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Research Note

Effect of selected fungicides on *in vitro* vegetative growth of *Beauveria bassiana* (Balsamo) Vuillemin, a pathogen of rice leaf folders

V. AMBETHGAR[1*], M. SWAMIAPPAN[2], R. J. RABINDRA[3] and R. RABINDRAN[2]

[1]*Regional Research Station, Tamil Nadu Agricultural University, Vridhachalam 606001, Tamil Nadu, India.*
[2]*Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India.*
[3]*National Bureau of Agriculturally Important Insects, Post Bag No. 2491, H. A. Farm Post, Hebbal, Bellary Road Bangalore 560024, Karnataka, India.*
[*]*Corresponding author E-mail*: drva_1965@yahoo.co.in

ABSTRACT: The toxic effect of 10 fungicides on mycelial growth of *Beauveria bassiana* (isolate BbCm KKL 1100), a pathogen of rice leaf folders, was evaluated *in vitro* at three concentrations each: the normal field application rate (I.0X), 10-fold lower rate (0.1X) and 10-fold higher rate (10.0X) on agar plates. The fungicides tested were antagonistic to *B. bassiana* and inhibited the mycelial growth of either partially or completely depending on the concentration. At 10-fold higher concentration, all 10 fungicides caused total inhibition of mycelial growth. Two fungicides, *viz.*, benomyl and hexaconazole, caused total inhibition of the fungus in all the three concentrations tested. Three fungicides *viz.,* edifenphos, iprobenphos and mancozeb, appeared to be fungistatic at normal field dose (1.0X). Benomyl, carbendazim, hexaconazole, propiconazole and tricyclazole caused total inhibition of mycelial growth at 1.0X, while chlorothalonil, copper oxychloride and edifenphos were comparatively less toxic to *B. bassiana* causing 63.0, 72.6 and 76.3 per cent mycelial inhibition, respectively at 0.1X.

KEY WORDS: *Beauveria bassiana*, fungicides, mycelial growth, rice leaf folders

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The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has been extensively used for the control of many important pests of various crops across the world. In rice ecosystem, *B. bassiana* is an important natural mortality factor on a wide range of insect pests, particularly leaf folders, *Cnaphalocrocis medinalis* (Guenee), *Marasmia patnalis* Bradley and *Marasmia ruralis* Walker (Ambethgar, 1997). The fungus may be applied in the form of conidia or mycelia which sporulate after application. Prior field trials have proven that *B. bassiana* can effectively reduce target insect populations in rice ecosystem (Rama Mohan Rao, 1989; Hazarika and Puzari, 1990). Fungicides are routinely applied to control plant diseases, but many fungicides with broad spectra of activity may adversely affect the efficacy of *B. bassiana* that occurs in natural environment (Aguda *et al.,* 1988). *In vitro* growth inhibition of *B. bassiana* in the presence of fungicides is a useful criterion for testing its sensitivity. Several laboratory experiments followed by field tests on compatibility between fungicides and *B. bassiana* have been conducted (Srivastava *et al.,* 1991; Gupta *et al.,* 2002). Knowledge of compatibility between *B. bassiana* and fungicides is crucial to select suitable compounds and scheduling treatments to minimize deleterious effects on *B. bassiana* in integrated pest management programmes. The objective of the present study was to evaluate *in vitro* fungicide compatibility to serve as a guideline for field use of the fungus.

Beauveria bassiana isolate BbCm KKL 1100 used in this experiment was originally derived from a larval cadaver of *C. medinalis* from endemic area of Karaikal, Puducherry Union Territory, India (Ambethgar, 1997). The isolate was selected due to its high virulence towards *C. medinalis* larvae, which was determined through two-way screening using an initial single-dose assay with a standard concentration of 1 x 10^8 conidia/ml in 0.02% Tween 80®, followed by multiple-dose mortality assay with six different conidial concentrations containing 1 x 10^4, 10^5, 1^6, 10^7, 10^8 and 10^9 conidia/ml in 0.02% Tween 80® as surfactant.

Ten commonly used fungicides for control of crop diseases were selected for the present study. The field application rates were obtained from the Crop Production Guide recommendations for field crops. The effects of these fungicides on the radial vegetative growth of *B. bassiana* were evaluated in the laboratory. The fungicides were tested on Sabouraud dextrose agar slants fortified with 1% yeast extract (SDAY) at three

concentrations each, *viz.,* the normal field application rate (1.0X), 10-fold lower rate (0.1X) and 10-fold higher rate (10.0X) on inhibition of radial growth of the fungus. Twenty ml of SDAY medium was sterilized separately, cooled to luke-warm condition (55ºC) and the required concentration of each fungicide containing streptomycin (0.5g/l) was incorporated separately and plated into 9 cm diameter sterile Petri dishes. The agar plates were allowed to set overnight under laminar flow cabinet. Ten-day-old mycelial mat of *B. bassiana* cored out (10mm dia.) from the periphery of the colony was transferred to plates of SDAY amended with different concentrations of respective fungicides. Fungus inoculated SDAY plates without fungicides served as control. The plates were sealed and incubated at 25 ± 2ºC in a BOD incubator for 14 days after which the diameter of radial growth of the culture was measured. The experiment was repeated three times. The replicated radial growth data were averaged and expressed as percentage of growth inhibition in comparison to control following the formula:

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

where X, Y and Z stand for percentage of growth inhibition and radial mycelial growth of fungus in control and poisoned medium, respectively. The toxicity of the fungicides was further rated based on a 1-4 scoring: 1 = harmless (< 50% reduction in beneficial capacity), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-90%), 4 = harmful (> 90%) in toxicity tests (Hassan, 1989). The data were analyzed using the Statistical Analysis System (SAS Institute Inc., 1982) programme.

The results on the toxicity of 10 fungicides to the vegetative growth of *B. bassiana* isolate (BbCm KKL 1100) are shown in Table 1. The fungicides inhibited the mycelial development of *B. bassiana* either partially or completely at the three concentrations tested. At 10-fold higher concentration, all the fungicides tested caused total inhibition of mycelial growth. Benomyl, carbendazim and hexaconazole entirely inhibited the mycelial growth at all the three concentrations indicating very high sensitivity of the fungus to these chemicals. At normal field dose (1.0X), benomyl, carbendazim, hexaconazole, propiconazole and tricyclazole caused complete inhibition of mycelial growth, while chlorothalonil, copperoxychloride, edifenphos, iprobenphos and mancozeb inhibited 81.7-84.8 per cent mycelial growth. However, such extreme toxic effect was not observed in sub-normal concentrations (0.1X) except for benomyl, carbendazim and hexaconazole, which caused complete inhibition of mycelial growth. Other fungicides

Table 1. Effect of some fungicides on vegetative growth of *B. bassiana* in SDAY at 14 DAT

Fungicides	Field dose (ml/g L^{-1})	Mean mycelial colony diameter in mm			
		Lower dose (0.1X)	Field dose (1X)	Higher dose (10X)	Scoring at (0.1X)
Benomyl 50 WP	1.0	0.0 (100.0)^{f}	0.0 (100.0)^{e}	0.0 (100.0)^{b}	4
Carbendazim 50 WP	1.0	0.0 (100.0)^{f}	0.0 (100.0)^{e}	0.0 (100.0)^{b}	4
Chlorothalonil 75 WP	1.0	33.3 (63.0)^{b}	16.5 (81.7)^{b}	0.0 (100.0)^{b}	2
Copperoxychloride 50 WP	2.0	24.7 (72.6)^{c}	16.0 (82.2)^{b}	0.0 (100.0)^{b}	2
Edifenphos 50 EC	2.0	21.3 (76.3)^{c}	15.6 (82.6)^{b}	0.0 (100.0)^{b}	2
Hexaconazole 5 EC	2.5	0.0 (100.0)^{f}	0.0 (100.0)^{e}	0.0 (100.0)^{b}	4
Iprobenphos 3G	2.0	16.3 (81.8)^{d}	10.7 (88.2)^{d}	0.0 (100.0)^{b}	3
Mancozeb 75 WP	2.0	17.3 (80.7)^{d}	13.7 (84.8)^{c}	0.0 (100.0)^{b}	3
Propiconazole 20 EC	2.0	17.6 (80.4)^{d}	0.0 (100.0)^{e}	0.0 (100.0)^{b}	3
Tricyclazole 75 WP	1.0	10.0 (88.9)^{e}	0.0 (100.0)^{e}	0.0 (100.0)^{b}	3
Control (Blank SDAY)	–	90.0 (0.0)^{a}	90.0 (0.0)^{a}	90.0 (0.0)^{a}	–

Means followed by a common letter are not significantly different at 5% level by DMRT; values in parentheses are per cent inhibition of mycelial growth over control; 1 = harmless (<50% inhibition); 2 = slightly harmful (50-79% inhibition); 3 = moderately harmful (80-90% inhibition); 4 = harmful (>90% inhibition)

such as chlorothalonil, copper oxychloride and edifenphos were comparatively less toxic to *B. bassiana* with mean mycelial radial inhibition of 63.0, 72.6 and 76.3 per cent respectively, as against 90 mm normal growth in poison free SDAY. The present study indicates that co-application of fungicides at higher than normal concentration is not desirable due to their fungitoxic nature.

The sensitivity of *B. bassiana* to various pesticides has been examined in the laboratory (Gupta *et al.,* 2002; Prabhu *et al.,* 2007). Several fungicides including benomyl, mancozeb, copperoxychloride, chlorothalonil and carbendazim intended for control of foliar diseases were reported to be fungistatic to *B. bassiana* (Srivastava *et al.,* 1991; Gupta *et al.,* 2002). Fungal sporulation was less affected at low pesticide concentrations (Todorova *et al.,* 1998). However, in the present study, benomyl, carbendazim and hexaconazole exhibited very strong inhibition on the mycelial growth of *B. bassiana* even at lower concentration. Therefore, joint application of entomopathogenic fungi with fungicides may not be a desirable practice. Clark *et al.* (1982) clarified that any pesticides which are innocuous under *in vitro* conditions need not be safe in the open field conditions and *vice versa*. Therefore, fungicides harmful in sensitivity tests *in vitro* should be treated *in vivo* as well with the biocontrol pathogen on the target pests to predict their interference in natural epizootics. The results of the present experiment have clearly demonstrated the high sensitivity of *B. bassiana* to commonly used fungicides. However, *B. bassiana* is one of the predominant fungal entomopathogens elsewhere in diverse cropped ecosystems. Consequently, it would be expected that negative effects of fungicides on *B. bassiana* would occur during epizootics either by killing or limiting fungal colonization. Selective fungicides that can be used to control plant diseases without any adverse effects on the biological properties of entomopathogenic fungi need to be screened. Systematic field studies, complemented by parallel laboratory experiments are essential for clear understanding of the ecological impact of fungicides on the augmented fungal entomopathogens. Continued research should also be concentrated on the characterization and improvement of *B. bassiana* for selecting fungicide resistant strains.

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