International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 04-01-2024, Accepted: 21-01-2024, Published: 05-02-2024
Volume 9, Issue 2, 2024, Page No. 10-16

A laboratory assessment of the potential of entomopathogenic fungi against rice moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae)

S Gowri Lakshmi[1*], Levin Anbu Michele Gomez[2], Vinohar Stephen Rapheal[3], S Muthu Kumar[4]
[1] Assistant Professor, Department of Zoology, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India
[2] Division of Biotechnology, School of Agriculture & Biosciences, Karunya Institute of Technology & Sciences, Karunya Nagar, Coimbatore, Tamil Nadu, India
[3] Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India
[4] Department of Zoology, Avvaiyar Government College for Women, (Affiliated to Pondicherry University), Karaikal, Puducherry UT, Tamil Nadu, India

Abstract
Aim of the study: The ubiquitous grain pest *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) damages godowns and warehouses. Compared to *Ephestia kuehniella* and *Plodia interpunctella*, *C. cephalonica* pest potential is sometimes underestimated. Recent research shows that non-chemical management measures can successfully replace chemical pest control for farms. Thus, the most common fungal entomopathogens can control insects of diverse orders.
Methods: The plants *B. bassiana, M. anisopliae, L. lecanii* and *P. lilacinum* were taken from the ground and used at different amounts to treat *C. cephalonica* larvae in the lab. Statistical Package for the Social Sciences (SPSS), ver.16.00 SPSS Inc., USA, was used to do probit analysis on the concentration mortality rates and make correlations.
Results: Entomopathogenic fungus *B. bassiana, M. anisopliae, L. lecanii* and *P. lilacinum* were isolated from soil and treated on *C. cephalonica* larvae. *B. bassiana* proved effective. All four plates had protease, amylase, lipase, and chitinase clearance zones. This zone of clearance showed that *B. bassiana* culture filtrate produced protease, amylase, lipase, and chitinase. Negative impacts included short-lived adults with malformed bodies.
Conclusion: The study's findings indicate that *B. bassiana* effectively controlled *C. cephalonica*, suggesting its importance in an integrated pest management approach.

Keywords: Biological parameters, *Corcyra cephalonica*, entomophathogenic fungi, enzymatic analysis

Introduction

Asia and Africa will account for most of the 9.7 billion people by 2050 [1, 2]. In addition to population growth, climate change is expected to increase food consumption by 32% from 30% to 62% and the population at risk of famine by 39% from 91% to +30%. If the world is to survive population expansion and climate change, it must adopt new and improved agricultural practices for high sustainability and production. Biopesticides and other organic treatments like manure can boost crop output, as can minimizing yield losses from harsh environmental circumstances such abiotic and biotic stressors [3].

Corcyra cephalonica (Stainton) is a Lepidopterous insect that infests stored grains and products, commonly known as the rice moth. The insect pest has primarily been documented in continents like Europe, Africa, Asia, and North America [4]. However, it is also expanding its presence to other countries worldwide due to the transit of affected crops and commodities. The larval phase is highly damaging and typically exhibits a preference for infesting compromised or fractured cereal grains. Feeding persists beneath the silken fibers that are generated, gradually causing the contaminated items to clump together as the fibers grow more compact [5]. The presence of larval feces, webbings, exuviae, and cadavers diminishes human acceptance and hence lowers the market value of the products.

Pests of numerous types devastate crops and forests worldwide. Recent widespread use of chemical pesticides has damaged soil and aquatic ecosystems and caused insect resistance. Chemical pesticides are less effective due to insect resistance in over 400 species [6]. Chemical pesticides are an essential global pest control tool [7]. Many commercially significant insect pests in stored grains have developed resistance to popular insecticides, including phosphine [8, 9]. Repeated synthetic insecticide use causes insect resistance [10]. Integrated pest management systems that involve biological control can help overcome insect resistance [11, 12]. Biopesticides also have various advantages, such that many target pests are susceptible [13]. Biopesticide programmes must be utilized to prevent insect pests from developing biopesticide toxicity and synthetic chemical pesticide resistance [14].

Entomopathogenic fungi are being evaluated as viable alternatives to chemical pesticides. Their ability to cause disease has been the main subject of numerous research investigating the use of pathogens for controlling stored products. Entomopathogenic fungi are microorganisms that are widely present in nature and therefore have minimal impact on the environment and pose low toxicity to mammals. In addition, they have the capability to proliferate on the cadavers, so perpetuating the release of further inoculum into the system. The enduring impact of fungus, as opposed to the lasting presence of chemical pesticides, is a favorable characteristic in biological control [15].

The genera Beauveria, Metarhizium, and Isaria are considered highly significant microbial species utilized as biological control agents. These species have been extensively studied for their ability to combat various insect hosts [16]. The most extensively researched fungal species

for their efficacy against insect pests in stored products are *Beauveria bassiana* (Balsamo) *Vuillemin* (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metschinkoff) *Sorokin* (Hypocreales: Clavicipitaceae), and *Isaria fumosorosea* Wize (Hypocreales: Clavicipitaceae) [17]. The virulence of entomopathogenic fungi is influenced by various factors, including the timing of the initial host infection, the duration of the disease incubation period, which leads to delayed host development, the rate of spread of the entomopathogenic fungus, and other environmental factors that promote epizootics. The goal of the current study was to assess *B. bassiana* potential as a biological control agent against *C. cephalonica*.

Materials and methods

Isolation of Fungi

Soil samples were taken from the field of Tamilnadu Agricultural University Campus, located in Coimbatore, Tamil Nadu, India. A soil sample weighing 0.2 grams was placed in a micro tube with a volume of 1.5 milliliters. Then, 1.3 milliliters of a solution containing 0.02% Tween-80 was added to the tube. The contents of the tube were mixed vigorously for a duration of 15 minutes using a vortex mixer. The suspension was diluted in a series of steps till reaching a dilution of 10-7. SDA plates were prepared and 100 µl of the samples, which were diluted from 10-1 to 10-7, were distributed on sterile SDA plates using the spread plate technique [18]. The individual colonies from the mixed or mother culture were transferred to fresh SDA plates in order to obtain a pure culture of each isolate. Following a 6-day incubation period at a temperature of 25˚C, the pure culture was separated in order to undergo further identification using the LPCB staining procedure.

Selection of Culture Media

The following agar media were used in the study: Potato Dextrose Agar (PDA) [19], Sabouraud Dextrose Agar (SDA), Potato Carrot Infusion Agar (PCA) [20], Czapekdox Agar [21], Complete Medium Agar (CMA) [22], and Rice infusion [23]. The six distinct media were assigned the designations 1, 2, 3, 4, 5, and 6. Each of the six media, totaling 250 ml, were individually inoculated with entomopathogenic fungus A (BB), B (MA), C (LL), and D (PL) in separate 250 ml Erlenmeyer flasks. The flasks were then incubated for a period of nine days. Considering the mat's growth and thickness, Media 5 (CMA media) was chosen. Each of the four fungus was then separately introduced into CMA (media: 5) broth and cultured for a period of nine days.

Culture filtrate preparation

The culture filtrate was generated using the methodology described by Kim *et al* [24]. The user filtered two hundred and fifty milliliters of four fungal culture using sterilized filter paper. The filtrate was then passed through a Whatman filter paper No. 2 and a syringe filter with a pore size of 0.2 µm to remove spores and hyphae. This resulting liquid is referred to as the "culture filtrate". The four culture filtrates were utilized for bioassay within 24 hours of filtration. Subsequently, these culture filtrates were employed to assess their larvicidal activity.

Insect rearing

C. cephalonica larvae were procured from ICAR-National Bureau of Agricultural Insect Resources, Bangalore, India). Larvae were maintained at 25±1°C; and 65±5% relative humidity (RH) under a 16L:8D photoperiod in a growth chamber. Larval instars I–VI and pupae were used for acute toxicity experiments. Newly emerged male and female adults were tested for longevity and fecundity experiments.

Bioassay to select one effective fungus

2.5 milliliters of purified culture filtrate was diluted to a total volume of 10 milliliters. This solution was then sprayed over 50 grams of broken pearl millets. After allowing the millets to dry, ten newly moulted third instar larvae of *C. cephalonica* were introduced. The same process was applied to culture filtrates of 5 ml and 7.5 ml. The items were stored in plastic containers equipped with lids that had small holes. The control was represented by the broth that was not infected. Each concentration was replicated three times. Everyday mortality was documented over a period of up to seven days. Out of the four selected fungi (*B. bassiana*, *M. anisopliae*, *L. lecanii* and *P. lilacinum*), the fungus with the highest effectiveness in terms of death rate, *B. bassiana*, was selected for future study.

Enzymatic Plate Assay

The culture filtrate was first subjected to screening for protease, amylase, lipase, and chitinase activity, which was subsequently confirmed by observing the zone of inhibition.

Protease assay

The activity of protease in a solid medium was assessed by measuring the area of clearance resulting from the breakdown of milk protein in a pH indicator medium (consisting of 0.01% yeast extract, 2% agar, and 0.01% bromocresol purple, adjusted to pH 5.2) containing 1% skimmed milk [25]. A ten millimeter well was created at the center of the medium using a car borer. Then, 50 µl of crude culture filtrate was applied to the well. After 24-48 hours of incubation, the zone of clearance around the well was measured. Three instances of replication were conducted.

Amylase assay

The strain was tested for amylase production using starch agar media. The culture was introduced into the petriplate containing solidified starch agar medium. The petri dish was placed in an incubator and maintained at a temperature range of 28-30°C for a duration of 48 hours. Following incubation, the plate was immersed in a solution of Gram's iodine (consisting of a 2:1 ratio of potassium iodide and iodine in 300 ml of distilled water). A favorable result was determined by observing a distinct area surrounding the colony against the blue background [26].

Lipase assay

Lipase in solid medium was measured by the clearing zone produced by degradation of lipids in tributyrin agar (0.5% peptone, 0.3% yeast extract, 2% agar, 0.01% methyl red, pH 7.5 with 1% tributyrin) [27]. One percent sterile tributyrin was added to the media after cooling to 80°C and mixed thoroughly to emulsify the tributyrin completely and poured to maintain uniform turbidity. Ten millimetre well was cut in the centre of tributyrin agar plate and 50µl of crude culture filtrate was added to the well and the zone of clearance around the well was measured after 24-48 hrs of incubation. Each plate served as a replicate with three replications per treatment.

Chitinase assay

Colloidal chitin was synthesized using the procedure outlined by Gulati method 1997. Chitin powder, weighing 5 mg, was gradually introduced into 90 ml of strong hydrochloric acid (HCl) while vigorously stirring for a duration of 2 hours. Subsequently, 1 liter of distilled water was added. The fine white precipitate formed was collected at a temperature of 4°C using centrifugation at a speed of 6,000 revolutions per minute for a duration of 10 minutes. The colloidal chitin was washed multiple times with distilled water until it reached a neutral pH of 7. It was then dried at a temperature of 50°C and subsequently utilized for additional research purposes. Chitin agar plate was supplemented with colloidal chitin at a concentration of 0.5% and congo red at a concentration of 0.001%. The plate was injected with the fungi (5A) and examined for the presence of a clean area surrounding the Fungus colony following incubation.

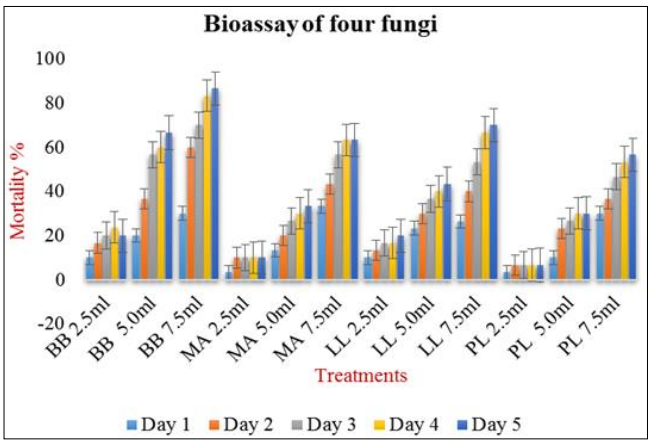
Statistical Analysis

The per cent mortality data from laboratory investigations were adjusted using Abbott's algorithm [28]. The percentages acquired from various trials were converted to arcsine percentage values, while the optimization of media for fungal growth was subjected to a square root transformation using the formula (x+0.5). The analysis of variance was conducted using AGRES (ver 7.01 Pascal International Software Solutions, USA) in various experiments. The means were then distinguished using the least significant difference (LSD) provided by the software package. The concentration mortality responses underwent probit analysis, as described by Finney [29], and correlations were computed using the Statistical Package of Social Sciences (SPSS), version 16.00, developed by SPSS Inc., USA. The collected data were statistically analyzed by combining the data from all the collections.

Results and Discussion

Percent mortality of *C. cephalonica* at different concentrations of four entomopathogenic fungi *B. bassiana, M. anisopliae, L. lecanii* and *P. lilacinum*

The efficacy of entomopathogenic fungi in the biological control of insect pests is largely attributed to their capacity to trigger widespread outbreaks from infected corpses [30]. The deceased bodies function as a reservoir of inocula, facilitating the dissemination of microbial agents in the surroundings [31, 32]. The mortality rates of *C. cephalonica* at different concentrations of culture filtrates from four distinct fungi were recorded and presented in Table 1. Additionally, a bar graph illustrating the percentage of deaths was shown in Figure 1. On the first day, the combination of 2.5 ml of *M. anisopliae* and *P. lilacinum* resulted in the lowest mortality rate of 3.33%, whereas using 7.5 ml of *M. anisopliae* resulted in the highest mortality rate of 33.33%. The CD value (11.33) signifies that there is a substantial difference between the treatments at a 5% level of significance. On the second day, the mortality rate was highest (60%) when using 7.5 ml of *B. bassiana* at a greater concentration, while the lowest mortality rate (6.66%) was seen with 2.5 ml of *P. lilacinum*. The critical difference (CD) score of 11.73 indicates that there is a significant difference between the treatments at a significance level of 5%. On the third day, the application of 7.5 ml of *B. bassiana* resulted in the highest death rate of 70%, while the use of 2.5 ml of *P. lilacinum* resulted in the lowest mortality rate of 6.66%. The control group did not experience any mortality. At a significance level of 5%, the treatments showed significant differences with a CD value of 13.91. On day 4, the mortality rate was highest for *B. bassiana* at a dosage of 7.5 ml (83.33%), while *P. lilacinum* at a dosage of 2.5 ml had the lowest mortality rate (6.66%). The treatments exhibited a significant difference at a 5% level of significance, as shown by a CD value of 13.30. On the fifth day, the concentration of *B. bassiana* at 7.5 ml exhibited the highest mortality rate of 86.66%, whereas the concentration of *P. lilacinum* at 2.5 ml had the lowest mortality rate of 6.66%. The critical difference (CD) score of 13.77 demonstrates that the treatments exhibited statistically significant differences at a significance level of 5%. In the combined investigation, the concentration of *B. bassiana* at 7.5 ml resulted in the highest mortality rate of 65.99%, while *P. lilacinum* at 2.5 ml had the lowest mortality rate of 5.99%. The critical difference (CD) score of 11.50 demonstrates a substantial difference between the treatments at a 5% level of significance. The findings of the experiment indicate that the *B. bassiana* culture filtrate, containing 7.5 ml, exhibited the highest larvicidal activity among the three fungi. The entomopathogenic fungi *L. lecanii, P. lilacinum, B. bassiana* and *M. anisopliae* were obtained from the soil during this study. Based on the studies conducted by Klingen *et al*. [33], Shapiro-llan *et al*. [34], Meyling *et al*. [35], and Batalla-Carrera *et al*. [36], this was found to be true. Entomopathogenic fungi, which occur naturally, were found in the soil.



BB-*B. bassiana*; MA-*M. anisopliae*, LL-*L. lecanii*; PL-*P. lilacinum*

Fig 1: Percent mortality of *C. cephalonica* larvae treated with different concentrations of culture filtrate of four entomopathogenic fungi

Table 1: Percent mortality of *C. cephalonica* larvae treated with different concentrations of culture filtrates of four entomopathogenic fungi

Treatment	Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Pooled Mean
B. bassiana	2.5 ml	10	16.66	20	23.66	20	18.06
	5.0ml	20	36.66	56.66	60	66.66	47.99
	7.5ml	30	60	70	83.33	86.66	65.99
M. anisopliae	2.5 ml	3.33	10	10	10	10	8.66

	5.0ml	13.33	20	26.66	30	33.33	24.66
	7.5ml	33.33	43.33	56.66	63.33	63.33	51.99
L. lecanii	2.5 ml	10	13.33	16.66	16.66	20	15.33
	5.0ml	23.33	30	36.66	40	43.33	34.66
	7.5ml	26.33	40	53.33	66.66	70	51.26
P. lilacinum	2.5 ml	3.33	6.66	6.66	6.66	6.66	5.99
	5.0ml	10	23.33	26.66	30	30	23.99
	7.5ml	30	36.66	46.66	53.33	56.66	44.66
Control		0	0	0	0	0	0
SE		5.51	5.70	6.77	6.47	6.70	5.84
CD (5%)		11.33	11.73	13.91	13.30	13.77	11.50

Enzymatic activity of *B. bassiana* on agar plate with substrates

In all four plates, the zone of clearance for the activities of protease, amylase, lipase, and chitinase could be seen. This zone of clearance demonstrated that *B. bassiana* produced the enzymes protease, amylase, lipase, and chitinase in the culture filtrate (Figures 2, 3, 4, and 5). The current investigation demonstrated that *B. bassiana* culture filtrate displayed enzyme secretion that was consistent with Kim *et al*. [37] findings. He discovered that chitinase and cotton aphids' degraded hemocoel were present in the supernatant of an isolate of *B. bassiana*. Similarly, Narasaiah, [38] examined the amylase and lipase enzymes in *Streptomyces albus* CN-4. This was also in tune with Patel *et al*. [39]. They screened the fungal endophtes from indigenous monocotaceous and dicotaceous plants for enzymes amylase and protease. Vining *et al*. [40], Hamill *et al*. [41], Suzuki *et al*. [42], Roberts, [43] Jeffs and Khachatourians [44], Strasser *et al*. [45] and Quesada-Moraga and Vey, [46] found that *Beauveria* sp. produce toxic metabolites including bassianin, beauvericin, bassionalidae, beauveroiolide, bassacridin, oosporein and tenellin.

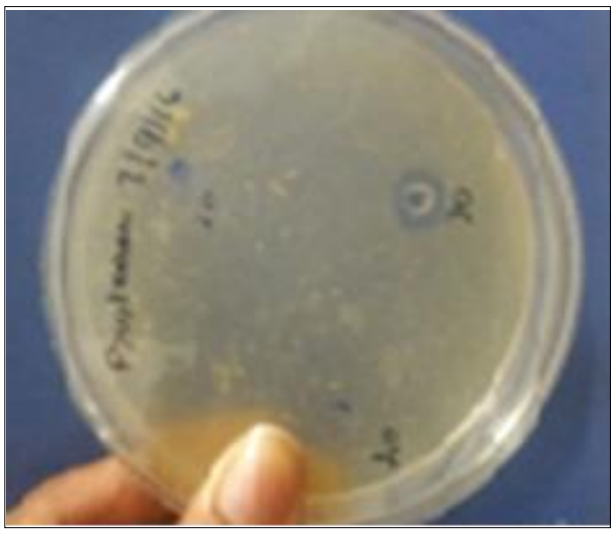


Fig 2: Protease activity *B. bassiana* on agar plate containing casein as substrate

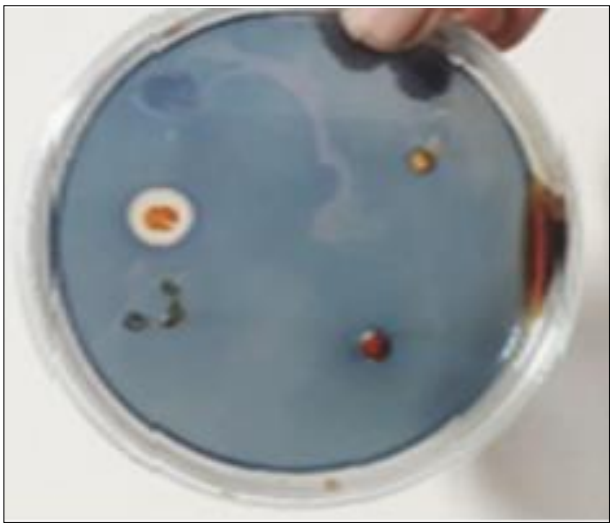


Fig 3: Amylase activity *B. bassiana* on agar plate containing starch as substrate

Fig 4: Lipase activity *B. bassiana* on agar plate containing olive oil as substrate

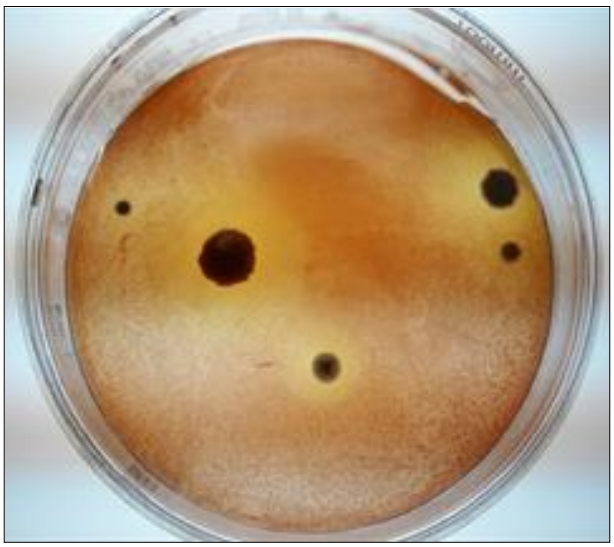


Fig 5: Chitinase activity B. bassiana on agar plate containing chitin as substrate

Fig 6: Bioassay tested against *C. cephalonica*

Fig 7: Dead larvae of *C. cephalonica* after the tratement

Conclusion

The results presented indicated that the persistency of virulence for *B. bassiana* was longer efficacy than *M. anisopliae L. lecanii* and *P. lilacinum* when tested against *C. cephalonica* larvae. *Beauveria bassiana* seemed to sustain pathogenicity better than *M. anisopliae*, *L. lecanii* and *P. lilacinum* in a drier environmental condition, and this concurs with the report by Hallsworth and Magan (1999). We conclude that the approach of *B. bassiana* is an effective microbial control tactic against *C. cephalonica*. *Beauveria bassiana* provided good protection against *C. cephalonica*, inflicting in excess of 86% larval mortality 5days after

treatment. The storage insects can absorb lethal doses of the entomopathogen from sporulating cadavers. Use of this fungus can potentially benefit the environment and lead to more effective protection of stored grains. So from these results it is evident that *B. bassiana* may become an integral part of safe IPM program of stored grain pests. To assess the practical value of these results further experiments are required to evaluate the efficacy of fungal treatment on a large scale.

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