



A novel method for the management of mealybug in Cotton.

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Introduction

- Due to large scale adoption of *Bt* cotton, there is a considerable reduction of pesticide usage.
- Emergence of sap feeders (jassids, aphids, thrips, whiteflies, mealybugs, mirids and strainers).
- Sucking pests pose potential threat to *Bt* cotton cultivation in India.
- Mealybugs (Hemiptera : Psuedococcidae) are small sap sucking insects and are responsible for significant yield loss in many agricultural and horticultural crops.
- There are about 2000 species, some of which are major pests of agricultural crops.

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- **The cotton mealybug, *Phenacoccus solenopsis* Tinsley attacks more than >194 plant species in India.**
 - **Both nymphs and adults of mealybugs suck sap from leaves, stems, bolls and twigs.**
 - **Severe infestation leads to stunted growth and drying.**
 - **Black sooty moulds grow due to honeydew secretions.**
 - **It spreads naturally by wind, water, ants and human activities in the form of intercultural operations and movement of infested material leading to its quick colonization in new areas.**
 - **It is a polyphagous pest attacking many field and horticultural crops.**
 - **Persistent increase in the population of mealybugs, has threatened the economical production of many crops including cotton.**

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- **Papaya mealybug, *Paracoccus marginatus* believed to be originated in Central America.**
 - **Recorded in a severe form for the first time on cotton in Coimbatore.**
 - **Severe infestation lead to stunted growth and drying of the sympodial branches.**
 - **The mealybug infestation can be seen as clusters of cotton-like masses on the leaf, squares and bolls.**
 - **Papaya mealybug caused havoc in agricultural and horticultural crops ever since its first report from Coimbatore in 2007.**
 - ***P. marginatus* found to infest over 84 plant species belonging to 35 families in south India**

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- **The control of mealybug is very difficult because they are characterized by waxy covering of the body which hinders the penetration of pesticides.**
 - **This feature of mealybug makes the application of only a few systemic insecticides for the control of mealybug.**
 - **Repeated application of chemical insecticides has disrupted biological control by natural enemies leading to pest outbreak and sometimes in the development of resistance.**
 - **These problems high lightened the need for the development of novel insect control alternatives.**

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- **Use of entomopathogenic fungi, is a viable alternative to conventional insecticides, by virtue of being ecofriendly nature and compatibility with IPM practices.**
 - **Fungal isolates from different geographical regions will have varying virulence and adaptability to environmental conditions like temperature and humidity.**
 - **Identification of native isolates will pave way for the development of biopesticides with high virulence and temperature adaptability.**
 - **The objectives of this study were to search for novel native fungal pathogens of mealybug under natural field conditions and to evaluate their pathogenicity to mealybug infesting cotton in India.**



Phenacoccus solenopsis Tinsley





Paracoccus marginatus
Williams and Granara de
Willink





Materials and methods

- **Survey -- 2012 in Coimbatore district.**
- **No. of samples-100.**
- **Cadavers were superficially sterilized - 70% ethanol - sterile distilled water - 0.5% sodium hypochlorite - sterilized distilled water.**
- **Sabouraud Dextrose Agar with Yeast extract (SDAY) - $25 \pm 1^\circ\text{C}$ and high humidity ($80 \pm 10\%$ RH)**
- **The single spore technique was employed to obtain fungal isolates in pure culture.**
- **Identification- Mycology Division of Agharkar Research Institute, Pune, Maharashtra**

Pathogenicity studies

- ❑ **The mealy bugs, *P.solenopsis* and *P. marginatus* were collected from the naturally infested cotton plants.**
- ❑ **Pure cultures of the fungi were subcultured and spores were harvested**
- ❑ **Spore count was determined using a double ruled Neubauer haemocytometer using phase contrast microscope (Goettel and Inglis, 1997).**



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- Insect : Nymphs and adults of *P.solenopsis* and *P. marginatus*
- Fungus : 5×10^5 , 1×10^6 , 5×10^6 , 1×10^7 , 1×10^7 and 1×10^8 spores/ml
- Control : Sterile distilled water containing Tween 80 solution (0.2 mL L^{-1})
- Observations: Numbers of dead insects at 24 hours interval.
- Dead insects were transferred to a Petri dish containing moistened filter paper to observe fungal growth on the insects.
- No. of replication: ten
- No. of insect/replication: 25

Results and discussion

**Survey –occurrence 55-90
per cent in different
fields.**



Infection process

24 hrs



96 hrs



48 hrs



72 hrs



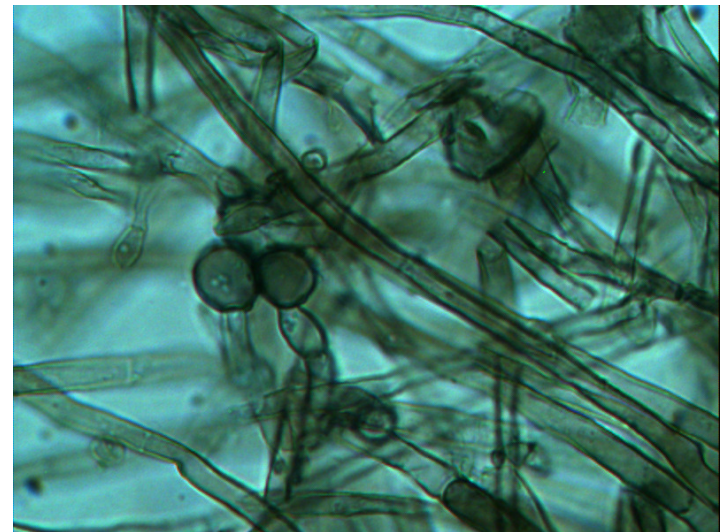
Results and discussion

Colonies on media on 7th day appeared first olive green later turned olive brown, floccose, reverse fuscus black.



Results and discussion

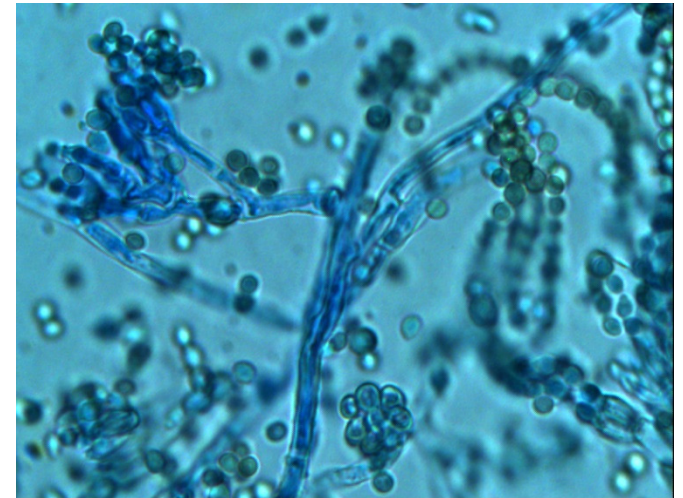
Hyphae were subhyaline to light olivaceous and smooth.



Results and discussion

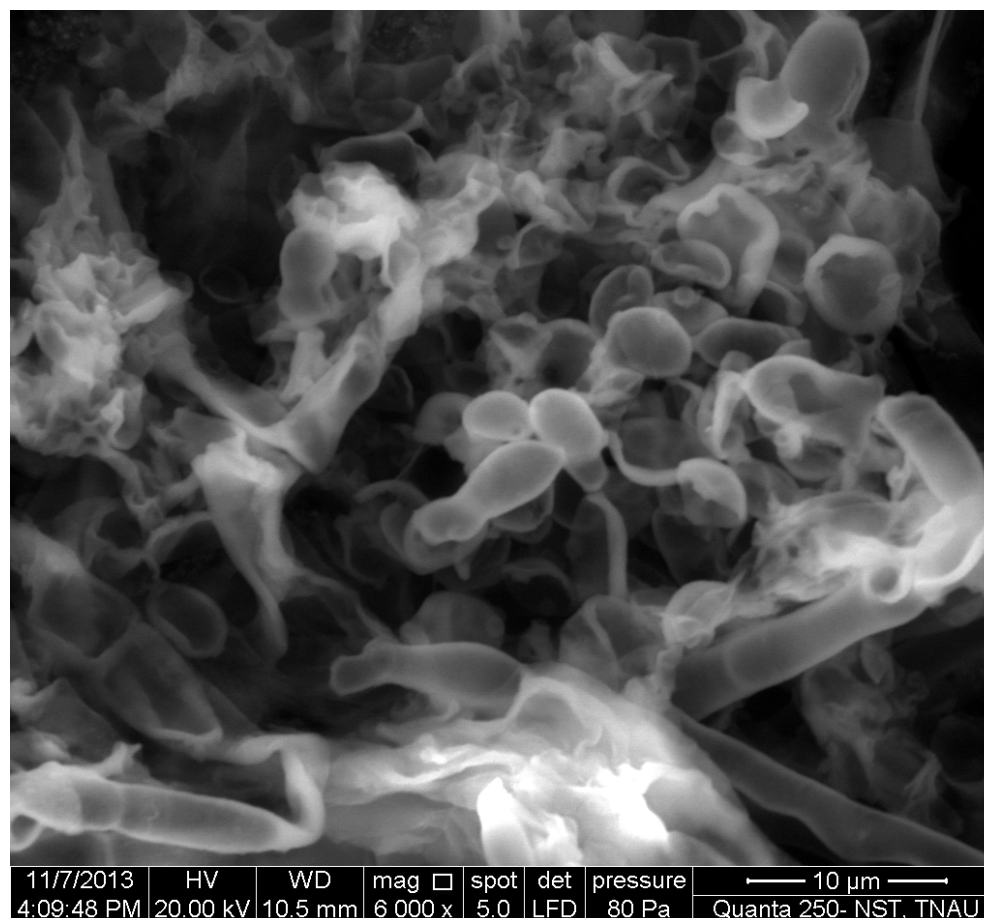
Conidiophores were macronematous to micronematous, small to large, flexuous, wall thickened and darkened, smooth, olivaceous brown.

Conidia were catenate, simple, formed in long chains, aseptate to septate (mostly non septate), limoniform to ellipsoidal, smooth, base thickened and darkened, variable in size up to 2.5-33.5 x 3.75 μ m.

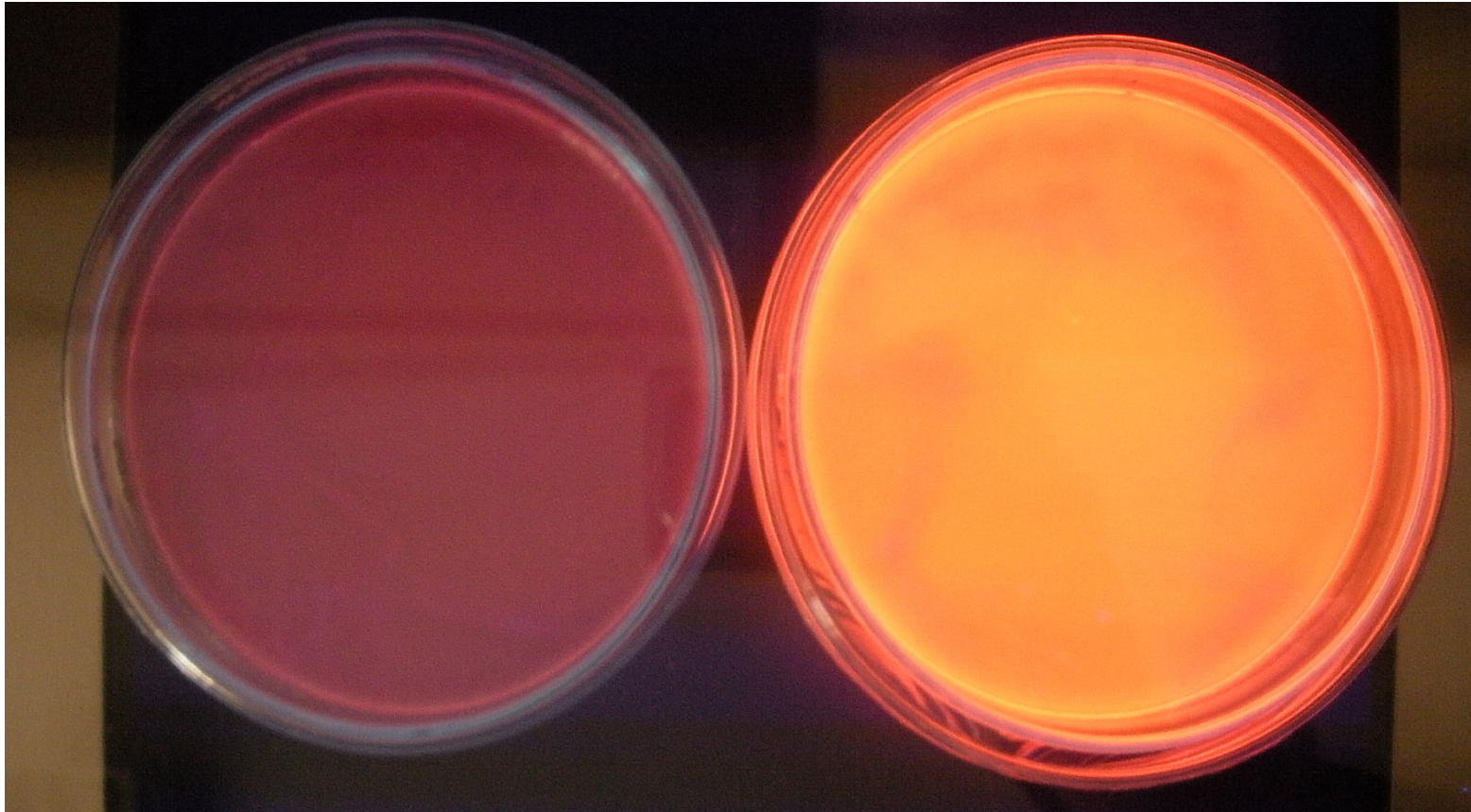


Results and discussion

The isolated fungus was morphologically identified as *Cladosporium cladosporioides* (Fresenius) de Vries (NFCCI-2958).



Lipase production



**Concentration - Mortality response of different stages of
P. solenopsis to *C.cladosporioides***

Stage	X ²	Regression Equation	LC ₅₀ (conidia ml ⁻¹)	95 % Fiducial limit	
				LL (conidia ml ⁻¹)	UL (conidia ml ⁻¹)
Nymph	1.79	Y= 1.19-0.86x	1.6 x 10 ⁷	1.0X10 ⁷	2.7X10 ⁷
Adult	1.11	Y=0.46+0.60x	3.5 x 10 ⁷	2.0x10 ⁷	6.0x10 ⁷

**Concentration - Mortality response of different stages of
P. marginatus to *C.cladosporioides***

Stage	X ²	Regression Equation	LC ₅₀ (conidia ml ⁻¹)	95 % Fiducial limit	
				LL (conidia ml ⁻¹)	UL (conidia ml ⁻¹)
Nymph	1.516	Y=2.45+0.34x	4.5 x 10 ⁷	1.1X10 ⁶	1.8X10 ⁷
Adult	1.099	Y= 0.91+0.53x	5.2 x 10 ⁷	1.2 x 10 ⁷	1.9 x 10 ⁸

**Time - Mortality response of different stages of
P. solenopsis to *C.cladosporioides***

Stage	X ²	Regression Equation	LT ₅₀ (h)	95 % Fiducial limit	
				LL	UL
Nymph	0.90	Y=1.84+3.85x	158.40	142.8	175.68
Adult	0.18	Y=1.37+4.21x	174.96	154.56	198.00

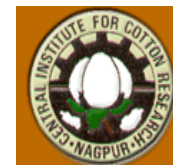
**Time - Mortality response of different stages of
P. marginatus to *C.cladosporioides***

Stage	X ²	Regression Equation	LT ₅₀ (h)	95 % Fiducial limit	
				LL	UL
Nymph	0.12	Y=0.99+4.49x	188.66	152.20	216.00
Adult	0.06	Y= 0.25+5.28x	191.04	168.48	216.96

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- ❑ **The entomogenous fungi *Cladosporium* spp. have been isolated 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.**
 - ❑ **Natural occurrence of *Cladosporium oxysporum* (Berk. and Curt.) was also reported on *Planococcus citri* (Risso) (Samways and Grech, 1986).**
 - ❑ **Natural infection of *T. urticae* by *C. cladosporioides* (50.95 to 74.76%) was also reported by Eken and Hayat (2009) in Turkey Jeyarani *et al.*, 2012 in India.**
 - ❑ ***Leanicillium lecanii* (Banu *et al.*, 2009)**
 - ❑ ***Fusarium pallidoroseum* (Monga *et al.*, 2010)**
 - ❑ **54 isolates of entomopathogenic fungi from mealy bug.**

Conclusion

- ❑ This is the first record on the natural infection caused by *C.cladosporoides* on mealy bug from India.
- ❑ Present investigation demonstrates the effectiveness of the fungus ,*C. cladosporioides* against both *P.solenopsis* and *P. marginatus* under laboratory condition.
- ❑ Histopathological changes due to *C. cladosporioides* on mealy bug was described.
- ❑ Further, research on the field efficacy of this fungus could wide open their potential for the biosuppression of *P solenopsis* and *P. marginatus* so as to reduce the dependence on chemical control.



Thank you