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Liquid bioformulations for the management of root-knot nematode, *Meloidogyne hapla* that infects carrot

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ABSTRACT

The field efficacy of *Pseudomonas fluorescens*, *Purpureocillium lilacinum* and *Trichoderma viride* liquid formulations was investigated against natural populations of root-knot nematode, *Meloidogyne hapla*. Two field experiments were conducted to test the effect of these biocontrol agents by seed treatment (ST) at 100 mL/kg of seed and by soil drenching (SD) at 5 L/ha. Their effect was compared with that of carbofuran 3G at 1 kg a.i. ha^{-1}. Results showed that all the tested biocontrol agents were capable of reducing *M. hapla* juvenile (J2) population in soil, infection of female population in roots and egg numbers per gram of root at various levels. The biocontrol efficacy also varied according to the delivery method. The *P. fluorescens*-ST caused greatest reduction of J2 populations (67–69%) in soil, female infection (67–69%) in roots and egg numbers per gram of root (68–69%). Similar efficacy was observed in *P. lilacinum*-SD, which reduced J2 population by 64–67%, infection of female populations by 62–63% and egg numbers by 63–66%. The carrot plants treated with *P. fluorescens*-ST and *P. lilacinum*-SD were 33–36% taller with 28–36% more leaves and 27–30% longer leaves than untreated plants. It resulted in 20–21% higher root tuber yield in *P. fluorescens*-ST and 19% in *P. lilacinum*-SD plots. Root colonization of the introduced *P. fluorescens* was 6 times higher when it was applied as ST than as SD. The trend was opposite in *P. lilacinum,* which colonized 4–5 times higher in the SD method than as ST. The efficacy was comparable with that of carbofuran application. It is concluded that *P. fluorescens*-ST at 100 mL/kg of seed and *P. lilacinum*-SD at 5 L/ha are highly effective for the management of *M. hapla,* which infects carrot under field conditions.

1. Introduction

Carrot (*Daucus carota* subsp *sativus* (Hoffm.) Schubl. & G.Martens: Apiaceae), an important root crop, is one of the top ten vegetable grown worldwide in tropical and subtropical areas (FAOSTAT, 2014). Roots are used for making a variety of cuisines including salad, pickle and juice. In addition, the seeds are used for extraction of essential oil. Carrot roots are rich source of provitamin A (alpha and beta carotene), vitamin K and vitamin B6. It is cultivated commercially in more than 130 countries in 1.59 million ha with 49.35 million tonnes of production globally. India is one of the major producers of carrots with an area of 0.32 million ha and produced in 0.49 million tonnes annually (FAOSTAT, 2014). Root parasitism by the root-knot nematode, *Meloidogyne* spp. remains a major hurdle in commercial carrot production, which causes severe yield loss in terms of quality and quantity. Worldwide, the carrot growers are confronted by six different species of root-knot nematodes including *Meloidogyne hapla* Chitwood, *Meloidogyne incognitia* (Kofoid & White) Chitwood, *Meloidogyne javanica* (Treub) Chitwood, *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, *Meloidogyne fallax* Karssen and *Meloidogyne polycephannulata* Charchar, Eisenback, Vieira, Fonseca-Boiteux & Boiteux (Huang, 1984; Wesemael and Moens, 2008; Charchar et al., 2009).

The northern root-knot nematode, *M. hapla* is a key pest of carrots in India (Devrajan et al., 2003; Seenivasan, 2017b). It is a sedentary endoparasitic nematode. The second-stage infective juveniles (J2) penetrate through growing tips of roots and forms several multinucleate giant cells in vascular tissues. This leads to the formation of galls, hairiness, digitation and constriction in taproots, which results in forked and defective carrots. Loss of quality by means of malformation (forking) happens even at low infestations, whereas the formation of taproot is drastically arrested under severe infestations (Belair and Fournier, 1997; Nagachandrabose and Baidoo, 2017). The estimated yield loss due to *M. hapla* in carrot is 24–55% by quantity and 13–77% by quality (Belair and Fournier, 1997; Gugino et al., 2006;

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Nagachandrabose and Baidoo, 2017).

Chemical nematicides, either organophosphate or carbamate, are the primary ways of management of *M. hapla* on carrot (Belair and Fournier, 1997; Gugino et al., 2006). However, it requires a high dose or repeated application to maintain nematode population density below threshold levels (Seenivasan, 2017a). Frequent use of toxic chemicals poses concerns of potential residues, ground water contamination, lethal effect on non-target organisms, and the application safety. Crop rotation with antagonistic or non-host plants is also a standard practice against *M. hapla* (Belair and Parent, 1996; Devrajan et al., 2003). Crop rotation is commonly practiced by Indian carrot growers with plants such as potato, beans, cabbage, cauliflower and peas, which are all susceptible hosts of *M. hapla*. Only a few varieties or germplasms have been identified as resistant to *M. hapla* in carrots (Gugino et al., 2006), and none of the commercial cultivars grown in India have been found to be tolerant or resistant to *M. hapla* (Nagachandrabose and Baidoo, 2017). Under these circumstances, there is a huge demand among carrot growers for a potential nonchemical alternative approach for *M. hapla* management.

Biological control with fungal or bacterial organisms that effectively antagonise the nematodes has tremendous potential to control nematode population build up and thereby reduce the crop damage. Currently, the field application of bio-agents for nematode management is becoming popular among farmers. If the introduced bio-agent establishes well with the nematode populations, the infestation can last for the complete season and more than that period (Jacobs et al., 2003). The root colonizing plant-growth promoting rhizobacterium, *Pseudomonas fluorescens* Migula, has shown better results for the management of various plant parasitic nematodes such as *Heterodera schachtii* Schmidt on sugarbeet (Oostendorp and Sikora, 1989), *Hirschmanniella oryza* (Van Breda de Haan) Luc & Goodey on rice (Seenivasan and Lakshmanan, 2002), *Globodera rostochiensis* (Wollenweber) Behrens on potato (Devrajan et al., 2004), *Meloidogyne graminicola* Golden & Birchfield on rice (Seenivasan, 2011), *Radopholus similis* (Cobb) Thorne on banana (Seenivasan et al., 2013), *M. javanica* on tomato (Siddiqui and Shaukat, 2004), and *M. incognita* on tomato, medicinal coleus and jasmine (Siddiqui et al., 2001; Seenivasan and Devrajan 2008; Seenivasan and Poornima, 2010). The facultative egg parasitic fungus, *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson *(Paecilomyces lilacinus*), has been reported to be effective against *Meloidogyne* spp. and many other plant parasitic nematodes in various crops (Rao, 2008; Rao et al., 2012; Crow, 2013; Mohd et al., 2009). The nematode antagonistic fungus, *Trichoderma viride* Weindling and Fawcett, has also been shown as a potential bio-agent against *Meloidogyne* spp. in various crops (Affokpon et al., 2011). However, the efficacies of *P. fluorescens, P. lilacinum,* and *T. viride* have not been yet tested against *M. hapla* on carrots.

In recent years, the liquid formulations of these bio-agents have been developed and reported to be more effective than solid carrier formulations such as peat and talc formulations (Selvaraj et al., 2014; Song et al., 2016; Kolombet et al., 2008). Liquid bioformulations have the following advantages over the other solid carrier formulations, namely, more cell counts, free from contamination, long storage life, and more virulence. It is well established that in liquid formulations the microbial bio-agents remain in the dormant stage and they become active when applied to soil rhizosphere regions of the field. This phenomenon helps to increase their shelf life in liquid formulations. The extended storage life of *P. fluorescens*, *P. lilacinum,* and *T. viride* for more than 1 year has already been demonstrated (Kolombet et al., 2008; Manikandan et al., 2010; Song et al., 2016). The application of liquid *P. fluorescens* significantly reduced the spiral nematode, *Helicotylenchus multicinctus* (Cobb) Golden by 89% in banana fields (Selvaraj et al., 2014), and the application of liquid *P. lilacinum* parasitized eggs and female population of *M. incognita* efficiently in tomato under glass house conditions (Song et al., 2016). Considering the potential advantages, the present investigation was carried out to test the field efficacy of liquid bioformulations of *P. fluorescens*, *P. lilacinum,* and *T. viride* for the management of *M. hapla* infection on carrot.

2. Materials and methods

2.1. Experimental site

Two field experiments were conducted during 2014–2015 at Kodaikanal, Tamil Nadu, India. Experiment I was conducted in a farmer's field during October 2014–January 2015 at Shenbaganur village, Kodaikanal, Tamil Nadu, India which lies at 10.24° N latitude and 77.52° E longitude at 2200 m above MSL. The soil is peaty and laterite type with texture of sandy clay loam (15% clay, 35% silt, and 55% sand), pH: 6.1, cation exchange capacity: 9.0 c mol (p^+)/kg, organic carbon: 1.8 g/kg, electrical conductivity: 0.30 dS/m, available N: 390 kg/ha, P: 32 kg/ha, K: 150 kg/ha, Fe: 125 ppm, Cu: 1.5 ppm, Mn: 1.4 ppm, and Zn: 2.8 ppm. Experiment II was conducted during June 2015–September 2015 at a research farm, Horticultural Research Station, Tamil Nadu Agricultural University, Kodaikanal, Tamil Nadu, India, which lies at 10.36° N latitude and 77.98° E longitude at 2300 m above MSL. The soil is peaty and a laterite type with the texture of sandy clay loam (16% clay, 34% silt, and 50% sand), pH: 6.0, cation exchange capacity: 8.1 c mol (p^+)/kg, organic carbon: 1.5 g/kg, Electrical conductivity: 0.40 dS/m, available N: 410 kg/ha, P: 30 kg/ha, K: 130 kg/ha, Fe: 105 ppm, Cu: 1.3 ppm, Mn: 1.4 ppm and Zn: 2.9 ppm. Both fields were naturally infested with *M. hapla*.

2.2. Pseudomonas fluorescens

The bacterial biocontrol agent, *P. fluorescens* strain Pf1 used in this study was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India and was maintained on Kings B (KB) agar slants at 4 °C. The bacteria were developed as an antibiotic resistant strain as per Seenivasan et al. (2012), and they grew well in KB media amended with streptomycin (30 μg mL^{-1}), actinomycin D (100 μg mL^{-1}), penicillin (90 μg mL^{-1}) and rifampicin (190 μg mL^{-1}).

The liquid formulation was prepared using nutrient broth (HiMedia Laboratories, Mumbai, India) amended with 2% glycerol. A two-day-old fresh culture of Pf1 was grown in KB broth and used as the mother culture. Forty eight hours later, the well-grown mother culture was centrifuged at 5000 rpm for 10 min at 4 °C. The bacterial pellets were collected by discarding the supernatant and suspended in double distilled water using a sterile glass rod. From the log phase culture of bacterial suspension that has 3×10^{10} colony forming units (CFU), 1 mL was inoculated in 300 mL of nutrient broth containing 2% glycerol, and this was incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28 ± 3 °C). The multiplied bacterium in the liquid formulation was collected in 1 L capacity plastic containers and sealed aseptically. The packed *P. fluorescens* was stored at room temperature (28 ± 3 °C) and used for two consecutive field experiments. The bacterial population at the time of packing was 5×10^9 CFU/mL.

2.3. Purpureocillium lilacinum

The *P. lilacinum* strain Ooty1 was obtained from Horticultural Research Station, Tamil Nadu Agricultural University, Ooty, India, cultured in potato dextrose agar slants and maintained at 4 °C. The *P. lilacinum* was subcultured on potato dextrose broth (PDB) for 15 days, and 1 mL of spore suspension containing 5×10^6 CFU/mL was used as the mother culture. The *P. lilacinum* was multiplied by inoculating 1 mL of mother culture into 300 mL of PDB containing 0.06 g of ferrous sulphate and 2% glycerol in 500 mL conical flasks. The flasks were incubated on a rotary shaker at 150 rpm for 20 days at room temperature (28 ± 3 °C). The multiplied fungus in liquid formulation was collected in 1 L capacity plastic containers and sealed aseptically. The