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Integrated management of root-knot and reniform, nematodes singly and concomitantly on linseed

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Abstract
The root-knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis* caused significant reduction in plant growth of linseed (*Linum usitatissimum* L.). Consequently water absorption capability of roots was impaired. In concomitant infections the reduction in plant growth as well as in water absorption capability of roots was more pronounced. The reduction of plant growth in combined inoculations was, however, relatively less than the sum total of reductions caused by the same levels of the single species. Seed dressing with culture filtrate of *Paecilomyces lilacinus* resulted in a significant control of root-knot nematodes, *Meloidogyne incognita* and reniform nematode, *Rotylenchulus reniformis* singly or concomitantly, with a corresponding increase in plant growth, length and weight of plants and number of pods, chlorophyll content of leaves, water absorption capacity of roots of linseed. In another experiment soil application with oil cake of neem (*Azadirachta indica*) showed significant suppression of the nematodes, both singly as well as concomitantly, with the consequent improvement in different growth parameters of the plants as above. A combination of seed dressing treatment with culture filtrate of *P. lilacinus* and soil amendment with neem cake gave synergistic effect with respect to nematode management and improvement in plant growth parameters.

Keywords: *Meloidogyne incognita, Rotylenchulus reniformis, Paecilomyces lilacinus,* Neem cake, *Linum usitatissimum* L

Introduction
Linseed (*Linum usitatissimum* L.) is a significant oilseed plant belonging to the genus *Linum* in the family Linaceae. It is also known as flax or flaxseed. Each part of the linseed plant, either directly or after processing, is used commercially. The stem produces high-quality fibres with high strength and durability [1]. Linseeds have nutritious as well as medicinal properties and are abundant in source of omega-3 fatty acid (α-Linolenic Acid), soluble and insoluble fibres, ligans, proteins, and a number of antioxidants [2, 3]. Linseed was valued as a food and medicine in Ancient and early modern times. Omega-3 fatty acids, which are very crucial fatty acids because they are necessary for maintenance of health but not synthesized by humans from any dietary precursors, so they must be present in the diet. Linseed has been regarded to be one of the powerful nutritional fibres that are achieving demand day by day. Linseed is also regarded as a rich source of a-linolenic acid (belongs to class of omega-3 fatty acid) [4, 5] with an enriched amount of polyunsaturated fatty acids that surpasses all other vegetable oils together with fish oil [6]. It is widely cultivated in India, primarily in U.P., Mahatashtra, Bihar, Rajasthan, and Madhya Pradesh. It is sown in India in the month of October-November and harvested in March-April. Linseed is use for both fibres as well as oil purpose. Scientific evidence promotes linseed intake of high-grade omega-3 fatty acids (α-linolenic acid), lignans, proteins and fibres, biologically effective compounds for the avoidance of certain chronic diseases such as many types of cancers, diabetes, cardiac diseases and cerebrovascular stroke.

According to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT, 2019) India has two fold changes in the yield of the linseed during the period of 2008 to 2017. Therefore, total linseed production is not sufficient to meet the demands of the population and it affects the cost in international market, India has an average of 613.3 kg/ha against world average of 1005.9 kg/ha. Some pathogens include fungi, bacteria, viruses and nematodes, which are independent to each other and are capable of producing diseases in linseed.

Since a large number of fungi occur naturally in the soil, they might be expected to exert an influence on other soil micro-organisms including nematodes. Culture filtrates of several soil borne fungi are known to exhibit nematicidal action besides inhibiting larval emergence of plant parasitic nematodes [7, 8, 9]. This has opened a new field for further exploration of nematode pathogenic fungi to be used as successful biological agents. In the present study, the efficacy of the culture filtrate of fungus *Paecilomyces lilacinus* (Thom.). Samson was tested against *Meloidogyne incognita* and *Rotylenchulus reniformis* attacking on linseed in presence and absence of neem cake.

Material and Methods
Paecilomyces lilacinus was cultured in Richards's liquid medium. Mycelial mats of 15-day-old culture was removed and the liquid medium was filtered through a Whatman No. 1 filter paper. Culture filtrate was centrifuged at 6000 rpm for 15 min and used undiluted's' for seed dressing. Seeds of the linseed thoroughly mixed with the culture filtrate to give a uniform and smooth coating over the seeds. The treated seeds were then spread in an enamel tray and allowed to dry in shade before sowing. The treated seeds were sown in earthen pots containing sterilized oil manure mixture separately.

Another experiment on the above lines was also established by using organic amendment. In this experiment manure, however, was not mixed with soil. The pots were treated with the oil-seed cake of neem/margosa (*Azadirachta indica* A. Juss.) at the rate of 1.0 g N/kg soil. The pots were

watered after treatment to ensure proper decomposition of the soil amendments and after a week, four surface sterilized seeds of linseed were sown in each of the pots separately. Untreated pots served as control. Each treatment including controls were replicated six times. The pots were then placed on a greenhouse bench in a randomized block. After emergence thinning was done to keep only one plant per pot. The plants were inoculated with 5000 2nd stage juveniles of *Meloidogyne incognita* or immature females of *Rotylenchulus reniformis* separately and concomitantly. Weeding and watering were done as and when required.

The experiments were terminated three months after inoculation. The plants were uprooted and the roots were thoroughly washed with running water. The water absorption capability of roots was determined by the method described by Alam [10]. The chlorophyll content of leaves was determined by the method described by Hiscox and Israelstam [11].

The degree of root-knot infection was assessed by counting the root galls per root system. In the case of soil population of the reniform nematode, soil from each treatment was processed after the termination of the experiment using Cobb's siewing and decanting and the modified Baermann funnel techniques [12].

Statistical analysis of the data for critical difference (C.D.) at $p = 0.05$ and $p = 0.01$ levels was done as per procedure described by Pansey and Sukhatme [13]. After separating them from extraneous material, samples of seeds were crushed to obtain a meal for extracting the oil. To assess their oil content, 25 g of seed powder was transferred to a soxhlet apparatus to which sufficient quantity of pure petroleum ether was added. The apparatus was kept in a hot water bath, running at 60°C, for about 6 h, for complete extraction of the oil. The petroleum ether, containing the extracted oil, was evaporated. The extracted oil was expressed as a percentage by weight and was calculated using the following formula:

$$\frac{m}{m_o} \times 100$$

Where m is the sum of the weight of oil in g and m_o, the weight of the seed sample in g.

Results

It is clear from the results presented in Tables 1 and 2, that when plant was inoculated with 5000 specimens per plant of the root-knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis* singly or concomitantly, there was significant reduction in different growth parameters (length and weight of plants), chlorophyll content of leaves, water absorption capacity of roots more being in concomitant inoculations. However, the reduction due to concomitant inoculation was relatively less than the sum total of the reductions caused by either of them singly at the same inoculum level. Similar effects of test nematodes were also noted in plants raised from culture-filtrate of *Paecilomyces* treated seeds but to a lesser extent. These reductions in different parameters due to the test nematodes were also found to have positive correlation with root-gall development (in *M. incognita* - inoculated plant) and nematode multiplication (in *R. reniformis* - inoculated plants), thus, indicating an inhibitory effect of culture filtrate root dressing on the nematodes. It was also observed that both the nematodes were mutually inhibitory in concomitant inoculations.

The culture filtrate of the *Paecilomyces lilacinus* was found to be highly effective in reducing the damage caused by either of them *M incognita* and *R. reniformis* and did not have significant effect on the plant growth. The number of galls and multiplication rate of *R. reniformis* was reduced in plants raised from *P. lilacinus* treated seeds. Damage caused by both the nematodes was further reduced when organic amendments were also added along with culture filtrate of *P. lilacinus.* The overall growth of plants was comparatively improved in presence of neem cake.

Discussion

Efficiency of the fungus, *P. lilacinus* as bio-control agent of nematodes has been tested in many countries with fruitful results. In India, work with *P. lilacinus* is meagre and literature available on the effect of *P. lilacinus* on *M. incognita* and *R. reniformis* is very scanty. A successful bio-control agent must be capable adopting to or tolerating varying environmental conditions. It should be capable of affecting a diverse number of nematode species and must not be pathogenic to plants, human and other animals. Keeping the above points in view, it is necessary to test the potentially effective nematode control under varying climatic conditions with different types of nematode species and on different crops. In the present study, the efficacy of culture filtrate of the fungus *P. lilacinus* was tested against M incognita and *R. reniformis* singly and concomitantly attacking on linseed.

It was found from the results that culture filtrate of *P. lilacinus* was not pathogenic to the test plants (Tables 1 and 2). However, it was highly effective in reducing plant damage caused by test nematodes. *P. lilacinus* was found to be more effective against root-knot than the reniform nematode. This could be due to the fact that these nematodes have differential pathological/ecological behaviour with different morphology and biology.

Adverse effect of the culture filtrates of several fungi on hatching and survival of plant parasitic nematodes [14, 15, 16]. Nematicidal action of culture filtrates against plant parasitic nematodes may be attributed to the production of certain toxic metabolites by fungi. *P. lilacinus* culture filtrate, in the present study, also showed nematicidal action. The fungus is known to produce certain toxic metabolites and/or enzymes, like β(1-3) gluconase, chitinase, leucostatin lilacin [17, 18, 19, 20]. Thus, this presence in the fungal filtrate may be responsible for deleterious effect on nematode.

When culture filtrate of *P. lilacinus* was used along with neem cake the level of nematode control increased. It is understandable because both the components of the integrated nematode control strategy are known to be highly effective against plant-parasitic nematodes [21, 22, 23, 24, 25]. The application of oil seed cake provide more and more inducing substrate (nitrate) for enzyme (nitrate reductase) to accelerate its activity, which results ultimately in increased metabolic activity of plant then plant growth. *P. lilacinus* being an opportunistic fungus is capable of not only colonizing and destroying reproductive organs of females, eggs and cysts of nematodes but is also a good competitor of soil microbes growing easily on organic substances in absence of its natural host (e.g. nematodes). Thus it may

will have first multiplied on organic substrate before attacking the nematodes.

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