact in the current study. Shah *et al.* [12] reported variation in the inhibitory effect of the fungicides on different fungi. While captan appears to be detrimental to multiple entomopathogenic fungi, strobilurin fungicides, azoxystrobin and pyraclostrobin seem to be compatible with *B. bassiana*, but not with *Metarhizium* spp [9, 12].

Kouassi, Coderre, and Todorova [13] evaluated the timing between the application of *B. bassiana* and metalaxyl, mancozeb, and cooper oxide against the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). These fungicides inhibited the growth of *B. bassiana* in vitro and reduced the mortality of *L. lineolaris* when applied with or 2-4 days before the application of fungus. Compared to 84% mortality caused by *B. bassiana* alone, the mortality was 20, 25, and

30% from metalaxyl, mancozeb, and copper oxide, respectively, when they were applied 2-4 days earlier. These results are somewhat different from those observed by Jaros-Su, Groden, and Zhang [7] and Loria, Galaini, and Roberts [11] with metalaxyl, mancozeb, and copper hydroxide. However, Kouassi, Coderre, and Todorova [13] found that application of fungicides 2-4 days after the insects were treated with *B. bassiana* had a synergistic effect in the case of metalaxyl and copper oxide due to the insecticidal effect of these fungicides. The time interval of 2-4 days allowed *B. bassiana* infections in *L. lineolaris* along with additional mortality caused by the insecticidal effect of the fungicides.

Strobilurin fungicides are very popular and widely used in cropping systems around the world as reported by Bartlett, Clough, Godwin, Hall, Hamer, and Parr-Dobrzanski [14]. Their compatibility with *B. bassiana*, as demonstrated in the current or other studies, is promising for the use of *B. bassiana* for pest management in cropping systems where such fungicides are used against plant pathogens. This is the first study evaluating the compatibility of *B. bassiana* with some of the fungicides commonly used in California strawberries. However, as some of these fungicides are used on other crops, these results are applicable wherever these fungicides are used. Results promote sustainable agriculture by providing an opportunity to use a non-chemical alternative to chemical insecticides or acaricides and address the concern of growers in selecting appropriate fungicides while using *B. bassiana*. Additional studies with other fungicides and different application intervals between *B. bassiana* and fungicides would be more useful to further evaluate their compatibility.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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