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Myco-Biocontrol of Red Spider Mite (Oligonychus Coffeae,Nietner) Using Metarhizium Anisopliae.

Novonita Baruah[1], A. C. Deka[2]
[1]*Department of Biotechnology, Cotton University, Guwahati-781001*
[2]*he Energy and Resources Institute, NE Regional Centre, Guwahati-781036*

Abstract: ***The red spider mite Oligonychus coffeae, Nietner is considered to be one of the major pest of tea in North East India which leads to considerable loss of crop yield. Considering ill effects of chemical pesticides on human health and environment it is essential for exploration of biological solution to solve the problem of tea red spider mite. Entomopathogenic fungi are a group of fungal organisms associated with insects and extensively used in various parts of the world as biopesticides against economic important insect pests. The present study is intended to find potential indigenous entomopathogenic fungi from highly diverse tea growing area and further to develop as a biocontrol agent. Entomopathogenic fungi were isolated from Tea growing soil by using Insect Bait technique. Further, from the isolated entomopathogenic fungus Metarhizium anisopliae was selected for its wide availability and prior knowledge as the natural enemies of a number of different pests. In this study, laboratory evaluation was made on the impact of the entomopathogenic biocontrol agent Metarhizium anisopliae on various stages of red spider mite. In the pathogenicity experiments, Oligonychus coffeae were exposed to fungal spore concentrations (10^6, 10^7, 10^8, and 10^9spores/ml). The isolate of Metarhizium anisopliae shows higher degree of virulence against tea red spider mite. The total mortality percentage of red spider mite caused by Metarhizium anisopliae ranged from 60% to nearly 90% in all the stages within 2 to 3 days. After the application of Metarhizium anisopliae spore suspension, the highest percentage of egg 90.4%, nymph 92% and adult 90% mortality was recorded within 2-2.6days at the spore load of 10^8 spores/ml. Dose dependent variability also observed in the mortality of Oligonychus coffeae. Metarhizium anisopliae spore load 10^8 has detrimental effects on mortality of Oligonychus coffeae. The laboratory findings confirms the biocontrol properties of the tarhizium anisopliae against all stages of tea red spider mite and suggested the feasibility for effective biocontrol of the pest in Tea crop. The Metarhizium anisopliae considered to be a most valuable biocontrol agent in the tea growing region of North East India. FTIR studies show the position of the hydroxyl group and carbonyl (C=O) groups play important roles in insecticidal activity of Metarhizium anisopliae***.

I. INTRODUCTION

India is the largest producer of tea in the world and the share of North East India is about 75% of total Indian production. Besides earning valuable foreign exchange tea industry in India provides employment to 1.5 million skilled personnel. Tea plants are attacked by insects and mite pests which incur significant loss to production of Tea. Among the mite pests, Red spider mite O. coffeae Nitner is a serious pest of tea plants(*Camellia sinensis* L(O) Kunz) in North East India(Muralidharan et al., 2001). It has been estimated that about 75% of tea growing area in North East India suffer from attack of red spider mite with variable intensity(Baruah et al, 2008).

Tea red spider mite(RSM) feeds on the upper surface of foliage at tipping level and reduces photosynthesis efficiency of tea plants to a great extent.

In severe infestation they feed on lower surfaces and leaf turn copper brown due to feeding damage and lead to defoliation and mortality of tea plants. It causes significant losses to yield of tea as high as 10% (Das et al., 1985). Several strategic and tactical measures are used for control of RSM in North East India.

Among these use of resistant cultivars, cultural control, shade managements, drainage improvement practices and biointensive pest control methods are considered important. Mite pest in NE India is predominantly controlled by application of acaricides which varies from 2-4 lit/ha/yr (Barbora and Biswas, 1996) which is several fold more than the national average(Atwal, 1976).

Adverse impact of chemical pesticides on environment in general and tea ecosystem in particular have been well documented by different workers (Rajendran, 2003, Gurusubramanian et al., 2005, Muralidharan et al., 2001).

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Therefore it needs to evolve alternative strategy for control of tea pest in India. Integrated pest management(IPM)has been perceived as a viable alternative to reduce dependence on chemical pesticides in agriculture as it brings about environmental, social and economic benefits. It is increasingly recognized that the biodiversity in agro-ecosystems deliver significant ecosystem services to agricultural production such as biological control of pest(Meyling and Eilenberga, 2007).

Naturally occurring entomopathogenic fungi are one of the important components of IPM which regulate pest population in nature. More than 1000 species of fungi are reported from insect's body all over the world and a few are registered for commercial utilization as biocontrol agent.

NE India is recognized as a paradise for fungi because of prevalence of high relative humidity(80-85%) and other topographical factors. However, till date few works had been reported on entomopathogenic fungi of this region excepting a few earlier reports (Hazarika et al, 2009, Hazarika and Puzari, 2001, Debnath, 2004).

The fungi Metarhizium anisopliae is an entomopathogenic fungus that has evolved specialized strategies to infect insect hosts. The efficacy of M. anisopliae have been studied for the control of tetranychid mites(Alves et al, 2002). Rodrigo et al(2004) studied the effect of several strains of M. anisopliae against cassava green mite. It was reported that M. anisopliae able to degrade the host components and actively secrete proteins to manage the physiology of the host(da-Silva et al, 2014). The present study has therefore been undertaken to evaluate the pathogenicity of indigenous Metarhizium anisopliae isolate against tea red spider mite.

II. METHODS

A. Source of mite and rearing

Various stages of RSM were collected from Tea Estate of Kamrup(Rural), Assam. The collected pest were cultured in laboratory condition following the pre established technique (Baruah et al, 2008). Field collected RSM were maintained in mid-stage suitable tea leaves, which were managed in tap water. The petiole was wrapped in a plug of moist cotton to keep the leaf afresh for a longer period and leaf was placed in moist petridish. The cotton wool was kept wet by adding water periodically and replaced with fresh leaf at 3 days interval.

B. Source of Fungal isolates

A number of fungal strains were isolated from dead adult red spider mite collected from different tea estates of Assam. These strains were isolated using Potato Dextrose Agar(PDA) medium following single spore isolation method.

After preliminary screening *Metarhizium anisopliae* was found to be effective against RSM.

Conidia from these cultures served as inocula for further experiments.

These strains were maintained and multiplied in culture tube and glass plates at 26±1°C for seven days to encourage sporulation.

C. Bioassay

Fungal spore suspension prepared by adding sterilized distilled water containing 0.005% (v/v) Tween 20 to one weak old PDA plates. Spores were scraped off from the media surface and transferred into screw capped glass bottle. After vortexing for 2 min the spores werer separated from debries by filtering through pre-sterilized glass wool.

Spore concentration was estimated using an improved Neubauer Haemocytometer and adjusted to different concentration ranging from $1x10^5$ to $1x10^8$ conidia/ml by dilution with 0.005%(v/v) Tween 20. One hundred RSM were used to bioassay each isolates of fungus.

Sterile distilled water with 0.005% Tween 20 was used as control.

stages of RSM were exposed to M. anisopliae isolate by coating them with a liquid spore suspension of various concentration. After treatment, the pests were maintained in humid condition and were incubated at 26±1°C for fungal growth and examined daily to study the pest mortality.

D. FTIR(Fourier transform infrared)

The fungal pure culture established in PDA media was incubated at 27°C for 5 days for proper sporulation. Potato Dextrose (PD) broth was prepared and inoculated with fungal spore from the pure culture plates. The incubated flask with spore suspension was incubated on incubatred shaker at 25±2°C and agitated at 120 rpm for 96h. The biomass was harvested after incubation by centrifugation at 5000 rpm for 10min. The biomass was again washed with de-ionized water to remove any medium component from the biomass. 10g(wet wt.) of fungal biomass dispensed in 100ml pre-sterilized de-ionized water and incubated for 48h at 30°C in a 250ml Erlenmeyer flask and agitated at 120rpm. After incubation the cell filtrate i.e. extracellular fungal secretes was obtained

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by filtering it through Whatman Filter paper No 1. The filtrates were again centrifuged at 5000 rpm for 10 min to remove all the cell debris. The fungal secrete concentrate and proceed for FTIR analysis. FTIR spectra was analyazed 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 wavelength between 2.5 and 12.5µm.

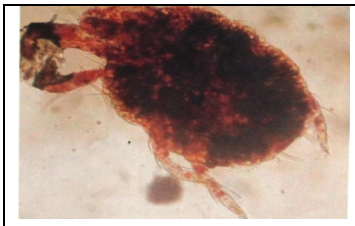
III. RESULTS AND DISCUSSION

Rapid external fungal hyphal development and sporulation was observed under moist condition. Initially, hyphal strands emerged from the mouth parts followed by anal parts of the mite cadaver and then quickly covered the cadaver with profused hyphal growth. One to two days later mycelia emerged around the mouth parts, legs and abdominal regions of the treated pest. After three days of maintaining death mite at 27°C the mite become covered with mycelia which was followed by sporulation within 4 to 6 days. Sporulation of the fungus was observed after complete colonization by the mycelium. *M. anisopliae* appeared greenish in colour. After that the cuticle of the mite starts to degraded and become reddish-green in colour before finally turned into dark blackish-green(Plate -1).

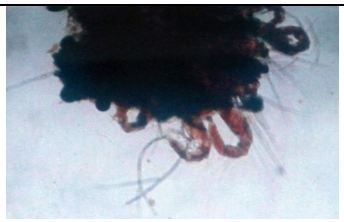
Mortality rates of mites at different growth stages due to fungal infection at different fungal spore concentrations used at 5 days post infection (DPI) are summarized in Table-1. The highest percentage of egg (90% after 2.0days), nymph(92% after 1.6days) and adult(90% after 2.0days) mortality of red spider mite was recorded at the spore load 10^8(Table-1). From the mortality rates Metarhizium anisopliae was found to be highly potent to control the red spider mite. Mortality of the treated mite probably due to the toxic fungal metabolites or depletion of nutrients from the insect body during the fungal growth. In the untreated control treatments the mite population increased progressively and there was no mortality observed.

Table 1: Effectiveness of *Metarhizium anisopliae* against tea red spider mite

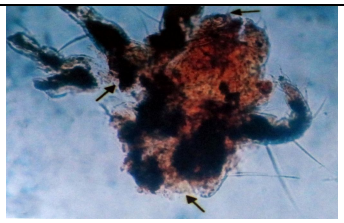
Dosage spore/ml	Stage of the pest	Time taken to kill after treatment(days)	% mortality
10^5	Egg	3.90±0.20	60.00±1.14
10^6		3.60±0.22	84.30±1.62
10^7		2.80±0.25	90.00±2.12
10^8		2.00±0.12	90.00±1.58
10^5	Nymph	4.00±0.23	70.00±1.14
10^6		3.80±0.18	83.00±0.90
10^7		3.30±0.22	90.00±1.64
10^8		1.60±0.11	92.00±1.14
10^5	Adult	4.30±0.13	66.90±1.10
10^6		3.30±0.17	83.20±0.97
10^7		2.60±0.03	90.00±1.20
10^8		2.00±0.14	90.00±1.10
SD±		0.25	2.44
CD5%		0.51	4.91



Effect of *M. anisopliae* on nymph after 3 days of infection



Mycelial outgrowth over the cadavers of Red spider mite after 5 days of infection



Degradation of adult red spider mite after 7 days of treatment

Plate 1: Effect of Metarhizium anisopliae on different stages of Red spider mite.

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The FTIR studies show the position of the hydroxyl group and carbonyl (C=O) groups which may play an important roles in the insecticidal activity of the Metarhizium anisopliae studied(Fig-1).

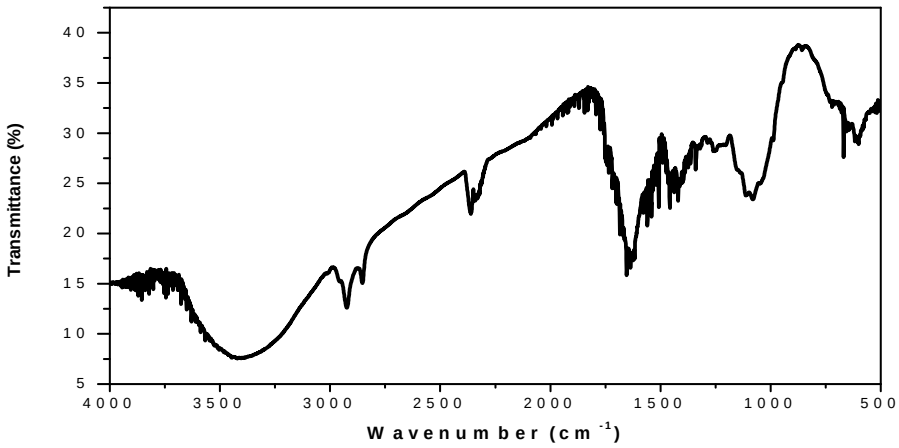


Fig 1: FTIR Spectrum of Metarhizium anisopliae

Studies on the efficacy of entomopathogenic fungi against the phytophagous mite under laboratory conditions showed high per cent mortality of mites. The results obtained in the present study are comparable with findings of other researches using fungal pathogens against mite pests. Per cent mycosis of mites due to entomopathogenic fungi under laboratory condition revealed higher mortality (90%) in M. anisopliae treatment which was at par with the findings of other workers.

Mortality response of M. anisopliae, at 10^8 conidia ml^{-1} against red mite Oligonychus yothersi (McGregor) caused 12.00% to 45.00%, and LT50 values that ranged from 8.6 to 18.4 days whereas another entomopathogenic fungi Beauveria bassiana Isolates showed 77.00% to 98.00% mortality (de Oliveira et al. 2002). The differences in virulence of entomopathogenic fungal strains are probably associated with the presence of enzymes that influence the penetration process of the fungus. The variation in virulence among the different fungal isolates to insect pest may be associated with the enzymes produced by each isolate (de la Rosa et al. 1997). A high enzymatic activity of cuticle-degrading enzymes was reported in the more virulent isolates of entomopathogenic fungi M. anisopliae (Tiago et al, 2001). The present findings showed that the epizootic of entomopathogenic fungus can regulate the population of red spider mites.

A. SIGNIFICANCE

Metarhizium anisopliae isolates tested are more efficient for utilization in integrated management of Tea pest including tea red spider mite. These results support the possibility of exploring the highly virulent indigenous entomopathogenic fungi Metarhizium anisopliae of Noth East India as a biological acaricide against tea red spider mite with great potentiality of commercial exploitation.

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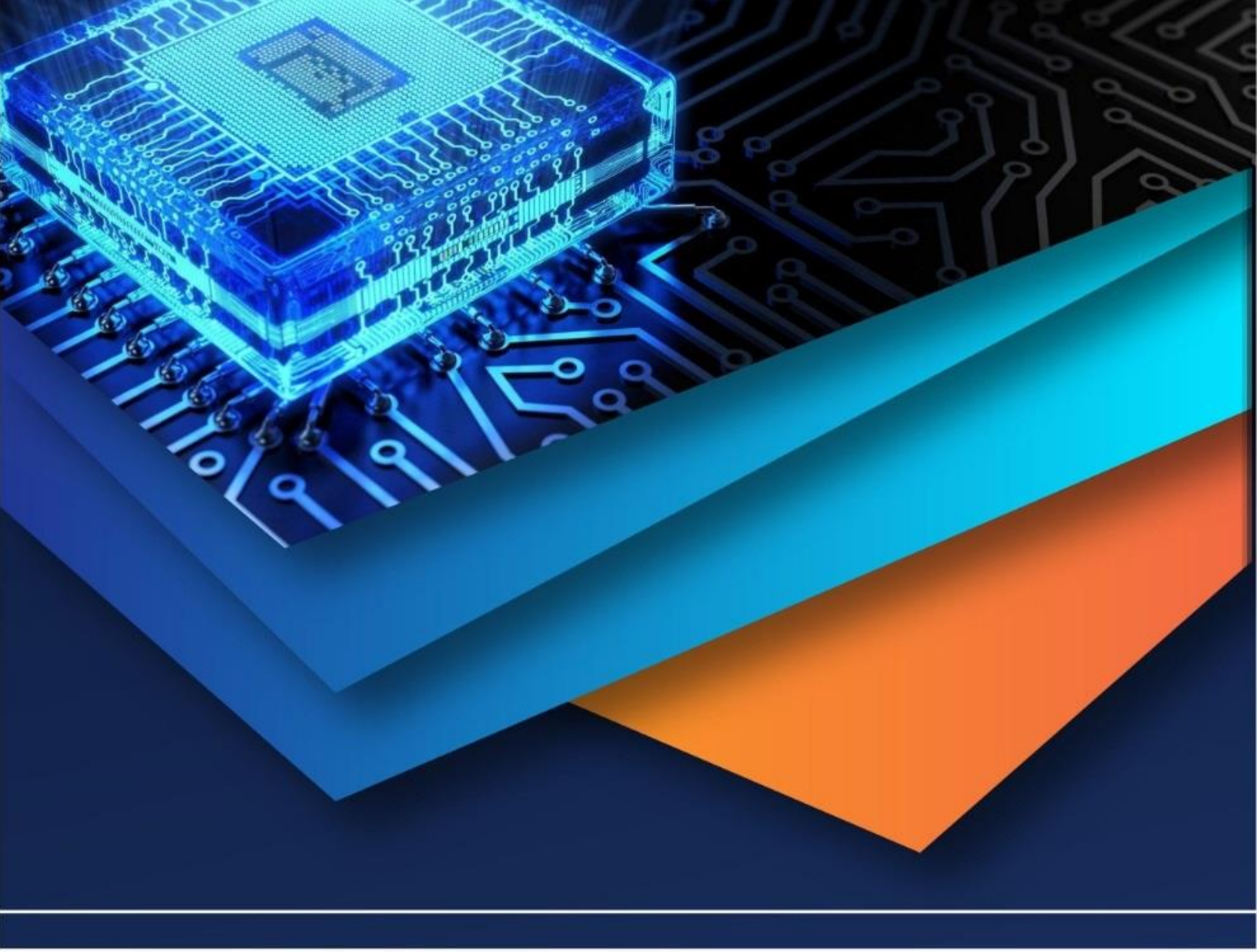
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