Histopathology of the root-knot nematodes and interaction of *Meloidogyne javanica and Fusarium* sp. and *Thielaviopsis paradoxa* on date palm, *Phoinex dactylifera* seedlings

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Abstract: The pathological effects of *Meloidogyne javanica* and *M.incognita* on seedlings obtained by germinating seeds of Khistawi,Zahdi and Braim date palm cultivars and the interaction of *M.javanica* , *Fusarium sp.,* and *Thielaviopsis paradoxa* on two date palm cultivars was studied. *M. incognita* caused more reduction in seedlings immergence of cv.Khastawi and cv.Braim than *M.javanica. M.incognita* produced significantly more galls on the root of both cultivars compared with those caused by *M.javanica*. The different nematode inoculum densities used in this study caused no significant differences in seedlings shoot height or root length compared with that in the control. Combined treatments of *Fusarium* sp.,*T. paradoxa* and *M.javanica* caused increased percentages of root necrosis in cv. Zahdi and cv. Braim compared with necrosis caused by inoculation with any one of these pathogens alone. *M.javanica-T.paradoxa* combined inoculation cause the greatest root necrosis on both cultivars when seedlings were inoculated with the nematodes 3 weeks prior to fungal inoculation. Nematode infection caused marked disorganization of root anatomy. Characteristic nematode induced feeding giant cells were observed in only the stealer tissues and in close association with xylem vessels. Several normal size females each associated with 3-6 giant, multinucleate cells and large egg masses were often observed in relatively small areas of root tissue.

***Keywords*: Date palm, *Meloidogyne* spp, *Fusarium* sp., *Thielaviopsis paradoxa*, disease complex, histopathology.**

دراسه نسيجيه امراضيه لبادرات نخيل التمر المصابه بديدان تعقد الجذوروالتداخل بين *Meloidogyne javanica* والفطرين *Thielaviopsis paradoxa* و *Fusarium* sp.

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ألخلاصه: درست التاثيرات المرضيه التي يحدثها نوعا ديدان تعقد الجذور *Meloidogyne javanica* و *M. incognita* على بادرات نخيل ناتجه من بذور الاصنا ف خستاوي و زهدي وبريم والتداخل بين النوع *M.javanica* والفطرين *Fusarium* sp. و *Thielaviopsis paradoxa* على الصنفين زهدي وبريم. كان النوع *M.incognita* اكثر تاثيرا من النوع *M.javanica* في خفض بزوغ بادرات الصنفين خستاوي وبريم في تربه غيرمعقمه. سبب النوع *M.incognita* احداث زياده معنويه بعدد العقد في بادرات كلا الصنفين مقارنة مع العقد التي سببها النوع *M.javanica*. ولم تسبب مختلف الكثافات اللقاحيه من الديدان المستعمله في هذه الدراسه اختلافات معنويه في ارتفاع البادرات او اطوال الجذورفيما بينها ومقارنة مع البادرات غير الملقحه بالديدان. وسببت المعامله المشتركه بالفطرين *Fusarium* sp. و*T. paradoxa M.javanica* زياده معنويه في النسبه المؤيه لتقرح جذوربادرات الصنفين زهدي وبريم مقارنة بالتقرحات التي تحدثها اي من هذه الممرضات كلا على انفراد. واحدث التلقيح المشترك بيافعات الطور الثاني للنوع *M.javanica* و *T.paradoxa* النسبه المؤيه الاكبرمن التقرحات عند ما سبق التلقيح بالديدان التلقيح بالفطر مدة ثلاثة اسابيع. واحدثت الاصابه بالديدان تشوهات مميزه في انسجة الجذور. وتكونت الخلايا العملاقه في منطقة الدائره المحيطيه للجذورالمصابه بالديدان على مقربه كبيره من اوعية الخشب. وغالبا ما جرى ملاحظه عدد من الاناث بحجم طبيعي مع ٣ - ٦ خلايا عملاقه للانثى الواحده من الديدان متصله باكياس بيض كبيرة الحجم في مساحه صغيره من نسيج الجذر.

Introduction

Date palm, *Phoinex dactylifera* L. is the most important fruit tree in Iraq and in recent years, date palm trees have exhibited a decline syndrome of unknown cause(s). The trees are reported to be subjected to different important plant diseases such. Among the most important vascular diseases which are known to affect the date palms is lethal yellowing caused by *Phytoplasma*, Bayoud caused by *Fusarium oxysporum* f.sp. *albiedinis* and terminal bud rot caused by *Thielaviopsis paradoxa* (Djerbi,1983). Of these diseases only the latter is reported to occur in Iraq (Al-Hassan & Abbas,1987). Various surveys have reported the occurrence of important genera of plant parasitic nematodes including the root knot nematodes associated with date palm trees (Lamberti *et al*., 1975; Al-Hassan & Habib, 1986; Al-Khoury,1987). Griffith and Koshy (1990) reported that up-to 90% young seedlings of date palm were killed in soil heavily infested by root-knot nematodes. Furthermore, *Meloidogyne javanica* has been reported to be widely distributed and cause severe damage to vegetables, tobacco and date palm trees in Iraq (Ibrahim, 1985). There is limited information on the pathological effects of the root-knot nematodes on date palms other than the work of Carpenter (1964) in the US and Al-Shamary (1979) in Iraq.

This study was undertaken to provide further information on the histopathological effects of the root knot nematodes, *M.javanica* and *M.incognita* and the possible interaction of *M.javanica* with *Fusarium* sp. and *T.paradoxa* on date palm seedlings.

MATERIALS AND METHODS

Effect of the root knot nematodes on seedlings immergence

In this experiment, rhizosphere soil was used from tomato plants that were heavily infected by large numbers of *Meloidogyne* sp. egg masses on their roots. The soil was equally divided into two parts . One part was sterilized by autoclaving twice on successive days at 1kg/cm^2 at 121 C for 1h, and was used for the control treatments. Two species of the root-knot nematodes, *M. javanica* and *M .incognita*,identied according to r perineal patterns of adult females (Hartman & Sasser, 1985) were each raised from a single egg mass and maintained as pure cultures on susceptible tomato plants. Date palm seeds (10/pot) obtained from fruits of Khastawi and Braim cultivars were planted in plastic pots containing 1kg of autoclaved or unautoclaved soil and infested 1000 freshly hatched second stage juveniles of the two nematode species. Each treatment was replicated four times and completely randomized and maintained in a green house (30 ± 5 C). One month later emerged seedlings were counted and seedlings were uprooted to determine the number of nematode galls per seedling, shoot height and root length.

Effect of nematode inoculums density on seedling growth

Three date palm seeds, each of cv. Braim and cv. Zahdi, were planted in autoclaved 2:1 peat moss: sandy loam soil (soil mixture) in 19 cm diam. plastic pots. The seeded pots were maintained under greenhouse conditions and 80 d later, seedlings were inoculated with 0, 1000, 5000, or 10000 eggs per pot. Six months later root length, plant height, root weight and number of galls per plant were determined. The treatments were completely randomized and replicated four times.

Interaction of *M. javanica* and two fungal pathogens on date palm seedlings

Two fugal pathogens, *Fusarium* sp. and *T. paradoxa* were isolated from declined date palm offshoots and trees, respectively. The fungi were added to potted seedlings derived from seeds obtained from fruits of two date palm cultivars, cv. Zahdi and cv. Braim growing in autoclaved soil mixture and replicated four times. Fungal inoculation were done either at the time of nematode inoculation or 3 wk after nematode inoculation. Fungal inoculums were applied as 1g per pot of fungal culture grown on barley seeds. The treatments were as follow: 1) Nematodes only, 2) *Fusarium* only, 3) *T. Paradoxa* only, 4) Nematodes + *Fusarium*, 5) Nematodes + *T. Paradoxa*, 6) *Fusarium* + *T. paradoxa*, 7) *Fusarium* + *T. paradoxa* + Nematodes. Nematode inoculums used in each pot in this experiment were 40000 eggs per pot. The experiment was terminated 45 d after inoculation with the pathogens, disease index of roots was determined according to the following scale: 0 = no root necrosis, 1 = 1-25% of the roots necrotic, 2 = 26 - 50% of the roots necrotic, 3 = 51- 75% of the roots necrotic, 4 = 76- 100% of the roots necrotic. Number of galls per plant and shoot and root dry weights were determined. Disease severity percentages were calculated according to Mckinney equation (Mckinney,1923).

Histopathology of date palm seedlings roots infected with root -knot nematodes

For this study, root samples of date palm seedlings obtained from heavily root-knot nematodes, *M. javanica* and *M. incognita* infected cv. Zahdi and cv. Braim were used. Roots were washed free of soil, 0.5 cm pieces were cut off using a sharp blade, fixed in

FAA (Formalin acetic acid ethanol) for 24- 48 h, dehydrated through a graded ethanol – xylol series, embedded in paraffin wax (56-58 C melting point) and sectioned at 10 µm thickness with a rotary microtome. The sections were stained with saffranin and fast green (Johanson, 1940) and mounted in Canada balsam for light microscope examination. Deparaffined sections (10 µm) were Sputter coated with gold for examination under a Joels scanning electron microscope (Gaudet and Kokko, 1984).

RESULTS AND DISCUSSION

Nematode infection significantly (p=0.05) reduced the emergence of date palm seedlings, cv. Khistawi and cv. Braim (Table 1). *M. incognita* appeared to reduce seedlings emergence of both cultivars more than *M.javanica. Meloidogyne incognita* reduced Braim seedling emergence twice as much as did *M. javanica* (Table 1).The high reduction in seedlings emergence was likely enhanced by the soil microorganisms because seedlings were grown in natural, unsterilized soil. This was evident by the clear necrosis of nematode infected seedlings (Fig. 1). Carpenter (1964) reported that fungi and other secondary organisms play an important role in root damage of palm seedlings infected by the root-knot nematodes. The interaction of various fungi and nematodes has been very well demonstrated (Powel, 1971; Bergeson, 1972). *M. incognita* significantly (p=0.05) reduced seedlings height of cv. Khistawi. However, in other treatments, nematode infection did not cause significant growth reduction (Table, 1). *M. incognita* produced significantly (p=0.5) more galls on the seedlings roots of both date cultivars compared with that caused by by *M. javanica.* Gall formation is a typical host response to

Meloidogyne infection but the degree of galling varies with host plant and nematode species (Fattah and Webster, 1984).

The effect of *M. javanica* and *M.incognita* on seedling growth and galling on cv. Braim and cv. Zahdi is presented in table 2. The different inoculums densities of *M. javanica* showed no significant intertreatment differences in shoot height and root length compared with the control. Gall number increased with increased concentration of egg inoculums. However, it was significant only between 1000 and 10000 eggs per pot treatments of *M. javanica* inoculation of Braim cultiva seedlings. Inoculation of Braim seedlings with 1000 eggs per pot significantly (p=0.05) reduced root weight compared with the control treatment (Table 2). With cv. Zahdi, different inoculum densities of *M. javanica* caused no significant intertreatment differences in shoot height or root weight but 10000 eggs per pot produced significant difference in root length compared with control treatment. *M. javanica* caused generally similar galling on seedlings of Braim and Zahdi cultivars.

With *M .incognita* treatments, the different nematode densities appeared to cause no significant effect on gall number per plant in either cultivar. However, gall number was much greater on cv. Braim than on cv. Zahdi (Table 2). None of the treatments caused a significant effect on root weight of the two date palm cultivars. *M. incognita* inoculums density of, 1000 eggs per pot significantly (p=0.05) decreased plant seedling height compared with that of the control treatment (Table 2). If gall number is considered an indication of susceptibility cv. Braim can be considered as equally susceptible to *M.* incognita and *M. javanica*. While cv. Zahdi is more susceptible to *M. javanica* than to *M. incognita*. Because of the possibility of high variability among seedlings produced from

seed propagation, results of this experiment can provide only a general view of the reaction of date palm cultivars to root knot nematodes.

Fusarium sp. and *T. paradoxa* treatment caused root necrosis on seedlings of Zahdi and Braim date cultivars. The degree of necrosis was generally similar regardless of the use fungal species and date palm cultivars (Table 3 and 4). These fungi are recognized as important plant pathogens and especially, *T. paradoxa, which* was reported to cause a serious terminal bud rot of date palm in Iraq (Djerbi, 1983).The combined inoculation of each of these fungi or either of them with *M. javanica* increased the percentage of root necrosis of seedlings derived from seeds of both date cultivars (Table 3 and 4). The *M. javanica–T. paradoxa* combination produced the greatest root necrosis on both cultivars when the nematode inoculation exceeded that of fungal pathogens. Similar results were obtained with *Meloidogyne–Fusarium* interaction (Pitcher, 1965; Powel, 1971; Bergeson, 1972). This confirms the notion that the relationships between two or more parasites on a single host is often more complex than expected (Webster, 1985). The increased disease development due to the association of nematodes with other pathogens indicates and supports the importance of nematodes in plant disease complexes. Powel (1971) suggested that interaction of nematodes with other pathogens may be the major economic hazard posed by the plant parasitic nematodes.

It can be observed from tables 3 and 4 that when nematode inoculation exceeded fungal inoculation, significantly ($p=0.05$) more reduction in root fresh and dry weight of seedlings of both cultivars occurred. A significantly greater reduction in dry weight was observed also when nematodes and *Fusarium* and *T. paradoxa* were simultaneously added to Zahdi seedlings compared with treatment of *Fusarium* alone. In a greenhouse

experiment Greco *et al*., (1980) investigated a possible interaction between *F.oxysporum* sp. *albidinis*, the causal pathogen of the serious date palm bayoud disease, and the root-knot nematode *Meloidogyne incognita*. They reported initial symptoms of decline three months after dual inoculation with both pathogens. However, the concomitant presence of nematode and fungus did not further enhance the disease.

Light and scanning electron microscope examination of sections of artificially infected date palm seedlings by root knot nematodes revealed the presence of well developed giant cells associated with *M. javanica* and *M. incognita* females. These nematode feeding cells were typical and similar to those induced by *Meloidogyne* spp in other host plants (Endo, 1971; Fattah and Webster,1983 and1984 ; Lamberti *et al.,* 1975).

Results of this study confirmed Previous studies (Al-Shamary, 1979; Eissa *et al.*, 1998; Lamberti *et al.*, 1977) on the giant cells induced by the root- knot nematodes in date palm and provided further information by scanning electron microscopy. This scanning electron microscopy technique (Gaudet and Kokko, 1984) used in this study is the first to examine nematode infected date palm roots.

Nematode infection caused severe disorganization of the date palm seedlings root tissues (Fig.2, b, e-g) compared with that of uninfected root tissues (Fig.2, d and e). The feeding giant cells were only observed within stele area of the roots (Fig.2, a,b and Fig.3, a) and were closely associated with xylem elements (Fig.3, b and d) which showed marked distortion (Fig.2, h and Fig.3 b) . Xylem disorganization was previously observed in roots of other plant species infected with root -knot nematodes (Siddiqi & Taylor, 1970; Jatala and Jensen, 1976; Fattah &Webster, 1984). The formation of abnormal xylem is thought to be due to stimulation of nematode feeding or injury to xylem

parenchyma (Jatala & Jensen, 1976). Xylem distortion (Fig.2, h) may impede the upward movement of water and nutrients in the roots of infected date palm seedlings. The endodermis was observed to be suppressed in almost all examined sections (Fig.2, a and b, Fig.3, a). Sections of uninfected seedlings, however, revealed well developed endodermis and normally organized root tissues (Fig.2, c and d). The development of suppressed endodermis was previously reported in the roots of barley (Edis & Dikerson, 1976) and sorghum (Orr & Morey, 1978) infected by *M. nassi* and *M. incognita* respectively.

The giant cells contained dense granular cytoplasm and large numbers of irregular nuclei with one or more prominent nucleoli (Fig.3, e and f).The cell walls of these giant cells showed considerable thickenings (Fig.3,b and d) and wall ingrowths which were especially extensive adjacent to xylem vessels (Fig.3, e). Groups of 3-6 giant cells were observed associated with their female nematodes and several nematodes associated with giant cells clusters were often observed in relatively small area of the root (Fig.2, e – g ,Fig.3, a). The nematodes appeared structurally and normal histologically and were associated with large numbers of apparently normal eggs (Fig.3g and h). No histopathological differences were observed in the response of cv. Zahdi and cv. Braim to infection by *M. javanica* or *M. incognita.* The giant cells in the roots of both cultivars appeared similar to each other regardless of nematode species.

Results of this work indicates that date palm seedlings are good hosts for these two species of the root knot nematode and that these nematodes may have an impact on the decline of date palm in Iraq.

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Figure Legends

Fig.1. *Phoenix dactylifera* L. seedlings infected with *Meloidogyne* sp. (B,C,D, and, E) showing root necrosis and stunted growth, and uninfected control (A).

Fig.2 a-h. Sections in *Phoenix dactylifera* L. seedlings roots stained with saffranin and fast green. (a) Infected with *Meloidogyne*, note that giant cells are confined to stellar tissue (large arrow),and deformed endodermis (small arrow).**(b)** Enlargement of Fig.3.**(c)** Uninfected cross section, note the well developed endodermis(arrows). **(d)** Longitudinal section in uninfected root showing epidermis (large arrow), and normal looking endodermis large arrow). **(e)** Large number of giant cells clusters and 2 feeding female nematodes**.(f)** Feeding females associated with giant cells clusters. Note the cell hyperplasia (asterisks). **(g)** Five females in cortical tissues. Note root ruptures (arrows) close to egg laying females. **(h)** Scanning electron micrograph of deformed xylem in nematode infected root (asterisks) ,C,cortex, E,eggs,Gc,giantcell(s), N,nematode,V,vascular tissue, X, xylem. Bars = 100µm **(a-d)**; 500µm **(e-f)**; 500µm **(g)**; 20µm **(h)**.

Fig.3a-h. Sections in *Phoenix dactylifera* L. seedlings roots stained with saffranin and fast green. (a) Scanning electron micrograph showing clusters of giant cells within stellar tissue (Small arrow) and eggs (arrow head) close root rupture (large arrow). **(b)**Close association of giant cells with xylem tissue. **(c)** Large number of giant cells with dense cytoplasm and the typical cell wall thickenings (arrows).**(d)** Scanning electron micrograph showing part of a thick wall (arrow) giant cell associated with xylem tissue.**(e)** Tow adjacent giant cells with dense cytoplasm, large number of nuclei containing one or more nucleoli (small arrows) and thick walls(large arrow).**(f)**Scanning electron micrograph showing multinucleate giant cells (arrows).**(g)** Three females with egg masses containing large number of eggs in the cortex close to ruptured (arrow) root surface.**(h)** Scanning electron micrograph showing mature female with egg mass in cortical tissue (asterisks), C,cortex, E,eggs,Gc,giant ell(s), N,nematode, X,xylem. Bars = 100µm **(a-c)**; 10µm **(d-f)**; 500µm **(g)**; 100µm **(h)**.

Table 1.Effect of the root-knot nematode on emergence and growth of date palm, *Phoenix dactylifera*L. seedlins

Treatment	cv.Khistawi				cv.Braim			
	Emergence (%)	Number of galls/plant	Plant height (cm)	Root length (cm)	Emergencee (%)	Number of galls/plant	Plant height (cm)	Root length (cm)
M.javanica Autocolaved soil (control)	100 a	0.0 bc	19.45 a	7.98 abc	80 ab	0.0 bc	17.75 Abc	11.40 ab
M.incognita Autocolaved soil (control)	100 a	0.0 bc	18.55 abc	11.3 ab	87.5 a	0.0 bc	18.4 Abc	9.18 abc
M.javanica Non-Autocolaved soil (control)	70 b	0.68 b	18.63 ab	12.75 a	65 abc	0.03 b	18.8 a	10.20 abc
M.incognita Non-Autocolaved soil (control)	10 c	5.25 a	8.75 d	8.10 abc	32.5 d	3.28 a	18.7 ab	12.20 a

[1] Means within a column with the same letter are not statistically different (P=0.05) according to DMRT.

Table 2. Effect of the root-knot nematodes on *Phoenix dactylifera*L. seeding growth

Treatment (Eggs/pot)	Cv. Braim				Cv. Zahdi			
	Shoot height (cm)	Root length (cm)	Number of galls/plant	Root weight (g)	Shoot height (cm)	Root length (cm)	Number of galls/plant	Root weight (g)
M.javanica (control) 0	34.38 abc	62.88 abc	0.00 a	6.50 a	29.45 a	58.23 a	0.00 c	5.25 a
1000	33.50 abc	64.45 ab	20.23 b	4.50 bc	28.03 ab	49.28 abc	30.53 ab	4.9 ab
5000	34.45 ab	64.55 a	24.05 abb	4.83 abc	29.45 a	54.53 ab	38.08 a	4.75 abc
10 000	35.45 a	59.08 abc	33.73 a	5.83 ab	25.5 abc	31.63 bc	20.38 b	3.85 abc
M.incognita (control) 0	35.70 a	56.05 ab	0.00 d	6.48 a	30.08 abc	57.88 a	0.00 bc	4.3 abc
1000	30.38 bc	52.60 abc	31.15 a	5.93 ab	34.13 a	52.88 ab	3.26 abc	5.83 A
5000	34.50 ab	37.98 bc	29.23 ab	5.35 abc	28.90 abc	38.03 abc	9.5 a	4.10 abc
10 000	34.10 abc	56.30 a	26.03 abc	5.65 abc	33.36 ab	51.15 abc	6.25 ab	4.56 ab

[1] Means within each column with the same letter are not statistically different (P=0.05) according to DMRT.

Table 3. Effect of nematode-fungal interaction (simultaneous inoculation) on disease percentages and growth of *Phoenix dactylifera* L. seedlings

Treatment	cv.Zahdi				cv.Braim			
	Disease severity (%)	Number of galls/plant	Root weight (g)		Disease severity (%)	Number of galls/plant	Root weight (g)	
			Fresh	Dry			Fresh	Dry
Fusarium sp.	31.25 bc 1	0.0 a	1.75 a	0.50 a	25.0 d	0.0 d	2.05 a	0.47 a
Thielaviopsis	37.5 bc	0.0 a	1.3 abc	0.37 abc	37.50 c	0.0 d	1.27 abcdef	0.42 ab
Fusarium+ *Thielaviopsis*	25.0 c	0.0 e	1.5 ab	0.40 ab	43.75 b	0.0 d	1.75 abcd	0.42 ab
M. javanica	0.0 d	15.0 bc	1.27 abc	0.35 bcd	0.0 e	14.75 ab	1.57 abcd	0.42 ab
M. javanica + *Fusarium*	68.75 a	15.32 ab	1.3 abc	0.35 bcd	37.50 c	20.0 a	1.82 abc	0.42 ab
M.javanica + *Thielaviopsis*	43.75 b	12.25 cd	1.1 abcd	0.30 bcd	31.25 b	14.65 abc	1.77 abcd	0.47 a
M. javanica+ *Fusarium*+ *Thielaviopsis*	68.75 a	18.65 a	1.3 abc	0.35 bcd	62.50 a	13.75 bc	2.0 ab	0.47 a

[1] Means within each column with the same letter are not statistically different (P=0.05) according to DMRT.

Table 4. Effect of nematode-fungal interaction on disease and growth of *Phoenix dactylifera* L. seedlings

Treatment	cv.Zahdi				cv.Braim			
	Disease severity (%)	Number of galls/plant	Root weight (g)		Disease severity (%)	Number of galls/plant	Root weight (g)	
			Fresh	Dry			Fresh	Dry
Fusarium sp.	25.0 cd	0.0 d	2.65 Abc	0.72 a	31.25 c	0.0 d	3.85 abc	0.92 ab
Thielaviopsis	18.75 d	0.0 d	1.97 cde	0.57 bcde	8.75 e	0.0 d	3.82 abcd	0.92 ab
Fusarium+ *Thielaviopsis*	31.25 c	0.0 d	2.77 ab	0.70 ab	18.75 d	0.0 d	4.05 a	0.97 a
M. javanica	0.0 e	21.50 bc	3.02 A	0.67 abc	0.0 f	26.5 bc	3.05 cdef	0.82 abc
M. javanica+ *Fusarium*	50.0 b	16.25 c	1.92 cdef	0.45 de	31.25 C	36.25 ab	3.17 cde	0.70 bcd
M. javanica+ T*hielaviopsis*	81.25 a	30.25 a	1.95 cdef	0.57 bcde	87.50 a	20.0 c	2.52 ef	0.72 abcd
M. javanica+ *Fusarium*+ *Thielaviopsis*	37.5 c	32.32 a	2.62 abcd	0.62 abcd	50.0 b	39.0 a	3.95 ab	0.97 a

[1] Means within each column with the same letter are not statistically different (P=0.05) according to DMRT. Fungal inoculations were 3 wks after nematode inoculations.

Figure 1

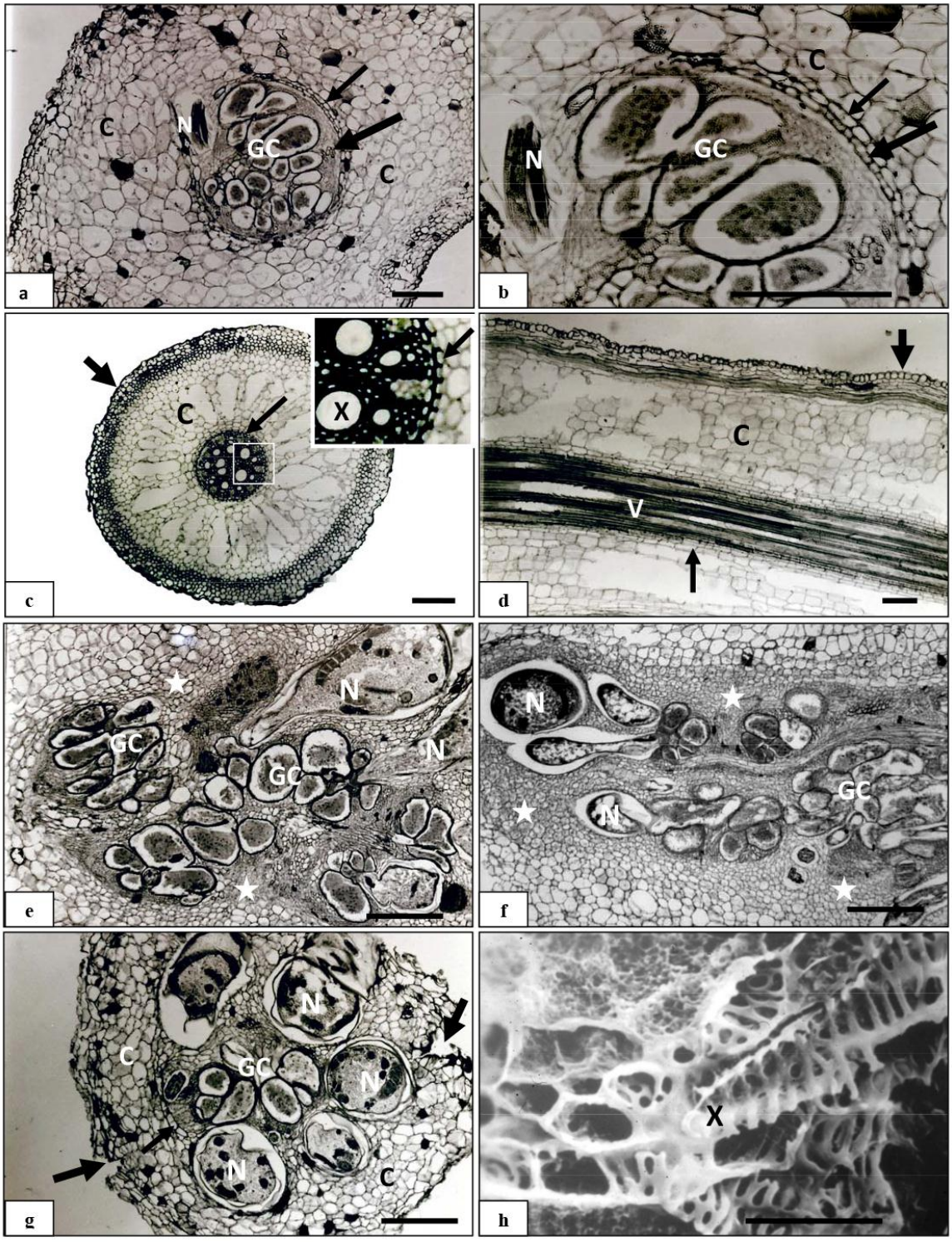

Figure 2

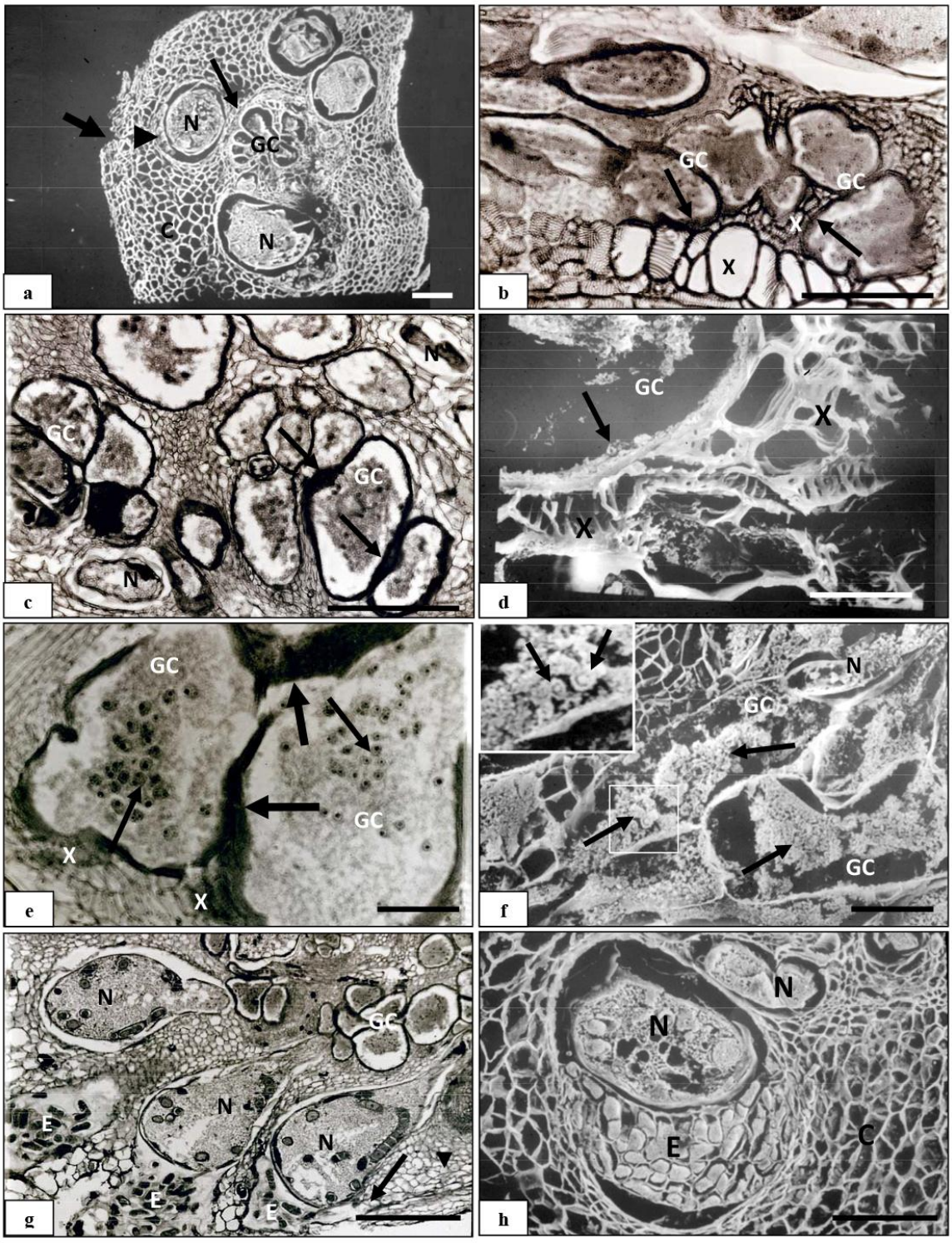
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Figure 3