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Variability in Morphology and Growth Characteristics of Different Isolates of Entomopathogenic Fungi Managing the Mealy Bugs *Maconellicocus hirsutus*

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A B S T R A C T

Current research efforts are directed towards native entomopathogenic fungi which are highly virulent to insect pests to develop efficient and eco-friendly bio-pesticides. From the insect cadavers fifteen different fungal isolates were isolated on DOC2-50% selective media and were identifying as isolates of *Aspergillus tamari, A. niger* and *A. flavus.* All the fifteen isolates showed variation in all the morphological characters studied. Highest mean colony diameter (mm) was reported in isolate EPF-14 at all the time intervals. The lowest mean colony diameter (mm) was reported in isolate EPF-13 at 24, 72 and 96 hr interval while at 48 hrs the lowest mean colony diameter (mm) was reported in isolate EPF-9. The most of the isolates were not produced any colony pigmentation on PDA media. The isolates EPF-12 and EPF-15 were grayish green color, while EPF-1 and EPF-7 observed light grayish green color. The isolate EPF-5 was dark grayish green color while, EPF-13 was yellowish grayish green color. The isolates EPF-9 & EPF-11 were dull whitish green color and isolate EPF-6 & EPF-8 were dark green and bluish green color respectively. The isolates EPF-2 & EPF-14 were black in color while, isolates EPF-4, EPF-3 & EPF-10 were dark black, light black and bluish black in color respectively. Among all the isolates, the isolates EPF-1, EPF-4, EPF-5, EPF-6, EPF-7, EPF-9, EPF-11, EPF-12, EPF-13 & EPF-15 produced the concentric rings while, in isolate EPF-2, EPF-3, EPF-8, EPF-10 & EPF-14 concentric rings were absent. Isolates showed variation in the spore's shape, size and colours. The spore shape was varying from round to globose. While, spore size was varying from to 10.1 x 9.7 = 97.97µ to 4.3 x 4.2 = 18.06 µ and length width ratio varies from 1.06 to 1.00. The colour of spores was varies from brown to yellow except in isolate EPF-1, EPF-11 and EPF-13.

Introduction

The knowledge of entomopathogenic fungi dates back for several centuries (McCoy *et al.*, 1988). Pasteur (1874) was one of the first to suggest that microorganisms could be used to control insect pests. Numerous groups of entomopathogenic fungi were described during the 19th century. One of the earliest successes in biocontrol was the use of *Aschersonia aleyrodes* to control citrus white flies in Florida (Berger, 1921).

Many genera of entomopathogenic fungi are being used in agricultural crop pest management such as Lower fungi *i.e.* Mastigomycotina, Ascomycotina, Basidomycotina and fungi imperfecti which includes several genera like *Aspergillus, Beauveria, Metarhizium, Nomuraea, Paecilomyces, Penicillium, Trichoderma, Verticillium* etc which suppress the diverse group of insect pest such as coleopterans, lepidopterous, sucking pest. Amongst these, several asexual stages of fungi are associated with insect infection. There are approximately 750 species of fungi from 56 genera that infect arthropods. These are ubiquitous and in appropriate hosts are capable of natural recycling (Hajek and Leger, 1994; Alexopoulos *et al.*, 1996).

Recently increased use of conventional chemical pesticides over the years has not only contributed to an increase in food production, but also has resulted in adverse effects on the environment and non-target organisms. In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. Hence, the present investigation was planned and carried out, to study the morphology and growth characteristics of different isolates of entomopathogenic fungi.

Materials and Methods

Survey

The field survey was conducted in Dhule, Nandurbar, Jalgaon, Nasik and Beed districts of Maharashtra (India) during *kharif*, 2014 to collect the insect cadavers from fields and forest areas and nineteen insect cadavers infected with fungus were collected and placed in separate plastic containers of 6 x 4 cm size. Collected insect cadavers were brought to section laboratory for further study.

Isolation of entomopathogenic fungi

The selective media DOC2-50% (Shin *et.al.*, 2010) was prepared for the isolation of pure cultures entomopathogenic fungi. The infected portion of each insect cadaver was cut into small bits and a small portion of infected tissue was transferred aseptically to a culture plate containing DOC2-50% selective media having Bactopeptone 3.0 g, CuCl2 0.1 g, Crystal violet 2.0 mg, Agar 15.0 g distilled water 1000 ml pH with HCl 4. The inoculated culture plates were incubated at 28±2°C in BOD incubator and kept under constant observation for the growth and development of fungus. Three to five days after incubation, the fungus growth was purified by sub-culturing and slants of each purified fungus culture were prepared.

Pathogenicity test

To determine the pathogenicity of isolated fungal isolates over the insect, the mealy bugs (*Maconellicocus hirsutus*) were reared on their natural diet (pumpkin) in Biocontrol Laboratory, Agril. Entomology Section, College of Agriculture, Dhule. Surface sterilization of rearing containers were carried with 10 % formaldehyde to prevent bacterial contamination of the healthy stock.

The spore suspension of 10^{-3} spores/ml of each fungus isolate was prepared by mixing harvested spores with distilled water and 0.2 per cent Tween-80. The spore suspensions of all isolates were applied on adult mealy bug by direct dipping method. The adult mealy bugs were dipped in spore suspension for 30 seconds.

For the pathogenicity test of each fungus isolate 10 adult mealy bugs were used and another set was kept without addition of spores as control. The inoculated mealy bugs were placed on surface sterilized sprouted

potato in Petri plate lined with wet blotting paper and incubated at 28±2°C in BOD incubator. Dead mealy bugs were transferred into humidity chamber to monitor any fungal out-growth as detected on insect cadavers collected during the survey. Then the fungus isolates were reisolated from the inoculated mealy bugs on DOC2-50% selective media.

Identification of entomopathogenic fungi isolates

The purified coded fungus isolates were sent to Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi – 110 012 for identification.

Morphology and growth characteristics of entomopathogenic fungi isolates

Morphology and growth characteristics of entomopathogenic fungi isolates were studied on PDA media. Observations on morphological and growth characteristics of individual isolates of Radial growth, Colony color, Colony diameter, Concentric rings/circles (Zonetion), Colony surface layer, Colony pigmentation, Appearance of growth, Shape of spores, Colour of spores, Size of spores, Length and width ratio of spores were recorded after 7 days incubation at 28±2°C.

Results and Discussion

During the survey, different locations were surveyed and nineteen insect cadavers infected with fungus were collected and brought to section laboratory. Out of nineteen samples inoculated only fifteen samples showed the growth of fungus on DOC2-50% selective media. No any fungus was isolated from samples EPF-16, EPF-17, EPF-18 and EPF-19. Therefore, the fungal isolates EPF-1 to EPF-15 were taken for the further study and were purified by sub-culturing and maintained on Potato Dextrose Agar (PDA) slants.

The variations in colony diameter of all fifteen isolates of entomopathogenic fungi on PDA media at 24, 48 and 72 hrs were found statistically significant. There was significant variation between isolates and time interval. The results are presented in (Table 1; Plate 1; Fig. 1).

At 24 hrs all the fifteen isolates show statistically significant variation in colony diameter on PDA media. While, comparing the highest growth rate, the isolate EPF-14 (22mm) had recorded the highest colony diameter on PDA media and the lowest colony diameter was recorded in EPF-13 (14mm).

At 48 hrs all the fifteen isolates showed statistically significant variation in colony diameter on PDA media. The isolate EPF-14 (38.66mm) had recorded the highest colony diameter on PDA media and the lowest colony diameter was recorded in EPF-9 (26.66 mm).

At 72 hrs all the fifteen isolates showed statistically significant variation in colony diameter on PDA media. The isolate EPF-14 (60.00mm) had recorded the highest colony diameter on PDA media and the lowest colony diameter was recorded in EPF-13 (44.00 mm). At 96 hrs all the fifteen isolates showed statistically significant variation in colony diameter on PDA media. The isolate EPF-14 and EPF-2 (86.33mm) had recorded the highest colony diameter on PDA media and the lowest colony diameter was recorded in EPF-13 (59.00mm). The results presented in Table 2 showed that radial growth was present in all fifteen isolates of entomopathogenic fungi isolates on PDA media. The colony color of each isolate was recorded at 96 hrs on PDA media by visual

observation. The results presented in Table 2 showed that all the fifteen isolates showed variation in colony color on PDA media. All the fifteen isolates were visually differentiated in three main color categories *viz.*, grayish green, green and black. The concentric rings of each isolate were recorded at 96 hrs on PDA media. The results presented in Table 2 showed that all the fifteen isolates showed variation in concentric rings on PDA media. Colony pigmentation of seven days old cultures grown on PDA media was recorded. The result was presented in Table 2 showed that in most of the isolates pigmentation was absent. Appearance of growth of all the isolates of entomopathogenic fungi was recorded at 96 hrs on PDA media. Results were presented in Table 2 showed the variation in appearance of growth on PDA media.

After incubation up to seven days, the shapes of ten spores per isolate were recorded under microscope. The results are presented in (Table 3) showed that the shape of spores varies from round to globose. After incubation up to seven days, the colours of ten spores were recorded by visual observations. The result is presented in Table 3 showed that the colours of spores varies from brown to yellow except in isolate EPF-1, EPF-11 and EPF-13. The data presented in Table 3 showed variation in size of spores among all the fifteen isolates on PDA media. The isolate EPF-15 produced the biggest size spores (10.1 x 9.7µ) followed by EPF-1 (9.1 x 9.1µ) while smallest size spores were produced by the isolate EPF-8 fallowed by EPF-10 and EPF-9. On the basis of data presented in Table 3, the spores were grouped in three categories *viz.*, small size spores (≤33µ), medium size spores (>33 to ≤66µ) and large size spores (>66µ). The data presented in Table 3 showed the variation in length/width ratio of spores among all the fifteen isolates.

Table.1 Variability in colony diameter of entomopathogenic fungi isolates

Sr. No.	Isolates	Colony diameter (mm) at different time intervals			
		24 hr. (Mean)	48 hr. (Mean)	72 hr. (Mean)	96 hr. (Mean)
1	EPF-1	17.33	33.33	45.00	65.66
2	EPF-2	16.00	37.00	56.33	86.33
3	EPF-3	19.33	33.33	57.33	81.66
4	EPF-4	15.00	32.83	55.00	80.16
5	EPF-5	18.33	34.33	47.33	64.66
6	EPF-6	16.67	33.66	45.33	60.50
7	EPF-7	18.33	36.66	47.00	63.50
8	EPF-8	19.00	35.66	58.66	73.00
9	EPF-9	15.67	26.66	47.00	63.83
10	EPF-10	15.33	30.66	52.00	69.83
11	EPF-11	17.33	35.66	45.00	60.66
12	EPF-12	17.00	30.66	47.66	67.33
13	EPF-13	14.00	30.00	44.00	59.00
14	EPF-14	22.00	38.66	60.00	86.83
15	EPF-15	19.00	33.00	45.00	61.83
	SE±	**0.49**	**0.94**	**1.41**	**0.63**
	CD @5%	**1.42**	**2.71**	**4.06**	**1.81**

Table.2 Variability in colony characteristics of entomopathogenic fungi isolates

Isolates	Colony characteristics					
	Radial growth	**Colony color**	**Concentric rings**	**Colony surface layer**	**Colony pigmentation**	**Appearance of growth**
EPF-1	Present	Light grayish green	Present	Mass like mat	Absent	BLMG
EPF-2	Present	Black	Absent	Flat but mass like mat	Light yellow	CTkMG
EPF-3	Present	Light black	Absent	Flat but mass like mat	Absent	CTkMG
EPF-4	Present	Dark black	Present	Flat but mass like mat	Absent	CTkMG
EPF-5	Present	Dark grayish green	Present	Mass like mat	Absent	BLMG
EPF-6	Present	Dark green	Present	Mass like mat	Light golden yellow	BLMG
EPF-7	Present	Light grayish green	Present	Mass like mat	Absent	BLMG
EPF-8	Present	Bluish green	Absent	Completely Flat	Light yellow	CTnMG
EPF-9	Present	Dull whitish green	Present	Cottony fussy	Absent	TM
EPF-10	Present	Bluish black	Absent	Flat but mass like mat	Absent	CTkMG
EPF-11	Present	Dull whitish green	Present	Cottony fussy	Light golden yellow	TM
EPF-12	Present	Grayish green	Present	Mass like mat	Absent	BLMG
EPF-13	Present	Yellowish grayish green	Present	Mass like mat	Absent	BLMG
EPF-14	Present	Black	Absent	Flat but mass like mat	Yellow light	CTkMG
EPF-15	Present	Grayish green	Present	Mass like mat	Absent	BLMG

CTkMG: Clear thick mycelial growth TM: Tuft of mycelium
CTnM : Clear thin mycelial growth BLMG : Bread like mycelial growth

Table.3 Variability in conidia characteristics of entomopathogenic fungi isolates

Sr. No	Isolates	Conidia characteristics			
		Shape	Color	Size (µ) (Mean)	L/W ratio
1	EPF-1	Round	Light grayish yellow	9.1 x 9.1 = 82.81	1.00
2	EPF-2	Round	Dark brown	5.2 x 5.2 = 27.04	1.00
3	EPF-3	Globose	Dark brown	5.0 x 4.9 = 24.50	1.02
4	EPF-4	Globose	Dark Brown	4.6 x 4.3 = 19.78	1.06
5	EPF-5	Globose	Light yellow	9.0 x 8.8 = 79.20	1.02
6	EPF-6	Globose	Light yellow	6.4 x 6.1 = 39.04	1.05
7	EPF-7	Globose	Light brown	5.7 x 5.6 = 31.92	1.01
8	EPF-8	Globose	Yellowish	4.3 x 4.2 = 18.06	1.02
9	EPF-9	Round	Dark brown	4.4 x 4.4 = 19.36	1.00
10	EPF-10	Globose	Dark brown	4.4 x 4.3 = 18.92	1.02
11	EPF-11	Round	Light green	5.0 x 5.0 = 25.00	1.00
12	EPF-12	Round	Light brown	8.1 x 8.1 = 65.61	1.00
13	EPF-13	Round	Light yellow green	5.4 x 5.4 = 29.16	1.00
14	EPF-14	Round	Dark brown	6.0 x 6.0 = 36.00	1.00
15	EPF-15	Globose	Light yellow	10.1 x 9.7 = 97.97	1.04

L / W ratio = Length to Width ratio

Fig.1 Variability in colony diameter of entomopathogenic fungi isolates

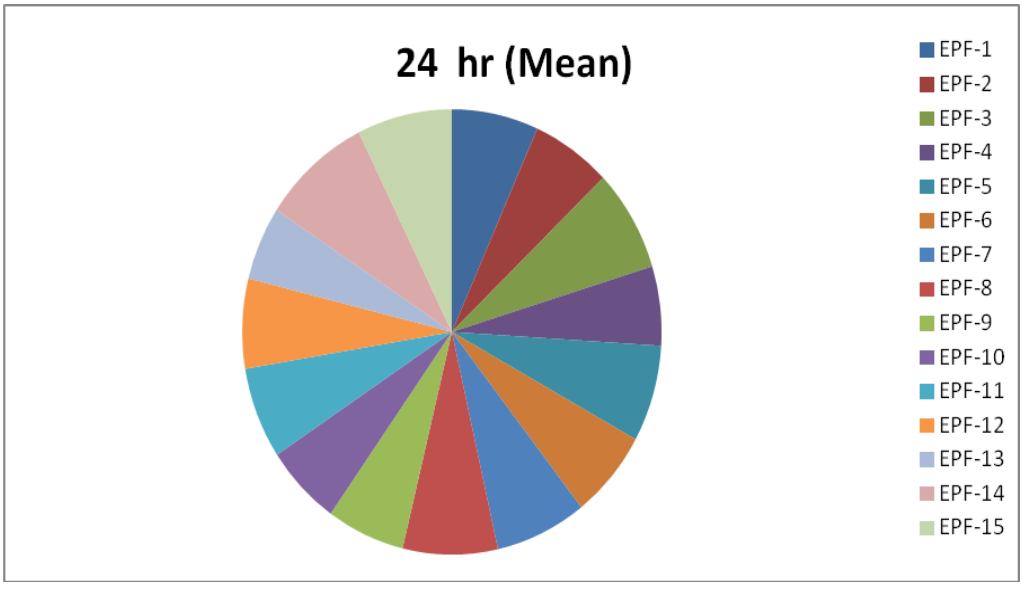
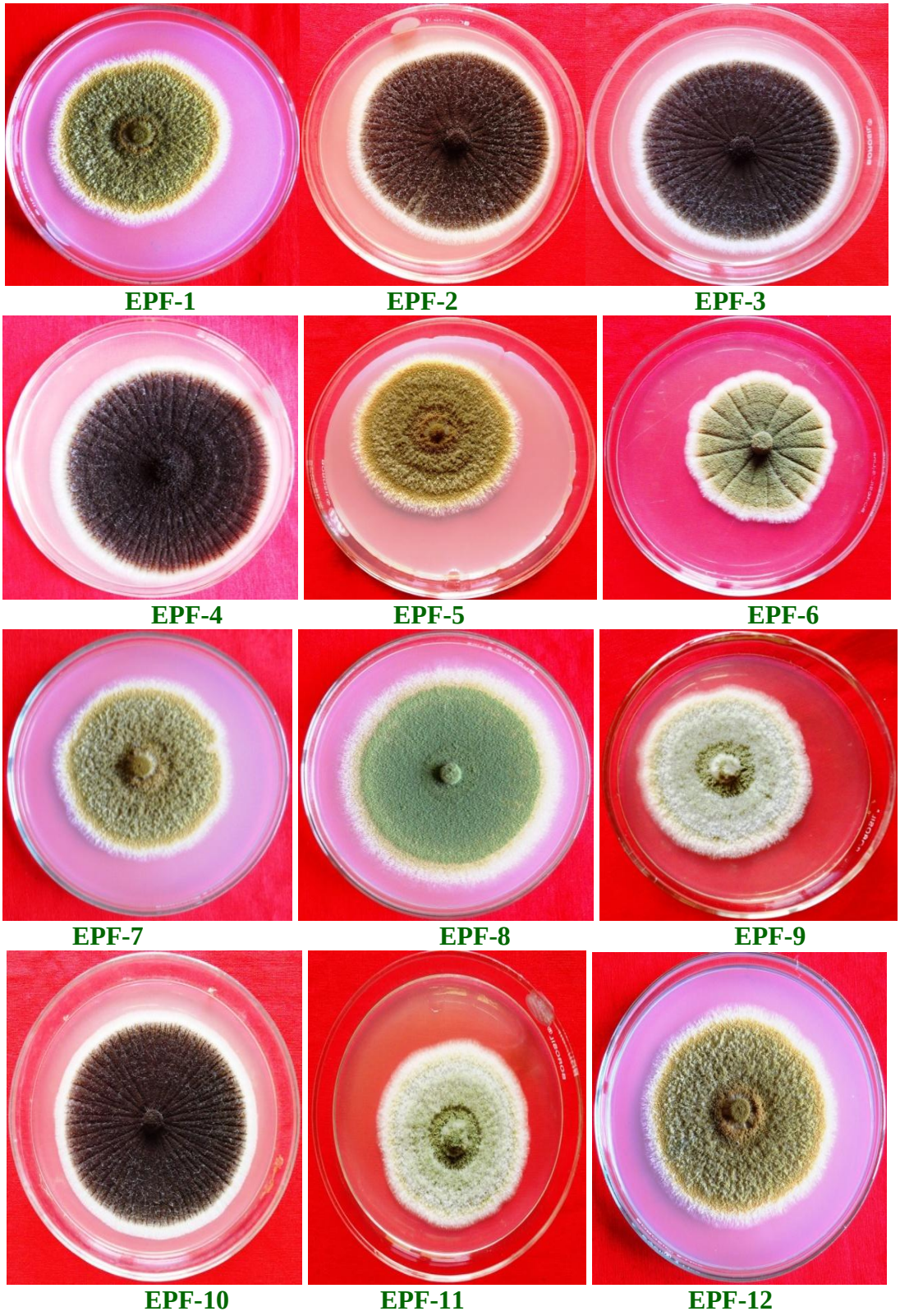


Plate.1 Variability in colony characteristics of entomopathogenic fungi isolates



EPF-13 **EPF-14** **EPF-15**

The highest length/width ratio of spores were observed in isolate EPF-4 (1.06). In addition to the fifteen isolates of entomopathogenic fungi were tested for their virulence against mealy bugs (*Maconellicoccus hirsutus*) *in vitro* conditions at 10^3, 10^6 and 10^9 spore concentrate.

Studied entomopathogenic fungi isolates were evaluated at different spore concentration against mealy bugs and insect mortality was observed at 24 hr interval after inoculation up to 10 days on red pumpkin in laboratory at room temperature.

The percent mortality was calculated by using following formula.

$$\text{Percent mortality} = \frac{\text{Total no. of dead mealy bug}}{\text{Total no. of inoculated mealy bug}} \times 100$$

Similar results with respect to variation in colony diameter and growth rate are reported by many workers. Nyongesa *et al.,* (2015) and Odhiambo *et al.,* (2013) observed the colonies of *A. niger* on MEA were date brown with white While, the colonies of *A. flavus* on MEA were yellow green with white mycelia at the edges; formed sporulation rings; did not produce exudates and soluble pigments; *A. flavus* strains had similar surface colour of olive green with whitish margins and reverse colour of creamish to yellow on PDA.

The spore shape was varying from round to globose. While, spore size was varying from to 10.1 x 9.7 = 97.97µ to 4.3 x 4.2 = 18.06 µ and length width ratio varies from 1.06 to 1.00. The colour of spores was varies from brown to yellow except in isolate EPF-1, EPF-11 and EPF-13. The spores of these isolates were light grayish yellow, light green and light-yellow green in colour respectively. The spores of isolate EPF-5, EPF-6 and EPF-15 were light yellow in colour while, spores of isolate EPF-8 were yellowish in colour. Ulhan *et al.,* (2006) observed that conidia of *Aspergillus spp.* were 2.5-3.5 µm in diameter, globose to sub-globose, with wall smooth to slightly rough. While, Abdei *et al.,* (2012) recorded conidia diameter of 3.2 µm in *A. tamarii*.

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