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Salma Mazid
Dept. of Zoology, Gauhati University, Guwahati-781014, Assam.

R.C. Rajkhowa
Dept. of Zoology, Cotton College, Guwahati-781001, Assam.

J.C. Kalita
Dept. of Zoology, Gauhati University, Guwahati-781014, Assam.

Correspondence:
Salma Mazid
Dept. of Zoology, Gauhati University, Guwahati-781014, Assam.

Pathogenicity of *Aspergillus Niger* and *Aspergillus flavus* on red spider mite (*Oligonychus coffeae* Nietner), a serious pest of tea

Salma Mazid, R.C. Rajkhowa, J.C. Kalita

Abstract
Red spider mite, *Oligonychus coffeae* Nietner is one of the most serious pest of tea plantation in Northeast India. In order to evaluate the pathogenicity of entomopathogenic fungi against this pest, mite populations were maintained in the laboratory by detached leaf culture method. *Aspergillus niger* and *Aspergillus flavus* were isolated from dead spider mites collected from Tongani tea garden located in Mangaldai, Assam and identified. The bioassay was carried out by spraying the mites with three different conidial concentrations of $1x10^6$, $1x10^7$, $1x10^8$ conidia/ml and 0.01% Tween 80 which was used as blank control. Each concentration was replicated three times comprising of 20 mites in each replicate. Results showed that *A. niger* was highly pathogenic and recorded highest mortality of 91.11% after 96 hrs. LC_{50} and LT_{50} values for *A. niger* was recorded to be lower than that of *A. flavus* thereby indicating that *A. niger* was more pathogenic to red spider mite population.

Keywords: *Oligonychus coffeae*, entomopathogenic fungi, pathogenic, *Aspergillus niger* and *Aspergillus flavus*

Introduction
India is the second largest producer and fourth largest exporter of tea in the world. The major tea producing regions in India are Assam, West Bengal, Karnataka, Tamil Nadu and Kerala out of which Assam produces more than 50% of the total tea in the country [1]. A number of pests are found to occur on tea crops that damage the tea plants and lead to considerable loss of productivity. The red spider mite (*Oligonychus coffeae* Nietner) is one of the most destructive pests of tea in Northeast India [2]. It normally attacks the upper surface of the young and mature tea leaves. Under conditions of severe infestation, the leaves turn brown and dry up and this ultimately leads to defoliation. Among the different tea pests found, this pest is known for its destructive nature as it has been reported that it alone causes over 18% loss of tea production in India [3, 4]. Various acaricides such as dicofol, ethion, propargite, sulphur etc. are being successfully used to control the outbreak of this pest. However, it is known that continuous use of chemicals leads to various problems such as pest resurgence, development of pesticide resistance, outbreak of secondary pests, harmful effects on human health and environment and presence of undesirable residue in processed tea [5, 6]. Thus, to overcome such constrains it is necessary to search for a suitable alternate and the best suited for this purpose would be the biological control.
Microbial control, a type of biological control is an alternative to chemical control of pests. Microbial pesticides such as entomopathogenic fungi has been reported by various workers to provide effective control of a large variety of pests [7, 8, 9, 10, 11].The present study was therefore aimed to search for suitable entomopathogenic fungi and determine its efficacy in controlling red spider mite populations.

2. Materials and methods
2.1. Rearing of red spider mite
Studies on the pathogenicity of *A. niger* and *A. flavus* on red spider mites were conducted during the year 2013-2014. Red spider mites were collected from Banglagarh division of Tongani tea estate of Mangaldai located in Darrang district of Assam and were maintained in the laboratory at 25 ± 2 ºC and 75 ± 10 % RH. Rearing of the mites was done by following detached leaf culture method [12]. Leaves were placed with their surface facing upwards on cotton bed kept in petridish (9 cm diameter). The cotton bed was kept moist by adding water

so that the leaves remained fresh for a longer period. The petioles of the tea leaves were also wrapped with moist cotton. Dried leaves were replaced with fresh ones usually after 3 to4 days interval.

2.2. Isolation and culture of *Aspergillus niger* and *Aspergillus flavus*

A. niger and *A. flavus* was isolated from the dead red spider mites collected from the tea estate of Mangaldai. Pure culture of the fungal isolates was obtained through single spore isolation technique and was sent to National Centre of Fungal Taxonomy (NCFT), New Delhi for its proper identification. The two isolated fungi were then grown separately on Potato Dextrose Agar (PDA) and incubated at 25 ± 1 °C. For bioassays, conidia were harvested by scraping them from the surface of 14 days old culture. The conidial clumps thus obtained were then suspended in sterile distilled water containing 0.01% Tween 80 (used as blank control) and spore suspensions were standardized at $1x10^6$, $1x10^7$ and $1x10^8$ conidia/ml.

The average conidial viability of each isolate used in the tests was determined using the standard technique of Goettel and Inglis[13]. Conidial suspension at concentration of $1x10^6$ conidia/ml was spread-plated on plates containing PDA and incubated for 24 hrs at 25 ± 2 ºC. The percentage of germination was determined by counting the number of conidia germinated per 100 conidia at 200x magnification. Spores were considered to be viable only if the germ tube length was found to correspond with the conidium width. The percentage of germination was estimated at >95% for all experiments.

2.3. Bioassay

Spore suspension of each isolate was applied on red spider mites and its effect was studied. For each isolate, three different concentrations (**1**$x10^6$, $1x10^7$ and $1x10^8$ conidia/ml) were prepared and 0.01% Tween 80 was used as blank control. For each treatment, 20 mites placed on a tea leaf kept in a petridish (replicated three times) were kept at the bottom of a plastic bucket (60 cm in height and 48 cm in diameter). The mites were then sprayed with spore suspension using a hand held sprayer from the top of the bucket. Post treatment, the tea leaves carrying the treated mites were kept in an incubator at 25 ± 1 °C and examined to record daily counts of mortality. The dead mites were then kept in moist chambers for 2 to 3 days to observe fungal growth and confirm death due to fungal infection.

2.4. Data analysis

Statistical analysis was performed using software SPSS 21. Cumulative mortality rates of the mites were determined using one way analysis of variance (ANOVA) and Tukey's HSD procedures. Probit analysis [14] was conducted to determine the lethal concentration (LC_{50}) and lethal time (LT_{50}) of the fungal isolates. All the percentage mortality values were corrected based on the mortality in the control using Abbott's formula [15].

3. Result and discussion

The efficacy of *A. niger* and *A. flavus* was evaluated against red spider mites in the laboratory using three different concentrations of conidia ($1x10^6$, $1x10^7$ and $1x10^8$ conidia/ml) at different interval of time. Results showed that both the isolates were effective against *O. coffeae* populations. However, efficacy of *A. niger* was much higher than *A. flavus* (Table 1) It was found that mortality percentage increased with the increase in conidial concentration. Highest mortality of 91.11% was recorded after 96 hours of treatment with *A. niger* at concentration of $1x10^8$ conidia/ml. *A. flavus* resulted in mortality percentage as high as 62.22 % after 96 hours of treatment with the highest concentration. Pathogenicity studies showed that *A. niger* resulted in mortality that was statistically significant compared to control ($F=65.66$, $df = 3, 16$, $P=0.0004$).

Table 1: Mortality percentage of red spider mites when treated with different concentrations of *Aspergillus niger* and *Aspergillus flavus*

Treatment	Dose (conidia/ml)	Mean % mortality ± SEM			
		24 hrs	48 hrs	72 hrs	96 hrs
Aspergillus niger	$1x10^6$	25±0.55**	30.91±0.33**	42±0.46**	48.89±0.56***
	$1x10^7$	33.33±0.40**	49.09±0.46***	52±0.58***	64.44±0.65***
	$1x10^8$	40±0.42***	76.36±054***	82±0.65***	91.11±0.42***
Aspergillus flavus	$1x10^6$	18.33±0.44*	20.91±0.46*	23±0.54**	33.33±0.56**
	$1x10^7$	25±0.46*	29.09±0.48**	34±0.33**	44.22±0.52**
	$1x10^8$	38.33±0.52**	46.36±0.48**	57±0.50***	62.22±056***
Control	-	1.66±0.20	8.33±0.22	16.66±0.28	25±0.36

Mean ± SEM, Tukey's HSD test ($P<0.05$). *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$, (n=20)

Table 2: LC_{50} values for *Oligonychus coffeae* after 24, 48, 72 and 96 hrs of treatment with *A. niger* and *A. flavus* at concentrations of $1x10^6$, $1x10^7$ and $1x10^8$ conidia/ml

Fungal Isolate	LC_{50} value (95% confidence limit) (conidia/ml)			
	24 hrs	48 hrs	72 hrs	96 hrs
Aspergillus niger	$8.1x10^7$ ($5.9x10^7$-$1.1x10^8$)	$4.2x10^7$ ($2.2x10^7$-.$4x10^7$)	$2.6x10^7$ ($6.3x10^6$- $4.7x10^7$)	$7.1x10^6$ ($1.5x10^6$-$2.7x10^7$)
Aspergillus flavus	$1.21x10^8$ ($9.4x10^7$-$1.5x10^8$)	$9.6x10^7$ ($7.3x10^7$-$1.3x10^8$)	$6.1x10^7$ ($3.9x10^7$-$8.5x10^7$)	$2.3x10^7$ ($1.9x10^6$-$4.3x10^7$)

Table 3: LT_{50} values for *Oligonychus coffeae* after treatment with *A. niger* and *A. flavus* at concentrations of $1x10^6$, $1x10^7$ and $1x10^8$ conidia/ml

Fungal Isolate	LT_{50} value (95% confidence limit) (hrs)			
	Control	$1x10^6$	$1x10^7$	$1x10^8$
Aspergillus niger	134.13 (118.24 – 155.57)	85.33 (75.15 – 97.60)	66.90 (57.16 – 77.41)	31.94 (19.11 – 42.89)
Aspergillus flavus	152.60 (130.31 – 186.35)	116.25 (100.27 – 139.52)	99.08 (85.19 – 118.11)	67.49 (54.98 – 81.17)

The LC_{50} and LT_{50} value of both *A. niger* and *A. flavus* was determined (Table 2 and Table 3). It was found that both LC_{50} and LT_{50} of *A. niger* was lower than that of *A. flavus* thereby indicating its pathogenicity. Lowest LC_{50} value was recorded to be $7.1x10^6$ conidia/ml whereas lowest LT50 value was calculated to be 31.94 hours.

Although in this experiment it was found that *A. niger* was much more effective against red spider mites than *A. flavus*, it was found that various workers had earlier reported the efficacy of *A. flavus* against different insect pests [16]. Efficacy of *A. niger* and *A. flavus* was earlier evaluated against *Helopeltis* sp. in tea and cacao plantation [17, 18] and was found to be effective where 80% and 90% mortality was recorded with *A. niger* and *A. flavus* respectively. Singh and Prakash [19] reported *A. niger* to be highly effective against mosquito vectors of filaria, malaria, and dengue.

4. Conclusion

The results of the present study indicate the potentiality of the two fungal isolates, *Aspergillus niger* and *Aspergillus flavus* for use in control of red spider mite populations. Conidial suspensions of *A. niger* were found to be more effective against red spider mites than *A. flavus*. However, further research is to be conducted to determine its efficacy by conducting field trials against *O. coffeae* in tea plantations.

5. References

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