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EFFICACY OF SOME BIOAGENTS, CHITOSAN COMPOUNDS AND ORANGE PEELS EXTRACT IN CONTROLLING *MELOIDOGYNE HAPLA* ON STRAWBERRY IN EGYPT

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ABSTRACT: ***Five treatments (Bacillus subtilis, Trichoderma harzianum, chitocar product, soft guard and orange peels extract) with different concentrations were used to control root-knot nematode, Meloidogyne hapla under laboratory as well as greenhouse and field conditions on strawberry. Also, application number of the treatments were studied under both greenhouse and field conditions.***

The most effective treatment in controlling root-knot nematode was soft guard, while Bacillus subtilis, Trichoderma harzianum, chitocar product occupied an intermediate position in the effectiveness whereas, the least effective one was orange peels extract under both laboratory and greenhouse conditions. So the treatment of orange peels extract was neglected under field conditions. The most effective treatment under field conditions in controlling Meloidogyne hapla was soft guard, whereas the least effective was Trichoderma harzianum.

Under laboratory conditions the five treatments were used to study their effect on percentage of juveniles mortality. All treatments led to high percentage of juvenile mortality especially at the highest concentration and after 72 hours exposure.

Under greenhouse conditions using soft guard and Bacillus subtilis were more effective in reducing numbers of developmental stages, females, galls, egg-masses and eggs/ egg-mass as well as number of 2 nd stage larvae in soil, whereas, the least effective was orange peels extract.

Adding the treatments as soil drench led to increase the fresh weights of both the root and shoot system of strawberry seedlings at all used concentrations especially at the highest concentration. Using three times of addition from each treatment achieved high decrease in nematodes in both roots and soil.

Also, all the treatments individually as soil drench decreased the number of nematodes in both roots and soil. In addition the fruit yield of strawberry

increased after adding the treatments at all three times and at high concentration under field conditions.

Key Words: ***Non chemical control, root-knot nematodes, Meloidogyne hapla, Bacillus subtilis, Trichoderma harzianum, chitocar product, soft guard, orange peels extract and strawberry.***

INTRODUCTION

Strawberry (*Fragaria* × *ananassa L*.), is one of the most economic crops in Egypt. Several species of root- knot nematodes, *Meloidogyne* spp., have been recognized as a potentially serious problem to the crop productivity. (Annonymus, 1986) indicated that nematodes are a particular problem in strawberry crop in some countries, severe nematode infestations force grape growers to destroy strawberry plants and let the land lie fallow for many years before replanting.

In recent years, the awareness of the nematicides hazards to human and environment has directed the attention towards soil-borne antagonists as an alternative method to chemical control.

Biological control is gaining increasing role throughout the world for nematode suppression.

***Bacillus subtilis* is reported as a bio-control agent against root-knot nematodes. Nematode mortality was observed from 8 hours incubation. A concentration of at least 10^{8} cfu / ml were necessary to cause nematode mortality higher than 30%. *Bacillus subtilis* act through production of number of antibiotic as bacterocin (Ferreiro *et al*., 1991; Asaka and Shoda, 1996; Farahat, 1998; Khan *et al*., 2002 and Shawky & Abd El- Moneim, 2005).**

***Trichoderma harzianum* act through different mechanisms including mycoparasitism, also through production of antibiotic substances (Abd El Moity & shatla, 1981and Banhamoud & Chet, 1993). *Trichoderma harzianum* also act through production of destructive enzymes i.e, chitenase (Padares *et al*., 1992 ; Bolar *et al*., 2000 ; Sharon *et. al.* ,2001 ; Faruk *et al.* , 2002; Siddiqui and Shaukat., 2004 ; Shawky & Abd El- Moneim, 2005 and Sahebani & Hadavi ,2008). Abd El-Moity *et al.* (1998) said that application of *Bacillus subtilis* and *Trichoderma harzianum* to nematode infested soil twice was always more effective compared with single treatment.**

Loumedjiinon *et al.* (2007) used dried skin peels of orange to control root-knot nematode (RKN), *Meloidogyne spp*. in *solanum macroatponl* under field condition. They found significantly reduction in root galling and nematode soil population density and increasing in plant biomass as compared to control. Tsai (2008) stated that the extract of fresh orange peels showed significantly nematostatic effect against *M. incognita* second stage juveniles after 48 h of treatment. The nematicidal activity was very low in the extracts of fresh peels but was greatly enhanced in the extracts of stored extract peels with 93.5% mortality of nematodes. He explained that by the possibility that essential oils from the citrus peels might have released in the extract

during storage of the orange peels. He also mentioned that infection of *M. incognita* second stage juveniles on mungbean root was significantly inhibited by the extracts of the refrigerator-stored peels of orange.

(Mulawarman *et al*. 2001) said that chitosan compound is a natural product stimulate microbial activity in the soil and promote plant growth. They found positive effects of natural products in stimulating soil microbial activity and thereby the antagonistic potential in soils leading to a reduction in nematode infestation and improved plant growth.

(Vasyukova *et al.* 2001) stated that chitosan play an elicitor activity by inducing the local and systemic of resistance inside plant tissues against different pathogens and nematodes. They added that chitosan induced the accumulation of phytoalexins in tissues of host plants; decreased the total content; changed the composition of free sterols producing adverse effects on infesters; activated chitinases, -glucanases, and lipoxygenases; and stimulated the generation of reactive oxygen species.

(Hallmann *et al* 1999) said that the addition of chitin to soil at 1% (w/w) eliminated plant-parasitic nematodes in a first planting of cotton cv. 'Rowden' and significantly reduced *Meloidogyne incognita* infestation in a second planting, confirming long-term nematode suppressiveness induced by this organic amendment. They found that chitin amendment was associated with an increase in fungal and bacterial populations, especially those with chitinolytic activity.

The present work was carried out to study the efficacy of some bioagents, chitosan compound and extract of orange peels in controlling *Meloidogyne hapla* under laboratory, greenhouse and field conditions

MATERIALS AND METHODS

In this study five different treatments were used. These treatments included:-

1- *Bacillus subtilis* + Nematode (N).
2- *Trichoderma harzianum* + N.
3- Soft guard compound as a commercial product + N.
4- Chitocare compound as a commercial product + N.
5- Orange peels extract + N.
6- Check treatment with nematode only

All treatments were prepared in Central Laboratory of Organic Agriculture, Agricultural Research, Center, Giza.

1- Preparation of the treatments:-

A- Bacteria:-

***Bacillus subtilis* was grown on nutrant glucose broth (NG) for 48 hours. The bacterial suspension was adjusted to be containing (30 x 10^6) cells /ml (Dowson, 1957).**

B- Fungi:-

Trichoderma harzianum **was grown in gliotoxin fermentation media (GFM) under complete darkness just to stimulate toxin production (Abd El- Moity and Shatla, 1981) for 9 days. The suspension of *T. harzianum* was prepared by adjusting the number of *T. harzianum* propagules, in the suspension to be (30 x 10^6) cfu /ml using sterilized distilled water.**

C- Soft guard:-

It contains chitosan oligo Saccharin (molecular weight ≤ 3000) .Importer: Technogreen Group for agricultural production .Exporter: Leili; Agro Chemistry CO. LTD; China.Registration No. 4585. Application rates: for watering dilute 1000 times.

D- Chitocar product:-

It contains chitosan oligomers, and cheleated elements: Fe, Zn, Mn, Cu, B and NPK. Sentic Company. Application rates: for watering dilute 1000 times.

E- Orange peels:-

Extract of orange peels was prepared by mixing 10 g of dry powder orange peels with 100 ml of water using electric blender for 5 minutes.

I- Laboratory experiment:-

Efficacy of some bioagents, chitosan compounds and orange peels extract treatments against active nematode juveniles under laboratory conditions:-

To test the efficacy of the five different treatments were used in inhibiting the activity of *M. hapla* juveniles *in vitro*, 1 ml of each (*Bacillus subtilis*, containing (30 x 10^6) cells /ml. , *Trichoderma harzianum* containing (30 x 10^6) cfu /ml were added at three concentrations of(1:50, 1:100, 1:150) and I ml. of each (chitocar product , soft guard and orange peels extract) were added at three concentrations of (1:500, 1:800, 1:1000) to 1 ml of nematode suspension containing (200 active juveniles of *M. hapla*) in glass vials and compared with check treatment of 1 ml distilled water containing (200 active juveniles of *M. hapla*). The glass vials were examined after 24, 48 and 72 hours from exposure. The numbers of active and non active juveniles were counted microscopically. New blue R (0.05% aqueous solution), potassium permanganate (0.062-0.50% KMnO4 in aqueous solution) and chrysoidin have been used to stain dead nematodes (Barker *et. al*. 1985).

II- Greenhouse experiments:-

1- Evaluation the different concentrations of some bioagents, chitosan compounds and orange peels extract treatments to control *M. hapla* under greenhouse conditions:-

In all greenhouse experiments nematodes inoculation procedure were prepared as

Nematodes inoculation procedure:-

After strawberry seedlings (*Fragaria x ananassa L.*) Camarosa variety, were transplanted individually in 20 cm diameter clay pots each containing 2.5 Kg steam –sterilized soil. (1:2) loam and sand respectively, each pot was inoculated with 3000 newly hatched juveniles from pure culture of *M. hapla* by making 3 holes at different depths (2-3 cm) around the roots and immediately after inoculation the roots were covered with soil.

One week after inoculation different treatment which include *Bacillus subtilis*, *Trichoderma harzianum*, chitocar product, soft guard and orange peels extract were added. The five different treatments were prepared by diluting (1:50, 1:100, 1:150) for *Bacillus subtilis*, *Trichoderma harzianum* and orange peels extract and by diluted suspensions of (1:500, 1:800, 1:1000) for both chitocar product and soft guard. Pots were treated with nematode active larvae without the treatments served as control treatment.

All treatments received the same agricultural treatment such as amount of water, number of seedling /pot and amount of fertilizers. All pots were arranged in completely randomized design, and kept under greenhouse conditions.

2-Effect of soil drench application number of different treatments to control *M. hapla* under greenhouse conditions:-

This experiment was conducted to determine the effect of application numbers of the previous five treatments in controlling *M. hapla* under greenhouse conditions.

All inoculated strawberry seedlings were divided into four groups.

1- The first group received one time of 100 ml/kg. soil from the highest concentrations of the different treatments.
2- The second group received two times of 100 ml/kg. soil from the highest concentrations of the different treatments.
3- The third group received three times of 100 ml/kg. soil from the highest concentrations of the different treatments.
4- The fourth group received nematode only as control.

In all greenhouse experiments, after 60 days, all plants were carefully uprooted. Root and shoot systems were weighted. Nematode populations in soil and roots were recorded according to (Franklin & Goodey, 1957). Eggs of *M. hapla* were extracted from galls of strawberry roots using sodium hypochoride (NaOCl) method as described by Husssey and Baker (1973).

III- Field experiments:-

Evaluation the different treatments in controlling *M. hapla* under field conditions:-

This experiment was conducted in sandy loamy naturally infested soil with *M. hapla* to determine the effect of numbers of application of four

treatments (*B. subtilis*, *T. harzianum*, chitocar product and soft guard) to control *M. hapla* under field conditions at the high concentration of (1:50) for *B. subtilis* and *T. harzianum*, and the concentration of (1:500) for both chitocar product and soft guard. At the end of the experiment nematodes in both soil and root were determined. Also, the totally fruit yield were determined. Each treatment include 10 replicates and every replicate contains 100 plants

Statistical Analysis:-

All obtained data were subjected to statistical analysis proposed by Gomez and Gomez (1984)

RESULTS AND DISCUSSION

I- Laboratory experiment:-

Efficacy of some treatments against active nematode juveniles under laboratory conditions:-

Data in Table (1) illustrated that the five tested treatments (*B. subtilis*, *T. harzianum*, chitocar product, soft guard and orange peels extract) had various degrees of effectiveness toward the nematode juveniles. Moreover, the percentage of mortality increased with increase of the treatments concentration and exposure period.

The highest concentration of (1:50) for *B. subtilis*, *T. harzianum* and orange peels extract and the concentration of (1:500) for both chitocar product and soft guard in all the tested treatments achieved the highest percentage of juvenile mortality during all exposure periods.

After 24 hours, the mortality caused by all used treatments (*B. subtilis*, *T. harzianum*, chitocar product, soft guard and orange peels extract ranged between 23.5 to 87.3% compared with control of mortality % (0.4 %). Also, after 72 hours at the concentration (1:50) the highest percentage of mortality % caused by soft guard was (95.4%) while the lowest percentage of mortality caused by orange peels extract was (57.2 %) compared with control of (1.5%). *B. subtilis* was occupied the second rank of effectiveness on mortality % by (92.5%). Chitocar product was occupied the third rank of effectiveness on mortality % by (87.2%) followed by *T. harzianum* treatment which achieved a percentage of mortality of (70.1%).

***Bacillus subtilis* acts through production of number of antibiotics (Ferreiro, 1991; Asaka and Shoda, 1996; Abd El Moity *et. al.*, 1998 and Farahat., 1998). *Bacillus subtilis* can grow and multiplicate very fast under this circumstance.**

***Trichoderm harzianum* act through different mechanisms including mycoparasitism, also through production of antibiotic substances (Abd El Moity & shatla, 1981 and Banhamoud & Chet, 1993). *Trichoderma harzianum* also act through production of destructive enzymes i.e., chitenase (Padares *et al.*, 1992 ; Bolar *et al.*, 2000).**

Table (1): Effect of exposure period to some treatments on mortality percentage of *M. hapla* juveniles under laboratory conditions.

Treatments	Conc.	Mortality %		
		Exposure period (in hours)		
		24	48	72
Bacillus subtilis + N	1:150	50.7	55.2	68.5
	1:100	71.9	75.6	81.4
	1:50	83.5	88.3	92.5
Trichoderma harzianum + N	1:150	35.2	38.5	46.8
	1:100	48.9	55.7	64.2
	1:50	55.5	62.8	70.1
Soft guard + N	1:1000	57.2	64.9	79.3
	1:800	76.9	82.5	85.8
	1:500	87.3	91.7	95.4
Chitocar product + N	1:1000	46.6	50.4	69.2
	1:800	61.3	68.2	75.1
	1:500	74.4	81.6	87.2
Orange peels extract + N	1:150	23.5	26.9	35.6
	1:100	28.7	33.8	38.2
	1:50	41.3	46.7	57.2
Nematodes in distilled water		0.4	1.1	1.5
L.S.D. (5 %)		2.11	2.23	3.56

Loumedjiinon *et al* (2007) used dried skin peels of orange to control root-knot nematode (RKN), *Meloidogyne spp*. in *solanum macroatponl* under field conditions. Data obtained showed that significantly reduction in number of galls and nematode population density in soil and increased plant biomass as compared to control. Tsai (2008) stated that the extract of fresh peels of orange showed significantly nematostatic effect against *M. incognita* second stage Juveniles after 48 h treatment. The nematicidal activity was very low in the extracts of fresh peels but was greatly enhanced in the extracts of stored orange peels with 93.5% mortality of nematodes. (Hallmann *et al* 1999) said that addition of chitin to soil at 1% (w/w) eliminated plant-parasitic nematodes in a first planting of cotton cv. 'Rowden' and significantly reduced *M. incognita* infestation in a second planting, confirming long-term nematode supressiveness induced by this organic amendment. The chitin amendment was associated with an increase in fungal and bacterial populations, especially those with chitinolytic activity.

II- Greenhouse experiments:-

1- Evaluation the different concentrations of some bioagents, chitosan compounds and orange peels extract treatments to control *M. hapla* under greenhouse conditions:-

1- Effect on nematode population:-

a- Number of root galls:-

However all the treatments showed remarkable decrease of the number of root galls Fig.(1). Soft guard performed the highest reduction of galls number compared with the other treatments. *B. subtilis* ranked on the second place, whereas orange peels extract resulted in the lowest reduction of root galling. *T. harzianum* and chitocar product occupied an intermediate position in decreasing the root galling. Increase the concentration of soft guard from (1:1000) to (1:500) resulted in decrease the root galling from 46 to 29 galls/root system. Similar decrease was found in the case of all the other treatments.

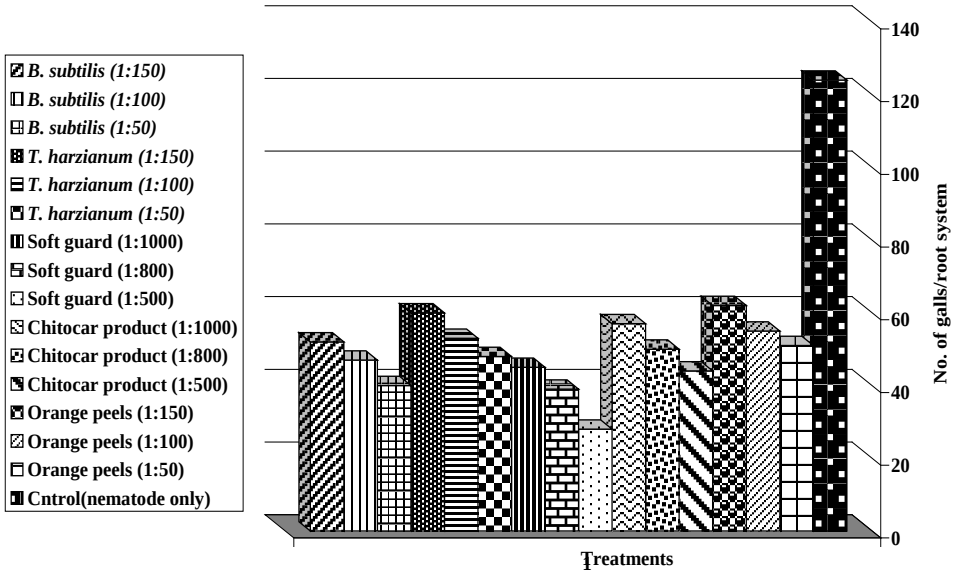

Fig. (1): Effect of some bioagents, chitosan compounds and orange peels extract treatments on number of galls /root of strawberry plants infected by *M. hapla* under greenhouse conditions.

b- Number of juveniles in soil, developmental stages, females, egg masses ,eggs numbers and rate of build up of *M. hapla* :-

Data in Table (2) illustrated that all tested treatments were effective in decrease the final nematode population and rate of build up of root knot nematode in both soil and roots especially at the highest concentrations. Soft guard was the most effective treatment whereas the least effective treatment one was orange peels extract. *B. subtilis*, *T. harzianum*, and chitocar product occupied an intermediate position. Also, data showed that positive correlation between efficacy of the treatments and concentrations.

The same trend obtained with the effect on juveniles in soil, and developmental stages, females, egg -masses and eggs numbers in roots.

Table (2): Efficacy of some bioagents, chitosan compounds and orange peels extract treatments on reproduction of *M. hapla* infecting strawberry plants under greenhouse conditions.

Treatments	Conc.	Nematode population in					*Final nematode population (PF)	Rate of build- up (PF/PI)
		No. of juveniles in 250g soil	Root					
			No. of developmental stages	No. of females	No. of egg-masses	No. of eggs/ egg-mass		
Bacillus subtilis + N	1:150	180	63	52	48	341	16663	5.55
	1:100	120	57	47	41	335	13959	4.65
	1:50	100	50	40	37	317	11919	3.97
Trichoderma harzianum +N	1:150	340	71	60	55	360	20271	6.75
	1:100	300	64	53	50	345	17667	5.88
	1:50	220	58	48	42	333	14312	4.77
Soft Guard + N	1:1000	140	58	45	40	320	13043	4.34
	1:800	100	51	39	35	308	10970	3.65
	1:500	80	45	28	26	295	7823	2.60
Chitocar Product + N	1:1000	260	66	57	54	352	19391	6.46
	1:800	200	60	50	47	340	16290	5.43
	1:500	140	52	44	40	331	13476	4.49
Orange Peels + N extract	1:150	400	73	62	60	380	23335	7.78
	1:100	320	67	55	52	351	18684	6.23
	1:50	280	62	51	49	343	17200	5.73
Control (nematodes only) (N)		2400	112	124	119	430	53806	17.93
L.S.D. (5 %)		18.36	1.23	1.54	1.12	11.87	465.61	0.23

Each value presented the mean of four 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.**

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

All data are in agreement with those obtained by Hanna *et. al*. (1999) who mentioned that *B. subtilis* was effective against root-knot nematode *M. incognita* on tomato plants. Also agree with Abd El-Moity, *et. al.* (1998) who mentioned that the most effective in controlling the root-knot nematode was *Bacillus* spp., whereas the least effective was *T. harzianum*. Also, Khan *et. al*.(2002) said that treatment with *B. subtilis* or *Beijerinckia indica* reduced galling by 33-34 % and increased the dry weight of shoots by 22-24 % , respectively. *Trichoderma* spp. can produce various toxin metabolites and different enzymes that improve photolytic activity of the antagonist and control of nematodes. ((Sharon *et. al.*, 2001; Faruk *et. al.*, 2002; Siddiqui and Shawkat,2004 and Shawky & Abd El- Moneim, 2005).

2 - Effect on the vegetative growth of the treated seedlings:-

The effect of the treatments (*B. subtilis*, *T. harzianum*, chitocar product, soft guard and orange peels extract) on fresh weights of the treated plants were illustrated in Fig.(2).

All the treatments enhanced increase in fresh weights of the whole plants compared with control plants. The maximum increasing % at the highest concentration was recorded in treatment with soft guard (223%) followed by *B. subtilis* with (199%), chitocar product (171%) followed by *T. harzianum* with (152%). The treatment of orange peels showed the lowest shoot weight increasing %, which reached to (83 %) compared with the control.

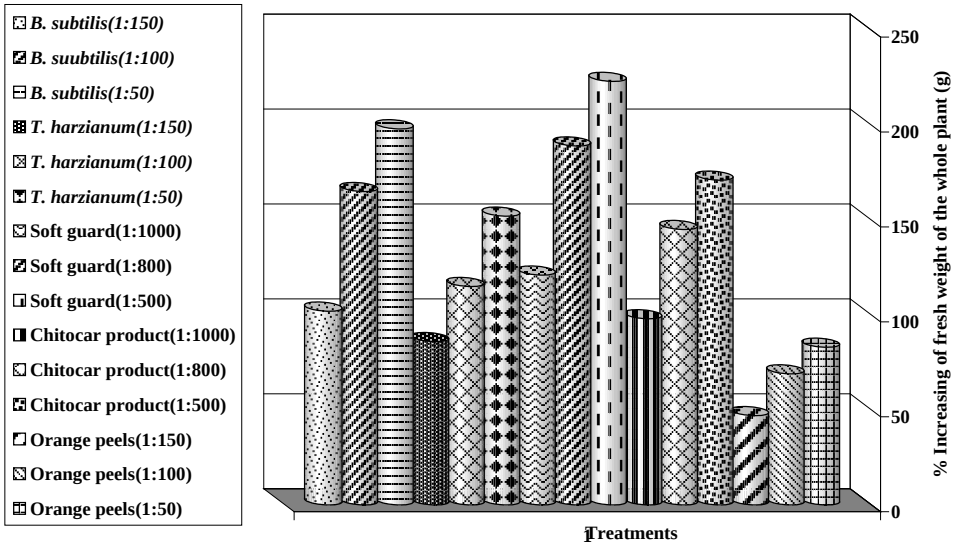

Fig. (2) : Effect of some bioagents, chitosan compounds and orange peels extract treatments on increasing % of fresh weight of the whole plant of strawberry infected by *M. hapla* under greenhouse conditions.

2- Effect of application number of treatments at different preparation to control *M. hapla* on strawberry under greenhouse conditions:-

A -Evaluation the application number on the nematode infecting strawberry plants under greenhouse conditions:-

1- Effect on nematode population:-

a- Number of root galls:-

However all the treatments showed remarkable decrease of the number of root galls Fig. (3), soft guard performed the lowest galls numbers compared

with the other treatments especially after three times of applications at the highest concentration (1:500). *B. subtilis* ranked on the second place, whereas orange peels extract resulted in the lowest decrease in the number of root galls. Both Chitocar product and *T. harzianum* were occupied an intermediate position in decrease number of root galling. The decrease in root galls was affected greatly with the number of applications of different treatments.

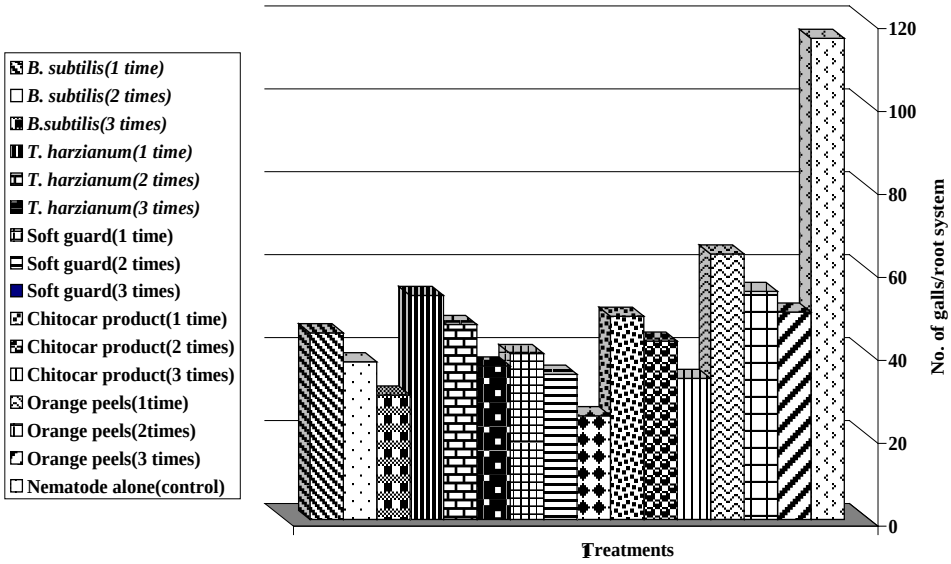

Fig. (3) : Effect of application number of some bioagents, chitosan compounds and orange peels extract treatments on number of galls /root of strawberry plants infected by *M. hapla* under greenhouse conditions.

b- Number of juveniles in soil, developmental stages, females, egg masses ,eggs numbers/ root and rate of build up of *M. hapla* :-

Data in Table (3) showed that all tested treatments were effective in decrease the final nematode population and rate of build up of root knot nematode in both soil and roots especially after three times of application. Soft guard was the most effective treatment, whereas the least effective one was orange peels extract. *B. subtilis*, *T. harzianum* and chitocar product occupied an intermediate position. Also, data showed that positive correlation between efficacy of the treatments and applications number of the treatments.

The same trend obtained with the effect on juveniles in soil, and developmental stages, females, egg -masses and eggs numbers in roots.

Table (3): Effect of application number of some bioagents, chitosan compounds and orange peels extract treatments on reproduction of *M. hapla* infecting strawberry plants under greenhouse conditions.

Treatments	Number of application	Nematode population in						Rate of build-up (PF/PI)
			Root				*Final nematode population (PF)	
		No. of juveniles in 250g soil	No. of developmental stages	No. of females	No. of egg-masses	No. of eggs/ egg-mass		
Bacillus subtilis + N	One time	160	56	45	41	360	15021	5.00
	Two times	120	51	38	35	340	12109	4.03
	Three times	80	43	30	28	310	8833	2.94
Trichoderma harzianum + N	One time	280	65	54	50	385	19649	6.55
	Two times	240	59	47	45	375	17221	5.72
	Three times	180	50	37	34	354	12303	4.10
Soft guard + N	One time	120	52	40	38	337	13018	4.34
	Two times	100	48	35	32	315	10263	3.42
	Three times	60	40	25	22	280	6285	2.09
Chitocar product + N	One time	200	62	49	46	378	17699	5.90
	Two times	180	57	43	41	366	15286	5.09
	Three times	140	46	34	30	337	10330	3.44
Orange peels extract + N	One time	360	72	64	61	405	25201	8.40
	Two times	300	65	55	51	389	20259	6.75
	Three times	240	54	50	47	373	17875	5.95
Control (nematodes only) (N)		2600	112	116	111	430	50558	16.85
L.S.D. (5 %)		15.86	1.23	0.89	0.81	10.12	415.16	0.06

Each value presented the mean of four 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.**

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

2 - Effect on the vegetative growth of the treated seedlings:-

The effect of number of applications of soil drench of the treatments (*Bacillus subtilis*, *Trichoderma harzainum*, chitocar product, soft guard and orange peels extract) on fresh weight of the treated plants were studied in Fig.(4).

All the treatments enhanced the increase in fresh weight of the whole plants compared with the control. The maximum increasing % after three times of applications was recorded with the seedlings treated with soft guard (255%) followed by *B. subtilis* with (232%), chitocar product (221%) followed by *T. harzianum* with (198%). The treatment of orange peels showed the lowest shoot weight increasing%, which reached (112 %) compared with control.

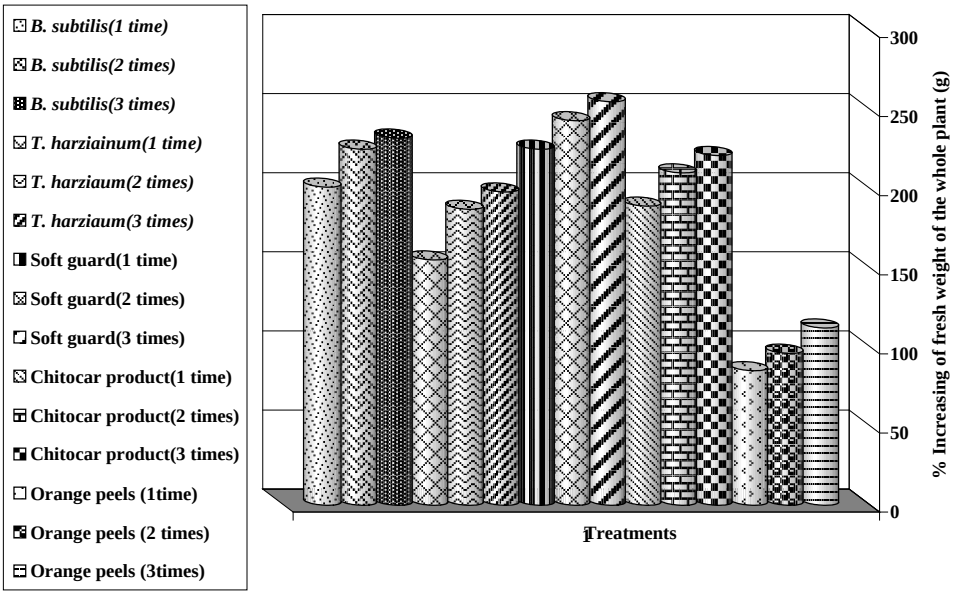

Fig. (4) : Effect of applications number of some bioagents, chitosan compounds and orange peels extract treatments on increasing of fresh weight of the whole plant of strawberry infected by *M. hapla* under greenhouse conditions.

III- Field experiments:-

Effect of some bioagents and chitosan compounds treatments to controlling *M. hapla* in naturally infecting strawberry plants under field conditions:-

1- Number of root galls:-

However all the treatments showed remarkable reduction of the number of root galls as shown in Table (4), the soft guard performed the lowest galls numbers as reached to 24 galls/root compared with the other treatments at (1:500) with three times of application. *B. subtilus* ranked on the second place resulting in reduce galls to28 galls/root, whereas *T. harzianum* resulted in the lowest reduction in the number of root galls reached to35 galls/root. Chitocar product occupied an intermediate position in decrease number of galls/ root reached to31 galls/root in compared with the control 113 galls/root.

2- Number of juveniles in soil, developmental stages, females, egg masses and eggs numbers in roots:-

The same trend on number of root galls also was obtained with the effect on juveniles in soil, developmental stages, females, egg -masses and eggs numbers.

Table (4): Efficacy of some bioagents and chitosan compounds treatments on reproduction *M. hapla* in naturally infecting strawberry plants under field conditions.

Treatments	Nematode population in					
	No. of juveniles in 250g soil	Root				
		No. of galls	No. of developmental stages	No. of females	No. of egg-masses	No. of eggs/ egg-mass
Bacillus subtilis + N	100	28	45	31	29	330
Trichoderma Harzianum + N	200	35	53	38	36	372
Soft Guard +N	80	24	42	26	24	310
Chitocar product + N	160	31	48	35	30	356
Control (nematodes only) (N)	2800	113	117	118	117	460
L.S.D. (5 %)	18.91	3.22	2.33	2.75	1.71	18.32

Data in Fig.(5) illustrated the effect of the treatments on fruit yield weights of strawberry after treatments under field conditions. All the treatments showed remarkable increasing in yield weights. Using soft guard was the highest effective treatment in the increasing in fruit yield weights as reached to 8640 Kg/F. While, *T. harzianum* was the lowest effective one to increase fruit yield as reached to

7920 Kg/ F.

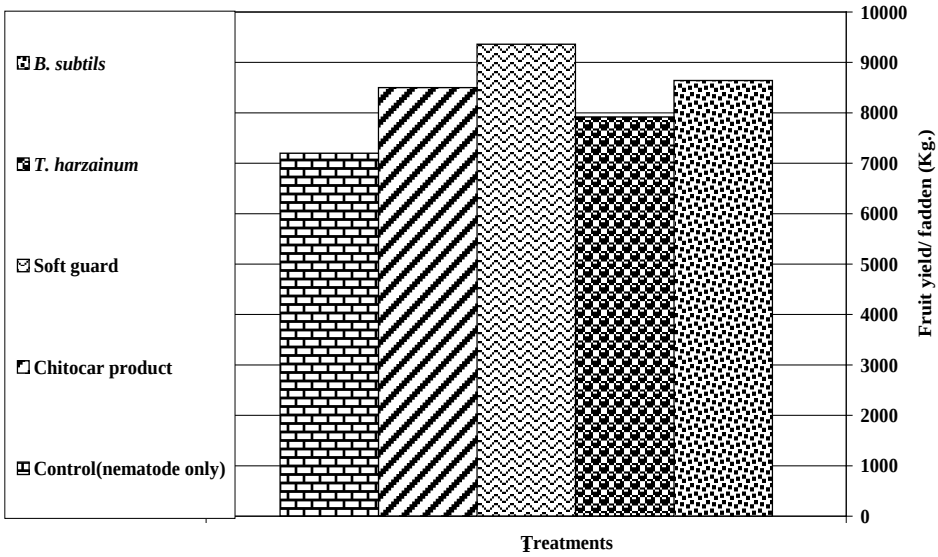

Fig. (5): Effect of some bioagents and chitosan compounds on fruit yield weight of strawberry infected naturally by *M. hapla* under field conditions.

Conclusion:-

From the above mentioned results, it can be concluded that soft guard compound was the most effective treatment; whereas *T. harzianum* was the lowest effective one three times soil drenching in comparing with control to control *M. hapla* under both greenhouse and field conditions and improved fruit yield of strawberry under field conditions.

REFERANCES

Abd El-Moity, T. H., M. H. El-Hamawi, T. El-Sharkawy and K. Tiilikkala (1998). Effect of some biologica agents on reproduction of *Meloidogyne incognita* on tomato plants. Egyptian journal of Agricultural Research. 76(1): 51-62.

Abd El- Moity, T. H. and M. N. Shatla (1981). Biological control of white disease of onion *Sclerotium cepivorum by Trichodermn harzianum*. Phytopathology (4):1187-1194.

Anonymous (1986). Manual for field trail in plant protection. Edited and Published By Werner Puntener, Agricultural Divsion, Ciba-Gaigy Limited, Basle, Switzerland.

Asaka, O. and M. Shoda (1996). Biocontrol of *Rhizoctonia solani* damping off of tomato with *Bacillus subtilis* Rb14. Applied and Environmental Microbiology , 62 (11) : 4081-4085 .

Barker, K. R., C. C. Carter and J. N. Sasser *(*1985). An advanced treatise on *Meloidogyne* Vol.2 Methodology pag. 41

Banhamoud, W. and I. Chet (1993). Hyphal interaction between *Trichoderma harzianum* and *Rhizoctonia solani* ultrastructure and 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 .

Dowson, W. G. (1957). Plant diseases due to bacteria. Second Ed., Cambridge University, Press., London, pp. 231.

Farahat, A. (1998). Biological control of some potato bacterial diseases. PHD. Thesis, Fac. Agric. Monofia Univ. PP118.

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: (1), 33-37,

Ferreiro, J. H. S., F.N. Matthee and A. C. Thomas (1991). Biological control of Eutypalota on grapevine by an antagonistic strain of *Bacillus subtilis*. 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 and ED. John Wiley and sons ltd , New York 680P.

Hallmann, J., R. Rodriguez-Kabana and J.W. Kloepper (1999). Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. Soil Biology and Biochemistry volume 31(4): 551-560.

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.

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.

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.

Loumedjiinon, S., I. Godonou, C. Atcha-Ahowe, B. James, H. Baimey, D. Coyne and A. Ahanchede (2007). Mangement of Root-Knot nematodes on *solanum macrocarpon* using Botanicals in Benin.ISHS Acta Horticulturae 752:1 International conference on indigenous vegetables and legumes. Prospects for fighting porerty ttunger and malnutrition.

Mulawarman, J., D. Hallmann, B. Bell, Kopp-Holtwiesche and R. A. Sikora (2001). Effect of natural products on soil organisms and plant health enhancement. Meded Rijksuniv Gentfak Landbouwkd Toegep Biol wet.; 66(2b): 609-617.

Padares, D. F., J. Hockenbull, D. F. Jensen and S. B. Mathus (1992). In vivo screening of *Trichoderma* isolates for antugonism against *Sclerotium rolfsii* using rice seedlings. Bulletin oil 15 (1) : 33-35 . (C.P. Rev. PL . Pathol. , vol . 72 No. 1, 208

Sahebani N. and N. Hadavi (2008). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum.* Journal of Soil Biology &Biochemistry, 40: 2016-2020.

Sharon, E., M. Bar-Eyal, I. Chet, A. O. Herrera – Estrella, Kleifeld and Y. Spiegel (2001).Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum.*Phytopathology.,91:7, 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., 30(6): 1685- 1708.

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 fluorescens* in tomato. Letters in Applied Microbiology ,38(2): 169-175.

Tsai, B.Y. (2008). Effect of peels of lemon, orange, and grapefruits against *Meloidogyne incognita*. Plant Pathology Bulletin 17: 195-201.

Vasyukova, N. I., S. V. Zinova, L. Ilinskaya, E. A. Perkhod, G. I. Chalenko, N. G. Gerasimova, A. V. Ilina, V. P. Varlamova and O. L. Ozeretskovskaya (2001). Modulation of plant resistance to diseases by water-soluble chitosan. Applied Biochemistry and Microbiology volume 37, Number 1,103-109

كفاءة استخدام بعض الكائنات الحية و مركبات الشيتوزان و مستخلص قشر البرتقال في مكافحة نيماتودا ميلودوجين هابلا على الفراولة المصابة بمرض تعقد الجذور في مصر

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الملخص العربي

تم اختبار خمسة معاملات و هي : بكتريا باسيلس ساتلس و فطر تريكودرما هارزيانم ومركب شيتوكير و مركب السوفت جارد ومستخلص قشر البرتقال. استخدمت ثلاثة تركيزات مختلفة من المعاملات لمقاومة نيماتودا تعقد الجذور من النوع ميلودوجين هابلا في المعمل و الصوبة و الحقل على شتلات الفراولة (صنف كاماروزا). كذلك تم دراسة تأثير عدد مرات الاضافة من المعاملات على مقاومة نيماتودا تعقد الجذور تحت ظروف الصوبة.

كانت اكثرالمعاملات تأثيرا تحت ظروف كلا من المعمل و الصوبة في مقاومة نيماتودا تعقد الجذور هو مركب السوفت جارد بينما كانت المعاملة بمستخلص قشر البرتقال اقلهم تأثيرا لذلك تم استبعادها في التجربة الحقلية. أظهرت المعاملة بالسوفت جارد أعلى كفاءة في مقاومة نيماتودا تعقد الجذور بينما كانت المعاملة بفطر تريكودرما هارزيانم اقلهم كفاءة تحت ظروف الحقل.

تحت ظروف المعمل أظهرت جميع المعاملات كفاءة في زيادة نسبة موت اليرقات خاصة عند التركيزات العالية و بعد ٧٢ ساعة.

اظهر استخدام كلا من المعاملتين السوفت جارد و بكتريا باسيلس ساتلس أعلى كفاءة في خفض أعداد العقد النيماتودية و الإناث و الأطوار الغير مكتملة النمو و كتل البيض و عدد البيض على النبات الواحد في الجذور و كذلك تعداد اليرقات من العمر الثاني في التربة بينما كانت المعاملة بمستخلص قشر البرتقال اقلهم تأثيرا تحت ظروف الصوبة.

أظهرت جميع المعاملات المستخدمة تحت ظروف الصوبة زيادة في الوزن الخضري سواء المجموع الخضري أو الجذري للشتلات خاصة عند التركيزات العالية. أدى استخدام الجرعات العالية بمعدل ثلاثة مرات إلى كفاءة عالية في خفض تعداد النيماتودا في كلا من التربة والجذور.

أظهرت جميع المعاملات تحت ظروف الحقل كفاءة فى خفض تعداد النيماتودا فى كلا من التربة و الجذور بالاضافة الى زيادة في وزن محصول الفراولة عند إضافة المعاملات ثلاثة مرات كل اسبوع باستخدام التركيز العالي.