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PATHOGENICITY OF *METARHIZIUM ANISOPLIAE* VAR. *ACRIDUM* STRAINS ON PINK HIBISCUS MEALY BUG *(MACONELLICOCCUS HIRSUTUS)* AFFECTING COTTON CROP

AZIZ AHMED UJJAN AND SALEEM SHAHZAD

Pest and Disease Research Laboratory,
Department of Agriculture, University of Karachi,
Karachi, Pakistan

Abstract

Metarhizium anisopliae var. *acridum* (Metschnikoff) Sorokin strains Ma1912, Ma1729 and Ma3605 were found to be pathogenic to the pink hibiscus mealy bug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae) affecting cotton crop. Effects of these strains on survival of adults, instars and egg hatching were assessed. The strains were able to infect adults within 2 days after inoculation and showed 90% mortality by 8th day, whereas, 100% mortality of instars was observed by 4th day of inoculation when females were inoculated. *Metarhizium anisopliae* strain Ma1912 reduced the egg hatching of *M. hirsutus* by up to 60% and other two strains reduced egg hatching less than 30%, whereas, direct inoculation of eggs on glass slides by all strains resulted in more than 70% reduction in egg hatching.

Introduction

Metarhizium anisopliae (Metschnikoff) Sorokin is a well-known, broad-range arthropod pathogen, which has been evaluated for the biological control of several insect pests, including pests and vectors for plant and human diseases (Goetal & Hajek, 2001). In addition, the entomopathogenic fungus *M. anisopliae* var. *acridum* is easily produced on a large scale and has been developed as a mycoinsecticide in several countries (Hoffman *et al.*, 1993; Hoffman, 1997). This fungus is tolerant to high temperatures, an important characteristic for insect pathogens developed for tropical agroecosystems (Lomer *et al.*, 2001).

The pink hibiscus mealy bug 'PHMB' (*Maconillicoccus hirsutus* Green) has become a severe pest of cotton crop in Southern regions of Sindh and other parts of Pakistan (Hakro & Buriro, 2005). In year 2006, PHMB infestation increased from two districts to eleven districts of Sindh province where Mirpurkhas and Sanghar districts were the worst affected. PHMB is known to cause economic damage to many crops. For example, cotton (Dhawan *et al*., 1980; Murlidharan & Badaya, 2000), the fibre crops *Hibiscus sabdariffa*, *Hibiscus cannabinus* and *Boehmeria nivea* (Ghose, 1961, 1971; Singh & Ghosh, 1970; Raju *et al.*, 1988), grapevine (Manjunath, 1985), mulberry (Rao *et al.*, 1993), pigeonpea (Patel *et al*., 1990) and *Zizyphus maritiana* (Balikai & Bagali, 2000). Presumably, many ornamental woody plants are also affected. Management of this bug is difficult because of its wide host range, quick and easy spread to other areas; wax coating on the body and nature of hiding. Therefore, PHMB is very difficult to control with pesticides since over-doses of pesticides would develop resistance in the pest and in long run pesticides might not be effective. From the environmental view point, pesticide residues are also of great concern and many countries are trying to reduce the use of pesticides. Biological control

provides an alternative method for the management of PHMB (Latge & Papeirok, 1988; Hajek & Leger, 1994).

Paecilomyces fumosoroseus (Wize) Brown & Smith has been reported as the pathogen of mealy bug in Apopka, Florida (Osborne & Landa, 1992). The present paper describe the pathogenicity of *Metarhizium anisopliae* var. *acridum* strains to PHMB that is amongst the most threatening pests of cotton crop in Pakistan.

Materials and Methods

Fungi and test insects: Three strains of *Metarhizium anisopliae* var *acridum viz.*, ARSEF Ma1912, ARSEF Ma1729, and ARSEF Ma3605 were obtained from Curator ARS Collection of Entomopathogenic Fungal Cultures, U.S. Plant, Soil, and Nutrition Laboratory, Tower Rd., Ithaca, NY 14853–2901. The fungal cultures were stored on SDA medium in slants. Insects were collected from naturally affected cotton plants growing in screen house of Pest and Disease Research Laboratory, University of Karachi.

Bioassay: The bioassays were carried out on three stages of mealy bug *viz.* adult females, instars and eggs. Spores of *M. anisopliae* strains were harvested from 15-20 days old culture and suspended in 0.1% solution of Tween 20 in water *(v/v)*. Spores were counted under microscope using haemocytometer and spore concentration was adjusted to $1.5X10^6$ spores ml^{-1}.

Bioassay on adults: The adult female insects were inoculated by pipette through dropping 1ml spore suspension on the body of insect. Insects were transferred in sterilized Petri plates lined with moistened filter paper and autoclaved cotton leaves were used as diet. Control insects were treated with 0.1% Tween 20 in water *(v/v)* as described by Hall (1976). During inoculation, a temperature of 25 ± 2ºC, 15:9 (Light: Dark) photoperiod and 100% humidity were maintained. The movement of insect, nymph production, fungal growth, and sporulation parameters on the body of adult insects were observed at 24h intervals; for pathogenicity test the insect cadavers were surface sterilized in 2% Sodium hypochloride solution for 2 minutes and placed on PDA medium in Petri plates.

Bioassay on instars: The instars (crawlers) were obtained from adult females by keeping them in sterilized Petri plates with moistened filter paper and cotton leaves as diet. The adult females were removed and instars in each Petri plates were counted and sprayed with 3ml of spore suspension. Control Petri plates were sprayed with 0.1% Tween 20 in water *(v/v)*.

Bioassay for eggs hatching: Adult females were surface sterilized with 1% Sodium hypochloride for 10 minutes and kept in Petri plates containing moistened filter paper and cotton leaf as diet. Spore suspensions were sprayed on adult females. Control plates were sprayed with 0.1% Tween 20 in water and the production of instars counted.

In another experiment surface sterilized adult females were placed on a microscopic slide and softly teased with needle. The body parts except egg masses were picked up from the slides, 1ml spore suspension was added on each slide, the slides were kept in Petri plates lined with moistened filter paper and extract from autoclaved ground of

cotton leaves was added as diet. Control slides were treated with 0.1% Tween 20 in water *(v/v)*.

Each bioassay consisted of three replicates. Adults, nymphs and eggs were examined every day. The data on mortality and egg hatching were recorded after each 24 hour for consecutive ten days and time interval for 50% mortality (LT_{50}) was recorded. Each experiment was repeated once.

Results and Discussion

Strain 1729 caused 100% mortality after 4 days, Strain 3605 caused 100% mortality after 8 days whereas Strain 1912 produced 100% mortality after 7 days of inoculation on adult females of PHMB (Fig. 1A). All strains showed hyphal penetration in body of instars (1^{st} & 2^{nd}), the strain 1729 grew with thick network of hyphae on the insect cadavers (Fig. 3E). The developmet of wax on the body of growing instar female adults hinders the hyphal penetration in the body. Therefore, the mortality of adults PHMB recorded during bioassay was lower than mortality of instars (Fig. 1A&B). All strains showed 100% mortality after 4 days of inoculation on instars (Fig. 1B). Strain 1912 showed maximum effect on eggs hatching in Petri plates than other strains (Fig. 1C). However, all strains showed lethal effects on egg hatching in glass slides test. Although glass slide bioassay revealed the hyphal growth on eggs and egg masses but it was observed that fungus could not stop egg hatching by 100% (Fig. 1D).

For the successful introduction of a fungal biocontrol agent, information is not only needed on the biology and feeding activity of the control agent but also on the most susceptible stage of pest species (Cuthbertson *et al.*, 2003). The lethal effects of *M. anisopliae* were most significant on instars followed by egg hatching and then on the adults.

During fungal penetration through the host cuticle, hydrolytic enzymes such as proteases, chitinases and lipases are produced that are proposed to be important for the initiation of the infection process leading to cuticle transposition. The conidia of *M. anisopliae* were found to germinate on cuticle of termites through the penetrating germ tube and establish a systemic infection which ultimately kills the insect (Milner & Staples, 1996). During the present studies, the fungal spores were able to germinate and penetrate the treated adult females, instars and eggs of mealy bugs (Fig. 2). Scanning electron micrographs also confirmed these results (Fig. 3). Microscopic observations confirmed these findings and results show that use of *M. anisopliae* var. *acridum* as biological control agent against PHMS holds promise and needs elucidation.

It has been reported that the relative humidity (RH) and temperature are known to be limiting environmental factors for fungal development on insects (Glare & Milner, 1991; Ferron *et al.*, 1991). High rates of infection and a rapid kill of bugs by the hyphomycetous fungi were obtained at humidities close to saturation (Silva & Messias, 1985; Romana & Fargues, 1987; Luz, 1990; Romana, 1992; Luz *et al.*, 1994).

Methyl Bromide (Chemical pesticide) has been reported to show the highest mortality effects against PHMB in the egg stage followed by instars and adults (Larry *et al.*, 2002). In view of the ban that is going to be imposed on Methyl bromide, microbial pesticides containing *M. anisopliae* would be a valuable tool for integrated pest management strategies against PHMB.

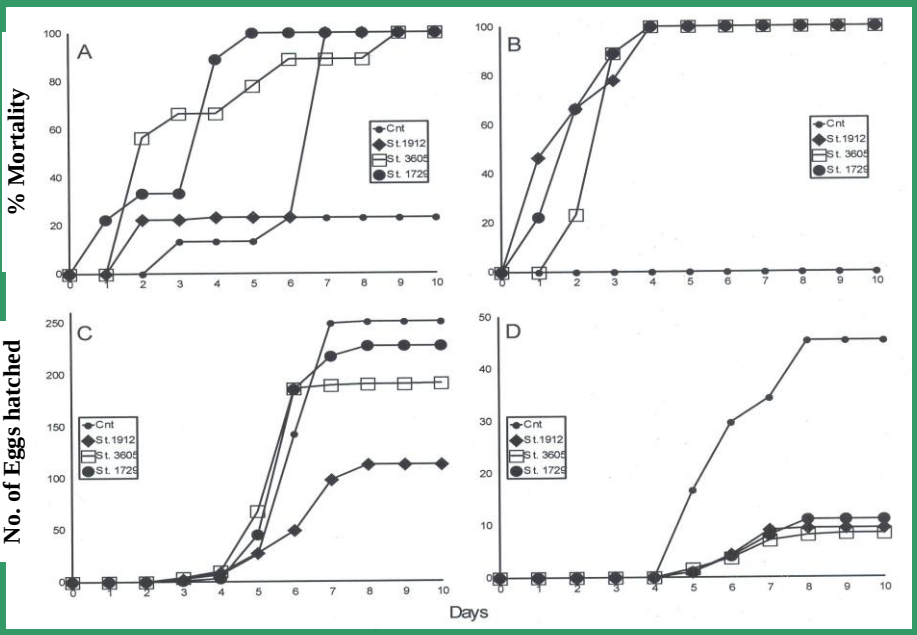

Fig. 1. A: Mortality of adult females of PHMB. B: Mortality of instars. C: No. of crawlers from the eggs laid by the adult female and effects on egg hatching. D: No. of crawlers hatched from the egg masses on microscopic slide..

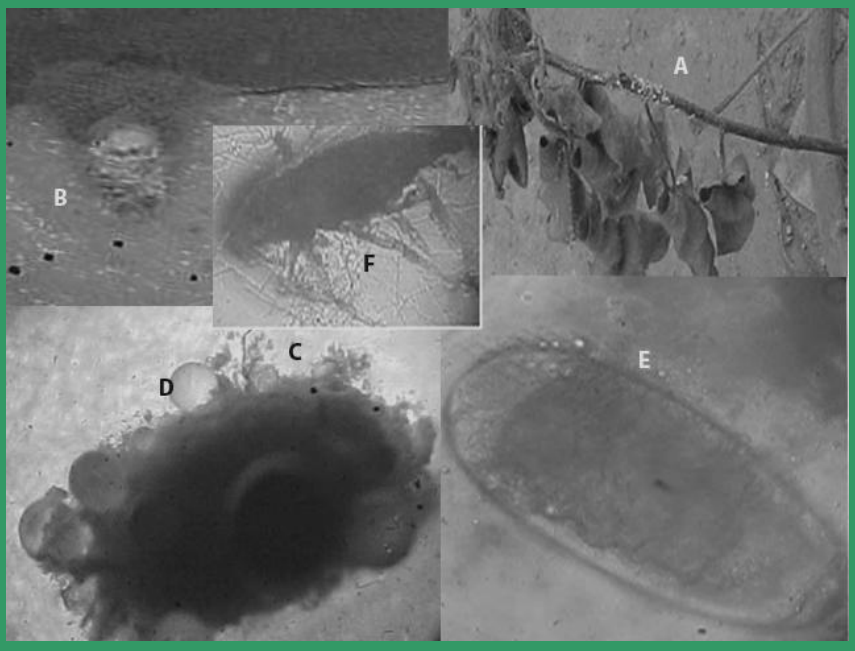

Fig. 2. A: Cotton plant affected by PHMB. B: Growth of *M. anisopliae* strain 1729 on the adult female PHMB. C: Hyphal growth on the body of PHMB instars. D: Contents squeezed out from the ruptured body of infected insect. E: Egg of PHMB surrounded by the fungal hyphae. F: Cadaver of PHMB instars surrounded by hyphae of *M. anisopliae* Strain 1912.

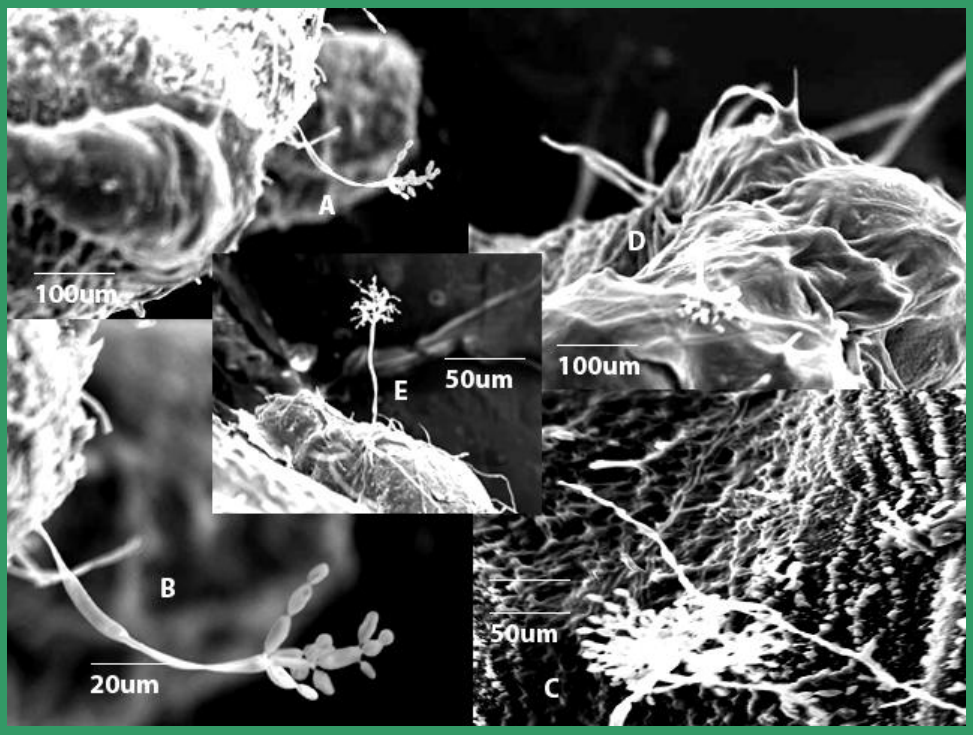

Fig. 3. A: Growth of strain 3605 hyphae on dorsal side of insect head shows phialides and spores; B. magnified micrograph of 'A'. C: Growth of fungal strain 1912 spores and hyphae on the posterior end of PHMB. D: Growth of strain 1912 on the body of insect. E. Growth of Strain 1729 on insect.

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