Efficacy of different entomopathogenic fungal isolates against chilli aphid, *Myzus Persicae* (Sulz.)

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Abstract
In recent years, microbial control of insect pests is becoming popular as insect pathogens such as bacteria, viruses, fungi and nematodes serve as potential bioagents in pest management. Among the different microbial agents, entomopathogenic fungi (EPF) are gaining importance in pest control. They can be easily mass cultured on artificial media without affecting their virulence at a cheaper cost. They are highly species specific with minimal impact on non-target organisms. The current study aimed to study the efficacy of entomopathogenic fungal isolates against aphid, *Myzus Persicae (Sulz.)* in chilli. Laboratory and field experiments were conducted to evaluate the pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* against chilli aphid. From the study it is revealed that *B. bassiana* @ 10^8 spores ml^{-1} were found to be effective and found to be more superior to the other entomopathogenic fungal isolates *viz*., *Metarhizium anisopliae* and *Lecanicillium lecanii* against chilli aphid.

Keywords: Entomopathogenic fungi, Chilli, Aphid, *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*

I. INTRODUCTION

Chilli is the most common spice crop. Among the different pests, chilli aphid, *Myzus persicae* (Sulz.) alone causes 20 to 40 per cent yield loss [1, 2]. It is a polyphagous pest and besides chilli it attacks a large number of host plants *viz.*, Tomato, broccoli, cabbage, carrot, cauliflower, eggplant, green beans, lettuce, mustards, papaya, peppers and sweet potato. The aphid causes both qualitative and quantitative losses in the seed yield and crop production by different ways include: Nutrient drain which cause direct reduction of plant productivity, transmission of viruses, phytotoxicity as a result of saliva toxins and excretion of honeydew leading to the development of black sooty mold and leaf shedding [3].

M. persicae is highly susceptible to chemical insecticides and some of the recommended chemicals for its management are malathion, phosalone, monocrotophos, dimethoate, methyl demeton and acephate [4, 5, 6]. However, their effectiveness is temporary and the aphid reappears after 2-3 weeks of spraying, besides, these insecticides are also highly toxic to the predators. Due to the negative impact of the chemical insecticides, the need for effective, safer, specific and sustainable method of aphid management arose. Biopesticides such as virus, bacteria and fungi play a major role in insect pest management. Unlike virus and bacteria, the fungal biocontrol agents do not have to be ingested to infect their host but invade directly through the cuticle and so can potentially be used for the control of sucking pests like aphid. In addition, due to their high degree of specificity, potential activity and environmental safety, recently more attention has been given to insect pest management with entomopathogenic fungi *viz.*, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchinkoff) Sorokin and *Lecanicillium lecanii* (Zimm.) Zare and Gams. Keeping this in view, the present study has been focused to study the effect of various entomopathogenic fungal isolates against chilli aphid.

II. MATERIALS AND METHODS

A. Maintenance of fungal isolates

Pure cultures of the entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae* and *Lecanicillium lecanii* were obtained from the National Bureau of Agricultural Insect Resources (NBAIR). These fungi were then subcultured in SMA + Y media, incubated at 25 ± 1°C for ten days and stored in refrigerator at 5°C. All the fungal isolates were subcultured once in three weeks. To maintain the virulence, after six subculturing all the fungal isolates were subjected to pathogenicity test and again reisolated for further studies [7].

B. Preparation of spore concentrations of the fungal isolates

Fungal isolates were cultured in 100ml SMA+Y liquid medium in 250ml conical flask and incubated at room temperature for 10 days. After sporulation of the fungal isolates, it was ground in ordinary mixer and made into liquid spore suspension. This was filtered through double layered muslin cloth to remove the mycelial mat. The suspension was shaken thoroughly with a drop of teepol solution in order to disperse the spores in the solution. The spore count in the suspension was assessed by using a haemocytometer [8].

C. Pathogenicity test

For bioassays against chilli aphid, the chilli leaves were placed individually on wet cotton swab with filter paper in a Petri dish. Thirty apterous aphids were released separately in each piece of leaf. The fungal

spore suspension was collected from ten day old respective cultures by scraping the surface of the culture plates with a sterile scalpel and suspending them in 0.05 per cent aqueous Tween 80 [9]. The spore load was assessed using Neubauer haemocytometer. From the stock solution, further dilutions were made to obtain the required concentrations for further studies. Spore concentration of $1x10^8$ spores ml^{-1} was prepared and ten ml was sprayed separately using atomizer. Aphids sprayed with 0.05 per cent Tween 80 solution served as check.

Mortality of aphids were recorded separately at 24 h interval up to seven days. Dead aphids were collected daily, placed in Petridish containing a moist filter paper and kept in humid chamber. The dead aphids that produced mycelial growth were considered for the mortality count. Mortality data was corrected with control mortality by using Abbott's formula [10]. The data was then analysed by probit analysis [11] and the Median Lethal Concentration (LC50) and the Median Lethal Time (LT50) values were computed by using the statistical computer programme, Statistical Package of Social Sciences (SPSS).

D. Field efficacy of fungal pathogens on chilli aphid

A field experiment was conducted to evaluate the pathogenicity of fungal pathogens *viz*., *B. bassiana*, *M. anisopliae* and *L. lecanii* on *M. persiace*. In the experiment, monocrotophos 36 EC and malathion 50 EC (2ml/lit) were included as standard checks. The following treatments were imposed

	Treatments	Dose
T_1	*B. bassiana*	$1x10^8$ spores/ml
T_2	*M. anisopliae*	$1x10^8$ spores/ml
T_3	*L. lecanii*	$1x10^8$ spores/ml
T_4	Monocrotophos 36 EC	2ml/lit
T_5	Malathion 50 EC	2ml/lit
T_6	Untreated check	-

Two rounds of treatments were given at 14 days interval with the help of a knapsack sprayer. The first spray was given when the aphid population was high. The pre-treatment and post-treatment observations on aphid population were assessed on 0, 1, 3, 7 and 14 days after treatment. Ten clumps were randomly selected per plot and they were treated and tagged. Observations were made on three consecutive leaves of treated plants from top and they were stapled together for easy identification and assessed for live aphid population in three places of $4cm^2$ area and the mean was calculated.

III. RESULTS AND DISCUSSION

A. Pathogenicity of fungal pathogens against *M. persicae*

Among the three fungal pathogens tested, *B. bassiana* and *L. Lecanii* caused mortality of *M. persicae* at 33.75, 16.25 per cent, respectively and *M. anisopliae* was found to be less effective as compared to other two isolates. The standard checks monocrotophos and malathion showed mortality of 84.30 and 78.75 per cent, respectively (Table 1).

B. Determination of LC_{50} and LT_{50}

The data on dose-mortality and time-mortality response of *M. persicae* to *B. bassiana* and *M. anisopliae* showed significant differences in the LC_{50} and LT_{50} values (Table 2). The LC_{50} value of *B. bassiana* was $4.58x10^6$ spores/ml followed by $3.47x10^6$ spores/ml for *M. anisopliae*. Similarly, the LT_{50} values of *B. bassiana* and *M. anisopliae* were 73.15 and 83.11 hours, respectively. Low LC_{50} value of $1.2x10^4$ spores ml^{-1} for *L. lecanii* against *Brevicoryne brassicae* and $2.7x10^4$ spores ml^{-1} against *Aphis gossypii* [12, 13]. The difference in the LC_{50} values might be due to the difference in the virulence of fungal isolates and the host species.

Similar results for *B. bassiana* with LT50 value of 3.17 days [14]. The LT50 value of 3.31 days obtained for *L. lecanii* against *Aphis fabae* [15] also agree with the present finding. *M. anisopliae* and *C. oxysporum* recorded higher LT50 values of 5.54 and 5.24 respectively.

C. Field efficacy of entomopathogenic fungi against *A. craccivora*

The data on the field efficacy of fungal pathogens, *B. bassiana*, *M. anisopliae* and *L. lecanii* in comparison with the standard checks *viz*., monocrotophos 36 EC and malathion 50 EC against *M. persicae* showed significant variations among pre-treatment and post treatment counts (Table 3 and 4). The pre-treatment population ranged from 52.62 – 67.73/ 4 cm^2 leaf area (Table 3). After the first spray, at 1 DAT, a significant reduction in the aphid population was observed in *B. bassiana* treatment. The *M. anisopliae* showed significantly less reduction of 1.23 per cent and was on par with the untreated check. The standard checks monocrotophos and malathion recorded significantly more reduction in aphid population than other treatments.

At 3 DAT, the same trend was observed in all the treatments. *B. bassiana* recorded 10.57 per cent reduction and *L. lecanii* recorded 2.34 per cent reduction. However, at 7 DAT, aphid population slowly increased in all the treatments. At 14 DAT, the population build up was relatively more than at 7 DAT in all the treatments. The mean percent reduction in the population at 14 DAT after first round of treatments was 13.32, 11.25, 81.43 and 79.87 per cent, respectively in *B.bassiana*, *M. anisopliae*, monocrotophos and malathion. From

the data obtained, *B. bassiana* was found to be superior among the three fungal pathogens applied under field conditions and the standard checks gave best results against *M. persicae* (Table 3).

The aphid population on second round of treatments ranged between 23.72 to 73.86 aphids/4 cm^2 (Table 4). After second spraying, *B. bassiana* showed a reduction of 12.23 per cent and *L. lecanii* showed significantly less reduction of 3.72 per cent at 1 DAT. The population was significantly reduced to about 71.13 and 80.28 per cent in standard checks endosulfan and malathion, respectively. Similar trend was observed on 3 and 7 DAT. Though, there was increase in population at 14 DAT, the standard checks were superior to untreated check with a percent reduction of 82.23 and 81.26 in monocrotophos and malathion, respectively. After second round of treatments, the mean percent reduction in aphid population at 14 DAT observed in *B. bassiana*, *M. anisopliae*, *L. Lecanii*, monocrotophos and malathion were 27.37, 18.73, 2.75, 41.34 and 42.24 per cent, respectively (Table 4). The cadavers collected from *B. bassiana* treated plants alone produced characteristic mycelial growth in SMA+Y medium on incubation and it was not so in the cadavers collected from *L. lecanii* treatment.

The result obtained with respect to the effect of *B. bassiana* in reducing the population of chilli aphid is supported by the findings of *B. bassiana* on banana aphid *Pentalonia nigronervosa*, which caused 37.0 to 96.66 per cent mortality of adult aphids and established that this fungus showed the highest mortality of nymphs and adults [16]. *B. bassiana* isolate CPD11 was highly pathogenic to cowpea aphid, *A. craccivora* causing a mortality range of 58 to 91 per cent, seven days after treatment [17]. *B. bassiana* was highly effective in controlling *C. lanigera* population at 14 days after treatment [18].

IV. CONCLUSION

The use of entomopathogenic fungi for the control of agricultural pests has long been recognized [19]. Despite their huge potential in biocontrol processes, the use of entomopathogenic fungi has been underestimated due to a lack of knowledge on their abilities. Moreover the efficacy of fungal pathogens in the field largely depends on extreme temperatures in the environment [20]. For future prospects, strategies to standardize the risk assessment of these fungal species as biopesticides are needed. Proper selection of strains with specific host target without having negative influence on non-target organisms is another major point of concern. Overall entomopathogenic fungi hold a promising role as a potential biopesticide for sustainable use in agriculture.

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Table 1. Pathogenicity of fungal pathogens on *M. persicae*

S. No	Fungal pathogen	% mortality*
1	*B. bassiana*	33.75 (31.67)[b]
2	*M. anisopliae*	11.25 (12.73)[d]
3	*L. lecanii*	16.25 (18.45)[c]
4	Monocrotophos	84.30 (83.24)[a]
5	Malathion	78.75 (79.45)[a]
6	Untreated check	0.0 (0.93)[d]

In a column, means followed by a common letter are not statistically different by DMRT (p = 0.05) Values in parentheses are *arc sine* transformed values ***** Mean of four observations

Table 2. Probit analyses of concentration, time - mortality response of *M. persicae* to fungal pathogens

Fungal pathogen	Concentration- mortality response				Time-mortality response			
	χ^2 * (p = 0.05)	Slope b ± SE	LC_{50} ($x10^6$spores/ml)	Fiducial limits	χ^2 * (p = 0.05)	Slope b ± SE	LT_{50} ** (Hours)	Fiducial limits
B. bassiana	8.41	1.42 ± 0.06	4.58	2.27 - 9.25	3.50	2.21 ± 0.75	73.15	72.98 - 94.65
M. anisopliae	9.52	0.34 ± 0.12	3.47	3.38 - 7.23	2.64	1.21 ± 0.45	83.11	70.28 - 97.12
L. lecanii	6.72	0.52 ± 0.59	2.84	2.11 - 4.52	5.24	3.50 ± 0.63	87.52	53.62 - 72.23

* All lines are significantly a good fit at p = 0.05
****** Tested at the higher dose of $1x10^8$ spores/ml

Table 3. Efficacy of fungal pathogens against *M. persicae* - I spraying – Field Trial

Treatments	Aphid population (No./4 cm^2) * Days after first spraying										
	Pre Treatment count	1		3		7		14		Mean	
		No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check
B.bassiana (1×10^8spores/ml)	61.52	57.39	5.82 (12.02)^{c}	62.27	10.57 (10.74)^{c}	58.71	23.52 (27.83)^{c}	84.13	15.27 (27.32)^{c}	76.49	13.32 (23.26)^{c}
M. anisopliae (1×10^8spores/ml)	57.37	54.93	1.23 (7.21)^{d}	57.24	1.92 (5.59)^{d}	51.28	17.24 (24.24)^{d}	73.96	19.22 (26.24)^{d}	62.31	11.25 (20.32)^{d}
L. lecanii (1×10^8spores/ml)	52.62	53.87	1.73 (7.68)^{d}	62.43	2.34 (6.59)^{e}	62.53	0.24 (2.81)^{e}	87.48	1.24 (5.48)^{e}	72.23	1.42 (6.47)^{e}
Monocrotophos (2ml/lit)	64.20	32.36	74.90 (62.12)^{a}	23.42	72.82 (68.34)^{a}	11.48	77.20 (61.47)^{a}	32.82	62.40 (52.30)^{a}	25.35	81.43 (73.54)^{a}
Malathion (2ml/lit)	52.68	38.54	72.50 (57.67)^{b}	28.54	76.40 (65.26)^{b}	12.25	70.50 (57.10)^{b}	34.26	71.62 (60.23)^{b}	22.47	79.87 (61.82)^{b}
Untreated check	67.73	70.27	-	62.43	-	65.40	-	85.14	-	73.45	-

In a column, means followed by a common letter are not statistically different by DMRT (p = 0.05)
Values in parentheses are *arc sine* transformed values ***** Mean of four observations

Table 4. Field efficacy of fungal pathogens against *M. persicae* - II spraying

Treatments	Aphid population (No./4 cm^2) * Days after first spraying										
	Pre Treatment count	1		3		7		14		Mean	
		No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check	No. of Aphids	% reduction over untreated check
B.bassiana (1×10^8spores/ml)	71.23	62.36	12.23 (20.64)^{c}	76.42	13.42 (21.27)^{c}	82.58	17.37 (19.47)^{c}	64.26	27.37 (22.46)^{b}	75.75	15.24 (21.94)^{b}
M.anisopliae (1×10^8spores/ml)	73.86	69.55	9.62 (18.24)^{d}	74.24	11.42 (26.12)^{d}	65.36	11.24 (23.76)^{d}	66.82	18.37 (19.26)^{c}	73.84	10.23 (18.52)^{c}
L.lecanii (1×10^8spores/ml)	77.38	73.33	3.72 (8.74)^{e}	77.38	3.46 (8.78)^{e}	75.13	3.26 (9.23)^{e}	72.45	2.75 (6.36)^{d}	78.33	4.32 (8.56)^{d}
Monocrotophos (2ml/lit)	23.72	8.44	82.23 (66.42)^{b}	6.77	75.53 (63.44)^{a}	8.35	64.27 (50.16)^{a}	24.58	41.34 (40.36)^{a}	10.67	64.23 (54.82)^{a}
Malathion (2ml/lit)	27.36	6.27	81.26 (64.62)^{a}	7.52	72.52 (56.27)^{b}	10.42	59.57 (52.29)^{b}	26.34	42.24 (41.83)^{a}	12.73	64.57 (56.47)^{a}
Untreated check	75.64	81.33	-	73.78	-	83.52	-	83.36	-	85.67	-

In a column, means followed by a common letter are not statistically different by DMRT (p = 0.05)
Values in parentheses are *arc sine* transformed values ***** Mean of four observations