Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

Effect of Intercropping of Nematotoxic Plants on Root- Knot Nematode, *Meloidogne incognita* Infecting some Vegetable Plants in the Screenhouse

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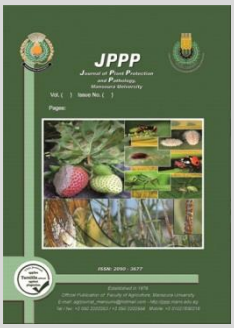

ABSTRACT

Plant parasitic nematodes (PPNs) especially root-knot nematodes cause significant economic damage and considered as a limiting factor in some vegetables production all over the world. Due to the harmful effect of synthetic nematicides on human health, non-target organisms as well as on environment and agricultural ecosystem, we need to use alternative management practices that are effective, cheap, safe, economically acceptable, and environmentally friendly. Intercropping nematicidal plants may be represented as an alternative efficient practice. Our research was aimed to investigate the suppressive effect of *Crotalaria juncea, Tagetes erecta*, and *Brassica juncea* intercropping with tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), and pepper (*Capsicum annum* L.), on *Meloidogyne incognita* and their effect on the host plant growth. In the screenhouse, the experiment was conducted using 1000 IJs/pot, after 2 months the data were recorded. The highly effective intercropping plant was *Crotalaria* followed by *Tagetes* and *Brassica* in suppressing the nematode galls, egg masses, final population, and rate of reproduction significantly as follow: 153.7, 160.3, 570.0, 0.57 in tomato, and 205.7,158.3, 692.3, 0.69 in cucumber and 197.0, 189.7, 746.3, 0.75 in pepper, respectively. Moreover, plant growth parameters were enhanced in the three host plants and so, such intercropping system could be of a considerable merit in integrated pest management programs.

Keywords: Root- knot nematodes, Intercropping, Crotalaria, Tagetes, Brassica.

INTRODUCTION

Plant parasitic nematodes (PPNs) are considered one of the most biotic factors which effect negatively on vegetables production. The loss was estimated statistically by US to be $157 billion worldwide (Singh *et al*., 2015). One of the most important PPN genus is the root-knot nematode (RKN) (*Meloidogyne*) (Jones *et al*., 2013), which has more than 3000 host species, affecting vegetables, fruits, and ornamental crops, (Perry *et al*., 2009; and Khalil, 2013).

Tomato, cucumber, and pepper considered of the most popular versatile vegetables worldwide, because of their diversified use and their high nutritive value such as minerals, vitamins, sugars, essential amino acids, dietary fibers. Moreover, they contain high concentration of anti-carcinogenic and antioxidant substances (Bosland and Votava, 2012), In addition to that the duration crop of these vegetables is relatively short (U.S. Department of Agriculture, 2005).

Because of the pesticide's hazardous effect on human health, as well as non- target organisms and on the environment and their high costs, therefore there is an increased interest for alternative strategies especially the non-chemical nematode management methods (Samaliev, 2009; Wang and Brent, 2009; Nagaraju *et al*., 2010; and Lamov¡sek *et al*., 2013). Each method of plant parasitic nematode management has a limited effect, to achieve a highly efficient management of PPN. So, we need to carefully use a combination of different methods.

Using of intercropping plants, viz, Sunn hemp (*Crotalaria juncea*), Marigold (*Tagetes erecta*), and Mustard (*Brassica carinata*) offers an alternative to nematicidal chemical (Noling and Becker, 1994; Youssef *et al*., 2011; Kas¸ kavalci *et al*., 2009 and Ploeg, 2008). Intercropping method is an eco-friendly management and has no hazardous effect on plant growth. These plants are a very important source of natural compounds which have nematicidal properties, such as: alkaloids, isothiocyanates, diterpenes, glucosinolates, fatty acids, phenols, thienyls, sesquiterpenes and polyacetylenes (Chitwood, 2002). *Crotalaria, Tagetes*, and *Brassica* have been proved to have a reduction effect on nematode population of RKN and can be used as intercropping plants with other plants to reduce the root-knot nematodes population (Bridge, 1996), which may release during decomposition nematicidal plant materials (Halbrendt,1996).

Varma *et al*., (1978) reported that, Marigold reduced *Meloidogyne* final population and their root galls and increased the plant growth parameters when intercropping with tomato and with eggplants (Ferraz and Grassi de Freitas, 2004) and potato (Chen *et al*., 2007). So, the usage of mustard can decrease the nematode populations because of its nematicidal activity (Haider *et al*., 2004; Curto 2008).

According to the previous literatures, this research was conducted to evaluate the nematicidal effect of *Crotalaria, Tagetes*, and *Brassica*. as intercropping plants to control the root-knot nematode, *Meloidogyne incognita* activity on tomato, cucumber, and pepper as host plants and their effect on the host growth properties in the screenhouse.

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DOI: 10.21608/jppp.2021.58913.1013

MATERIALS AND METHODS

A- Materials:

1- Root-knot nematode culture:

Culture of *Meloidogyne incognita* (Mi) was maintained in the screenhouse on tomato plants (*Solanum lycopersicum* L. cv. Castel Rock) in sterile sandy loam soil. Nematodes were extracted from tomato galled roots; washing, cutting into pieces, and placed in the mist chamber for egg hatching. Hatched J_2s were collected daily and refrigerated at 6 ^oC for the experimental use.

2- The host plant:

Screenhouse experiments were carried out using seedlings of 21-days-old of tomato (*S. lycopersicum* L. cv. Castel Rock), cucumber (*Cucumis sativus* L.), and pepper (*Capsicum annum* L.). They were transplanted singly in 20-cm-diam pots which filled with sterilized sandy clay soil.

B- Experimental methods:

Effect of intercropping cultivation of nematotoxic plants on the pathogenicity and reproductivity of *M. incognita* on tomato, cucumber, and pepper seedlings in screenhouse:

Seeds of sunn hemp, *Crotalaria juncea* L., marigold, *Tagetes erecta*, and mustard, *Brassica carinata* were planted directly in 20-cm-diam pots filled with sterilized sandy clay soil under outdoor screenhouse conditions. After 10 days from germination, the plants were thinned to two per pot and 21-days-old of tomato, cucumber, or pepper seedlings were transplanted singly in each pot. Forty pots of tomato, cucumber, and pepper were planted and divided into four groups as follow: first group (10 replicates) was intercropped with *Crotalaria* and 1000 2IJs/pot of Mi were added to 5 replicates of them after one week. Second group (10 replicates) was intercropped with marigold and 1000 2IJs/pot were added to 5 replicates of them. Third group was intercropped with mustard and 1000 2IJs/pot were added to 5 replicates of them. The remaining pots were left as control: five replicates received nematodes and the other five without nematodes. The experiment was ended after 2 months.

Determination of plant growth and nematode parameters:

At the end of the experiment, roots were washed, and fresh weight of roots and shoots were measured. Thereafter, roots were stained with acid fuchsine in cold lacto-phenol (McBeth *et al*., 1941) and stored in it. Stained roots were rinsed in water and cut into pieces to facilitate counting of galls, egg masses, and final population (the final population were extracted from the soil by Burman funnel technique and from the roots by mist chamber) were recorded, the rate or reproduction was calculated from the ratio of Pf (final population density) to Pi (initial population density).

3- Statistical analysis:

The data of all experiments were statistically analyzed using analysis of variance to check the significance of the differences between treatments using SAS program (2005) and separation between means was applied by Tukey test.

RESULTS AND DISCUSSION

Results of the present study demonstrated that intercropping with selected nematicidal plants recorded significant reduction in nematode parameters and increased the host plant growth parameters.

Tables (1& 2) proved that, intercropping with *Crotalaria* was the highest suppressive effect on nematode parameters. The mean number of nematode galls, egg masses, nematode final population and the rate of reproduction were decreased significantly in the three host plants comparable with the infected plants without intercropping followed by *Tagetes* and *Brassica* intercropping. The data agreed with those obtained by Kowalska and Sonalinska (2001); Kumar and Khanna (2006); Singh and Sitaramaiah (1993) when used *Brassica* intercropping in the greenhouse to decrease the plant parasitic nematode populations. It is worth noting that there was no significant effect of *Brassica* on galls and egg masses numbers when intercropping with pepper, while the effect was significant on the nematode final population and the rate of reproduction (Table 1&2).

In addition, Anaya (2006) and Akhtar and Malik (2000); Wilson and Caveness (1980) considered *C. juncea* as a non-host to *M. incognita* and has an antagonistic exudate that suppress *Meloidogyne* in pineapple. Moreover, Esparago *et al*., (1999) reported that *C. juncea* suppressed the Pf of three species of *Meloidogyne* in vegetables

The effect of *Tagetes* and *Brassica* on the nematode parameters were high and these data agreed with those obtained by Ferraz and Grassi de Freitas (2004); Hooks *et al*. (2010); Evenhuis *et al*. (2004) who reported that *Tagetes* spp. are very efficient in nematode control, especially for *Pratylenchus* and *Meloidogyne* species in intercropping technique. And, agreed with Abid and Maqbool (1990) who reported that the number of root galls due to *M. javanica* in tomato plants grown side by side with *T. erecta* was significantly lower when compared to tomato grown alone. On the other hand, there were no significant differences between intercropping with *Tagetes* and *Brassica* in almost cases.

Table 1. Mean of galls and egg masses in tomato, cucumber, and pepper intercropping with *Crotalaria, Tagetes*, and *Brassica* plants in the screenhouse.

Treatments	No. of Galls			No. of Egg masses		
	Tomato	Cucumber	Pepper	Tomato	Cucumber	Pepper
Intercropping with *Crotalaria*	153.7 c	205.7 c	197.0 c	160.3 c	158.3 c	189.7 c
Intercropping with *Tagetes*	251.7 bc	311.0 b	305.3 b	233.7 bc	220.7 c	295.0 b
Intercropping with *Brassica*	332.7 ab	374.0 b	363.7 ab	288.7 b	325.3 b	371.7 a
Without Intercropping	429.7 a	474.0 a	408.7 a	387.7 a	431.7 a	411.0 a
F value	21.83 ** < 0.0003	28.37 ** < 0.0001	31.79** < 0.0001	28.94 ** < 0.0001	41.70 ** < 0.0001	102.17** <0.0001
LSD	113.83	95.681	73.48	80.626	84.274	43.687

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance. LSD (5%) = least significant difference, while different letters had a statistically significant differences. *: mean significance. **: mean highly significance.

Table 2. Final population (Pf) and rate of reproduction (Rr) of *Meloidogyne incognita* infecting tomato, cucumber, and pepper intercropping with *Crotalaria, Tagetes,* and *Brassica* plants in the screenhouse

Treatments	Pf			Rr		
	Tomato	Cucumber	Pepper	Tomato	Cucumber	Pepper
Intercropping with *Crotalaria*	570.0 c	692.3 d	746.3 c	0.57 c	0.69 d	0.75 c
Intercropping with *Tagetes*	875.3 b	1054.7 c	1250.3 b	0.88 b	1.05 c	1.25 b
Intercropping with *Brassica*	1099.3 b	1180.0 b	1076.3 b	1.10 b	1.18 b	1.08 b
Without Intercropping	2175.7 a	2276.3 a	2179.0 a	2.18 a	2.28 a	2.18 a
F value	180.68** <0.0001	704** <0.0001	90.98** <0.0001	180.68** <0.0001	704** <0.0001	90.98** <0.0001
LSD	235.27	116.46	291.53	0.235	0.116	0.29

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance. LSD (5%) = least significant difference, while different letters had a statistically significant differences. *: mean significance. **: mean highly significance.

Moreover, intercropping *Crotalaria*, *Tagetes*, and *Brassica* increased the plant growth of the main host plants (Figures 1,2,3)

Fig. (1) showed that the different growth parameters of tomato were increased significantly by using the intercropping technique in the case of *Crotalaria* and insignificantly when *Tagetes* and *Brassica* were used.

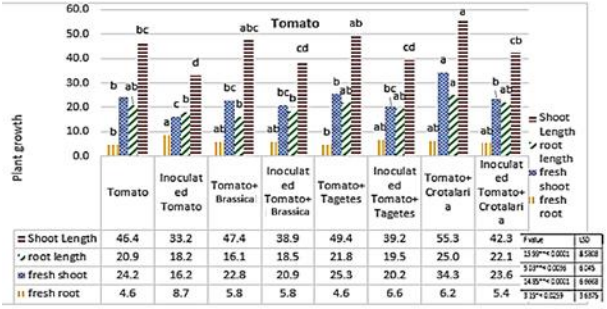

Figure 1. Effect of intercropping *Crotalaria, Tagetes,* and *Brassica* with tomato on the plant growth parameters.

The same letters above bars have no significant differences, while different letters above bars indicate statistically significant differences in their effects.

The growth of cucumber demonstrated that there was efficient increase in the plant growth without any significant differences between the effect of *Crotalaria* and *Tagetes* followed by *Brassica* (Fig.2).

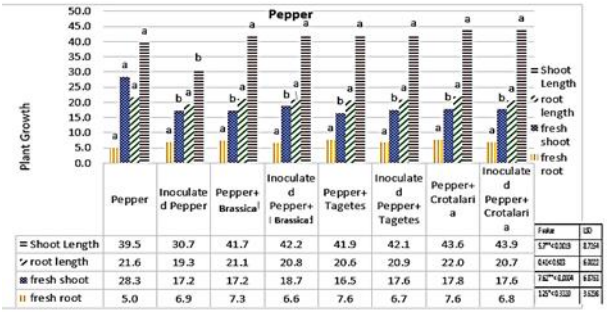

Figure 2. Effect of intercropping *Crotalaria, Tagetes,* and *Brassica* with pepper on plant growth parameters.

The same letters above bars have no significant differences, while different letters indicate statistically significant differences in their effects.

Data in Figs. (2&3) recorded that all plant growth parameters of pepper and cucumber were improved with the intercropping of *Crotalaria, Tagetes*, and *Brassica* and the differences were insignificant between them.

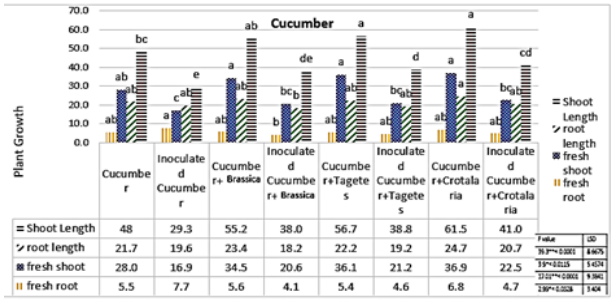

Figure 3. Effect of intercropping *Crotalaria, Tagetes,* and *Brassica* with cucumber on plant growth parameters.

The same letters above bars have no significant differences, while different letters above bars indicate statistically significant differences in their effects.

Generally, the previous data stated that, the intercropping of some nematotoxic plants i.e. *Crotalaria, Tagetes*, and *Brassica*. decreased the nematode parameters while increased growth. These data were supported by Ferraz and Grassi de Freitas (2004) or at least did not suppress the host plant. Bello *et al*., (2014) reported that, *T. erecta* and *C. juncea* improved the plant growth and yields when intercropped with tomato plants.

As typified before many researches explained the effect of the plants which have a nematicidal effect on plant parasitic nematodes such as: *Crotalaria*, *Tagetes*, and *Brassica*.

Crotalaria has a nematicidal active ingredient byproduct property on *M. incognita* which can inhibit the growth of the nematodes. This effect may be better than some nematicidal chemicals due to the continued effect in suppression even after the host is planted (Huang et. al., 1981; Sharma and Scolari, 1984), as well as safety and improving the host yield. *Crotalaria* effect may be attributed to their nematicidal root exudates (Villar andZavaleta-Mejía, 1990) which contain allelopathic compounds that called monocrotaline and pyrrolizidine alkaloids (PA) which can affect the nematode (Jourand *et al*., 2004; and Wang and McSorley, 2004), or penetrate larval body and inhibit their metabolic activity and some enzymes functions such as: acetyl cholinesterase enzyme and respiratory enzymes (Aguillera, *et al*., 1984). *Crotalaria* can be used as a non-crop plants for some plant parasitic nematodes especially root knot nematodes (Luc *et al*., 1990). Also, it is a highly resistant plant to some *Pratylenchus* species and *Rotylenchulus reniformis* (Silva *et al*., 1989a, 1989b; and Ferraz and Grassi de Freitas, 2004).

Intercropping of marigold is very effective (Ferraz and Grassi de Freitas, 2004; and Hooks *et al*., 2010) on

Pratylenchus pratensis (Oostenbrink, 1960; and Conijn *et al*., 1996) and *Meloidogyne* by multiple mechanisms; as a poor or a non-host (Hooks *et al*., 2010), producing allelopathic nematicidal compounds (alpha-terthienyl) (Topp *et al*., 1998; and Ferraz and Grassi de Freitas, 2004), enhancing some nematode antagonistic fauna (Wang *et al*., 2001) or using as a trap host (Pudasain *et al*., 2008). Ferraz and Grassi de Freitas (2004) explained that *T. patula* allowed J2 penetration, but the nematode did not develop beyond the early second stage and some necrotic reaction was observed in the feeding sits of the infective juveniles and may be few and very small giant cells and galls are produced (Suatmadji, 1969). Moreover, the using of marigold may be more effective in nematode control than some fumigants and chemical nematicides in some instances (Hooks *et al*., 2010), and the effect may have greater depths because of the debris which remained in the soil after the crop rotation that continue to exert its nematicidal effect on PPNs (Evenhuis *et al*., 2004).

All *Brassica* spp. can release bio-toxic compounds that showed activity against fungi, bacteria, insects, nematodes (Bohinc *et al*., 2013; Laznik *et al*., 2014; Grubišić *et al*., 2018). The nematocidal effect of brassicas is due to their ability to produce glucosinolate compounds which are b-D-thioglucosides, that are accumulated in the different plant parts (roots, stems, and leaves). Brassicas plants attract the juvenile larvae of endoparasitic nematodes as a trap, after root penetration, they are poisoned by hydrolysis products and fail in completing their life cycle during the intercropping time. Consequently, the population density in soil will be decreased (Curto, 2008). Penfold and Collins, (2012) reported that, *Brassica* can be used in rotation crops and the results depend on the geographic regions, and the toxic chemical compounds in *Brassica* species (Chen *et al*., 2007; Hartz *et al*., 2005; Monfort *et al*., 2007; and Edwards and Ploeg 2014), and correlated to the soil moisture at the time of the application (Matthiessen *et al*., 2004).

In conclusion, the intercropping of the previous plants which have nematotoxic effect on root knot nematode *M.incognita* without any suppressive effect on the plant growth, can be an efficient method in the integrated pest management programs after further studies.

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تأثير تحميل النباتات التى لها تأثير ابادى على نيماتودا تعقد الجذور فى بعض نباتات الخضر بالصوبة السلكية

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تتسبب النيماتودا الممرضة للنبات وخاصة نيماتودا تعقد الجذور فى أضرار اقتصادية بالغة على النباتات وخاصة نباتات الخضر على مستوى العالم. وبسبب الاضرار السلبية لاستخدام المبيدات النيماتودية ذات الاصل الكيميائى على صحة الانسان وعلى الحيوانات وما تسببه لما تسببه من تلوث فى البيئة الزراعية، كانت هناك ضرورة ملحة لايجاد وسائل اخرى غير كيميائية لمكافحة النيماتودا بحيث تكون فعالة وامنة ومقبولة اقتصاديا. يعتبر تحميل النباتات التى لها تأثير مثبط لنشاط النيماتودا احد هذه البدائل المطروحة، وعليه كانت فكرة البحث لدراسة تأثير تحميل نباتات الكروتالاريا *Crotalaria juncea* والقطيفة *Tagetes erecta* والخردل *Brassica carinata* منفصلة على بعض نباتات الخضر الهامة اقتصاديا مثل الطماطم *Lycopersicon esculentum* L. var. Castel Rock، الخيار*Cucumis sativus* والفلفل *Capsicum annum* لدراسة قدرتها على خفض اعداد نيماتودا تعقد الجذور (بتركيز 1000 يرقة نيماتودا/اصيص مع مراعاة تاثيرها على نمو النباتات، أجريت التجربة فى الصوبة السلكية ووأنهيت بعد حوالى شهرين وأخذت النتائج وكانت كالتالى: سجلت النباتات الثلاثة المستخدمة فى التحميل تأثر ابادى على النيماتودا حيث تسببت فى خفض أعداد الاورام على الجذور، أعداد البيض، وخفضت أعداد النيماتودا المسجلة فى نهاية التجربة وايضا معدل تكاثر النيماتودا وكانت أشدها فى التأثير الكروتالاريا والتى سجلت: 153.7 – 160.3- 570.0-00.57 فى الطماطم، و 205.7-158.3- 692.3- 0.69 فى الخيار وأخيرا 197.0- 189.7- 746.3- 0.75 فى الفلفل على الترتيب ثم تلاها نبات القطيفة فى التاثير ثم نبات الخردل. كما سجلت النباتات الثلاثة المستخدمة فى التحميل تحسين معنوى فى نمو النباتات المنزرعة عند مقارنتها بالنباتات المنزرعة بدون تحميل. من النتائج المتحصل عليها يتضح انه من الممكن استخدام نباتات الكروتالاريا، القطيفة والخردل فى التحميل كوسيلة جديدة فعالة وأكثر أمان فى مكافحة نيماتودا تعقد الجذور فى نباتات الخضر ضمن برامج المكافحة المتكاملة.