

Effect of different bioformulations of *Paecilomyces lilacinus* against root-knot nematode (*Meloidogyne incognita*) infecting tomato (*Solanum esculentum*)

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ABSTRACT

Biocontrol potential of the parasitic fungus *Paecilomyces lilacinus* (PI-181) strain was isolated from the egg masses of the nematode affected tomato (*Solanum esculentum* L.; syn *Lycopersium esculentum* Mill.) crops was evaluated for the management of root-knot nematode (*Meloidogyne incognita* Chitwood). The fungal isolate grown on starch rich grains was tested for an ideal formulation with different natural and synthetic inert carrier material. Keeping in view the parameters like colony count, shelf-life coupled with easy dispersibility out of Attapulgitite-based clay dust powder + peat powder, heavy loam soil powder + peat powder, talc fine powder + peat powder, kaolin light powder + peat powder, boric acid powder + peat powder, bentonite powder + peat powder and *Paecilomyces lilacinus* + alone were tried separately. Attapulgitite-based clay dust powder + peat powder + *Acacia* gum powder showed best performance in respect to all the three parameters and also suppressed *M. incognita* population to a greater extent.

Key words: Biocontrol, Bioformulation, Crop disease management, *Paecilomyces lilacinus*, Root-knot nematode, Shelf-life

Root-knot nematode (*Meloidogyne* spp) a polyphagous of global significance cause immense reduction in crops, like tomato (*Solanum esculentum* L. syn *Lycopersicon esculentum* Mill.) both alone and in association with soil-borne fungi in tropical and sub-tropical countries (Seenivasan and Devrajan 2008).

Since 1982, DBCP, MBr, BHC, DDT etc. used for the management of root-knot nematode have been banned due to their alarming adverse side effects causing environmental and groundwater pollution, toxic residual problem and deleterious effect on soil microflora (Stirling 1991). Considering these limitations the use of eco-friendly, bio-efficacious, economical, biodegradable and environmentally safe methods are preferred and could be ideal for control of plant parasitic nematodes and other pathogenic microorganisms. Consequently the use of antagonistic fungi known to produce nematicidal or nematostatic compounds (Khan and Goswami 2001) against root-knot nematode, *M. incognita* could be an alternative may be referred to

as novel nematicides (Kanai 2004). *P. lilacinus* a soil-inhabiting, saprophytic culturable and surviving well up to 35°C has exhibited considerable potential against root-knot nematode under *in-vitro* and *in-vivo* conditions (Kiewnick and Sikora 2006). First isolated from soil on potato in Peru to control root-knot nematodes (Jatala *et al.* 1979) is reported widely and found parasitizing eggs of *Meloidogyne* spp and *Globodera pallida* (Stone) Behrens (Goswami and Singh, 2002). This fungus also invades the females or cysts of a number of nematode species (Park *et al.* 2004).

Hence, it was felt to develop an ideal, cost-effective and easily deliverable formulation to control two most economically important endoparasitic root-knot (*Meloidogyne* spp) and cyst-forming (*Globodera* spp) nematodes (Sun *et al.* 2002).

The present study is an attempt to develop an ideal bioformulation with easy, cost-effective with an excellent dispersal properties and long shelf-life in combating the nematodal diseases.

MATERIALS AND METHODS

The study was undertaken at the experimental farm ACB and PDM, Amity University Uttar Pradesh and farmers field during 2006 and 2007 the antagonist *P. lilacinus* (Thom) Samon, isolate-PI-181 from culture collection of ACB-Amity

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University was selected on the basis of larvicidal, hatching inhibition and egg parasitizing capacity for the present investigations. The fungal antagonist maintained on PDA slants at 5°C after seven days of growth at 25±2°C was multiplied on pre-boiled sterilized sorghum grains (solid medium) supplemented with 5% dextrose in conical flask and incubated at 25±2°C for 12 days. Colonized grains were air-dried, powdered and sieved with 80-mesh sieve under aseptic conditions and the concentration of conidia and chlamydospores was determined using a haemocytometer prior to preparations test formulation fungus. After estimation of spore load in fine powder of *P. lilacinus*, a concentration of 2×10⁸ CFU/g was added to the pre-sterilized carrier substrate. The formulations used in the present study were: Attapulgitite based clay dust (ABCD) powder + peat powder (PP) (1:1), heavy loam soil powder (HLSP) + peat powder (PP) (1:1), talc fine powder (TFP) + peat powder (PP) (1:1), kaolin light powder (KLP) + peat powder (PP) (1:1), boric acid powder + peat powder (PP) (1:1), bentonite powder (BP) + peat powder (PP) (1:1) and PL+ acacia alone @ 4 g w/w.

With each of these seven carrier/substrate powdered 'gum arabica' from *Acacia arabica* 5% w/w as an adjuvant/adhesive was added, followed by sealing 250 g of each above formulation containing *P. lilacinus* spores and the adjuvant under a laminar air flow bench.

Spore suspension from each formulation was separately assayed after one month. In each prepared bioformulations spore load was maintained with initial concentration 2×10⁸. In each formulation/treatment one ml of spore suspension was mixed with 18 ml of potato dextrose agar medium and poured in to Petri-plates separately. Spore suspension of *P. lilacinus* alone served as check. An observation on spore germination count was recorded after 48 hr of incubation at 25±1°C for each preparation including check.

To study the shelf-life (longevity) of *P. lilacinus* in each formulation was kept separately at ambient/room temperature (approx. 25°C) along with check comparative powder of *P. lilacinus* + acacia powder. Spore viability in each treatment was checked periodically at two-month interval. For finding the CFU value of each formulation 1 g powder-based formulation of the fungus was diluted at 10⁻⁴ and was plated with 1% potato dextrose agar medium in triplicate. Observations on spore germination were made after 48 hr of incubation at 25±2°C.

The experiments were conducted under glasshouse conditions having temperature 25–28°C, 60–70% relative humidity and 66–70% soil moisture. Earthen pots (15 cm) were filled with infested sandy loam soil (2-larvae of *M. incognita* /g soil). Ten healthy seedlings of highly susceptible 'Pusa Ruby' tomato were transplanted in pot for eight treatments including check in triplicate after amending the soil with seven formulations 10g w/w separately.

Data on seedling emergence, nematode population and plant growth were recorded 45 days after transplantation.

Field experiment was also conducted under micro-plot in a nematode sick with 4 larvae/g soil at farmer's field. The trial was laid out in a randomized block design with three replications with individual plot size of 3m–2.5m Diammonium phosphate 150 kg/ha and nitrate of potash 50 kg/ha was added in each plot. Seedlings treated with the formulations 15g/100 seedlings were transplanted in each micro-plot at a distance of 30 cm between plant and 30–40 cm between row. Plant growth parameters, galls/plant, egg-masses/plant, eggs/egg-masses and soil nematode population were estimated after 60 days of transplantation. Yield/micro-plot was recorded by harvesting the each micro-plot separately. Data were analyzed through the AGRES statistical software package.

RESULTS AND DISCUSSION

Five formulations were found compatible between the ingredients as confirmed by their viable spores (Table 1). However, boric acid with peat powder was found as it incompatible formulation showed least cfu count (53.8%) after one month of storage at normal temperature. The maximum proliferation of *P. lilacinus* (CFU count 92.3%) was noticed in Attapulgitite-based clay dust (ABCD) with peat powder ratio of 1:1 formulation, followed by kaolin light powder with peat (92.1% CFU count).

Almost same performances were also observed with Talc fine powder (91.9%), heavy loam soil powder (89.6%). The bentonite powder with peat powder failed to show significant compatibility (76.9) and was also expensive and not easily available. The least compatible formulations were boric acid and acacia alone.

The shelf-life of *P. lilacinus* in four formulations showed

Table 1 Formulations compatibility with *P. lilacinus* at room temperature (25°C)

Formulation	Spore viability (%)	
	0 month	1 month
ABCD powder + peat powder (PP) (1:1)	94.9	92.3
heavy loam soil powder (HLSP) + peat powder (PP) (1:1)	93.6	89.6
talc fine powder (TFP) + peat powder (PP) (1:1)	94.7	91.9
kaolin light powder (KLP) + peat powder (PP) (1:1)	94.8	92.1
boric acid powder + peat powder (PP) (1:1)	83.7	53.8
bentonite powder (BP) + peat powder (PP) (1:1)	85.8	76.9
PL+ acacia alone @ 4 g w/w	87.6	71.6
SEM±	1.20	1.35
LSD (P= 0.05)	2.86	3.29

SEm, Standard error mean; LSD, least significant difference

gradual decline (>43.8%) up to six month with maximum spore viability in Attapulgitite-based clay dust (ABCD) powder + peat powder for-mulations (94.9, 86.5, 81.2, 56.8 and 38.3) followed by kaolin light powder (KLP) and peat powder (94.8, 80.4, 72.5, 47.6 and 34.4), Talc fine powder (TFP) + peat powder (94.7, 83.2, 78.5, 49.0 and 36.4) and heavy loam soil powder (HLSP) + peat powder (93.6, 78.2, 69.4, 43.8 and 21.5), on 0, 2, 4, 6 and 8 months, respectively at 25°C on the other hands a decline was noticed in Plant + *Acacia* alone (87.6, 71.6, 51.5, 34.8 and 12.7), bentonite powder (BP) + peat powder (85.8, 73.4, 59.2, 34.5 and 08.2) and boric acid powder + peat powder (83.7, 52.4, 45.8, 36.1 and 05.9) over a period of four months at 25°C (Table-2). The maximum spore viability was observed in ABCD powder + peat powder formulation (94.9 to 38.3%) up to period of eight months, as compared to *P. lilacinus* alone (87.6 to 12.7%). The present investigations showed that the ABCD powder + peat powder (94.9 to 56.8), kaolin light powder (KLP) and peat powder formulations (94.8 to 47.6), talc fine powder (TFP) + peat powder (94.7 to 49.0) and heavy loam soil powder (HLSP) + peat powder (93.6, 78.2, 69.4, 43.8 and 21.5) formulations significantly improved spore viability and longevity of *P. lilacinus* up to eight months at 25°C in comparison to other (Table 2).

Effect on plant growth

The highest significant increase in plant growth parameter, viz, shoot and root length, shoot and root weight were found with treated formulation ABCD powder + peat powder, kaolin light powder and peat powder, talc fine powder + peat powder and heavy loam soil powder + peat powder respectively (Table 3).

Similar significant observations were revealed in plant growth and yield with above mentioned formulations under field conditions also (Table 4).

Effect on development of eggs/egg mass, gall formation/index, egg parasitization/egg infection and nematode population

Maximum significant reduction in eggs/egg masses, number of gall formation, and nematode population with highest egg parasitization/egg infection in soil were observed in treatments ABCD powder + peat powder, followed by talc fine powder + peat powder, kaolin light powder and peat powder, and heavy loam soil powder + peat powder respectively in both pot and field experiments (Table 4).

The egg parasitization/egg infection of *M. incognita* by *P. lilacinus* were found maximum treated with formulation of ABCD powder + peat powder (97.50), followed by talc fine powder + peat powder (96.65), kaolin light powder and peat powder (95.30) and heavy loam soil powder + peat powder (94.64), respectively.

The final soil nematode population also showed the similar trend of inhibition in both pot and field experiment with above mentioned formulation. Minimum disease infestation was observed in formulation ABCD powder + peat powder, followed by talc fine powder + peat powder, kaolin light powder and peat powder etc. compared to other two formulations and control.

Maximum significant protection and production of the crop was also observed in the same formulations as compared to other non-significant bioformulations including *P. lilacinus* alone as control under pot and field experiments possible because acacia gum provided better coating and food base for the antagonist. A similar observation on the use of acacia gum for efficient use of biocontrol agents was also demonstrated by Prasad and Rangeshwaran (2000) wherein they also reported proliferation of biocontrol agent on seed coat (Table 4). Considering the economy and also for using a natural plant product, powdered was selected as a substitute of carboxymethyl cellulose salt (CMC) with each formulation.

Table 2 Longevity of *P. lilacinus* in different formulations during eight month storage at room temperature (25°C)

Treatment	Viability (%)				
	Storage time (months)				
	0	2	4	6	8
ABCD powder + peat powder (PP) (1:1)	94.9	86.5	81.2	56.8	38.3
heavy loam soil powder (HLSP) + peat powder (PP) (1:1)	93.6	78.2	69.4	43.8	21.5
talc fine powder (TFP) + peat powder (PP) (1:1)	94.7	83.2	78.5	49.0	36.7
kaolin light powder (KLP) + peat powder (PP) (1:1)	94.8	80.4	72.5	47.6	34.4
boric acid powder + peat powder (PP) (1:1)	83.7	52.4	45.8	36.1	05.9
bentonite powder (BP) + peat powder (PP) (1:1)	85.8	73.4	59.2	34.5	08.2
PL+ acacia alone @ 4 g w/w	87.6	71.6	51.5	34.8	12.7
SEM±	1.4	2.2	4.6	5.1	2.0
LSD (P= 0.05)	2.1	3.7	8.9	10.4	2.6

SEM, Standard error mean; LSD, least significant difference

Table 3 Efficacy of bioformulations (*P. lilacinus*) on *Meloidogyne incognita* infecting tomato in pot (45 days of transplantation)

Treatment	Plant growth parameter*				Nematode multiplication				
	Shoot		Root		Root system				
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Number of galls	Number of egg masses	Number of eggs/egg mass	Egg infection (%)	Larval nematode population/250g soil*
ABCD powder + peat powder (PP) (1:1) 0gw/w	44.6	19.42	36.87	12.20	04.75	01.58	60.28	96.72	78.82 (8.91)
Heavy loam soil powder (HLSP) + peat powder (PP) (1:1) 10gw/w	41.24	16.16	33.62	09.61	08.96	04.81	92.60	85.50	128.45 (11.32)
Talc fine powder (TFP) + peat powder (PP) (1:1) 10gw/w	43.86	18.26	35.90	11.32	05.25	02.50	65.50	89.67	90.21 (8.52)
Kaolin light powder (KLP) + peat powder (PP) (1:1) 10gw/w	43.10	17.40	35.06	10.72	07.80	03.23	78.21	88.91	113.20 (7.66)
Boric acid powder + peat powder (PP) (1:1) 10gw/w	36.47	11.35	27.67	05.82	13.50	08.75	127.83	58.34	144.62 (12.05)
Bentonite powder (BP) + peat powder (PP) (1:1) 10gw/w	40.13	14.60	31.43	07.23	10.68	07.25	108.62	74.50	130.50 (11.45)
PL+ <i>Acacia</i> 5gw/w	37.82	11.87	28.90	06.15	9.62	8.87	116.80	62.58	122.62 (11.1)
Un-inoculated check	25.46	8.12	16.70	4.67	208.65	130.75	223.50	0.0	2150.85 (46.38)
Normal check	45.10	17.67	35.62	11.40	0.0	0.0	0.0	0.0	0.0
SEM±	1.60	1.10	2.1	2.0	4.5	5.3	2.6	3.6	(4.5)
LSD (P= 0.05)	3.20	2.13	4.60	2.48	6.69	8.21	7.45	7.25	(8.6)

*Figures in parentheses are $\sqrt{n}+0.5$ transformed angular values; SEM, Standard error mean; LSD, least significant difference

Results of field trials showed, in general, significantly improved plant vigour and yield of tomato in all the formulations, except boric acid powder + peat powder as compared control (Table 4).

Biological control of root-knot nematode infecting wide range of crops including tomato has been achieved by combined application of potent strains of *Trichoderma viride* and *P. lilacinus* (Goswami *et al.* 2006). However, the successes of a bio-control agent depends upon its long viability in formulation, ability to produce; peat is preferred as ideal component of a formulation also because of its good dispersive property and lower adhesive property of inoculum in excess, and proliferate well on an ideal substrate around rhizosphere of the growing plants. The microbial biomass as alginate priUs and granules, starch granules (Lewis *et al.* 1995), wheat bran alginate pellet, pyrax (Lewis and Fravel 1996), talc powder (Sankar and Jeyrajan 1996) have been successfully used against soil-borne plant patho-gens as seed, seedling and soil treatment on various crops. Significant reduction in the incidence of chickpea damping-off (*R. solani*) was observed by the application of modified granular formulation containing powder wheat bran, kaolin, acacia powder and biomass of *T. harzianum*, *T. viride* and *Glocladium deliquescens*. The population of antagonist in formulation declined after 30 days of storage, but should be retained substantial number of viable propagules even at 90

days (Prasad and Rangeswaran 2000). Various formulations of antagonists have been developed and tried with various efficacy and shelf-life (Goswami *et al.* 2006).

In the present investigations most promising results were obtained with ABCD powder + peat powder formulation of *P. lilacinus* exhibited highest increase in yield tomato with remarkable reduction in population of root-knot nematode (*M. incognita*). In same formulation, viability and compatibility were maximum (>50%) after six month storage period which is remarkable achievement of this finding. Similar results were obtained by Goswami and Devi (1993) who reported that ABCD possess nematicidal properties against *M. incognita*, in addition to its use as cost effective carrier material. Application of *P. lilacinus* with different formulations into soil, seed and seedlings is proposed to be exploited, especially against soil-borne root-knot nematode and *F. oxysporum* f. sp. *lycopersici*, *F. solani*, *Rhizoctonia bataticola*, *R. solani*, and *Sclerotium rolfsii* which is expected to more effective and useful approach as it provides better coating and food base on seed surface for better proliferation of bio-control agents. The root-knot nematode (*M. incognita*) is known to predispose the host many soil-borne pathogenic fungal attack. Through the management of root-knot nematode by potential bioformulations of antagonistic fungal bioagent the predisposing factor for the infection secondary pathogens (soil-borne pathogenic fungi) could also be

minimized. Fungal bioagent *T. viride* is also known to be effective against many fungal pathogens (Pandey *et al.* 2005) our formulations in addition to reducing the incidence of *M. incognita* would also keep in reducing the incidence and invasion of soil-borne fungi causing root-wilt and root-rot diseases.

The best performance of ABCD + peat powder in view of compatibility, longer shelf-life being till eight month which was much more than all other formulations under present study is attributed towards the most inert property of ABCD as compared to talc fine powder and kaolin, although the same was statistically at par with heavy loam soil. However, since ABCD has been reported to possess nematicidal properties (Goswami and Devi 1993), in the present study the formulation constituting ABCD + peat powder is most preferred and recommended to be used by the farmers. Further, all the components of this particular formulation containing ABCD, peat powder and acacia gum leaves no toxic residues and thus help to retain the sustainability (Neethling 2002). Bansal *et al.* (1992) observed that wood charcoal was a good carrier of *P. lilacinus* for applying in the field. The carrier material in low-density polyethylene pouch could support up to 10^6 spores/g for at least six months.

Kiewnick and Sikora 2006, evaluated agro-industrial wastes for mass production of *P. lilacinus*.

A problem associated with the establishment of a potential mycoparasite, is to maintain the levels of inoculum so that a mycoparasite can initiate its action effectively (Jeffries and Young 1994). Secondly the development of a stable cost effective and easy to apply biocontrol formulation is critical for the advancement of biological control of plant pathogens with introduced antagonists (Lisanky 1985). Carboxymethyl cellulose has been most commonly used as a sticker for seed treatment of different crops with antagonists (Mihuta Grimm and Rowe 1986). Lewis *et al.* (1996) studied the proliferation of *Gleocladium virens* and *T. hamatum* using various food such as wheat bran, maize, cob groundnut hulls, cocoa hulls and chitin. Hagedorn *et al.* (1993) reported that peat-based formulations applied to cotton seeds @110/kg effectively checked the seedling disease of cotton in field trials. Gandhi Kumar (1996) studied gypsum formulation to be equally effective as talc formulation in maintaining rhizosphere population and cost-effective than talc-based formulation.

Selvakumar *et al.* (2001 a) reported that peat-based bioformulation retained viability of ascospores of *C. globosum* and maintained initial level (6×10^7 cfu/g) at 5°C

Table 4 Efficacy of bioformulations (*P. lilacinus*) on *Meloidogyne incognita* population infecting tomato in micro-plot (60 days of transplantation)

Treatment	Plant growth parameter				Nematode multiplication					
	Shoot*		Root*		Yield (g/6m ²) or (kg/ha)	Root system				Larval nematode population/ 250g soil**
	Length (cm)	Weight (g)	Length (cm)	Weight (g)		Number of galls	Number of egg masses	Number of eggs/ egg mass	Egg infection (%)	
ABCD powder + peat powder (PP) (1:1)	58.25	27.60	39.50	18.40	236.00 (393.33)	01.42	01.58	60.28	97.50	108.56 (10.44)
Heavy loam soil powder (HLSP) + peat powder (PP) (1:1)	54.50	25.75	37.62	17.12	216.20 (360.33)	04.56	04.81	92.60	94.60	140.70 (11.88)
Talc fine powder (TFP) + peat powder (PP) (1:1)	57.86	27.00	39.00	18.10	225.80 (376.33)	02.68	02.50	65.50	96.65	102.00 (10.98)
Kaolin light powder (KLP) + peat powder (PP) (1:1)	56.10	26.80	38.56	17.43	219.80 (366.33)	02.94	03.23	78.21	95.30	121.20 (11.03)
Boric acid powder + peat powder (PP) (1:1)	42.60	20.50	27.67	11.82	188.80 (314.67)	09.15	08.75	127.83	78.20	167.75 (12.97)
Bentonite powder (BP) + peat powder (PP) (1:1)	53.45	26.15	36.65	16.50	213.20 (355.33)	5.20	07.25	108.62	89.65	140.63 (11.88)
PL+ acacia alone @ 4 g w/w	48.82	25.62	35.80	14.87	192.60 (321.00)	4.10	8.87	116.80	88.90	143.15 (11.99)
Un-inoculated check	29.75	12.70	14.45	14.50	161.00 (268.33)	218.45	130.75	223.50	0.0	2360.85 (48.94)
Normal check	50.56	24.29	36.50	17.10	188.80 (314.67)	0.0	0.0	0.0	0.0	0.0
SEm±	3.62	2.10	2.90	2.52	(4.21)	2.90	6.7	5.40	6.10	(4.95)
LSD (P= 0.05)	4.21	2.81	3.67	2.94	(7.52)	3.04	7.30	6.94	7.25	(11.90)

*Mean of five plant from central row of the micro-plot

**Figures in parentheses are "n+0.5 transformed angular values
SEm, Standard error mean; LSD, least significant difference

up to 60 days of storage. The viability of *C. globosum* on treated seeds at different period and temperatures revealed that the viability remained significantly at par with initial population up to 45 days at 5°C. However at 15°C it reduced to 3.52×10^7 cfu/g seed. There was drastic reduction in the population at 25° and 35°C even after 15 days and on 60 days, respectively. Wheat seed treated with bioformulation of *C. globosum* significantly increased per cent seed germination over control. Treatment 4g and 8g/kg of seeds resulted in considerable increase of seed germination, shoot and root growth of plants. The rhizosphere of seed germination, shoot and root growth of plants. The rhizosphere competence of *C. globosum* was determined in pot culture and the population was estimated at 20 days intervals up to 80 days. The results of the experiment showed increase in population of the bioagent up to 40 day and then declined from 60 to 80 day.

Besides the *in vitro* observations of the formulations in respect to compatibility and shelf-life, the plant growth and nematode population both in soil and roots as presented in Tables 3,4 also reflects positive response when the sources of inoculum were done from each of the above formulations. It is thus very clear that the response of plant growth parameters and nematode multiplication was variable when the source of bioformulation was either of above seven formulations under study. Here also the observations clearly indicate best performance of ABCD + peat powder combined formulations in respect to the improvement of the plant growth with reduction of *M. incognita* population both in roots and in soil, thus further confirming the merit of the selected formulation which in addition to its being cost-effective and ABCD having been reported to possess nematicidal properties is one of its constituents (Goswami *et al.* 1993).

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