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Advances in Applied Science Research, 2017, 8(4):21-29

ISSN : 0976-8610
CODEN (USA): AASRFC

Biocontrol Prospects of Entomopathogenic Fungi for Management of Mustard Aphid (*Lipaphis erysimi* Kalt.) on Rapeseed-Mustard

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ABSTRACT

Mustard aphid, Lipaphis erysimi (Kalt.), is the most serious insect-pest of rapeseed- mustard and responsible for causing the yield losses ranging from 35.4 to 96 percent depending upon weather condition. Entomopathogenic fungi are natural enemies of various pests and are considered to be valuable bio-control agents in sustainable crop management. In this study, laboratory evaluations were made to assess bio-control potentiality of few entomopathogenic fungi for mustard aphid management. Analysis of the virulence of 4 fungal strains against Mustard aphid was carried out in laboratory bioassays at 1 × 10^8 CFU ml^{-1} for 3 and 7 days. The pathogenicity study revealed two most efficient fungal strains Metarhizium anisopliae followed by Nomuraea releyi as bioagent with least incubation period against Mustard aphid. The FTIR spectrum of extracts for the fungal isolates such as Metarhizium anisopliae, Beauveria bassiana, Verticillium lecanii and Nomuraea releyi were analyzed. FTIR spectrum was blown up for the selected fungal strains to locate specific wavenumber cm-1 with regard to find out the specific stretch.

Keywords: Mustard aphid, Bio-control, Entomopathogenic fungi, Virulence, FTIR

INTRODUCTION

India is the 4th largest edible oil economy in the world after the USA, China and Brazil with about 10 percent oil seed production of the world. India contributes around 6.7 m of rapeseed – mustard production in the world. A wide range of oilseed crops is produced in different agro climatic zones of the country. Among the seven edible oilseeds cultivated in India rapeseed-mustard contributes 28.6 percent in the total oil seeds production and ranked second after groundnut with a sharing of 27.8% in the India's oilseed economy.

Rapeseed-mustard (*Brassica* sp.) is a major group of oilseed crop which constitutes a major source of edible oil for the human consumption and cake for animals. It is grown on an area of about 6.4 m ha with production of 8.02 m and productivity is 1262 kg/ha [1]. Different species of rapeseed- mustard are grown in different parts of the country and in Assam the Yellow sarson (*B. rapa* var. *trilocularis*) is usually cultivated. Among various biotic factors responsible for reducing the yield of rapeseed-mustard, insect pests are the major one. Pests and diseases can dramatically reduce crop yield, quality and subsequent returns. Thirty- eight insect pests are known to be associated with rapeseed-mustard crop in India [2]. Many pests attack these crops during its different growth stages among which mustard aphid, *Lipaphis erysimi* (Kalt.) is one of the major pest in all the mustard growing regions of the country [1,3]. *Lipaphis erysimi* causes 35.4 to 96% yield loss, 30.9 percent seed weight loss and 2.75 percent oil loss [2]. It has a rapid reproduction which causes a serious damage. Both nymphs and adults of the mustard aphid suck cell sap from the leaves, inflorescences and immature pods resulting into very poor pod setting and yield. Aphids also transmit plant viral diseases, i.e., turnip mosaic virus, which can be managed by effective control of aphid [4,5].

Rapeseed-mustard cultivation under marginal management becomes less productive for the farmers which results in a big gap between the national requirement and production in India. Rapeseed - Mustard is the principal oilseeds grown in the State Assam which occupied about 8.00 percent of the total crop area. Effective management of natural resources with an adaption of integrated nutrient and pest-management practices will play a major role in increasing

the productivity of rapeseed-mustard in India. Though the introduction of high yielding varieties and advancement in technology results in productivity improvement of food grains however in oilseed sector still India have to import about half of domestic requirement.

A wide range of insecticides is being used to control this pest, which in turn contributes to environmental pollution. The growing demand for reducing chemical inputs in agricultural practices influence to search for some alternative eco-friendly methods of pest control. Myco-biocontrol agents are naturally occurring organisms which offer an alternative to the use of chemical pesticides for eco-friendly pest management. The exploitation of biocontrol agents is considered as a suitable alternative to the use of chemical pesticides [6].

Among the various biocontrol agents, entomopathogenic fungi are being a major component of an integrated approach that can provide significant and selective insect control. Entomopathogenic fungi are important natural regulators of insect populations and are preferred to kill insects at various stages of its life cycle [7]. Entomogenous fungi are the promising myco-biocontrolling agent for a number of crop pests. Several species belonging to order Lepidoptera, Coleoptera, Homoptera, Hymenoptera and Diptera are susceptible to various fungal infections. These entomopathogens have potential as bio-control agents against diverse insect pests in agriculture due to their eco-friendliness and bio-persistence [7]. Because of the host specificity of entomopathogenic fungi they become the most versatile biological control agents. The effectiveness of the myco-biocontrol agents mainly depends on the susceptibility of the insect and also virulence of the fungus. The virulence of entomopathogenic fungi varies considerably with respect to pest and other environmental factors.

The anamorphic entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* are natural enemies of a wide range of insects including aphids [8,9]. Various researchers conducted bioassays on different crop pest and recorded one or two isolates of entomopathogenic fungi to be highly effective against mustard aphid [10]. Similarly, *Paecilomyces fumosoroseus*, *P. farinosus* and *P. lilacinus* have been reported as entomopathogenic on a variety of insect pests [11]. Limited information is available on the use of indigenous entomopathogenic fungi for the control of insect pests in India. In view of combating the serious mustard pest, the present investigation was undertaken to evaluate pathogenicity of four indigenous fungal strains viz., Metarhizium, Beauveria, Verticilium and Nomuraea in the laboratory against mustard aphid for their utilization in an integrated pest management program.

MATERIALS AND METHODS

The investigation was carried out in Environmental and Industrial Biotechnology Division, Biotechnology area, TERI (The Energy and Resources Institute), Guwahati, during 2014-2016.

Screening of entomopathogenic fungi

Samples of insects both alive and dead, were collected from agricultural fields of Khetri, Kamrup (Rural) using brush and forceps. The samples were aseptically placed in sterile glass vials and taken to the laboratory for isolation of natural pathogens. The fungal hyphae were directly isolated from the insect cadavers and inoculated to Potato Dextrose Agar (PDA) medium [12]. Other diseased insects with no fungal growth on their body were surface sterilized with 0.1% sodium hypochlorite, rinsed with sterile water, placed in PDA poured Petri Plates and the pure cultures of the fungi growing out from the insects were maintained on Sabroud dextrose agar (SDA) slants. All the locally isolated strains were identified by using key for identification as described by Humber [13], Humber and Steinkraus [14]. The fungal culture regularly maintained and revived on mycological media [12].

Insect culture

For preparation of nucleus culture, aphid adults were collected from mustard field of Khetri, Kamrup (Rural) and rearing was done in the laboratory at 26 ± 2°C and 70-80% RH. The 25 days old mustard plant raised on pots were used as food for freshly emerged adults. Potted Mustard plants were placed inside the rearing cages for nymph lying along with at least 60 pairs of aphid per pot. After 2-3 days the females starts nymph lying on mustard plants. After the emergence of nymphs on plants aphid adults were transferred to another plant with the help of aspirator for nymph lying. When newly emerged nymphs reached to the first and second instars they were used for pathogenicity and virulence study of different plant extracts. As aphids are homopteran having ovoviviparous type of reproduction, its multiplication is easy as compared to others.

Insect rearing and bioassay preparation

Mustard aphids were collected from different mustard crop fields and reared on mustard plants in experimental insect

rearing unit for use in further experiments. Petri Plates of 9 cm diameter were used as bioassay chambers. Freshly collected mustard leaves were surface sterilized with 0.005% Sodium hypochlorite for 2 min, dipped in 200 ppm solution of penicillin in sterilized water for 1 min and then rinsed with sterilized water. The pre-sterilized test leaf was placed at the center of each Petri plate and numbers of Aphids were gently transferred to the leaves. Wet and sterilized cotton was placed around the midrib initial end of the leaf for humidity and protection of leaf from dehydration in Petri plate chamber. These leaves containing 10 numbers of aphids were then transferred to bioassay chambers.

Preparation of Fungal Spore suspension: Ten ml of sterilized water with 0.001% Tween 80 was added to each 10 days old culture plate and spore suspension was prepared by gently rubbing the culture surface with the help of a sterilized spatula. Suspension from all plates of a fungus was collected in a 100 ml beaker and mixed thoroughly to get a homogenized mixture. Spore suspension prepared at the range of 10^5 to 10^8 spores ml^{-1} by adding distilled water.

Laboratory bioassays

The viability of spores was determined by standard method [12] by plate counts of spores on SDA after 18 h of incubation. In initial experiments, spore suspensions containing $>10^{10}$ c.f.u. ml^{-1} were used to select the potential entomopathogenic isolates. With the help of a 1cc insulin syringe, a drop of spore suspension was placed on each insect in bioassay chambers. Water with 0.001% Tween80 but without fungal spores was used to inoculate the insects in control treatments. After 72 h incubation, the insect cadavers were surface sterilized and inoculated in PDA poured Petri plates for re-isolation of the inoculated fungi. On the basis of results of this experiment, strains showing entomopathogenic potential were selected for use in future experiments whereas non-entomopathogenic strains were discarded. In another experiment, the Spore suspensions of all the selected isolates were diluted to get final, concentrations of 10^9, 10^7, 10^5 and 10^3 spores ml^{-1} and used to evaluate their efficacy by the method described above. The mortality of insects was recorded at 24 h interval for 5 days in comparison to control insects. LC50 and LT50 values for each fungal strain were calculated by using probit analysis for % mortality [15]. The experiment was performed at 22 ± 1°C with 10:14 h L:D photoperiod. The relative humidity of bioassay chambers was kept at 99 ± 1%.

FTIR Analysis of the potent entomopathogenic fungal isolates

FT-IR spectrum also studied to investigate the functional group of the screened potent entomopathogenic fungal isolates against mustard aphids. FTIR (Fourier transform infrared) spectra was analyzed by KBr pellets methods using FTIR spectrophotometer [Perkin Elmer, Spectrum two FTIR, Standard DTC (Dithiocarbamates), KBr (Potassium bromide)] to investigate the functional groups present in the MIR (Mid Infrared) region of 400-4000 cm^{-1} in the fungal secrete [16]. The functional groups were assigned referring FTIR data available in published literature. The samples were prepared by harvesting fungal biomass after incubation at Potato Dextrose (PD) broth by filtering through filter paper followed by repeated washing with de-ionized water to remove any medium component from the biomass. For the FTIR spectra study the vacuum dried samples (2.0 mg) were mechanically mixed with 20 mg KBr in reduced pressure and performs analysis.

STATISTICAL ANALYSIS

The data were analyzed for ANOVA and Duncan's Multiple Range Test (DMRT) to analyze the efficacy of different strains and conidial concentrations. Time-dose mortality determination for LC50 and LT50 was also analyzed by Finney's test [15] using Regression/Probit analysis.

RESULTS

Entomopathogenic fungal pathogens like *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea releyi* and *Verticillium lecanii* were isolated from mycosed cadavers of mustard aphids and soil sample on agar plate. Pure culture was established with repeated subculture on PDA medium and maintained a set of culture on SDA medium.

Pathogenicity of *Metarhizium anisopliae*

The application of *Metarhizium anisopliae* on *Lipaphis erysinbi* showed significant result. Higher mortality was observed at higher concentration of spores and recorded ≥ 50% mortality from the 5th day onwards. Highest mortality per cent was recorded in the treatment 1×10^8 and 1×10^9 and recorded 69.2% mortality. In contrast no mortality was observed in the control. The LT50 decreased as the conidial concentration increased. It was observed that *M. anisopliae* strains were more virulent with lower LT50 values than the other entomopathogenic fungal strains studied, LT50-3.04, LC50-1.71×10^7 (Figure 1).

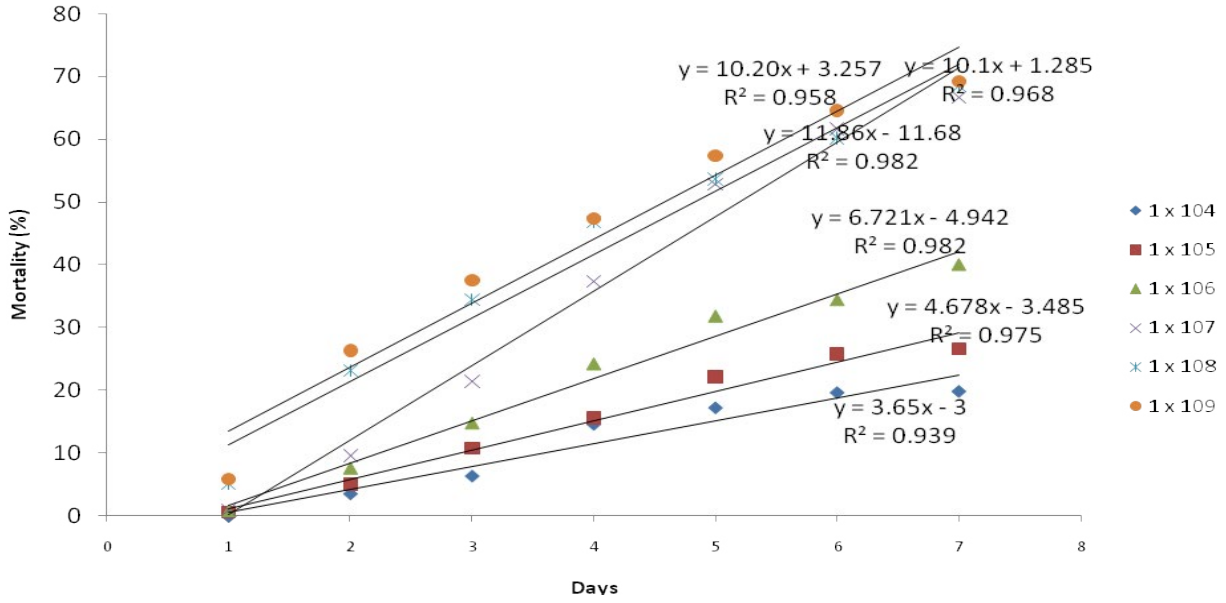


Figure 1: Pathogenecity of *Metarhizium anisopliae* against Mustard aphid

Pathogenicity of *Beauveria bassiana*

The fungal spore application of *Beauveria bassiana* on *L. erysimi* showed effectiveness after 2nd day onwards. Higher mortality was observed at higher concentration of spores and recorded ≥ 50% mortality from the 5th day onwards. Highest mortality per cent of 69.6% was recorded in the treatment 1×10^9 which was followed by 65% in the treatment of 1×10^9 spore load after 7days of post treatment. Lowest pest mortality (21.6%) was recorded in the lower concentration (1×10^4) of fungal spores. There was no mortality recorded in the control, the LT50-4.88, LC50-2.8×10^8 (Figure 2).

Pathogenicity of *Verticillium lecanii*

The effect of entomopathogenic fungus *Verticillium lecanii* against Mustard aphid showed significant results from the 4th day and more than 60% mortality was obtained at 6th day at the spore load 1×10^8 and above. The results showed that mortality was dose dependent which increased with enhancing the concentration while no mortality was recorded in control, LT50-5.69, LC50-5.1×10^8 (Figure 3).

Pathogenicity of *Nomuraea releyi*

The effect of entomopathogenic fungus *Nomuraea releyi* against Mustard aphid showed significant results from the 4th day and more than 60% mortality was obtained at 6th day at the spore load 1×10^8 and above. The results showed that mortality was dose dependent which increased with enhancing the concentration while no mortality was recorded in the control. At the highest concentration of 10^8 spores ml^{-1}, *Nomuraea releyi* also gave appreciable reduction in mustard aphid population with a LT50 values of 4.35, the estimated LC50 was recorded as 2.8×10^8 (Figure 4 and Plate 1).

FTIR spectrum of selected fungal isolate extract

The FTIR spectrum of extracts for four fungal isolates such as *Penicillium pinophilum, Acremonium cellulolyticus, Aspergillus tamarii, Fusarium culmorum, Metarhizium anisopliae* and *Beauveria bassiana* are shown. The FTIR spectrum was categorised based on published reference into four zones such as: i) 4,000 to 2,500 cm^{-1} single bonds, ii) 2,500 to 2,000 cm^{-1} triple bonds, iii) 2,000 to 1,500 cm^{-1} double bonds and iv) 1,500 to 400cm^{-1} fingerprint region single bonds. The zone 4,000 to 2,500 cm^{-1}single bonds is further sub-classified into 3500-3300 cm^{-1} for N-H, 3100-3010 cm^{-1} for C-H and 3000-2500 cm^{-1} for O-H single bond. Likewise 2,500 to 2,000 cm^{-1} zone into C≡N 2240-2280 and C≡C 2100-2200; 2,000 to 1,500 cm^{-1}double bonds into C=O 1680-1750, C=N 1615-1700, C=C 1640-1680 and 1,500 to 400 fingerprint region single bonds into 1494-1357, 1182-995 and 680-530 cm^{-1} (Table 1).

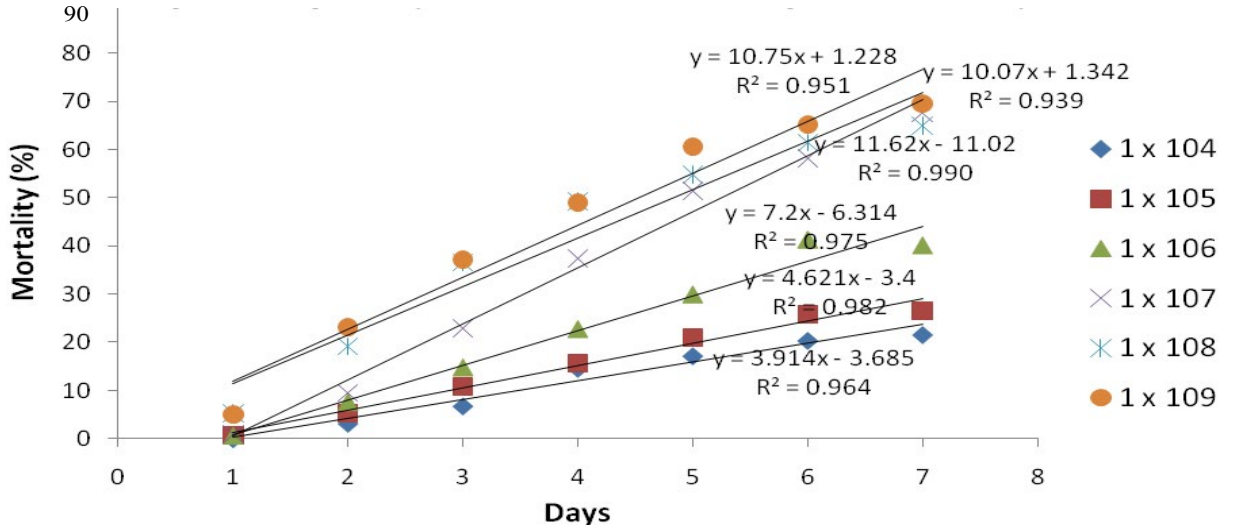


Figure 2: Pathogenecity of *Beauveria bassiana* against Mustard aphid

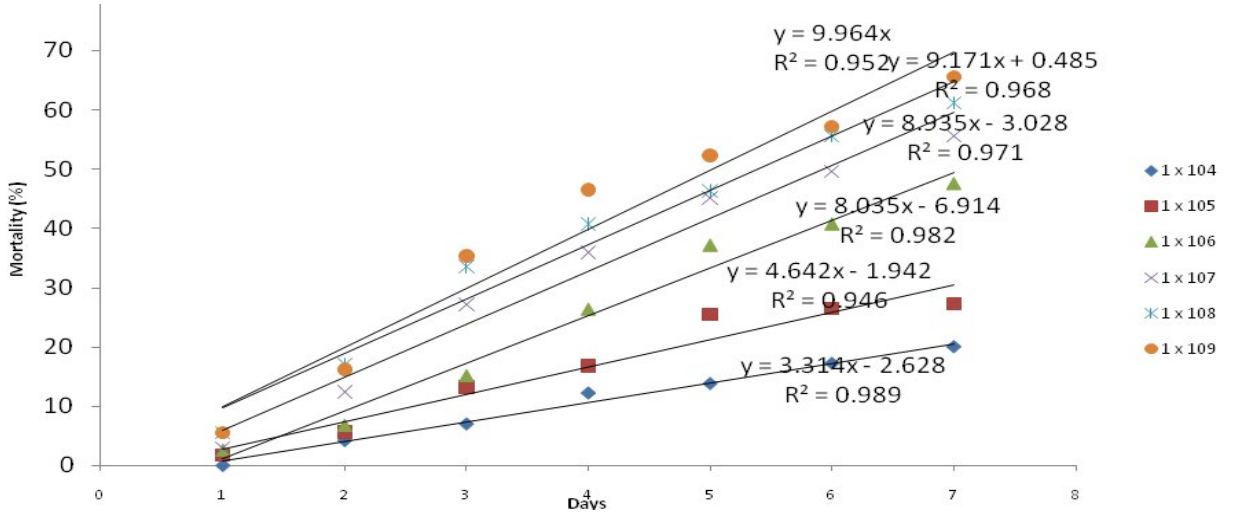


Figure 3: Pathogenecity of *Verticillium lecanii* against Mustard aphid

Table 1: FTIR observed bands for 4 entomopathogenic fungi

Sl no	Strains	Lowest point of Transmittance % in wave number cm-1										
		4,000 to 2,500, single bonds			2,500 to 2,000 triple bonds		2,000 to 1,500 double bonds			1,500 to 400		
										Fingerprint region single bonds		
		N-H 3500-3300	C-H 3100-3010	O-H 3000-2500	C≡N 2240-2280	C≡C 2100-2200	C=O 1680-1750	C=N1615-1700	C=C 1640-1680	1494-1357	1182-995	680-530
1	*Metarhizium anisopliae*	12.28	15.72	13.95	21.48	21.78	18.25	17.1	17.12	19.5	21.92	25.42
2	*Beauveria bassiana*	6.45	14.37	15.56	27.37	28	18.22	14	14.21	22.4	23.27	26.52
3	*Verticillium lecanii*	3.37	9.31	9.88	26.2	27.33	17.15	11	11.08	18	17.6	24.17
4	*Nomuraea releyi*	10.85	14.41	10.91	20.64	20.93	14.69	14.7	14.72	16.9	18.63	24.65

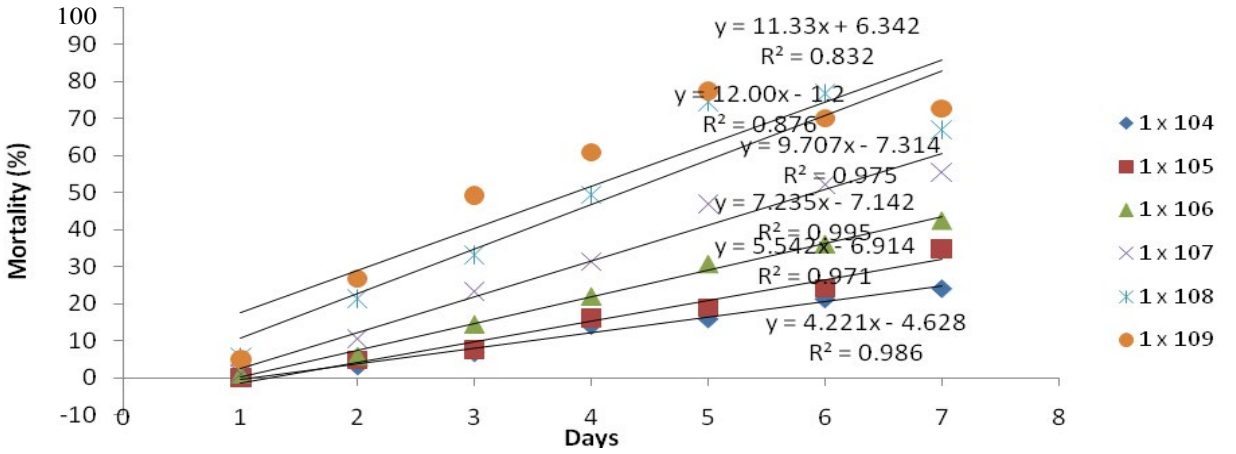
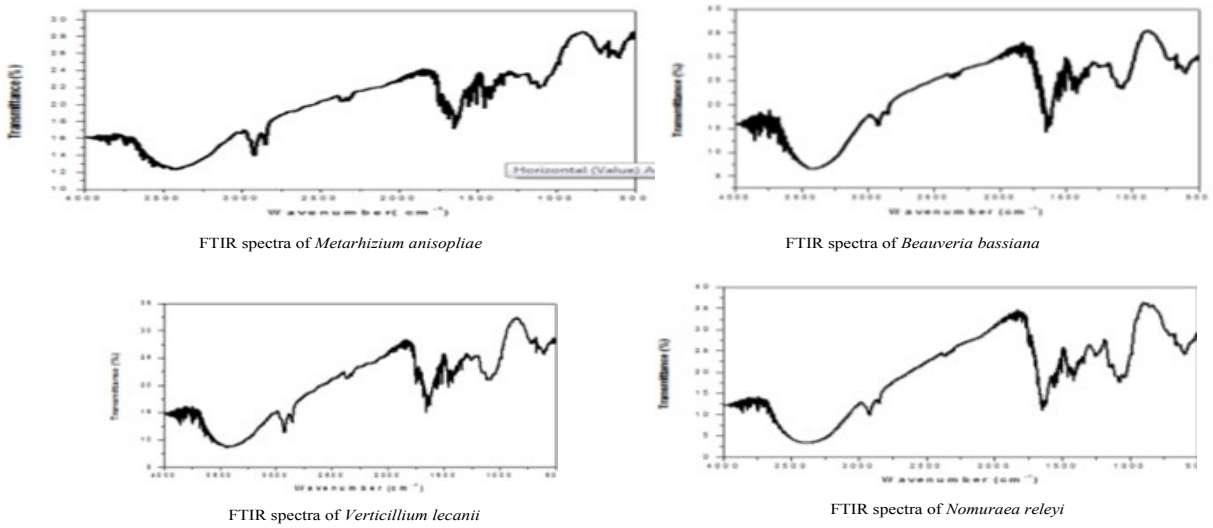


Figure 4: Pathogenecity of *Nomuraea releyi* against Mustard aphid



FTIR spectra of *Metarhizium anisopliae*

FTIR spectra of *Beauveria bassiana*

FTIR spectra of *Verticillium lecanii*

FTIR spectra of *Nomuraea releyi*

Figure 5: FTIR spectra of four virulent entomopathogenic fungi against Mustard aphid

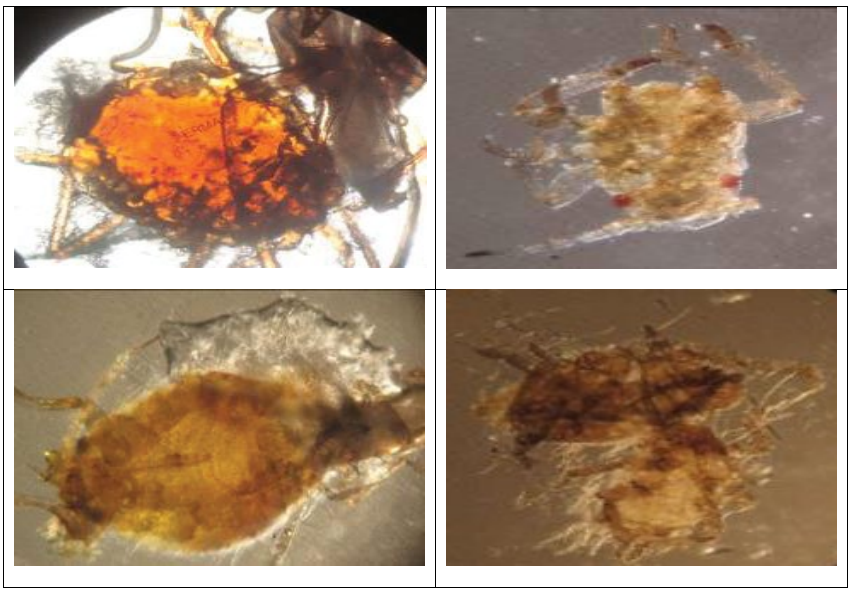


Plate 1: Infected and degraded Mustard aphid of different stages after treatment with four entomopathogenic fungi - *Metarhizium anisopliae* (A), *Beauveria bassiana*(B), *Verticillium lecanii*(C), *Nomuraea releyi* (D) after 7 days of post treatment

DISCUSSION

Pathogenicity of four entomopathogenic fungi on mustard aphid was found to be effective in the laboratory and field condition. Mortalities of Mustard aphid exposed to spore suspension of four entomopathogenic fungi like *Metarhizium anisopliae*, *Nomuraea releyi*, *Verticillium lecanii* and *Beauveria bassiana* indicate different degree of susceptibility of the pathogens. All the fungal isolates in the highest spore concentration (10^8 spores ml^{-1}) produced high mortality. The estimated LC50 and LT50 indicated that *Metarhizium anisopliae* was more virulent followed by *Nomuraea releyi* to control mustard aphids. Mycelial growth observed in various body parts and even sporulation in the body causing degradation of mustard aphids. Fungal isolates originated from the mustard aphid host were found to be pathogenic to mustard aphid *L. erysmi* as indicated by low LT50 values in the single-dose isolate selection assays. The fungal strain *Metarhizium anisopliae* was found most effective and recorded higher mortality at the spore load 1×10^8. In the present study *M. anisopliae* isolate showed 69.2% mortality which was at par with the findings of the other workers [10]. Araujo et al. also reported that some isolates of *M. anisopliae* showed 64% virulence against mustard aphid population after 3.8 days [17] whereas, in another study, *M. anisopliae* isolate showed 72% mortality within 3 days [10]. In the present study the entomopathogenic fungi *B. bassiana* have shown effective virulence against the mustard aphid and caused ≥ 50% mortality from the 5th day onwards whereas other workers reported 90% mortality of mustard aphid after 4.4 days at 10^7 spore ml^{-1} concentrations [17]. It was reported that *L. lecanii* showed 77.16% mortality using 10^5 spore ml^{-1}after 10 days against *L. erysimi* whereas in the present study more than 60% mortality was obtained at 6th day. Other authors have also noted similar differences in susceptibility to aphid host for the same fungi [18].

Among the four fungal isolates, *M. anisopliae* caused 50 per cent mortality at the lowest concentration of 1.71×10^7 spores ml^{-1}. This was followed by *N. releyi* ($2.8 \times 10^{8,}$ spores ml^{-1}), *B. bassiana* (2.8×10^8 spores ml^{-1}) and *V. lecanii* (5.1×10^8 spores ml^{-1}). Higher the LC50 values indicate that there will be higher relative toxicity. This is in conformity with the findings of other workers on different pest [19].

Ujan and Shahzad, also reported the efficacy of *L. lecanii*, *M. anisopliae* and *B. bassiana* and recorded 98, 72 and 88% mortality of mustard aphid after 3 days at 10^7 spore ml^{-1} [10]. Saranya et al. reported *L. lecanii*, *M. anisopliae* and *B. bassiana* strains caused 100, 83.3 and 61.5% mortality, respectively of cow pea aphid (*Aphis craccivora* Koch) after 7 days when used at10^7 spores ml^{-1} [20]. The difference in virulence of the fungal isolates may be attributed to genetic variation in fungal strains, difference in bioassay methods, aphid species and to different abiotic and biotic factors [21-23].

The results of the present studies show that *Metarhizium anisopliae* and *Nomuraea releyi*, when used at 10^8 c.f.u. ml^{-1} were highly virulent against mustard aphid population. A dose dependent positive relationship was found between inoculums density from 10^5 to 10^8 spores ml^{-1}. The pest mortality response increases with the increasing spore concentration. Similar results were also recorded by different workers in different pests [24,25]. The present findings support the possibility of exploiting the entomopathogenic strains *M. aniusopliae* and *N relyi* as biocontrol agents against mustard aphids.

CONCLUSION

This study discusses selection and optimization of low cost fermentation medium nutrient components and renewable raw substrates sources in glucose oxidase production processes and their role in reducing the production cost. In future, our research on fermentation medium nutrients components for optimized glucose oxidase production must be targeted on the economics of the fermentation processes of glucose oxidase production, predominantly carried out through the practice of alternative low-cost effective production media and recovery processes.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Department of Science and Technology, Government of India for financial support. The authors also like to acknowledge the Director General, TERI for inspiration and support. The authors also acknowledge the Head of the Department Chemical Sciences, Institute of Science and Technology, Guwahati University for providing the FTIR facility.

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