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EFFICACY OF SOME BIOAGENTS AND NEMASTOP COMPOUND IN CONTROLLING ROOT KNOT DISEASE ON PEANUT.

Howida A. Metwaly[1] and Hanaa S. Zawam[2]

1- Central Laboratory of Organic Agriculture, Agricultural Research Center,Giza, Egypt.

2- Nematode Research Department,Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

ABSTRACT

Biological control of plant diseases especially root knot nematode has been handled in many scientific papers. *Bacillus subtilis*, *Trichoderma harzianum* (T1), *T. viride* (T2), mixture of both (T1andT2) in addition to the commercial product Nemastop were used under field conditions to control root knot nematode, *Meloidogyne javanica* Chitwood on peanut plants which was the most frequent extracted from soil and roots .

The efficacy of the treatments at different concentrations was also assayed under laboratory conditions as percentage of juvenile mortality and inhibition of egg hatching. All treatments revealed good effect in controlling root-knot at the highest concentrations, Nemastop was the most effective at(1:10), whereas, *B. subtilis* was the lowest one. Results obtained from field experiments were in harmony with those obtained from laboratory compared with control. Efficacy of the treatment also positively correlated with number of application time. The plot treated with any one of the bioagents tested three times achieved highest effect in controlling root knot nematode and increased peanut yield. Under field condition mixture of *Trichoderma* isolates was the most effective followed by Nemastop.

Keywords: Biological agents, Root-knot nematode, *Meloidogyne javanica*, Peanut, *Bacillus subtilis*, *Trichoderma harzianum*, *T. viride*.

INTRODUCTION

Peanut *(Arachis hypogaea* L.*)*, is very important annual herb legume crop of tropical and subtropical areas of the world. The importance of peanut as a food and oil crop and as a cash source in the semi-arid tropics is well known. Peanuts are rich in energy and contain health benefit nutrients ,minerals ,antioxidants and vitamins that are essential for optimum health (Umesh 2009).The production of peanut is negatively affected by soil borne fungi and nematodes. Root-knot nematodes (*Meloidogyne* spp.),are considered the most important plant parasitic nematodes attacking more than 2000 plant species(Jung and Wyss 1999)..Reduction in yield of peanut was recorded due to more than 55 pathogens including fungi, bacteria, viruses, mycoplasma, nematodes and parasitic flowering plants (Podile and Kishore 2002). Plant parasitic nematodes cause significant damage to agriculture throughout the world. The damage caused by the root-knot nematodes are much higher in tropical and sub-tropical countries compare with cold ones (Sasser and Freckman 1987).The presence of root-knot nematodes in the roots induces galls that restrict nutrient, water uptake and peanut growth, while also facilitating fungal infections .Root-knot nematodes are the most

significant nematode pest of Egyptian peanut and can cause up to 60% yield loss (Sharma and McDonald 1990).

In recent years, the awareness of the fungicides and nematicides hazards to human and environment has directed the attention towards soil-borne antagonists and natural products to control nematodes in replacing of chemicals. Biological control is gaining increase role throughout the world for nematode suppression .The genus, *Trichoderma* is common saprophytic filamentous imperfect fungus and considered as the most common fungi in the rhizosphere and found in almost all soil type. Sharon *et al*., 2001 reported that *T. harzianum* was antagonistic organism to root knot pathogen *M. javanica* in soil. Other *Trichoderma* species and isolates have also exhibited significant biocontrol activity against *M. javanica* in growth chamber experiments.

Biological control is gaining increasing role throughout the world for soil pathogens and nematodes suppression. Mixing antagonists with each others may be lead to antagonistic effect consequently decrease efficacy of treatment or lead to synergistic effect and increase the efficacy (Robinson *et al*., 2009).

Bacillus subtilis is reported as a biocontrol agent against root-knot nematodes. (Khan *et al.,*2002,Huang *et al.,* 2005 and Huang *et al.,* 2009).Several reports clarified that the basic mechanisms of *B. subtilis* included production of extracellular antibiotics metabolites or enzymes (e.g. proteases, chitinases and glucanases), stimulation of host defenses, incensement of plant growth, induced systemic resistance in plants, suppression of the plant diseases and secreting volatile nematicidal substances (Abd- El- Moneim,2005, Ji *et al.,*2006,Kloepper and Ryu 2006 and Lahlali *et al.,*2013).

The present study is conducted to control the root knot nematode on peanut plants, different biocontrol agents *T.harzianum, T. viride*, mixture of both and *B. subtilis* as well as Nemastop are used to improve protection effect against this disease and to increase the peanut yield. Also, to determine the effect of these biological treatments compared with control under laboratory as well as field conditions.

MATERIALS AND METHODS

Bioagents and biocide:

Different biocontrol agents, *B. subtilis*, *T. harzianum* (T1), *T. viride* (T2), mixture of both T1and T2 and a commercial biocide, Nemastop were kindly obtained from central lab of Organic Agricultural Research Center, Giza, Egypt. *B. subtilis* was grown on nutrient glucose broth (NGB) suggested by Dowson (1957) *T. harzianum* (T1)*, T. viride* (T2) were grown in liquid gliotoxin fermentation medium (GFM) (Brian and Hemming 1945).The bioagents were allowed to grow under complete darkness for nine days just to stimulate toxin production at 28^{o}C (Abd-El-Moity and Shatla 1981). Different bioagents were formulated as suspension using method developed by Abd-El-Moity(1985). Prepared suspension was adjusted to contain 30 x 10^{6}cfu /ml and mixture of them was added as (1:1).Nemastop was used as

commercial biocide to compare its effect with other bioagents against root knot nematode on peanut. Peanut shoot weight, shoot length, root weight, root length and yield were determined.

Extraction and classification of Nematodes:-

Nematode infestation was verified by analysis of soil samples by a laboratory specialist. Different kinds of nematode infested the soil samples associated with rhizosphere of roots and root galls which showed identical galls root knot symptoms from peanut plants collected through the experimental duration in two seasons in El-Bustan Research Station, Nubaria–Behira Governorate, Egypt. An aliquot of 250cm^3 from each soil sample was processed for nematode extraction by sieving and modified Bearman technique (Goodey, 1957). After 48 hours the extracted nematodes were counted using 1 ml Hawksly counting slide under a stereo microscope and classified according to (Mai and Lyon 1975 and Siddiqi 1986) females of root knot nematode were excised from large galls on the roots of peanut plants and individually macerated and accomplished with automated apparatus(Phast System Pharmacia, Uppsala,Sweden) to have esterase phenotype (Tomaszewski,et al.,1994)

Effect of biocontrol agents on *M. javanica* under laboratory and field conditions

I- Laboratory experiment:-

To study the efficacy of biocontrol agents on *M. javanica* under laboratory conditions, one ml of each biocontrol agents (*B.subtilus* ,*T. harzianum* (T1), *T. viride* (T2) and mixture of (T1and T2) were containing (30 x 10^6) cfu /ml and Nemastop were added to nematode suspension at different concentrations (1:10, 1:25 and1:50) in glass vials for each of them. Each of biocontrol agent suspension was added to five handpicked egg-masses and to 500 active nematode juveniles to detect their effect on hatching and juveniles mortality of *M. javanica*. The same number of egg-masses and juveniles received distilled water and served as control. Each treatment was applied in three replicates. The percentage of inhibition in egg hatching was recorded after 3 days and the percentage of Juveniles mortality was recorded after 48 hours under a stereoscopic microscope.

II- Field experiments:-

Field experiment was carried out in naturally heavy infested field with *M. javanica* at El-Bustan Research Station, Nubaria–Behira Governorate, Egypt in April 2012 / 2013 growing seasons. A randomized complete block design with 5 replications was used in each season. A field experiment consisted of plots (7 × 6 m); each comprised of 10 rows 20 cm distance and 30 holes/row at which each treatment was conducted with five plots as replicates as well as check treatment. Gliotoxin fermentation medium (GFM) used to grow the bioagents. *B. subtilis*, *T. harzianum* (T1), *T. viride* (T2), mixture (T1andT2) and Nemastop were used as seed coating before sowing and sprinkled as a liquid at rate of 10 L/fed. after 15 days of sowing(two times applications) and after month of sowing (three times applications).

Plants only treated with water act as control treatment. Peanut seeds Giza 6 obtained from Field Crop Research Institute, Agricultural Research

Centre, Giza. All treatments received the same agricultural treatment such as amount of water, number of seeds /plot and amount of fertilizers.

Effect of applications time using biocontrol agents against (*M. javanica*) in peanut.

Different bioagents were used as one, two and three applications with interval 15 days between applications. Biocontrol agents were used in liquid form and the first treatment was applied as seed coating in the suspension for 20 minutes, where the second and third treatments were applied as soil drench, at five liters of each bioagent / plot. Treated plants were examined at the end of seasons. The roots were then washed to get rid of the adhering and particles to determine the number of root knot galls, juveniles in soil, eggs /5 gm roots, developmental stages in the roots. The number of nematode larvae (in 250 cm^3 soil) and RF (Reproduction Factor) was determined according to Norton (1978).

Reproduction Factor (RF) =

$$\frac{\text{No. Eggs + Developmental stages + Free Nematode in soil+ adult females}}{\text{Initial Population}}$$

Developmental stages (DS) =
number of developed juveniles (third and fourth stages) embedded in the roots.

Efficiency of each treatment recorded according to the equation:-

$$\text{Efficiency} = \frac{\text{RF in control- RF in treatment}}{\text{RF in control}} \times 100$$

At harvest some peanut parameters such as shoot length (cm), shoot weight (g), root length (cm), root weight (g) and yield of pods with kilogram per plot were determined and the efficacy of each bioagents were recorded as average of two seasons.

Statistical Analysis:-

Data obtained were subjected to statistical analysis of variance for completely randomized design (CRD) and randomized complete block design (RCBD) as outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Nematode associated with peanut :-

Data in Table (1) show the percentage frequency of the nematodes recovered species from El-Bustan Research Station, Nubaria–Behira governorate, *Criconemella* sp.,*M. javanica, Paratrichodorus minor, Pratylenchus* sp. and *Tylenchorhynchus* sp. Lamberti (1981) stated that, plant parasitic nematodes account worldwide, for an average estimated by 10-20% yearly loss of agricultural peanut plants which multiplication and spread over thousands of feddans under best conditions. The most dominant root knot nematode was *M. javanica*. Root knot nematodes cause varying degrees of stunting, chlorosis depending on the initial population. (Taylor and Sasser, 1978) showed that more than 80% of the major *Meloidogyne* population belonged to *M. javanica*. Root-knot nematodes (*M. javanica*) are among the most damaging nematodes in agriculture, causing an estimated US$100 billion loss/year on peanut plants worldwide (Oka *et al.*, 2000). *M. javanica* Chitwood

and other root-knot nematodes cause galls in roots of many crops impeding normal uptake of water and nutrients.

Table (1): The percentage frequency of nematodes attacked peanut plants.

Nematode genera	Frequency of occurrence
Criconemella sp.	1.37
Meloidogyne javanica	70.09
Paratrichodorus minor	3.50
Pratylenchus sp.	3.43
Tylenchorhynchus sp.	21.61

Data in Table (1) indicate that, *M. javanica* was the most frequent species of overall the seasons of peanut with frequent percentages of (70.09%) while, *Criconemella* sp. was the least frequent species with frequent percentages of (1.37%).

Effect of different bioagents on the viability of *M. javanica*:-

The morphological change of *M. javanica* juveniles was examined using an inverted microscope during 7 days of incubation at 27 °C. Deforming of juveniles was showed in Figures (1: 5), for treatments and the figure (6) for untreated control. Some juveniles appeared thin with destruction in some parts, but no deforming was observed in the control. Observations through the inverted microscope demonstrated that the microorganisms were widely attached the juveniles of *M. javanica*. The morphological changes of *M. javanica* juveniles agreed with the study of Westcott and Kluepfel (1993) who reported that chitinases produced by bacteria was potent in attacking the cuticle of *M. javanica*. Regarding to *Trichoderma* species (Ghisalberti 2002) reported that chitinolytic enzymes produced by these fungi are thought to be responsible for the degradation of cell walls; they also had ability to produce a wide range of secondary metabolites with diverse biological actions including mycoparasitism also through production of antibiotic substances. *T. viride* and *T. harzianum* destructed and lysis the juveniles body after seven days. The effect of Nemastop on root knot nematode might be due to alkyl cysteine sulphoxides which released a mixture of volatile alkyl thiols and sulphides, these volatile compounds override the inhibitory effect on parasitic nematode (Coley-Smith, 1976)). Results of an antagonism test showed in (Table 2) had significant inhibition and mortality as affected by treatments of *B. subtilis*, *T. harzianum, T. viride*, mixture of them (T1,T2) and Nemastop compound .

Fig. (2): Interaction between bioagents and juvenile of pathogenic nematode

These bioagents had various degrees of effectiveness toward the nematode juveniles. Moreover, the percentage of mortality increased as increase of the concentration. Results also revealed there is positive correlation between different concentrations of treatments and efficacy of application. The obtained results might be due to the increase in the amount of the active component of bioagent. Nemastop was the most effective treatment of the three concentrations. The highest concentration (1:10) for all the tested treatments achieved the highest percentage of juvenile mortality and inhibition of eggs hatching. The inhibition in egg-hatching caused by all the used treatments ranged from 100% for both Nemastop and *T. harzianum* to 56% for *T. viride* . The lowest concentration (1:50) recorded the lowest percentage of egg hatching inhibition . Rahman Khan *et al*., (2005) mentioned that *B. subtilis* produce nematoxic metabolites that involved in the nematode suppression. The morphological change of *M.javanica* juveniles agreed with the study of Westcott and Kluepfel (1993) who reported that chitinases activity produced by PGPR were more potent in attacking eggs than the cuticle of *M.incognita*, this might have resulted from the direct damage caused by the bacterial chitinase.

Table (2): The inhibition and mortality of *M. javanica* as affected by biocontrol agents.

Treatments	Concentrations	Inhibition of egg hatching%	Mortality of juvenile %
B. subtilis	1/10	59	75
	1/25	42	47
	1/50	19	30
T. harzainum (T1)	1/10	100	96
	1/25	96	79
	1/50	79	68
T. viride (T2)	1/10	56	87
	1/25	33	61
	1/50	20	36
Mixture of (T1 +T2)	1/10	94	95
	1/25	87	89
	1/50	73	78
Nemastop	1/10	100	98
	1/25	95	95
	1/50	88	83
Control		0	0
L.S.D. at 5%(TREATMENT)		2.625	1.977
L.S.D. at 5 %(CONCN.)		1.856	1.398

Effect of applications time using biocontrol agents against (*M. javanica*) in peanut.

To determine the suitable time for adding different antagonist to get the highest effect, different bioagents were added at three different times. These adding times were at planting time, fifteen days after planting and one month after planting. The early treatment showed the highest effect in plant protection and vice versa. Time of application was reflected on the yield and percentage of dry matter in treated plants. This is due to that early treatment,

at planting time, allows propagules of used antagonist to spread out and surround newly developed roots causing protection against root knot nematode that may be found in court of infection. On the contrary, adding antagonist fifteen days and one month after planting this means that, the developed roots before this period, already attacked by pathogens which negatively affect plant growth and developing (Mannanov and Sattar 2009). That is why plants treated very early, at planting time, gave the highest yield and highest percentage in dry matter.

Data in Table (3)showed that , significant differences in the number of developmental stages and eggs /5g roots, Juveniles/ 250 cm^3 soil between peanut plants treated one time and those treated two or three times. In plants treated at three times, the percentage of nematodes was found to be less than those recorded after one or two times. This might be due to appearance of new roots on the treated plants free from the nematode infection. Obtained data also showed that, all antagonists significantly reduced the number of root galls, developmental stages and juveniles in soil. Mixture of *Trichoderma* spp. isolates showed good effect in controlling nematode. Mixing different isolates may increase the scope on mode of action consequently increase efficacy of the treatment this agree with Zhang and Zhang (2009) who showed that combination of isolated *T. viride* strain and *T. harzianum* reduced root galling and the number of new infection by nematode. *T. harzianum* parasitized eggs and larvae of nematode. The hyphae penetrate the eggs and larval cuticle by dissolving the chitin layer through enzymatic activity. They proliferate within the organism and produce toxic metabolites (Dos Santos *et al.,* 1992). Thus, the enzymes produced by *Trichoderma* spp. such as chitinases ,glucanases and proteases seem to play an important role in parasitism (Haran *et al*., 1996). *Trichoderma* has not only been proved to parasitize nematodes and inactivate pathogen enzymes but also help in tolerance by enhanced root development (Harman, 2000).Sharma and Pandey(2009) found that *T. harzianum* alone can reduce gall formation and improved plant growth. As known by Li *et al. (*2007) and (Kim *et al.,* 1997) that *B. subtilis* produce antibiotics and antifungals that performed as bio-pesticide against nematode, as bacillomycin, iturin, surfactin, and agrocin. The reduction of nematode plant parasitic nematodes associated with *B. subtilis* may be attributed to diverse mechanisms which involve phytohormones production, mineral solubilisation, reduction of the activity of egg hatching factors, alteration of root exudates and inhibition of nematode penetration into the roots thereby interfering with host finding process and reducing galls

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Data in Table (4) showed that positive correlation between numbers of application and plant growth improvement. All treatments either bioagent alone or in combination with each other as well as commercial biocide led to high significant effects on yield, shoots and roots (weight and length). Increase number of application to be two or three times led to significant increase in efficacy due to increase establishment of the antagonist which led to increase in yield of treated plants. This may be due to repeated treatment with bioagents which increase growth regulators and increase growing roots and also may increase plant nutrients uptake. Data also recorded that the mixture of *Trichoderma* isolates occupied the highest rank (20.71kg/plot) compared with control treatment at the third application. This may be explain as compatible relation between mixture of *Trichoderma* isolates, led to synergistic effect between them and increasing the uptake of macro and micro nutrients and improving all vegetative characters .This increase in yield may be due to that rhizosphere organisms produced greater amount of organic acids, such as tartaric, citric acid and lactic acid which may improve plant productivity and increase the root system growth.

Table(4):Effect of applications number using biocontrol agentsin improving agronomic characteristic on peanut plants.

Yield kg / plot		Root Length (cm.)		Roots Weight(g.)		Shoot Length(cm.)		Shoots Weight(g.)		Application time	Treatments
2013	2012	2013	2012	2013	2012	2013	2012	2013	2012		
11.2	9.28	22	25	39.03	25	29	27	73.7	32.98	One time	*Bacillus subtilis*
55.4	12.41	25	18	42.32	27	30	29	147.4	26.89	Two time	
72	14.17	25	30	57.52	30	44	43.3	203.1	53.84	Three time	
42.8	8.12	29	25	44.39	20	30	25	119.2	41.9	One time	*Trichoderma harzianum* (T1)
53	9.47	30	27	66.05	20	38	26.8	140.3	42.87	Two time	
55.34	11.64	30	20	71.47	30	39	28.3	142.1	37.5	Three time	
44.41	3.59	28	30	50.4	20	31	27	100.6	39.94	One time	*Trichoderma viride* (T2)
49.14	7.81	30	20	50.49	30	35	30	121.2	36.2	Two time	
91.2	11.69	33	30	71.03	36.7	39	37.7	182.3	48.76	Three time	
66.5	13.12	30	20	36.24	25	30	27.7	138.4	31.5	One time	Mixture of (T1+T2)
67.8	13.57	35	36.7	41.88	30	34	32	148.6	62.04	Two time	
79.13	20.71	40	25	59.07	35	44	45	162.3	47.28	Three time	
70.15	12.61	30	35	43.82	30	31	22.7	140	55.31	One time	Nemastop
81.49	13.34	32	30	50.64	30	39	35	153.1	38.01	Two time	
107.69	14.25	45	35	84.03	35	47	42	192.5	79.33	Three time	
13.5	4.33	21	33.5	33.5	29	27.5	33.7	33.5	18	Control	
1.171	0.45	1.077	0.88	0.689	0.90	1.131	0.747	1.774	2.92	LSD at 5 %	

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تأثير بعض العوامل الحيوية ومركب نيماستوب في مكافحة مرض تعقد الجذور علي الفول السودانى

هويدا عبد الوهاب متولي[١] و هناء سيدهم زوام [٢]

١-المعمل المركزي للزراعة العضوية- مركز البحوث الزراعية الجيزة – مصر

٢- قسم بحوث النيماتودا معهد بحوث أمراض النباتات-مركز البحوث الزراعية الجيزة-مصر

المكافحة الحيوية لمرض نيماتودا تعقد الجذور تم تداولها في أبحاث علمية عديدة .أستخدمت باسيلس ستلس وتريكودرما هارزيانم وتريكودرما فيردي وخليط منهما ونيماستوب بتركيزات مختلفة لمكافحة نيماتودا تعقد الجذور،ميلودوجيني جافينكا (والتي كانت أكثر استخلاصا من التربة والجذور المصابة) في المعمل وفي الحقل علي الفول السوداني. وكان أفضلهم تأثيرا هو النيماستوب وأقلهم باسيلس ستلس في المكافحة تحت ظروف المعمل وقد أعطت كل المعاملات تأثيرا جيدا عند أعلي تركيز١(/ ١٠)علي نسبة موت الطور اليرقي الثاني ونسبة فقس البيض. وجد ت علاقة ايجابية بين زيادة التركيز وتأثير المعاملة عند التقييم.كما توافقت النتائج المتحصل عليها فى الحقل مع النتائج المتحصل عليها من المعمل حيث أعطت المعاملات الحيوية أفضل تاثيراً بالمقارنة بالكنترول. سجلت علاقة طردية بين خفض نسبة المرض و عدد مرات الاضافةحيث وجد ان المعاملة بعد ثلاث مرات من الاضافة كان لها أعلى تأثير في تقليل اعداد النيماتودا في الجذوروالتربة و زيادة وزن محصول الفول السوداني .وأعطت المعاملة بخليط من عزلات التريكودرما أفضل تأثيرسواء على وزن المحصول أو خفض اعداد النيماتودا فى التربة و الجذور يليها النيماستوب تحت ظروف الحقل بالمقارنة بالكنترول.

Table (3): Effect of applications on number of using biocontrol agents against (*Meloidogynejavanica*) in peanut plants.

Effecacy%		Rf		Galls/ root		Juveniles /250 cm^3 soil		Eggs /5 g roots		Number of developmental stages/5 g roots		Application time	Treatments
2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012		
87.6	85.5	3.23	3.65	459	275	1800	2100	159	184	199	312	One time	*B.subtilis*
96.8	94.7	.83	1.34	153	113	730	1120	89	92	163	122	Two time	
98.8	97.9	.30	.53	99	64	250	640	31	43	63	55	Three time	
90.0	69.5	2.59	7.69	147	256	840	2730	113	95	213	98	One time	*T. harzianum* (T1)
95.3	87.5	1.23	3.15	109	164	560	1250	39	39	109	67	Two time	
98.6	93.2	0.36	1.72	104	98	280	840	24	27	89	31	Three time	
85.6	87.6	3.75	3.14	152	184	1750	1450	152	189	218	156	One time	*T. viride* (T2)
93.5	94.3	1.70	1.44	124	87	950	860	102	114	115	107	Two time	
97.8	97.6	0.56	0.61	31	63	620	580	41	76	55	52	Three time	
94.2	77.5	1.50	5.68	203	215	380	1920	103	95	179	81	One time	Mixture of (T1and T2)
96.6	89.8	0.89	2.57	83	109	190	620	71	60	97	43	Two time	
98.5	97.3	0.38	0.68	21	38	83	210	21	51	18	22	Three time	
95.9	92.5	1.05	1.89	234	378	350	1500	234	236	317	64	One time	Nemastop
96.0	93.9	1.03	1.55	127	121	220	380	62	80	121	27	Two time	
98	98.6	0.52	1.36	29	34	90	130	29	40	23	11	Three time	
		25.98	25	664	681	3900	2400	218	265	498	942	Control	
				2.126	4.293	58.9	15.28	1.275	5.5	2.751	5.6	LSD at 5%	