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Influence of *Meloidogyne javanica* and *Rotylenchulus reniformis* alone on nodulation of some leguminous crops

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Abstract

The effect of root-knot nematode, *Meloidogyne javanica* and the reniform nematode, *Rotylenchulus reniformis* alone on nodulation of four Rhizobium species on roots of 27 cultivars related to six leguminous species were evaluated. The formation of nodules on their roots varied greatly in their numbers according to host species, host cultivars, rhizobal species and nematode species. The greater nodulation reduction in infected roots with nematode species, *M. javanica* and *R. reniformis* was observed on roots of common bean cvs. Exira, Nebraska, Polista and Savana inoculated with rhizobial species *Rhizobium phaseoli* and lupine cv. Giza 2 inoculated with rhizobial species *R. lupine.* In general, inoculation of *M. javanica* or *R. reniformis* with rhizobial species greatly reduced root nodulation as compared to leguminous species inoculated with *Rhizobium* species alone. Also, it is important to note that the reduction in the number of bacterial nodules was more pronounced with *R. reniformis* infection than with *M. javanica* infection. It is noteworthy that the number of nodules on roots of broad bean cv. Wade 1 inoculated with *R. phaseoli* increased by both nematode species infections.

Key words: *Rhizobium*, nodulation, *Meloidogyne javanica, Rotylenchulus reniformis*, leguminous.

Introduction

Nodulation is an ecologically and economically important plant phenomenon, in which the symbiosis between plants from the family Fabaceae and *Rhizobium* results in the development of nitrogen fixing nodules on the roots of the host plant. In this organ, the bacteria differentiate into bacteroids, fix nitrogen for the plant host and in return are provided with carbon by the host plant **(Crespi & Gálvez, 2000; Hirsch *et al*., 2001).** Root-knot nematode infection is typically associated with the formation of multinucleate cells that usually develop from vascular parenchyma cells **(Davis, 1959)** Histological effects induced by root-knot nematode in nodular tissues of legumes have been reported in common bean (**Yousif, 1972**), cowpea (**Kassab, 1974**), and peanut (**Taha & Yousif, 1976**). The previous studies have shown that *M. Javanica* and *M. incognit*a drastically inhibited nitrogen fixation (**Taha *et al*., 1974**) and nodulation of four leguminous crops: horse bean (*Vicia faba*), lupine (*Lupinus termis*), pea (*Pisum sativum*), and clover (*Trifolium alexandrinum*). Nematode galls on the roots may affect nodulation by causing nutrient deficiency in

host plants and by occupying space on the root system. **(Masefield, 1958)** and **(Malek & Jenkins 1964).** The formation of giant cells in vascular bundles of nodules and roots, and the basal connection of the nodule, were usually associated with abnormal xylem and/or deformed xylem strands. However, giant cells did not disturb or prevent the development of nodular tissues **(Yousif, 1978)**. Nodule formation was hindered only when *R. reniformis* infection preceded rhizobia inoculation. **(Taha & Kassab 1980)**. Severe nitrogen deficiency and retarded growth than plants inoculated with nematodes only or un-inoculated plants. Nematode invasion reduced the numbers of nodules and inhibited nitrogen fixation by about 63% in nodular tissue **(Ali *et al*., 1981)**. *M. incognita* reduced the leghaemoglobin and bacteroid content of nodules thus adversely affecting the functioning of nodules of mungbean (*Vigna radiate)* **(Chahal & Chahal, 1989).** In the tropical climate of Venezuela, *Rotylenchulus reniformis* reproduced rapidly on soybean (*Glycine max)* roots and the nematode also, infected the *Rhizobium* root nodules. **(Meredith *et al*., 1983)**. The root-knot nematode, *M. javanica* reduced nodulation on roots of broad bean **(El-Bahrawy & Salem, 1989)**. Lentil seedlings (*Lens culinaris* Medic.) suffered greater damage from *M. incognita* and *R. reniformis* individually or concomitantly, when plants were not infected with *Rhizobium* **(Fazal *et al*., 1992)**. With the exception of common bean genotype M 28, *Meloidogyne* infection caused significant reductions in nodulation **(Kimenju *et al*., 1999)**. Inoculation of 100 and above *R. reniformis* reduced the nodule, fresh and dry weight of shoot and root of French bean **(Vadhera & Dave, 2000)**. *M. incognita* resulted in significant decrease in the growth of black gram, root-nodule development, nitrogen contents of root and shoot, and nitrogenase activity at all inoculation levels **(Bhat *et al.,* 2012)**. Application of *M. incognita* or *Xanthomonas campestris* prior to *Rhizobium* sp. caused a greater reduction in chickpea plant growth than *Rhizobium* sp. applied prior to *M. incognita* or *X. campestris* **(Siddiqui *et al*., 2013).**

Materials and Methods

Seeds of six cultivars broad bean (*Vicia faba* L.) viz Giza 3, Giza 40, Giza 843, Maser 1, Maser 3 and Wade 1, seven common bean (*Phaseolus vulgaris*) cvs. Bronco, Exira, Giza 6, Nebraska, Neuten, Polista and Savana, two fenugreek (*Trigon foenum-graecum*) cvs. Giza 2 and Giza 3, six lentil (*Lens culinaris*) cvs. Giza 4, Giza 9, Giza 29, Giza 37, Giza 51 and Siena 1, three lupine (*Lupinus termis*) cvs. Dagon 2, Giza 1 and Giza 2 and three pea (*Pisum sativum*) cvs. Master Be, Victory and Lincoln obtained from the Agricultural Research Center (Giza - Elmenia, Mallawe - New Valley, AlKharga). Rhizobial inoculum was obtained from Microbiology Department of Agricultural Research Center in Giza.

Seed cultivars of (broad bean & common bean, lentil & pea, fenugreek and lupine were mixed with rhizobial species; *Rhizobium phaseoli*, *R. leguminosarum*, *R. meliloti* and *R. lupini* respectively, and immediately planted in pots (25 cm diameter). After two weeks plants were inoculated with 1000 newly hatched

juveniles of *Meloidogyne javanica* or 1000 immature females of *Rotylenchulus reniformis* per plant by pipetting the nematode suspension in five holes around the root system. Each of the treatments was replicated four times. The various treatments were: *Rhizobium* alone, *M. javanica* inoculation two weeks after rhizobial inoculation, and *R. reniformis* inoculation two weeks after rhizobial inoculation.

Then the pots were arranged in randomized block design on a bench in a greenhouse. All plants were grown during the normal growing seasons at a greenhouse temperature 20 ± 5C^{o}. after forty five days of inoculation, plants were gently uprooted from the pots and shaken to remove adhering soil. The total number of nodules on each plant was recorded.

Results and Discussion

Data presented in Tables (1 & 2) and Figs. (1 & 2) indicate that, the formation of nodules on their roots varied greatly in their numbers according to host species, host cultivars, *Rhizobium* species and nematode species.

The root-knot nematode, *M. javanica* infection was found to cause significant reduction in the number of nodules on roots of broad bean cvs. Giza 40 and Giza 843 and common bean cv. Bronco inoculated with rhizobial species *Rhizobium phaseoli* with rates of 38.23, 29.41 and 42.85 % respectively, lentil cvs. Giza 4, Giza 29 and Giza 51 inoculated with rhizobial species *R. leguminosarum* with rates of 25.00, 71.87 and 52.56 % respectively, lupine cv. Degon 2 inoculated with rhizobial species *R. lupine* with rate of 44.44 % and pea cvs. Lincoln, Master Be and Victory inoculated with rhizobial species *R. leguminosarum* with rates of 64.58, 62.00 and 30.90 % respectively. Table (1) and Fig. (1).

Also, the reniform nematode, *R. reniformis* infection was found to cause significant reduction in the number of nodules on roots of broad bean cvs. Giza 3, Maser 1 & Maser 3 and common bean cvs. Giza 6 and Neuten inoculated with rhizobial species *Rhizobium phaseoli* with rates of 49.47, 68.03, 12.06, 52.00 and 45.16 % respectively, fenugreek cvs. Giza 2 and Giza 3 inoculated with rhizobial species *R. meliloti* with rates of 61.29 and 68.30 % respectively, lentil cvs. Giza 9, Giza 37 and Siena 1 inoculated with rhizobial species *R. leguminosarum* with rates of 78.68, 50.00 and 64.22 % respectively, lupine cv. Giza 1 inoculated with rhizobial species *R. lupine* with rate of 57.69 %. Table (1) and Fig. (1).

On the other hand, a similar greater nodulation reduction in infected roots with nematode species, *M*eloidogyne *javanica* and *Rotylenchulus reniformis* was observed on roots of common bean cvs. Exira, Nebraska, Polista and Savana inoculated with rhizobial species *Rhizobium phaseoli* with rates of 48.83, 54.09, 48.88 and 77.77 % respectively and lupine cv. Giza 2 inoculated with rhizobial species *R. lupine* with rate of 68.29 % (Tables 1 - 2 and Figs. 1 - 2).

Table (1): Effect of *Meloidogyne javanica* on nodulation of four inoculated *Rhizobium* species for some cultivars of six leguminous species.

Leguminous crops	Cultivars	No. of nodules on roots.		Decrease	% reduction
		Rhizobium* + *Meloidogyne	***Rhizobium* Alone**		
		Rhizobium phaseoli			
Broad bean (*Vicia faba* L.)	Giza 3	63 *	95	32	33.68
	Giza 40	63 *	102	39	38.23
	Giza 843	48	68	20	29.41
	Maser 1	51 *	122	71	58.19
	Maser 3	55	58	3	5.17
	Wade 1	51	46	-	-
		Rhizobium phaseoli			
Common bean (*Phaseolus vulgaris*)	Bronco	16	28	12	42.85
	Exira	22 **	43	21	48.83
	Giza 6	14 *	25	11	44.00
	Nebraska	28 *	61	33	54.09
	Neuten	23 *	31	8	25.80
	Polista	23 *	45	22	48.88
	Savana	20 **	90	70	77.77
Fenugreek (*Trigon foenum-graecum*)		***Rhizobium meliloti***			
	Giza 2	75 *	155	80	51.61
	Giza 3	55 **	142	87	61.26
		Rhizobium leguminosarum			
Lentil (*Lens culinaris*)	Giza 4	21 *	28	7	25.00
	Giza 9	78 *	122	44	36.06
	Giza 29	9 **	32	23	71.87
	Giza 37	25 **	44	19	43.18
	Giza 51	37 **	78	41	52.56
	Siena 1	41 **	109	68	62.38
		Rhizobium lupini			
Lupine (*Lupinus termis*)	Degon 2	15 *	27	12	44.44
	Giza 1	17 *	26	9	34.61
	Giza 2	13 *	41	28	68.29
		Rhizobium leguminosarum			
Pea (*Pisum sativum*)	Lincoln	17 **	48	31	64.58
	Master be	19 **	50	31	62.00
	Victory	38 *	55	17	30.90

*Significant at 0.05 level of probability by student t-test. **Highly Significant at 0.01 level of probability.

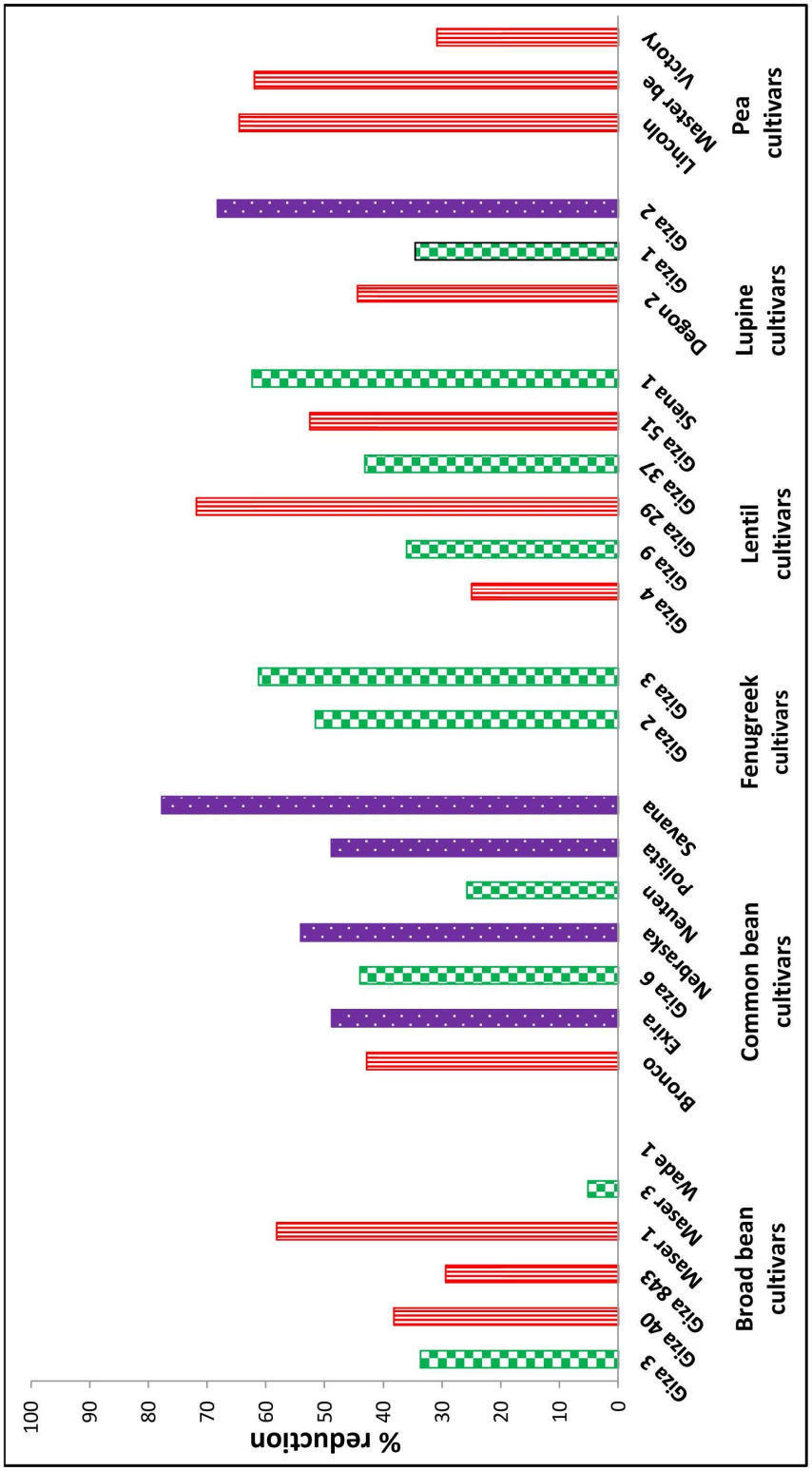

Fig. (1): Percentage of reduction in nodule number on some cultivar roots of six leguminous species as influenced by the infection of the root-knot nematode, *Meloidogyne javanica* under greenhouse condition.

Table (2): Effect of *Rotylenchulus reniformis* on nodulation of four inoculated *Rhizobium* species for some cultivars of six leguminous species.

Leguminous crops	Cultivars	No. of nodules on roots.		Decrease	% Reduction
		Rhizobium* + *Rotylenchulus	***Rhizobium* alone**		
		Rhizobium phaseoli			
Broad bean (*Vicia faba* L.)	Giza 3	48 *	95	47	49.47
	Giza 40	90	102	12	11.76
	Giza 843	49 *	68	19	27.94
	Maser 1	39 **	122	83	68.03
	Maser 3	51	58	7	12.06
	Wade 1	61	46	-	-
		Rhizobium phaseoli			
Common bean (*Phaseolus vulgaris*)	Bronco	17 *	28	11	39.28
	Exira	22 **	43	21	48.83
	Giza 6	12	25	13	52.00
	Nebraska	28 *	61	33	54.09
	Neuten	17	31	14	45.16
	Polista	23 *	45	22	48.88
	Savana	20 **	90	70	77.77
		Rhizobium meliloti			
Fenugreek (*Trigon foenum-graecum*)	Giza 2	60 *	155	95	61.29
	Giza 3	45 *	142	97	68.30
		Rhizobium leguminosarum			
Lentil (*Lens culinaris*)	Giza 4	23	28	5	17.85
	Giza 9	26 **	122	96	78.68
	Giza 29	14 *	32	18	56.25
	Giza 37	22	44	22	50.00
	Giza 51	51 *	78	27	34.61
	Siena 1	39 **	109	70	64.22
		Rhizobium lupini			
Lupine (*Lupinus termis*)	Degon 2	20	27	7	25.92
	Giza 1	11 *	26	15	57.69
	Giza 2	13 *	41	28	68.29
		Rhizobium leguminosarum			
Pea (*Pisum sativum*)	Lincoln	18 **	48	30	62.50
	Master be	22 *	50	28	56.00
	Victory	43 *	55	12	21.81

*Significant at 0.05 level of probability by student t-test. **Highly significant at 0.01 level of probability.

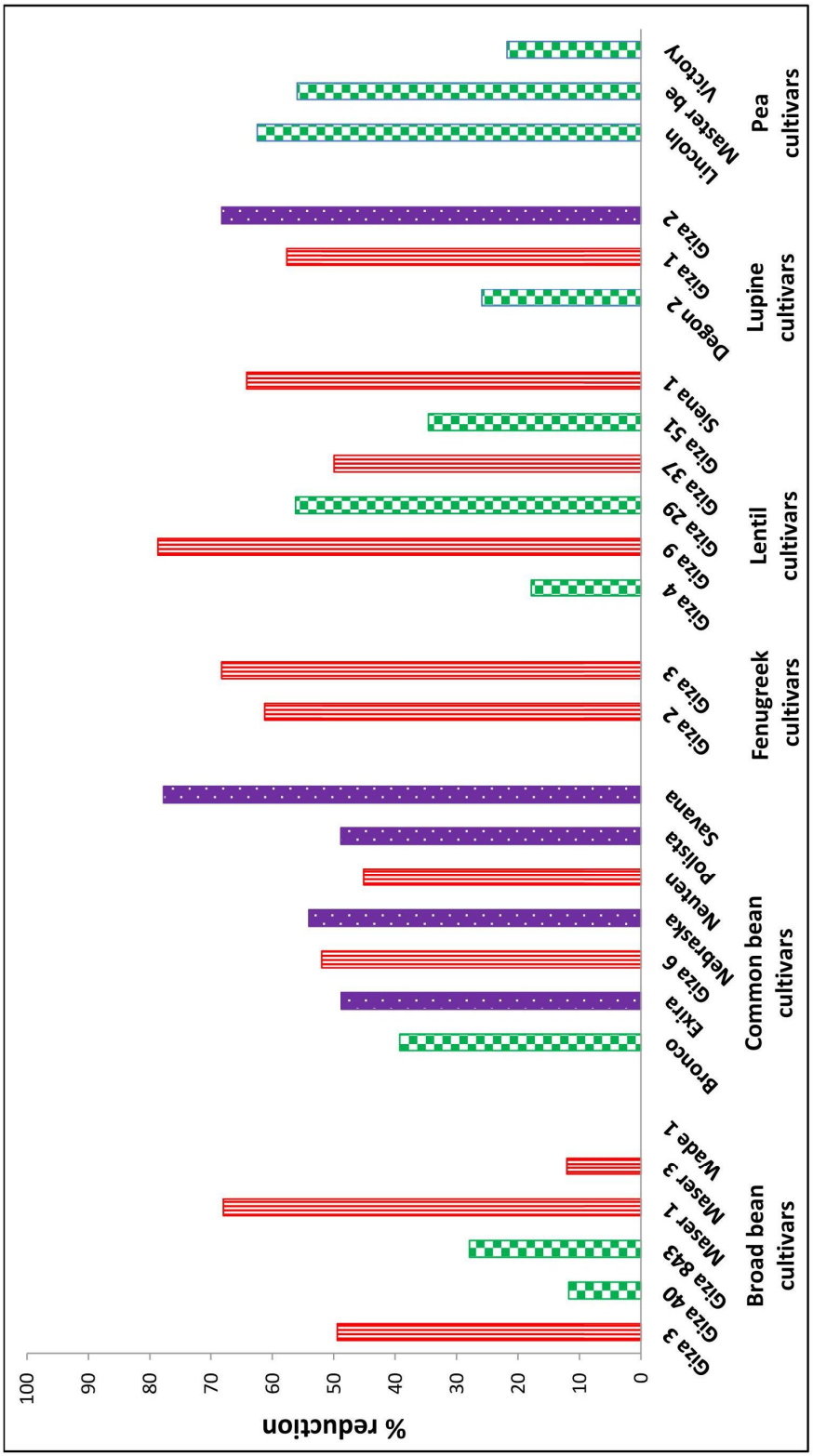

Fig. (2): Percentage of reduction in nodule number on some cultivar roots of six leguminous species as influenced by the infection of the reniform nematode, *Rotylenchulus reniformis* under greenhouse condition.

In general, inoculation of *M. javanica* or *R. reniformis* with rhizobial species greatly reduced root nodulation as compared to leguminous species inoculated with Rhizobium species alone. Also, it is important to note that the reduction in the number of bacterial nodules was more pronounced with *R. reniformis* infection than with *M. javanica* infection. It is noteworthy that the number of nodules on roots of broad bean cv. Wade 1 inoculated with *Rhizobium phaseoli* increased by both nematode specie infections.

These findings are in agreement with reports of several investigators who found that the root-knot nematode and reniform nematodes caused reduction in nodulation on roots of some leguminous crops **(Ayala, 1962; Raut and Sethi, 1980 and Verdejo *et al*., 1988).** Several plant parasitic nematode have been found to cause great reduction in root nodulation of some leguminous crops **(Romaniko, 1961; Wardojo *et al*., 1963 and Karanja, 1988)**. Also, **Taha & Raski, (1969); Hussey & Barker, (1976); Singh & Reddy (1981) and Siddiqui & Mahmood (1994)** showed that, early destruction of nodules as a result of nematode infection deprived the plants of some nitrogenous materials. Reduction in nodulation might be due to the formation of *Meloidogyne* galls thus occupying space in the roots **(Malek & Jenkins, 1964; Barker & Hussey, 1976 and Hyens & O'Connl, 1990)** and due to suppression of lateral root formation by *R. reniformis* which might cause reduction in numbers of sites for nodule initials (**Taha & Kassab, 1980; Chahal *et al*., 1988 and Mohanti *et al*., 2001).**

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الملخص العربي

تأثير نيماتودا تعقد الجذور والنيماتودا الكلوية على تكوين العقد البكتيرية في بعض المحاصيل البقولية

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تلعب بكتريا العقد الجذرية ***Rhizobium*** دورًا حيويًّا في الزراعة حيث تكون العقد البكتيرية **Nodules** على جذور نباتات العائلة البقولية والتي تقوم بدورها في تقليل كمية الأسمدة الكيماوية النيتروجينية المضافة للتربة خلال فترة نمو هذه المحاصيل.

ولقد استهدف هذا البحث دراسة تأثير إصابة كل من نيماتودا تعقد الجذور ميليودوجين جافانيكا والنيماتودا الكلوية روتيلينكيولس رينيفورمس منفردين على عدد العقد المتكونة على جذور نباتات الفول البلدي والفاصوليا الملقحة حبوبها قبل الزراعة بالنوع البكتيري ***Rhizobium phaseoli*** والحلبة بالنوع البكتيري ***Rhizobium meliloti*** والعدس والبسلة بالنوع البكتيري ***Rhizobium leguminosarum*** والترمس بالنوع البكتيري ***Rhizobium lupini*** .

ولقد أشارت النتائج إلى وجود اختلافات معنوية متفاوتة لعدد العقد البكتيرية المتكونة على جذور أصناف البقوليات المختبرة تبعًا لاختلاف نوع المحصول، وأصنافه، ونوع البكتريا، ونوع النيماتودا. حيث سجلت أصناف الفاصوليا (**Nebraska – Polista – Exira – Savana**) أقل عدد من العقد البكتيرية المتكونة علي جذورها والمصابة بكلا نوعي النيماتودا منفردة كما أظهرت النتائج أن الإصابة بالنيماتودا قد أدت إلى انخفاض عدد العقد البكتيرية المتكونة على جذور أصناف البقوليات المختبرة وإن كان تأثير النيماتودا الكلوية أشد في خفض عدد العقد البكتيرية مقارنةً بتأثير نيماتودا تعقد الجذور، ومن ناحية أخرى أوضحت النتائج أن صنف وادي ١ من الفول البلدي زادت عدد العقد البكتيرية المتكونة على جذوره بالإصابة بالنيماتودا الكلوية أو نيماتودا تعقد الجذور.