# Comparison of concomitant and sequential inoculation of *Steinernema* sp. in the management of Reniform (*Rotylenchulus reniformis*) nematode infecting eggplant

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**ABSTRACT:**
Culture of *Steinernema* sp. was maintained on *Corcyra cephalonica. Steinernema* sp. was then formulated on calcium alginate capsules. *Steinernema* sp. were concomitantly inoculated @ 50, 500, 1000, 2500, 5000, 10,000 and 20,000 ij's / 500 g soil with 500 immature females of *R. reniformis* / 500 g soil in pots having eggplant seedlings. The simultaneous inoculation of *R. reniformis* with either of the inoculum levels (1000, 2500, 5000 and 10,000 $J_3$ / 500 g soil) of *Steinernema* sp. significantly reduced the damage caused by *R. reniformis* in terms of plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. Moreover, the highest improvement in plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits was recorded in plants inoculated with 5000 $J_3$ of *Steinernema* sp. / 500 g soil followed by 2500, 1000 and 10,000 $J_3$ / 500 g soil. The highest reduction in the reproduction factor (Rf) was recorded in the plants treated with 5000 $J_3$ *Steinernema* sp. / 500 g soil followed by 2500, 1000 and 10,000 $J_3$ / 500 g soil. Comparison of concomitant and sequential inoculations showed that the sequential inoculation (both prior and post) of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000 ij's / 500 g soil) were more effective in the management of reniform nematode than the concomitant inoculation. Therefore, the application of *Steinernema* sp. might be useful for suppression of nematode pest on eggplant and may be used as an alternative to chemical use
*Keywords: - Rotylenchulus reniformis, Steinernema sp., Eggplant, Corcyra cephalonica*

## 1. Introduction

Plant-parasitic nematodes (PPN's) account for worldwide losses of between 5% and 12% annually in various crops (Barker and Koenning, 1998).Tropical and subtropical climates provide ideal conditions for PPN populations and consequently, the damage caused by them. Among these PPN's , the genus *Rotylenchulus* with 10 species are the most prevalent and important group of plant parasitic nematodes occurring throughout the world but found more frequently and in greater numbers in areas having tropical climate. Among these *Rotylenchulus* species, *R. reniformis* with at least 314 plant hosts is the most economically important species (Robinson, 1997). Reniform nematodes not only deprive nutrients of infected plants but also reduce the quantity and market value by affecting the quality of the fruit. Symptoms include reduced root systems, leaf chlorosis, overall stunting of host plants and reduction in plant longevity. Successful and economical management of reniform nematodes in view of their worldwide distribution and extensive host range particularly of the

species *R. reniformis* have always been a necessity of vegetable growers across the world. Chemical nematicides, which were effective to combat nematode pests and in use for the management for a long time, have been found to cause pollution. Increasing awareness of environmental and human health concerns associated with chemical nematicides and removal of several efficacious products from the world market in recent years provide impetus for a search of environmentally compatible products for nematode management. Biological control of reniform nematode, as for other pathogens, parasites and pests is currently in focus and is thrust area, which is receiving attention in almost all parts of the world. Biological control in addition to being cheap and effective is the safest in terms of environmental consideration and needs relatively less technical skill for their application. Keeping in view of these aspects, the present study was undertaken to search for effective and alternate biocontrol agents, which can be developed as biopesticides. Various biocontrol agents like fungi, bacteria and viruses which have already been used to control nematodes since long back. Another important group which is emerging as potent biocontrol agent of pests is of entomopathogenic nematodes (EPN's).

EPN's have shown some potential as antagonists to PPN's. Applications of EPN's to soil have been found to reduce a number of important PPN species including *Meliodogyne* spp. (Grewal *et al*. 1997; Ishibashi and Choi, 1991; Ishibashi and Kondo, 1987; Smitley *et al.* 1992), *Belonolaimus* spp. (Grewal *et al*. 1997), *Tylenchorhynchus* spp. (Smitley *et al.* 1992), *Rotylenchulus reniformis* (Shahina and Tabassum, 2009) *Rotylenchus* (Jagdale *et al*. 2002 and Perez and Lewis, 2006) and *Criconematidae* (Grewal *et al*. 1997, Ishibashi and Kondo, 1987). Jagdale *et al*. 2002 reported that both live as well as dead (heat killed ) infective juveniles of entomopathogenic nematode *Steinernema carpocapsae* significantly reduced the populations of *Rotylenchulus* in all the treatments relative to the control both after 15 and 30 days after treatment on boxwood.

This paper throws light on the efficacy of native species and strains of entomopathogenic nematode *Steinernema* sp. isolated from different regions (U.P. and J and K) of India in the control of *Rotylenchulus reniformis* infecting eggplants. Comparison was also made between concomitant and sequential inoculation of *Steinernema* sp. in the management of *Rotylenchus reniformis* infecting eggplant.

## 2. Materials and methods

The rice moth, *Corcyra cephalonica* larvae was reared on sterilized and chopped maize grains. These larvae were then used for baiting of entomopathogenic nematodes (EPN's) i.e. *Steinernema* from the soil samples collected from various localities in India. The Steinernema sp. isolated were then mass multiplied *in vivo* on *Corcyra cephalonica* larvae by using White trap method (White, 1927). Counting of *Steinernema* sp. was done under Stereomicroscope. One ml from the original concentration was diluted by adding 3ml water and counted under Stereomicroscope. Total number of nematodes was counted based on the average of 5 counts multiplied by the total volume. *Steinernema* sp. was then formulated on calcium alginate capsules as per Kaya and Nelsen procedure (1985). Survival of encapsulated EPN's was checked after every 15 days by dissecting samples of five capsules. The nematodes encountered in the microscopic field were examined to determine whether they were alive or dead. At the time of application, alginate capsules were then taken and dissolved in 0.5 M sodium citrate containing 0.1% Triton×-100. The capsules were stirred with magnetic spin bar until dissolution (about 30 minutes), and the nematode in 1 ml of suspension were counted using stereoscopic microscope. Total number of nematodes was counted based on the average of 5 counts multiplied by the total volume. Volume of water in the nematode suspension was so adjusted that each

ml contained about 400 ij's, which was done by either adding more water or decanting the excess water.

Mass rearing of *Rotylenchulus reniformis* was done on susceptible eggplants Cv. Pusa purple long to get the regular supply of inoculum for the experiment. The egg masses from heavily infected roots of eggplant on which pure culture of *R. reniformis* multiplied were handpicked with the help of sterilized forceps. These egg masses after being washed in distilled water were placed in a sieve with a layer of double tissue paper. The sieve was placed over Petridish (10 cm diameter) containing water. The water level was kept such that it just touched the lower portion of the sieve having egg masses. A series of such assemblies was kept in BOD at 25 ± 2 $^0$C to obtain large number of juveniles required for inoculations in the experiments. After every 24 hrs, the hatched out larvae were collected up to 4 days along with water from Petridish in a beaker and fresh water was added to the Petridish. The water suspension containing juveniles in such a way were kept at room temperature (28$^0$C ± 2$^0$C) for about 7 days so that all the stages of juveniles converted into immature females. From the water suspension of immature females of *R. reniformis*, 2 ml suspension was transferred in counting dish for counting the number of nematodes under the stereoscopic microscope. An average of five counts was taken to determine the density of nematodes in the suspension. Volume of water in the nematode suspension was so adjusted that each ml contained about 100 nematodes, which was done by either adding more water or decanting the excess water so that 5 ml of this suspension contained 500 immature females of *R. reniformis*. The reniform nematode *R. reniformis* so obtained were used for inoculating experimental pots containing fresh eggplant seedlings grown in 8 inch earthen pots containing 4 kg sterilized soil. Holes of 5-7 cm depth around the plants, within a radius of 2 cm from the plant, were made in which required number (500 *R. reniformis* immature females per 500 g soil) were transferred with the help of sterilized pipette. The holes were then plugged with sterilized soil.

The schedule of inoculation of *Steinernema* sp. (@ 50, 500, 1000, 2500, 5000, 10,000 and 20,000 ij,s / 500 g soil) consisted of concomitant and sequential inoculations. *Steinernema* sp. was applied to the eggplants inoculated concomitantly with 500 *R. reniformis* / 500 g soil. In sequential inoculation, *Steinernema* sp. was applied to the eggplants one week prior or after the inoculation of 500 immature females of *R. reniformis* per 500g soil. In all the sets of experiment, healthy plants were kept as control. Each treatment was replicated five times.

After 60 days, the eggplants were removed from the pots and the root balls were shaken until most of the soil had been dislodged from the roots. Then parameters of growth like shoot length, root length, shoot dry weight, root dry weight, number of flowers and weight of fruits were recorded. Reproduction factor ($R_f$) of the nematode was also calculated. Data were analyzed stastically by one way analysis of variance (Anova) using SPSS 12.00 software (SPSS Inc., Chicago, IL, USA). C.D. was calculated at P= 0.01 and P = 0.05 to test for significant differences (Panse and Sukhatme, 1964).

## 3. **Results**

The data presented in Tables 1.1, 2.1 and 3.1 revealed that inoculation of eggplant seedlings with *R. reniformis* caused significant reduction in plant growth parameters as compared to control. The percentage reduction caused by *R. reniformis* in plant length, dry weight, number of flowers and weight of fruits was recorded as 39.78 %, 45.52 %, 53.10% and 44.71% respectively.

The simultaneous inoculation of *R. reniformis* and either of the inoculum levels (1000, 2500, 5000 and 10,000 $J_3$ / 500 g soil) of *Steinernema* sp. significantly reduced the damage caused by *R. reniformis* in terms of plant growth parameters. Moreover, the highest significant improvement in plant growth parameters was recorded in plants inoculated with 5000 $J_3$ of *Steinernema* sp. / 500 g soil followed by 2500, 1000 and 10,000 $J_3$ / 500 g soil.

The multiplication was found to be highest when *R. reniformis* was inoculated alone in eggplants. The reproduction factor was found to be as 13.87 (Tables 1.2, 2.2, 3.2).

However, on the other hand, the inoculation of eggplants with *Steinernema* sp. at inoculum levels i.e. 1000, 2500, 5000 and 10,000 significantly reduced the multiplication of *R. reniformis*. Highest reduction in the reproduction factor (Rf) was recorded in the plants treated with 5000 $J_3$ *Steinernema* sp. / 500 g soil followed by 2500, 1000 and 10,000 $J_3$ / 500 g soil. In the corresponding treatments, the reproduction factor (Rf) was recorded as 6.65, 9.35, 12.05 and 12.55. Moreover, the inoculation of *Steinernema* sp. at lower (50 and 500 $J_3$) and higher (20,000 $J_3$) inoculum levels neither significantly improved growth parameters and nor reduced the reproduction factor. However, the sequential inoculation of *Steinernema* sp. with either of the inoculums levels (1000, 2500, 5000, 10,000 and 20,000) one week before the inoculation of *R. reniformis* in eggplants significantly reduced the damage in terms of plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits caused by *R. reniformis*. The highest significant improvement in plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits was recorded in the plants treated with 5000 $J_3$ *Steinernema* sp. / 500 g soil followed by 2500, 1000, 10,000 and 20,000 $J_3$ *Steinernema* sp. / 500 g soil. In the corresponding treatments the percentage reduction in length was recorded as 12.25%, 20.14%, 27.13%, 29.66% and 32.19% and dry weight 13.75%, 22.57%, 31.00%, 35.79% and 37.31% and number of flowers as 11.91%, 26.80%, 37.97%, 42.18% and 42.93% and weight of fruits was found as 17.06%, 26.47%, 34.12%, 35.88% and 38.24% in comparison to control.

The data presented in table 2.2 revealed that reniform nematode, *R. reniformis* multiplication was found to be significantly higher in case of *R. reniformis* inoculated egg plants. However, the inoculation of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) one week before the inoculation of *R. reniformis* in eggplants significantly reduced the *R. reniformis* multiplication. The highest reduction in the reproduction factor (Rf) was recorded in the plants treated with 5000 $J_3$ *Steinernema* sp. / 500 g soil was followed by 2500, 1000, 10,000 and 20,000 $J_3$ / 500 g soil. In the corresponding treatments the reproduction factor was recorded as 9.86, 7.55, 11.75, 12.20 and 12.55. However on the other hand, the lower inoculum (50,500) of *Steinernema* sp. applied one week before the inoculation of *R. reniformis* didn't significantly improve the eggplant growth parameters and reduce the reproduction factor as compared to the plants individually inoculated with *R. reniformis.*

Moreover, the sequential inoculation of *Steinernema* sp. with either of the inoculum levels (1000, 2500, 5000, 10,000 and 20,000) one week after the inoculation of *R. reniformis* in eggplants significantly reduced the damage caused by *R. reniformis* in terms of plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. The highest improvement in plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits was recorded in the plants treated with 5000 $J_3$ *Steinernema* sp. / 500 g soil followed by 2500, 1000, 10,000 and 20,000 $J_3$ *Steinernema* sp. / 500 g soil. The significant improvement in

the plant growth parameters was noticed in all these treatments as compared to plants inoculated with *R. reniformis* alone. In the corresponding treatments the percentage reduction in length was recorded as 15.18%, 21.96%, 28.14%, 30.26% and 33.20% and dry weight as 17.17%, 25.84%, 33.13%, 36.93% and 39.06% and number of flowers as 13.65%, 28.29%, 38.21%, 42.93% and 43.92% and weight of fruits as 20.00%, 28.84%, 35.29%, 36.47% and 37.64% in comparison to control. The data presented in table 3.2 revealed that reniform nematode multiplication was found to be significantly higher in case of eggplant seedlings inoculated with *R. reniformis*. The reproduction factor was found to be as 13.87.

However, the inoculation of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) one week after the inoculation of *R. reniformis* in eggplants significantly reduced the *R. reniformis* multiplication. The highest reduction in the reproduction factor was reported in the plants treated with 5000 $J_3$ *Steinernema* sp. / 500 g soil followed by 2500, 1000, 10,000 and 20,000 $J_3$ *Steinernema* sp./ 500 g soil. In the corresponding treatments the reproduction factor was found to be as 5.05, 8.75, 11.85, 12.30 and 12.60. However on the other hand, the lower inoculum (50,500) of *Steinernema* sp. one week after the inoculation of *R. reniformis* didn't significantly improve the eggplant growth parameters and reduce the reproduction factor as compared to the plants inoculated individually with *R. reniformis.* Comparison of concomitant and sequential inoculations also showed that the sequential inoculation (both prior and after) of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) were significantly more effective in the management of *R. reniformis* than the concomitant inoculation.

## 4. Discussion

The present study revealed that entomopathogenic nematode *Steinernema* sp. significantly reduced the populations of *Rotylenchulus reniformis* which in turn improved plant growth parameters. our results are in coincidence with results of Shahina and Tabassum, 2009 who reported the significant reduction in population of *Rotylenchulus reniformis* on Bermuda grass by the application of live or dead (killed) infective juveniles of *Steinernema pakistanese*. EPN's role as an efficient control agent of *Rotylenchulus reniformis* is also in conformity of various other previous studies showing reduction of PPN's by entomopathogenic nematodes ( Bird and Bird, 1986; Ishibashi and Kondo, 1986, Ishibashi and Kondo,1987; Ishibashi and Choi, 1991; Smitley *et al*. 1992; Gouge *et al*. 1994; Grewal *et al*. 1997, Perry *et al*. 1998; Grewal and Georges, 1998; Grewal *et al*. 1999; Lewis *et al*. 2001; Jagdale *et al*. 2002; Somasekhar *et al*. 2002; Lewis and Grewal, 2005; Perez and Lewis, 2006; and Shahina and Tabassum, 2009).

The present study also revealed that the application of *Steinernema* sp. at different levels (50, 500, 1000, 2500, 5000, 10,000 and 20,000 $J_3$ / 500 g soil) in eggplant seedlings inoculated with *Rotylenchulus reniformis* showed that lower (50 and 500) as well as higher (20,000) inoculum levels of *Steinernema* sp. were insignificant in the reduction of reniform nematode population and consequently did not improve plants growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. These findings were also found to be in agreement with Fallon *et al.* (2002) and Sherbiny *et al.* (2007). Fallon *et al.* (2002) reported that *Steinernema* sp. did not affect the growth or development of *M. javanica* infected tomatoes at higher (20,000 ij's i.e. 44 ij's/$cm^3$) concentration levels. Sherbiny *et al*., (2007) reported that the application of Steinernema *feltiae* and *Heterorhabditis bacteriophora* @1000 to 8000 $J_3$ / pot almost had no effects on improving visual growth parameters and pod weights of common bean in treated plants as compared to *M. javanica*- infected ones. Our results revealed that only the inoculum levels (1000,

2500, 5000, 10,000 J3 / 500 g soil) of *Steinernema* sp. were significant in reduction / suppression of the reproduction factor of *Rotylenchulus reniformis* which consequently improved the plant growth parameters viz., plant length, dry weight, number of flowers and weight of fruits. However, our findings are in disagreement with the findings of Lewis *et al*. (2002). They reported that both the lower and higher rates of *S. riobrave* suppressed *M. incognita*. The reason for higher inoculum levels to be non-significant in improving the plant growth of *M. incognita* infected plants is due to greater numbers of ij's of *Steinernema* sp. entering the root and consequently increasing entry points for subsequent entry of root knot nematode. This behavior might have resulted in increased *R. reniformis* root penetration at higher ij application levels of *Steinernema* sp. and consequently had a negative impact on plant growth parameters.

The suppressive effects of entomopathogenic nematodes on plant parasitic nematodes may be due to several factors. Bird and Bird (1986) demonstrated the attraction of *Steinernema glaseri* Steiner to tomato roots and suggested that suppression of plant parasitic nematodes by entomopathogenic nematodes may be due to competition between the two nematode groups for space. However, this mechanism does not explain the suppression of plant parasitic nematodes by application of dead EPN's observed in various studies. Ishibashi and Kondo (1986) attributed the suppressive effects of entomopathogenic nematodes to the increased density of predators resulting from the application of nematode biomass to the soil. However, based on the observations of suppressing populations of plant parasitic nematodes by entomopathogenic nematodes in sterile soil, and the suppression of root penetration of *Meloidogyne incognita* by heat killed entomopathogenic nematodes, Grewal *et al*. (1999) suggested that behavioral response and increased natural enemies are unlikely to account for the entire effect observed in the field. More recent evidence attributes the suppressive effects to the production of allelochemicals by the entomopathogenic nematode-symbiotic bacteria complex (Grewal *et al*., 1999; Hu *et al*., 1999; Lewis *et al*., 2001; Samaliev *et al*., 2000; Jagdale *et al*., 2002). Nematicidal properties of metabolites of symbiotic bacteria *Xenorhabdus* spp. associated with *Steinernema* spp. have been demonstrated in several laboratory / greenhouse studies (Grewal *et al*., 1999; Hu *et al*., 1999; Samaliev *et al*., 2000). EPN- associated bacteria, *Xenorhabdus* spp. or *Photorhabdus* spp., produce endotoxins composed of lipopolysaccharides that are toxic and could kill or affect in another way the evaluated stages (Dunphy and Webster, 1988). According to Hu *et al*., 1995, 1996, 1999, the bacteria produce Stilbene and Indole metabolites that are nematicidal to a range of nematode species.

It was also reported that the sequential inoculation of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) one week before or after inoculation of *R. reniformis* showed the significant improvement in plant growth parameters and reduction in reproduction factor. These findings also showed that the sequential inoculation (both prior and after) of *Steinernema* sp. at different inoculum levels (1000, 2500, 5000, 10,000 and 20,000) were more effective in the management of reniform nematode than the concomitant inoculation. These findings were found to be in agreement with Perez and Lewis (2002); Grewal, (1997); Sasnarukkit *et al*., (2002); Evan & Haydock, (1993). Perez and Lewis (2002) found that *S. riobravis* and *S. feltiae* applied at the rate of 25ijs / $cm^2$ before or after and *H. bacteriophora* applied before *M. incognita* were most efficient in suppression of penetration and egg production on tomatoes than the concomitant application. They also reported that pre- or post *M. incognita* infestation application of 25 ij/$cm^2$ of *H. bacteriophora*, *S. riobrave* or *S. feltiae* suppressed *M. incognita* on tomato plants grown in green house. Sasnarukkit *et al.* (2002) reported that antagonism was observed only when

inoculation of root-knot nematode ij's was done after the application of EPN's. EPN's tend to die rapidly after their inundative application to soil (Curran 1993). The entomopathogenic nematodes dying and serving as substrate for the growth of their symbiotic bacteria resulted in the production of antagonistic metabolites. Evan and Haydock (1993) also reported that the prior application of EPN's may lead to the production of nematicidal metabolites. Moreover, our results were in disagreement with the finding of Perez and Lewis (2004), who reported that not only pre-application of EPN's are effective but the application at the same time with root knot nematodes is equally effective.

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Table 1.1. Effect of different inoculum levels of *Steinernema* sp. during concomitant inoculation on the growth of eggplant infected with *Rotylenchulus reniformis*.

| Treatments Inoculum level/500g soil | Plant length (cm) | | | | Dry weight (g) | | | | No. of flowers per plant | % reduction over control | Fruit wt./ plant (g) | % reduction over control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | % reduction over control | Shoot | Root | Total | % reduction over control | | | | |
| **Control (uninoculated-untreated)** | 69.5 | 29.3 | 98.80 | - | 11.75 | 1.44 | 13.20 | - | 40.30 | - | 850.0 | - |
| ***R.reniformis* (Rr)** | 40.2 | 19.3 | 59.50 | 39.78 | 6.19 | 0.98 | 7.20 | 45.52 | 18.90 | 53.10 | 470.0 | 44.71 |
| **50$J_3$ [a]EPN + [b]Rr** | 40.8 | 19.4 | 60.20ns | 39.07 | 6.22 | 0.99 | 7.20ns | 45.21 | 19.00ns | 52.85 | 475.0ns | 44.12 |
| **500$J_3$ EPN + Rr** | 41.5 | 19.6 | 61.10ns | 38.16 | 6.30 | 1.03 | 7.30ns | 44.30 | 20.80ns | 47.64 | 485.0ns | 42.94 |
| **1000$J_3$ EPN + Rr** | 46.6 | 20.7 | 67.40* | 31.78 | 7.20 | 1.14 | 8.30** | 36.63 | 23.50** | 41.69 | 540.0** | 37.06 |
| **2500$J_3$ EPN + Rr** | 50.9 | 22.9 | 73.80** | 25.30 | 8.10 | 1.23 | 9.30** | 29.10 | 27.20** | 32.51 | 595.0** | 30.00 |
| **5000$J_3$ EPN + Rr** | 56.3 | 24.5 | 80.80** | 18.32 | 9.07 | 1.33 | 10.40** | 21.50 | 33.20** | 17.62 | 665.0** | 21.76 |
| **10000$J_3$ EPN + Rr** | 46.2 | 20.3 | 66.50* | 32.69 | 7.08 | 0.97 | 8.10* | 38.83 | 21.35* | 42.93 | 525.0* | 38.24 |
| **20000$J_3$ EPN + Rr** | 44.0 | 19.9 | 63.90ns | 35.32 | 6.75 | 1.04 | 7.80ns | 40.81 | 21.20ns | 47.39 | 500.0ns | 41.18 |
| **C.D. at *P*=0.05** | - | - | 5.80 | - | - | - | 0.83 | - | 2.40 | - | 47.14 | - |
| **C.D. at *P*=0.01** | - | - | 7.99 | - | - | - | 1.00 | - | 3.20 | - | 64.95 | - |

Values are mean of five replicates.
* Significant over control at 5%.
** Significant over control at 5% and 1%.

[a]EPN = *Steinernema* sp.
[b]Rr = *Rotylenchulus reniformis*

Table 1.2 Effect of different inoculum levels of *Steinernema* sp. during concomitant inoculation on the multiplication of *Rotylenchulus reniformis* infecting eggplant.

| **Treatments Inoculum level/500g soil** | **Nematode population of *R. reniformis*** | | | |
|---|---|---|---|---|
| | **Females/root system** | **Larvae/500g soil** | **Total** | **[c]R.f.** |
| **Control (uninoculated-untreated)** | – | – | – | – |
| ***R.reniformis* ( Rr)** | 205 | 6730 | 6,935 | 13.87 |
| **50$J_3$ [a]EPN + [b]Rr** | 200 | 6635 | 6,835ns | 13.67ns |
| **500$J_3$ EPN + Rr** | 180 | 6495 | 6,675ns | 13.35ns |
| **1000$J_3$ EPN + Rr** | 170 | 5855 | 6,025** | 12.05** |
| **2500$J_3$ EPN + Rr** | 145 | 4530 | 4,675** | 9.35** |
| **5000$J_3$ EPN + Rr** | 120 | 3205 | 3,325** | 6.65** |
| **10000$J_3$ EPN + Rr** | 175 | 6100 | 6,275** | 12.55** |
| **20000$J_3$ EPN + Rr** | 180 | 6295 | 6,475ns | 12.95ns |
| **C.D. at *P*=0.05** | – | – | 476.93 | 0.95 |
| **C.D. at *P*=0.01** | – | – | 657.13 | 1.31 |

Values are mean of five replicates.
* Significant over control at 5%.
** Significant over control at 5% and 1%.

[a]EPN = *Steinernema* sp.
[b]Rr = *Rotylenchulus reniformis*
[c]R.f. = Reproduction factor

Table 2.1. Effect of different inoculum levels of *Steinernema* sp. applied during sequential inoculation, one week prior to *Rotylenchulus reniformis* on the growth of eggplant.

| Treatments Inoculum level/500g soil | Plant length (cm) | | | | Dry weight (g) | | | | No. of flowers per plant | % reduction over control | Fruit wt./ plant (g) | % reduction over control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | % reduction over control | Shoot | Root | Total | % reduction over control | | | | |
| **Control (uninoculated-untreated)** | 69.5 | 29.3 | 98.8 | - | 11.75 | 1.44 | 13.16 | - | 40.30 | - | 850 | - |
| ***R.reniformis* (Rr)** | 40.2 | 19.3 | 59.5 | 39.78 | 6.19 | 0.98 | 7.17 | 45.52 | 18.90 | 53.10 | 470 | 44.71 |
| **50$J_3$ [a]EPN → [b]Rr** | 41.8 | 20.4 | 62.2ns | 37.04 | 6.50 | 1.03 | 7.53ns | 42.78 | 20.50ns | 48.88 | 485ns | 42.94 |
| **500$J_3$ EPN → Rr** | 42.5 | 21.0 | 63.5ns | 33.70 | 6.62 | 1.08 | 7.70ns | 41.49 | 20.90ns | 47.15 | 495ns | 41.76 |
| **1000$J_3$ EPN → Rr** | 50.0 | 22.0 | 72.0** | 27.13 | 8.10 | 0.90 | 9.00** | 31.00 | 24.50** | 37.97 | 560** | 34.12 |
| **2500$J_3$ EPN → Rr** | 54.5 | 24.4 | 78.9** | 20.14 | 8.92 | 1.27 | 10.19** | 22.57 | 29.50** | 26.80 | 625** | 26.47 |
| **5000$J_3$ EPN → Rr** | 60.2 | 26.5 | 86.7** | 12.25 | 10.00 | 1.35 | 11.35** | 13.75 | 35.50** | 11.91 | 705** | 17.06 |
| **10000$J_3$ EPN → Rr** | 47.5 | 22.0 | 69.5** | 29.66 | 7.42 | 1.13 | 8.55** | 35.79 | 23.60** | 42.18 | 545** | 35.88 |
| **20000$J_3$ EPN → Rr** | 45.2 | 21.8 | 67.0* | 32.19 | 7.18 | 1.12 | 8.30** | 37.31 | 22.50** | 42.93 | 525* | 38.24 |
| **C.D. at *P*=0.05** | – | – | 6.01 | – | – | – | 0.76 | – | 2.19 | – | 48.39 | – |
| **C.D. at *P*=0.01** | – | – | 8.28 | – | – | – | 1.05 | – | 3.02 | – | 66.68 | – |

Values are mean of five replicates.
. * Significant over control at 5%.
** significant over control at 5% and 1% .

[a]EPN = *Steinernema* sp
[b]Rr = *Rotylenchulus reniformis*

Table 2.2 Effect of different inoculum levels of *Steinernema* sp. applied during sequential inoculation, one week prior to *Rotylenchulus reniformis* on the multiplication of *R.reniformis* infecting eggplant.

| **Treatments Inoculum level/500g soil** | **Nematode population of *R. reniformis*** | | | |
|---|---|---|---|---|
| | **Females/root system** | **Larvae/500g soil** | **Total** | **[c]R.f.** |
| **Control (uninoculated-untreated)** | – | – | – | – |
| ***R.reniformis* (Rr)** | 205 | 6730 | 6,935.0 | 13.87 |
| **50$J_3$ [a]EPN → [b]Rr** | 185 | 6540 | 6,725.0ns | 13.45ns |
| **500$J_3$ EPN → Rr** | 165 | 6430 | 6,595.0ns | 13.19ns |
| **1000$J_3$ EPN → Rr** | 145 | 5730 | 5,875.0** | 11.75** |
| **2500$J_3$ EPN →Rr** | 120 | 3655 | 3,775.0** | 7.55** |
| **5000$J_3$ EPN → Rr** | 90 | 4840 | 4,930.0** | 9.86** |
| **10000$J_3$ EPN → Rr** | 145 | 5960 | 6,105.0** | 12.20** |
| **20000$J_3$ EPN → Rr** | 155 | 6120 | 6,275.0** | 12.55** |
| **C.D. at *P*=0.05** | – | – | 477.291 | 0.955 |
| **C.D. at *P*=0.01** | – | – | 657.625 | 1.315 |

Values are mean of five replicates,
* Significant over control at 5%.
** Significant over control at 5% and 1%.

[a]EPN = *Steinernema* sp.
[b]Rr = *Rotylenchulus reniformis*
[c]R.f. = Reproduction factor

Table 3.1 Effect of different inoculum levels of *Steinernema* sp. applied during sequential inoculation, one week after *Rotylenchulus reniformis* on the growth of eggplant.

| **Treatments Inoculum level/500g soil** | **Plant length (cm)** | | | | **Dry weight (g)** | | | | **No. of flowers per plant** | **% reduction over control** | **Fruit wt./ plant (g)** | **% reduction over control** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Shoot** | **Root** | **Total** | **% reduction over control** | **Shoot** | **Root** | **Total** | **% reduction over control** | | | | |
| **Control (uninoculated-untreated)** | 69.5 | 29.3 | 98.80 | - | 11.75 | 1.44 | 13.16 | - | 40.30 | - | 850 | - |
| ***R. reniformis* (Rr)** | 40.2 | 19.3 | 59.50 | 39.78 | 6.19 | 0.98 | 7.17 | 45.52 | 18.90 | 53.10 | 470 | 44.71 |
| **50$J_3$ [a]EPN ← [b]Rr** | 41.5 | 20.2 | 61.70ns | 37.55 | 6.45 | 1.01 | 7.46ns | 43.31 | 20.30ns | 49.63 | 480ns | 43.53 |
| **500$J_3$ EPN ← Rr** | 42.3 | 21.2 | 63.00ns | 35.73 | 6.55 | 1.04 | 7.59ns | 42.33 | 21.00ns | 47.39 | 490ns | 42.35 |
| **1000$J_3$ EPN ← Rr** | 48.0 | 22.0 | 70.00** | 28.14 | 7.80 | 1.10 | 8.80** | 33.13 | 24.90** | 38.21 | 550** | 35.29 |
| **2500$J_3$ EPN ← Rr** | 53.2 | 23.8 | 77.00** | 21.96 | 8.50 | 1.26 | 9.76** | 25.84 | 28.90** | 28.29 | 610** | 28.24 |
| **5000$J_3$ EPN ← Rr** | 58.5 | 25.6 | 84.10** | 15.18 | 9.60 | 1.30 | 10.90** | 17.17 | 34.80** | 13.65 | 680** | 20.00 |
| **10000$J_3$ EPN ← Rr** | 47.1 | 21.8 | 68.90** | 30.26 | 7.30 | 1.00 | 8.30** | 36.93 | 23.25** | 42.93 | 540** | 36.47 |
| **20000$J_3$ EPN ← Rr** | 46.0 | 21.3 | 67.30* | 33.20 | 7.22 | 0.95 | 8.17* | 39.06 | 22.80** | 43.92 | 530* | 37.64 |
| **C.D. at *P*=0.05** | – | – | 5.45 | – | – | – | 0.70 | – | 2.18 | – | 47.88 | – |
| **C.D. at *P*=0.01** | – | – | 8.19 | – | – | – | 1.03 | – | 3.01 | – | 65.97 | – |

Values are mean of five replicates.
***** Significant over control at 5%.
** significant over control at 5% and 1%.

[a]EPN = *Steinernema* sp.
[b]Rr = *Rotylenchulus reniformis*

Table 3.2 Effect of different inoculum levels of *Steinernema* sp. applied during sequential inoculation, one week after *Rotylenchulus reniformis* on the multiplication of *R. reniformis* infecting eggplant.

| **Treatments Inoculum level/500g soil** | **Nematode population of *R. reniformis*** | | | |
|---|---|---|---|---|
| | **Females/root system** | **Larvae/500g soil** | **Total** | **[c]R.f.** |
| **Control (uninoculated-untreated)** | – | – | – | – |
| ***R. reniformis (Rr)*** | 205 | 6730 | 6,935 | 13.87 |
| **50$J_3$ [a]EPN ← [b]Rr** | 195 | 6580 | 6,775ns | 13.55ns |
| **500$J_3$ EPN ← Rr** | 175 | 6450 | 6,625ns | 13.25ns |
| **1000$J_3$ EPN ← Rr** | 155 | 5770 | 5,925** | 11.85** |
| **2500$J_3$ EPN ← Rr** | 130 | 4245 | 4,375** | 8.75** |
| **5000$J_3$ EPN ← Rr** | 105 | 2420 | 2,525** | 5.05** |
| **10000$J_3$ EPN ← Rr** | 160 | 5990 | 6,150** | 12.30** |
| **20000$J_3$ EPN ← Rr** | 165 | 6135 | 6,300* | 12.60* |
| **C.D. at *P*=0.05** | – | – | 466.58 | 0.93 |
| **C.D. at *P*=0.01** | – | – | 642.86 | 1.29 |

Values are mean of five replicates.
* Significant over control at 5%.
** Significant over control at 5% and 1%.

[a]EPN = *Steinernema* sp.
[b]Rr = *Rotylenchulus reniformis*
[c]R.f. = Reproduction factor