



Interrelationships of root- knot nematodes with root- rot fungi and their effect on common bean grown in natural infestation

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Abstract : Interaction between the root-knot nematode, *Meloidogyne arenaria* and root-rot fungi were studied on common bean *phaseolus vulgaris* L. cv. Giza -6 grown in natural infested field. Four root- rot fungi, *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium sp.* were isolated from bean root- roted. *F.solani* was the most frequent fungi, occupying the first order with average of 57.5% frequency followed by *R.solani* with average of 18.68%, *Pythium sp.* with 14.43% and *S. rolfsii* with 9.38%. Relationship between nematode damage (root galling) and yield of bean, regardless of root- rot disease severity, was highly significant and negative ($r = - 0.97$). Correlation between root – rot disease severity and yield of bean, regardless of nematode damage was also highly significant and negative ($r = - 0.99$). Relationship between nematode damage and root-rot disease severity was highly significant and positive ($r = 0.97$) indicating a synergistic interaction occurred between them and producing a disease complex.

Key words : root – knot nematode, root- rot fungi, interaction, common bean, natural infestation.

Introduction

Common bean, *phaseolus vulgaris* L. is one of the most widely cultivated food legume species in the world (Baudoin *et al.*,¹). It is major source of low cost calories, protein, dietary fibers, minerals and vitamins for poor populations (Pachico,²; Hillocks,³). So, U.S. Department of Agriculture considered bean to be both a vegetable and protein source (Long *et al.*,⁴).

The root knot and root – rot are probably the major diseases of common bean in tropics and subtropics. Root- knot nematodes (*Meloidogyne species*) have been reported causing damage in the Americas, Africa and Asia and perhaps no country in the tropics and subtropics in which bean are not affected by root- knot nematodes (Sikora and Greco,⁵). The root – knot nematode, *Meloidogyne arenaria* was found to infect common bean plants in Egypt causing a significant yield loss. The loss in pod yield was more than 31% when plants were severely infected (Korayem *et al.*,⁶). The root - rot fungi, *Fusarium*, *Rhizoctonia* and *Pythium* are also common in tropics and subtropics infecting beans and causing severe root- rot disease (Nekesa *et al.*,⁷; Miklas,⁸; Long *et al.*,⁴). Yield loss of up to 70% in commercial bean cultivars was reported in Rwanda and Kenya (Rusuku,⁹; Otsyula *et al.*,¹⁰).

Interaction of nematodes with other microorganisms are common in nature. Since Atkinson¹¹ observed that the incidence and severity of Fusarium wilt of cotton was greater in the presence of root- knot nematode infection than when the nematodes were absent, the importance of these interactions have received more attention.

Meloidogyne incognita, *M. javanica* and *M. arenaria* have been found to increase severity of root – rot fungi, *Macrophomina phaseolina*, *Fusarium solani* f. sp. *phaseoli* and *Rhizoctonia solani* on bean plants (Al-Hazmi, ¹²; France and Abawi, ¹³; Al Hzmi *et al.*, ¹⁴). Most of these interactions were done under artificial infestation, so they are unsuitable for determining the full extent of interaction which occur in the natural infestation. As "the nature does not work with pure culture" Fawcett, ¹⁵, so more effort should be directed for enhancing our understanding of these interactions which occur in natural agroecosystem. Good information about this matter will help for developing a good disease complex management. The objectives of the present study were to (i) determine the relationship of both nematode damage and root- rot disease severity of soil borne fungi to yield of common bean, (ii) determine possible interaction between nematode infection and root-rot disease severity in the natural infestation, (iii) quantify the yield loss of bean caused by the joint action of both nematodes and fungi.

Materials and Methods

The work was conducted during 2015 season in loamy sand soil naturally infested with both root- knot nematode, *Meloidogyne arenaria* and root – rot fungi at Nobaria region, Egypt. Land of the experiment was prepared in rows with 70cm spacing between the rows. Seeds of common bean, *Phaseolus vulgaris* L. cv. Giza - 6 were manually planted in singly rows at rate of about three to four seeds per 50 cm of row in 25-8-2015. Nitrogen, phosphorus and potassium (NPK) fertilizers, were added as soil application at the recommended dose for bean plants which were watered by drip irrigation system. At harvest in 12-12-2015, more than one hundred plants were randomly selected for estimating each of nematode damage, root – rot disease severity and yield components for each plant.

Nematode damage assaying: The nematode root – gall index (GI) of each plant root was estimated as follows: 1=no galls (healthy), 2= 1-20% root galling, 3= 21-40%, 4= 41-60%, 5= 61-80% and 6= 81- 100% root galling according to Barker, ¹⁶.

Fungi root – rot severity assaying: Root – rot severity ratings were based on a scale of 0 to 4 described by Medvecky *et al.*, ¹⁷ as follows: 0= no infection, 1= 1-25%, 2= 26-50%, 3= 51-75%, 4= 76-100% infected roots.

Frequency of root – rot fungi: Roots of bean plants were washed with tap water to remove the adhering soil particles, after that roots were surface disinfected using sodium hypochlorite solution (3%) for 3 minutes, and washed with sterilized water several times. Then roots were dried using sterilized filter paper and transferred into Petri- plates containing water agar medium. Plates were incubated at 25°C for 5 days. Frequency of the isolated fungi was recorded using the following equation:

$$\text{Frequency \%} = \frac{\text{Number of isolated fungus}}{\text{Total number of isolated fungi}} \times 100$$

Determination of yield components: The vegetative fresh weight, yield of pods and dry seeds per each plant were recorded.

Statistical analysis: Data were subjected to analysis of variance. Tukey test was used for multiple comparisons among means Neler *et al.*, ¹⁸. Relations between both nematode root gall and root – rot disease severity and between bean yields were also depicted as regression lines. Nematode root gall indices were also plotted against root – rot severity to indicate the correlation between them.

Results

Relationship between nematode and bean growth and yield:

The relation between nematode damage (root galling) and weights of shoots and seeds are presented in Table (1). Data indicated that both fresh shoot (including pods) and dry seed weights decreased with increasing nematode damage. Significant reductions ($p= 0.05$) 28.9%, 51.0% and 62.9% in fresh shoot weight and 20.9%, 27.3% and 38.% in seed weight were occurred at 4,5 and 6 root galling (GI), respectively. When root- gall indices were plotted against fresh shoot weights and seed weights, a highly significant and negative correlation

was found between them as correlation coefficient (r) was – 0.99 and -0.97 for the fresh shoot and seed weight, respectively (Fig. 1 and 2).

Table 1. Relationship between *M. arenaria* root galling and yield of common bean, regardless of root – rot severity.

Root gall index (GI)	Fresh shoot weights g/plant	Reduction %	Dry seed weights g/plant	Reduction
1	192.9 a	-	89.9 a	-
2	180.3 a	6.4	89.9 a	0.1
3	152.6 a	20.8	81.4 a	9.5
4	137.0 b	28.9	71.1 b	20.9
5	94.4 b	51.0	65.4 b	27.3
6	71.5 c	62.9	55.3 c	38.5

Means having different letters are significantly different at p= 0.05 according to Tukey test for comparison among means.

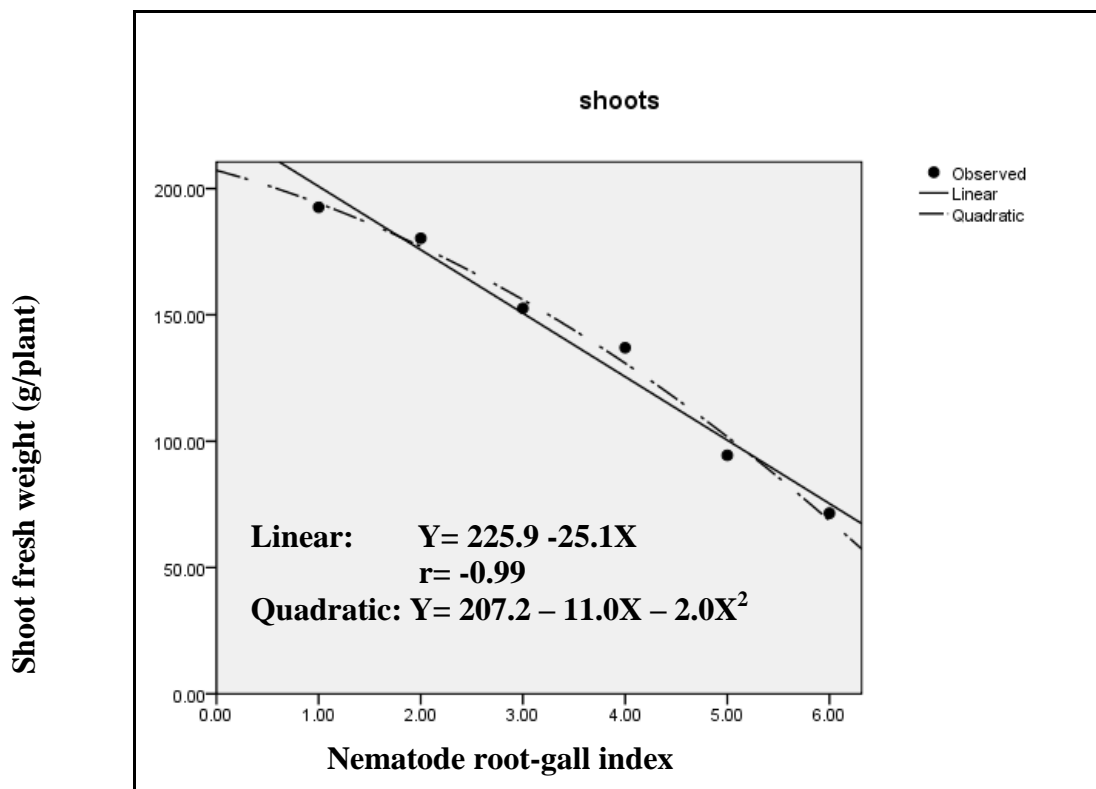


Fig. 1: Relationship between nematode root gall index and weight of the fresh shoot of common bean

Relationship between root – rot disease severity and yield of bean plants:

Results in Table (2) revealed that seed yield decreased with increasing root- rot disease severity. Significant reductions in the yield 21.9%, 33.9%, 51.1% and 61.3% were obtained at 1,2,3 and 4 disease severity, respectively. Depicting the relation between bean yield (seeds) and root- rot severity as regression lines, indicated that a highly significant and negative correlation was found between them, as correlation coefficient (r) was – 0.99 (Fig. 3).

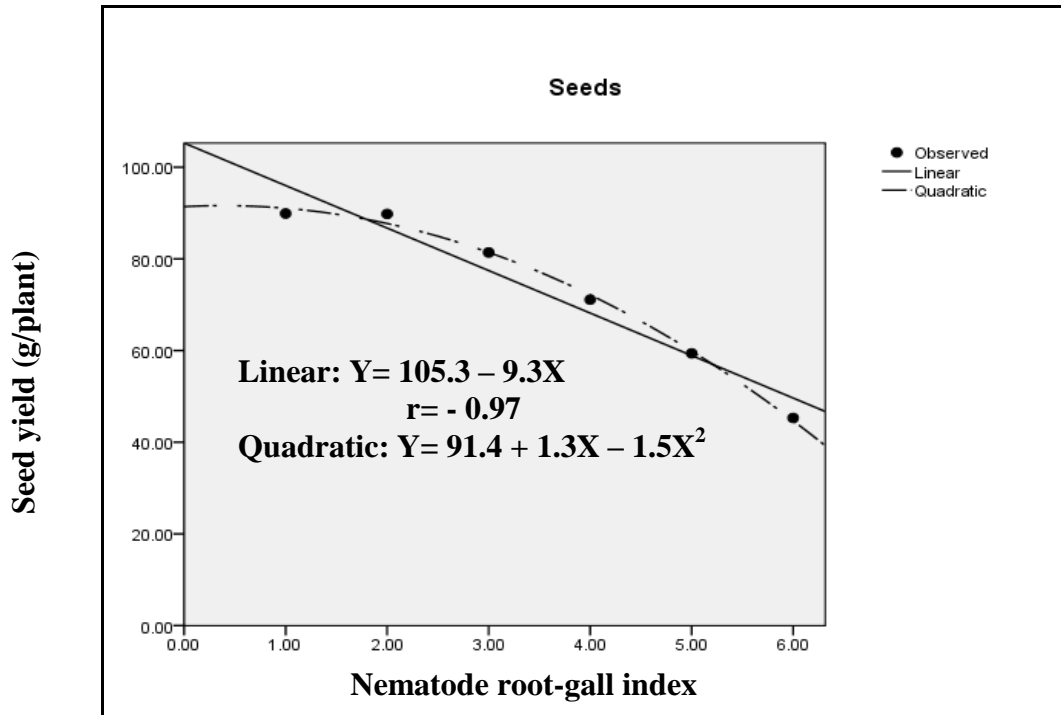


Fig.2: Relationship between nematode root-gall index and seed yield of common bean.

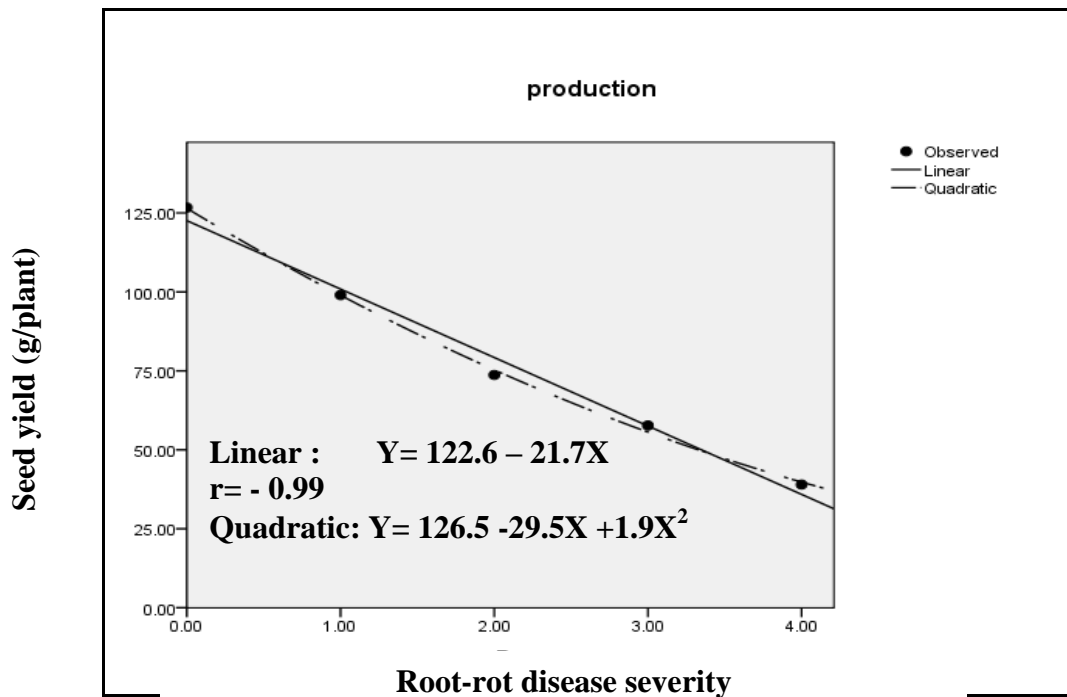


Fig.3: Relationship between root-rot severity and seed yield of common bean

Relationship between disease severity and frequency of root – rot fungi isolated from bean roots:

Four root – rot fungi ie *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium sp.* were isolated from the infected bean roots. *F. solani* was the most frequent isolated fungi from diseased roots as it occupied the first order with 82.0, 71.3, 45.2 and 31.5% for frequencies for degree of 1,2,3 and 4 disease severity, respectively with average of 57.5% (Fig. 4).

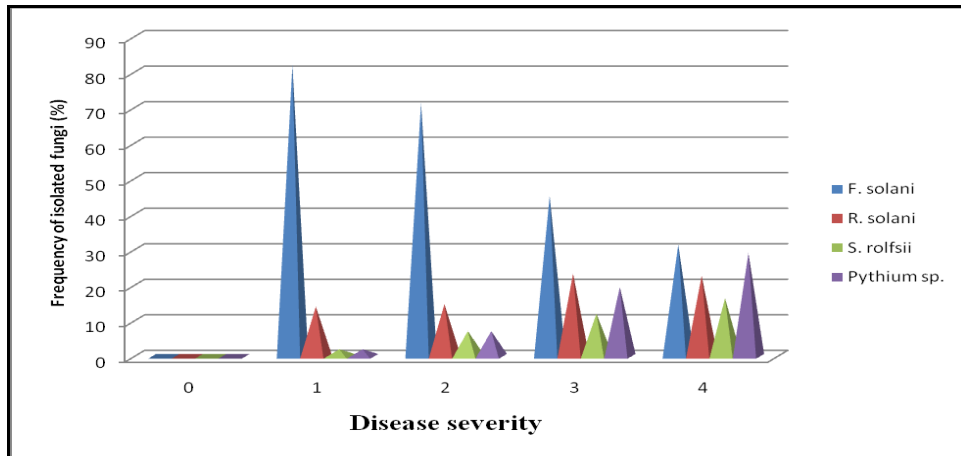


Fig (4) Relationship between disease severity and frequency of isolated fungi of bean plants under field conditions

Table 2. Relationship between root – rot disease severity and yield of common bean, regardless of nematode damage.

Root –rot severity	Seed yield g/ plant	Reduction %
0.0	126.7 a	-
1.0	99.0 b	21.9
2.0	83.7 b	33.9
3.0	61.9 c	51.1
4.0	49.0 d	61.3

Means having different letters are significantly different (P= 0.05) according to tukey test.

R. solani occupied the second order, with 14.0%, 14.7%, 23.3% and 22.7% frequency of occurrence at 1,2,3 and 4 disease severity, respectively with average of 18.68%. *Pythium sp.* occupied the third order, still it was more frequent (29.1%) at 4- disease severity. While *S. rolfsii* was least frequency with average of 9.38%.

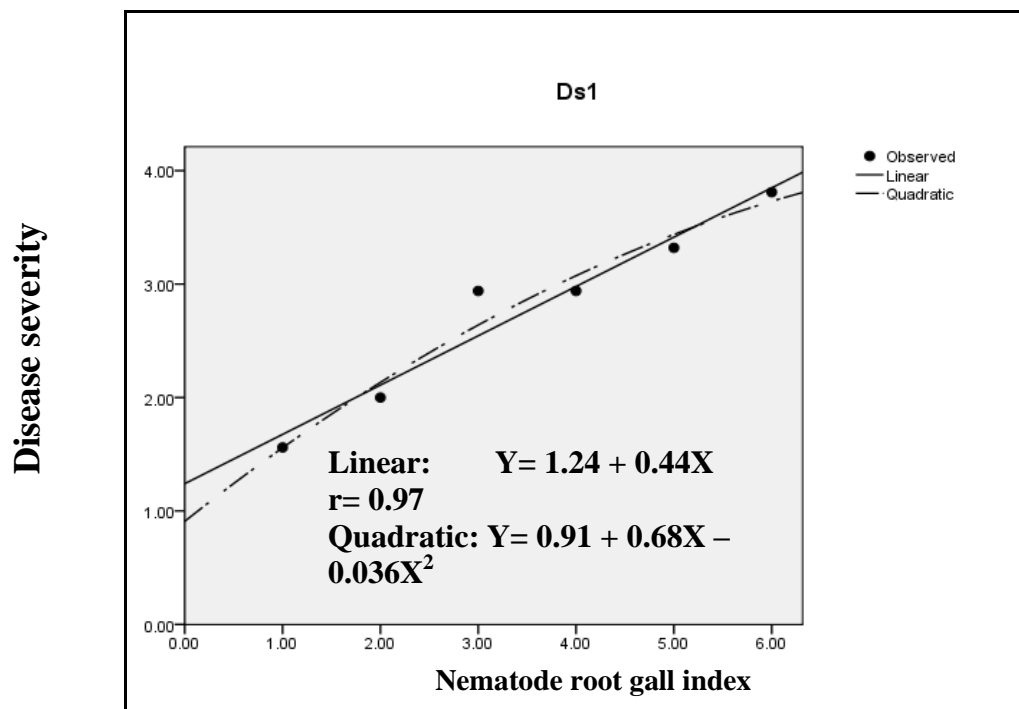


Fig. 5: Relationship between nematode damage and root-rot disease severity

Relationship between nematode damage and root – rot disease severity:

Data presented in Figure (5) indicated a positive and significant correlation ($r= 0.97$) was found between nematode damage and root- rot severity. Root- rot severity increased with increasing nematode damage.

The Combined effect of nematodes and root- rot fungi on yield of bean:

Data presented in table (3) indicated that yield of bean decreased by 25.1% when plants were infected with nematodes only, and it decreased by 52.4% when plants were infected with fungi only. When plants were infected with both nematodes and fungi, their yield decreased by 80.1% compared with yield of healthy plants, that is the joint effect of both nematodes and fungi was greater than the sum of individual effect, indicating a synergistic interaction occurred between them, and producing a disease complex.

Table 3. The combined effect of the root- knot nematode (*M. arenaria*) and root – rot fungi on common bean yield under natural infestation.

Treatments (type of infection)	Seed yield* g/ plant	Reduction %
Nematode only	89.1	25.1
Root rot fungi only	56.6	52.4
Nematode x fungi	23.7	80.1
Control (healthy plants)	119.0	-

Data are average of 15 to 20 replicates.

* Yield of infected plants with nematode at GI= 6 and with fungi at 4- root – rot severity.

Discussion

Our results emphasized that common bean *P.vulgaris* L. is severely damaged with both of root- knot nematode, *M. arenaria* and root- rot fungi, *F. solani*, *R. solani*, *S. rolfsii* and *pythium sp.* Damage of root- knot nematodes to common bean was also reported by many investigators either in natural or artificial infestation (Osman *et al.*,¹⁹ and²⁰; Korayem *et al.*,²¹ and²²). Also, the root- rot fungi *Fusarium solani*, and *Rhizoctonia solani* were also found to infect common bean causing severe damage (Buruchara and Camacho,²³; El-Mougy *et al.*,²⁴; Naseri,²⁵).

Our results indicated that correlation between *M.arenaria* and root- rot disease - fungi was highly significant and positive, as severity of root – rot disease increased with increasing nematode damage. These increases in severity of root- rot disease in the presence of root – knot nematodes were also found on several host plants, and reported by several investigators (Anwar and Khan,²⁶; Poornima and Subramanian,²⁷; Bhagawati *et al.*,²⁸; Mokbelet *et al.*,²⁹).

Back *et al.*,³⁰ indicated that a disease complex is produced when synergistic interaction occur between two organisms, an interaction is synergistic if the association between two organisms result in plant damage greater than the sum of individual damage ($1+1>2$). Our results indicated that the nematode (*M.arenaria*) was positively interacted with the root – rot fungi on bean plants, and the join effect of both nematodes and fungi was more than the sum of individual effect. Therefore, the interrelationship between *M.arenaria* and root- rot disease fungi was a synergistic interaction, producing a disease complex on bean plants. Our results are also in accordance with that of Wallace³¹, who concluded that interaction between nematodes and other factors occur when the combined effect of both in the same time is not additive.

Interaction of phytonematodes with other microorganisms is a common phenomenon in the natural rhizosphere. It has been documented in several host plants by many investigators (Powell,³²; Bergeson,³³; Webster,³⁴; Mai and Abawi,³⁵; Sikora and Carter,³⁶; Shahzad and Ghaffar,³⁷; Evans and Haydock,³⁸; Back *et al.*,³⁰; Manzanilla- Lopez,³⁹). It was concluded that interactions between two or more organisms, may be synergistic, antagonism, symbiotic or nutral (Khan,⁴⁰). Major mechanisms involved in the interaction

between phytonematodes and soil fungi were listed as vectors of fungal pathogens, mechanical wound agents, host modifiers, rhizosphere modifiers and resistance breakers (Ravichandra, ⁴¹).

Briefly good understanding of the interaction between pathogenic nematodes and other pathogenic fungi in natural soil ecosystem will much help for developing a successful management, especially biological control. An unsuccess of disease management practices in several instances may be due to incomplete diagnosis of disease complexes, resulting in inappropriate management.

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