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Cellular Alteration of Root-Knot Nematode *Meloidogyne incognita*-Infected Squash Plant and Intercropping Sesame Plant or Sesame Oil Seed Cake as Control Measures

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The feeding sites (giant cells) of *Meloidogyne incognita*-infected roots of squash are mainly confined in the endodermis and stele regions. Giant cells have dense and multinucleated cytoplasm and surrounded by hyper-plastic cells. When sesame plant was intercropped with susceptible squash, it significantly suppressed *M. incognita* on squash as indicated by the number of nematode galls and egg-masses compared to those on squash grown in pure stand. There was a positive correlation between number of sesame plants and the percentages of reduction in nematode galls and egg-masses on squash. The percentages of nematode reduction in squash roots intercropped with one, two, three and four sesame plants were 48.1, 74.1, 84.4 and 88.8% for root galling and 20, 50, 70 and 80% for egg-masses, respectively. Sesame oil seed cake was used, at the rates of 0, 10 and 15 g/pot, to study its effect on reproduction and development of the root-knot nematode on squash. All treatments have significantly reduced the nematode galls and egg-masses. In addition, sesame oil seed cake insignificantly improved plant growth criteria in proportional to the tested rates.

Key words: Cellular alterations, intercropping, *Meloidogyne incognita*, root-knot, sesame and squash.

Squash (*Cucurbita pepo* var. *Melopepo* L.) represents the most important cucurbitaceous vegetable crop in Egypt. Several species of *Meloidogyne* have been reported in cucurbit fields, however, *M. incognita* being the most frequent (Moussa *et al.*, 1981). Unfortunately, cucurbits have been grouped among crops with no successful breeding program for *Meloidogyne* resistance (Cook and Evans, 1987). Hence, it is a focal point to study the root-knot nematode symptoms on squash roots and to study the management tactics against this nematode.

The high toxicity of the currently used nematicides and their adverse effects on other forms of life have recently prompted research for new means of nematode management. In this concern, some plants are known to produce nematicidal compounds that inhibit nematode penetration or motility or that plants do not provide essential elements for nematode development (Hackney and Dickerson, 1975; Haroon and Smart, 1983; Korayem and Osman, 1992; Yassin and Ismail, 1993 and Ameen, 1996). Meredith and Perez (1973) reported that sesame (*Sesamum indicum*) could reduce numbers of *Meloidogyne* spp. In addition, Araya and Caswell-Chen (1994) reported that sesame and certain plants could reduce *Meloidogyne javanica* in soil.

Similarly, the use of oil seed cakes in the management strategies has shown great promise in the control of plant parasitic nematodes (Alam *et al.*, 1980; Alam *et al.*, 1982; Siddiqui and Alam, 1999 and Mittal and Goswami, 2001). The objectives of this study are to investigate the cellular alterations of the root-knot nematode, *M. incognita*-infected squash and to study the management of the nematode through intercropping with sesame plants and sesame oil seed cake.

Materials and Methods

Histological studies:

Two-month-old roots of squash (cv. Iskandarani), infected by *M. incognita*, were cut into small portions and fixed in formalin-glacial acetic acid-ethyl alcohol (FAA). Root portions dehydrated by n-butyl-alcohol as described by Sass (1951). Transverse and longitudinal sections (20μ thick) were cut by means of a rotary microtome and fixed on slides according to Johanson (1941). After staining with safranin and fast green (Sass, 1951), sections were mounted in Canada balsam for microscopic examination.

Managing M. incognita on squash:

Thirty six clay pots (20-cm-diam.) filled with 1 kg solarized sandy loam soil (1:1 w/w) were arranged in six groups, each one consists of six replicates. Squash seeds were sown in each pot and thinned to one plant per pot. At the same time, one to four seeds of sesame (*Sesamum indicum* L.), cv. Giza 32, were sown in the same pots at the periphery of each pot. One week after emergence for each plant species, plants were inoculated with 500 freshly hatched juveniles (J2) of *M. incognita* per pot. Squash and sesame plants in pure stands served as checks 1 and 2, respectively. The treatments were arranged in a completely randomized design. Sixty days after nematode inoculation, plants were taken off and number of galls and egg-masses were estimated and rated according to Sharma *et al.* (1994).

In the second experiment, pots were filled with solarized soil. Sesame oil seed cake were mixed with the soil at the rate of 10 or 15 g per pot. The pots were daily watered for oil seed cake decomposition. Fifteen days later, tested squash seeds were sown in each pot as previously mentioned. Fifteen days after seed germination, each pot was inoculated at a level of 500 individuals of the root-knot nematode, *Meloidogyne incognita*. Other pots free of the tested materials and inoculated with nematodes served as check. Forty days after nematode inoculation, plants were cut at the base level and detracted. Roots were carefully removed from soil and examined for nematode assay including numbers of galls and egg-masses. Also, shoots and roots length, as well as fresh and dry weights were determined.

Results

Stained longitudinal and transverse sections of *M. incognita*-infected roots of tested squash plants were microscopically examined. Squash roots exhibited severe infection of *M. incognita* and some alterations in the tissues were noticed (Fig. 1, plates 2, 3, 4 and 5) when compared with that of the healthy one (Fig. 1, plate 1). Moreover, Figure 1 (plate 2) indicates evidently the occupation of the meristemic or elongated root region with a nematode female. A mature female lays in the cortex with egg-mass in a gelatinous matrix protruding on the outer root surface was observed (Fig. 1, plate 3). Numerous giant cells having dense and granular cytoplasm were located in the endodermis. The giant cells were surrounded by hyper-plastic cells and they were in close vicinity to the nematode female (Fig. 1, plate 4). In some cases, multinucleated giant cells occupy a part of the stele region with displaced and deformed xylem. The nuclei are scattered or aggregated within the giant cells that have boundary walls (Fig. 1, plate 5).

Data presented in Table (1) reveal that when sesame (cv. Giza 32) plants were intercropped with susceptible squash (cv. Iskandarani) plants, they decreased reproduction and development of *M. incognita*. Numbers of galls and egg-masses were significantly reduced on roots of squash. Such reduction was positively correlated with number of sesame plants/pot. The average percentage of reduction in root galling reached 48.1, 74.1, 81.4 and 88.8% on squash roots intercropped with one, two, three and four sesame plants, respectively. Also, reduction in egg-masses recorded was 20, 50, 70 and 80% in the respective treatments. However, root galls on sesame were too small and degenerated.

Table 1. Effect of sesame intercropped with squash on *Meloidogyne incognita*

Treatment	Number of galls						Number of egg-masses			
	On squash	Red. (%)	Index	On sesame	Red. (%)	Index	On squash	Red. (%)	Index	On sesame
Squash in pure stand (check 1)	27 *	-	5.0	-	-	-	10	-	3.3	-
Sesame in pure stand (check 2)	-	-	-	3	-	2	-	-	-	-
Squash + 1 sesame plant	14	48.1	3.6	2	33.3	2	8	20	3.0	-
Squash + 2 sesame plants	7	74.1	3.0	1	66.7	2	5	50	2.3	-
Squash + 3 sesame plants	5	81.4	2.3	1	66.7	2	3	70	2.0	-
Squash + 4 sesame plants	3	88.8	2.0	0	100	1	2	80	2.0	-
L.S.D. at 5%	9.07	-	-	N.S.	-	-	2.29	-	-	-
L.S.D. at 1%	12.90	-	-	N.S.	-	-	3.39	-	-	-

* Each figure represents the mean of 6 replicates.

Gall or egg-mass index (according to Sharma *et al.*, 1994): 1= no galls or egg-mass; 2= 1-5; 3= 6-10; 4= 11-20; 5= 21-30; 6= 31-50; 7= 51-70; 8= 71-90 and 9 > 100.

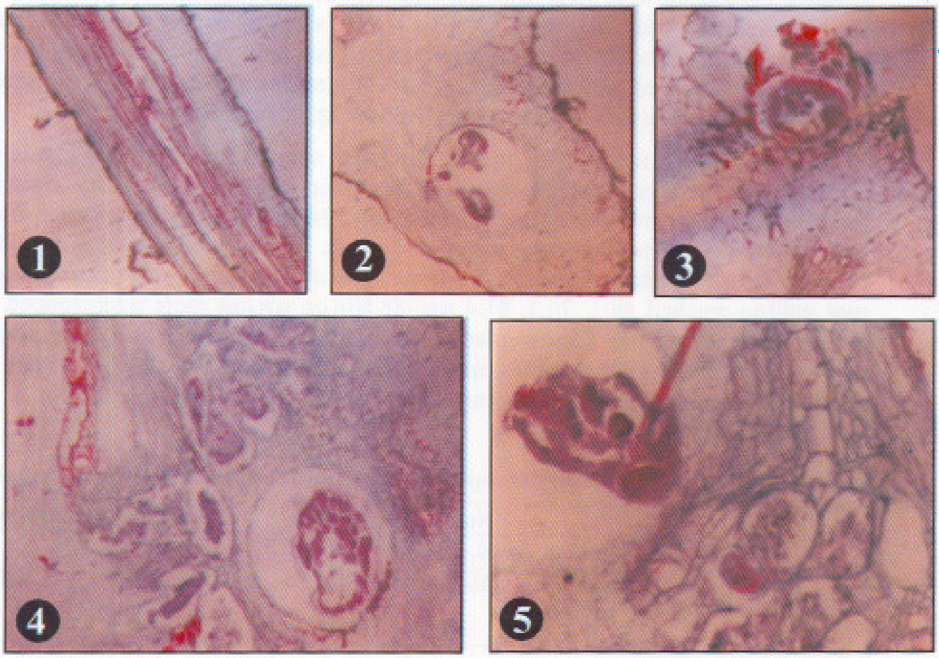

Fig. 1. Longitudinal and transverse sections of squash (cv. Iskandarani) showing anatomical changes in root tissues infected by *M. incognita*.

Plate 1. Healthy squash root showing the stele region.

Plate 2. Infected squash root showing a female in the meristemic region.

Plate 3. Infected squash root showing a female in the cortex with its eggmass on the outer root surface.

Plate 4. Infected squash root showing a female and numerous giant cells surrounded by hyperplastic cells in the endodermis.

Plate 5. Infected squash root showing a female and a group of multinucleated giant cells surrounded by hyperplastic cells in the stele.

Data presented in Table (2) show that sesame seed oil cake at the rates of 10 and 15 g/pot caused significant decrease in the number of galls and egg-masses developed on squash plants in comparison to those in the check (untreated). The highest nematode reductions (being 87.5 & 96.8%) were obtained at the highest rate (15 g/pot) for the respective nematode criteria followed by (85.4 and 93.5%) at the rate of 10 g/pot for the same nematode criteria.

Table 2. *Meloidogyne incognita* reproduction as influenced by sesame oil seed cake

Sesame oil seed cake rate (g/plot)	No. of galls	Red. (%)	Gall index	No. of egg-masses	Red. (%)	Egg-mass index
10	7 *	85.4	2	2	93.5	1
15	6	87.5	2	1	96.8	1
Check	48	-	4	31	-	4
L.S.D. at 5%	24.6	-	-	5.4	-	-
L.S.D. at 1%	37.2	-	-	8.2	-	-

* Each figure represents the mean of 6 replicates.

In case of squash growth, data presented in Table (3) reveal that in most cases, length, fresh and dry weights of shoots and roots were insignificantly increased in proportional to the tested rates when compared with those in the check treatment.

Table 3. Squash plant growth infected with *Meloidogyne incognita* as affected by sesame oil seed cake

Sesame oil seed cake rate (g/plot)	Shoot						Root			
	Length (cm)	Incre. (%)	Fresh wt. (g)	Incre. (%)	Dry wt. (g)	Incre. (%)	Fresh wt. (g)	Incre. (%)	Dry wt. (g)	Incre. (%)
10 g	54.0	3.3	63.3	5.5	6.7	34	2.40	18.2	0.17	30.8
15 g	67.7	29.4	80.0	33.3	12.7	154	3.50	72.4	0.23	76.9
Check	52.3	-	60.0	-	5.0	-	2.03	-	0.13	-
L.S.D. at 5%	N.S.	-	17.6	-	N.S.	-	N.S.	-	N.S.	-
L.S.D. at 1%	N.S.	-	N.S.	-	N.S.	-	N.S.	-	N.S.	-

Discussion

It was observed that *M. incognita* invaded squash root tissues and causing severe damage to its tissues. The nematode females were detected in the cortex and endodermis. Giant cells were detected in endodermis and stele regions, which appeared as distorted layers. The presence of the large multinucleated giant cells in the stele with compressed and dislocated xylem and vessel elements may result in poorer squash fruit formation and interruption of the host nutrient translocation. These results agree with those obtained by Osman, 1974 and Korayem, 1979.

The decrease in nematode galls and egg-masses of *M. incognita* on roots of susceptible squash as affected by growing resistant sesame plant(s) may be attributed to the toxic nature of resistant sesame root exudates. Similar results have been obtained from other intercropping systems (Alam *et al.*, 1977; Sartaj *et al.*, 1986; Huang, 1984; Korayem and Osman, 1992; Ameen, 1996 and Youssef and Soliman, 1997). Coinciding with the present results, Araya and Caswell-Chen (1994) reported that *Sesamum indicum* was resistant to penetration of *M. javanica*. Low nematode penetration may be partially responsible for reducing nematode numbers when this plant was grown in the field. Sesame roots may contain or secrete toxins that inhibit nematode penetration or motility of those nematodes invading the roots, but leave them after finding them unsuitable. Accordingly, resistant sesame cultivars are recommended to be used in rotations with root-knot susceptible crops in infested soil. Comparative efficacy of sesame oil seed cakes varied according to the amount of oil seed cake added to soil planted to squash. Sesame oil seed cake has been known to inhibit penetration and development of nematodes (Mehta *et al.*, 1994 and Zid, 2002). Also, these results are in harmony with those obtained by many researchers (Alam and Khan, 1974; Alam, 1991; Yassin and Ismail, 1993; Abid *et al.*, 1992 and Mittal and Goswami, 2001). They reported that application of oil seed cakes as soil amendment, has significantly suppressed the population of certain plant parasitic nematodes on the tested crops. Alam and Khan (1974) reported that with the liberal supply of water, oil seed cakes decompose and release many compounds including ammonia, phenols and aldehydes, which have nematicidal nature as proved by many other workers (Abrar *et al.*, 1974; Whitehead, 1976 and Alam *et al.*, 1978 & 1979). In addition, oil seed cakes produce during the decomposition of water-soluble fractions, some compounds that are highly toxic to nematodes (Alam *et al.*, 1982).

Better growth of plants in oil seed-amended soil appears to be due to reduction in nematode population as well as to the effect of oil seed cakes as manure. Besides, the roots of the amended plants may gain physiological changes, which make them unfavourable for nematode feeding and reproduction. In other words, such materials may induce certain degrees of resistance to plants against the nematodes (Alam *et al.*, 1980).

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التغيرات التشريحية في جذور الكوسة المصابة بنيماتودا تعقد الجذور *ميلودوجين انكوجنيتا* ، واستخدام التحميل مع السمسم أو إضافة كسب السمسم في مكافحة المرض

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تبين من دراسة القطاعات التشريحية لجذور الكوسة صنف أسكندراني المصابة بنيماتودا تعقد الجذور *ميلودوجين انكوجنيتا* أن الخلايا العملاقة التي تتكون نتيجة الإصابة بهذه النيماتودا قد تركز وجودها في طبقتي البشرة الداخلية والاسطوانة الوعائية للجذور ، و قد تميزت الخلايا العملاقة بوجود سيتوبلازم كثيف متعدد الأنوية و تكون الخلايا محاطة بخلايا اصغر متعددة الانقسام.

تمت دراسة تأثير تحميل نباتات السمسم علي الكثافة العددية لنيماتودا تعقد الجذور التي تصيب نبات الكوسة (صنف حساس للإصابة) تحت ظروف الصوبة الزجاجية. و بناء علي ذلك تم تحميل نباتات السمسم صنف جيزة ٣٢ بكثافة مختلفة (١، ٢، ٣، ٤ نباتات / أصيص) مع نبات الكوسة صنف أسكندراني (نبات واحد / أصيص). وقد أدى تحميل نباتات السمسم إلى حدوث نقص معنوي في الكثافة العددية لنيماتودا تعقد الجذور متمثلا في عدد العقد النيماتودية و عدد كتل البيض علي نبات الكوسة ، و تناسب النقص في النيماتودا طرديا مع كثافة نباتات السمسم المستخدمة. وكانت النسبة المئوية للنقص في عدد العقد النيماتودية علي جذور الكوسة ١و٤٨ ، ١و٧٤ ، ٤و٨٤ ، ٨و٨٨% ، وكذلك النسبة المئوية للنقص في عدد كتل البيض ٢٠ ، ٥٠ ، ٧٠ ، ٨٠% و ذلك بالترتيب مع كثافة نباتات السمسم المستخدمة في التحميل.

وفي تجربة أخرى تم دراسة تأثير إضافة كسب بذرة السمسم علي نيماتودا تعقد الجذور التي تصيب الكوسة صنف أسكندراني. أظهرت النتائج أن إضافة كسب السمسم تسبب معنويا في حدوث نقص لعدد العقد النيماتودية بنسبة ٤و٨٥ ، ٥و٨٧% وكذلك كتل البيض بنسبة ٥و٩٣ ، ٨و٩٦% في المعدلات المستخدمة ١٠ ، ١٥ جم/أصيص علي الترتيب. وأدت هذه المعدلات إلى زيادة غير معنوية في الصفات الخضرية لنباتات الكوسة تحت الدراسة متناسبة طرديا مع زيادة المعدلات المستخدمة. وحيث إن الطرق المستخدمة سهلة الاستخدام وغير ملوثة للبيئة ، لذلك ينصح باستخدامها في مجال المكافحة الحيوية للنيماتودا ضمن إطار المكافحة المتكاملة.