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RESEARCH ARTICLE

FIRST RECORD ON OCCURRENCE OF EPIZOOTICS OF *METARHIZIUM ANISOPLIAE* (METCHNIKOFF) SOROKIN ON GROUNDNUT LEAF MINER, *APROAREMA MODICELLA* (DEVENTER)

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ABSTRACT

In *kharif* groundnut, in Tirupati region, 100 per cent of groundnut leaf miner population was found mycosed during II FN October and I FN November 2018. The blotches of leaf miner were looked milky white with cadavers protruding from the blotch. Such leaves were carried to the laboratory and fungus from blotches and cadavers was inoculated onto SDAY. The plates were incubated at 23°C. Whitish colonies with green centre sporulation was observed after 5 days. The culture was reinoculated onto plates and slants. After attaining good growth and sporulation (one week), the characters of the fungus was studied. The hyphae, conidiophores, conidiogenous cells (phialides) and conidia were studied. Hyphae aseptate with 8-9 microns width. Small branches of hyphae ranged 4-5 microns in width. Conidiophores are septate and like candle holders at branching. Phialides are cylindrical. Conidia arranged in chains. Two types of conidia, viz., globose and elongated were studied. Length of conidia ranged from 10 to 62 microns and width ranged 5 to 44 microns. The fungus was identified as *Metarhizium anisopliae* var *anisopliae* (Metchnikoff) Sorokin. This is first report on Occurrence of Epizootics of Entomopathogenic fungus on groundnut leaf miner (Fungus is *Metarhizium anisopliae* var *anisopliae*).

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INTRODUCTION

The groundnut leaf miner, *Aproarema modicella* (Deventer), a Gelechiid lepidopteran is one of the key insect pests of Groundnut in South and South East Asian countries (Wightman and Amin, 1988). It also reported from Africa (Page et al., 2000, Subramanyam et al., 2000 and Plessis 2003). Yield losses crosses 50% with severe infestation. The main host crops for *A.modicella* are groundnut, soybean and pigeon pea and more than 10 other host crops were reported. The brownish grey small moths of *Aproarema* lays shiny white eggs on lower surface of leaflets. In India, it attacks both *kharif* and *rabi* groundnut under congenial weather conditions. The larvae are greenish grey with dark head region and prothorax. Early larva mines below the epidermal layer of leaflets and feeds on mesophyll content. As a result, blotches are formed. Grownup larva web the adjacent leaflets and feed inside the folds. In case of severe incidence, the field looks like a burnt one. In India, it completes 4 generations in a season, densities fluctuate in seasons (ICRISAT, 1986).

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A no. of Groundnut leaf miner predators and pathogens have been identified Most of the natural enemies are parasitoids belonging to different families of Hymenoptera (Godse and Patil 1981, Shanower and Ranga rao 1990). In India, parasitisation may cross 90% in some rainy seasons (Aug – Sep) (Shetgar and Thombre 1984). Yadav et al., 1987 reported that *Apanteles* species was found abundant in rainy season and *Goniozus* species was abundant in post rainy season (Dec – April) that leads to 70 – 90% parasitisation. Rajagopal et al., (1988) reported that the bacterium, *B.t.*, the fungus, *Beauveria bassiana* and an unidentified virus caused 5.60 – 36.40, 11.60 – 30.80 and 1.20 – 5.20 mortalities of larvae respectively. Shanower et al., (1992) stated that viral and fungal pathogens causes 30% kill of GLM larvae but, pathogens not identified. Rao and Reddy (1997) isolated the fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin from field collected cadavers and pathogenicity was tested in the laboratory. During October second fortnight and November first fortnight of 2018, in *Kharif* groundnut, in Tirupati region i.e in the fields of Regional Agricultural Research station and surrounding villages viz, Cherlopalli, Perumallapalli, Sreenivasamangapuram, Kuchivaripalli, Rangampeta and Kotala, 100 per cent of groundnut leaf miner population was found infected with some fungus. The blotches of GLM looked milky white with brownish larval cadavers inside the blotch.

Some cadavers looked protruding from the blotch. The groundnut leaves having infected larvae and blotches were carried to the laboratory in sterilized vials and fungus was inoculated onto Sabourauds Maltose Agar Medium with Yeast extract (SDAY). The plates were incubated at 23°C. After 5 days, observed whitish colonies with green centre sporulation. The culture was again reinoculated onto plates and slants. After attaining good growth and sporulation(one week), the characters of the fungus was studied. The hyphae, conidiophores, conidiogenous cells (phialides) and conidia were studied and measured with the help of Research Microscope (Olympus, BX41). With literature, the fungus was identified as *Metarhizium anisopliae* var *anisopliae* (Metchnikoff) Sorokin.

Characters of the fungus studied

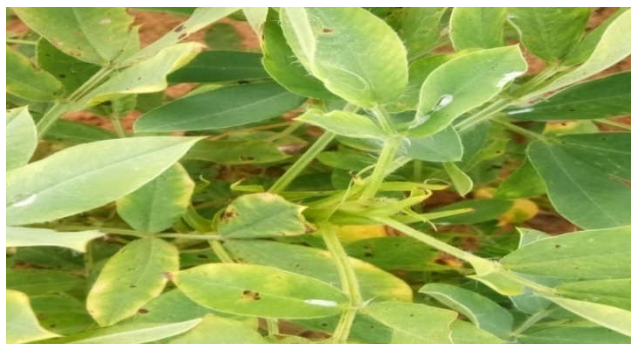
Culture on Medium: Mycelium appeared pure white in colour, spreading fastly in the plate, covered the most part of the plate in 1 week to 10 days. The mycelia growth was found with medium texture, neither very puffy nor very adhesive. The sporulated portion appeared light leaf green in colour first. After few days, turned into dark leaf green colour. After 1 week to 10 days, the sporulated portions turned into very thick (pine) green colour.

Mycelium: Pure white in colour on Sabourauds Maltose Agar Medium with yeast Extract. Hyphae aseptate with 8 -9 microns width. Small branches of hyphae ranged 4 – 5 microns in width.

Conidiophores: More wider than hyphae with upright branching looking like candle holders at branching. Repeated branching noticed. Conidiophores are septate. The Segments are measured in the lengths of ranging 119 to 132 microns. The width of conidiophores ranged from 9 to 15 microns. The dark moving granules are clearly seen inside the segments at 400X magnification.

Conidiogenous cells (Phialides): These are seen rod like, cylindrical, wider at bases and somewhat narrowed terminally arised at tips of conidiophore branches. The length of phialides ranged from 25 to 38 microns and width 5 to 7microns.

Conidia (Spores): The conidia arised from conidiogenous cells i.e phialides. Conidia were seen in chains. The conidia appeared in two main different shapes i.e globose (almost lemon shaped) and elongated (cylindrical) with blunt ends. In some elongated spores, endings somewhat narrowed. Inside the cylindrical spores, 1- 3 septa like structures are seen and spore looked as segmented. In Lemon shaped and cylindrical spores, dark internal contents are observed at 400X. The length of conidia ranged from 10 to 62 microns and width of ranged 5 to 44 microns.



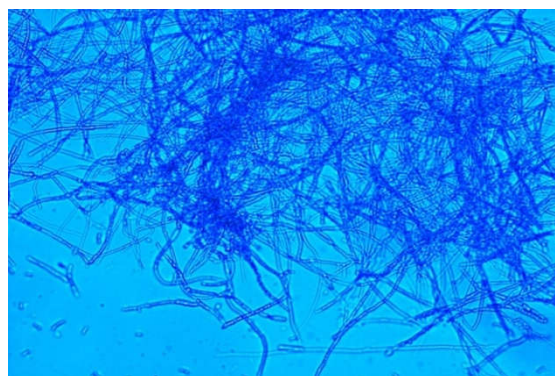
Groundnut leaf miner larval blotches on the leaves. Inside, larvae found dead. Entire blotch looked white due to spread of Fungus



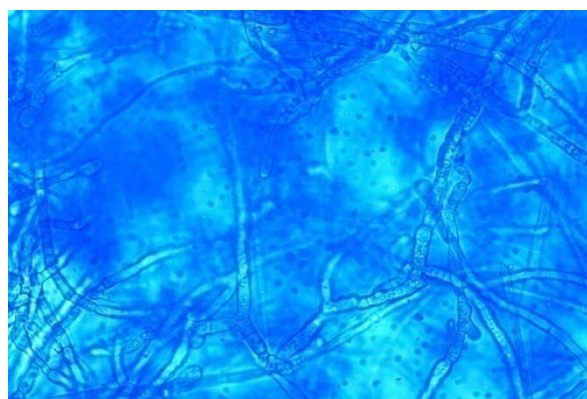
Growth and sporulation of *Metarhizium anisopliae* on SMAY (White mycelium, leaf green sporulation)



Sporulation turned very thick (Pine) green colour after 7 to 10 days

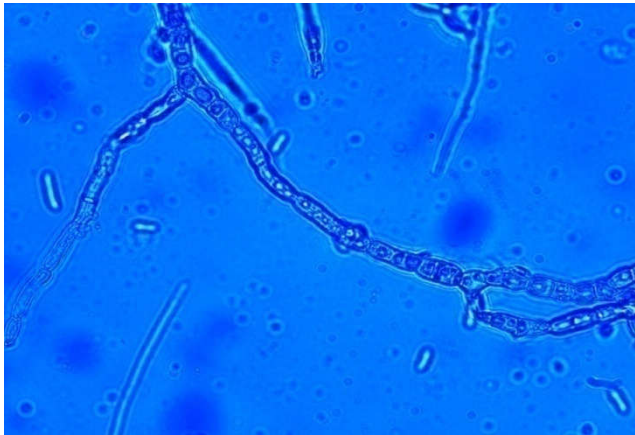


Mycelium, Conidiophores, Conidia of *M.anisopliae* (100X)

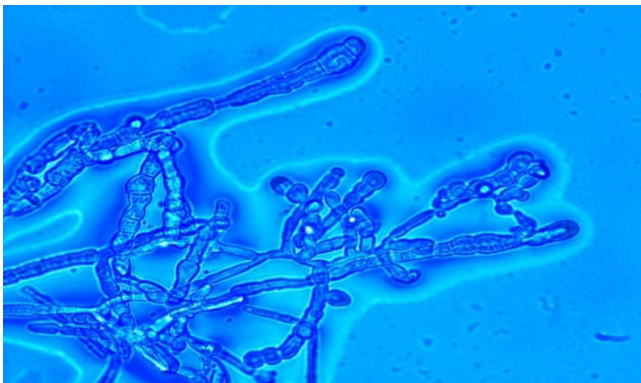


Conidiophores and chains of conidia of *Metarhizium anisopliae* (400X)

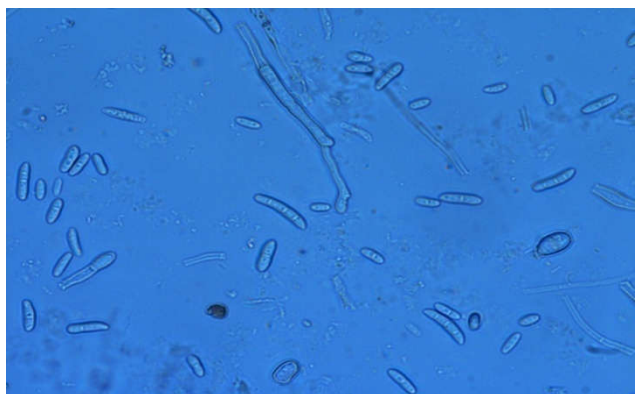
Month	Max. Temp. (°C)	Min.Temp. (°C)	Morn. RH (%)	Eve. RH (%)	Rainfall (mm)	Sunshine hours (per day)	Wind speed (Km/hr)
October '18	27.80 – 37.50	18.00– 24.50	71- 96	36 -89	90	6.4	5.2
November '18	24.00 -33.80	16.80 -24.50	75 -97	28-89	114.8	5.7	6.4



Repeated branching of Conidiophores (400X)



Chains of Conidia of *M.anisopliae* (400X)



Conidiophore segments, conidia

Weather conditions prevailed during November – December 2018: The weather data recorded at Regional Agricultural Research Station, Tirupati during November – December, 2018 is furnished. The temperatures, relative humidity and rainfall are found very congenial for infection by Entomopathogenic fungi. Maximum temperatures were ranged from 24.00 to 33.80°C whereas Minimum temperatures were from 16.80 to 24.50°C. Morning relative humidity was in the range of 71 to 97 Per cent. Total rainfall received during October – November was 204.80mm. Average sunshine hours were 6.4 in October and 5.7 in November. The *M. anisopliae* inoculum might be abundantly available in the field of RARS

and surrounding villages of Tirupati for which prevailed weather factors very much suited to cause Epizootics. The temperatures ranging around 25°C and relative humidity above 70% highly favours the growth and sporulation of any Entomopathogenic fungi. Good amount of rainfall received coupled with lower sunshine hours created high atmospheric relative humidity during the above period. This is first report on occurrence of epizootics of a disease to Groundnut leaf miner. The disease causing organism is Green Muscardine fungus, *Metarhizium anisopliae* var *anisopliae*. This is Sordariomycetes fungus under the order Hypocreales and Clavicipitaceae family.

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