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Management of the root-knot nematode *Meloidogyne graminicola* infesting rice in the nursery and crop field by integrating seed priming and soil application treatments of pesticides

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ABSTRACT

The study evaluates the effects of seed priming (5 g or ml kg^{-1} seed) and soil application (2 kg or l ha^{-1}) of eight organophosphate pesticides on rice root-knot disease caused by *Meloidogyne graminicola*. Seed priming (SP) or soil application (SA) of phorate, carbofuran and chlorpyriphos (1000 J_2 of *M. graminicola* kg^{-1} soil) suppressed galling in the rice nursery by 92 and 99%, 80 and 88% and 76 and 80%, respectively, over control. Relatively similar decreases in the galling were recorded when this nursery was grown for four months in the sterilized soils in earthen pots. Rice cv. PS-5 grown in naturally infested soil in earthen pots (1000 J_2 kg^{-1} soil) became stunted, showing chlorotic foliage, and terminal galls developed on the roots. The treatment of SP + SA 15 + 30 days after planting (DAP) with phorate, carbosulfan, and chlorpyriphos significantly suppressed the root-knot development and improved the plant growth of rice over the controls ($P \leq 0.05$). The overall effect of the SP + SA 15 DAP treatments was marginally weaker than the SP + SA 15 + 30 DAP treatments but statistically on par. Under field conditions, the greatest decrease in the galling occurred due to SP + SA 15 + 30 DAP of phorate (69–71%) and SP + SA 15 DAP (65–67%) followed by carbosulfan and chlorpyriphos. The yield of rice plants was also highest with phorate (32–36% and 29–34%) over the control during the two years of the study. The soil population of *M. graminicola* decreased by 58–84% over four months due to the phorate treatments. The study demonstrates that seed priming with phorate effectively controls nematode infections in the nursery and that soil application at 15 DAP (2 kg ai ha^{-1}) prevents root-knot development in an infested field under irrigated conditions. Use of SP + SA 15 DAP may enable to avoid one soil application of phorate in the field.

1. Introduction

Root-knot nematode, *Meloidogyne* spp. is one of the most devastating and widespread nematode pests of agricultural crops (Sasser, 1989). The nematode has exceedingly wide host range and attacks almost all cereal, vegetable, pulse, fiber, fruit and beverage crops (Bridge et al., 2005). Rice is the world's most important staple food and is cultivated in around 162 mha annually with an annual global production of 464 mmt (FAOSTAT, 2013). Rice is cultivated in five major ecosystems viz., irrigated, deep water, upland, lowland and rainfed rice. About 53% of the world's rice is grown under irrigated conditions (having good water control) that provides 75% of total global production (Bridge et al., 2005). Rainfed lowland rice (31% of the world rice area) is entirely dependent on rainfall, whereas the deep water areas (35%) occurs in the river deltas. Upland rice area (13%) is also rainfed but without surface water accumulation (Bridge et al., 2005).

Rice is quite susceptible to root-knot nematode and is attacked by *Meloidogyne incognita*, *Meloidogyne graminicola*, *Meloidogyne triticoryzae*, *Meloidogyne javanica*, *Meloidogyne oryzae* and *Meloidogyne arenaria* (Gaur and Pankaj, 2010). Amongst these species, *M. graminicola* is a primary pest of rice and poses a substantial threat to rice cultivation in particular Southeast Asia where around 90% of the world rice is grown and consumed (Dutta et al., 2012). Rice root-knot nematode, *M. graminicola* causes terminal, hook shaped or spiral galls which are characteristic symptoms of the infection of this nematode species (Khan et al., 2012). In India, rice is grown in all five major ecosystems (Jairajpuri and Baqri, 1991). *M. graminicola* is highly damaging to upland, rain-fed lowland

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(Prot et al., 1994) and irrigated rice cultivation (Netscher and Erlan, 1993). In India, this nematode has been found to cause yield losses of 16–32% in rainfed and upland rice (Prasad et al., 2010).

There are various methods of nematode management that may prove effective against rice root-knot nematodes (Gaur and Pankaj, 2010). Despite the known deleterious effects of chemicals, pesticides are still the most effective means of nematode management in rice ecosystems (Khan et al., 2012). In view of limited availability of rice cultivars resistant to *M. graminicola* (Dutta et al., 2012), pesticides are preferred by farmers, as they provide instant results, whereas other disease management practices only begin to have a recognizable impact over considerable time. Pesticides can function through contact and/or via systemic action, and are applied through different modes, such as seed (Jain and Gupta, 1990), bare root-dipping (Jain and Bhatti, 1991) and nursery bed treatments (Jain and Gupta, 1990), but soil application is a universal method (Jain and Bhatti, 1988).

Pesticides from the organophosphate group have been found to be effective against *M. graminicola*, though the degree of control achieved may vary greatly with the mode of application (Khan and Jairajpuri, 2010). The application of carbosulfan and chlorpyriphos at concentrations of 0.1 and 0.2%, respectively, to rice seedlings through root-dipping treatments for 6 h was found to significantly suppress galls and increase plant growth (Khan et al., 2012). Seedlings grown from carbosulfan-treated seeds show fewer galls and egg masses, and this treatment reduces the nematode population by up to 82% (Rahman and Das, 1994). Soil application of phorate and carbosulfan at 1 kg a.i. ha^{-1} or more also significantly reduces galling on rice plants (Prasad and Rao, 1976, 1977a,b).

A critical analysis of the available information reveals that most of the studies in this area have been carried out using phorate and carbosulfan with multiple treatments, whereas other organophosphate pesticides have seldom been tested against *M. graminicola* in rice. In addition, the effects of different modes and times of application have not been adequately studied. In the present study it has been attempted to target the chemicals precisely and specifically against *M. graminicola* by applying the pesticides on seeds. Farmers in India who realize the significance of rice root-knot nematode generally apply pesticides at 2–3 kg a.i. ha^{-1} atleast twice, one or two days before the irrigation (Prasad et al., 2010). Despite of giving two high doses of pesticides, the root-knot disease persists in the field because of rapid percolation of the chemicals beyond the root zone of plants under saturated conditions resulting from irrigation. In the present study, we attempted to avoid the loss of pesticides in soil by giving the soil treatments 2–3 days after irrigation when the soil is only wet not flooded. However, a major objective of the study was to reduce the cost of management and to prevent environmental contamination by reducing the dose and the number of treatments of the pesticides. In the present study, we first evaluated the effectiveness of seed priming (SP) and soil application (SA) of the pesticides to control *M. graminicola* in the rice nursery. Thereafter, persistence of SP and SA treatments was tested by allowing the nursery to grow in the nematode infested and non-infested soil for four months. Management of root-knot in a nursery shall prove a much effective tactic in preventing the spread of *M. graminicola* as well as controlling the nematode attack in rice cultivation. To improve effectiveness, soil application treatments were integrated with SP. Hence, in the present study, the effectiveness of different combinations of seed priming and soil application (after 15 and 30 days of planting of seedlings) using eight organophosphate pesticides, viz., phorate, carbosulfan, malathion, dimethoate, monocrotophos, dichlorvos, methyl parathion and chlorpyriphos was examined against *M. graminicola* Golden and Birchfield. The effect of the SP and soil application (SA) treatments was first evaluated in relation to the control of root-knot in a rice nursery. Thereafter, the evaluation was done under pot conditions, followed by a field trial in two consecutive years to ascertain the effectiveness of the three pesticides found to be most effective pesticides tested in the pot trial.

2. Materials and methods

2.1. Rice nursery

Certified seeds of rice *Oryza sativa* L. cv. PS-5 (Pusa Sugandha-5) were procured from an authorized dealer in Aligarh, India. This cultivar is widely cultivated in the northern India and has been found to be highly susceptible to *M. graminicola* (Khan and Anwer, 2011). The seeds were soaked in water for 12 h and then transferred to a clean muslin bag. The bag was hung in the shade for 24 h to facilitate seed germination. Thereafter, the seeds were sown in 25 cm-diameter earthen pots containing 2 kg of autoclaved soil (four parts sandy loam plus one part farm yard manure). The pots were watered daily. The seedlings reached transplantation stage (four leaves and 12–15 cm height) in 4 weeks.

2.2. Pesticides and their doses

The chemical pesticides phorate (Ambuja Agrochem, India), carbosulfan (FMC, India), malathion (Insecticides India Ltd), dimethoate (Vallabh Pesticides Ltd., India), monocrotophos (Unique Farm Aid, India), dichlorvos (Crop Life Science Ltd., India), methyl parathion (Crop Chemicals India Ltd.) and chlorpyriphos (Century, India) were procured from an authorized pesticide dealer in Aligarh. The pesticides were applied to the pot soil (1 kg) at the recommended dose: 25 mg phorate 10G (equivalent to 2 kg a.i. ha^{-1}); 10 μl carbosulfan 20 EC (2 l a.i. ha^{-1}); 720 μl malathion 50 EC (0.06%, 3 l a.i. ha^{-1}); 100 μl methyl parathion 50 EC (0.08%, 4 l a.i. ha^{-1}); 100 μl dimethoate 30 EC (0.05%, 1.5 l a.i. ha^{-1}); 400 μl dichlorvos 76 EC (0.05%, 1.6 l a.i. ha^{-1}); 1000 μl monocrotophos 36 SL (0.06%, 1.7 l a.i. ha^{-1}) and 25 μl chlorpyriphos 20 EC (0.1%, 2 l a.i. ha^{-1}) per pot. In the field trial, phorate, carbosulfan and chlorpyriphos were applied at 2 kg or l a.i. ha^{-1}. For SP, the pesticides were applied at 5 g or ml kg^{-1} dry seeds.

2.3. Pot trial

Bulk soil was collected in July–August in two consecutive years from the same rice fields, which showed symptoms of root-knot caused by *M. graminicola*. Infected rice roots with galls were also collected. Ten females were excised from the galled tissue, and their perineal patterns were prepared to identify the *Meloidogyne* species associated with the galls (Yik and Birchfield, 1979). The soil was thoroughly mixed, and the population of *M. graminicola* and other nematodes was estimated by processing five soil samples of 500 g each soil using Cobb's decanting and sieving method (modified), followed by the Baermann funnel technique (Southey, 1986). Nematode larvae were picked from each soil isolate, and temporary mounts were prepared to identify *M. graminicola* and other nematode genera on the basis of the morphological characters of juveniles and to count their relative populations (Siddiqui, 2000). The average populations of *M. graminicola* estimated in the bulk soil were 1267 ± 79 and 1306 ± 54 J_2 kg^{-1} soil in the two study years, respectively. The population of other nematodes present in soil ranged from 131 ± 26 and 107 ± 38 larvae kg^{-1} soil, which included *Radopholus, Helicotylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Rotylenchus*, *Rotylenchulus*, *Hirschmanniella*, *Pratylenchus*, *Ditylenchus*, *Trichodorus* and some free-living nematodes (fast moving), among others. A mixture of 1 kg of nematode-infested soil (1267 ± 79 and 1306 ± 54 J_2 kg^{-1} soil) and 250 g of autoclaved farm yard manure