



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(4): 694-698

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Received: 07-05-2020

Accepted: 09-06-2020

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Bio-efficacy of entomopathogens on major sucking pests in cowpea (*Vigna unguiculata* L.)

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Abstract

An experiment was conducted to study the bio-efficacy of entomopathogens on cowpea sucking pests viz., *Aphis craccivora* and *Riptortus pedestris*. *Fusarium pallidoroseum* and *Serratia marcescens* proved very effective entomopathogen against *A. craccivora* and *R. pedestris* respectively showing consistently higher mortality with increase in exposure time. *Beauveria bassiana* and *Metarhizium anisopliae* were found to be moderately effective. All the entomopathogens tested found to be promising in controlling these sucking pests.

Keywords: Cowpea, entomopathogens, bio-efficacy, *Aphis craccivora*, *Riptortus pedestris*

1. Introduction

Cowpea, *Vigna unguiculata* (L.) Walp, is an ancient Neolithic African crop grown throughout the tropics and subtropics as vegetable, pulse, fodder and cover crop. In India, cowpea is mainly cultivated in the states of Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. It is a nutritionally rich and highly priced vegetable and pulse in the domestic markets of Kerala.

The crop is damaged intensively by a large number of insect pests at various stages of its growth. Though the crop invites an array of pests, sucking pests, predominantly aphids and pod bugs often inflict severe damage to the economically viable parts. Cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae), is one of the most common aphid species in the tropics and is cosmopolitan, polyphagous pest with marked preference for leguminous plants and is a serious pest of cowpea, resulting in 20 to 40 percent yield loss^[15]. It is a sporadic pest, serious throughout the crop season. The colonies and scattered aphids feed on leaves, flower buds, pods and branches of cowpea^[16]. Serious damage occurs at high populations. Infestation greatly reduces pod formation and the entire plant may even be destroyed^[6].

Pod bug, *Riptortus pedestris* (Fabricius) (Heteroptera: Coreidae), the most destructive of leguminous crops, desaps tender shoots and pods of cowpea leads to the damage to pods and seeds up-to 60 to 70 percent^[7]. Management of *A. craccivora* and *R. pedestris*, the two most destructive sucking pests that severely curtail yield is of paramount importance for successful production of cowpea. Farmers often resort to application of chemical pesticides as a single track measure to contain them. This strategy, though provides initial relief, is not only counterproductive on the long run but also leaves toxic residue in the produce posing health hazards to consumers, warranting development of viable, sustainable and environmentally benign alternatives.

With increasing awareness of eco-friendly approach of pest management, microbial control employing application of entomopathogens particularly fungi found to be promising and several attempts proved as success against several sucking pests^[14]. Approximately 700 species of fungi in 90 genera are known to be entomopathogenic^[1]. Entomopathogenic fungi are reported from most of the insect taxa like Lepidoptera, Isoptera, Coleoptera, Hemiptera, Diptera and Orthoptera. These fungi have a wide host range including many important pests of cowpea. Widely studied entomopathogenic fungi belong to genera *Beauveria*, *Metarhizium*, *Verticillium*, *Hirsutiella*, *Erymia* (*Zoophthora*), *Nomuraea*, *Aspergillus*, *Aschersonia*, *Paecilomyces*, *Tolypocladium*, *Leptolegnia*, *Culicinomyces*, *Coelomomyces*, and *Lagenidium*^[10]. Broad spectrum fungal pathogens viz., *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorok were reported to be effective against a number of sucking pests^[5, 4, 11].

The present study is an attempt to biologically manage major sucking pests in cowpea by utilizing potential entomopathogens producing epizootics.

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2. Materials and Methods

The experiment was carried out at the Department of Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, India. The initial culture of the

entomopathogens was obtained from Department of Entomology, College of Agriculture, Vellayani. The cowpea variety Kanakamony was used to raise the seedlings.

Maintenance of cultures of entomopathogens

Entomopathogens	Isolated from	Media
<i>Fusarium pallidoroseum pallidoroseum</i>	<i>Aphis craccivora</i>	Potato Dextrose Agar (PDA)
<i>Beauveria bassiana</i>	<i>Odoiporus longicollis</i> Oliver	PDA
<i>Metarhizium anisopliae</i>	<i>Odoiporus longicollis</i>	PDA
<i>Serratia marcescens</i>	<i>Paradasynus rotratus</i> Dist.	Nutrient agar (NA)

200 ml of each culture medium was taken in separate 500 ml conical flasks, autoclaved at 121 °C (15 lbs) for 20 minutes. The plates were prepared by pouring 20 ml of media per plate and each isolate was inoculated in separate plates and were incubated at 25 °C.

The virulence of the entomopathogens were maintained by passing them periodically through *A. craccivora* and *R. pedestris* and reisolating them in fresh cultures. For this purpose spore suspension of the entomopathogens were prepared aseptically by pouring 10 ml of sterile distilled water into heavily sporulated one-week old culture plates. After shaking the plates the resulting spore suspension was sprayed on host insects. The mortality of host insects was noticed after two to four days. Later the dead insects showing fungal growth were collected, surface sterilized with 0.1 per cent

mercuric chloride, washed in sterile water three times and placed at the center of Petri dishes containing medium and incubated at room temperature. When growth was visible, it was subcultured and maintained in plates and slants for further studies.

2.1 Bio-efficacy of entomopathogens on sucking pests

Three entomopathogenic fungi and one entomopathogenic bacteria (Fig. 1) were tested for their pathogenicity to *A. craccivora* and *R. pedestris*. LC₅₀ value was fixed with the help of literature available.

1. *Beauveria bassiana* - 6.8×10^5 spores/ml [4]
2. *Metarhizium anisopliae* - 3.2×10^6 spores/ml [9]
3. *Fusarium pallidoroseum* - 7×10^6 spores/ml [17]
4. *Serratia marcescens* - 2.9×10^9 cells/ml.

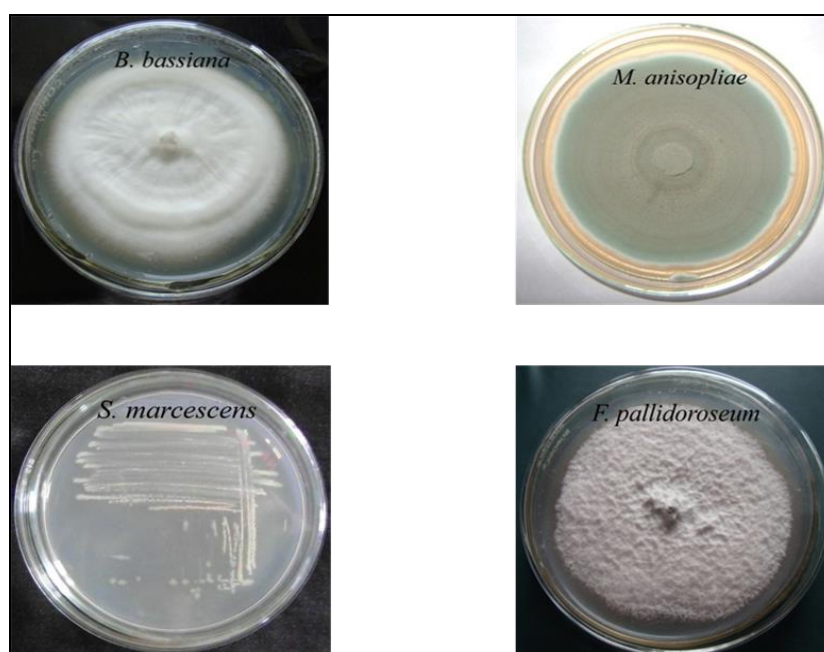


Fig 1: Entomopathogens used in bio-efficacy experiments

2.1.1 Preparation of spore suspension of entomopathogens

The fungi viz., *B. bassiana*, *M. anisopliae* and *F. pallidoroseum* were grown on PDA. From seven days old cultures, stock suspension of spores were prepared. The spores of fungi were harvested by flooding the plate with 10 ml sterile distilled water containing a little soap powder and scraping the surface with sterile spatula. The required spore concentration was adjusted with the help of haemocytometer. The bacterium, *S. marcescens*, was grown on NA. Two day old culture was used. For preparing the spray suspension, 10 ml of sterile distilled water was poured into culture plate and scraped using spatula. The required cell concentration was adjusted with the help of haemocytometer.

2.1.2 Application of spore suspension on *A. Craccivora*

Cowpea plants were raised in plastic cups of diameter 6 cm filled with soil in the glass house. Twelve replications were maintained for each treatment. Field collected *A. craccivora* was maintained on cowpea plants. Ten aphids were released to each plant, allowed for multiply for seven days. Pre-count of aphid was recorded. The spore suspension was sprayed uniformly on aphids using atomizer. The control was maintained by spraying aphids with sterile water.

2.1.3 Application of spore suspension on *R. pedestris*

Uniform staged bugs collected from field were used for the experiment which was conducted with five replications each

with five insects. Ten ml of spore suspension was sprayed using atomizer. After 20 minutes, treated insects were transferred into fresh cowpea pods placed in plastic jars secured with muslin cloth at the top. An untreated control was maintained by spraying the bugs with sterile water.

2.1.4 Observations

The treated insects were examined daily for their mortality. Observations on mortality of aphids were recorded at two, four and seven days after spray. Observations on mortality of bugs were recorded at five, seven, nine, eleven and thirteen days after spray. Dead insects were transferred to Petri plates containing moist tissue paper and observed for mycelial growth on the cadavers or symptoms of bacterial infection. Pathogenicity was further confirmed by Koch's postulates. The percent mortality was worked out using the following formulae.

$$\text{Per cent mortality} = \frac{\text{Initial population} - \text{Final population}}{\text{Initial population}} \times 100$$

2.2 Statistical analysis

The data was analysed for statistical significance by analysis of variance (ANOVA), to determine whether differences between the treatments on the basis of determining critical difference (CD) at 5% level of significance. The population data was converted into square root transformed value $\sqrt{x+1}$ and then subjected to statistical analysis.

3. Results

3.1 Bio-efficacy of entomopathogens on *A. craccivora*

The percentage mortality of the *A. craccivora* sprayed with different entomopathogens is presented in Table 1.

3.1.1 Two days after spray

F. pallidoroseum proved to be significantly superior to all other treatments recording 25.06 percent mortality. *B. bassiana* (11.83%) and *M. anisopliae* (8.57%) were found statistically on par with each other. *S. marcescens* caused least percent mortality of 4.62%. All the treatments were significantly superior to the control.

3.1.2 Four days after spray

All the treatments viz., *F. pallidoroseum*, *B. bassiana*, *M. anisopliae* and *S. marcescens* were significantly different from each other and from the control with mortality percent of 62.87, 18.82, 11.53 and 7.29 respectively.

3.1.3 Seven days after spray

F. pallidoroseum recorded the highest mortality (70.97%) which was significantly superior to rest of the treatments. This was followed by *B. bassiana* (35.34%), *M. anisopliae* (23.22%) and *S. marcescens* (11.34%). All the treatments differed significantly from each other and from the control.

3.2 Bioefficacy of entomopathogens on *R. pedestris*

The percentage mortality of the *R. pedestris* sprayed with different entomopathogens is presented in Table 2 (Fig.2).

3.2.1 Five days after spray

B. bassiana and *S. marcescens* recorded the highest percent mortality (16.60%) which was superior to other treatments. *M. anisopliae* (11.52%) was significantly different from the rest. *F. pallidoroseum* recorded least percent mortality (2.65%). All the treatments except *F. pallidoroseum* were significantly superior over the control.

3.2.2 Seven days after spray

S. marcescens showed highest percent mortality (28.66) which was on par with *B. bassiana* (26.22%). Next highest value was recorded by *M. anisopliae* (16.79%). Least percent mortality was recorded with *F. pallidoroseum* (2.65). All the treatments except *F. pallidoroseum* were significantly superior over the control.

3.2.3 Nine days after spray

S. marcescens caused highest percent mortality (51.88), which was on par with *M. anisopliae* (41.90) and *B. bassiana* (41.18). *F. pallidoroseum* recorded least value (5.09%) and was on par with the control.

3.2.4 Eleven days after spray

S. marcescens recorded highest percent mortality (61.23), which was on par with *B. bassiana* (50.43) and *M. anisopliae* (47.46). *F. pallidoroseum* recorded least percent mortality (9.14) and was on par with the control.

3.2.5 Thirteen days after spray

S. marcescens recorded highest percent mortality (81.09) and was on par with *B. bassiana* (73.03). Next highest percent mortality was recorded with *M. anisopliae* (63.69). *F. pallidoroseum* was having least mortality (18.35%) and was significantly different from all other treatments and the control.

Table 1: Percent mortality of *Aphis craccivora* treated with different entomopathogens

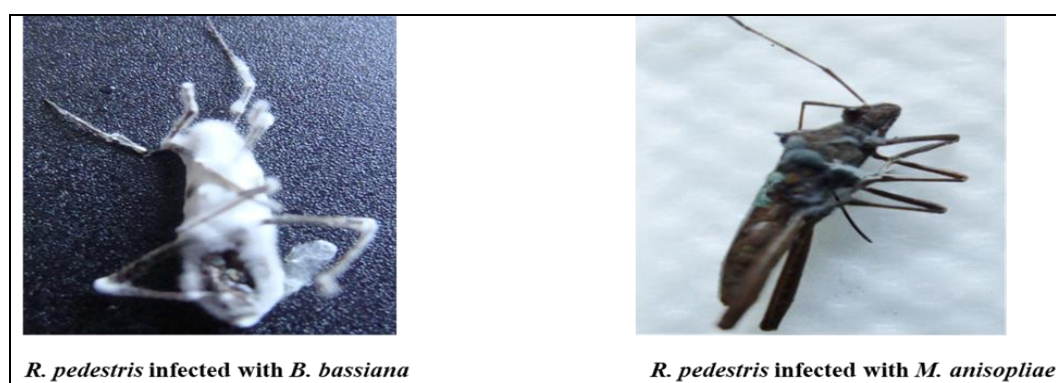
Entomopathogens		Mean per cent mortality		
		2 DAS	4 DAS	7 DAS
T1	<i>Fusarium pallidoroseum</i>	25.06 (5.11)	62.87 (7.99)	70.97 (8.48)
T2	<i>Beauveria bassiana</i>	11.83 (3.58)	18.82 (4.45)	35.34 (6.03)
T3	<i>Metarhizium anisopliae</i>	8.57 (3.09)	11.53 (3.54)	23.22 (4.92)
T4	<i>Serratia marcescens</i>	4.62 (2.37)	7.29 (2.88)	11.34 (3.51)
T5	Control (Water spray)	0.71 (1.31)	1.21 (1.49)	1.79 (1.67)
CD values (0.05)		0.611	0.414	0.551

Figures in parenthesis are $\sqrt{x+1}$ transformed values. DAS: Days after sowing

Table 2: Percent mortality of *Riptortus pedestris* treated with different entomopathogens

Entomopathogens		Mean per cent mortality				
		5 DAS	7 DAS	9 DAS	11 DAS	13 DAS
T1	<i>Beauveria bassiana</i>	16.60 (4.19)	26.22 (5.22)	41.18 (6.49)	50.43 (7.17)	73.03 (8.60)
T2	<i>Metarhizium anisopliae</i>	11.52 (3.54)	16.79 (4.22)	41.90 (6.55)	47.46 (6.96)	63.69 (8.043)
T3	<i>Fusarium pallidoroseum</i>	2.65 (1.91)	2.65 (1.91)	5.09 (2.47)	9.14 (3.18)	18.35 (4.39)
T4	<i>Serratia marcescens</i>	16.60 (4.19)	28.66 (5.45)	51.88 (7.27)	61.23 (7.89)	81.09 (9.06)
T5	Control (Water spray)	1.91 (1.71)	3.25 (2.06)	5.85 (2.62)	9.14 (3.18)	12.99 (3.74)
CD values (0.05)		1.325	1.387	1.219	1.306	0.806

Figures in parenthesis are $\sqrt{x+1}$ transformed values. DAS: Days after sowing

**Fig 2:** *R. pedestris* infected with entomopathogens

4. Discussion

Naturally occurring entomopathogens are important regulatory factors in insect populations and many species are employed as biocontrol agents of insect pests primarily from the perspective of safety to non target organisms. Under natural conditions, fungi are frequent natural mortality factor in insect populations. Unlike other potential biocontrol agents, fungi do not have to be ingested to infect their host but invade directly through the cuticle and hence can be used for the control of all insects including the sucking pests. Biological control with entomopathogenic fungi offers a sound management strategy for reducing yield losses caused by insect pests on cowpea [3].

Fungal diseases are regular feature among natural populations of sucking pests. Epizootics are noticed at times, though usually low incidences prevail. The possibility of controlling sucking pests by microorganisms is probably restricted to fungi since they are less amenable to control by others such viruses and bacteria.

All the four entomopathogens viz., *B. bassiana*, *M. anisopliae*, *F. pallidoroseum* and *S. marcescens* tested against *A. craccivora* under laboratory condition were found to be pathogenic but their virulence varied greatly. *F. pallidoroseum* proved to be very effective against *A. craccivora* as shown by consistently increasing mortality of aphids with increase in exposure period viz., 25.06, 62.87 and 70.97 percent mortality after two, four and seven days post treatment. *B. bassiana* and *M. anisopliae* were moderately effective with 18.82 to 35.34 percent and 11.53 to 23.22 percent mortality at four and seven days post treatment respectively. *S. marcescens* proved to be least effective among the entomopathogens tested by recording only 11.34 percent mortality seven days post treatment. Similar results

were previously recorded, which proved high virulence of *F. pallidoroseum* on *A. craccivora* in Kerala [8]. Laboratory bioassay of different fungal isolates of *B. bassiana*, *M. anisopliae* and *V. lecanii* showed mortality ranging from 16.70 to 60.45, 20.00 to 60.00 and 20.00 to 74.00 percent, respectively against cowpea aphid, *A. craccivora* [12]. However, in Agricultural Research Farm, Zari, Nigeria while studying the bioassay of fungal pathogens on *A. craccivora*, recorded 58 to 91 and 66 to 100 per cent mortality of aphids exposed to *B. bassiana* and *M. anisopliae*, respectively at seven days post treatment [4]. This difference from the results of present study may be due to variation in the pathogenicity of different isolates of the same fungus. Our results was supported by Nirmala *et al.* (2006) [12] in Bangalore where the pathogenicity of twelve fungal isolates belonging to *B. bassiana*, *M. anisopliae* and *V. lecanii* against *A. craccivora*, *Aphis gossypii* (Glov.) and *R. maidis* using the detached leaf bioassay technique. All the twelve isolates of the fungi were found to be pathogenic to *A. craccivora* and *A. gossypii* at a concentration of 1×10^7 spores per ml and the mortality ranged from 2 to 74 per cent in *A. craccivora*.

All entomopathogens tested against the cowpea pod bug, *R. pedestris* were found to be effective and consistent in bringing mortality of *R. pedestris* with prolonged time of exposure. *S. marcescens* proved to be more pathogenic to *R. pedestris* with highest mean mortality of 81.09 percent 13 days post treatment. This was followed by *B. bassiana* (73.03%) and *M. anisopliae* (63.69%). However, *F. pallidoroseum* showed its ineffectiveness in infesting *R. pedestris* as compared to *A. craccivora* with least mortality of 18.35 percent 13 days post treatment. Similar results were recorded on the pathogenicity of *B. bassiana* to the coreid bug *R. linearis* under laboratory conditions, where all the life stages of the bug was shown to

be susceptible to *B. bassiana* [5]. At different concentrations, *B. bassiana* and *M. anisopliae* caused mortality in *Clavigralla tomentosicollis* ranging from 58 to 97 percent and 53 to 100 percent, respectively at seven days post treatment [2]. Prayogo and Suharsono (2005) [13] observed that control of pod sucking bug *R. linearis* using entomopathogenic fungus, *V. lecanii*, as the most promising biocontrol tactic due to environmental safety.

6. Conclusion

In the present investigation, entomopathogen *F. pallidoroseum* proved to be very effective against *A. craccivora* showing consistently increasing mortality of aphids with increase in exposure time. *B. bassiana* and *M. anisopliae* were found to be moderately effective. *S. marcescens* was found to be pathogenic to *R. pedestris* exhibiting highest mean mortality percent at 13 days post treatment. *B. bassiana* and *M. anisopliae* showed satisfactory results in containing *R. pedestris*. Employing these entomopathogens in IPM modules or in organic farming can be promising in controlling these sucking pests.

7. Acknowledgment

We would like to thank Kerala agricultural university for providing necessary facilities to carry out the experiment. We are thankful to Dr. Anith, K.N for the help rendered in proving cultures and laboratory facilities.

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