Evaluation of fungal pathogens, *Fusarium semitectum* Berk. and Ravenel and *Hirsutella thompsonii* Fisher against red spider mite, *Tetranychus neocaledonicus* (Andre) of okra under laboratory and greenhouse conditions

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Abstract: Efficacy of fungal pathogens, *Fusarium semitectum* Berk. and Ravenel and *Hirsutella thompsonii* Fisher was evaluated against red spider mite, *Tetranychus neocaledonicus* (Andre) of okra under laboratory and greenhouse conditions. Among the treatments evaluated under laboratory conditions, the highest mortality of 78.20 and 88.34 per cent adults and immatures, respectively was recorded at 2.10 x 10⁸ spores per ml, whereas it was 82.05 and 92.31 per cent adults and immatures, respectively in case of *spores per ml,* *spores per ml. Among the twelve treatments evaluated under greenhouse conditions, *H. thompsonii* 4.60 x 10⁸ spores per ml + dicofol 0.025% and *F. semitectum* 2.10 x 10⁸ spores per ml + *H. thompsonii* 4.60 x 10⁸ spores per ml, *H. thompsonii* 4.60 x 10⁸ spores per ml + dicofol 0.025% and *F. semitectum* 2.10 x 10⁸ spores per ml + *H. thompsonii* 4.60 x 10⁸ spores per ml + dicofol 0.025% were found to be on par with each other with a cumulative percent mortality of 81.20, 81.48, 81.21 and 82.59 percent, respectively. By testing the field efficacy of *F. semitectum* and *H. thompsonii* against *T. neocaledonicus,* these fungal pathogens can be used as potential biocontrol agents for the sustainable management of mites on okra. Hence the present study evaluates the potentiality of *F. semitectum* for the management of *T. neocaledonicus* by comparing with the well-known acaridical fungus, *H. thompsonii.*

Keywords: *Fusarium semitectum, Hirsutella thompsonii, Okra, Pathogenicity, Tetranychus neocaledonicus*

INTRODUCTION

India is a major vegetable growing and consuming country in the world. It is cultivated in an area of 9.21 million hectares with an annual production of 162.19 million metric tons during 2013-14 (Anonymous, 2014). Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable of India and form an integral part of Indian culinary. Among many factors responsible for low production of okra, the damage inflicted by insect and mite pests has been considered important. Mites of the family Tetranychidae are undoubtedly among the many destructive pests of okra in many parts of the world. The okra mite, *Tetranychus neocaledonicus,* a serious spider mite pest in India. It is well distributed throughout tropical and sub-tropical areas of the world including Hawaii, Fiji, Venezuela, Puerto Rica, Mauritius, Bahamas, South America and Southern United States (Goldsmid, 1962). The mites suck the sap from the leaves producing white spots that gradually coalesce as feeding continues; leaves lose their green colour, gradually wilt, dry and drop. Webbing caused by them ultimately hampers the process of photosynthesis and thus limiting the yield potential of okra (Rachana, 2007).

Okra mite is a difficult pest to manage by spraying chemical acaricides because of its polyphagous nature with very short life cycle. Major emphasis is given on biological control approaches in the present context of environmental safety. Biological control of mites using fungal pathogens is gaining importance due to their target specificity, self-perpetuity and environmental safety. The use of fungal pathogens for biological control is a rapidly developing field and is increasingly being adopted and accepted worldwide for the management of agricultural pests (Hajek and Delalibera, 2010). Fungi have been considered as an important group of mite pathogens. Pathogenicity of *Hirsutella thompsonii* against mites of different crops had already been studied and confirmed (Murali Gopal and Alka Gupta, 2001). The infectivity of *Fusarium semitectum* against chilli mite, *Polyphagotarsonemus latus* (Banks) (Mikunthan, 2004) has been reported. Keeping this in view, the present study was carried out in order to evaluate the efficacy of the fungus *F. semitectum* as compared to *H. thompsonii* against *T. neocaledonicus* under laboratory and greenhouse conditions. Large
discrepancies between field and laboratory results have made it difficult to predict the real effects of these fungi on both target and non-target insects due to several factors like differences in exposure stages and environmental conditions. Hence present investigation was targeted to compare the effect of laboratory and greenhouse conditions on pathogenicity of Fusarium semitectum and Hirsutella thompsonii against Tetranychus neocaledonicus. Thus by testing the field efficacy of F. semitectum and H. thompsonii against, T. neocaledonicus, these fungal pathogens can be used as potential biocontrol agents for the sustainable management of mites on okra.

MATERIALS AND METHODS

The experiments were conducted in the laboratory and greenhouse of Department of Entomology, College of Agriculture, Shimoga, Karnataka, India.

Culture of Fusarium semitectum and Hirsutella thompsonii: For standardizing the effective dosage of the fungi, Saboraud Maltose Agar Yeast (SMAY) media was used as the medium. Two hundred ml of culture medium was taken in a 500 ml conical flask, autoclaved at 121°C (15 lbs.) for 20 minutes. The plates were prepared by pouring 20 ml of SMAY media per plate and inoculated with culture of the fungi in separate plates. The inoculated plates were incubated at room temperature (25°C) for a week for the development of the fungi, which developed as a fluffy mass. The mass production of the fungi was done on sorghum grains. One Kg of sorghum grains was half boiled and shade dried. The grains were sprayed with 20 ml distilled water after spreading them on a paper and mixed evenly by repeated heaping to retain moisture on grains. The grains were divided into four equal parts by weight and kept in polythene bags. The mouth of the bags was closed with an elastic rubber band. These bags were sterilized in an autoclave at 121°C (15 lbs.) for 30 minutes. These bags were inoculated separately with six mm discs of two week’s old F. semitectum and H. thompsonii grown on SMAY media. The bags were incubated at room temperature 25±1°C and 71±2 per cent RH for 20 days. After prescribed period, the bags were cut open under the laminar flow chamber and further utilized for laboratory and greenhouse studies.

Preparation of F. semitectum and H. thompsonii suspension: Spores were harvested with the help of a small metal spatula, from the culture grown in SMAY media. The spore suspension was prepared using distilled water and filtered through a double layered muslin cloth to remove the mycelial mat in order to get a clear spore suspension. Tween 80 (0.02%) was used to disperse the conidia uniformly in the solution. One ml of spore suspension was poured on to hemocytometer to count the fungal spores using phase contrast microscope. From the stock spore suspension, serial dilutions were made to obtain the required concentrations for bioassay studies.

Laboratory bioassay studies: Spore concentrations of 2.10 x 10^2 to 2.10 x 10^4 and 4.60 x 10^4 to 4.60 x 10^8 spores per ml of F. semitectum and H. thompsonii were evaluated, respectively. Spore suspensions were sprayed using hand atomizer. The different concentrations of spore suspensions were sprayed on red spider mite population present on okra leaves kept on moistened cotton wad in plastic containers (size 5 x 9 cm) which had opening on top of size 8 x 4.50 cm for aeration to maintain high relative humidity to enhance fungal growth. Mortality was obtained at two, four and six days after spraying. The pre count and post spray counts were recorded to work out the percent mortality. The effective concentration of the fungi, which showed the highest mortality were taken for future greenhouse studies.

Greenhouse studies: For green house studies, okra plants were grown in pots (one plant/pot) making twelve treatments, which were randomly arranged. Agronomic practices like watering, fertilizer application and weeding were practiced in greenhouse under semi natural conditions. The mite culture was maintained until all experiments were completed. Treatment details are given in Table 3. Three replications were maintained for each treatment. One control treatment was maintained alongside. Pre-count of red spider mite was taken a day before and post sprays counts at three, six, nine and fifteen days after spraying with the help of a hand lens (15x). The greenhouse temperature was maintained at 30±2°C.

Determination of LC50: Daily observations on mite mortality were continued for 6 days under laboratory conditions. The cadavers showing mycosis were considered to be dead. Mortality data was corrected with that in control by using the Abbott’s formula (Abbott, 1925).

Statistical analysis: The laboratory and greenhouse experiments were designed under Completely Randomized Design (CRD) and Randomized Block Design (RBD), respectively. The means for the corrected mortality were separated by Duncan’s Multiple Range Test (DMRT). All the statistical analysis was performed by using SAS (SAS, 2002), while probit analysis (Finney, 1971) was used to calculate the LC 50 values. One way analysis of variance (ANOVA) was conducted on the mortality data to test the level of significance of the difference in response between the treatments under both laboratory and greenhouse conditions.

RESULTS AND DISCUSSION

In the present study fungal pathogens that parasite mites are valuable tools for biological control and play an important role in encouraging integrated pest management. Till date, H. thompsonii has been used to manage mites (Yadav Babu, 2004; Aghajanzadeh et al., 2006; Fernando et al.,2007).
Pathogenicity of *F. semitectum* against *T. neocaledonicus* under laboratory conditions: In present study, the results revealed that at two days after spray, *F. semitectum* at 2.10×10^8 spores/ml differed significantly (P=0.01) from all other treatments in causing the mortality of immatures of *T. neocaledonicus* with a mortality of 37.19 per cent, whereas the lowest concentration of 2.1×10^4 spores/ml recorded a mortality of 8.97 per cent (Table 1). At four days after spray higher mortality (44.87%) was observed at 2.10×10^5 spores per ml, followed by treatments viz., 2.10×10^6 and 2.10×10^7 spores per ml which recorded 33.32 and 25.62 per cent mortality, respectively. However the fungus at 2.10×10^8 spores per ml recorded a mortality of 20.51 and 16.67 per cent, respectively. Whereas, it was minimal (12.82%) at lower concentration of 2.10×10^2 spores per ml. At fourth day immature showed sluggish movement, immobility, internal and external growth of mycelium and sporulation. At six days after spray all the treatments differed significantly (P=0.01) with each other and a maximum of 83.34 per cent mortality was observed at 2.10×10^5 spores per ml concentration, which was significantly superior (P=0.01) compared to all other treatments. In case of adults of *T. neocaledonicus* at two days after spray, mortality of 20.51 per cent was recorded by *F. semitectum* at 2.10×10^8 spores/ml. At four days after spray, a significantly higher mortality (58.14%) was observed at 2.10×10^9 spores per ml followed by 2.10×10^8 and 2.10×10^7 spores per ml. Mortality of 12.82 and 8.92 per cent of was registered by *F. semitectum* at 2.10×10^6 and 2.10×10^7 spores per ml respectively. However, the lowest concentration of 2.10×10^5 spores per ml recorded a mortality of 6.27 per cent which was on par with control. At six days after spray all the tested treatments (*F. semitectum* 2.10×10^5 to 2.10×10^8 spores/ml) were significantly different from each other with the highest concentration (2.10×10^8 spores per ml) recorded a mortality of 78.20 per cent. These results under laboratory conditions revealed that there was a significant reduction (P=0.01) in the mite population at all the tested concentrations of the fungus. Of the six different concentrations of *F. semitectum*, the treatment 2.10×10^8 spores per ml recorded the highest mortality of both immatures and adults of *T. neocaledonicus* after six days of spraying. Increased mortality was observed with an increase in spore concentrations. Moreover, the per cent mortality was increased with increased exposure time. Studies revealed that immatures were found to be more susceptible to fungus than adults. The results described herein are in agreement with Mikunthan and Manjunatha. (2006) who reported *F. semitectum* was pathogenic against chilli mite, *Polypagotarsonemus latus*. Similar results were also observed by Navik et al. (2015) who reported that *F. semitectum* at 2.30×10^8 spores/ml was effective in killing mite, *Calepitirmerus azadiractae* Channa Basavanna with a mortality of 82.00 per cent.

Pathogenicity of *H. thompsonii* against *T. neocaledonicus* under laboratory conditions: In present study, the results revealed that at two days after spray, significantly higher (P=0.01) mortality (34.60%) of immatures of *T. neocaledonicus* was recorded by *H. thompsonii* at 4.60×10^8 spores per ml (Table 2). The mortality of *H. thompsonii* at 4.60×10^4 and 4.60×10^6 spores per ml was 25.64 and 19.23 per cent, respectively. Similarly, 4.60×10^4 and 4.60×10^6 spores per ml recorded a mortality of 15.29 and 12.82 per cent, respectively. At four days after spray, higher mortality (50.00%) was observed at 4.60×10^8 spores per ml, followed by the treatments viz., 4.60×10^4 and 4.60×10^6 spores per ml, which recorded 37.19 and 30.77 per cent mortality, respectively and were on par with each other. However the fungus at 4.60×10^4 and 4.60×10^6 spores per ml, yielded a mortality of 20.51 and 14.11 per cent, respectively. At fourth day of infestation immatures showed sluggish movement, immobility, internal and external growth of mycelium and sporulation. Similar trend was observed at six days after spray. At six days after spray, maximum of 92.31 percent mortality was observed at 4.60×10^8 spores per ml concentration, which was superior as compared to all other treatments. In case of adults of mites at two days after spray, mortality of 25.64 percent was recorded by *H. thompsonii* at 4.60×10^8 spores per ml which was on par with 4.60×10^7 spores per ml. This was followed by other fungal treatments. At four days after spray, a
significantly higher (P=0.01) mortality (44.87%) was observed at 4.60 \times 10^8 spores per ml followed by 4.60 \times 10^7 spores per ml and was on par with each other. A mortality of 17.95, 15.39 and 12.82 percent was registered by the fungus at 4.60 \times 10^6, 4.60 \times 10^5 and 4.60 \times 10^4 spores per ml, respectively and were on par with each other. At six days after spray, all the treatments were significantly different (P=0.01) from each other with the highest mortality of 82.05 per cent was recorded by the fungus at 4.60 \times 10^8 spores per ml. These results under laboratory conditions revealed that there was a significant reduction in the mite population at all the tested concentrations of the fungus. Of the six different concentrations of \textit{H. thompsonii}, the treatment 4.60 \times 10^8 spores per ml recorded the highest mortality of both immatures and adults after six days of spraying. Increased mortality was observed with an increase in spore concentrations. Immatures were more susceptible compared to adults. The effectiveness of \textit{H. thompsonii} is in conformation with the report of Yadav Babu (2004) who recorded 85 per cent mortality on arecanut mite and also with the study of Navik \textit{et al} (2015) who reported that \textit{H. thompsonii} at 4.60 \times 10^8 spores was effective in killing mite, \textit{Calepitrimerus-sazadirachtae} Channa Basavanna with a mortality of 86 per cent whereas the present study recorded a mortality of 82.05 per cent.

\textbf{Determination of LC}_{50} for \textit{T. neocaledonicus}: In present study, the LC_{50} values of \textit{F. semitectum} and \textit{H. thompsonii} against \textit{T. neocaledonicus} were 2.70\times10^8 and 1.72\times10^8 spores/ml, respectively and were calculated using the data of four days after treatment.

\textbf{Greenhouse study:} In present study, the performance of \textit{F. semitectum} and \textit{H. thompsonii} alone and in combination with dicofol and Econeem on active forms of \textit{T. neocaledonicus} was compared with water spray and evaluated after three, six, nine and fifteen days of spray. After third day of spray, the lowest mortality was observed in \textit{F. semitectum} at 2.10 \times 10^9 spores/ml + Econeem 0.002% with 12.86 percent mortality and it was the highest in dicofol 0.05% with 69.86 per cent mortality (Table 3). The treatments \textit{H. thompsonii} at 4.60 \times 10^8 spores/ml alone, \textit{F. semitectum} at 2.10 \times 10^9 ml + \textit{H. thompsonii} at 4.60 \times 10^8 spores/ml and \textit{F. semitectum} at 2.10 \times 10^9 spores/ml + \textit{H. thompsonii} at 4.60 \times 10^8 spores/ml + Econeem 0.002% were statistically on par with each other with a mortality of 23.16, 22.82 and 23.15 per cent, respectively. \textit{F. semitectum} at 2.10 \times 10^9 spores/ml alone recorded a mortality of 17.70 percent which was on par with \textit{H. thompsonii} 4.60 \times 10^8 spores/ml + Econeem 0.002%. The same trend remained but at increasing rate of mortality in all the treatments even at sixth and ninth day after spray. After 15 days of spray, the highest mortality of 81.20 per cent was recorded in treatment \textit{H. thompsonii} 4.60 \times 10^8 spores/ml which was on par with \textit{F. semitectum} at 2.10 \times 10^9 spores/ml + \textit{H. thompsonii} at 4.60 \times 10^8 spores/ml. \textit{H. thompsonii} at 4.60 \times 10^8 spores/ml + dicofol 0.025% and \textit{F. semitectum} at 2.10 \times 10^9 spores/ml + \textit{H. thompsonii} at 4.60 \times 10^8 spores/ml + dicofol 0.025% showing a mortality of 81.48, 81.21 and 82.59 percent, respectively. The lowest mortality was observed in dicofol 0.05% with 30.15 per cent. The treatments \textit{F. semitectum} at 2.10 \times 10^9 spores/ml alone and \textit{F. semitectum} at 2.10 \times 10^9 spores/ml + dicofol 0.025% were on par with each other recording a mortality of 59.60 and 60.71 percent, respectively. The present findings are in agreement with Pena \textit{et al} (1996) who reported effective control of \textit{Polyphagotarsonemus latus} using \textit{H. thompsonii} under greenhouse conditions. Effectiveness of fungus is also in confirmation with Odongo \textit{et al} (1998) who recorded 85.20 percent mortality of cassava mite using \textit{H. thompsonii} at 8.00 \times 10^5 conidia/ml under greenhouse conditions. Similarly Yadav Babu (2004) reported 81.65 per cent mortality of \textit{Oligonychus indicus} Hirst using \textit{H. thompsonii} 4.60 \times 10^8 spores/ml + dicofol 0.025% under greenhouse conditions. Previous reviews showing the efficacy of \textit{F. semitectum} against \textit{T. neocaledonicus} were scarce to discuss the present findings critically. Combination of fungi (\textit{F. semitectum} and \textit{H. thompsonii}) + sub lethal dose of pesticides (Econeem and dicofol) suppressed the mite population compared to fungi alone. This was in conformity with the report of Mikunthan (2004) who reported the higher efficacy of \textit{H. thompsonii}, when it is used in combination with Monocrotophos and Neem Seed Kernel Extract (NSKE) under field conditions. The

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Concentration (spores/ml) & 2DAS* & 4DAS & 6DAS & \\
\hline
4.60 \times 10^8 & 34.60 (36.01)* & 25.64 (30.41)* & 50.00 (45.00)* & \\
4.60 \times 10^7 & 25.64 (29.56)* & 20.51 (26.91)* & 37.19 (37.57)* & \\
4.60 \times 10^6 & 19.23 (26.01)* & 15.39 (22.99)* & 30.77 (33.66)* & \\
4.60 \times 10^5 & 15.29 (22.99)* & 12.82 (20.94)* & 20.51 (26.91)* & \\
4.60 \times 10^4 & 12.82 (20.94)* & 10.26 (18.61)* & 14.11 (22.02)* & \\
Control & 0.00 (4.05) & 0.00 (4.05) & 0.00 (4.05) & \\
S.Em ± & 0.01 & 0.87 & 1.00 & \\
CD (P=0.01) & 3.53 & 2.58 & 3.50 & 1.63 & 4.25 & 2.38 \\
\hline
\end{tabular}
\caption{Efficiency of \textit{Hirsutella thompsonii} the immatures and adults of \textit{Tetranychus neocaledonicus} under laboratory conditions.}
\end{table}
It is evident from the results of the present study that even though immatures and adults of *T. neocaledonicus* were susceptible to infection of both *F. semitectum* and *H. thompsonii* under laboratory and greenhouse conditions, *H. thompsonii* was found to be more effective than *F. semitectum* in managing them. The mortality observed was low at 2 days after treatment in both fungal concentrations, it increased gradually and maximum mortality was obtained at 6 days after treatment. Further, the mortality of infected mites with both fungi increased with an increase in spore concentration of conidial suspensions and exposure time. So from the study, it is clearly evident that different concentration of both fungi against *T. neocaledonicus* is both dose and time dependent ie., mortality of mites increased with increased fungal concentration as well as increased exposure time. Both the fungi were found to be compatible with the tested acaricides, dicofol and Econeem. By testing their field efficacy, they can be used as potential fungal pathogens for the sustainable management of *T. neocaledonicus* of okra. Hence potentiality of the above mentioned fungi can be utilized for the ecofriendly management of mites. Considering the sustaining nature of these fungi it is suggested to incorporate them in the management strategy of red spider mite, *T. neocaledonicus*.

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REFERENCES


