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In vitro Sensitivity of Entomopathogenic Fungi to Pre mix Herbicides

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ABSTRACT

A laboratory experiment was carried out at the Soil and Water Testing Laboratory of Krishi Vigyan Kendra, Kerala Agricultural University, Sadanandapuram, Kollam, Kerala to evaluate the sensitivity of entomopathogenic fungi (EPF): *Metarhizium anisopliae*, *Lecanicillium lecanii* and *Beauveria bassiana* to pre-mix herbicides. The herbicides used for the in vitro study were Metsulfuron methyl + Chlorimuron ethyl, Cyhalofop-Butyl + Penoxsulam, and Pretilachlor + Bensulfuron methyl which are the popular herbicides among the rice farmers. The experiments were laid out in a completely randomized block design with 4 treatments and 4 replications separately for each entomopathogen. Radial growth and percentage inhibition of entomopathogenic fungi observed in the media poisoned with respective herbicides were recorded. Percentage inhibition was rated using Hassan's classification chart. During all stages of observation, the growth inhibition of *M. anisopliae* ranged between 1.79% to 10.65% and it was rated as harmless. The radial growth of the fungus was higher in control plates which were statistically similar to fungal growth in petridishes poisoned with herbicides. With respect to *L. lecanii* it was seen that percentage inhibition was below 50% in the treatments involving the herbicide Metsulfuron methyl +Chlorimuron ethyl and Cyhalofop-Butyl + Penoxsulam, whereas Pretilachlor +Bensulfuron methyl treated plates showed maximum growth inhibition of 57.70%. The growth of *B. bassiana* was slow irrespective of the herbicide treatments and the treatment involving Metsulfuron methyl +Chlorimuron ethyl registered the lowest growth inhibition compared to Cyhalofop-Butyl+ Penoxsulam and Pretilachlor+Bensulfuron methyl. The percentage inhibition ranged from 22.33% to 87.57%. The results indicated that *M. anisopliae* was compatible with all the three tested herbicides and *L. lecanii* was compatible with Metsulfuron methyl +Chlorimuron ethyl and Cyhalofop-Butyl + Penoxsulam whereas Pretilachlor +Bensulfuron methyl was slightly harmful to *L. lecanii.* With respect to *B. bassiana*, Metsulfuron methyl +Chlorimuron ethyl was slightly harmful to the fungal growth while Cyhalofop-Butyl + Penoxsulam and Pretilachlor +Bensulfuron methyl were moderately harmful.

Key Words: Invitro sensitivity, herbicides, entomopathogenic fungi,radial growth, inhibition percentage

INTRODUCTION

The ecosystem balances are influenced by the below-ground microbial diversity and its population dynamic which plays a significant role in sustainable agriculture through the enhancement of soil productivity. Soil productivity maintains the soil and plant health. However indiscriminate use of chemical fertilizers and pesticides leads to various problems *viz.,* pesticide resistance, pest resurgence, shift in weed flora, and environmental hazards. These challenges lead the scientific world to emphasize the adoption of integrated pest management and nutrient management which includes the judicious use of biofertilizers, biocontrol agents, and other eco-

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friendly management options along with chemical fungicides, insecticides, herbicides, and fertilizers at recommended doses. Entomopathogenic fungi (EPF) for pest management in agriculture is a novel tool that is highly effective for the management of lepidopteran larvae, thrips, and aphids hence the manipulation of these organisms as an eco-friendly management option in sustainable agriculture is started nowadays in order to reduce the pesticide load in the environment. Entomopathogenic fungi can act as a parasite and kill or seriously disable the pest of crops which are considered natural mortality agents and are environmentally safe (Yashaswini and Vijay Kumar, 2016). The advantages of EPF over conventional insecticides are economic, safe to plant and soil, limited residues in the environment, and improved biodiversity in ecosystems. *Metarhizium. anisopliae, Leanicilliumlecanii* and *Beauveria bassiana* are the important EPFsthat are slowly gaining popularity among the farmers of Kerala for the management of various agricultural pests. *Metarhizium. anisopliae* is used as a biocontrol agent against rice plant hoppers, leaf folder, beetles, bugs, and locusts (Peng *et al,* 2014 Rizwan,2019) while *Leanicilliumlecanii* and *Beauveria bassiana* infect insects like aphids and whiteflies, sporulates and eventually kills them. The mode action of these beneficial organisms on insect pests is mainly through the production of toxic metabolites, and excretion of mycotoxins like beauvericin, cyclodepsipeptide, destruxin, and desmethyldestruxin (Isaac, 1992 and Wang et al., 2018). The farmers are nowadays using these EPF for various crops and the Kendra popularised the low cost mass multiplication techniques for on farm production in *Metarrhizium anisoplia* (Manu *et al,* 2019).The combined use of EPF and various insecticides and fungicides have been reported earlier which indicated the synergistic or antagonistic effects of chemicals on the growth of EPF. Herbicides are an integral component in commercial rice cultivation for the management of weeds as traditional hand weeding becomes costly and laborious. Many premixed herbicides are being used widely for the management of weeds in cereals (Shaktawa *et al.*, 2019). The application of herbicide may sometimes adversely affect or positively influence the existing microflora and fauna present in the soil and plant system. Several in vitro studies have been performed to evaluate the impacts of herbicides on the growth and development of several biocontrol agents while the studies on the effect of EPF on herbicides are limited. Hence, an in vitro study had been performed to assess the compatibility of three commonly used pre-mixed herbicides *viz.,* Metsulfuron methyl 10% +Chlorimuron ethyl 10%, Cyhalofop-Butyl 5.1% + Penoxsulam1.02% and Pretilachlor 0.6% +Bensulfuron methyl 0.06% to entomopathogenic fungi *Lecanicillium lecanii*, *Metarhizium anisopliae*, and *Beauveria bassiana*.

MATERIALS AND METHODS

In vitro, studies were conducted to evaluate the compatibility of *Metarhizium anisopliae, Beauveria bassiana* and *Lecanicillium lecanii*to pre-mix herbicides Metsulfuron ethyl +Chlorimuron ethyl, Cyhalofop-Butyl + Penoxsulam, and Petrilachlor + Bensulfuron methyl. The experiments were conducted separately for each EPF and each experiment included four treatments and four replications for each EPF, and it was set up using a totally randomized block design. The experiments were carried out at the soil and water testing laboratory of Krishi Vigyan, Kerala Agricultural University Kollam Kerala during the year 2020 (m (8°58' N, 76°48' E and 76 m above mean sea level).

The first experiment was done to test the in vitro sensitivity of *Metarhizium anisopliae* to different herbicides at field doses recommended by KAU. The treatments were M_1- Control (without herbicide), M_2-Metsulfuron ethyl 10% +Chlorimuron ethyl 10% @ 4gai/ha, M_3- Cyhalofop-Butyl 5.1% + Penoxsulam1.02% @130gai/ha and M_4- Pretilachlor 0.6% +Bensulfuron methyl 0.06% @ 0.6+0.6 kg ai/ ha. The second and third experiments consisted of the herbicides mentioned above and the EPF were *Lecanicillium lecanii and Beauveria bassiana*. The treatments were L_1- Control (without herbicide),

L_2- Metsulfuron ethyl 10% +Chlorimuron ethyl 10% [@4 g ai/ha], L_3- Cyhalofop-Butyl 5.1% + Penoxsulam 1.02% [130 gai /ha], L_4- Pretilachlor 0.6% +Bensulfuron methyl 0.06% [0.6 +0.06 Kg ai/ha), B_1- Control (without herbicide), B_2- Metsulfuron ethyl 10% +Chlorimuron ethyl 10% [@4 g ai/ha], B_3- Cyhalofop-Butyl 5.1% + Penoxsulam 1.02% [130 gai /ha], B_4- Pretilachlor 0.6% +Bensulfuron methyl 0.06% [0.6 +0.06 Kg ai/ha].

The in vitro sensitivity test was conducted by following the poisoned food method (Zentimeyer, 1955). The necessary amount of herbicide was added to a conical flask of 250 ml, stirred properly, and then put onto sterile petri plates with 50 ml of double-strength melted Potato Dextrose Agar (PDA). Then the medium was allowed to solidify. After solidification, a 5 mm-sized agar disc of the respective EPF was inoculated at the centre of the plate with and without herbicides. Inoculated plates were sealed with cling film. Plates containing non-poisoned media (without herbicide) served as control. For each herbicide, 4 replications were kept. The petri dishes were kept warm throughout the incubation process. The observations on the radial growth of fungi were recorded on alternate days starting from the first day after inoculation (DAI) to the day when the full growth of the fungus was observed in the control plate (8 cm or more than 8 cm). This duration varied according to the fungus. The percentage inhibition of mycelial growth of the fungi was calculated by using the formula suggested by Vincent (1927) ie., I= [(C-T)/C]*100 Where I is the percent inhibition of growth, C- colony diameter (cm), T-colony diameter in treatment(cm). The percentage inhibition was rated using Hassan's classification scheme (1989) *viz.,* Harmless (≤ 50% inhibition), Slightly harmful (50-79% growth inhibition), Moderately harmful (80-90% growth inhibition), Harmful (≥ 90% growth inhibition). Using a completely randomized design and online statistical program WASP, an analysis of variance (ANOVA) was performed on the collected data. Wherever the findings were significant, a 5 percent probability was calculated for the key difference.

RESULTS AND DISCUSSION

In vitro sensitivity of *Metarhizium anisopliae* to different herbicides

The effect of different tested herbicides at recommended field doses on radial growth and percentage inhibition of *Metarhizium anisopliae* is given in Table 1. All the herbicides tested significantly inhibited the radial growth of fungus at the initial days of inoculation i.e., 1 and 3 DAS. The lowest radial growth of fungus (2.4cm) was observed in the media poisoned with herbicide Pretilachlor +Bensulfuron methyl and Cyhalofop-Butyl + Penoxsulam at 3DAI which was statistically comparable with Metsulfuron methyl +Chlorimuron ethyl. The growth of fungus in the control plates recorded the highest radial growth of

Table 1. *In vitro* sensitivity of *Metarhizium anisopliae* to different herbicides on the radial growth.

Treatment	Radial growth of *Metarhizium anisopliae* (cm)				
	1 DAI	3 DAI	5 DAI	7 DAI	9 DAI
M_1	1.50	2.70	4.90	6.95	8.25
M_2	1.42	2.50	4.80	6.70	8.20
M_3	1.40	2.40	4.60	6.65	8.05
M_4	1.40	2.40	4.65	6.80	8.10
CD	0.039	0.220	NS	NS	NS
SE(m)	0.012	0.071	0.080	0.084	0.061
CV	1.747	5.657	3.371	2.484	1.502

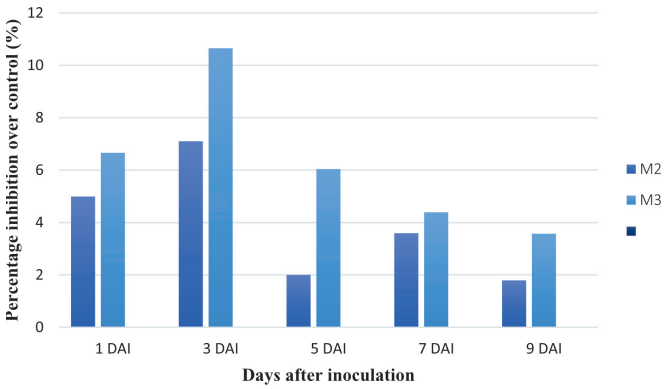

Fig 1 Effect of different herbicides on the percentage inhibition (%) of *Metarhizium anisopliae* over control

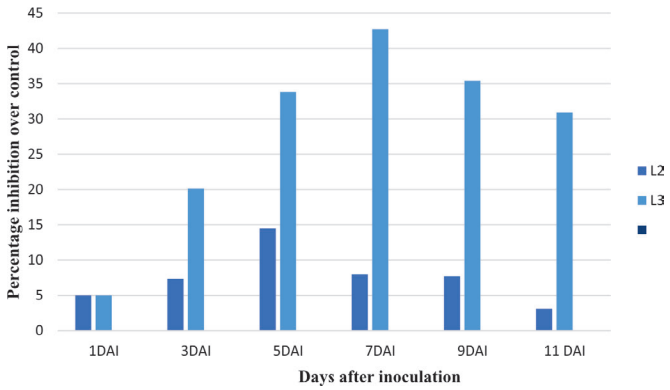

Fig 2. Effect of different herbicides on the percentage inhibition (%) of *Lecanicillium lecanii*over control

2.7 cm but was statistically similar to metsulfuron methyl +Chlorimuron ethyl. From 5 DAI onwards ie., 5, 7, and 9 DAI, the presence of herbicides did not significantly affect the growth of *Metarhizium anisopliae*. The control plates recorded the highest radial growth of 8.25 cm at 9 DAI. The inhibition percent of tested herbicides ranged from 4.99 to 6.99 at 1 DAI and 7.1 to 10.65 at 3 DAI. During all stages of observation, the growth inhibition of *Metarhizium anisopliae* ranged between 1.79% and 10.65%. Maximum percent growth inhibition was noticed at 3 DAI ie., 10.65%. It was observed that all the herbicides tested were harmless to the EPF *Metarhizium anisopliae* as per Hassan's classification scheme in which the chemical that produces less than or equal to 50 % growth inhibition in that range is categorized as harmless. Hence, the study's findings showed that all the tested herbicides were not harmful to EPF *Metarhizium anisopliae*. As per Li and Holdom (1994) *M. anisopliae* isolates demonstrated more tolerance to herbicides and insecticides than to fungicides. The activity of *Metarhizium anisopliae* was not affected by the application of herbicides at normal doses (Silva *et al*,2013; Neves *et al*,2001 Accinelli *et al,* 2002). Accordingly, the EPF *Metarhizium anisopliae* was compatible with herbicides which were in agreement with the results of the present study.

In vitro sensitivity of *Lecanicilliumlecanii* to different herbicides

The effect of different herbicides on radial growth and percentage inhibition of *Lecanicilliumlecanii* is presented in Table 2. There was an increasing trend in the radial growth of fungus irrespective of

Table 2. *In vitro* sensitivity of *Lecanicillium lecanii*to different herbicides on the radial growth.

Treatment	Radial growth of *Lecanicilliumlecanii*(cm)					
	1 DAI	3 DAI	5 DAI	7 DAI	9 DAI	11 DAI
L_1	1.00	2.02	3.10	5.32	6.50	8.10
L_2	0.95	1.87	2.65	4.90	6.00	7.85
L_3	0.95	1.61	2.05	3.07	4.20	5.60
L_4	0.80	1.45	1.85	2.25	2.95	4.05
CD	0.064	0.139	0.149	0.183	0.308	0.407
SE(m)	0.020	0.044	0.048	0.059	0.099	0.131
CV	4.413	5.107	3.969	3.016	4.029	4.084

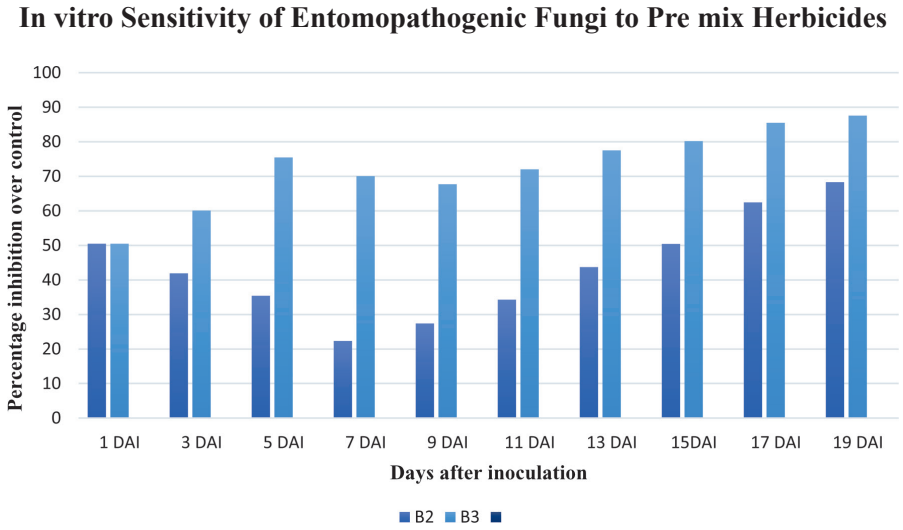

Fig. 3. Effect of different herbicides on percent inhibition (%) of *Beauveriabassiana* over control

herbicide treatments and control (without herbicide) from the first day after inoculation to the eleventh day after inoculation. The highest radial growth of fungus was observed in the treatment L1 (control) during all days of observation and was considerably different from all other treatments except at 11 DAI. The fungus attained maximum growth at 11 DAI in the control plates (8.1 cm) and it was comparable with L2 (7.85cm) *i.e.,* metsulfuron methyl +Chlorimuron ethyl. The lowest radial growth of fungus (4.05 cm) was recorded by L4 (Pretilachlor + Bensulfuron methyl) which was significantly different from the control treatments. Presence of Pretilachlor 0.6%+ Bensulfuron methyl 0.06% (L4) inhibited the growth of EPF over control and the percentage inhibition ranged from 20 to 57.70% at various stages of observation. The data on the percentage inhibition of herbicide on *Lecanicillium lecanii* over control had ranged from 5 to 42.71 for Cyhalofopbutyl+Penoxulam. Inhibitory effect of herbicides on the growth of *Lecanicillium lecanii* to a significant extent at the later stages of observation ie., 5,7,9 DAI with respect to L2 and L3. The percent inhibition was more than 50% in media poisoned with L4 (Pretilachlor + Bensulfuron methyl) and it came under the category of slightly harmful according to Hassan's classification. The percent inhibition of the fungus in the presence of herbicides upto 5 DAI was below 50% which was rated as harmless according to Hassan (1989). Hence, it can be concluded that the herbicides Metsulfuron methyl 10%+Chlorimuron ethyl 10%, Cyhalofop-Butyl 5.1%+ Penoxsulam 1.02% were harmless while Pretilachlor 0.6%+Bensulfuron methyl 0.06% was

Table 3. *In vitro* sensitivity of *Beauveria bassiana* to different herbicides on the radial growth.

Treatment	Radial growth of *Beauveria bassiana*(cm)									
	1 DAI	3 DAI	5 DAI	7 DAI	9 DAI	11 DAI	13 DAI	15DAI	17 DAI	19 DAI
B_1	1.01	1.38	2.25	2.70	3.10	3.57	4.45	5.05	6.70	8.05
B_2	0.50	0.80	1.45	2.10	2.25	2.35	2.50	2.50	2.55	2.55
B_3	0.50	0.55	0.55	0.80	1.00	1.00	1.00	1.00	1.00	1.00
B_4	0.50	0.50	0.50	0.70	1.00	1.00	1.00	1.00	1.00	1.00
CD	0.016	0.102	0.101	0.298	0.119	0.087	0.174	0.174	0.174	0.110
SE(m)	0.005	0.033	0.032	0.096	0.038	0.028	0.056	0.056	0.056	0.035
CV	1.594	8.121	5.436	12.158	4.157	2.822	4.997	4.683	3.975	2.244

slightly harmful to *Lecanicillium lecanii* according to Hassan's classification scheme. The present result was supported by previous findings of Saito and Yabuta (1996) and Khalil *et al* (1985). In the view of previous work on agrochemicals and also from the result of the present study the tested herbicides *viz;* Metsulfuron methyl 10% +Chlorimuron ethyl 10%, Cyhalofop-Butyl 5.1%+ Penoxsulam1.02% were harmless and Pretilachlor 0.6% +Bensulfuron methyl 0.06% was slightly harmful to the growth of entomopathogenic fungi *Lecanicillium lecanii.*

In vitro sensitivity of *Beauveria bassiana* to different herbicides

The effect of different herbicides on radial growth and percentage inhibition of *Beauveria bassiana* is given in Table 3. From the data, it was seen that the EPF *Beauveria bassiana* was a slow-growing fungus. The radial growth of fungus in the control plate gradually increased from 1.01 cm (at 1 DAI) to 8.05 cm (at 19 DAI). The fungus in media without herbicide *i.e.,* control plate attained full growth (8.05 cm) at 19 DAI while its growth in the medium treated with herbicide ie., B2 (Metsulfuron methyl 10%+Chlorimuron ethyl 10%), B3 (Cyhalofop-Butyl 5.1%+ Penoxsulam1.02%) and B4 (Pretilachlor 0.6% +Bensulfuron methyl 0.06%) were 2.55cm, 1cm and 1cm at 19DAI respectively which was considerably lower than the control treatment. Hence, the effect of herbicide Metsulfuron methyl 10%+Chlorimuron ethyl 10% on EPF was rated as slightly harmful and from 15 DAI onwards. The herbicide Cyhalofop-Butyl 5.1%+ Penoxsulam 1.02% and Pretilachlor 0.6%+Bensulfuron methyl 0.06% moderately inhibited the growth of fungus as the percentage growth inhibition ranged from 80 to 90%.

The inhibition percentage of *Beauveria bassiana* on treatments ranged from 22.33% to 87.57%. According to Hassan's classification scheme,metsulfuron methyl 10% +Chlorimuron ethyl 10% were slightly harmful (50-79% of growth inhibition) whereas Cyhalofop-Butyl 5.1%+ Penoxsulam1.02% and Pretilachlor 0.6%+Bensulfuron methyl 0.06% were moderately harmful (80-90% of growth inhibition) to EPF *Beauveria bassiana*. The negative effect of herbicides on *Beauveria bassiana* was reported by Franci and Katarina (2016). According to Katarina Kos and Franci (2013), the effect of the herbicide Dicamba on *Beauveria bassiana*was slightly harmful for germination and moderately harmful for sporulation, and herbicides like pyridate, metribuzin, tembotrione, andS-metolachlor, were harmful to vegetative growth, sporulation, and germination.

CONCLUSION

It can be concluded that all three tested herbicides at recommended dose were safe for the tested EPF *Metarhizium anisopliae*. The herbicides Metsulfuron methyl 10%+ Chlorimuron ethyl 10% and Cyhalofop-Butyl 5.1%+ Penoxsulam 1.02% were harmless to *Lecanicilliumlecanii*while the fungus was sensitive to Pretilachlor 0.6% +Bensulfuron methyl 0.06%. With respect to *Beauveria bassiana* all three herbicides tested Metsulfuron ethyl 10%+ Chlorimuron ethyl 10%, Cyhalofop -Butyl 5.1%+Penoxsulam 1.02% and Pretilachlor 0.6%+ Bensulfuron methyl 0.06% (at the recommended dose) were not compatible as they were rated as slightly harmful to moderately harmful. Hence it is advisable to use the tested three herbicides viz; Metsulfuron methyl 10%+ Chlorimuron ethyl 10%, Cyhalofop -Butyl 5.1% +Penoxsulam 1.02%, and Pretilachlor 0.6%+ Bensulfuron methyl 0.06% along with EPF *Metarhizium anisopliae*. Combined application of herbicides Cyhalofop -Butyl 5.1% +Penoxsulam 1.02% or Pretilachlor 0.6%+ Bensulfuron methyl 0.06% with EPF *Lecanicillium lecanii*or *Beauveria bassiana* was not advisable as these were slightly harmful to moderately harmful to fungal growth.

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