# Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

Journal of Entomology 8 (3): 274-279, 2011
ISSN 1812-5670 / DOI: 10.3923/je.2011.274.279


# First Record of Natural Occurrence of *Cladosporium cladosporioides* (Fresenius) de Vries and *Beauveria bassiana* (Bals.-Criv.) Vuill on Two Spotted Spider Mite, *Tetranychus urticae* Koch from India

[1]S. Jeyarani, [2]J. Gulsar Banu and [1]K. Ramaraju
[1]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[2]Central Institute for Cotton Research, Coimbatore 641 003, Tamil Nadu, India

*Corresponding Author: S. Jeyarani, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India*

## ABSTRACT

A survey for natural occurrence of entomopathogenic fungi of two spotted spider mite *Tetranychus urticae* Koch was made in Coimbatore District of Tamil Nadu, India during 2009. Occurrence of two entomopathogenic fungi viz., *Cladospoirum cladosporioides* (Fresenius) de Vries to the tune of 75.25, 87.00 and 96.75% and *Beauveria bassiana* (Bals.-Criv.) Vuill to the tune of 7.50, 12.00 and 5.25% were recorded on *T. urticae* infesting cowpea, red gram and okra, respectively. Both the fungal isolates were assessed for their pathogenicity against the spider mite, *T. urticae* and the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink commonly occurring on okra and cotton, using leaf disc bioassay. The results revealed that the fungus, *C. cladosporioides* was more effective followed by the *B. bassiana* against two spotted spider mite and for mealybug, *B. bassiana* was found to be more effective. *C. cladosporioides* and *B. bassiana* recorded the $LC_{50}$ values of $4.30\times10^{6}$ and $5.27\times10^{6}$ conidia $mL^{-1}$ with $LT_{50}$ values of 63.80 and 110.30 h, respectively against *T. urticae*. Against *P. marginatus*, *C. cladosporioides* and *B. bassiana* recorded the $LC_{50}$ values of $5.20\times10^{7}$ and $3.60\times10^{7}$ conidia $mL^{-1}$ with $LT_{50}$ values of 191.04 and 176.64 h, respectively. This is the first record on natural infection of *T. urticae* by *C. cladosporioides* and *B. bassiana* in Tamil Nadu, India.

**Key words:** Entomopathogenic fungi, *Cladosporium cladosporioides*, *Beauveria bassiana*, *Tetranychus urticae*, *Paracoccus marginatus*

## INTRODUCTION

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is responsible for significant yield losses in many horticultural, ornamental and agricultural crops worldwide (Zhang, 2003). *T. urticae* is of major concern in vegetables causing heavy damage leading to 7-48% yield loss (Srinivasa and Sugeetha, 1999). One of the major problems in the control of *T. urticae* is its ability to rapidly develop resistance to many important acaricides after only a few applications (Nauen *et al*., 2001). To circumvent the problems of acaricide resistance and also to enable farmers and growers to respond to consumer concerns about pesticide residues, there is a need for an effective method of phytophagous mite control that does not involve chemicals. This is most likely to be achieved with a suite of natural enemies that complement one another's activities at different times during crop and pest development. Several insect pathogens viz., *Hirsutella*

*thompsonii* Fisher, (Aghajanzadeh *et al*., 2006), *Beauveria bassiana* (Bals.-Criv.) Vuill (Irigaray *et al*., 2003) and *Metarhizium anisopliae* (Metschn.) Sorokin (Chandler *et al*., 2005) are found promising both under laboratory and field conditions against the phytophagous mites. Entomopathogenic fungi are more advantageous as they are capable of infecting them directly through the integument and are amenable for easy culturing. In recent years, more attention has been given to fungal pathogens of insects, but less attention has been given to the exploitation of fungal pathogens for the control of acarine pests. Hence, there is a scope for the use of fungal pathogens against plant mites. Fungal isolates from different geographical locations will have varying virulence and adaptability to environmental conditions like temperature and humidity. Identification of such type of isolates will pave way for the development of biopesticide with high virulence and temperature adaptability. Moreover, not much work has been done on these aspects in India, against mites. With this view, the present investigation was undertaken to isolate and identify strains of entomopathogenic fungi infecting *T. urticae* in nature and test the pathogenicity of these fungi under the laboratory conditions

## MATERIALS AND METHODS

**Survey for natural occurrence of spider mite fungal pathogens:** Survey was undertaken during 2009 to isolate and identify the fungi associated with *T. urticae* on cowpea, redgram and okra in Coimbatore district of Tamil Nadu. Ten plants in each crop viz., cowpea, red gram and okra were selected at random and the number of live and mycosed mites per square cm area from top, middle and bottom leaves were counted and the percentage mortality due to fungi was worked out. Mite cadavers showing natural external growth of fungi were collected and transferred to the laboratory for isolation and identification. Sabouraud Dextrose Agar with yeast extract (SDAY) medium was used for isolation of the fungi and the slants were incubated in BOD incubator at 25±2°C and 80±10% RH until sporulation. After sporulation, *T. urticae* adults were inoculated with the fungi and reisolated in pure form from the cadavers showing typical mycosis as per the procedure outlined by Goettel and Inglis (1997). The fungal species were identified with the experts at Indian Agricultural Research Institute, New Delhi and USDA, ARS, USA. The isolated cultures were maintained at 25±2°C in an incubator on SDAY. The pure stock cultures were sub-cultured at 15 days intervals in Petri plates (10 cm diameter). Pure stocks in slants were held under refrigerated condition until further use.

**Test insect cultures:** The red spider mites, *T. urticae* were collected from okra fields, mass reared and maintained in the okra plants at glass house of Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University and Coimbatore by following the method developed by Krishnamoorthy (1988). The mealybugs, *P. marginatus* were collected from the naturally infested okra and cotton plants, mass reared and maintained on potato sprouts. The mites and the mealybugs from the culture were used for the fungal bioassays.

**Bioassays:** Two fungi viz., C. cladosporioides and *B. bassiana* isolated from the *T. urticae* were assayed in the laboratory against *T. urticae* and *P. marginatus* for its pathogenicity. Pure cultures of the fungi were subcultured and the plates showing luxuriant fungal growth (11 to 15 days after inoculation) were selected for harvesting conidia and flooded with 20 mL of sterile distilled water containing 0.02% surfactant, Tween 80 (Feng *et al*., 1994). The conidia were liberated by gentle agitation and collected in sterile 250 mL Erlenmeyer flask. The final volume was made upto 100 mL with sterile distilled water. Conidia count was determined using a double ruled Neubauer haemocytometer using phase contrast microscope (Goettel and Inglis, 1997).

Adult mites and mealybugs were used for the bioassays. For bioassay against *T. urticae,* okra leaf discs were placed in a petri dish (10 cm diameter) lined with moist cotton wool and sprayed individually with conidial suspensions of *C. cladosporium* and *B. bassiana* at different doses viz., $5\times10^5$, $1\times10^6$, $5\times10^6$, $1\times10^7$, $5\times10^7$ and $1\times10^8$ conidia $mL^{-1}$ using hand atomizer and allowed to dry for 20 min in a laminar flow. Fifty mites were then transferred to the leaves using a camel hair brush. The control leaves were treated with sterile distilled water containing a 0.02% of Tween 80. All dishes were incubated at 25±2°C. Five replications were maintained for each treatment. Observations on the mortality of mites were taken at 24 h interval upto 8 days after treatment. All dead mites removed from the petridishes were kept in an incubator and the cadavers showing mycosis were considered to be dead as a result of infection by the fungus (Hall, 1984).

Similar procedures were adopted for bioassay against mealybugs using cotton leaf discs as substratum.

**Statistical analysis:** The concentration and time mortality responses were subjected to probit analysis (Finney, 1971) using a Statistical Package for Social Sciences (SPSS), Ver. 10.00 SPSS Inc., USA.

## RESULTS

**Survey for natural occurrence of spider mite fungal pathogens:** Survey for the natural infection of entomopathogenic fungi on *T. urticae* revealed the occurrence of two fungi viz., *Cladosporium cladosporioides* (Fresenius) de Vries (ITCC No. 7641; TNAU 2 - ARSEF 9616) with dirty green color and a white colored fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill (ITCC No.7654). Occurrence of *C. cladosporioides* to the tune of 75.25, 87.00 and 96.75% and 7.50, 12.00 and 5.25% by *B. bassiana* were recorded for the first time on the two spotted spider mite, *T. urticae* infesting cowpea, red gram and okra, respectively at Coimbatore, Tamil Nadu, India (Table 1).

**Bioassays:** Fungal pathogens viz., *C. cladosporioides* and *B. bassiana* isolated from the spider mite was assayed for its efficacy against the red spider mite, *T. uticae*, an important pest of okra and against the papaya mealybug, *P. marginatus* which is a growing concern over most of the crops including okra and cotton. Results of the bioassays revealed significant differences in the concentration and time mortality responses. *C. cladosporioides* was more effective with lowest $LC_{50}$ and shortest $LT_{50}$ values followed by *B. bassiana* against two spotted spider mite and for mealybug *B. bassiana* was found to be more effective. *C. cladosporioides* and *B. bassiana* recorded the $LC_{50}$ values of $4.30\times10^6$ and $5.27\times10^6$ conidia $mL^{-1}$ with $LT_{50}$ values of 63.80 and 110.30 h, respectively against *T. urticae* (Table 2, 3). Against *P. marginatus*, *C. cladosporioides* and *B. bassiana* recorded the $LC_{50}$ values of $5.20\times10^7$ and $3.60\times10^7$ conidia $mL^{-1}$ with $LT_{50}$ values of 191.04 and 176.64 h, respectively (Table 4, 5).

Table 1: Survey for the natural occurrence of fungal pathogens on two-spotted spider mite, *T. urticae* -in Coimbatore District (2009)

| | Mortality±SE (%) | |
|---|---|---|
| Crop | *C. cladosporioides* | *B. bassiana* |
| Cowpea | 75.25±1.32 | 7.50±0.32 |
| Red gram | 87.00±0.73 | 12.00±0.32 |
| Okra | 96.75±0.49 | 5.25±0.33 |

Table 2: Concentration-mortality response of two-spotted spider mite, *T. urticae* to fungal pathogens

| Fungal pathogen | $\chi^2$ | Regression equation | $LC_{50}$ (x $10^6$ condia $mL^{-1}$) | 95% fiducial limit | |
|---|---|---|---|---|---|
| | | | | LL (x $10^6$ condia $mL^{-1}$) | UL (x $10^6$ condia $mL^{-1}$) |
| *C. cladosporioides* | 1.38 | Y = 1.19+0.49x | 4.30 | 2.33 | 7.91 |
| *B. bassiana* | 1.10 | Y = 0.91+0.53x | 5.27 | 3.01 | 9.23 |

No. of mites used per treatment was 300

Table 3: Time-mortality response of two-spotted spider mite, *T. urticae* to fungal pathogens

| Fungal pathogen | $\chi^2$ | Regression equation | $LT_{50}$ (h) | 95 % fiducial limit (h) | |
|---|---|---|---|---|---|
| | | | | LL | UL |
| *C. cladosporioides* | 6.56 | Y = -2.01+0.31x | 63.80 | 58.95 | 69.61 |
| *B. bassiana* | 4.01 | Y = -2.24+0.20x | 110.30 | 102.16 | 121.50 |

No. of mites used per treatment was 300

Table 4: Concentration-mortality response of papaya mealybug, *P. marginatus* to fungal pathogens

| Fungal pathogen | $\chi^2$ | Regression equation | $LC_{50}$ (x $10^7$ condia $mL^{-1}$) | 95% fiducial limit | |
|---|---|---|---|---|---|
| | | | | LL (x $10^7$ condia $mL^{-1}$) | UL (x $10^8$ condia $mL^{-1}$) |
| *C. cladosporioides* | 1.58 | Y = 0.57+0.49x | 5.20 | 1.40 | 1.90 |
| *B. bassiana* | 0.26 | Y = 0.60+0.42x | 3.60 | 1.10 | 1.21 |

No. of mealybugs used per treatment was 300

Table 5: Time-mortality response of papaya mealybug, *P. marginatus* to fungal pathogens

| Fungal pathogen | $\chi^2$ | Regression equation | $LT_{50}$ (h) | 95% fiducial limit (h) | |
|---|---|---|---|---|---|
| | | | | LL | UL |
| *C. cladosporioides* | 0.06 | Y = 5.28 x + 0.25 | 191.04 | 168.48 | 216.96 |
| *B. bassiana* | 0.16 | Y = 4.83 x + 0. 81 | 176.64 | 158.40 | 197.28 |

No. of mealybugs used per treatment was 300

## DISCUSSION

Potential of entomopathogenic fungi for the management of phytophagous mites was reviewed by Van der Geest *et al.* (2000). Isolation and identification of indigenous fungal pathogens are the need of hour to manage the pests in an ecofriendly manner. In the present investigation, survey for the natural infection of entomopathogenic fungi on *T. urticae* revealed the occurrence of *C. cladosporioides* and *B. bassiana*. The entomogenous fungi, *Cladosporium* spp. have been isolated and reported from different insect (Abdel-Baky and Abdel-Salam, 2003; Perea *et al.*, 2003) and mite (Pena *et al.*, 1996; Van der Geest *et al.*, 2000) hosts in nature in different regions of the world. Occurrence of *C. cladosporioides* on *Bemisia* sp., (Abdel-Baky *et al.*, 1998), *Cladosporium. uridenicola* (Link ex Gray) on aphids and whiteflies (Abdel-Baky, 2000) and *Cladosporium oxysporum* (Berk. and Curt.) on *Planococcus citri* (Risso) (Samways and Grech, 1986) was also reported. Natural infection of *T. urticae* by *C. cladosporioides* was also reported by Eken and Hayat (2009) in Turkey. However, this is the first record of natural infection of fungal pathogens on *T. urticae* from Tamil Nadu, India.

Pathogenicity tests of the two fungal isolates revealed significant differences in the concentration and time mortality responses. *C. cladosporioides* was more effective with lowest $LC_{50}$ and shortest $LT_{50}$ values followed by *B. bassiana* against two spotted spider mite and for mealybug *B. bassiana* was found to be more effective. Similarly, Eken and Hayat (2009) reported that the *C. cladosporioides* isolates could cause 50.95 to 74.76% mortality of *T. urticae* with a $LT_{50}$ values ranged from 2.34 to 3.90 days in Turkey. Tamai *et al.* (1999) reported that the *B. bassiana* could cause 50% mortality at concentrations ranging from $5\times10^6$ to $1\times10^9$ conidia $mL^{-1}$. The isolate 447 (ATCC 20872) of *B. bassiana* applied at a concentration of $10^8$ conidia $mL^{-1}$ gave 74.4% mortality of *T. urticae* (Alves *et al.*, 2002). Chandler *et al.* (2005) reported that *B. bassiana* 432.99 (cultured from 'Naturalis-L', Troy Biosciences, Phoenix, TX, USA) recorded 98% reduction in the numbers of *T. urticae* adults, nymphs and eggs under glass house condition. Efficacy of *B. bassiana* against *P. marginatus* was also reported by Banu *et al.* (2010).

## CONCLUSION

Selection of successful and virulent isolates of fungal pathogens is the need of the hour for the biosuppression of the phytophagous mites. Present investigation demonstrates the effectiveness of the fungi viz., *C. cladosporioides* and *B. bassiana* against both *T. urticae* and *P. marginatus* under laboratory condition. Further, research on the field efficacy of these fungi could wide open their potential for the successful management of *T. urticae* and *P. marginatus* with less dependence on chemical control. Natural occurrence of other entomopathogenic fungal fauna against these pests needs to be explored.

## ACKNOWLEDGMENTS

The authors thankfully acknowledges help rendered by staff, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi and Dr. Richard Humber, USDA-ARS-NAA-BioIPM, Insect Mycologist and Curator, ARSEF Collection of Entomopathogenic Fungal Cultures, USA for their identification services.

## REFERENCES

Abdel-baky, N.F., Arafat, S. Nehal and A.H. Abdel-salam, 1998. Three *Cladosporium* spp. As promising biological control candidates for controlling whiteflies (*Bemisia* spp.) in Egypt. Pak. J. Biological Sci., 1: 188-195.

Abdel-Baky, N.F., 2000. *Cladosporium* spp. An entomopathogenic fungus for controlling whiteflies and aphids in Egypt. Pak. J. Biol. Sci., 3: 1662-1667.

Abdel-Baky, N.F. and A.H. Abdel-Salam, 2003. Natural incidence of *Cladosporium* sp. as a bio-control agent against whiteflies and aphids in Egypt. J. Applied Entomol., 127: 228-235.

Aghajanzadeh, S., B. Mallik and S.C. Chandrasheka, 2006. Bioefficacy of six isolates of *Hirsutella thompsonii* fisher against citrus rust mite, *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae) and two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidea). Pak. J. Biol. Sci., 9: 871-875.

Alves S.B., L.S. Rossi, R.B. Lopes, M.A. Tamai and R.M. Pereira, 2002. *Beauveria bassiana* yeast on agar medium and its pathogenicity against *Diatraea saccharalis* (Lepidoptera: Crambidae) and *Tetranychus urticae* (Acari: Tetranychidae). J. Invertebr. Pathol., 81: 70-77.

Banu, J.G., T. Surulivelu, M. Amutha and N. Gopalakrishnan, 2010. Susceptibility of cotton mealybug, *Paracoccus marginatus* to entomopathogenic fungi. Ann. Plant Protec. Sci., 18: 247-248.

Chandler, D., G. Davidson and R.J. Jacobson, 2005. Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. Biocontrol. Sci. Technol., 15: 37-54.

Eken, C. and R. Hayat, 2009. Preliminary evaluation of *Cladosporium cladosporioides* (Fresen.) de Vries in laboratory conditions, as a potential candidate for biocontrol of *Tetranychus urticae* Koch. World J. Microbiol. Biotechnol., 25: 489-492.

Feng, M.G., T.J. Poprawski and G.G. Khachatourians, 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status. Biocont. Sci. Technol., 4: 3-34.

Finney, D.J., 1971. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve: Probit Analysis. 3rd Edn., Cambridge University Press, London.

Goettel, M.S. and G.D. Inglis, 1997. Fungi: Hyphomycetes. In: Manual of Techniques in Insect Pathology, Lacey, L.A. (Ed.). Academic Press, New York, San Diego, USA, pp: 213-250.

Hall, R.A., 1984. Epizootic potential for aphids of different isolates of the fungus *Verticillium lecanii*. Entomophaga, 29: 311-321.

Irigaray, F.J.S.C., M.V. Mancebon and P.I. Moreno, 2003. The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: Effects on the two spotted spider mite, *Tetranychus urticae*. Biol. Control, 26: 168-173.

Krishnamoorthy, A., 1988. A simple method for mass rearing of an exotic predaceous Phytoseiid mite, *Phytoseiulus persimilis* A.H. J. Bio. Control, 2: 53-55.

Nauen, R., N. Stumpf, A. Elbert, C.P.W. Zebitz and W. Krans, 2001. Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). Pest Manage. Sci., 57: 253-261.

Pena, J.E., L.S. Osborne and R.E. Duncan, 1996. Potential of fungi as biocontrol agents of *Polyphagotarsonemus latus* (Acari: Tarsonemidae). Entomophaga, 41: 27-36.

Perea, A.E.I., M.E. Rojas and V.Y.A. Pineda, 2003. Evaluation of different media for isolation of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) molds. Rev. Colomb. Entomol., 29: 13-19.

Samways, M.J. and N.M. Grech, 1986. Assessment of the fungus *Cladosporium oxysporum* (Berk. and Curt.) as a potential biocontrol agent against certain homoptera. Agric. Ecosys. Environ., 15: 231-239.

Srinivasa, N. and J. Sugeetha, 1999. Bioeffectiveness of certain botanicals and synthetic pesticides against okra spider mite *Tetranychus macfarlanei*. J. Acarol., 15: 1-5.

Tamai, M.A., N.B. Alves and P.S. Neves, 1999. Patogenicidade de Beauveria bassiana (Bals.) Vuill. ao acaroTetranychus urticae Koch. Sci. Agric., 56: 285-288.

Van der Geest, L.P.S., S.L. Elliot, J.A.J. Breeuwer and E.A.M. Beerling, 2000. Diseases of mites. Exp. Applied Acarol., 24: 497-560.

Zhang, Z.Q., 2003. Mites of Greenhouses: Identification, Biology and Control. CABI, New York, pp: 240.