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ABSTRACT

The effect of root-knot nematode, *Meloidogyne incognita* on the severity of Fusarium wilt of strawberry was evaluated under greenhouse conditions. Inoculation of strawberry cultivar Sweet Charlie with 1,000 second stage juvenile of root-knot nematode in combination with *Fusarium* sp. resulted in greater incidence and severity of *Fusarium* wilt. However, this was not significantly different from plants inoculated with the fungus alone. Fresh root weight was significantly reduced when plants were inoculated with fungus alone and the combination of nematode and fungus. However, no significant effect was noted on the fresh top weight.

A significant number of second, third and forth stage juveniles of root-knot nematode was recovered from the roots of plants inoculated with nematode alone but they did not develop into adult females. On the other hand, significantly less number of J2, J3 and J4 was noted in the roots of plants inoculated with the combination of nematode and the fungus.

The results imply that strawberry cultivar Sweet Charlie is not a suitable host of M. *incognita*. Thus, the expected increase in the severity of *Fusarium* wilt due to

inoculation of nematode plus fungus was not observed in the present investigation despite the susceptibility of the strawberry cultivar to the fungal pathogen.

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INTRODUCTION

In Baguio and Benguet, particularly in La Trinidad strawberry, (*Fragaria x ananassa* Duch) production is one of the sources of farmers' income. It is grown for its edible red fruits which are either eaten as fresh and flavoring purposes. It is highly demanded by tourists, processing factories like Magnolia, Nestle as well as local consumers (Flores, 2007). It contains ellagic acid, which is a potent antioxidant. Strawberries are highly perishable, and vitamin C is present on the berries (Esiong, 2004).

Several strawberry varieties were earlier introduced in the highland; which includes Tioga, Solana, Royal Gem, Mission bells and Fukuba. These varieties are no longer planted in the locality. The most widely grown variety today is Sweet Charlie.

Recent survey showed the presence of *Fusarium spp*. in strawberry growing areas in Baguio and Benguet. This fungus usually enters its host through feeder roots, and then it multiplies and colonizes the vascular system. Infection may occur at any time during the life of the plant. The disease is more severe when air and soil temperature are 78˚ to 90˚ F and is more likely to occur in poorly drained soil (Tarr, 1972).

Meloidogyne has the greatest capability in predisposing plants to attack by *Fusarium* spp. Interactions between *Meloidogyne* spp. and *Fusarium oxysporum* have been reported in many crops (Mai and Abawi, 1987). Most papers on this interaction reported the use of inoculum at either specified concentrations or densities that were unnaturally high. Roots commonly were wounded, either by cutting, transplanting, or making inoculation holes in the soil. Studies of the effects of different inoculum densities have demonstrated an increased in susceptibility to *Fusarium* wilt at moderate or high levels of *Meloidogyne spp*.

and moderate levels of *F. oxysporum* in watermelon (Summer and Johnson, 1973) and cotton (Starr and Garbey *et. al*, 1989).

Objectives of the Study

1. To determine the influence of root-knot nematode (*Meloidogyne incognita*) on the severity of *Fusarium* wilt in strawberry, and

2. To determine the effect of *Fusarium* sp. on the reproduction/ population of *M. incognita.*

Time and place of the study

The study was conducted at the BIOCON laboratory and greenhouse of Horticultural Research and Training Institute, (HORTI) Benguet State University, La Trinidad, Benguet.

REVIEW OF LITERATURE

The Host Plant

Strawberry cultivar Sweet Charlie is from the University of Florida (UF). It is resistant to anthracnose fruit rot. The fruit has distinctly sweet flavor due to high sugar/ acid ratio. Its external fruit color is orange red and internal color is orange streaked with white. The fruit is generally less firm than the fruit of Oso Grande and have average size of 17 g, which is similar to that of Selva but less than that of Oso Grande. Fruit has a larger calyx compared to the fruit of other cultivars grown in Florida (Anonymous, 2002).

The Pathogen

Fusarium wilt. Fusarium wilt is a soil borne fungal disease that affects a wide variety of herbaceous plants, causing wilting and death.

Fusarium is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes and are relatively abundant members of the soil microbial community.

Disease cycle

The *Fusarium* fungus survives and may actually increase in a number of soil types for many years, independent of any host plants. This ability to survive eliminated any normal rotation program on general sanitation as an effective control measure. The appearance and severity of the disease are increased when air and soil temperature average at this rise at point. The fungus grows most rapidly and the disease is most severe at temperature 80 to 85˚F (26˚ to 29˚ C). The *Fusarium* wilt fungus invades the plant

through the young rootlets or wounds in the older roots at transplanting time or later. The fungus moves directly to the water- conducting tissues (xylem) and they progresses up the stem into the leaves. The fungus colonizes the xylem tissues and does not invade other tissues until part or the entire plant dies. The fungus produces its spore both inside and outside the affected stems.

Life Cycle

According to Agrios (1988), *Fusarium* produces one to two- celled thick- walled clamydospores that can withstand drought and low temperature. The fungus thrives on dead plant tissues and can over winter as mycelium or spores on infected tissues or seeds. The spores are early spread by air, contaminated equipment and water

Species of *Fusarium* produces two types of conidia that are termed macroconidia and microconidia (Alexopolus, 1996).

Persistence and transmission

Fusarium wilt can persist in most soils indefinitely because of its ability to colonize the roots of a number of weeds and to produce resistant spore structure.

Symptoms

Symptoms of black root rot usually begin in the first fruiting year. The injury is most noticeable in low or compacted soil of a field where drainage is poor. Strawberry plants with black root rot show a general lack of vigor with poor runner growth and small berries. Plants may collapse when water demand is high such as during spring growth, during or after fruiting, or during drought stress (Ellis *et al*., 2005).

Site Selection

To reduce infection of *Fusarium sp*., future planting site should be selected at least one year before planting. Soil drainage should be good. Avoid low- lying areas that have a tendency to be poorly drained (Pritts *et al*., 1991).

Control

Crop rotation is a great tool for controlling the disease as well, however, it is difficult to control if the space is limited because the spore may persist for as long as six years in the soil. In addition, the planting of host crops like eggplant, peppers, potatoes, strawberries and raspberries may not be avoided.Plant only certified, disease free transplants in fertile, well- drained soil .

Meloidogyne incognita Root- knot nematodes represent a relatively small but economically important group of obligate plant pathogens (Agrios, 1997). They are endoparasite with an interesting life cycle (Akthmar and Malik (N.D.)). They have evolved very specialized and established complex feeding relationship with their host plants. Juveniles hatch from egg after already having molted once. These second stage juveniles travel though the soil until they find plant tissue where they can penetrate and feed on, usually on tender root tip (Ferraz and Brown, 2002).

Symptoms

Plants infected with root-knot nematode exhibit leaf chlorosis, stunting and less blossom. Galling is also observed and the degree depends on the population of the nematode in the soil.

Survival of Nematodes

Nematode population is influenced by many factors. Soil moisture is one of the major factor affecting the nematode population, especially when rapid and extreme changes occur in the soil environment (HARRDEC, 1996). Since nematodes are aquatic animals, they require film of water coating soil particle for motility and survival (Agrios, 1997).

Control

Solarization can effectively suppress most species of nematode. This is an non-chemical technique in which transparent polyethylene film is laid over raised beds for 2-6 period to heat non- cropped soil to temperature lethal to nematodes and other soil borne pathogens (Noling,1995).

MATERIALS AND METHODS

The study was conducted at the BIOCON Laboratory and greenhouse of Horticultural Research and training Institute, Benguet State University, La Trinidad, Benguet. There were four treatments, which were replicated three times. Rice hull and sand were mixed and heat- sterilized prior to inoculation.

A. Root-Knot Nematode (*Meloidogyne incognita)*

Source of Nematode Inocula

Galled roots were collected from single egg mass culture and washed with tap water to remove the adhering soil particles. These galled roots containing egg masses of M. *incognita* were chopped into 1- 2 cm pieces and placed in 250 ml flasks. The eggs were allowed to disperse by dissolving the gelatinous matrix using 1% NaOCL solution (commercial bleach) in flasks which were vigorous by shaken (Shurtleff and Averre, 2000).

Standardization

Nematodes in water suspension were standardized by determining the number of eggs in a known volume. The suspension was agitated by stirring and blowing of pipette to make air bubbles while an aliquot was being withdrawn to ensure the even distribution of nematodes. When the tip of the pipette is midway between the top and the bottom of the liquid, an aliquot was immediately drawn and released into a counting dish. The number of eggs in a 1 ml aliquot was counted under a stereomicroscope and multiplied by the total volume of the suspension.

Inoculation

One thousand (1,000) eggs were used for inoculation per plant (Villanueva *et. al*, 2007).

B. *Fusarium* wilt.

Isolation of the Organism

Infected strawberries were collected from the field and washed thoroughly to remove the adhering soil particles. The roots were cut measuring 1 cm and surface-sterilized with 1% NaOCL, blot dry and washed three times with sterile distilled water. Sections of infected roots were placed in previously plated kidney bean agar.

Standardization of the Inoculum

Suspension containing conidia was prepared and spore concentration was estimated using a haemacytometer. One ml of conidial suspension was sucked using a micropipette and deposited on the ridge of the hemacytometer. After the suspension had flown automatically to the counting chamber, conidia counting was done in five squares of the nine squares labeled A which contains 16 small squares. Standardization was done three times. The average count of the five squares per trial was multiplied with 50,000 to obtain the total spore/ ml.

Inoculation

One week after the inoculation of the root- knot nematode (M. *incognita*), the plants were inoculated with Fusarium sp. using the inoculum level of $1x\ 10^6$ spore/ ml.

The different treatments were the following:

T_{1-} Uninoculated

T_2- Root-knot nematode (M. *incognita*) alone

T_3- *Fusarium* sp. alone

T_4- Root-knot nematode (M. *incognita*) + Fusarium sp.

Data Gathered

1. Disease severity. plants were assessed for Fusarium wilt severity using the rating scale below:

Rating	Description
1	normal; no infection
2	.5- 5 cm discoloration
3	6- 10 cm discoloration
4	11- 20 cm discoloration
5	31- 40 cm discoloration
6	41- 60 cm discoloration
7	61- 80 cm discoloration
8	81- 100 cm discoloration

2. Top weight (g). This refers to the weight of the upper portion of the plant after harvest.

3. Root weight (g). This refers to the weight of the root after harvest.

4. Number of nematodes in 3 g of root. Population counts of nematodes in feeding position were made from 3 g roots per treatment. Roots were stained in acid fuchsin glycerol to facilitate counting.

5. Number of nematodes in 200 g of soil (g). To determine whether the population of root knot decreased or increased, 200 g of soil samples were obtained from each pot and processed using the modified Baermann-tray technique. The nematodes were collected separately in 150-ml capacity Erlenmeyer flasks. The suspensions were allowed to stand at least 3 hours at room temperature and the excess water in the flasks was reduced to 50 ml by suction using a pipette. The nematodes were transferred to 50 ml capacity test tubes and were again allowed to stand at least 1 hour for nematodes to settle at the bottom. The nematodes were counted under a stereoscopic microscope.

RESULTS AND DISCUSSION

Disease Severity

Inoculation of *Fusarium* sp. and *Meloidogyne incognita* on strawberry cultivar Sweet Charlie resulted in wilting and sudden death of the plant (Fig. 1). However, it did not significantly differ with plants inoculated with *Fusarium* alone. Plants inoculated with nematode also showed vascular discoloration but significantly lower than those inoculated with the fungus and the combination of fungus and nematode. On the other hand, the uninoculated plants did not show vascular discoloration and wilting typical of *Fusarium* wilt infection. This was significantly different from the other treatments.

Fresh Root Weight

Plants inoculated with Fusarium wilt alone resulted in significantly lower fresh top weight (Figure 2). Although not significantly different from plants inoculated with the combination of the fungus and nematode and uninoculated control, those inoculated with root-knot nematode alone has the highest fresh root weight. Nevertheless, this was significantly higher than those plants inoculated with *Fusarium* sp. alone.

Fresh Top Weight

Inoculation of root-knot nematode alone, combination of nematodes and Fusarium sp. and fungus alone did not significantly affect the fresh top weight of strawberry (Fig. 3).

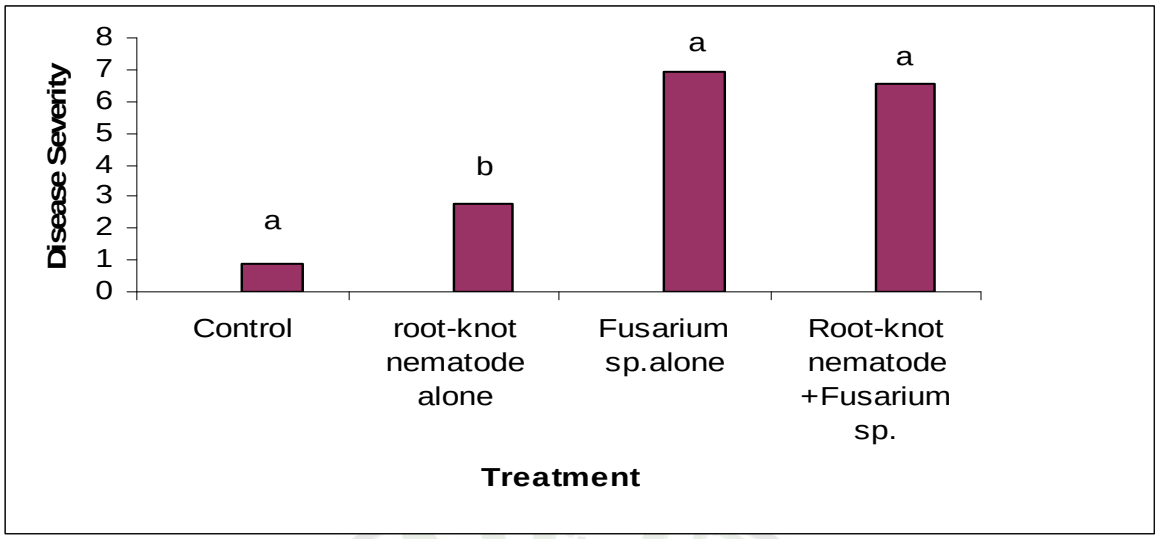


Fig. 1. Effect of root-knot nematode (*M. incognita*) and *Fusarium sp.* on disease severity.

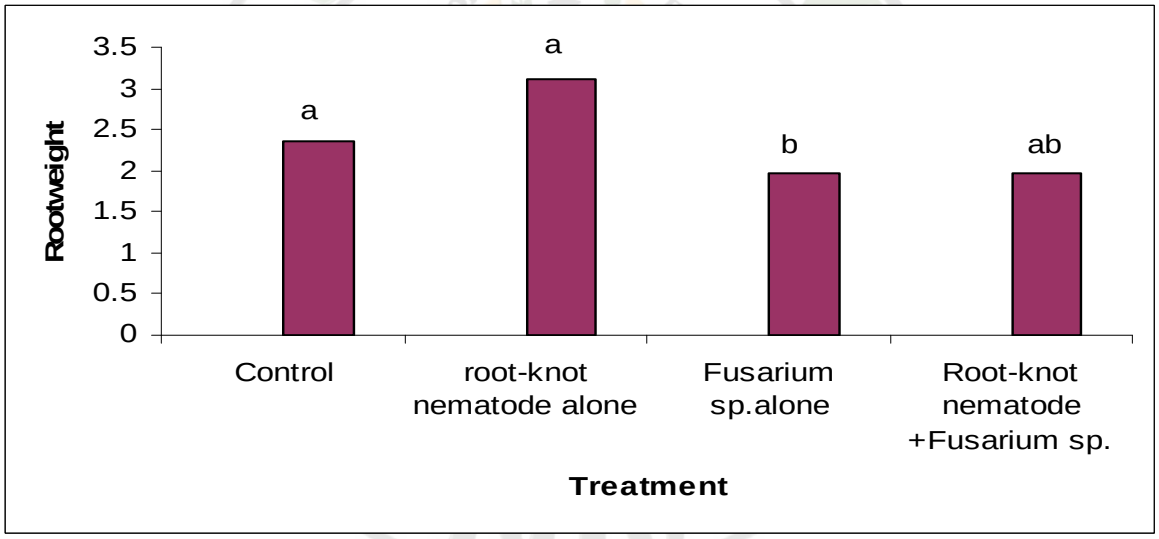


Fig. 2. Effect of root-knot nematode (*M. incognita*) and *Fusarium sp.* onweight.

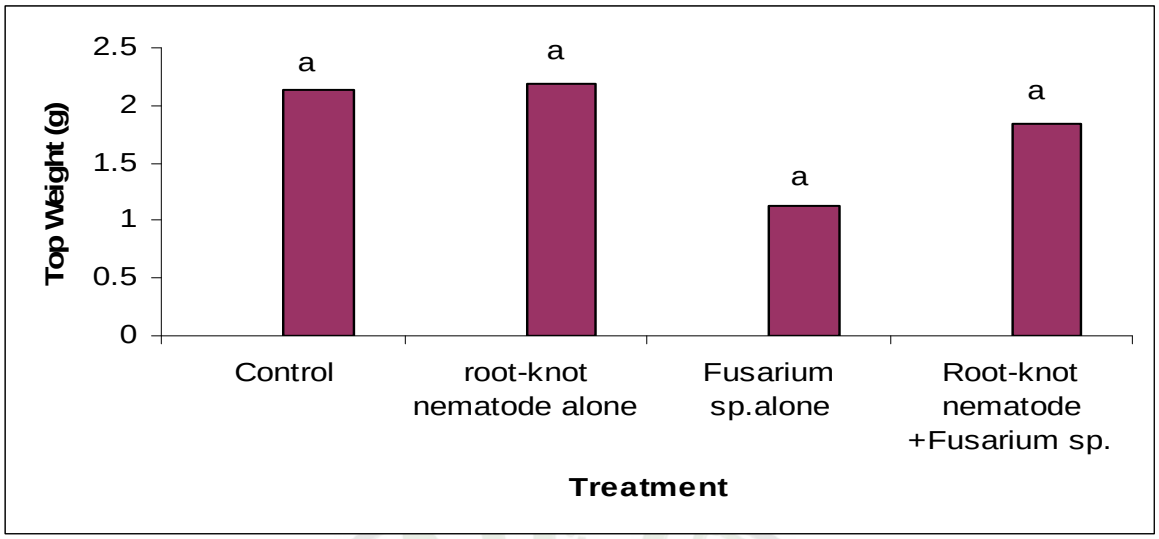


Fig. 3. Effect of root-knot nematode (*M. incognita*) and *Fusarium sp.* on fresh top weight

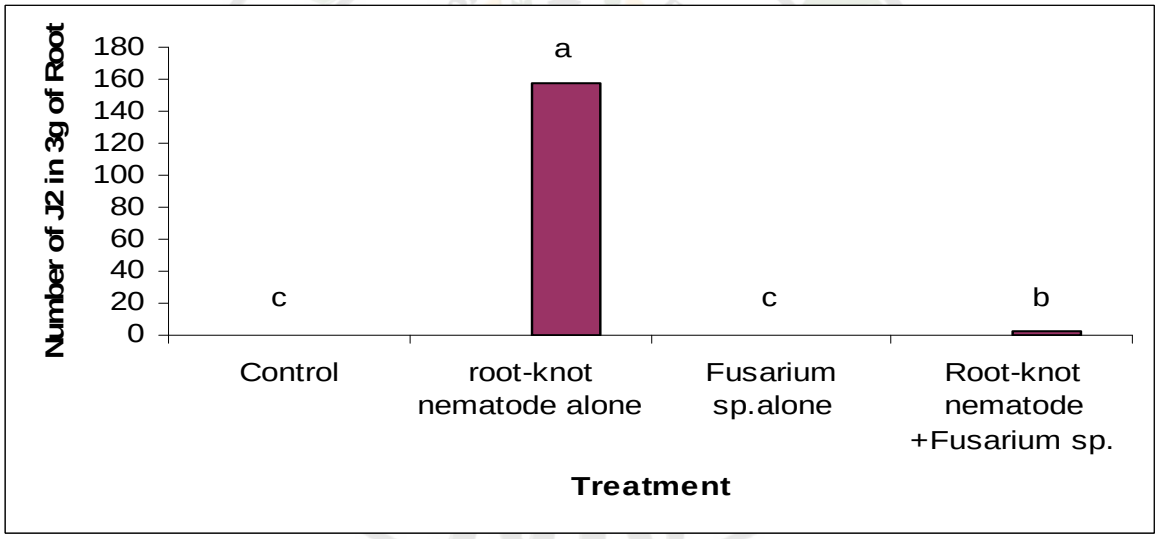


Fig. 4. Effect of Effect of root-knot nematode (*M. incognita*) and *Fusarium sp.* on the number of J2 in 3 g of roots

Number of J2 in the Root

Figure 4 shows the effect of the different treatments on the number of second stage juveniles in the root of strawberry. Apparently, the highest population of J2 was noted in plants inoculated with root-knot nematode alone. This was significantly different from those inoculated with the combination of the nematode and the fungus. No

nematode was recorded from the uninoculated plants and those inoculated with *Fusarium* sp. alone.

Number of J3 and J4 in the Root

The effect of the different treatments on the number of third and fourth stage juveniles in 3 g root is shown in Figure 5. The highest nematode count was noted in plants inoculated with root-knot nematode alone. This was significantly different from plants inoculated with the combination of nematode and the fungus. There was no nematode recovered from the control, plants and those inoculated with *Fusarium* sp. alone.

The absence of adult female nematode in the roots indicates that the nematode failed to develop up to maturity. According to Powell *et. al* (1960) fungal colonization of giant cells(the source of food for the nematode) and xylem is common in fungus-root knot interactions. The fact that the structure of the giant cell changes prior to fungal entry implies that translocatable fungal secretions rather than the penetrating hyphae initially modify and eventually kill the giant cells (Fattah and Webster, 1983). In addition to this fungus-induced/produced factor in the *Meloidogyne-Fusarium* disease complex on tomato, the developing nematode produces a factor or induces the plant to produce such a factor that is transmitted upward through the plant and breaks the fungal resistance (i.e. predisposes) of the host to *Fusarium*. The fungus develops and its secretions cause a progressible necrosis of the giant cell which, in turn, causes the nematode to stave to death.

Generally, the increase in the severity of *Fusarium* wilt in the strawberry due to *M. incognita* was not observed in the present study. This could be due to the following

reasons. First, strawberry is not a suitable host of *M. incognita* and second , *Fusarium sp.* has an adverse effect on the development and reproduction of the nematode.

Previous reports have shown that strawberry is not a suitable host of *M. incognita*. However, in Huelva, Spain *M. incognita* was one of the root knot nematodes recover from strawberry samples aside from *M.hapla,M.arenaria* and *Meloidogyne* sp.(Vega *et. al* 2002). Early observations of nematode- fungal interaction suggested that they were due to the nematode providing a ready means of entry into the host for the fungus. Undoubtedly, this occurs when root- browsing nematode cause superficial root injury and so enhance fungal asses and secondary pathogenicity of the roots (Webster, 1985).

The *Meloidogyne- Fusarium* interaction has been extensively studied because of its significance on major world crops, such as cotton (Sasser, 1972) and tobacco (Milne, 1972). The extent of the disease interaction is greatly influenced by range of factors. For instance, there is more extensive *Fusarium* colonization of the plant tissues as the number of developing *Meloidogyne* increases. Moreover, symptom expression, as a manifestation of disease interaction is greater in “young” than in “old” plants (Pitcher, 1974).

Total Number of Nematodes in the soil

Figure 6 shows the number of nematodes recovered from the soil. No significant difference was noted between those inoculated with nematode alone and the combination of root-knot nematode and *Fusarium* sp. As expected, no nematodes were obtained from the inoculated pots and those applied with *Fusarium* sp. alone.

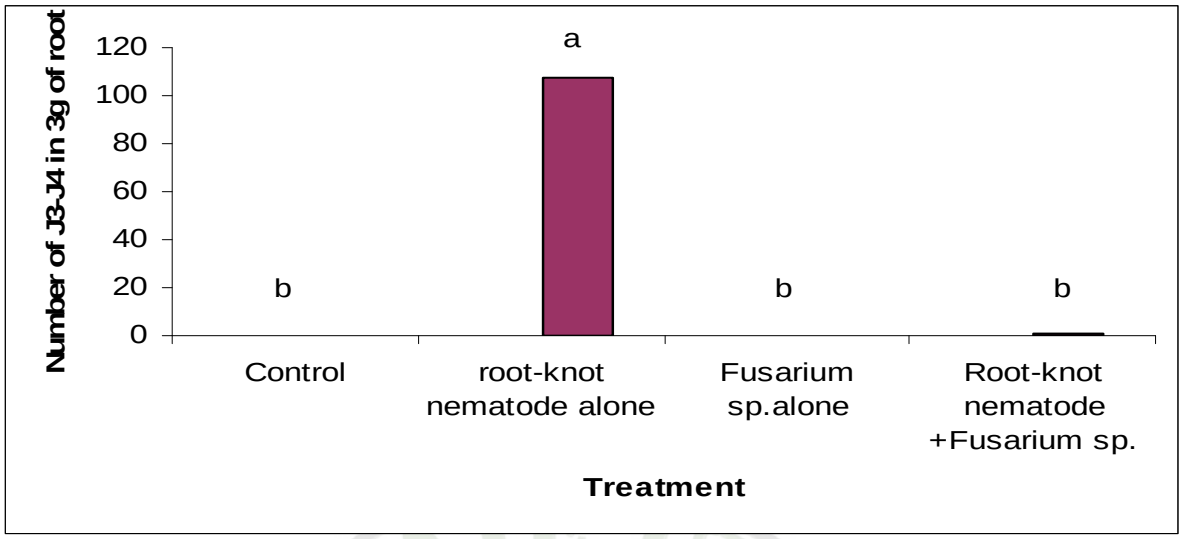


Fig. 5. Number of third and fourth stage juveniles in 3 g of roots as affected by the different treatments

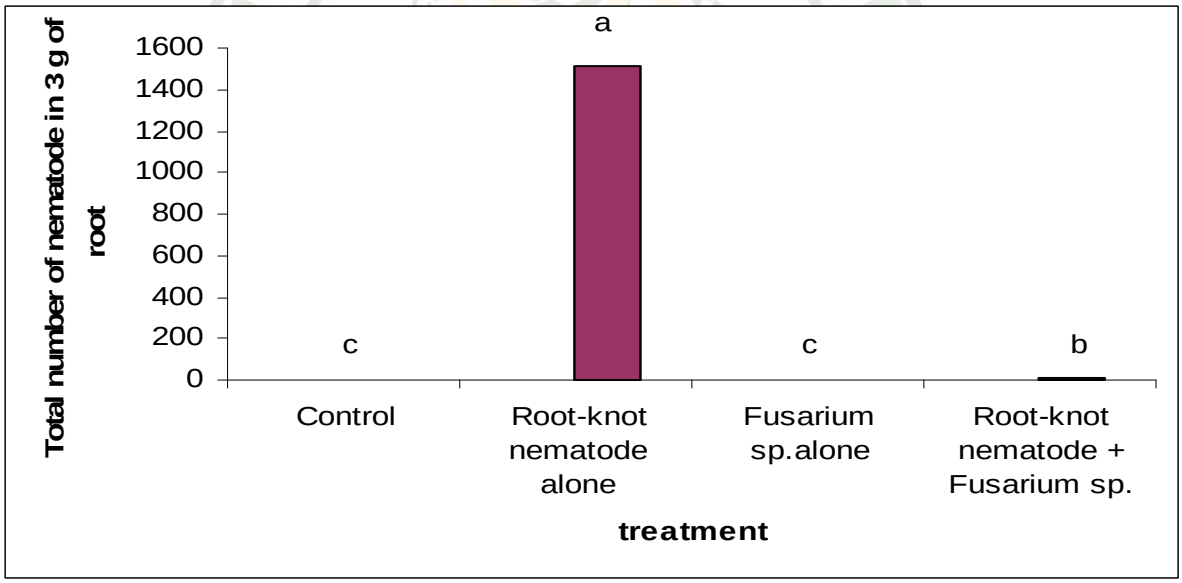


Fig. 6. Total number of nematodes in 3 g roots as affected by the different treatments.

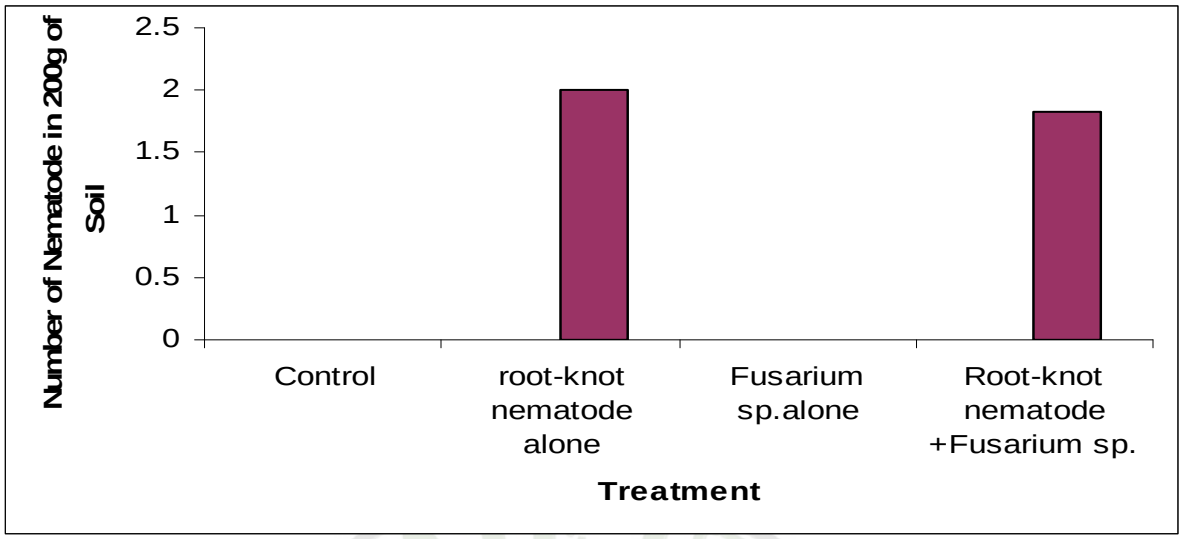


Fig.7. Number of nematodes in the soil

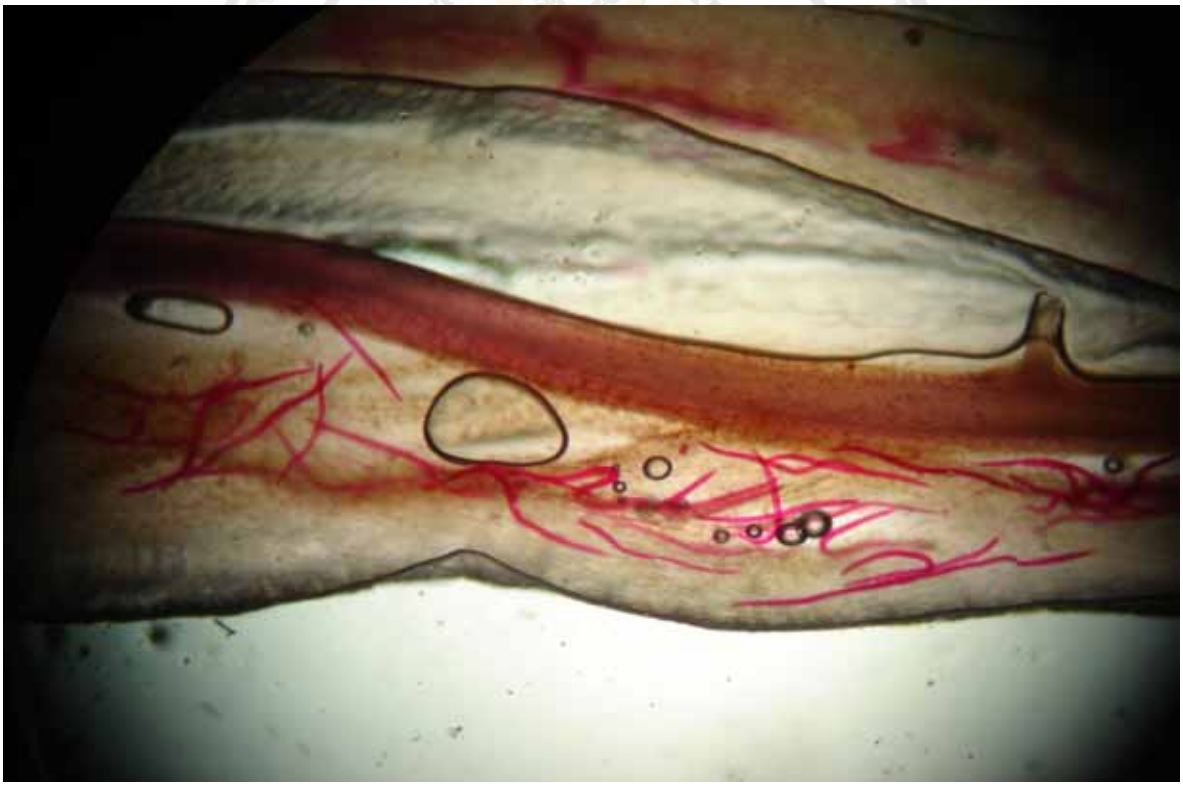


Fig. 8. Second stage juveniles (J_2) of *M. incognita* inside the roots of strawberry. Note the absence of mature females.

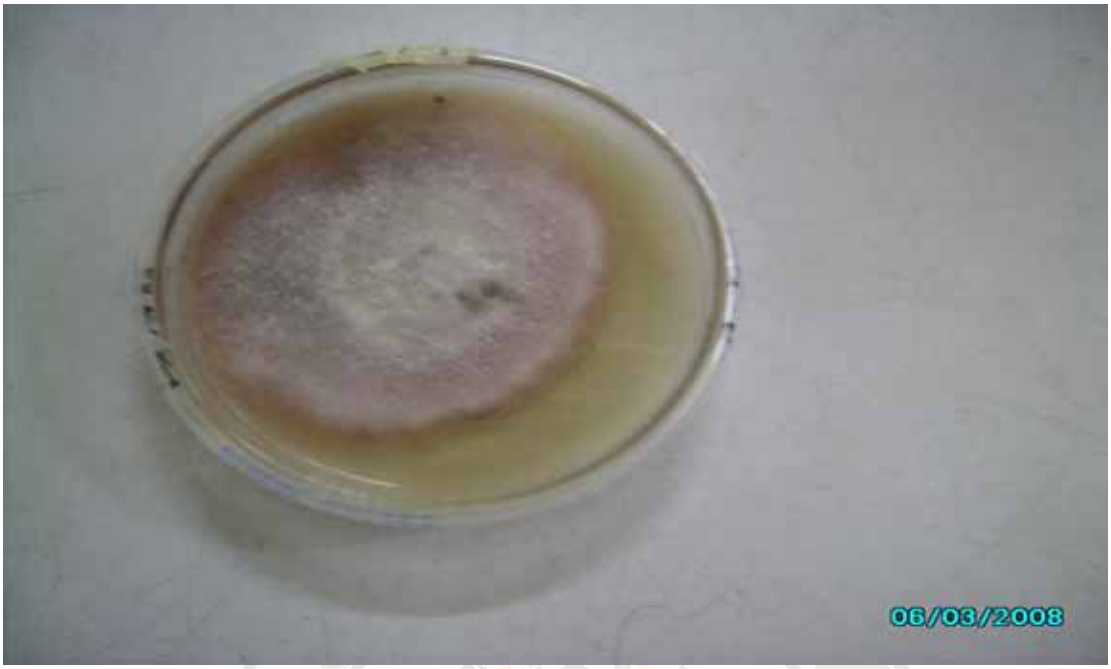


Fig.9. Pure culture of *Fusarium sp.* isolated from infected strawberry.

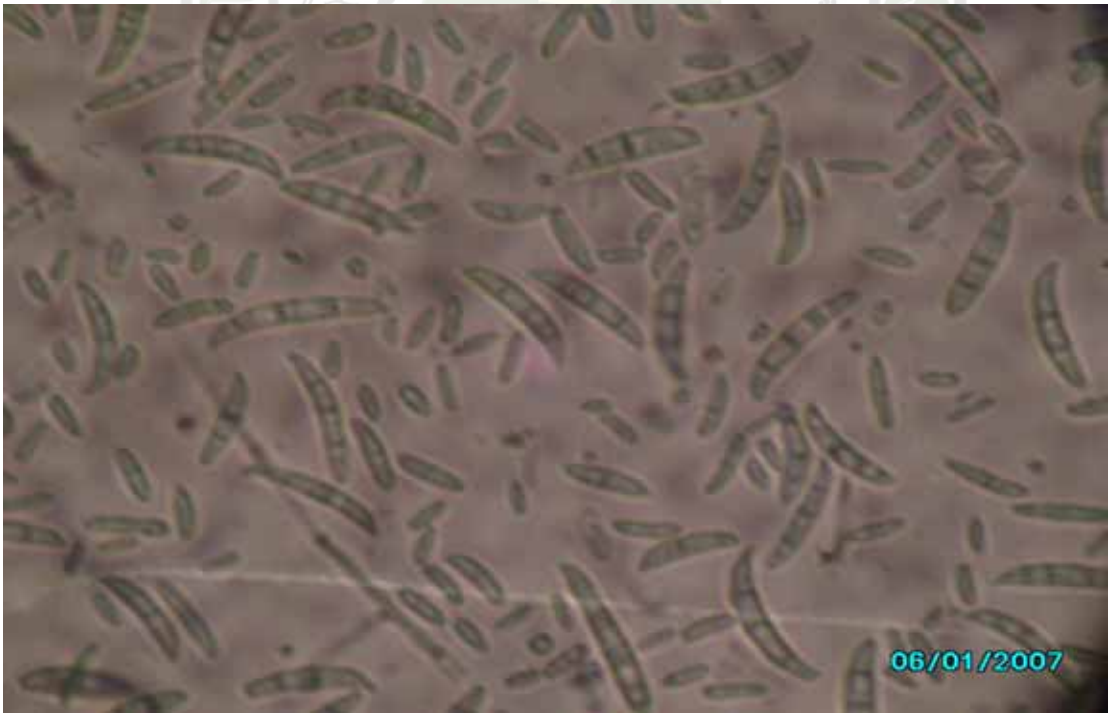
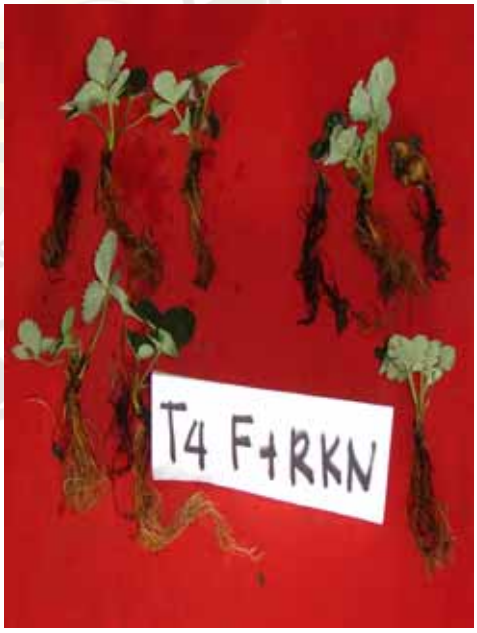


Fig.10. Macroconidia and microconidia of *Fusarium sp.*

A

B

C



D

Fig.11. Root systems of strawberry cultivar Sweet Charlie A – Uninoculated control, B – inoculated with *M. incognita* alone; C – Inoculated with *Fusarium sp.* alone and D – Inoculated with *Fusarium sp. + M. incognita*

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

A greenhouse experiment was conducted to determine the influence of root-knot nematode *(Meloidogyne incognita*) on the severity of *Fusarium* wilt in strawberry cultivar Sweet Charlie. Inoculation of 1,000 second stage juveniles (J2) of M. *incognita* did not significantly enhance the incidence and severity of Fusarium wilt of strawberry.

Inoculation of root-knot nematode alone and the combination of root-knot nematode and *Fusarium sp*. resulted in significant reduction in fresh root weight but not in fresh top weight.

A significant number of second, third and fourth stage juveniles of root-knot nematode was recovered from the roots of plants inoculated with nematode alone but they did not develop into adult females. On the other hand, significantly less number of J2, J3 and J4 was noted in the roots of plants inoculated with the combination of root-knot nematode and *Fusarium* sp. indicating the adverse effect of the fungus on the growth and development of the nematode.

Conclusion

Based on the results obtained, strawberry cultivar Sweet Charlie is not a suitable host to root-knot nematode. Because of the above reason, the nematode was not able to enhance the incidence and severity of Fusarium wilt despite the susceptibility of strawberry cultivar Sweet Charlie to *Fusarium* sp.

Recommendations

Further studies are therefore necessary using another species of *Meloidogyne* (preferably M. *hapla*) to fully evaluate the influence of the above nematode on the severity of Fusarium wilt in strawberry. Studies on the effect of varying nematode population densities and inoculation methods which minimize wounding are also recommended.

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APPENDICES

Table 1. Effect of root- knot nematode (*Meloidogyne incognita*) and *Fusarium sp*. on the disease severity

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
T_1	3	4	4	11	3.66
T_2	18	4	11	33	11
T_3 Fusarium sp.	31	27	25	83	27.66
T_4 RRN+Fusarium sp.	28	25	26	79	26.33

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F Value	Tabular 0.05	0
Replication	3	0.024	0.012	2.0308		
Treatment	3	0.785	0.262	44.6400	4.76	9
Error	6	0.035	0.006			
Total	11	0.844				

ns- not significant
**- Highly significant

Coefficient of Variation: 12.14%

Table 2. Effect of root- knot nematode (*Meloidogyne incognita*) and *Fusarium sp*. on fresh root weight

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
T_1 Control	7.8	8.24	12.31	28.35	9.45
T_2 RKN	10.84	13.04	13.5	37.38	12.46
T_3 Fusarium sp.	7.55	6.78	9.16	23.49	7.83
T_4 RRN+Fusarium sp.	7.55	6.78	9.16	23.49	7.83

ANALYSIS OF VARIANCE

Source	DF	SS	MS	F Value	Tabular 0.05	0
Replication	2	0.020	0.010	1.7857		
Treatment	3	0.078	0.026	4.6266	4.76	9
Error	6	0.034	0.006	0.006		
Total	11	0.131				

ns - not significant

Coefficient of Variation: 14.37%

Table 3. Effect of root- knot nematode (*Meloidogyne incognita*) and *Fusarium sp*. on fresh top weight

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
T_1 Control	8.02	7.97	12.4	28.39	9.46
T_2 RKN	6.09	15.55	4.69	26.33	8.77
T_3 Fusarium sp.	1.52	2.7	9.46	13.68	4.56
T_4 RRN+Fusarium sp.	4.02	6.57	8.08	18.67	6.22

ANALYSIS OF VARIANCE

Source	DF	DD	MS	F Value	Tabular 0.05	0
Replication	2	0.321	0.160	2.3192		
Treatment	3	0.096	0.032	0.4649	4.76	9
Error	6	0.415	0.069			
Total	11	0.832				

ns - not significant

Coefficient of Variation: 56.76%

Table 4. Effect of root- knot nematode (*Meloidogyne incognita*) and *Fusarium sp*. on the number of J2 in 3 g of root

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
T_1 Control	0	0	0	0	0
T_2 RKN	875	995	935	2805	935
T_3 Fusarium sp.	0	0	0	0	0
T_4 RRN+Fusarium sp.	13	0	0	13	433

ANALYSIS OF VARIANCE

Source	DF	SS	MS	F Value	Tabular 0.05 0
Replication	2	0.045	0.022	0.8144	
Treatment	3	17.605	5.868		4.76 9
Error	6	0.165	0.028		
Total	11	2.466		213.0443	

ns- not significant
**- Highly significant

Coefficient of Variation: 16.92%

Table 5. Effect of root- knot nematode (*Meloidogyne incognita*) and *Fusarium sp*. on the number of J3-J4 in 3 g of root

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
T_1 Control	0	0	0	0	0
T_2 RKN	508	643	575.5	1726.5	575.5
T_3 Fusarium sp.	0	0	0	0	0
T_4 RRN+Fusarium sp.	13	0	0	13	4.33

ANALYSIS OF VARIANCE

Source	DF	SS	MS	F Value	Tabular 0.05 0
Replication	2	0.275	0.137	1.9643	
Treatment	3	0.739	0.246	3.5222	4.76 9
Error	6	0.420	0.070		
Total	11	1.434			

ns- not significant
**- Highly significant

Coefficient of Variation: 43.27%

Table 6. Effect of root- knot nematode (*Meloidogyne incognita*) and *Fusarium sp*. on the total number of nematode in 3 g of root

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
T_1 Control	0	0	0	0	0
T_2 RKN	1383	1638	1510	4531	1510.3
T_3 Fusarium sp.	0	0	0	0	0
T_4 RRN+Fusarium sp.	29	13	0	42	14

ANALYSIS OF VARIANCE

Source	DF	SS	MS	F Value	Tabular 0.05 0
Replication	2	0.447	0.223	1.9208	
Treatment	3	0.378	0.459	3.9528	4.76 9
Error	6	0.697	0.116		
Total	11	2.522			

ns- not significant
**- Highly significant

Coefficient of Variation: 21.25%

Table 7. Effect of root- knot nematode (*Meloidogyne incognita*) and *Fusarium sp*. on the total number of nematode in 200 g of soil

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	III		
T_1 Control	0	0	0	0	0
T_2 RKN	8	8	8	24	8
T_3 Fusarium sp.	0	0	0	0	0
T_4 RRN+Fusarium sp.	5	10	7	22	733

ANALYSIS OF VARIANCE

Source	DF	SS	MS	F Value	Tabular 0.05 0
Replication	2	0.023	0.012	1.2396	
Treatment	3	0.637	0.212	22.4564	4.76 9
Error	6	0.057	0.009		
Total	11	0.717			

ns- not significant
**- Highly significant

Coefficient of Variation: 42.28%