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Effect of Different Spore Concentration of Entomopathogenic Fungi Isolates against Mealy Bug *Maconellicocus hirsutus*

S.B. Sable[1*], P.B. Deore[1], V.R. Bangar[2] and K.C. Kurhade[2]

[1]College of Agriculture, Dhule, Plant Pathology Section, MPKV, Rahuri, Maharashtra, India
[2]Department of Plant Pathology, PGI, MPKV, Rahuri, Maharashtra, India

**Corresponding author*

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A B S T R A C T

Entomopathogenic fungi suppress the diverse group of insect pest such as coleopterans, lepidopterous and sucking pest. Wide spread incidence of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus tamarii* was observed during extensive and repeated survey covering different agro ecological niches of Dhule, Nandurbar, Jalgaon, Nasik and Beed districts of Maharashtra (India) on insect cadavers infected with fungi. From the insect cadavers the fungal isolates were isolated on PDA media. Out of nineteen samples collected only fifteen samples showed the growth of fungus on DOC 2-50% selective media. Virulence of entomopathogenic fungi isolates against mealy bugs (*Maconellicoccus hirsutus*) revealed that as the spore concentration increased from 10^{-3} to 10^{-9} the insect mortality was decreased. Among all the fifteen isolates the highest insect mortality was recorded at 10^{-3} spores/ml concentration and the spore dilution of entomopathogenic fungi isolates increased from 10^{-3} to 10^{-9} spores/ml the time required for insect mortality was also increased. Among the isolates of entomopathogenic fungi the isolate EPF-14 was the most virulent. Among each isolate, it was observed that as the spore dilution increased the insect mortality decreased. *Aspergillus tamarii*, *A. niger* and *A. flavus* the isolate EPF-7, EPF-14 and EPF-6 were the most virulent, respectively against mealy bugs at 10^{-3} spores/ml concentration and isolates EPF-1, EPF-2 and EPF-6 were the most virulent isolates respectively against mealy bugs at 10^{-6} and 10^{-9} spores/ml concentration.

Introduction

Many genera of entomopathogenic fungi are being used in agricultural crop for 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. 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).

Microbial control of crop pest offers an environmentally acceptable strategy with lower cost and effect in longer run. Entomopathogenic fungi played a significant role in the history of insect pathology and especially in microbial control (Steinhous, 1956). 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 encompassing to study the virulence of different isolates of entomopathogenic fungi against mealy bugs *Maconellicoccus hirsutus.*

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. During the survey, different locations were surveyed and nineteen insect cadavers infected with fungus were collected and placed in separate plastic containers of 6 x 4 cm size.

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. 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.

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.

Preparation of spore inoculum and inoculation of insects

For the preparation of spore inoculum, the fifteen days mature old culture grown on PDA media were used. The spores of each fungal isolate were harvested from PDA plates and the spore suspensions of 10^{-3}, 10^{-6} and 10^{-9} spores/ml concentration of each fungus isolate were prepared by mixing harvested spores with distilled water and 0.2 per cent Tween-80. The spore concentration was determined by using a Neubauer hemocytometer. The spore suspensions of all isolates were applied by direct dipping method on twenty-five adult mealy bugs per treatment (isolate) and experiment was replicated twice.

The adult mealy bugs were dipped in spore suspension for 30 seconds. The inoculated mealy bugs were placed on surface sterilized sprouted potato and red pumpkin in laboratory at room temperature. Thereafter, inoculated mealy bugs were checked daily and mortality was recorded.

Virulence of entomopathogenic fungi

For the virulence study the 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. The percent mortality was calculated by using following formula.

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

Results and Discussion

During the survey, different locations were surveyed and nineteen insect cadavers infected with fungus were collected and brought to section laboratory. After fairly drying the collected insect cadaver samples were stored in refrigerator and coded for further study. Out of nineteen samples inoculated only fifteen samples showed the growth of fungus on DOC2-50% selective media. As the identification report of Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi the coded entomopathogenic fungal isolates were decoded as follows (Table 1).

The virulence studies of entomopathogenic fungi isolates against mealy bugs (*Maconellicoccus hirsutus*) revealed that as the spore concentration increases from 10^{-3} to 10^{-6} & 10^{-9} the insect mortality decreases. Among all the fifteen isolates the highest insect mortality was observed at 10^{-3} spores/ml concentration. Also, it was observed that as the spore dilution of entomopathogenic fungi isolates increases from 10^{-3} to 10^{-9} spores/ ml the time required for insect mortality increases (Fig. 1).

Table.1 Identification of entomopathogenic fungi isolates

Sr. No.	Isolate Code	Fungus identified	Remark
1	EPF-1	*Aspergillus tamarii*	EPF*
2	EPF-2	*Aspergillus niger*	EPF
3	EPF-3	*Aspergillus niger*	EPF
4	EPF-4	*Aspergillus niger*	EPF
5	EPF-5	*Aspergillus tamarii*	EPF
6	EPF-6	*Aspergillus flavus*	EPF
7	EPF-7	*Aspergillus tamarii*	EPF
8	EPF-8	*Aspergillus flavus*	EPF
9	EPF-9	*Aspergillus flavus*	EPF
10	EPF-10	*Aspergillus niger*	EPF
11	EPF-11	*Aspergillus flavus*	EPF
12	EPF-12	*Aspergillus tamarii*	EPF
13	EPF-13	*Aspergillus flavus*	EPF
14	EPF-14	*Aspergillus niger*	EPF
15	EPF-15	*Aspergillus tamarii*	EPF

*EPF=Entomopathogenic fungi

Table.2 The virulence of entomopathogenic fungi isolates at 10^{-3} spore concentration

Isolate	Per cent mortality at 10^3 spore concentration									
	1DAT	2DAT	3DAT	4DAT	5DAT	6DAT	7DAT	8DAT	9DAT	10DAT
EPF-1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	32.00 (34.45)	32.00 (34.45)	42.00 (40.40)	42.00 (40.40)	62.00 (51.94)	62.00 (51.94)	70.00 (56.80)
EPF-2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.43)	24.00 (29.33)	34.00 (35.67)	38.00 (38.06)	44.00 (41.55)	70.00 (56.79)	70.00 (56.79)
EPF-3	0.00 (0.00)	0.00 (0.00)	16.00 (23.58)	16.00 (23.58)	16.00 (23.58)	40.00 (39.23)	64.00 (53.13)	64.00 (53.13)	76.00 (60.67)	80.00 (63.44)
EPF-4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	20.00 (26.56)	20.00 (26.56)	20.00 (26.56)	34.00 (35.67)	40.00 (39.23)	40.00 (39.23)	72.00 (58.05)
EPF-5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.00 (29.33)	24.00 (29.33)	50.00 (45.00)	50.00 (45.00)	50.00 (45.00)	82.00 (64.90)	82.00 (64.90)
EPF-6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	12.00 (20.27)	12.00 (20.27)	30.00 (33.21)	30.00 (33.21)	50.00 (45.00)	50.00 (45.00)	58.00 (49.60)
EPF-7	0.00 (0.00)	0.00 (0.00)	16.00 (23.58)	16.00 (23.58)	36.00 (36.87)	36.00 (36.87)	36.00 (36.87)	64.00 (53.13)	64.00 (53.13)	74.00 (59.34)
EPF-8	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	20.00 (26.56)	26.00 (30.66)	54.00 (47.29)	54.00 (47.29)	54.00 (47.29)	80.00 (63.44)
EPF-9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.00 (29.33)	26.00 (30.66)	56.00 (48.45)	56.00 (48.45)	70.00 (56.79)	88.00 (69.73)
EPF-10	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.00 (29.33)	24.00 (29.33)	54.00 (47.29)	54.00 (47.29)	86.00 (68.03)	90.00 (71.56)
EPF-11	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.00 (23.58)	16.00 (23.56)	48.00 (43.85)	48.00 (43.85)	68.00 (55.55)	68.00 (55.55)	86.00 (68.03)
EPF-12	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	22.00 (27.97)	40.00 (39.23)	40.00 (39.23)	68.00 (55.55)	78.00 (62.03)	78.00 (62.03)
EPF-13	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	22.00 (27.97)	28.00 (31.95)	28.00 (31.95)	42.00 (40.40)	66.00 (54.33)
EPF-14	0.00 (0.00)	0.00 (0.00)	20.00 (26.56)	20.00 (26.56)	50.00 (45.00)	50.00 (45.00)	72.00 (58.05)	80.00 (63.44)	82.00 (64.90)	100.00 (90.00)
EPF-15	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.00 (29.33)	24.00 (29.33)	58.00 (49.60)	72.00 (58.05)	76.00 (60.67)	84.00 (66.42)
SE	0.00	0.00	0.81	0.81	0.93	1.24	1.45	1.64	1.66	2.12
CD@5%	0.00	0.00	2.44	2.45	2.83	3.76	4.37	4.96	5.02	6.40

Figures in parenthesis are arc sin values DAT= Day After Treatment

Table.3 The virulence of entomopathogenic fungi isolates at 10^6 spore concentration

Isolate	Per cent mortality at 10^6 spore concentration									
	1DAT	2DAT	3DAT	4DAT	5DAT	6DAT	7DAT	8DAT	9DAT	10DAT
EPF-1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	14.00 (21.97)	14.00 (21.97)	38.00 (38.05)	38.00 (38.05)	44.00 (41.55)
EPF-2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.00 (14.18)	10.00 (18.44)	20.00 (26.56)	34.00 (35.67)	38.00 (38.05)	50.00 (45.00)	50.00 (45.00)
EPF-3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.00 (23.58)	16.00 (23.58)	40.00 (39.23)	40.00 (39.23)	46.00 (42.71)	46.00 (42.71)	50.00 (45.00)
EPF-4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	12.00 (20.27)	12.00 (20.27)	20.00 (26.56)	34.00 (35.67)	40.00 (39.23)	40.00 (39.23)
EPF-5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	14.00 (21.97)	32.00 (34.45)	32.00 (34.45)	32.00 (34.45)	46.00 (42.71)
EPF-6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.00 (14.18)	10.00 (18.44)	18.00 (25.10)	24.00 (29.33)	30.00 (33.21)	46.00 (42.71)	46.00 (42.71)
EPF-7	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.43)	10.00 (18.44)	16.00 (23.58)	26.00 (30.66)	50.00 (45.00)	54.00 (47.29)	54.00 (47.29)
EPF-8	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	20.00 (26.56)	26.00 (30.66)	32.00 (34.45)	50.00 (45.00)	66.00 (54.33)
EPF-9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	24.00 (29.33)	26.00 (30.66)	38.00 (38.05)	44.00 (41.55)	46.00 (42.71)
EPF-10	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	16.00 (23.58)	22.00 (27.97)	32.00 (34.45)	48.00 (43.85)	66.00 (54.33)
EPF-11	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	14.00 (21.97)	46.00 (42.71)	46.00 (42.71)	56.00 (48.45)	66.00 (54.33)
EPF-12	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	20.00 (26.56)	40.00 (39.23)	40.00 (39.23)	56.00 (48.45)	68.00 (55.55)	68.00 (55.55)
EPF-13	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	12.00 (20.27)	12.00 (20.27)	22.00 (27.97)	36.00 (36.87)	54.00 (47.29)
EPF-14	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	40.00 (39.23)	54.00 (47.29)	72.00 (58.05)	82.00 (64.90)	88.00 (69.73)
EPF-15	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	24.00 (29.33)	50.00 (45.00)	60.00 (50.77)	72.00 (58.05)	80.00 (63.44)
SE	0.00	0.00	0.00	3.92	1.45	0.91	1.13	1.25	1.21	1.64
CD@5%	0.00	0.00	0.00	11.82	4.38	2.75	3.42	3.79	3.65	4.96

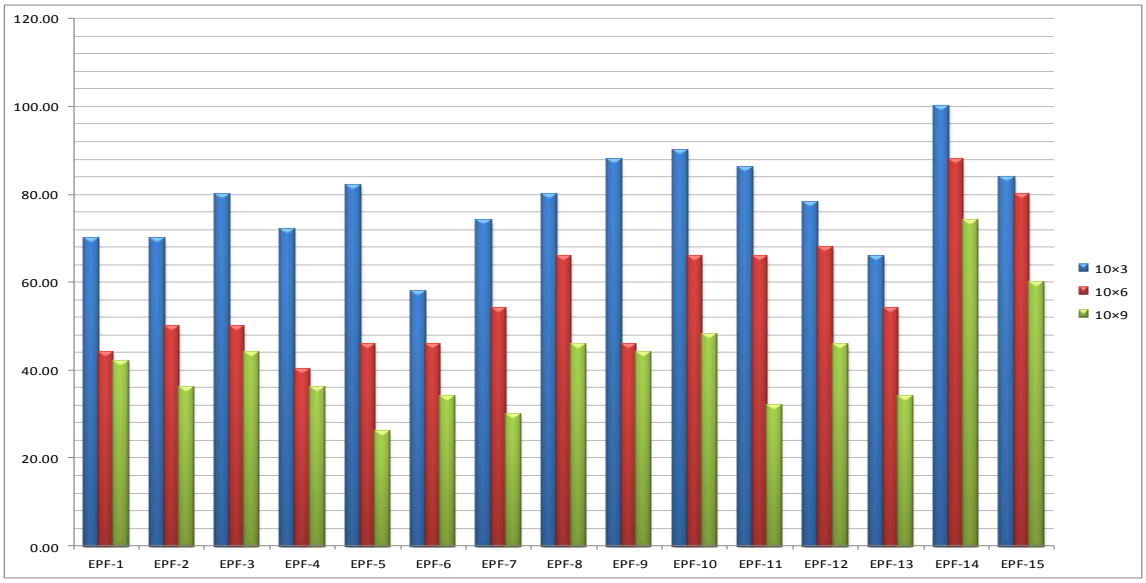
Figures in parenthesis are arc sin values DAT= Day After Treatment

Table.4 The virulence of entomopathogenic fungi isolates at 10^9 spore concentration

Isolates	Per cent mortality at 10^9 spore concentration									
	1DAT	2DAT	3DAT	4DAT	5DAT	6DAT	7DAT	8DAT	9DAT	10DAT
EPF-1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	28.00 (31.95)	28.00 (31.95)	42.00 (40.40)
EPF-2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.00 (14.18)	18.00 (25.10)	26.00 (30.66)	28.00 (31.95)	28.00 (31.95)	36.00 (36.87)
EPF-3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	14.00 (21.97)	32.00 (34.45)	32.00 (34.45)	44.00 (41.55)	44.00 (41.55)
EPF-4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	12.00 (20.27)	12.00 (20.27)	28.00 (31.95)	28.00 (31.95)	36.00 (36.87)
EPF-5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	4.00 (11.54)	4.00 (11.54)	10.00 (18.44)	10.00 (18.44)	20.00 (26.56)	20.00 (26.56)	26.00 (30.66)
EPF-6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.00 (14.18)	10.00 (18.44)	10.00 (18.44)	24.00 (29.33)	24.00 (29.33)	34.00 (35.67)
EPF-7	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.43)	10.00 (18.44)	26.00 (30.66)	26.00 (30.66)	26.00 (30.66)	30.00 (33.21)
EPF-8	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.00 (23.58)	22.00 (27.97)	32.00 (34.45)	38.00 (38.06)	46.00 (42.71)
EPF-9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	10.00 (18.44)	20.00 (26.56)	20.00 (26.56)	38.00 (38.06)	44.00 (41.55)
EPF-10	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.43)	14.00 (21.97)	14.00 (21.97)	28.00 (31.95)	48.00 (43.85)
EPF-11	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.43)	8.00 (16.43)	8.00 (16.43)	16.00 (23.58)	32.00 (34.45)
EPF-12	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.00 (21.97)	14.00 (21.97)	22.00 (27.97)	34.00 (35.67)	34.00 (35.67)	42.00 (40.40)	46.00 (42.71)
EPF-13	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.43)	8.00 (16.43)	28.00 (31.95)	34.00 (35.67)
EPF-14	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.00 (16.43)	10.00 (18.44)	36.00 (36.87)	36.00 (36.87)	36.00 (36.87)	68.00 (55.55)	74.00 (59.34)
EPF-15	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.00 (14.18)	6.00 (14.18)	30.00 (33.21)	30.00 (33.21)	60.00 (50.77)	60.00 (50.77)
SE	0.00	0.00	0.00	0.42	0.88	1.22	1.18	0.76	0.62	1.23
CD @5%	0.00	0.00	0.00	1.28	2.67	3.76	3.58	2.12	1.88	3.72

Figures in parenthesis are arc sin values DAT= Day After Treatment

Fig. No. 1 The virulence of entomopathogenic fungi isolates



Among each isolate at 10^{-3} spores/ml concentration the insect mortality was noted earlier compare to 10^{-6} and 10^{-9} spores/ml concentration, while at 10^{-6} spores/ml concentration the insect mortality was noted earlier compare to 10^{-9} spores/ml concentration. At all the three-spore concentration the isolate EPF-14 had recorded the highest mealy bug's mortality compare to rest of isolates. Form the present research it was pointed out that at $_{10}^{-3}$ spores/ml concentration, the isolate EPF-14 recorded the highest mealy bugs mortality followed by EPF-3 and EPF-7 at 3 DAI means EPF-14 was the most aggressive isolate compare to the rest of the isolates (Table 2).

At 10 DAI, the isolate EPF-14 had recorded the highest mealy bugs mortality i.e. 100%, 88% and 74% at 10^{-3}, 10^{-6} and 10^{-9} spores/ml concentration respectively means the isolate EPF-14 was the most virulent isolate compare to the rest of the isolates. While, at 10^{-3} spores/ml concentration EPF-10 was next most virulent isolate compare to the rest of the isolates and at 10^{-6} and 10^{-9} spores/ml concentration EPF-15 was next most virulent isolate compare to the rest of the isolates (Table 3).

Among the isolates of *Aspergillus tamarii*, the isolate EPF-7 was the most virulent against mealy bugs at 10^{-3} spores/ml concentration and at 10^{-6} and 10^{-9} spores/ml concentration the isolate EPF-1 was the most virulent. Among the isolates of *Aspergillus niger*, the isolate EPF-14 was the most virulent isolate against mealy bugs at 10^{-3} spores/ml concentration and at 10^{-6} and 10^{-9} spores/ml concentration the isolate EPF-2 was the most virulent isolate while, among the isolates of *Aspergillus flavus*, the isolate EPF-6 was the most virulent isolate at 10^{-3}, 10^{-6} and 10^{-9} spores/ml concentration.

Fungal isolates were isolated from the insect cadavers on PDA media and most of the isolates were resemble with *Aspergillus* spp. The selective media DOC2-50% was used for the isolation of entomopathogenic fungi (Shin *et al.*, 2010). Out of nineteen samples inoculated only fifteen samples showed the growth of fungus on DOC2-50% selective media.

The virulence studies of entomopathogenic fungi isolates revealed that, as the spore concentration increases from 10^{-3} to 10^{-9} the insect mortality decreases. Among all the fifteen isolates the highest insect mortality was observed at 10^{-3} spores/ml concentration. At all the three-spore concentration the isolate EPF-14 had recorded the highest mealy bug's mortality followed by EPF-3 and EPF-7 at 3 DAI means EPF-14 was the most aggressive isolate compare to the rest of the isolates. Among the isolates of *Aspergillus tamarii*, the isolate EPF-7 was the most virulent against mealy bugs at 10^{-3} spores/ml concentration and at 10^{-6} and 10^{-9} spores/ml concentration the isolate EPF-1 was the most virulent. Similar task is carried out by Fawrou *et al.*, (2014) in laboratory bioassay with five different concentrations of *Aspergillus clavatus* (Desmazières), *Aspergillus flavus* (Link) and *Metarhizium anisopliae* ((Metschnikoff) Sorokin) spores against the pea aphid, *Acyrthosiphon pisum* (Harris). *Aspergillus* isolates induced higher mortalities than *M. anisopliae*. Lethal concentrations (LC50 and LC90) were 1.23 × 103 and 1.34 × 107 spores/ml for *A. flavus*, 4.95 × 102 and 5.65 × 107 spores/ml for *A. clavatus*, and 3.67 × 103 and 9.71 × 107 spores/ml for *M. anisopliae* after 5 days of treatment. Mycelia development and sporulation on adult cadavers were observed at 48 hr after incubation.

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