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Management of root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood in chrysanthemum using *Paecilomyces lilacinus* (Thom) Samson in combination with neem cake

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ABSTRACT: **Talc and pesta granule formulations of *P. lilacinus* spores, obtained from solid and liquid media, at 12, 10, 8 and 6 per cent moisture levels were evaluated for their spore viability and their field efficacy, singly and in combination with neem cake against root-knot nematode, *Meloidogyne incognita* in the farmer's field. The preparation of *P. lilacinus* used in the field consisted mainly of spores. The fungus was cultured under two sets of defined conditions to produce aerial spores, i. e., cultured on sorghum grains, and submerged spores, grown in a liquid medium. Aerial spores were more robust (96, 87, 80 and 60% viability at 6, 8, 10 and 12% moisture levels, respectively, in talc formulation from sorghum grains), compared to submerged spores (83, 72, 64 and 54% viability at 6, 8, 10 and 12% moisture levels, respectively, in talc formulation from liquid broth), over a period of 60 days after formulation. Talc and pesta granules of *P. lilacinus*, applied at 2 doses, 10 and 15 kg/ha to the root-knot nematode infested chrysanthemum field correspondingly reduced root gall index to 1.4-2.7, nematode multiplication rate to 1.4-2.12, and enhanced floral yield by 12 per cent depending on the dose of formulation. Further, combined use of these formulations with neem cake enhanced fungal propagules in rhizosphere to 700-1070, fungal infectivity to 32-52 per cent and chrysanthemum flower yield (by 23-28 %) depending on the dose of formulation.**

KEY WORDS: Chrysanthemum, field evaluation, formulations, *Meloidogyne incognita*, moisture levels, *Paecilomyces lilacinus*

INTRODUCTION

Chrysanthemum is an important flower crop extensively grown for domestic and export market in many parts of the country. Root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1909) Chitw. 1949 being polyphagous, was observed to be one of the major soil-borne biotic limiting factors. The nematode causes 20-30 per cent pyrethrum yield losses, a decrease in flower size, yield, and pyrethrin content, stunting, chlorosis, wilting and predisposes infected plants to infection by root-rot and wilt fungal pathogens (Warui *et al.*, 1991). The high cost of nematicides and amendments with oil cakes, and the environmental hazards posed by the use of nematicides make biological control a viable alternative in horticultural ecosystems. Among several potential antagonistic fungi, *Paecilomyces lilacinus* (Sampson) Thoms. was found effective against root-knot nematodes under field conditions (Nagesh *et al.*, 2001). *Paecilomyces lilacinus* is mass multiplied on solid and liquid media and

formulated as talc formulations. However, moisture content in these formulations is an important factor for spore viability and field efficacy (Sterling *et al.*, 1998). The experiment under report was designed to evaluate the spore viability in two formulations of *P. lilacinus* at different moisture levels and evaluate their field efficacy singly and in combination with neem cake against *M. incognita* in chrysanthemum.

MATERIALS AND METHODS

Formulations of *Paecilomyces lilacinus* were developed at different moisture levels and their efficacy against *M. incognita* was evaluated in predominantly root-knot nematode-infested chrysanthemum field of a farmer at Muddenahalli of Kolar district, Karnataka during 2001-2002.

i. Preparation of formulations

Local isolate of *Paecilomyces lilacinus* (PDBC PL1) was mass produced under two sets of defined conditions to obtain (i) aerial spores, namely, cultured on agar plates initially and on sorghum grain later, and (ii) submerged spores, grown in a liquid medium (potato dextrose broth). Using these spores of *P. lilacinus*, two formulations, *viz.*, talc and pesta granules, were prepared and dried to 6, 8, 10 and 12 per cent moisture levels using the moisture analyzer (Sartorius MA100). The conidial mass from liquid and sorghum grains were mixed with proportionate amounts of autoclaved talc powder with blender and the spore load was maintained at 2-4x10^7/g talc formulation. Another set of spores was used to develop pesta granules as per the procedure described by Connick *et al.* (1991). A dough was prepared from the spores of *P. lilaceous,* wheat flour, mineral oil and water, was rolled into a thin sheet, air dried in the laminar hood and broken in to small pieces using autoclaved pestle and mortar. The ground pieces were sieved through 4-mesh (4.0 mm pore aperture) sieve and the retentate pieces were designated as granules. Pieces smaller than 4 mm and too large size were mixed with wheat flour, other ingredients and new batch of spores and the process was repeated to obtain as many granules. Finally four formulations of *P. lilacinus*, *viz.*, sorghum + talc (powder), sorghum+ pesta (granules), PD broth + talc (powder) and PD broth + pesta (granules), were prepared and dried to 6, 8, 10 and 12 per cent moisture levels. Granules were dried to desired moisture levels in a laminar flow. The lowest moisture level of 6 per cent was achieved by keeping the granules in vacuum desiccator in batches. The moisture content of the formulations was estimated using moisture analyzer (Sartorius MA100). Thus obtained formulations at specific moisture contents were maintained by packing them in high density and thick (0.2 mm) polythene packets at room temperature in desiccator.

Spore viability of *P. lilacinus* in talc and pesta formulations at 6, 8, 10 and 12 per cent moisture content was recorded at 60 days of storage at room temperature (26 ± 2° C). Formulations with 6 per cent moisture were utilized for field evaluation.

ii. Field evaluation

A farmer's field at Muddenahalli (40 km from Bangalore), Kolar, Karnataka, with high incidence of root-knot nematode, *Meloidogyne incognita* was earmarked for the study. The soil was red laterite with a pH of 6.7 and organic carbon of 2.6 per cent. The population of *M. incognita* recorded at planting ranged 184–238 per 100cc soil. Thirty-meter (length) rows were laid out with a spacing of 1.5 m (row) x 0.5m (intra row). The treatments, talc and pesta granule formulations of *P. lilacinus* with an average spore load of 2 x 10^7 spores/g at 10 and 15 Kg/ha, singly or in combination with neem cake (*Azadirachta indica* Juss.) at 500 kg/ha were incorporated in to the rows at the time of planting the chrysanthemum suckers. Each treatment was replicated thrice and randomized in rows. Further, 3 rows each were earmarked for two controls, *viz.*, untreated and Carbofuran treated (1.5 kg/ha).

iii. Observations recorded

The colony forming units (CFU) on root and in rhizosphere soil, and egg mass infection (Egg mass %) were recorded on *P. lilacinus* semi-selective medium (Mitchell *et al.*, 1987). Observations on root-knot index, per cent healthy root and chlorotic plants per row of 10m were recorded 60 days after imposing treatments. Chrysanthemum root samples

from treated rows were collected, cleared of soil and organic debris, and stained with acid-fuschin (Byrd *et al.*, 1983). Twenty root bits (1cm length) were randomly placed on glass slide and observed under microscope for presence of root-knot nematode growth stages. The number of chlorotic plants was enumerated per row of 10-meter length at 60 days after treatment. Flower yield was recorded for 4 harvests/pickings in 30 days duration and expressed in terms of per cent increase in flower yield over untreated check.

RESULTS AND DISCUSSION

Moisture levels of the talc and pesta formulations indicated a negative influence on the spore viability of *P. lilacinus* (Fig. 1). At 60 days of formulation, the spore viability of *P. lilacinus* in talc and granules was observed to decrease with increase in moisture levels of formulations (i.e., 6 to 12 %). The spore viability in Sorghum grain + talc was 96 per cent at 6 per cent moisture, while the spore viability was 60 per cent at 12 per cent in the same formulation. Similarly, the spore viability was 87 per cent at 6 per cent moisture level in sorghum grain + pesta granules, while it was 54 per cent at 12 per cent moisture level. The decrease in spore viability was more prominent in formulations (talc and pesta granules) developed from liquid broth. The talc formulation of *P. lilacinus* from liquid broth at 6 per cent recorded 83 per cent, which decreased to 54 per cent at 12 per cent moisture level. Similarly, the granular formulation made out of liquid broth at 6 per cent moisture recorded a spore viability of 76 per cent, while it was 45 per cent at 12 per cent moisture. Observations on viability of spores at different moisture levels suggest that aerial spores obtained from sorghum grain (96, 87 % spore viability at 6 % moisture; 87 and 78 % at 8 % moisture; 80 and 68 % spore viability at 10 % moisture; 60 and 54 % spore viability at 12 % moisture) were more robust when compared to submerged spores (produced in liquid broth) observed at 60 days of formulation. Between talc and pesta granules, talc exhibited higher spore viability at all moisture levels (96, 87, 80 and 60 % from sorghum grain, and 83, 72, 64 and 54 % from liquid broth at 6, 8, 10 and 12 % moisture levels, respectively) compared to pesta (87, 78, 68 and 54 % from sorghum grain 76, 68, 57 and 45 % from liquid broth at 6, 8, 10 and 12 % moisture levels, respectively). Earlier, Stirling *et al.* (1998) observed

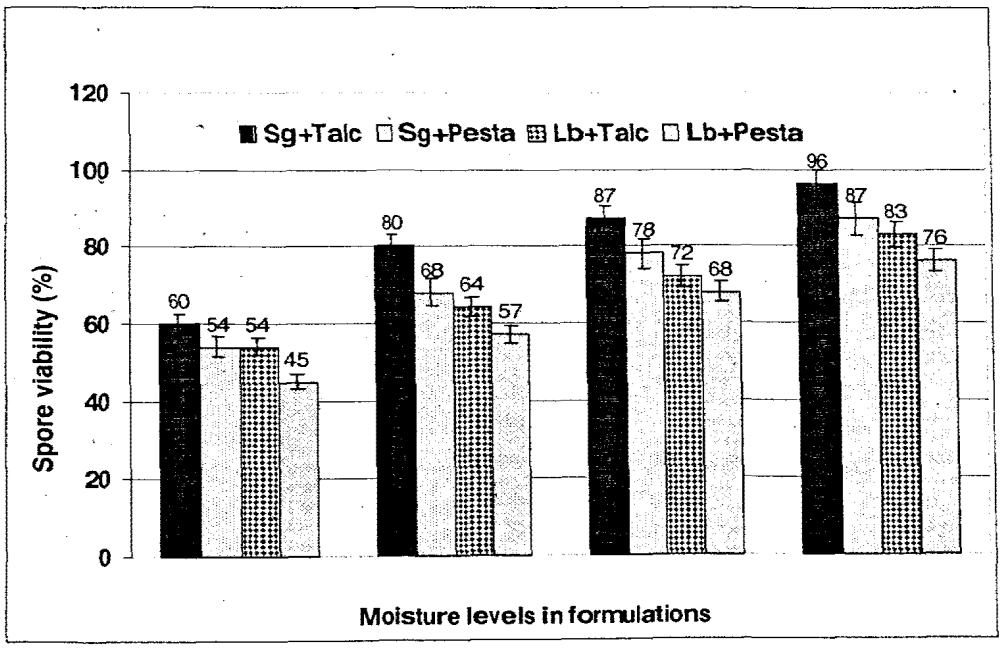

Fig. 1. Effect of moisture levels of *P. lilacinus* formulations on spore viability after 60 days of formulation

that the granular formulations of mycelia and conidia of *V. chlamydosporium* at 2 per cent moisture retained its viability at 25°C for 12 months. This study corroborates that the moisture levels in formulations influence the spore viability under different storage conditions. Further Stirling *et al.* (1998) used kaolin plus *Gum Arabica* with fungal propagules, while in the study under report; wheat flour was used for the preparation of granules. Inert material like talc, kaolinite, bentonite, china clay, charcoal powder could be abetting maintenance of stable moisture levels in formulations. Bansal *et al.* (1992) reported that wood charcoal powder was suitable as carrier material for *P. lilacinus* spores although they did not mention the moisture level in the formulation. In contrast, Cabanillas *et al.* (1989) found that fungal viability was high in wheat and diatomaceous earth granules compared to alginate pellets, soil and soil plus chitin combinations.

The talc and pesta granule formulations of *P. lilacinus* at 10 and 15 kg/ha applied to the root-knot nematode infested chrysanthemum field, singly or in combination with neem cake correspondingly reduced root gall index and nematode populations (Fig. 2a, b). Root gall index (RGI) and nematode multiplication rate (NMR) in untreated check were observed to be 3.2 and 2.9, respectively, while plants treated with aerial spores on sorghum grain formulated as talc in combination with neem cake recorded 0.8 and 1.15 RGI and NMR, respectively, at 10 kg of *P. lilacinus*/ha. At 15 kg of *P. lilacinus*/ha in combination with neem cake, the RGI and NMR were minimum (0.5 and 0.7, respectively). Similarly, *P. lilacinus* as pesta granules from sorghum and liquid broth, and talc powder from liquid broth, in combination with neem cake recorded significantly lower RGI and NMR at 10

And 15 kg/ha doses compared to uninoculated check and carbofuran. As indicated in earlier studies, neem cake as a supplement to antagonistic fungi exhibited synergistic effect against the nematodes in terms of parasitation and reduced soil nematode populations in subsequent generations. Talc formulations of aerial and submerged spores performed better in terms of reduced root-knot index and nematode populations compared to pesta granules (Fig. 2a, b). Per cent healthy root was comparatively higher in plants that received higher dose (15 kg/ha) of *P. lilacinus* formulations than that received 10 kg/ha of *P. lilacinus* (Fig. c). A maximum of 86 per cent healthy root was recorded in plants that received talc formulation of aerial spores (sorghum grain) at 15 kg/ha in combination with neem cake, while the pesta granules of aerial spores plus neem cake, the healthy root was 72 per cent, while, almost the same per cent of healthy root (72 and 70 %) was recorded in plants that received talc and pesta granules of submerged spores (liquid broth cultures) of *P. lilacinus* (15 kg/ha), respectively. Talc formulations of aerial and submerged spores of *P. lilacinus* recorded higher per cent of healthy root compared to the plants that received pesta granules. In contrast, Sankaranarayanan *et al.* (2000) observed that pesta granules of *Verticillium chlamydosporium* were on par with its talc formulation in controlling *M. incognita* on tomato under glass house conditions. The observed difference may be due to the variation in fungal species, crop and cultivation conditions i.e., glass house and field.

Increased application rate of the formulated *P. lilacinus* resulted in increased egg mass parasitation and percent healthy root, more so when integrated with neem cake (Fig. 2 d, e, f). A maximum (52 %) egg mass parasitization was recorded in plants that received talc formulation of aerial spores (sorghum grain) at 15 kg/ha, in combination with neem cake compared to other treatments under field conditions (Fig. 2d). A maximum (31 %) egg mass parasitization was observed in plants that received talc formulation of aerial spores without neem cake at 15 kg/ha. Similarly, the application of aerial or submerged spores of *P. lilacinus* without neem cake recorded egg mass parasitization between 21 and 31 per cent, while the same formulations in combination with neem cake recorded the egg mass parasitization between 30 and 52 per cent. The propagules of *P. lilacinus* in treated soil and on roots of chrysanthemum were highest in plants that received combinations of *P. lilacinus* formulations with neem cake (Fig. 2e, f). In general, the plants that received *P. lilacinus*

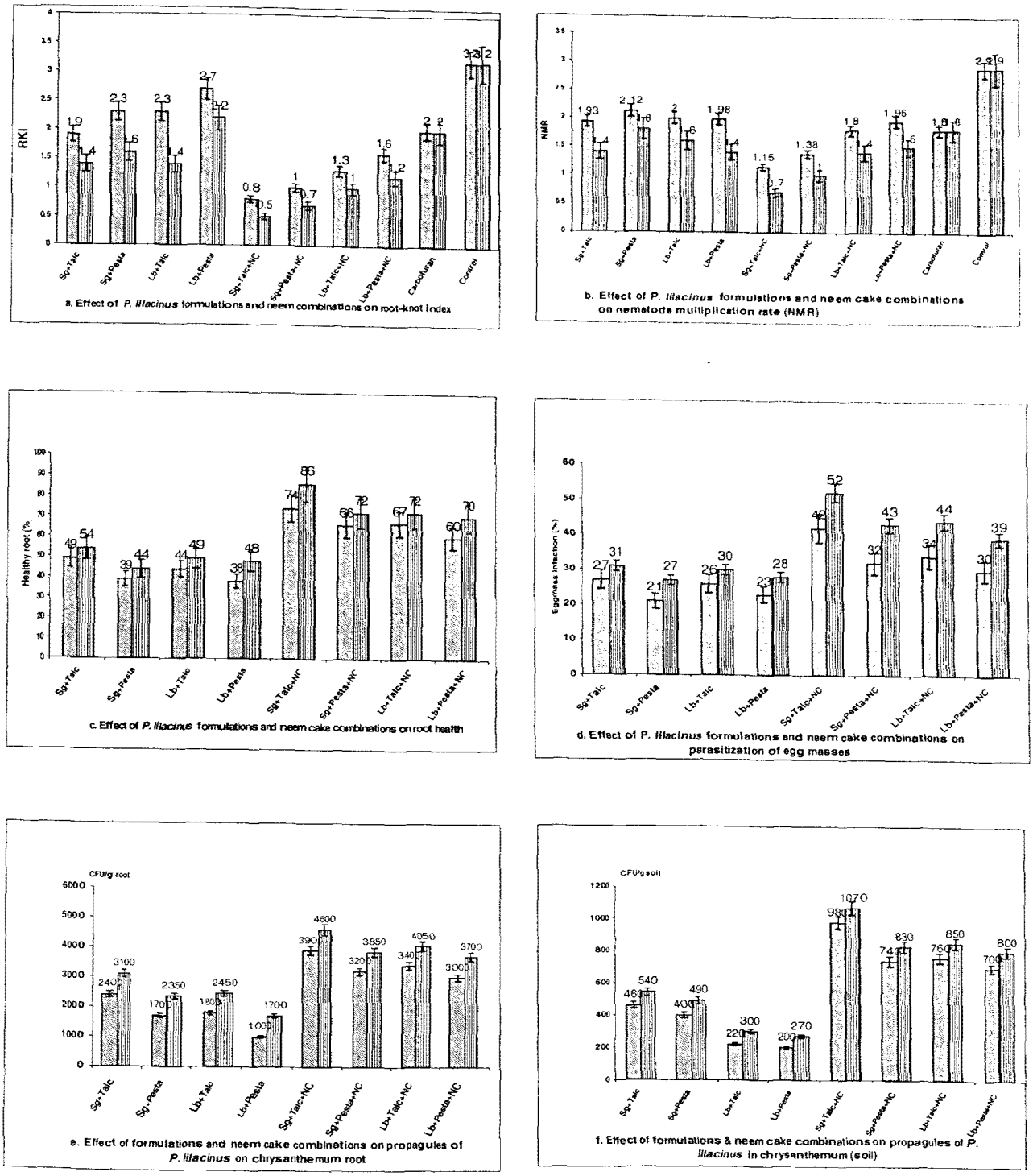

Fig. 2. Effect of *P. lilacinus* formulations and neem cake combinations on (a) root-knot index, (b) multiplication rate of *M. incognita*, (c) healthy root, (d) egg mass parasitization by *P. lilacinus*, (e) *P. lilacinus* propagules in root and (f) *P. lilacinus* propagules in soil

Table 1. Effect of *P. lilacinus* formulations and neem cake combinations on the flower yield and chlorotic plants

Formulation	Chlorotic plants after 60 days of application/10m row		Increase in flower yield/plot (%)	
	10kg/ha	15kg/ha	10kg/ha	15kg/ha
Sorghum grains + Talc	10	7	14	20
Sorghum grains + Pesta	10	8	13	16
Liquid broth + Talc	9	7	10	16
Liquid broth + Pesta	10	7	11	15
Sorghum grains + Talc + Neem cake	4	3	23	28
Sorghum grains + Pesta + Neem cake	8	6	18	20
Liquid broth + Talc + Neem cake	7	6	20	23
Liquid broth + Pesta + Neem cake	9	6	18	19
Carbofuran 3G (1.25kg a. i./ha)	8	8	16	16
Control	14	14	0	0
CD (P=0.05)	3.88	3.98	-	-

spores as pesta granules recorded lower CFUs on root (1000-1700 at 10 kg/ha dose and 1700-2350 at 15 kg/ha, without neem cake; 3000-3200 at 10 kg/ha dose and 3700-3850 at 15 kg/ha dose, with neem cake) and in soil (200-400 at 10 kg/ha dose and 270-490 at 15 kg/ha, without neem cake; 700-740 at 10 kg/ha dose and 800-830 at 15 kg/ha dose, with neem cake) compared to talc formulations. Stirling *et al.* (1998) reported that the formulated products of *V. chlamydosporium* at 10g/litre of soil under glasshouse conditions effectively checked *M. javanica* populations by colonizing the rhizosphere and parasitizing the egg masses of *M. javanica.*

Application of talc formulations of *P. lilacinus* and neem cake reduced the number of chlorotic plants/10 m row compared to untreated check and flower yield was higher (Table 1). Further, combined use of these formulations with neem cake enhanced fungal infectivity and flower yield by 23-28 per cent (Table 1).

The study demonstrated that higher moisture levels in formulations reduced the spore viability of *P. lilacinus.* The field efficacy of these formulations at the lowest moisture content (6%) when combined with neem cake, enhanced the antagonistic potential of chrysanthemum rhizosphere against *M. incognita,* besides increasing flower yield compared to carbofuran treatment. Periodic studies are needed to record the dynamics and behaviour of the nematode and the antagonists, for cataloguing long-term benefits.

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