Minufiya J. Agric. Res. Vol.35 No. 3:885-902 (2010) "http://agri.menofia.edu.eg/megla.html"

SEASONAL FLUCTUATION, INOCULUM LEVELS AND CONTROL Of THE ROOT- KNOT NEMATODES; *MELOIDOGYNE INCOGNITA* ON CASSAVA

Samaa M. Shawky, A. E. Khalil and Manal M. Soliman
Nematology Department, Plant Pathology Research Institute, Agricultural Research, Center, Giza, Egypt.

(Received: Apr. 18, 2010)

ABSTRACT: ***The experiments were conducted under both greenhouse and field conditions to determine seasonal fluctuation, inoculum levels of the root-knot nematode, Meloidogyne incognita and application of some bioagents to control this nematode on cassava. Seasonal fluctuation of the root knot nematode Meloidogyne incognita were studied on two cassava cultivars (Brasilia and Endonisy) during the period from January to December 2008. Results reveal that M. incognita population fluctuated in soil of two cassava cultivars (Brasilia and Endonisy) increased from 420 individuals per 250 g. soil in Brasilia cultivar and from 180 individuals per 250 g. soil in Endonisy cultivar as the initial population in January 2008 to 3220 or 2100 individuals per 250 g. soil in August 2008 and then declined down to 640 or 420 individuals / 250 g. soil in December 2008 where soil temperature reached 17± 4ºC, respectively.***

Results showed that Brasilia cultivar was more susceptible than Endonisy cultivar. Nematode population increased during July, August and September in the two cultivars and then nematode population decreased to from November to December. Two cultivars of cassava were examined for their relative susceptibility to the infection with M. incognita. Brasilia cultivar was more susceptible than Endonisy to the root knot nematode. The nematode population in 250g soil and in root (number of developmental stages, females, egg masses number and eggs/ egg mass) were significantly higher on Brasilia as compared to the same criteria on Endonisy cultivars. Also data indicated decreasing % of fresh weight of the whole plant in cultivar Brasilia at inoculum 3000 where the percentage of decrease reached (88.6%) while, in cultivar Endonisy at inoculum 1000 was (26.2%).

Five treatments (Psudomonas fluorescens, Trichoderma viridi, Bacillus thurngenthis, DiTera; Myrothecium verrucaria and Nemathoren were evaluated to control M. incognita. Using Nemathoren (10%) and Myrothecium verrucaria specially at the highest concentration performed the highest decrease in both soil. and root (developmental stages, females, egg-mass and number of eggs/ egg-mass) comparing with the other treatments. Psudomonas fluorescens and Bacillus thurngenthis occupied the intermediate rank in reducing the nematode populations, whereas Trichoderma viridi resulted the lowest number of nematode populations in both soil and roots. The results expressed as increasing % over control. Data

indicated percent increase of fresh weight of the whole plant were greatly improved in both treatments of Nemathoren(10%G) and Myrothecium verrucaria at the highest concentration where the percentage of increase reached (87.2, 72.1%) respectively while, the treatment of Trichoderma viridi was (42.5%)at the lowest concentration. Also data showed a positive correlation between the amounts of total or reducing sugars and concentration of the used treatments. Amounts of phenols increasing the concentrations of the treatments. Amounts of total amion acids were decreased by increasing the concentration of the used treatments.

Key Words***: Cassava cultivars, root knot nematode Meloidogyne incognita, seasonal fluctuation, Psudomonas fluorescens, Trichoderma viridi, Bacillus thurngenthis, DiTera; Myrothecium verrucaria , Nemathoren, sugars, phenols and amion acids.***

INTRODUCTION

Cassava yield contribute to food security and filling the food gap in Egypt, like all from Ghana and Nigeria, where the two countries are of the few countries which have succeeded in reducing the prevalence of food for more than 30% In Ghana, the reduction rate decreased from 62% to 10% while in Nigeria, it decreased from 44% to 8% .The importance of crop, "cassava" because it contains a large amount of starch than any other vegetable crop, where the roots contain about 25-30% of fresh weight,. and starch content of up to 3 times the content in potatoes. Cassava leaves containing papers on the proportion of protein, 8-10% (Lenis *et al*. (2005).

Cassava used by 65%for human consumption,21% for animal feeding, 14% in the manufacture of starch and other industries. Root knot nematode attack and damage the crop (Makumbi-Kidza *et al*.(2000).

***Psudomonas fluorescens* has been reported as a biocontrol agent against nematodes (Devi and Upma, 2002; Hamid *et al*., 2003; Mahapatra & Mohanty, 2003; Rao *et al*., 2004 ; Siddiqui & Shaukat, 2005 and Shawky *et al*. 2008).**

***Bacillus thurngenthis* is reported as a bio-control agent against root-knot nematodes. *Moreover it* act through production of number of antibiotics as bacterocin and subitisin antibiotics (Ferreira, 1991; Farahat,1998; Khan *et al*. 2002 and Shawky and Abd El- Moneim ,2005).**

***Trichoderm* spp. act through different mechanisms including mycoparasitism (Benhamoud and Chet, 1993) also through production of antibiotic substances (Hayes,1992). *Trichoderm viridi* also act through production of destructive enzymes i.e., chitenase (Bolar, 2000 ; Sharon *et al.*, 2001; Faruk *et al.*, 2002; Spiegel and Sharon, 2004;Siddiqui and Shaukat, 2004; Shawky and Abd El- Moneim ,2005 and Jegathambigai *et al.* 2008).**

Trichoderma* spp. can produce various toxic metabolites and different enzymes that improve photolytic activity of the antagonist and control of nematodes. In addition *T. harzianum* has ability to colonise (Tronsmo *et al .,

1993; Devi *et al*. ,2000; Sharon *et al.* ,2001; Faruk *etal.* ,2002 and Siddiqui and Shaukat, 2004)

DiTera; *Myrothecium verrucaria* (Valent U.S.A) which is known to be produced commercially as a new biological nematicides (Warrior *et al.*, 1999). DiTera used as biological control of root-knot nematode (*Meloidogyne* spp.) on tomato by using different formulations from fungal antagonists (Hagag,2009).

The aim of the present work was carried out to throw light on the seasonal fluctuation, inoculum levels and control on reproduction of *M. incognita*, infecting cassava as well as its plant growth.

MATERIALS AND METHODS

1-Seasonal fluctuation of root knot nematode *Meloidogyne incognita* :-

The seasonal fluctuation of the root knot nematode *Meloidogyne incognita* was studied on two cassava cultivars (Brasilia and Endonisy) from January to December 2008 in a naturally infested field at Nubaria. Each sample was replicated five times for each cultivar.Nematode populations in soil (number of juveniles/250g soil) were determined according to Franklin & Goodey, (1957). Roots were stained by acid fuchsin in acetic acid according to Byrd *et al*. (1983), and examined for number of developmental stages and egg laying females/1g root. Eggs /egg-mass of *Meloidogyne incognita* were extracted by using sodium hypochorite (NaOCl) method as described by Husssey and Baker, (1973).

2- Greenhouse experiments:-

1- Susceptibility of two cassava cultivars to the root knot nematode, *Meloidogyne incognita* :-

Cassava nodes of two cultivars i.e. Brasilia and Endonisy obtained from Aly Mobarak Farm Station (Nubaria), Egypt were examined for their relative susceptibility to the infection with root -knot nematode; *Meloidogyne incognita*. Cassava nodes of each cultivar were planted in 25 cm diameter clay pots filled with steam sterilized sandy loamy soil (18% clay, 10% silt and 72% sand) , each pot was inoculated with 1000, 3000 and 5000 newly hatched larvae of *Meloidogyne incognita* around the roots one month after planting. All cultivars received the same agricultural treatments. Each cultivar replicated five times as well as five nodes for each cultivar were kept without inoculation to serve as a check. All pots were arranged in complete randomized design, and kept under greenhouse conditions at about 25-28 C^o.

After 90 days from inoculation, all plants were carefully uprooted and fresh roots and shoots were weighted. Nematode populations in soil (number of juveniles/250g. soil) were determined according to Franklin & Goodey, (1957) Roots were stained by acid fuchsin in acetic acid according to Byrd *et al*. (1983), and examined for number of developmental stages and

egg laying females/1g root. Eggs /egg-mass of *Meloidogyne incognita* were extracted by using sodium hypochoride (NaOCl) method as described by Husssey and Baker,(1973).

2-Efficacy of some bioagents in controlling *Meloidogyne incognita*:-

In this study five different treatments were used:-

1- *Psudomonas fluorescens*

2- *Trichoderma viridi*

3- *Bacillus thurngenthis*

4-DiTera; *Myrothecium verrucaria*

5-Nemathoren(10% G),Fosthiazate

The concentrations of both *Psudomonas fluorescens* and *Bacillus thurngenthis* (*1×10^5, 1×10^8,5×10 ^{8}* cells)/ ml. were against *M. incognita*. The *Psudomonas fluorescens* and *Bacillus thurngenthis* were taken from Microbiology Department, Soils, Water and Environment Research Institute, Agriculture Research Center. The concentrations of *Trichoderma viridi* were (*1×10^5,1×10^8,5×10 ^{8}* cfu)/ ml. and was taken from Plant Pathology Department, Faculty of Agriculture, Cairo University. 150 ml. from *Psudomonas fluorescens* , *Bacillus thurngenthis* and *Trichoderma viridi* were added to each pot. The concentration of DiTera; *Myrothecium verrucaria* (Valent U.S.A) which is known to be produced commercially as a new biological nematicides were (0.5,1.0 and 1.5 g)/pot Warrior *et al.*, (1999). The nematicide product of DiTera was incorporated into the upper 10 cm. of soil as dry powder. Nemathoren G% (12.5 kg./feddan) (0.4 g/pot) was used as recommended concentration .

Cassava nodes cultivar (Brasilia) were transplanted individually in 25 cm diameter clay pots. Each pot filled with steam sterilized sandy loam soil (18% clay, 10% silt and 72% sand) . Pot were inoculated with 3000 newly hatched larvae of *M. incognita* around the roots. All treatments received the same agricultural treatments. Treatment replicated five times. Also, five cassava nodes were treated with newly hatched larvae of *Meloidogyne incognita* alone as check. All pots were arranged in complete randomized design, and kept under greenhouse conditions at about 25-28 C^o.

After 90 days, plants were then harvested, parameters of shoot and root growth were measured and recorded. Nematode populations in soil were extracted using method as previously mentioned and counted. Infected roots were stained as previously mentioned and number of developmental stages, females, egg-mass and eggs were then counted and recorded.

Chemical analyses:-

Chemical analyses of sugars, phenolic compounds and amino acids were detected in Food Technology Research Institute. Total and reducing sugars were measured by Thomas and Dutcher,(1924). Phenolic compounds were

determined by Snell and Snell, (1953). The total free amino acids were determined by Rason, (1959).

Statistical analysis procedure:-

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) and means were compared by using L.S.D. at 5 % level of significance.

This work was undertaken in the greenhouse of Nematology Division, Plant Pathology Res.inst.,Giza and a naturally infested field with *M. incognita* on cassava plants in Aly Mobarak Farm Station (Nubaria), Egypt.

RESULTS AND DISCUSSION

1-Seasonal fluctuation of the root knot nematode *Meloidogyne incognita* :-

The seasonal fluctuation of the root knot nematode *Meloidogyne incognita* on two cassava cultivars (Brasilia and Endonisy) during the period from January to December 2008 is illustrated in Fig(1).Results showed that Brasilia cultivar was more susceptible than Endonisy cultivar. Nematode population increased during July, August and September in the two cultivars and then nematode population decreased to from November to December. Results indicate that *M. incognita* population fluctuated in soil of two cassava cultivars (Brasilia and Endonisy), since its values were 420 or 180 individuals /250 g. soil as the initial population in January 2008, increased up to 3220 or 2100 individuals /250 g. soil in August 2008 then declined down to 640 or 420 individuals /250 g. soil in December 2008 due to temperatures at this time of the year that were recorded to be 12±5ºC, 34±3ºC and then 17±4ºC, respectively. The present findings are in agreement with those of Osman (1977) who reported that winter crops, which commonly precede tomato cultivated resulted in variable degrees of root-knot nematode infecting tomato plants. These findings support the evaluation of El-Sherif *et.al.* (2009) in respect to *Rotylenchulus reniformis*.

2- Greenhouse experiments:-

1-Host susceptibility of two cassava cultivars to the root knot nematode, *M. incognita*:-

Data in Table (1) indicate that Brasilia cultivar was the more susceptible than Endonisy to the root knot nematode The nematode population in 250g soil and in root (number of developmental stages, females, egg masses number and eggs/ egg mass) were significantly higher on Brasilia specially at the highest inoculum level,with rate of nematode build -up reached to 7.90 as compared to the paralled values on Endonisy cultivar(1.98).

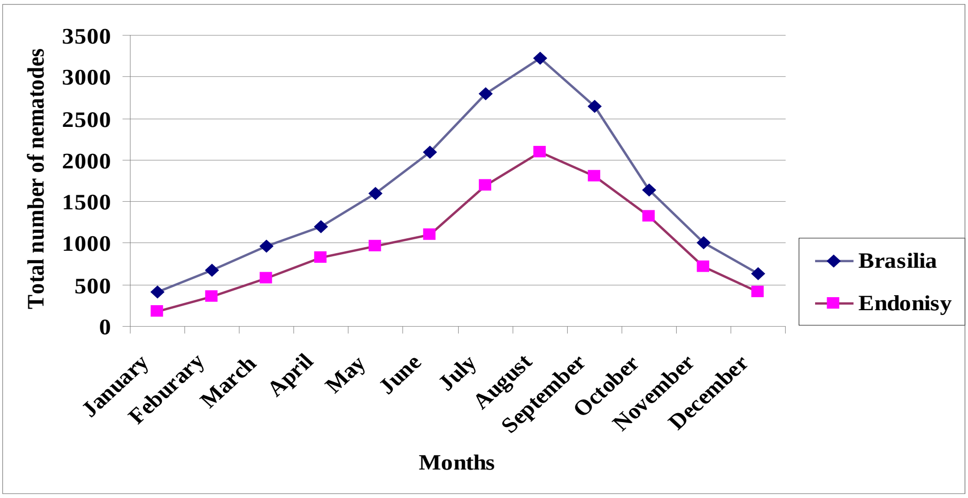

Fig. (1): Seasonal fluctuation of *M. incognita* on two cassava cultivars under field conditions.

Table (1): Impact of inoculum levels of *M. incognita* on two cassava cultivars cvs. Brasilia and Endonisy.

Cultivars	Inoculum levels	*Nematode population in					**Final nematode population (PF)	Rate of build-up (PF/PI)
		Soil/ pot	Root					
			developmental stages	females	Egg-mass	Eggs/ egg-mass		
Brasilia	1000	580	36	26	22	270	6582	6.58
	3000	960	62	55	53	396	22065	7.35
	5000	2240	112	79	75	494	39481	7.90
Endonisy	1000	80	17	10	7	170	1297	1.30
	3000	260	34	21	18	268	5139	1.71
	5000	640	45	37	32	287	9906	1.98
L.S.D 5%		52.3	1.8	4.5	2.7	15.6	1243.6	0.2

*Each value presented the mean of five replicates.
**Final nematode population (PF)= No. of egg-masses x no. of eggs/egg-mass + no. of females +no. of developmental stages+ no. of juveniles in soil/pot.

$$\text{Rate of build-up} = \frac{\text{Final population (PF)}}{\text{Initial population (PI)}}$$

Data in Fig (2) showed the number of galls in two cultivars of cassava (Brasilia and Endonisy). Brasilia cultivar was the more susceptible cultivar than Endonisy specially at the highest inoculum level. Brasilia cultivar resulted the highest number of root galls at the highest inoculum level (79) while, Endonisy cultivar showed a relatively lower number of galls at the same inoculum level of root galls at the highest inoculum level (37).

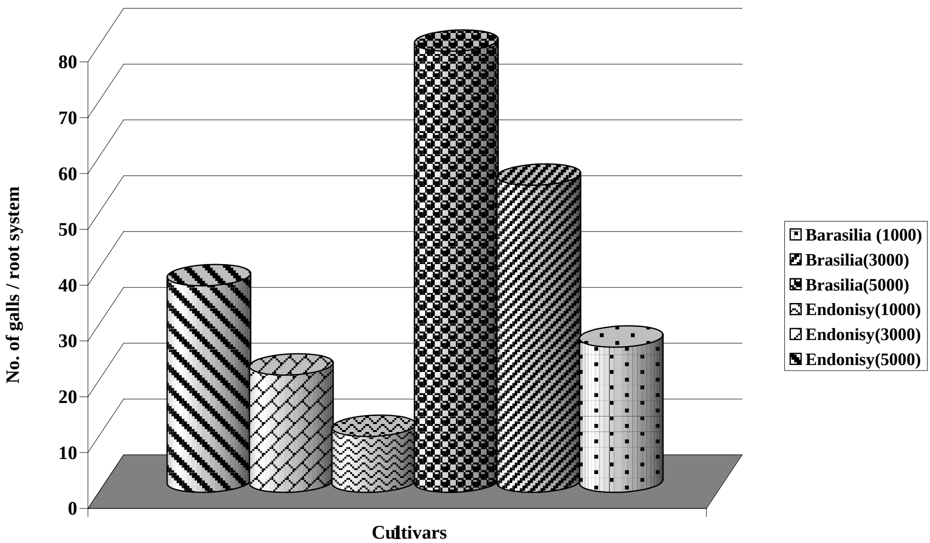

Fig. (2): Effect of inoculum levels of *M. incognita* on number of galls /root on two cassava cultivars under greenhouse conditions.

Data in Fig.(3) showed the effect of inoculum levels of nematodes on percent decrease in fresh weight of the whole plants of both Brasilia and Endonisy under greenhouse conditions. The results expressed as decreasing % over control (plant free of nematodes). Data indicated decreasing of fresh weight of the whole plant in cultivar Brasilia at inoculum 3000 where the percentage of decrease reached 88.6%, while in cultivar Endonisy at inoculum 1000 it was 26.2% .

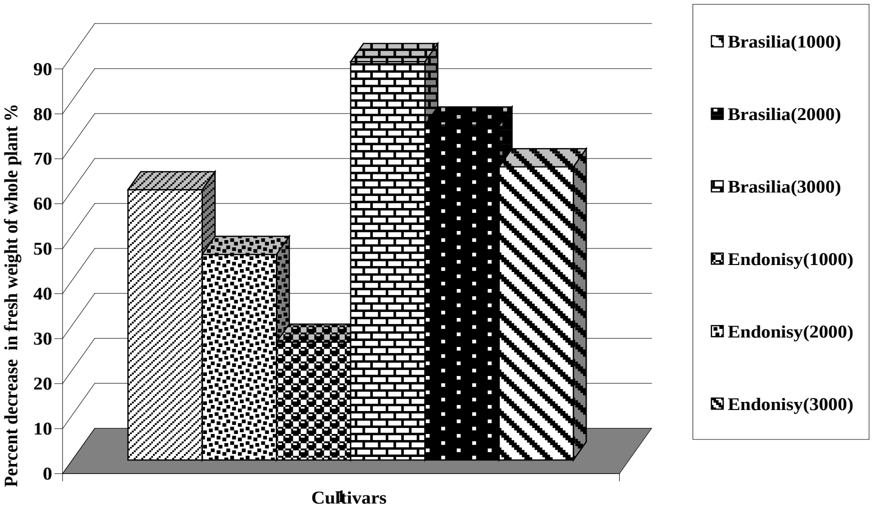

Fig.(3): Effect of inoculum level of *M. incognita* on fresh weight of the whole plants of two cassava cultivars under greenhouse conditions.

2- Efficacy of some bioagents on cassava (cv. Brasilia) infecting with *M. incognita* :-

Data in Table (2) illustrated that all tested bioagent treatments were effective in controlling root-knot nematode; *M. incognita* under greenhouse conditions. Nemathoren(10%G) and *Myrothecium verrucaria* treatments were the most effective treatment than the other treatments whereas *Psudomonas fluorescens* and *Bacillus thurngenthis* ranked the intermediate position in the efficacy. On the other hand *Trichoderma viridi* was the least effective treatment. Also, data showed that positive correlation between efficacy of the treatments and concentrations.

Data reveled that by using Nemathoren (10%) and *Myrothecium verrucaria* at (0.9 g/pot) performed the highest decrease in both soil/ 250g and root (developmental stages, egg laying females, number of eggs/ egg-mass) comparing with the other treatments. *Psudomonas fluorescens* (*5x10^8* cells/ml. per pot) and *Bacillus thurngenthis* (5×10^8 cells/ml per pot) occupied the intermediate rank in reducing the nematode populations, whereas *Trichoderma viridi* (5×10^8 cfu/ml per pot) resulted in the lowest number of nematode populations in both soil and roots.

Table (2): Efficacy of some bioagents on cassava (cv. Brasilia) infected with *M. incognita* under greenhouse conditions.

Treatments	Concentration	*Nematode population in					**Final nematode population (PF)	Rate of build- up (PF/PI)
		Soil/ pot	Root					
			developmental stages	females	Egg-mass	Eggs/ egg-mass		
Bacillus thurngenthis	1×10^6 cells/ ml.	840	41	31	28	286	8920	2.97
	3×10^8 cells/ml.	560	32	26	21	264	6162	2.05
	5×10^8 cells/ml.	320	21	19	16	225	3960	1.32
Psudomonas fluorescens	1×10^6 cfu/ml.	480	34	21	18	245	4945	1.65
	3×10^8 cfu/ml.	360	25	17	14	220	3482	1.16
	5×10^8 cfu/ml.	200	16	12	10	194	2168	0.72
Trichoderma viridi	1×10^6 cfu/ml.	960	46	37	31	315	10808	3.60
	3×10^8 cfu/ml.	880	37	29	25	295	8321	2.77
	5×10^8 cfu/ml.	680	26	22	19	280	6048	2.02
Myrothecium verrucaria	0.5 g/pot	220	24	16	13	210	2990	1.00
	1.0 g/pot	140	19	13	11	185	2207	0.74
	1.5 g/pot	100	12	10	8	170	1482	0.49
Nemathoren	0.4 g/pot	40	7	5	4	152	660	0.22
Nematode (check)/pot		2800	115	56	50	420	23971	7.99
L.S.D(5%)		35.2	1.7	0.9	1.4	3.8	112.3	0.11

***Each value presented the mean of five replicates.**
****Final nematode population (PF)= No. of egg-masses x no. of eggs/egg-mass + no. of females +no. of developmental stages+ no. of juveniles in soil/pot.**

$$\text{Rate of build-up} = \frac{\text{Final population (PF)}}{\text{Initial population(PI)}}$$

Data in Fig (4) showed the number of galls in cultivar Brasilia of cassava. The treatments of both Nemathoren(10%G) and *Myrothecium verrucaria* were the lowest in number of galls. Whereas *Psudomonas fluorescens* and *Bacillus thurngenthis* ranked the intermediate position in number of galls. On the other hand *Trichoderma viridi* was the highest in number of galls specially at the highest concentration. Also, data showed that negative correlation between number of galls of *M. incognita* and concentrations of the treatments.

Data in Fig.(5) showed the effect of some bioagents on percent increase in fresh weight of the whole cassava plants infected by *M. incognita* under greenhouse conditions. The results expressed as increasing % over control. Data indicated that increasing % of fresh weight of the whole plant were greatly improved in both treatments of Nemathoren(10%G) and *Myrothecium verrucaria* at the highest concentration(0.9 g/ pot) where the percentage of increase reached (87.2 , 72.1 %) respectively while, the treatment of *Trichoderma viridi* was (42.5%)at the lowest concentration(1×10^6 /pot).

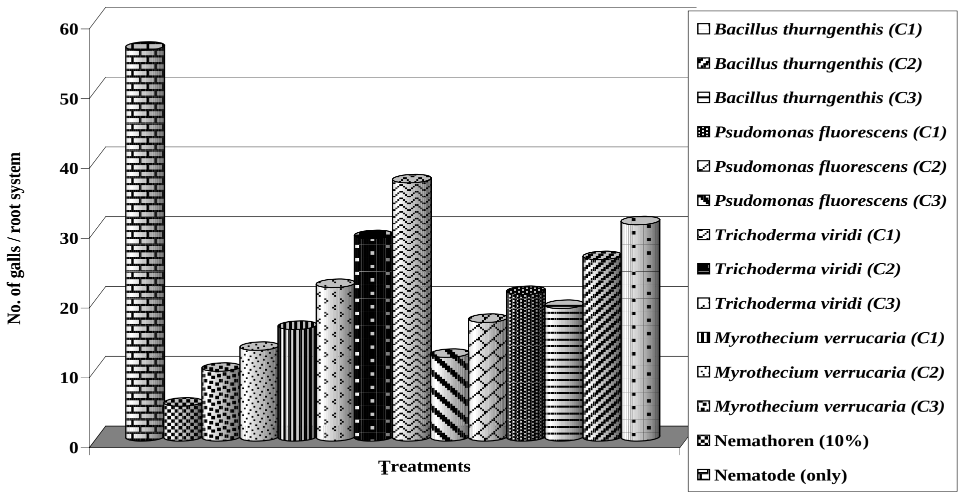

Fig. (4): Effect of some bioagents on number of galls of *M. incognita* on two cassava cultivars under greenhouse conditions. (C1=first concentration, C2=second concentration, C3=third concentration).

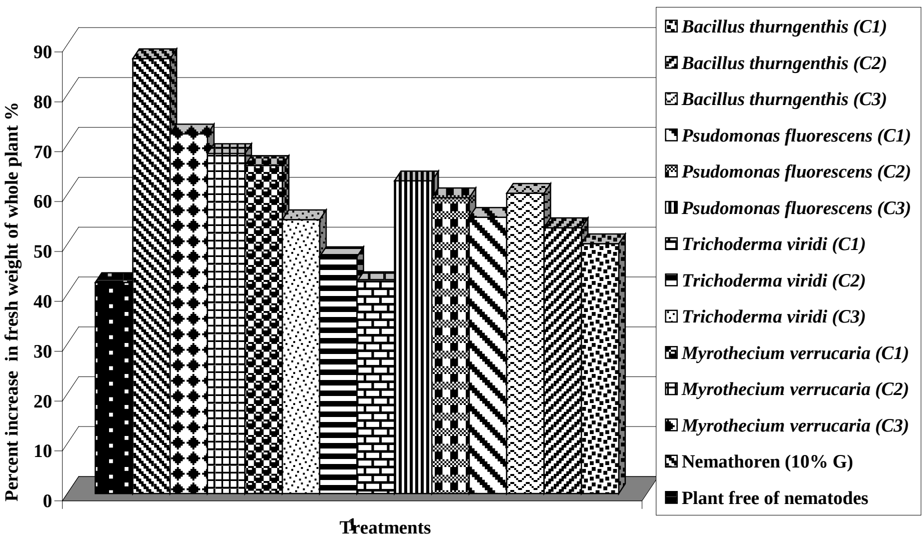

Fig.(5) : Effect of some bioagents on percent increase in fresh weight of the whole cassava plants infected by *M. incognita* under greenhouse conditions. (C1=first concentration, C2=second concentration, C3=third concentration).

Data in table (3) showed a positive correlation between the amounts of total or reducing sugars and concentration of the used treatments. Amounts of phenols increasing the concentrations of the treatments. Amounts of total amion acids were decreased by increasing the concentration of the used treatments.

Table (3): Efficacy of some bioagents on some chemical components of cassava (cv. Brasilia) infected with *M. incognita* under greenhouse conditions.

Treatments	Concentration /pot	Concentration (mg/g)				Total amino acids
		Total sugars	Reducing sugars	Total phenols	Free phenols	
Bacillus thurngenthis	1×10^6 cells/ ml.	1.666	0.265	10.945	7.982	0.097
	3×10^8 cells/ml.	1.910	0.273	11.369	8.147	0.089
	5×10^8 cells/ml.	2.100	0.299	16.100	8.321	0.072
Psudomonas fluorescens	1×10^6 cfu/ml.	1.950	0.290	13.000	9.359	0.110
	3×10^8 cfu/ml.	2.100	0.310	16.288	10.315	0.080
	5×10^8 cfu/ml.	2.388	0.312	18.220	10.684	0.056
Trichoderma viridi	1×10^6 cfu/ml.	1.230	0.219	9.965	6.897	0.213
	3×10^8 cfu/ml.	1.209	0.223	10.123	7.128	0.195
	5×10^8 cfu/ml.	1.311	0.330	10.341	7.691	0.176
Myrothecium verrucaria	0.5 g/pot	2.113	0.333	15.387	11.235	0.095
	1.0 g/pot	2.430	0.385	16.410	12.600	0.080
	1.5 g/pot	3.060	0.431	18.442	14.330	0.006
Nemathoren	0.4 g/pot	4.052	0.519	18.610	15.600	0.009
Nematode (check)/pot		1.631	0.114	9.231	1.220	0.378
L.S.D(5%)		0.011	0.009	0.213	0.194	0.002

These results may be due to that *Psudomonas fluorescens* produced various toxic metabolites and different enzymes that improve photolytic activity of the antagonist and control of nematodes (Hanna *et al*., 1999; Devi and Upma, 2002; Khan *et al*., 2002; Hamid *et al*., 2003; Mahapatra & Mohanty, 2003; Rao *et al*., 2004; Siddiqui & Shaukat, 2005 and El Gendy & Shawky, 2006).

***Trichoderma* spp. has ability to conolization (Tronsmo *et al .,* 1993; Devi *et al*. ,2000; Sharon *et al.* ,2001; Faruk *et al.* ,2002 and Siddiqui and Shaukat, 2004 and Jegathambigai *et al.* 2008).**

***Bacillus thuringiensis* can grow and multiplicate very fast under this circumstance and act through production of number of antibiotics (Farahat *et al.*, 1998; Chen *et al*.,2000 and Xiang *et. al.,* 2007).**

DiTera; *Myrothecium verrucaria* (Valent U.S.A) which is known to be produced commercially as a new biological nematicides (Warrior *et al.*, 1999).

DiTera used as biological control of root-knot nematode (*Meloidogyne* spp.) on tomato by using different formulations from fungal antagonistics (Hagag, 2009).

DiTera biological nematicide is based on a naturally occurring fungus, *Myrothecium verrucaria*. The fermentation process that yields DiTera produces multiple components, including water soluble molecules that either kill or impair nematodes. The nematicidal activity occurs on contact, while the nematostatic activity slows nematode movement, inhibits egg hatch, interferes with sensory perception and reduces feeding. Properly timed DiTera applications protect roots from nematodes during key root flushes-when it matters the most. In addition, studies have demonstrated that DiTera increases beneficial microbial populations in the soil, contributing to overall root health.

This pesticide active ingredient is a mixture of the killed fungus, *Myrothecium verrucaria*, and the liquid in which the fungus was grown. This dead fungus kills specific parasitic pests called nematodes, which attack, usually through their roots. The active ingredient is specific, being effective only against nematodes that parasitize plants; it does not harm free-living nematodes. Because the mixture may be toxic to aquatic organisms, it not approved for use in or near bodies of water. No harmful effects to humans are expected as long as applicators protect their eyes and skin from contact with pesticide products that contain this active ingredient.

The active ingredient is the mixture of substances that are in suspension and in solution when the fungus *Myrothecium verrucaria*, is grown in the laboratory. To prepare the active ingredient as a dry power, water is removed from the culture mixture, and the fungus is killed by exposure to high temperatures. The pesticidal activity as apparently not due to a single identifiable component, but requires the entire mixture. Researchers do not know the mechanism of action.

The increase in sugar by increasing the concentration of the used treatments could be due to enhancing the metabolism and accumulation of metabolites that contain sugars. Another possibility is that the increase of concentration gave higher nematode suppression and consequently less consumption of nutrient including sugars. The increased of free phenols by increasing the concentrations of the applied compounds could be attributed to increase of the plant defense against the invading nematode. The increase in total amino acids by increasing concentrations of used compounds could be attributed to lower infection and consequently lower replenish of proteins from the plant cell that cell that adjacent to infected ones (Abdel-Momen *et al.*, 2005).

In conclusion, it can be said that seasonal fluctuation of *M. incognita* were increased in months (July, August and September) on two cassava cultivars (Brasilia and Endonisy). Brasilia cultivar was the more susceptible cultivar than Endonisy to the root knot nematode especially at the highest inoculum

levels (3000 J_2) and decreasing % of fresh weight of the whole plant. All treatments retained their nematicidal effects in soil and possibility of using this trend for integrated control of nematode clearly needs further investigation.

REFERENCES

Abdel-Momen, S. M., Hanaa S. Zawam and A. E. M. Khalil (2005). Effect of certain mineral salts, organic acids, amino acids and growth regulators on reproduction of *Meloidogyne javanica* infecting sunflower. J. Agric. Sci. Mansoura Univ., 30(5): 2951-2961.

Benhamoud, W. and I. Chet (1993). Hyphal interaction between *Trichoderma Harzianum* and *Rhizoctonia solani* ultra structure and Gold cyto-chemistry of mycoparasitic process Phytopathology, 83: 1026-1071.

Bolar, J. P., J. L. Norelli, K. W. Wong, C. K. Hayes, Q. E. Harman and H.S. Aldwinckle (2000). Expression of endochitinase from *Trichodermn harziianum* intransgenic apple increase resistance to apple scab and reduces vigour. Phytopathology , 90 : 72-77.

Byrd, D. W., T. Kirkpatrick and K. R. Barker (1983). An improved technique for cleaning and staining plant tissues for detection of nematodes. J. Nematol., 15 (1): 142-143.

Chen, J., G. S. Abawi, and B. M. Zuckerman (2000). Efficacy of *Bacillus thuringiensis*, *Paecilomyces marquandii*,and *Streptomyces costaricanus* with and without organic amendments against *Meloidogyne hapla* infecting lettuce.J. Nematol., 32(1): 70–77.

Devi, L.S., S. Richa and R. Sharma (2000). Effect of *Trichoderma spp*. against root-knot nematode *Meloidogyne incognita* on tomato. Indian journal of Nematology, 32:2 , 227-228.

Devi, L. S. and D. Upma (2002). Effect of *Pseudomonas fluorescens* on root-knot nematode (*Meloidogyne incognita*) of okra plant. Indian Journal of Nematology; 32(2): 215-216.

El Gendy, S. S. R. and Samaa, M. Shawky (2006). Efficacy of some natural plants and bioagents to minimize the population of root-knot nematode ; *Melidogyne incognita* in superior seedless vineyards and its reflection on vine growth and yield. 1 st. International Egyptian-Jordanian Conference, pp. 281-293.

El-Sherif, A. G., A. E. M. Khalil and A. R. Refaei (2009). Role of crop rotation on *Rotylenchulus reniformis* population with reference to its management on cotton plant , *Gossypium barbadense* in cement binzes under outdoor conditions.. J. Agric. Sci. Mansoura Univ., 34(3):2289-2296.

Farahat, A. (1998). Biological control of some potato bacterial diseases. Ph.D., Thesis, Fac. Agric. Monofia Univ. 118 pp.

Faruk, M. I., M. L. Rahman and M. A. Bari (2002). Mangement of root-knot nematodes of tomato using *Trichoderma harzianum* and organic soil amendment. Bangladesh journal of plant Pathology, 18: 33-37.

Ferreira, J. H. S., F.N. Matthee and A.C. Thomas (1991). Biological control of Eutypalota on grapevine by an antigonistic strain of *Bacillus subtitis*. Phytopathology , 81: 283-287 .

Franklin, M. T. and J. B. Goodey (1957). A cotton-blue lactophenol technique for mounting plant parasitic nematodes. J. Helminthological abstracts, 23:175-178.

Gomez, K.W. and A.A. Gomez (1984). Statistical procedures for agricultural research 2 nd Ed. John Wiley and Sons inc, New York 680p.

Hagag, Eman S. F. (2009). Biological control of root-knot nematode (*Meloidogyne* spp.) on tomato by using different formulations from fungal antagonistics. Ph.D. Thesis, Fac. Agric. Kafrelsheikh University 112pp.

Hamid, M., I. A. Siddiqui and S.S. Shaukat (2003). Improvement of *Pseudomonas fluorescens* strain CHA0 biocontrol activity against root-knot nematode by the addition of ammonium molybdate. Letters in Applied Microbiology; 36(4): 239-244.

Hanna, A.I., F. W. Riad and A. E. Tawfik (1999). Efficacy of antagonistic rhizobacteria on the control of root-knot nematode, *Meloidogyne incognita* in tomato plants. Egyptian Journal of Agricultural Research. 77(4): 1467-1476.

Hayes, C. K. (1992). Improvement of *Trichoderma* and *Gliocladium* by genetic manipulation. Pp.227- 286, in: Biological control of plant disease progress and challenges for the future T. Jamos , E. C ., G.C. and R.J. cook (Eds) Plenum Press, New, York and London published in co operation with NATOAffairs Division 462pp.

Hussey, R. S. and K. R. Barker (1973). A comparison on methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Reptr., 57:1925-1928.

Jegathambigai, V., M.D. Karunaratne, A. Svinningen and G. Mikunthan (2008). Biocontrol of root-knot nematode, *Meloidogyne incognita* damaging queen palm, Livistona rotundifolia using *Trichoderma* species. Commun Agric Appl Biol Sci.;73(4):681-7.

Khan, M.R., K. Kounsar and A. Hamid (2002). Effect of certain rhizobacteria and antagonistic fungi on root-knot nodulation and root-knot nematode disease of green gram. Nematologia Mediterranea, 30(1): 85-89.

Lenis, J. I., F. Calle, G. Jaramillo, J. C. Perez, H. Ceballos and J.H. Cock (2005). Leaf retention and cassava productivity. Centro Internacional de Agricultura Tropical (CIAT), AA 67-13 Cali, Colombia

Mahapatra, S. N. and K. C. Mohanty (2003). Management of root-knot nematode in brinjal using *Pseudomonas fluorescens* and its compatibility with carbofuran. Proceedings of National Symposium on Biodiversity and Management of Nematodes in Croppin Systems for Sustainable Agriculture, Jaipur; 135-137.

Makumbi-Kidza, N. N., P. R. Speijer and R. A. Sikora (2000). Effects of *Meloidogyne incognita* on growth and storage root formation of cassava (*Manihot esculenta*). J. Nematol.; 32(4S): 475-477.

Osman, A. A. (1977). The role of crop sequence and rotation in the scop of nematode integrated control. Ph. D. Thesis , Fac. of Agric., Cario Univ. Giza, Egypt, 92pp.

Rao, M. S., N. Dhananjay and M. Shylaja (2004). Bio-intensive management of root-knot nematodes on bell pepper using *Pochonia chlamydosporia* and *Pseudomonas fluorescens*. Nematologia Mediterranea; 32(2): 159-163.

Rason, E. L. (1959). Modern methods of plant analysis, " non volatile Mon-, Di- and Tricarboxylic acids" Cd. Peak, K and M.C. Tracely, pp. 539-582.

Sharon, E., E. M. Bar, I. Cheti, A. E. Herrera, O. Kleifeled and Y. Splegel (2001). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology , 91: 687-693 .

Shawky, Samaa M. and Maisa, L. Abd El-Moneim (2005). Non chemical control of root - knot nematode*, Meloidogyne incognita* on grape vines. Minufiya J. Agric. Res. Vol. 30(6): 1685-1708.

Shawky, Samaa M., A. M. Shady and Manal M. Soliman (2008). Efficacy of some bioagents, plants and humex in controlling *Tylenchulus semipenetrans* on olive in Egypt. Journal of Biological Chemistry and Environmental Sciences, 3(1), 223-241.

Siddiqui, I. A and S. S. Shaukat (2004). *Trichoderma harzianum* enhances the production of nematicidal compounds *in vitro* and improves biocontrol of *Meloidogyne javanica* by *Pseudomonas fluorescents* in tomato. Letters in Applied Microbiology, 38: 2 , 169-175.

Siddiqui, I. A. and S. S. Shaukat (2005). Phenylacetic acid-producing *Rhizoctonia solani* represses the biosynthesis of nematicidal compounds *in vitro* and influences biocontrol of *Meloidogyne incognita* in tomato by *Pseudomonas fluorescens* strain CHA0 and its GM derivatives. Journal of Applied Microbiology. 98(1): 43-55.

Snell, F. D. and C.T. Snell (1953). Colorimetric Methods of Analysis. Vol. 3 Van Nostrand Company, New York, 606pp.

Spiegel, Y. E. and I. C. Sharon (2004). Mechanisms and improved biological of the root –knot nematodes by *Trichoderma spp*. International Symposium on Chemical and non-Chemical Soil and Substrate Disinfestation – SD

Thomas, W. and R. D. Dutcher (1924). Picric method for carbohydrate. J. Am. Chem. Soc. 45: 1662-1669.

Tronsmo, A.S.S., C.K. Klemsdal, M. L. Hayes and G. F. Hannan (1993). The role of hydrolytic enzymes produced by *Trichoderma harzianum* in biological control of plant diseases. *Trichoderma reesei* cellulases and other hydrolases enzyme structures, Biochemistry, Genetics and Applications (P. Suominen and T. Reinikainen, eds.) Foundation for

Biotechnical and Industrial Fermentation Research, Helsinki,Finland, vol. 8,Pp. 159-168.

Warrior,P., A. Linda, M. Beach, P.A. Grau, G.W. Kirfman and J. M. Conley (1999). Commercial development and introduction of DiTera ™, a new nematicide. Pestic. Sci. 55:343-389.

Xiang, Q. L., W. Jun-Zhi, T. Anderson and V. A. Raffi (2007). Resistance to root-knot nematode in tomato roots expressing a nematicidal *Bacillus thuringiensis* crystal protein. Plant Biotechnology Journal 5: 455–464.

التذبذب الموسمى ومستويات عدوى ومقاومة نيماتودا تعقد الجذور (ميلودوجين إنكوجنيتا)على الكاسافا

سماء محمود شوقي و أشرف السعيد خليل ومنال محمد جمال

قسم بحوث الأمراض النيماتودية– معهد بحوث أمراض النباتات- مركز البحوث الزراعية – الجيزة – مصر.

الملخص العربي

تم إجراء التجارب البحثية فى هذه الدراسة تحت ظروف كل من الصوبة و الحقل حيث تم تقييم صنفين من الكاسافا (الإندونيسى و البرازيلى) باستخدام ثلاثة مستويات عدوى لنيماتودا تعقد الجذور و كذلك تمت مقاومة النيماتودا باستخدام بعض المعاملات الحيوية . كما تم دراسة سلوك التذبذب الموسمى لنيماتودا ميلودوجين إنكوجنيتا كعدوى طبيعية على صنفى الكاسافا(الإندونيسى و البرازيلى) من شهر يناير إلى ديسمبر ٢٠٠٨ حيث أظهرت النتائج أن تعداد نيماتودا تعقد الجذور فى كلا الصنفين يزداد خلال العام خاصة خلال شهور يوليو وأغسطس وسبتمبر وينخفض من شهر نوفمبر إلى ديسمبر حيث تذبذب أعداد النيماتودا في ترب الصنفين و زاد من ٤٢٠ فرد/٢٥٠ جرام تربة للبرازيلى و من ١٨٠ فرد/٢٥٠ جرام تربة للاندونيسى فى يناير ٢٠٠٨ كتعداد بدائى الى ٣٢٢٠ او ٢١٠٠ فرد/٢٥٠ جرام تربة لكلا الصنفين على التوالى فى أغسطس ٢٠٠٨ ثم إنخفض إلى ٦٤٠ أو ٤٢٠ فرد/٢٥٠ جرام تربة فى ديسمبر ٢٠٠٨ عند درجة حرارة ١٧± ٤ ^{o}م على التوالى.

تم اختبار تقييم صنفين من الكاسافا صنفى (برازيلى وإندونيسى) بثلاثة مستويات عدوى من النيماتودا.أظهر الصنف البرازيلى قابلية عالية للإصابة عن الصنف الإندونيسى خاصة عند المستوى العالى من النيماتودا وذلك بالنسبة لتعداد النيماتودا فى الجذور (الأطوار اليرقية الغير مكتملة و الإناث و أكياس البيض و البيض) و كذلك التعداد فى التربة. كما أوضحت النتائج حدوث نقص فى النسبة المئوية لوزن النباتات الكلية فى الصنف البرازيلى عند مستوى ٣٠٠٠ يرقة نيماتودية وصلت إلى ٨٨.٦% بينما أظهر الصنف الإندونيسى عند مستوى ١٠٠٠ يرقة نيماتودية وصلت إلى ٢٦.٢% من النسبة المئوية لوزن النباتات الكلية.

تم دراسة تأثير إستخدام أربعة معاملات حيوية من (بكتريا السيدومونس فلوروسنس – فطر تريكودرما فيريدى – بكتريا باسيلس سيرنجينزس– فطر مايروسيكم فيروكاريا) بالمقارنة بمبيد النيماثورين ١٠% محبب على تعداد نيماتودا تعقد الجذور (ميلودوجين إنكوجنيتا) . أظهر إستخدام كل من فطر مايروسيكم فيروكاريا خاصة عند التركيز الأعلى و مبيد النيماثورين ١٠% محبب أعلى كفاءة فى خفض تعداد النيماتودا فى كل من التربة والجذور (الأطوار الغير مكتملة – الإناث– أكياس البيض و البيض)بالمقارنة بباقى المعاملات. أعطت كل من معاملة بكتريا السيدومونس فلوروسنس و بكتريا باسيلس سيرنجينزس كفاءة متوسطة فى خفض تعداد النيماتودا بينما كانت المعاملة بفطر تريكودرما فيريدى أقل المعاملات كفاءة فى خفض تعداد النيماتودا فى كل من التربة و الجذور. كما أظهر إستخدام كل من مبيد النيماثورين ١٠% محبب و فطر مايروسيكم فيروكاريا خاصة عند التركيز الأعلى زيادة فى النسبة المئوية الكلية لوزن النباتات الكلية وصلت إلى(٨٧.٢ و ٧٢.١%) على التوالى بينما كانت الزيادة فى النسبة المئوية الكلية لوزن النباتات الكلية للمعاملة بفطر تريكودرما فيريدى (٤٢.٥%) عند التركيز الأقل. أظهرت النتائج أيضا أن هناك علاقة طردية بين كل من السكريات الكلية و المختزلة والفينولات و الزيادة فى تركيز المعاملات بينما إنخفضت الأحماض الأمينية بزيادة التركيز فى المعاملات المستخدمة.