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INFLUENCE OF OIL CAKES IN COMBINATION WITH INORGANIC FERTILIZERS ON GROWTH AND SPORULATION OF *PAECILOMYCES LILACINUS* AND ITS ANTAGONISM ON *MELOIDOGYNE INCOGNITA* INFECTING TOMATO

by
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Summary. The supplementation of castor and neem oil cakes with nitrogen, phosphorus and potassium in the form of inorganic fertilizers, had an additive effect on the mycelial growth and sporulation of *Paecilomyces lilacinus*, and enhanced its mycelial mat weight, duration of mycelial growth and sporulation in *in vitro* conditions. Of the two oil cakes, neem was the better substrate for *P. lilacinus* in terms of amount and duration of mycelial growth and sporulation. Further, application of inorganic fertilizers along with oil cakes was beneficial to the endozoic antagonistic fungus (*P. lilacinus*), plant host (tomato) and also enhanced the antagonistic potential of *P. lilacinus* against root-knot nematode (*Meloidogyne incognita*) under nursery conditions.

Application of inorganic fertilizers and oil cakes is a normal practice in horticultrural crop production. But their combined effect on the behaviour of the nematode-antagonistic fungus, *Paecilomyces lilacinus* and in turn, its effect on the nematode and plant hosts has not been studied. This information is essential to establish the feasibility of integrating the bioagents with the normal agronomic practice of applying inorganic fertilizers and oil cakes in the field. Therefore, *in vitro* studies were conducted to evaluate the role of organic amendments (oil cakes) in combination with inorganic fertilizers on the growth and sporulation of the antagonistic fungus, *P. lilacinus* (Thom.) Samson, with a nursery trial to evaluate the epizootic effect of *P. lilacinus* against *Meloidogyne incognita* infecting tomato.

Materials and methods

One gram each of finely powdered and sieved oil cakes of castor (*Ricinus communis* L.), and neem (*Azadirachta indica* A. Juss.) were placed in 50 ml conical flasks containing 10 ml distilled water to make 10% oil cake suspensions. The following fertilizers were added to the suspensions (w/v of 10% oil cake suspensions), alone or in combination: nitrogen (N), 1% (in the form of ammonium nitrate); phosphorus (P), 0.5% (single super phosphate); potassium (K), 0.5% (murate of potash); N (1%) + P (0.5%); N (1%) + K (0.5%); P (0.5%) + K (0.5%) and N + P + K (1:0.5:0.5). All the flasks were then autoclaved. One loopful of *P. lilacinus* pure culture (IIHR isolate) was inoculated

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in each conical flask under aseptic conditions; and the flasks were incubated at 27 ± 1 °C. The growth of *P. lilacinus* in each flask (i.e. area of mycelial mat cover/area of suspension in the flask) was recorded at weekly intervals for seven weeks after inoculation and rated on a 0-100 scale. The mycelial mat from each flask was collected and weighed. Another set of *P. lilacinus* inoculated flasks with the same treatments were incubated for eight weeks and the spore count was recorded in each treatment with a haemocytometer after serial dilution. Each treatment was replicated five times.

Also studies were carried out under nursery conditions to ascertain the effect of these fertilizers and oil cake (castor and neem) combinations with *P. lilacinus* on root-knot nematode infection of tomato, *Lycopersicon esculentum* Mill., and the parasitisation of nematode egg masses by *P. lilacinus*.

Paecilomyces lilacinus grown on castor (CC) and neem (NC) oil cakes; CC + N; CC + P; CC + K; CC + NP; CC + NK; CC + PK and CC + NPK; NC + N; NC + P; NC + K; NC + NP; NC + NK; NC + PK and NC + NPK suspensions were incorporated in to 50x50 cm^2 nursery beds infested with the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., (at least 2 J_2 /g soil). Each suspension was of 50 ml volume and the application rate per nursery bed was 50 ml. Four g of tomato seed (cv. *Pusa Ruby*) were sown in each of the treated nursery beds. An untreated control and treatment with carbofuran 3 G (10 g/bed) were maintained as checks. Each treatment was replicated three times. Observations on plant height, root length, root gall index, nematode multiplication rate (final nematode population on root and soil/initial population in soil) and CFU (colony forming units) in soil and root were recorded at 45 days after emergence of seedlings. Tomato roots were carefully uprooted from the treated nursery beds and 100 egg masses were collected from each treated nursery bed to ascertain the percentage parasitism by *P. lilacinus*. Egg masses were washed in sterile distilled water, surface sterilized with 0.01% NaOCl and placed in Petri plates containing semi-selective medium for *P. lilacinus* (Mitchell *et al.*, 1987). Similarly, 1 g of surface sterilized root and 10 g of soil samples (through serial dilutions) from treated nursery beds were collected and placed on selective media to arrive at CFU/treatment.

All the data were subjected to statistical analysis of variance.

Results and discussion

The mycelial growth of *P. lilacinus* by the end of first week of inoculation was greater on neem cake suspension (40%) and its suspensions supplemented with NPK combinations (i.e., ≥40%), while the growth on castor cake suspension or its combinations with NPK was significantly less (Table I). The mycelial growth of *P. lilacinus* on castor cake suspension reached a maximum of 90% at the end of the fourth week and it then decreased to 20% by the sixth week. The mycelial growth was maximum after four weeks on neem cake suspension (100%) and declined from the fifth week to 40%. These observations indicate that of the two oil cakes, neem served as a better substrate for *P. lilacinus* mycelial growth. The declines in mycelial growth (expressed in terms of area covered in the conical flask) follow the normal growth curve pattern of log, exponential and lag phase.

Supplementing castor cake suspensions with NPK combinations not only increased the amount of mycelial growth (100%), but also enhanced the duration of mycelial growth of *P. lilacinus* by two weeks (Table I). Supplementing neem cake suspensions with NPK combinations had a similar effect on the growth of *P. lilacinus* which was more significant.

The greatest mycelial mat weight and numbers of chlamydospores/ml were recorded uniformly on cake plus N+P+K combinations followed by cake plus N+P and cake plus P combinations

(Table II). The mycelial mat weight and spores/ml were lowest in cake plus N combinations. Supplementing oil cakes with P and K in general improved the mycelial mat weights either individually (P or K) or in combination (P+K) indicating that supplementing oil cakes with N was not as beneficial as with P and K to *P. lilacinus*.

Further, the natural N, P and K contents in the oil cakes were estimated to be (i) castor; 4:1.9:1.4%, respectively, and (ii) neem, 5.2:1.7:1.5%, respectively. Of the two oil cakes, neem had greater NPK content which was probably better for the growth and sporulation of *P. lilacinus*. In crossandra, the root colonization and propagule number of *Glomus mosseae* differed with its oil cake combinations viz., castor, neem and pongamia, due to the inherent difference in NP and K composition in the oil cakes (Nagesh and Parvatha Reddy, 1997a). Further, supplementation of N, P and K to the oil cake suspensions not only improved the duration and amount of mycelial growth of *P. lilacinus* but also enhanced its sporulation. Similar observations of additive effects of NPK supplementation to oil cakes (castor, karanj or neem) on the growth and sporulation of *Verticillium lecanii* were reported earlier by Nagesh and Parvatha Reddy (1997b).

The additive effect observed under *in vitro* conditions between the use of oil cakes with inorganic fertilizers on *P. lilacinus* was also observed under nursery bed conditions in terms of increased levels of CFU and parasitisation of egg masses by *P. lilacinus*. Integrated use of these inorganic fertilizers not only promoted better plant growth, but also resulted in more CFU of *P. lilacinus* in the plant rhizosphere and its parasitism of egg masses resulting in reduction in root galls and nematode multiplication rates (Table III). However, the effect was more discernible in

TABLE I - *Growth of* Paecilomyces lilacinus *on aqueous suspensions of oil cakes and their combination with inorganic fertilizers.*

Treatment	Mycelial growth after incubation (0-100 scale)					
Weeks:	1	2	3	4	5	6
Castor cake (CC)	25	55	80	90	40	20
Neem cake (NC)	40	60	90	100	60	40
CC + Nitrogen (N)	30	45	60	80	100	80
CC + Potassium (K)	30	40	60	90	100	80
CC + Phosphorus (P)	35	45	60	90	100	80
CC + NP	40	50	65	90	100	70
CC + NK	40	45	60	95	100	80
CC + PK	35	55	70	95	100	80
CC + NPK	35	65	90	100	100	80
NC + N	40	55	75	90	100	60
NC + P	40	60	80	90	100	80
NC + K	40	60	80	90	100	80
NC + NP	40	65	80	90	100	80
NC + PK	45	65	85	90	100	80
NC + NK	45	70	85	95	100	80
NC + NPK	45	70	100	100	80	60
C.D. (P = 0.05)	4.65	3.88	6.79	8.88	10.12	8.99

TABLE II - *Effect of oil cakes and inorganic fertilizers on the mycelial mat weight and spore number of* P. lilacinus.

Treatment	Mycelial mat dry weight (mg)		Spore number (10^5 / ml)	
	Castor	Neem	Castor	Neem
Cake alone	100	110	9.0	12.0
Cake + Nitrogen (N)	85	90	8.0	11.0
Cake + Phosphorus (P)	165	180	14.0	19.0
Cake + Potassium (K)	160	180	12.0	18.0
Cake + NP	195	215	19.0	21.0
Cake + NK	120	145	18.0	19.5
Cake + PK	210	230	17.0	19.0
Cake + NPK	295	310	28.5	33.0
C.D. (P = 0.05)	13.44	18.21	2.78	3.45

TABLE III - *Effect of integrated use of oil cakes, inorganic fertilizers and* P. lilacinus *on tomato plant growth,* Meloidogyne incognita *multiplication and parasitisation by* P. lilacinus *under mursery conditions.*

Treatment	Shoot		Root		Root gall index (0-5 scale	Nematode multiplication rate	PL	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)			Parasitisation (% egg masses)	CFU
P. lilacinus (PL)	29.6	12.2	22.0	6.9	3.8	3.11	23.6	2.1×10^3
PL + Castor cake (CC)	32.1	13.0	24.6	7.4	3.4	3.00	32.1	2.6×10^3
PL + Neem cake (NC)	33.6	13.8	25.4	8.6	3.0	3.00	38.2	5.2×10^3
PL + CC + NP	34.2	14.6	26.0	9.7	3.0	2.81	39.4	2.3×10^4
PL + CC + PK	35.6	14.6	26.2	9.7	2.8	2.74	40.8	3.8×10^4
PL + CC + NK	34.8	14.1	26.3	9.6	2.8	2.61	40.0	3.6×10^4
PL + CC + NPK	38.6	17.8	29.4	11.9	2.0	2.02	48.6	6.7×10^4
PL + NC + NP	35.0	15.0	27.0	10.1	2.8	2.42	42.4	4.4×10^4
PL + NC + PK	35.8	16.2	28.2	10.8	2.4	2.20	44.2	4.8×10^4
PL + NC + NK	37.6	17.1	27.9	10.1	2.4	2.20	44.0	4.0×10^4
PL + NC + NPK	39.9	19.6	30.5	12.2	1.4	1.56	53.6	9.8×10^4
Control	24.9	9.2	16.5	6.1	4.3	3.74	10.4	0.8×10^2
C.D. (P = 0.05)	1.12	0.93	1.24	0.78	0.11	0.14	6.86	–

nursery beds treated with oil cake suspensions containing all the three nutrients, i.e., NPK, compared to oil cake suspensions with any one or two of the nutrient combinations. This indicated that balanced supplementation of nutrients to oil cakes was better to both the fungus and plant host compared to other combinations. A natural occurrence of 0.8×10^2 CFU of *P. lilacinus* and egg mass parasitism of 10% were observed in the untreated check.

Although there was significant reduction in nematode populations in treated beds com-

pared to untreated beds, the nematode population levels were higher at the transplantation stage (45 days). This could be due to low dosage of treatments given per bed in the nursery i.e., 100 ml of 10% oil cake suspension with the fungus and NPK combinations. Further, increased dosages in nursery beds may reduce the nematode populations. In the case of the fungal application rate under field conditions, Gomes Carneiro and Cayrol (1991) suggested a propagule density of 10^{11} spores/m^2 for significant nematode control. The highest propagule density used in our experiment was 3.3×10^8 spores/m^2 (NC + NPK) indicating that the fungal dosage used was less, than could be expected to achieve acceptable nematode control.

In summary the normal agronomic practice of application of inorganic fertilizers together with oil cakes was beneficial to the antagonistic fungus (*P. lilacinus*), plant host (tomato) and also enhanced the antagonistic potential of the introduced bio-agent against the root-knot nematode (*M. incognita*) under nursery conditions.

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