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Suitability of soil types for *Paecilomyces lilacinus* and *Pochonia chlamydosporia* and their performance against root-knot nematode, *Meloidogyne incognita* on *Lycopersicon esculentum* in glasshouse

M NAGESH[1], SALEEM JAVEED[2], B RAMANUJAM[3] and R RANGESWARAN[4]

National Bureau of Agriculturally Important Insects, PB No. 2491, HA Farm Post, Bangalore, Karnataka 560 024

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ABSTRACT

During 2007-09, five broad soil types, black cotton, red laterite, alluvial, loamy sand and mountain soils collected from Regional Centre, NBSSLUP, Bangalore, were examined for their suitability to NBAII isolates of antagonistic fungi, *Paecilomyces lilacinus* (Samson) Thomson and *Pochonia chlamydosporia* (Goddard) Zare, Gams and Evans in terms of their establishment; temporal survival and behaviour; pathogenicity to *Meloidogyne incognita* (Kofoid and White) Chitw. under glasshouse conditions and to arrive at the baseline data for field guidance. Except black cotton soil, other soil types examined were suitable for tomato root growth, root-knot nematode infection and effective parasitization of the egg masses by the fungi under report. The time-course behaviour of *P. lilacinus* and *P. chlamydosporia* in red laterite, alluvial, loamy and mountain soils were more or less similar. Both the fungi established in the first 2-3 weeks in soils tested, increased in number in the next 6-7 weeks with a plateau at 8-10 weeks after application, marginally declined and reached constancy at 12 weeks after application, following more or less a normal curve. Mountain soil with lower pH and higher organic carbon was best suited to both the fungi in terms of egg mass parasitization and propagules multiplication. Better suitability of mountain soil, red laterite and alluvial soils for these fungal establishment, proliferation and pathogenicity to root-knot nematodes in tomato rhizosphere was attributed to high to medium organic carbon status, near neutral pH and equitable soil physical components.

Key words: Behaviour, Biological control, *Meloidogyne incognita*, Organic carbon, *Paecilomyces lilacinus, Pochonia chlamydosporia,* Soil type, Suitability

The success of classical biological control of soil-borne crop diseases and nematodes using their antagonists through inundative introduction or enhancement depends on several critical factors such as soil properties (physical, chemical and biological), soil moisture conditions, nature of crop, crop management and cropping pattern, and inherent biotic competition. As the soils and soil factors are considered to be complex and multi-factorial, examining each component for their influence on the behavior/dynamics of antagonists, pathogens etc., become experimentally and statistically complex. Further, studies on fate of introduced antagonists and their interactions with the target pathogen(s) and host plant in specific soil types are meagre. Studies in this direction are seriously lacking to define field level database. Therefore, the current study focused on 5 major (broad) soil types with proximal pH, organic carbon status and mechanical components, for their suitability to two fungi, viz. nematode egg-parasitic and root colonizing fungi, *Paecilomyces lilacinus* (Samson) Thomson and *Pochonia chlamydosporia* (Goddard) Zare, Gams and Evans, prospective antagonists of cyst and root-knot nematodes (Bharadwaj and Trivedi 1996, de Leij *et al.* 1991, Freire and Bridge 1985, Nagesh *et al*. 2001, Nagesh *et al*. 2007).

[1] Principal Scientist (e mail: nagesh55@yahoo.com)

MATERIALS AND METHODS

Pure culture of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitw. was continually maintained on eggplants in earthen pots with autoclaved soil. Healthy egg masses were collected individually from the roots of eggplant, cleared of soil, washed in distilled water and then kept on Baermann funnels at 30 °C. Freshly emerging nematode juveniles (collected daily for six days and stored at 8 °C in distilled water) were used for the experiments.

Single spore colony cultures of *Paecilomyces lilacinus* (Thoms.) Samson and *Pochonia chlamydosporia* (Zare *et al.* 2001) (*Verticillium chlamydosporium* synonimized with *P. chlamydosporia*) of NBAII collections (Nagesh *et al.* 2007, Anonymous 2009) grown on PDA and CMA, respectively,

were used in the experiment. The fungi were mass produced on rice grain at 28 ± 1°C and harvested using mycoharvester (CABI, UK). The respective fungal inocula were prepared by mixing harvested spores with autoclaved talc (mesh 200) at 10^6 spores/g of talc.

Five broad soil types, viz. black cotton, laterite, alluvial, loamy sand and mountain soils collected from NBSSLUP Regional Center, Bangalore, with marginal variation in organic carbon (excepting high content in mountain soil) were used in the study. The physical, mechanical and chemical properties such as colour (Munshell color chart); sand silt and clay percent; water holding capacity; soil pH; inorganic nutrient content and organic carbon were recorded from the data base of Regional Centre, National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Bangalore. However, soil parameters such as, soil mechanical composition, soil pH and organic carbon status only was considered for the present study.

Thirty days-old healthy tomato (*Lycopersicon esculentum* L. cv Pusa Ruby) seedlings were transplanted into two sets of earthen pots (22 cm dia.) filled with autoclaved (15 psi for 20 minutes) soils of 5 broad types obtained from NBSS & LUP. One set of pots with tomato were maintained as untreated healthy check, while the second set was inoculated with freshly hatched *M. incognita* juveniles at 2 IJs/g soil. The pre-designated nematode inoculated pots (second set) with specific soil types at 10^2 spores/g of pot soil were treated with talc formulations of *P. lilacinus* and *P. chlamydosporia*.

During 2007-08, observations on root-gall index (on a 1-5 scale, according to Heald *et al.* 1989), propagules of *P. lilacinus* and *P. chlamydosporia* separately in soil, root colonization and per cent egg masses infected in treated pots were recorded using their respective semi-selective media (defined by Mitchell *et al*. 1984, for *P. lilacinus* and Kerry *et al*. 1993, for *P. chlamydosporia*). In the second year (2008-09), studies on the time course behaviour of the fungi in soil and on root were carried out. Fungal propagules g^{-10} soil in treated pots was recorded by serial dilution method. Ten grams of composite soil from each pot was weighed, suspended in 100 ml sterile dH_2O with a constant vortexing for 2 hours and serially diluted to 10^{-4}. One ml of diluted soil suspensions were pipetted into sterile Petri plates followed by addition of semi-selective medium for *P. lilacinus* or *P. chlamydosporia* by pour plate method. For root colonization by the fungi under test, viz. *P. lilacinus* or *P. chlamydosporia*, 10 root bits of 1cm each were collected from treated pots, washed thoroughly to clear the soil particles, rinsed with sterile dH_2O, air dried under laminar hood, and placed on respective semi-selective media. These Petri plates were incubated at 30±1 °C for 7-10 days. Number of root bits with typical fungal growth (matched with conidiospore and sporophore characters of the fungi) was counted for arriving at percentage root colonization. Further, the cfus of *P. lilacinus* and *P. chlamydosporia* in soil and root colonization by respective fungi in treated pots were recorded once in a week, starting from one week after establishment of transplants. Five replications were maintained for each soil type. The numbers of parasitized and healthy egg masses were counted per 10 root bits of 1cm each in order to calculate the percentage of egg masses parasitized. The number of parasitized egg masses was arrived at by randomly picking egg masses with a fine tipped forceps, surface-sterilized by placing them in 0.2% NaOCl for 1 minute, clearing NaOCl with sterile dH_2O and placing them on the semi-selective media in 9cm Petri-plates. All data were subjected to analysis of variance (CRD) and the means compared by Duncan's multiple-range test.

RESULTS AND DISCUSSION

For our studies we considered broad soil types appropriate for developing baseline data on suitability to arrive at some of the critical factors for further examination. Important soil factors considered for suitability were soil pH, soil silt, clay and organic carbon contents. The four typical soils under study exhibited variation in textural compositions such as sand, silt and clay, and chemical properties such as pH, organic carbon status etc. (Table 1). Red laterite and alluvial soil samples recorded a near neutral pH of 6.8 and 6.7, respectively, with a moderate organic carbon content of 1.1 to 1.3 percent, respectively. The pH was 5.6 with a high organic carbon status in case of mountain soil (Sulya). Excepting black cotton soil, other soils recorded a moderate to high organic carbon status (Table 1). Root weight of tomato in untreated healthy control was highest in mountain soil closely followed by alluvial, red laterite, loamy sand and black cotton soil (Table 1) indicating the general soil suitability to tomato root growth.

Root-knot index caused by *M. incognita* in untreated tomato roots was highest (3.6) in mountain and alluvial soils; closely followed by lateritic and loamy sand soils (3.4) and black cotton soil (2.5) (Table 1). In fungus treated soils, pooled root-knot index recorded significantly lower values in all soils, lowest being mountain and black cotton soil, followed by red laterite and alluvial soil, while the reduction in root-knot index values was medium in loamy sand soil (Table 1).

Parasitization of egg masses of *M. incognita* in tomato was highest in mountain soil by *P. lilacinus* and *P. chlamydosporia* (62 and 58%, respectively) followed by the fungal parasitisation in red laterite (52 and 46%, respectively), alluvial (54 and 44%, respectively) and sandy loam (38 and 51%, respectively) (Table 1). In black cotton soil parasitization of egg masses by *P. lilacinus* and *P. chlamydosporia* was lowest, 18 and 13%, respectively) (Table 1). Correspondingly, the propagules (g^{-10} soil) of *P. lilacinus* and *P. chlamydosporia* in mountain soil were highest (1 486 and 922, respectively) and lowest in black cotton soil (238 and 146, respectively)

Table 1 Effect of soil types on the pathogenicity of *P. lilacinus* (PL) and *P. chlamydosporia* (PC) against root-knot nematode on tomato in glasshouse (2007-08)

Soil type	Physical composition			Organic carbon	pH	Tomato (60 days old)			Egg mass parasitization (%)		Fungal propagules/ 10g soil	
	Sand (%)	Silt (%)	Clay (%)	(%)		Healthy root wt (g)	RKI*	RKI**	PL	PC	PL	PC
Red laterite (Soil A)	36	44	20	1.1 (moderate)	6.8	7.3^{c}	3.4^{b}	2.4^{b}	52c	46^{b}	1226^{c}	688^{b}
Alluvial (Soil B)	48	33	19	1.3 (moderate)	6.7	7.4^{c}	3.6^{b}	2.5^{b}	54c	44^{b}	1112^{b}	744^{c}
Loamy sand (Soil C)	72	16	12	0.98 (moderate)	7.6	6.9^{b}	3.4^{b}	3.0^{c}	38b	51^{b,c}	998^{b}	692^{b,c}
Black cotton soil (Soil D)	26	28	38	1.0 (Low)	8.7	5.6^{a}	2.5^{a}	2.0^{a}	18a	13^{a}	238^{a}	146^{a}
Sulya mountain soil (Soil E)	22	60	18	2.6 (High)	5.6	8.1^{d}	3.6^{b}	2.0^{a}	62d	58^{b}	1486^{d}	922^{d}
F-test						S	S	S	HS	HS	HS	HS
CD (P=0.01)						0.38	0.24	0.21	4.4	6.2	45.4	33.6

*RKI-Root-knot index in untreated root-knot nematode infected plants; ** Pooled RKI-Root-knot index in root-knot nematode infected plants treated with PL or PC; S- significant at 1%; HS- Highly significant at 1%. Values are means of 5 replicates. Means followed by the same letter within a column are not significantly different according to Duncan's multiple range test.

(Table 1). Propagules of *P. lilacinus* were higher in red laterite (1 226/g soil) compared to alluvial (1 112/g soil) and loamy sand (998/g soil) soils, while the propagules of *P. chlamydosporia* were higher in alluvial (744/g soil) compared to red laterite (688/g soil) and loamy sand (692/g soil). Nagesh and Parvatha Reddy (2001) recorded the propagules of *P. lilacinus* and *P. chlamydosporia* 2 years after fungal incorporation in carnation and gerbera rhizospheres in commercial poly houses, recording an increase of 11 to 57% over the propagules number after first year. Also the parasitization of egg masses and eggs of *M. incognita* by the fungi could be observed after 2 years of application in commercial poly house conditions. The carnation beds were enriched with high organic manure and had favourably influenced the fungi. However, the soil type was not considered typical as the plant beds/substratum was extremely enriched with organic manure.

Of the five broad soil types examined for suitability for fungal establishment and parasitisation of the nematode egg masses, mountain soil with high organic carbon status, favourable pH and textural composition, recorded better establishment of the fungi and their pathogenicity, followed by soils (red laterite, alluvial, loamy sand) with moderate organic carbon status, near neutral pH and optimal textural composition. Black cotton soil with high pH, low organic status and textural composition recorded significantly low levels of fungal establishment and parasitization indicating low level of suitability to both *P. lilacinus* and *P. chlamydosporia* isolates. Melakeberhan *et al.* (2010) observed that sandy loam soil favoured more *M. hapla* nematodes compared to sandy or muck soil with arugula (*Eruca sativa*), a high-end green vegetable crop, in pot studies.

de Leij *et al.* (1993) examined survival, multiplication and establishment of *V. chlamydosporium* on the rhizoplane of tomato in peat, loamy sand and sand. It was observed that the fungus survived, but did not multiply, in loamy sand or sand. Establishment of *V. chlamydosporium* on the rhizoplane of tomato plants was greater in peaty sand than in loamy sand or sand. Nematode control was in general greater in peaty sand (average 59% control) than in the other two soil types (average control in loamy sand 51% and in sand 39%). Subsequently in a microplot experiment on sandy loam, *V. chlamydosporium* controlled populations of *M. hapla* on tomato plants by more than 90%. The fungus multiplied and survived in soil for at least 123 days. More fungus was found in rhizosphere soil than in non-rhizosphere soil.

Behaviour of antagonistic fungi in 5 typical soil types

Propagules of fungi as influenced by soil types: Behaviour of *P. lilacinus* and *P. chlamydosporia* in terms of propagules (cfus/g soil) recorded in each soil type at weekly intervals for 12 weeks were more or less similar. In both the fungi the propagules number decreased from initial application of 100 cfus/g soil to a minimum of 8-10/g in the first and second week after application, which in subsequent weeks of observation exhibited increasing trend up to 9 and 10 weeks of application (Fig 1 and 2). The propagules number recorded a declining trend from 10 and 11 weeks which after 12 weeks stabilized in all the five soils. Among the 5 soils under study, the propagules number of *P. lilacinus* and *P. chlamydosporia* were highest at all intervals in mountain soil followed by red laterite, alluvial and loamy sand, with black cotton soil

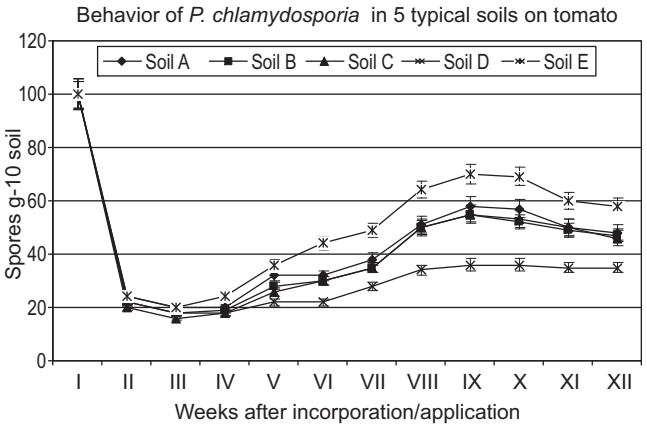

Fig 1 Time-course behaviour of *P. chlamydosporia* in 5 typical soils on tomato (2008-09). (Soil A- red laterite; Soil B- Alluvial; Soil C- sandy loam; Soil D- black cotton; Soil E- mountain soil).

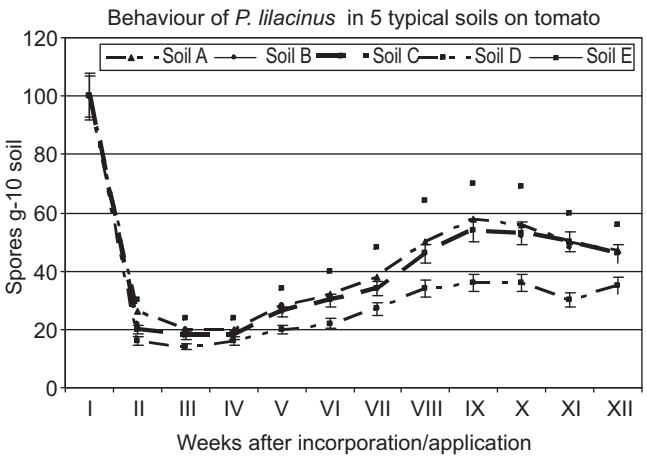

Fig 2 Time-course behaviour of *P. lilacinus* in 5 typical soils on tomato (2008-09). (Soil A- red laterite; Soil B- Alluvial; Soil C- sandy loam; Soil D- black cotton; Soil E- mountain soil).

recording the lowest cfus/g at all the intervals. In general, *P. chlamydosporia* propagules were marginally higher in number in corresponding soils compared to *P. lilacinus*.

These results indicate that the fungi under study established in soil in the first 2-3 weeks, increased in number in the next 6-7 weeks with a plateau at 8-10 weeks after application, marginally declined and reached a constancy at 12 weeks after application at the given *in vitro* (pot) conditions, following more or less followed a normal curve.

Root colonization by the fungi as influenced by soil types: Pattern of colonization of tomato roots by *P. lilacinus* and *P. chlamydosporia* were more or less similar in terms of percent root bits colonized (Fig 3 and 4). Tomato roots in fungus-treated soil types did not exhibit any fungal colonization till 3rd week after application (Fig 3 and 4). Colonization by the two fungi was detected in roots (3-5%) from 3rd week, which subsequently increased to 30-60% (9 to 10 weeks after application) depending on the soil type. Root colonization was highest in mountain soil followed by red laterite, alluvial and loamy sand, while it was lowest in black cotton soil for both the fungi. Root colonization by the fungi recorded a decline from 11 and 12 weeks after application in all the 5 soils. Mauchline *et al.* (2002) used molecular technique (cPCR) to study the behaviour of *V. chlamydosporium* in tomato rhizosphere against root-knot (RKN) and potato cyst (PCN) nematodes and observed increases in fungal growth in the rhizosphere of PCN-infested plants but not in the rhizosphere of RKN-infested plants after 14 weeks and provided evidence for nematode host specificity, survival and multiplication of the fungus that is highly relevant to the biological control efficacy of this fungus in a specific plant rhizosphere. Kerry *et al.* (2008) reported that isolates of *Verticillium chlamydosporium* and a sterile fungus added to soil on ground oat grain reduced the numbers of *Heterodera avenae* on wheat between 26 and 80%. The effect of the fungi on numbers of *H. avenae* eggs was similar in autoclaved

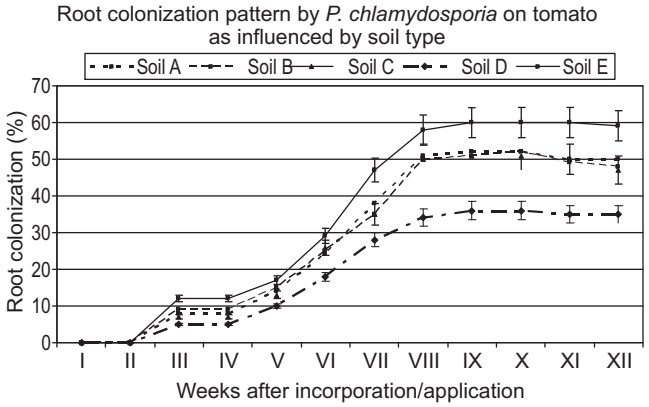

Fig 3 Colonization of tomato root by *P. chlamydosporia* as influenced by soil type (2008-09). (Soil A- red laterite; Soil B- Alluvial; Soil C- sandy loam; Soil D- black cotton; Soil E- mountain soil).

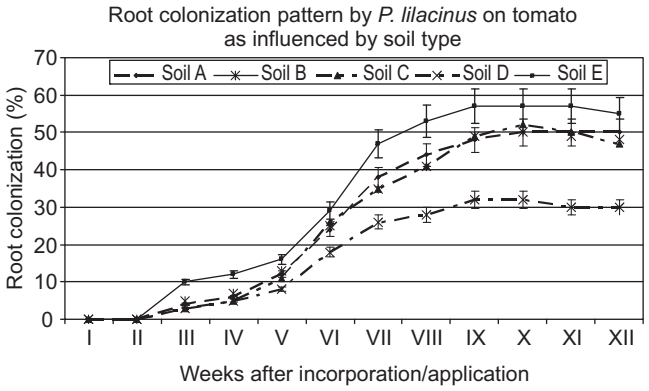

Fig 4 Colonization of tomato root by *P. lilacinus* as influenced by soil type (2008-09). (Soil A- red laterite; Soil B- Alluvial; Soil C- sandy loam; Soil D- black cotton; Soil E- mountain soil).

and non-sterilized soil. *V. chlamydosporium* added on attapulgite clay to calcareous sand and a calcareous silty loam could be re-isolated after at least 6 months. Some isolates colonised the roots of wheat without causing lesions or affecting the dry weights of shoots or roots.

In the present study the observed increase in tomato root colonization in the soil types tested with weeks after application in general, coincided with increased cfus of corresponding fungi, viz. *P. lilacinus* and *P. chlamydosporia* in soil. Although the root colonization and number of propagules in soil under *in vitro* (pot) conditions are generally observed to be higher than that in field due to confinement of root mass and spread, and higher levels of moisture, the behavior of the fungi follows similar pattern depending on the soil type. As discussed in the text, soil features such as organic status, pH and textural composition of the typical soils examined were critical factors for fungal establishment, parasitization and root colonization. The results of the study indicated that mountain soil followed by red laterite and loamy sand with high to moderate carbon status, moderate pH and equitable textural composition favoured these two fungi for their establishment and antagonism against *M. incognita*, which can be considered as guideline data for field application of these antagonists. Further, the fungal propagules in soil and in root in the initial 2-3 weeks declined, subsequently recorded an increase in the next 7 weeks and stabilizing at 11-12 weeks in all the 5 soil types used under glasshouse conditions. However, it is appropriate to consider that such studies need to be carried out intensively with more number of representative soils with variability in each factor and then corroborated with *in vivo* field monitoring over a longer period. Further, Atkins *et al.* (2005) employed advanced molecular tool, Real-time PCR in monitoring fungal spatio-temporal population changes in response to exogenous inputs, including changes in crops, farm practices, chemical inputs, and the presence of nematodes on roots and in soil. Glasshouse studies such as ours aid in developing baseline data, and observations on such time-course behavioural studies of the fungi in complex crop-soil conditions under *in vivo* systems could be best corroborated with the support from valuable molecular tools.

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