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Synergistic studies on interaction of nematode-fungal system of tea plant in Iran

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Identification of the role of each pathogen and their interactions are an important significance for the development of control strategies, and for understanding the formation disease-complex and the evolution of parasitism. The root lesion nematode, *Pratylenchus loosi* Loof is one of the most serious nematode pests of tea in Iran. The nematode causes die back and stunting of infested plants. In affecting plant, nematode is associated with pathogenic fungi. The root lesion nematode, *Pratylenchus loosi* and four species of fungi, *Rhizoctonia solani*, *Fusarium proliferatum*, *F. pallidoroseum* and *Sclerotium rolfsii*, were isolated together from nematode infecting tea plants in Guilan province in Iran. Interaction between nematode and fungi were studied in greenhouse experiments. In this study interaction between *P. loosi* and all species of fungi had a synergistic effect. Also, the pathogenic influence of *P. loosi* and all fungi increased greatly when they were applied together to the soil and produced a reduction in plant height, fresh and dry weight. In the treatments with *R. solani* or *F. proliferatum* and nematode together, the population of nematodes in root was significantly increased. But the fungus, *F. pallidoroseum* and *S. rolfsii* were not significantly affected on the population of nematodes. *R. solani* showed more synergistic effect than other fungi. This is the first report of pathogenic fungi *R. solani*, *F. pallidoroseum* and *F. proliferatum* on tea plants in Iran.

Key words: *Comellia sinensis*, *Pratylenchus loosi*, root infecting fungi, root lesion nematode, *Rhizoctonia solani*, *Fusarium proliferatum*, *F. pallidoroseum*, *Sclerotium rolfsii*

Introduction

Identification and management of tea disease is the important part of researches that is related to this industrial crop. Pests and disease were important factors affecting growth and development of the nurseries. Nematodes and fungi are common components of agroecosystems frequently forming active parasitic complexes. Nematode interactions are important biological phenomena and of great significance in agriculture. It is a

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fascinating subject which is multidisciplinary by nature, and concerns any scientist involved with plant health (Khan, 1993). Much experimental evidence indicated a biological interaction between nematodes and certain soil-born fungi (Botseas and Rowe, 1994; Jonathan *et al.,* 1996; Bhagawati *et al.*, 2000). In some interactions the nematodes are not essential for the establishment and development of fungal pathogens. However, the nematodes usually assist and enhance the pathogenicity mechanism of the fungus towards modifications in the host plants (Jordan, 1987; Mauza and Webster, 1992; Bowers *et al.,* 1996; Rahman *et al.,* 2000). The root-lesion nematode, *Pratylenchus loosi* is one of the most serious nematode pests of tea (*Camellia sinensis* (L.) Kuntze) in Sri Lanka, Philippines, India, Vietnam, Australia and America (Gnanapragasam *et al.,* 1993). This nematode has been recorded as a serious pest of tea in the northern of Iran (Maafi, 1993). The presence of these nematodes in the soil infested with root infecting fungi enhances the incidence, rate of disease development and severity of their disease in tea (Mehta *et al.,* 1992). Nematodes and fungi are common components of agroecosystems frequently forming active parasitic complexes (Khan, 1993). Identification of the role of each pathogen and their interaction has the most important significance for the development of the control management strategies and for understanding of the formation of disease-complexes and the evolution of parasitism (Khan, 1993). Evidence is presented here to show that *P. loosi* is capable of increasing the incidence of root diseases in tea plant.

Materials and methods

Pure population of *P. loosi* were isolated and maintained following the methods of Gnanapragasam *et al.,* (1993) and were sterilized by 2% stereptomycin and rinsed with distilled water and inoculated by pouring 10ml of this suspension containing 2000 nematodes to each pot containing 5 kg garden soil. Fungi inoculums, involving *Rhizoctonia solani*, *Fusarium proliferatum*, *F. pallidoroseum* and *Sclerotium rolfsii*, obtained from the tea gardens in Guilan province in Iran. A single spore (for *Fusarium* species) and hyphal tip culture (for *R. solani* and *S. rolfsii*) of the fungus was maintained on potato dextrose agar medium (PDA) and stored at 25ºC in an incubator. For preparing all fungi inoculum, potato dextrose broth was prepared in 500 ml Erlenmeyer flasks seeds of wheat with the fungus and incubated at 25 ± 2ºC for 20 days (Bhagawati *et al.,* 2000). The fungal mat from each flask was collected. Inoculations were made by mixing fungal mass to sterilized soil in the ratio of 5% (w/w). The six months tea seedlings were transplanted to 30 cm diameter plastic pots. After two weeks nematode and fungal inoculums were inoculated. There were 18 treatments, as follows:- 1) uninoculated check (C);

2) *P. loosi* alone (4 nematodes/10g soil) (P); 3) *F. pallidoroseum* alone (5 g mycelial mat/100 g soil) (F1); 4) *F. proliferatum* alone (5 g mycelial mat/100 g soil) (F2); 5) *R. solani* alone (5 g mycelial mat/100 g soil) (R); 6) *S. rolfsii* alone (5 g mycelial mat/100 g soil) (S); 7) F1 and P simultaneous (PF1); 8) P with F1 added 2 weeks later (P-F1); 9) F1 with P added 2 weeks later (F1-P); 10) F2 and P simultaneous (PF2); 11) P with F2 added 2 weeks later (P-F2); 12) F2 with P added 2 weeks later (F2-P); 13) R and P simultaneous (PR); 14) P with R added 2 weeks later (P-R); 15) R with P added 2 weeks later (R-P); 16) S and P simultaneous (PS); 17) P with S added 2 weeks later (P-S); 18) S with P added 2 weeks later (S-P); A factorial experiment was conducted based on completely randomize block design with 12 replications, each replicate consisting of a single potted seedling plant. The duration of the experiment was 130 days. Nematode populations were estimated by extracting the nematodes from soil by the method of Christie and Perry (1951) and from the roots by the method of Chapman (1957). The nematodes were suspended in a known volume of water and counts were made from 1 ml portion thereof. For dry weight determination, extraneous matter was washed from the roots and stems; free moisture was removed by blotting; the tissues were dried in an oven at 80ºC for 24 h. The dry weight of the foliage and roots were recorded separately. The experiments were conducted in a greenhouse in Tea Seedling Reproduction Station in Ezbaram, Lahijan, Guilan province in Iran. All data on nematode population were transformed into log (X+1). Other data before analyzing were normalized. Analysis of variance using SAS software (SAS Institute, 1988) was carried out on plant growth measurements. Treatment means were compared with Duncan test at the 5% level of probability.

Results and discussion

The result showed that the fungus *F. pallidoroseum* in combination with *P. loosi* affected the dry and fresh weight significantly. According to Table 1 the treatments F1 and F1-P did not differ significantly but P-F1 differed from other treatments. The treatments PF1 and P-F1 had no differences in all indexes. Then nematode inoculation time didn't have a significant effect on F1 pathogenicity. Presence of nematode in root prepare root for fungus invasion and synergistic effect was observed when F1 inoculated two weeks after nematode (Fig. 1 and Table 1). Thus fungus ability to penetrate the root is increased or nematode influence causes physiological changes that tea root was more susceptible to this fungus (Roy *et al.,* 1989). This fungus was no significantly effect on the number of nematode in root and soil inoculated in different time. Thus dry and fresh weight reduction related to increase fungus pathogenicity not for increasing nematode population that adapted with some researches (Mauza and Webster, 1992; Mehta *et al.*, 1992).

Table 1. Influence of *Pratylenchus loosi* alone and in combination with *Fusarium pallidoroseum* on growth rate of tea and nematode population.

Treatments	**Avg fresh wt (gr)**	**Avg dry wt (gr)**	**Number of nematode**	
			in 100 gr soil	**in 1 gr root**
C	19.58^{a}	7.82^{a}	-	-
F1	17.20^{b}	6.68^{b}	-	-
F1-P	16.58^{bc}	$6.48^{\mathbf{b}}$	4.96^{a}	7.29^{a}
P	15.48^{cd}	6.59^{b}	5.56^{a}	7.84^{a}
PF1	14.09^{de}	5.73^{c}	5.76^{a}	8.88^{a}
P-F1	13.81^{e}	5.54^{c}	5.52^{a}	8.82^{a}

Similar letter are not significantly different according to Student-Newman-Keuls test (P = 0.05)

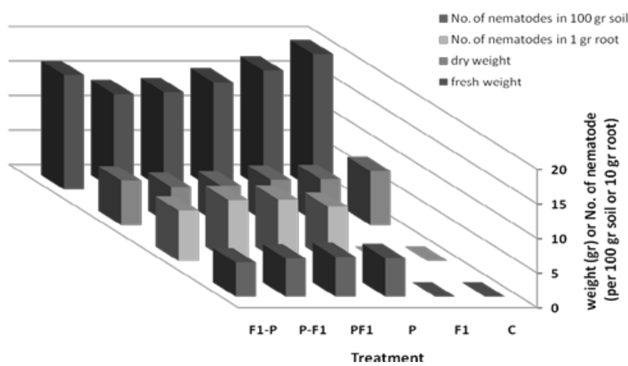

Fig. 1. Effect of *Fusarium pallidoroseum* and *Pratylenchus loosi* on dry and fresh weight and the number of nematodes in root and soil.

The results showed (Table 1 and 2) that nematode early inoculating in both fungi (P-F1, P-F2) increased nematode loss. In addition to, the nematode average in soil was significantly increased in the treatments PF2 and P-F2, thus this experiment showed a synergistic effect between two pathogens on tea. In this interaction, the loss of two pathogens is severed and it caused turnover reduction in conjugated treatments. These results were according to the researches about interaction between root lesion nematodes and wilt fungi (Szczygiel, 1989; Mac Guidwin and Rouse, 1992). It was noticed that the combined infection with the nematode two weeks after the fungus (F2-P treatment) decreased nematode reproduction in root and soil and this may be due to production of adverse effect of the fungus mass on the nematode penetration and/or fungal invasion of nematode feeding site (Mokbel *et al.*, 2007).

Table 2. Influence of *Pratylenchus loosi* alone and in combination with *Fusarium proliferatum* on growth rate of tea and nematode population.

Treatments	Avg fresh wt (gr)	Avg dry wt (gr)	Number of nematode	
			Soil (100 gr)	root (1 gr)
C	19.58[a]	7.82[a]	-	-
P	15.48[b]	6.59[b]	5.56[ab]	7.84[a]
F2	14.26[b]	5.66[c]	-	-
F2-P	14.58[b]	5.94[bc]	4.58[b]	6.14[b]
PF2	11.89[c]	4.82[d]	6.60[a]	9.47[a]
P-F2	11.32[c]	4.68[d]	6.40[a]	9.49[a]

Similar letter are not significantly different according to Student-Newman-Keuls test (P = 0.05)

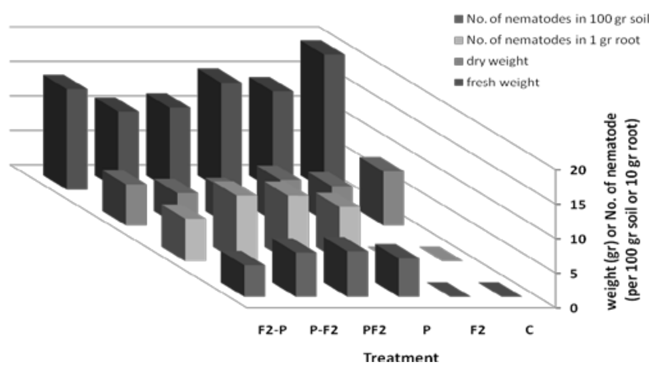

Fig. 2. Effect of *F. proliferatum* and *P. loosi* on dry and fresh weight and the number of nematodes in root and soil.

Studies on interaction between *F. proliferatum* and *P. loosi* showed that nematode and fungus inoculation together had significant effect on dry and fresh weight as compare to control. However nematode inoculation two week after fungus inoculation had no significant difference from fungus and nematode alone. These results demonstrated that root colonization with *F. proliferatum* prevents nematode losses on plant and caused a significant reduction of nematode in root and soil (Table 2, Fig. 2). Also, results showed that treatments with *F. proliferatum* and *F. pallidoroseum* either at the same time or two weeks after *P. loosi* inoculation resulted in significant reduction in growth parameters of tea plants and non-significant increasing in number of nematodes in root and soil. These results are in agreement with research findings of Zaidi and Tiyagi (1989).

Table 3. Influence of *Pratylenchus loosi* alone and in combination with *Sclerotium rolfsii* on growth rate of tea and nematode population.

Treatments	Avg fresh wt (gr)	Avg dry wt (gr)	Number of nematode	
			Soil (100 gr)	root (1 gr)
C	19.58^{a}	7.82^{a}	-	-
S-P	17.52^{b}	6.70^{b}	5.01^{a}	7.08^{a}
S	16.26^{bc}	6.48^{b}	-	-
P	15.48^{cd}	6.59^{b}	5.56^{a}	7.84^{a}
P-S	14.02^{de}	4.82^{cd}	4.97^{a}	7.83^{a}
PS	13.68^{e}	5.48^{d}	5.15^{a}	7.45^{a}

Similar letter are not significantly different according to Student-Newman-Keuls test (P = 0.05)

The interaction between *P. loosi* and *S. rolfsii* showed that the treatments S-P and S didn't have significantly differ in all parameter, thus initial infection of roots by *S. rolfsii* was not influenced by nematode activity in the root system (Table 3). These results were in agreement with findings of Zaidi and Tiyagi (1989), Anwar and Verma (1993) and Rahman *et al.* (2000) and in contrast with findings of Bowers *et al.,* (1996). The results revealed that infection with *P. loosi* plus *S. rolfsii* (P-S, PS, S-P) could induce significant reduction in fresh and dry weight of tea plants. But comparing the result of treatments P-S and PS showed insignificant differences in number of nematodes in soil and root those of P treatment (Fig. 3). Data on nematode population showed that the nematodes could entry into roots but in the presence of fungi, they didn't have high reproduction. Thus the fungus didn't affect on nematode reproduction. Finally, *P. loosi* in combination with *S. rolfsii* (P-S, PS, S-P) increased fungus disease losses presumably because fresh and dry weight were decreased significantly but the number of nematode in soil and root didn't differ significantly, then, the reduction of plant weight was related to fungus losses presumably. Moreover, the fungus didn't cause any effect on nematode reproduction. These results are in agreement with findings of researchers such as Faulkner *et al.*, 1970; Yassin, 1974.

Treatments with *P.* loosi in combination with *R. solani* resulted in significant reduction in fresh and dry weight but could increase greatly number of nematode in root in comparison with those in the control (Table 4). Also, treatments of *P. loosi* in combination with *R. solani* two week later (P-R) showed more nematode number in root than other treatments. Initial infection of roots by *R. solani* (R-P) was affected nematode activity and could reduced, when compared with other treatments co-inoculating (PR) and first nematode inoculation (P-R) (Fig. 4). The treatment P-R had most reduction in fresh and dry weight. Thus the relation between *P. loosi* and *R. solani* is a synergistic effect that the role of nematode is as a facilitator on fungus penetration into root by influence on host physic and physiology (Khan, 1993).

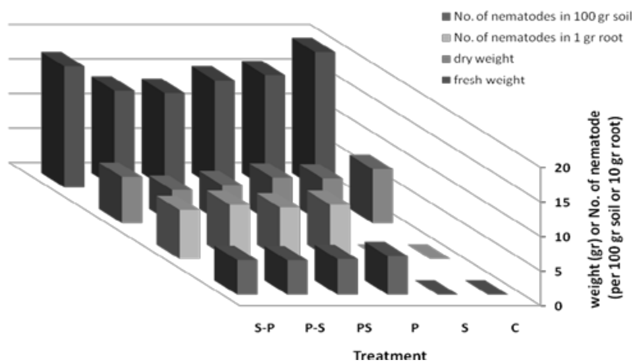

Fig. 3. Effect of *Sclerotium rolfsii* and *Pratylenchus loosi* on dry and fresh weight and the number of nematodes in root and soil.

Table 4. Influence of *Pratylenchus loosi* alone and in combination with *Rhizctonia. solani* on growth rate of tea and nematode population.

Treatments	Avg fresh wt (gr)	Avg dry wt (gr)	Number of nematode Soil (100 gr)	root (1 gr)
C	19.58^{a}	7.82^{a}	-	-
P	15.48^{b}	6.59^{b}	4.66^{b}	7.84^{b}
R	12.87^{c}	5.10^{c}	-	-
R-P	11.57^{cd}	4.70^{cd}	4.58^{ab}	6.71^{b}
PR	11.09^{d}	4.38^{d}	6.00^{ab}	10.49^{a}
P-R	10.89^{d}	4.24^{d}	6.35^{a}	11.22^{a}

Similar letter are not significantly different according to Student-Newman-Keuls test (P = 0.05)

The number of nematodes in soil in the treatment P was significantly differ from those PR, P-R and R-P that had no significant difference with each other, then the presence of fungus in combination with nematode caused nematode migration into soil. Some researchers believe when a plant root system is infected highly with fungi or nematode and injured, nematode population will be reduced in such a conditions. Also, nematode reproduction affected and reduced in rotting root and in this manner, nematodes exit from root and could enter into soil (Varain, 1987). Therefore, the role of fungus is to increase nematode population by influencing nematode entry into root and egg hatching (Khan, 1993). These results support other authors who reported that reproduction of *Pratylenchus* spp. was stimulated in some crops by simultaneous root infection of some root infecting fungi (Jordan *et al.,* 1987; Jin *et al.,* 1991; Pablo Castillo *et al.,* 1998). Several alternative hypotheses were consistent with these observations. Nematode feeding may increase root exudation and increase rhizosphere width, which in turn may increase the number of root contacts with micropropaguls and result in a higher percentage of root infecting (Bowers *et al.,* 1996). This synergistic response of tea to concomitant infection by *P. loosi* and *R. solani* is predictable and highly repeatable based on a number of independent studies on other fungi (Botseas and Rowe, 1994; Wheeler *et al*., 1994; Saeed *et al*., 1998).

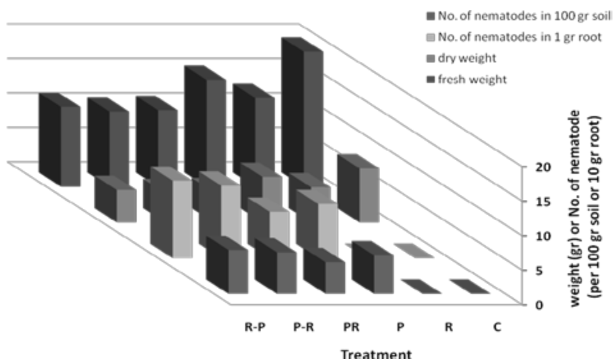

Fig. 4. Effects of *Rhizoctonia solani* and *Pratylenchus loosi* on dry and fresh weight and number of nematodes in root and soil.

In this study four fungi namely *Rhizoctonia solani*, *Fusarium proliferatum*, *F. pallidoroseum* and *Sclerotium rolfsii* were tested and data showed (Tables 1, 4) that the fungus *F. pallidoroseum* and *R. solani* were weak and strong parasites respectively. In all fungus-root system, we observed synergistic effects with various degrees. *R. solani* and *F. proliferatum* decreased great extent of fresh and dry weight in combination with *P. loosi* but *R. solani* increased nematode population in root (PR and P-R treatments) and in soil (P-R treatment) significantly. Experimental results supported the hypothesis that feeding injury by root lesion nematodes provides a direct avenue of entry of root infecting fungi into the root system (Bowers *et al.*, 1993; Khan, 1993). The physical and physiological activity of nematode feeding on plant roots was also related to entry of root fungi into roots. An alternative explanation is that the nematode enhanced host susceptibility to mycelia growth of the fungus. Increased susceptibility of the plant might allow root infecting fungi to move quickly into roots by enhancing their ability to colonize the plant roots. Nematode feeding causes vast changes on hormonal balance and biological changes on host that make host susceptible to fungi (Mai and Abawi, 1987).

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