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Growth characteristics and bio-efficacy of different isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin against certain key insect pests*

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ABSTRACT: An attempt was made to know the variation in field collected isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin under laboratory conditions. Isolates of *M. anisopliae* collected from different geographical regions were studied for various parameters like growth and sporulation on potato dextrose agar (PDA) medium and rice based medium, and exhibited considerable variation. Ma3 and Ma4 isolates took 6 days to initiate sporulation while Ma1 and Ma2 isolates took 2 to 3 days on PDA. Mycelial growth in Ma1 and Ma2 isolates, being on par, was significantly higher (38.60 and 42.30 mm, respectively) than the other two isolates (Ma3–32.30 mm and Ma4–33.19 mm). Ma1 and Ma2 isolates grew faster on rice based medium and yielded conidial yield of 8.93 and 9.32 g per 100 g of medium respectively. Isolate Ma2 was the most virulent against *Helicoverpa armigera* (Hübner) and *Plutella xylostella* (Linnaeus) with less LC_{50} values of 1.77 x 10^6 and 1.98 x 10^6 conidia per ml followed by Ma1, which was more virulent against *Oryctes rhinoceros* (Linnaeus). The lethal time (LT_{50}) was low in Ma2 for *H. armigera* and *P. xylostella* (126.15 h and 69.74 h, respectively) in contrast to Ma1 that recorded lower LT_{50} value against *O. rhinoceros* (294.47h). The results indicate that Ma2 was the best isolate against lepidopteran insects and Ma1 was the best against coleopterans.

KEY WORDS: Bio-efficacy, biological characters, *Helicoverpa armigera, Metarhizium anisopliae, Oryctes rhinoceros, Plutella xylostella*

INTRODUCTION

In nature, strains of the entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin display considerable specificity (Ferron *et al*, 1975) and diversity (Roberts, 1989) in biochemical and biological activity. Variation in the susceptibility of *Oryctes rhinoceros* L. and *Acanthoscelides obtectus* (Say) to different isolates of *M. anisopliae* have been documented (Ferron *et al*., 1975; Ferron and Roberts, 1975). Samuels (1986) screened 58 wild type isolates of *M. anisopliae* against *Nilaparvata lugens* (Stal) and categorized them as highly, moderately and poorly pathogenic isolates. Milner and Prior (1994) noticed high pathogenicity in certain isolates of *M. anisopliae* to previously un-encountered hosts and observed three times greater susceptibility of *Chortoicetes terminifera* (Walker) to isolate Ma9 of *M. anisopliae* than *Phaulacridium vittatum* Sjastedt. They also observed that locally derived isolates were more virulent than isolates derived from other origins. Theunis and Aloali (1998) selected MaTB101 out of 30 isolates of *M. anisopliae* as highly virulent against *Papuana uninodis* Prell based on LT_{50} values. Fernando *et al*. (1995) found no variation among three isolates against *O. rhinoceros.* Scan of literature reveals a lot of variation in isolates or strains from different locations and host insects. Keeping this in view, the present study has been attempted to assess the variations among some local isolates of *M. anisopliae* in order to select a virulent isolate for developing a mycoinsecticide for future pest management programme.

MATERIALS AND METHODS

The investigation was carried out in the laboratory of Department of Agricultural Entomology, University of Agricultural sciences, Dharwad.

Establishment of *M. anisopliae* isolates

Four isolates of *M. anisopliae* collected from different agroclimatic regions and hosts were used in the present study. Ma1 from *O. rhinoceros* grubs from Dharwad, Ma2 from *Aproaerema modicella* (Deventer) larvae from groundnut at Raichur, Ma3 from *Nilaparvta lugens* (Stål)

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from Gangavathi, and Ma4 from Kasargod (Kerala) on root grubs were isolated and purified by single spore isolation and were maintained on potato dextrose agar (PDA) slants at 26 ± 2°C for further use. After sporulation, conidial length and diameter for respective isolates were measured under a compound microscope. Studies on colony growth characters on PDA were done for each isolate. Fungal disc (3 mm dia.) of the respective isolates before sporulation was placed at the center of a plate containing sterilized PDA. After 24h of incubation, ten randomly selected plates from each isolate were observed continuously at an interval of 6h to record the initiation of sporulation visually and also by using a lens. Conidial colour was recorded through visual observations. Media pigmentation was examined at the bottom of agar plate. The colony diameter of the respective isolate was measured on 10th day of inoculation. For all observations 10 plates of each isolate were used.

Conidial production

Seventy-five gram of broken rice per 500 ml saline bottle containing 60 ml of 1 per cent yeast solution was soaked for 6h and sealed with cotton plug. The bottles were then autoclaved at 15 Psi pressure for 30 minutes. The rice medium was cooled and inoculated with 2ml of respective conidial suspension of *M. anisopliae* isolates (1 x 10^4 conidia ml^{-1}) per bottle under aseptic condition. The bottles were once again sealed and incubated at 26°C ± 2°C for 20 days for the production of aerial conidia. After incubation, rice grain with the growth of each isolate was taken out and dried. After drying, the conidial production of each isolate on rice grains was estimated using a haemocytometer. The rice grains with fungal sporulation were sieved through 300 mesh to get fine conidial powder. The quantity of conidia powder obtained per gram of rice medium in each isolate was estimated.

Bio-efficacy of *M. anisopliae* isolates

The four *M. anisopliae* isolates collected from four agro–climate regions were assayed on *H. armigera, P. xylostella and O. rhinoceros*. Pure cultures of respective isolates were maintained on PDA medium in slants and plates and sub–cultured once in three months. They were passed through original insect host to revive virulence after 5–6 sub–culturing. After complete sporulation, conidia were harvested by washing them with sterile distilled water containing Tween–80 (0.2%). Before inoculation, the colony forming units (CFUs) were assessed. Conidial spores were further extracted by passing the suspension through a double layered muslin cloth. Spore count was made using a Neubauer's haemocytometer after necessary serial dilution under phase contrast microscope. From the stock solution, further dilutions were made to obtain the desired concentrations.

Efficacy of *M. anisopliae* isolates against *H. armigera*

Newly moulted third instar larvae of *H. armigera* were topically applied under potter's tower with conidial suspensions of the four isolates each containing conidial load from 10^4 to 10^{10} ml^{-1} to find out LC_{50} values at 8th day of treatment. The LT_{50} value for each isolate was also assayed at 1 x 10^7 conidia ml^{-1}. After treatment, the larvae were transferred to multi–cavity trays containing sterilized artificial diet. For each treatment, 32 larvae were treated in four replications. Mortality was recorded at 12h interval from 3rd day after treatment to 10 days and per cent mycosis was corrected according to Abbott (1925).

Efficacy of *M. anisopliae* isolates against *P. xylostella*

Each isolate of *M. anisopliae* was sprayed on early third instar larvae (20 / replication) of *P. xylostella* released on cabbage leaf discs under a Potter's tower at a concentration of 1 x 10^7 conidia ml^{-1} for LT_{50} studies and from 10^4 to 10^{10} conidia ml^{-1} for LC_{50} studies. The treated leaf discs along with the larvae were later transferred to labelled Petri plates lined with moist blotting paper. The mortality count of the larvae was taken after two days to nine days.

Efficacy of *M. anisopliae* isolates against *O. rhinoceros*

Sterilized compost was taken as carrier for treating the isolates of *M. anisopliae* for bioassay studies against *O. rhinoceros* grubs. Each isolate was applied to sterilized compost @ 1 x 10^4 conidia g^{-1} for LT_{50} studies. For LC_{50} studies, six concentrations from 1x10^3 to 10^8 g^{-1} of sterilized compost was mixed and filled in to sterilized plastic tubs. The treated compost was moistened with sterile water. Second instar grubs of uniform size were selected from the stock culture maintained on FYM were washed with sterile water and released @ 20 per tub. Five replications for each isolate and untreated check were maintained for recording mortality, which was corrected according to Abbott (1925). Mortality of grubs was recorded commencing from the 6th day after treatment at an interval of 72 h. LT_{50} and LC_{50} values were analyzed by maximum likely hood method (Finney, 1971).

RESULTS AND DISCUSSION

Growth characters of *M. anisopliae* isolates

Colony growth characteristics of four isolates of *M. anisopliae* collected from different insects and locations showed that the Ma2 isolate produced dark green coloured spores, while the other three isolates produced green coloured spores. Distinct pigmentation of isolates was evident when the colony was examined at the bottom of the plate. Dull yellow, yellow and brown pigmentation was noticed in

Ma1, Ma4 and Ma3 isolates, respectively, while Ma2 appeared without any pigmentation. Ma3 and Ma4 took 5–6 days period to initiate sporulation while Ma1 and Ma2 isolates accomplished this in half of the time indicating faster growth and reproduction. Mycelial growth in Ma1 and Ma2 isolates was at par, but was significantly higher (38.60 and 42.30mm, respectively) than Ma3 and Ma4 isolates. Maximum conidial length (8.69µ) and width (3.31µ) was measured in Ma3 and minimum length (7.98µ) and width (3.16µ) in Ma2 (Table 1). In nature isolates of *M. anisopliae* contain a diverse assemblage of genotypes and probably comprise species complexes. Therefore, it is not surprising that within the taxa, individual isolates can exhibit variation in biological characters. Milner *et al*. (2002) observed variation in colony characters of *M. anisopliae* var. *anisopliae* collected from different geographical locations and host insects.

Conidia production

When multiplication was taken up in saline bottles (500 ml) on broken rice grains, the test isolates exhibited considerable variation in incubation period and spore yield. Ma1 and Ma2 isolates grew fast on rice based medium and attained full growth in about 10 days as against 16 days in the case of Ma3 and Ma4 (Table 1). In commensuration with

Table 1. Colony characters of *M. anisopliae* isolates on potato dextrose medium and growth characters on rice based medium

Isolate / Ecotype	Colony growth on PDA					Growth characters on rice medium		
	Conidial size (mm)	Initiation of sporulation (h)	Colour	Media pigmentation	Diameter on 10th DAI (mm)	Time taken to cover the medium (days)	Conidial yield	
							Per 100 g of diet (g)	x10^{9}conidia g^{-1} diet
Ma1	8.32x 3.26	48–72	Green	Slight yellow pigment	38.60^a	10.30^a	8.93^a	2.92^a
Ma2	7.98 x 3.16	48–72	Dark green	No pigment	42.30^a	10.00^a	9.32^a	3.21^a
Ma3	8.69 x 3.31	120–144	Green	Brown	32.30^b	15.60^b	5.62^b	1.53^b
Ma4	8.13x 3.21	120–144	Green	Yellow pigment	33.19^b	16.30^b	6.03^b	1.62^b

speed of growth and speediness in initiation of sporulation, spore production in the Ma2 isolate was 9.32g of spores per 100g of rice grain and 3.21 x 10^9 conidia per gram of diet, which was followed by Ma1 (8.93g / 100g diet and 2.92 x 10^9 conidia g^{-1} diet). However, these two isolates were on par with each other.

The LT_{50} values varied among the species of test insects. The Ma2 was quick in causing mycosis to *H. armigera* (LT_{50} = 126.15h) and *P. xylostella* (LT_{50} = 69.74h), while Ma4 and Ma3 had delayed action. However, with change in host insect, Ma1 exhibited faster action (LT_{50} = 294.47h) against *O. rhinoceros* (Table 3) followed by Ma2 (314.92h), Ma4 (439.85h). Rosa *et al*. (1995) tested the virulence of five strains of *M. anisopliae* against coffee berry borer under laboratory conditions. LC_{50} values for the three most virulent isolates, Ma4, Ma5 and Ma3 were 4.2, 5.9 and 6.7 x 10^6 conidia ml^{-1} of suspension, respectively. The median lethal time (LT_{50}) was between 9.7 and 13.8 days.

The Ma2 isolate caused the highest cumulative mortality in *H. armigera* and *P. xylostella*, whereas Ma1 excelled in causing the highest cumulative disease in *O. rhinoceros*. Distinct variation in cumulative mortality by different isolates of *M. anisopliae* in all the three test insects was noticed (Fig. 1). Ma1 isolate caused higher mortality in *H. armigera* (90%) and *P. xylostella* (83%), whereas Ma1 isolate caused highest mortality in *O. rhinoceros* (89%). The variation in the virulence of different isolates of *M. anisopliae* originating from different locations against *O. rhinoceros* (Ferron *et al.*, 1975); *Acanthoscelides obtectus* (Ferron and Roberts, 1975); *N. lugens* (Samuels, 1986); *Chortoicetes terminifera* (Milner and Prior, 1994); coffee berry borer (Rosa *et al*., 1995); and *Papuana uninodis* (Theunis and Aloali, 1998) has been well documented. Samuels (1986) catagorised 58 wild isolates from different geographical areas of England as highly, moderately and poorly pathogenic to *N. lugens*. Fernando *et al*. (1995) found no variation among the isolates against *O. rhinoceros*.

In the present study, Ma1 and Ma2 isolates collected from Dharwad and Raichur were the best isolates, which sporulated profusely and caused more than 80 per cent mortality in all the three test insects.

Table 2. Time–mortality response of insect pests to *M. anisopliae*

Insects	Isolates	Chi^2	Slope	LT_{50} (h)*	Fiducial limit (h)
Helicoverpa armigera	Ma 1	27.38	5.83	130.79	110.52 – 153.06
	Ma 2	29.27	6.71	126.15	107.11 –146.14
	Ma 3	23.27	6.32	183.15	160.15 – 206.19
	Ma 4	20.28	6.35	166.39	148.10 –181.68
Plutella xylostella	Ma 1	13.20	7.37	71.63	59.91 – 83.31
	Ma 2	14.41	6.80	69.74	58.01 – 81.84
	Ma 3	12.58	4.85	103.90	90.15 – 117.79
	Ma 4	13.00	4.41	107.71	94.08 – 122.39
Oryctes rhinoceros	Ma 1	15.58	6.91	294.47	245.47- 343.47
	Ma 2	13.80	5.93	314.92	271.14 – 358.70
	Ma 3	12.01	5.69	458.78	419.71 – 499.83
	Ma 4	10.39	6.04	439.85	412.60 – 470.19

* at 1 x 10^7 conidia ml^{-1} for *H. armigera*, *P. xylostella* and 1 x 10^5 conidia g^{-1} for *O. rhinoceros*

Bio-efficacy of M. anisopliae isolates

Four isolates of *M. anisopliae* were evaluated against three test insects *H. armigera, P. xylostella* and *O. rhinoceros.* The isolate Ma2 exhibited the highest virulence against *H. armigera* and *P. xylostella* by recording LC_{50} values of 1.77 x 10^6 conidia ml^{-1} and 1.98 x 10^6 conidia ml^{-1}, respectively (Table 2). Against *O. rhinoceros,* Ma1 proved to be virulent with the least LC_{50} value of 0.09 x 10^6 followed by Ma2 (0.26 x 10^6), Ma4 (0.38 x 10^6) and Ma3 (0.59 x 10^6 conidia g^{-1} of compost).

Table 3. Dose–mortality response of different insects to *M. anisopliae* isolates

Insects	Isolates	Chi^2	Slope	LC_{50} (x10^6)	Fiducial limit (… x 10^6)
H. armigera	Ma 1	8.31	1.21	2.03	1.69 – 2.37
	Ma 2	12.81	1.24	1.77	1.28 – 2.26
	Ma 3	10.36	2.03	4.86	3.99 –5.73
	Ma 4	7.82	2.01	3.91	3.01 – 4.87
P. xylostella	Ma 1	6.32	0.82	2.32	1.93 – 2.92
	Ma 2	7.11	0.99	1.98	1.61 – 2.43
	Ma 3	5.83	1.32	3.61	2.83 – 4.31
	Ma 4	5.01	1.61	4.22	3.63 – 4.95
O. rhinoceros	Ma 1	6.31	0.80	0.09	0.08 – 0.11
	Ma 2	5.82	0.67	0.26	0.21 – 0.34
	Ma 3	6.92	1.02	0.59	0.48 – 0.72
	Ma 4	7.02	1.09	0.38	0.31 – 0.49

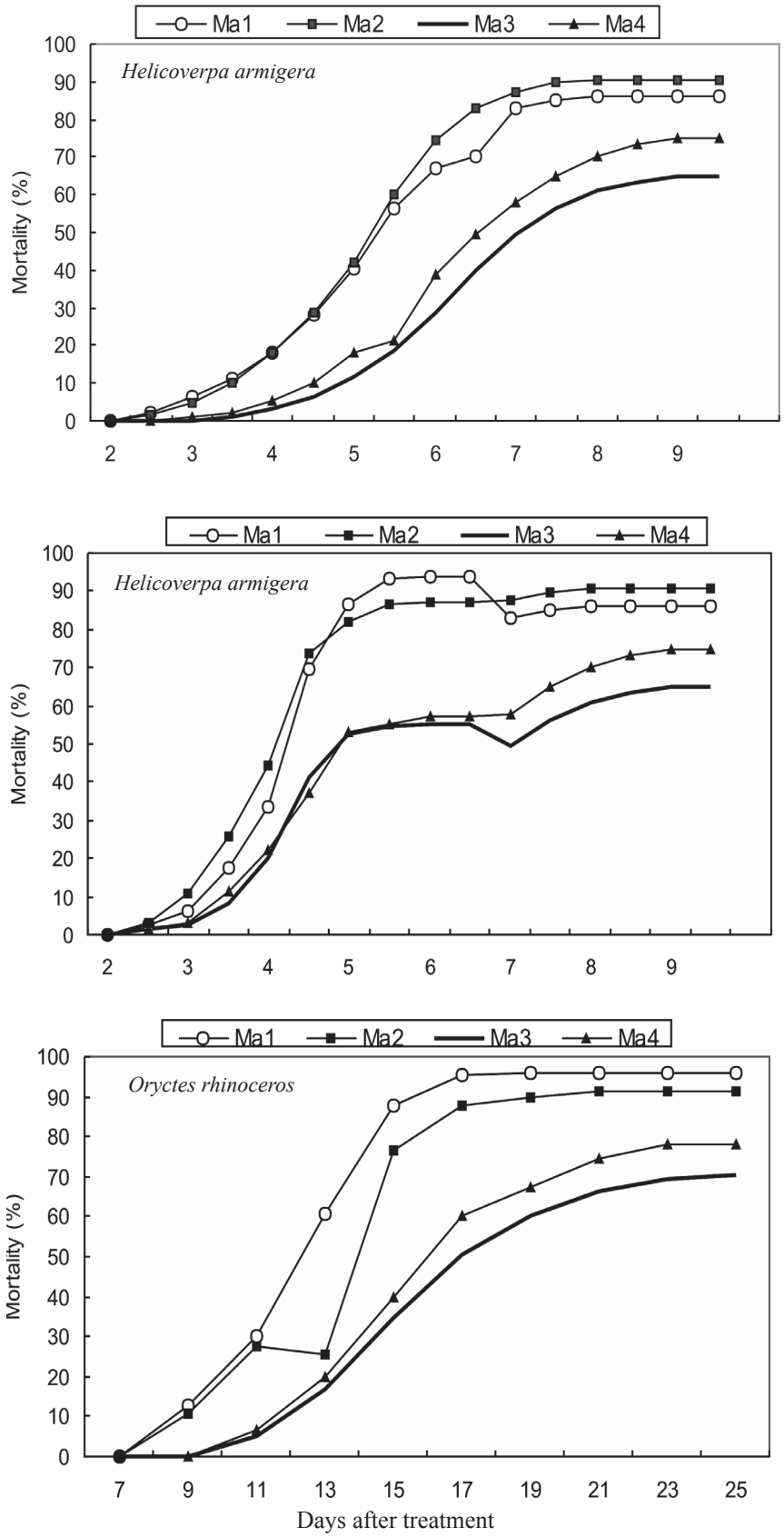

Fig. 1. Cumulative mortality of test insects treated with *M. anisopliae* isolates

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