Journal

J. Biol. Chem. Environ. Sci., 2009, Vol. 4(1): 445-461
www.acepsag.org

INTEGRATING SOME BIOCONTROL AGENTS ALONG WITH AGRISPON AND FURADAN (CARBOFURAN) TO CONTROL ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* KOFOID &WHITE (CHITWOOD) INFESTING SUGAR BEET CROP IN NUBARIYA

Gohar, I.M.A.[(1)]; K.M. Agami[(2)] and M.M. Abd-El Rahman[(3)]

[1] *Department of. Sugar Crops diseases& Pests. Sugar Crops Research Institute, Agricultural Research Center, 12619, Giza, Egypt*
[2] *Department of Agricultural Practices, Sugar Crops Research Institute, Agricultural Research Center, 12619, Giza, Egypt*
[3] *Department of Agricultural Physiology and Chemistry, Sugar Crops Research Institute, Agricultural Research Center, 12619, Giza, Egypt*

ABSTRACT

Two experiments were carried out in 2006/2007 and 2007/2008 seasons at West Nubariya region to study the effect of two fungal bioagents along with agrispon as bio-stimulant (bioregulator) and (carbofuran 10 % G) against root knot nematode *Meloidogyne incognita* infesting sugar beet under field conditions. Bioagents viz., *Trichoderma viride* and *Verticillium chlamydosporium* alone or in combination with agrispon and carbofuran 10 % G promoted plant growth, reduced number of galls/plant, egg masses/root system and number of juveniles/root system and juveniles/200 g soil to extent may be reached in some treatments to 100 % reduction in all aforementioned parameters. The fungal bioagents along with agrispon and nematicide showed the least nematodes build up rate as compared to untreated infested soil. Integration Agrispon as bio-stimulant for plants and micro-organisms made enhancement in the soil conditions of sugar beet growth and the bioagents at the same time, which improved quantity and quality of sugar beet crop as roots and sugar yields per feddan, sugar content per plant, sucrose and purity %. The present investigation thus, clearly shows the significant performance of the cumulative effect by agrispon, both the bioagents, one as egg parasitic (*V. chlamydosporium*) and the other as toxic (*T. viride*) with the little dosage of carbofuran 10%G as the best materials in reducing the nematode population and improving plant growth with preservation of agricultural environment and its biological balance.

Key words: Integrating, *Meloidogyne incognita*, sugar beet, *Trichoderma viride*, *Verticillium chlamydosporium*, carbofuran, agrispon, biocontrol agents, sucrose, purity, sugar yield.

INTRODUCTION

Currently, sugar beet (*Beta vulgaris* L.) is the second major sugar crop grown in newly reclaimed desert at West Nubariya and Al-Bostan regions as well as Al-Fayuom, Kafr El-sheikh and Al-Dakahlyia governorates. The total sugar beet cultivated area reached 257667 feddans with an average tonnage of 18.593 tons i.e., the total sugar beet production is 4790730 tons of sugar beets that contributed to about 30 % of total sugar production in Egypt (Annual Report of Sugar Crops Council, 2008).

Sugarbeet production faces powerful competitiveness lately from other field crops especially wheat, clover, beans and flax because these crops attain superior return soaring that of sugar beet. The decrease of sugar beet return is mainly due to the impact of the pests particularly nematodes on the final population of sugar beet plants in the field (which should be from 30,000 to 40,000 plants/ fed) and negatively affected its quality.

The root-knot nematodes, *Meloidogyne spp.* are economically important pests affecting sugar beet production (Altamn and Thomson, 1971 and Gohar and Maareg, 2005). Previously, soil fumigation was the most reliable technique to control nematodes. Root-knot nematode is now yield limiting due to its continuing loss and restriction of soil fumigants, thereby preventing farmers from growing sugar beet crop profitably. Host plant resistance to root-knot nematode, therefore, may be the most environmentally safe means to alleviate sugar beet production problems, but, the true that sugar beet resistant varieties to root-knot nematodes is still hard to pin down. Beside, nematicides due to their high costs, toxic effect on beneficial soil borne microorganisms and carcinogenic effect on human beings, alternative approaches are practiced mainly through environment-friendly means like biological control agents, organic amendments (Singh and Sitaramaiah, 1966).The present investigation was thus undertaken in West Nubariya, that have wide contaminated soil with plant parasitic nematodes for its suitability to this pest, to attempt an environment-friendly management of root knot nematode infecting

sugar beet fields through integrating potential and compatible management components viz. fungal bioagents (Pandey *et al.*, 2005), oil seed cake (Singh and Sitaramaiah, 1966) and carbofuran (Goswami and Mishra, 1994).

MATERIALS AND METHODS

Materials

Sugar beet (*Beta vulgaris saccarifera* L. var. Chems) is among various varieties evaluated by Sugar Crops Research Institute, characterized, as Sweden polygerm variety.

The root-knot nematode, *Meloidogyne incognita* Kofoid &White (Chitwood) which is the contaminant of the experimental area of sugar beet fields over the two seasons of the study in Nubariya district and genera identification was based on the morphology of adult and larval form as described by (Mai and Lyon 1975). Species of the root-knot nematode were identified on the basis of perineal pattern morphology of the adult females as described by Eisenback *et al.* (1980) and Eisenback (1985).

Fungi, *Trichoderma viride* and *Verticillium chlamydosporium* each obtained from sugar beet fields was prepared on a microscopic glass slide in temporary mounts using methylene blue or a drop of water and examined microscopically for grouping by features characterizing fungal classes. A type culture of each group was sent to Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, Egypt for insuring final identification of isolates.

Agrispon® is a non-toxic plant and mineral liquid extract containing zeatin, triacontanol and other components considered as bio-stimulant (bioregulator) that energizes the soil and makes nutrients more available to plants via enriching soil microorganism's activities (Dubravec *et al.*, 1995).

Carbofuran®, the chemical family, Carbamate has a chemical name as 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate. Pesticide type is insecticide, nematicide and acaricide, Adegbite and Adesiyan (2001).

Primary test, for compatibility of the three components used in present study, i.e. for the bioagents, bio-stimulant and the nematicide was made according to McLean *et al.* (2001) to validate the possibility of making combinations among them.

Methods

Experimental site description:

Field experiment was conducted in 2006/2007 and repeated in 2007/2008 at West Nubariya District that naturally had a contaminated soil with root-knot nematode, *Meloidogyne incognita*, after primitive soil survey to detect the objective nematode. The sites of the two experiments were in the same locality of sandy soil containing distinctly low percent of organic matter (0.37 %), and characterized by relatively low soluble cations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} with values of 2.76, 1.90, 4.35 and 0.82 meq L^{-1}, respectively) and anions (CO_3^{-2}, HCO_3^{-}, CL^{-} and SO_4^{-2} with values of 0.01, 1.71, 7.09 and 1.15 meq L^{-1}, respectively). The soil had electrical conductivity of 0.94 ds/m, and pH 8.05. Also, it had 9.74 % $CaCO_3$, and relatively low N, P and K with values of 32.09, 3.13 and 79.50 ppm, respectively. The soil temperature was with average of 25 ± 5.5 C° at 30 cm depth in the two seasons of the study.

Experimental design:

Completely randomized block design was used. The experimental field for each experiment in the two seasons was (462 m^2), divided into four blocks (replicates). Each replicate included eleven plots (3 m×3.5 m = 10.5 m^2 i.e. 1/400 Fed) with six ridges. Seeds of sugar beet, *Beta vulgaris saccarifera* L. var. Chems were sown on the last week of October in the two studied seasons. Five hills per meter were planted to provide a density of 40,000 plants / fed. The eleven treatments were allotted randomly in each block. With the exception of the treatments, all other agricultural practices for growing sugar beet were done as recommended by Sugar Crops Research Institute for this region.

Fungal mass production:

Each fungus was mass produced in conical flasks containing autoclaved mixture of coarse sand and milled barely (1:1 v/v). After two weeks of incubation at 25 °C, the cultures were washed through 250 and 53 µm sieves to remove the sand and barley and the fungal propagules were collected on a 10 µm sieve. The residue was further washed to remove conidia and hyphal fragments, leaving mainly spores. The inoculum was prepared by suspending the residue in autoclaved tap water and the concentration of spores was estimated in diluted samples using a haemocytometer (De Leij and Kerry, 1991)

and estimated to be 3.5×10^7 ml^{-1} for *T. viride* and 3.5×10^6 ml^{-1} for *V. chlamydosporium*. The production was done using the fungal isolates originated from soil for *T. viride* and from infected eggs of *M. incognita* for *V. chlamydosporium*.

Application of treatments procedure:

By sowing sugar beet seeds directly, a 100 ml suspension was drenched separately from each fungus in a groove that made in the seedbed along the 3 m sown ridge. In another set, soil was drenched with a mixture of both *T. viride* and *V. chlamydosporium* (50 + 50 ml per 3 m ridge) or drenched with 50 ml when any of fungus combined with any of other agents (i.e. *T. viride*, *V. chlamydosporium*, agrispon or/and carbofuran).Carbofuran was applied at 0.25 a.i kg/fed., alone or simultaneously with the half of this dose (0.15 a.i kg/fed.) in combination with any of other agents. Agrispon was applied at the rate of 400 ml/fed., that when applied alone in two equal rates, 200 ml in the previously described groove at sowing time and the second 200 ml at four true leaves stage (about 30- 40 days from sowing date). In the case of combination with the other agents at rate of 300 ml at two equal rates the first (150 ml fed.) at sowing and the second at four true leaves stage. The treatments used were (1) agrispon (Ag) alone at 400 ml/fed., (2) *Trichoderma viride* alone at 24 l/fed. (100 ml/ 3 m ridge), (3) *V. chlamydosporium* alone at 24 l/fed. (100 ml/ 3 m ridge), (4) carbofuran, alone at 0.25 a.i kg/fed., (5) *Trichoderma viride* at 12 l/fed. + agrispon 300 ml/fed (6) *V. chlamydosporium* at 12 l/fed. +agrispon at 300 ml/fed, (7) *Trichoderma viride* at 12 l/fed. + carbofuran at 0.15 a.i kg/fed., (8) *V. chlamydosporium* at 12 l/ fed + carbofuran at 0.15 a.i kg/fed., (9) *Trichoderma viride* at 8 L/fed.+ *V. chlamydosporium* at 8 l/fed +agrispon at 200 ml/fed., (10) *Trichoderma viride* at 8 l/fed.+ *V. chlamydosporium* at 8 l /fed + agrispon at 200 ml/fed + carbofuran at 0.15a.i kg/fed, (11) nematode alone with 100 juveniles per 200 g soil (control). Also, the rest of treatments were allotted at plots with the same average Pi equals to 100 juveniles/ 200 g soil. Four replicates were maintained for each treatment. Observations on number of galls/ plants, number of egg masses/ plant, number of juveniles / 200 g soil by sieving and decanding methods (Barker,1985) and number of juveniles/root system by incubation method according to (Young, 1954). As well as nematode build up rate which was deduced by formula adopted after

Maareg *et al.* (1998), whereas, build up = Total count of nematodes in root and soil / initial population at sowing time.

Also, the following characters of sugar beet yield and quality were determined at harvest (six months from planting). Number of survival plants/ plot was counted for each treatment in all replicates to estimate their number/ fed. A sample of 10 guarded plants represent each treatment in all replicates were collected to determine: root length and diameter (cm), plant weight (g), root and foliage weight (g) /plant, as well as, Total Soluble Solids percentage (TSS %) which was determined using Hand refractometer, sucrose was determined according to the method of Le Docte, 1927, purity % was estimated as it is equal (Sucrose % / TSS %) × 100 and sugar content g /plant was assessed as root weight (g) × sucrose %. Sugar beet plants of each plot were up-rooted, topped, cleaned and weighed to determine root yield in tons/fed. Whereas, sugar yield per feddan was estimated as it is = root yield (ton/ fed) × sucrose % × purity %.

Statistical analysis:

Data of the two seasons were combined for analysis of variance (ANOVA) using MSTAT version 4 (1987), followed by testing significant differences among the means of different treatments by Duncan's Multiple Range Test at 0.05 and 0.01 probability according to Duncan (1955).

RESULTS AND DISCUSSION

Effect on sugar beet root galling:

The analysis of variance for the combined data (the two seasons, 2006/2007 and 2007/2009) illustrated in Table (1) revealed at both P $\leq$0.05 and $\leq$0.01 of significance that the maximum reduction in root galling (100 %) occurred in plots treated with a combined of *Trichoderma viride* plus carbofuran and the same significant value (100 %) took place in plots received a combination of *Trichoderma viride*, *Verticillium chlamydosporium* and carbofuran. Also, the full absence of galling roots was observed in the plots treated with the combination of the two studied bioagents along with agrispon and carbofuran together. The same trend of severe reduction in sugar beet root galling obtained in plots treated with the two bioagents, *T. viride* plus *V. chlamydosporium* (95.7 %), also, with plots received the combination of agrispon plus *T. viride* (97.4 % reduction) and with that of agrispon plus the two bioagents (97.4 %). The solitary

application of any of the two bioagents (*T. viride* or *V. chlamydosporium*) achieved an outstanding reduction as 77.8 and 68.4 %, respectively, in regard to nematicide, carbofuran (83.8 %), Table (1).

Effect on development of females, egg masses, juveniles:

Data reported in Table (1) showed that there were significant variances for the combined data of the two seasons concerning number of females, egg masses, juveniles / root system and juveniles/ 200 g soil, whereas, the treatments of accompanying the *T. viride* with carbofuran, the two bioagents with carbofuran and/or the two bioagents along with carbofuran and agrispon exhibited a distinguished reduction in females estimated to 100 %, followed by agrispon plus the two bioagents (96.1 %), agrispon plus *T. viride* (95.1 %) and the treatment of applying the two bioagents together. Consequently, the same aforementioned treatments achieved the same trend of reduction on the number of eggmasses, juveniles per root system, and juveniles / 200 g soil, ranging from 81.8 for the treatment of applying the bioagent *T. viride* alone up to 100 % with same treatments that attained the same percentage reduction with females. Also, the case with juveniles/root system and juveniles/200 g soil. The second ranked effect on the previous parameters was exhibited by treatments of *T. viride* alone, *V. chlamydosporium* alone, carbofuran alone, the combination of *T. viride* plus *V. chlamydosporium*, agrispon with *T. viride* and agrispon along with *T. viride* plus V. *chlamydosporium* as shown in Table 1.

Effect on nematodes build up rate:

Also, from Table (1), both fungi *T. viride* and *V. chlamydosporium* showed a distinguished reduction on build up rates of *M. incognita* when they were applied singly as they were 80.6 and 70.4 %, respectively as compared with chemical nematicide carbofuran. While, the percentage of build up reduction soared upon the mentioned values whereas, they reached 90.8, 95.9 and 96.9 % in plots received *T. viride* + *V. chlamydosporium,* agrispon + *T. viride* and agrispon + *T. viride* + *V. chlamydosporium,* respectively. On the other hand, 100 % reduction was obtained in plots given *T. viride* + Furadan, *T. viride* + *V. chlamydosporium* + carbofuran and agrispon + *T. viride* + *V. chlamydosporium* + Carbofuran.

Account of the findings presented in the Table (1), it can be noticed that the master key of managing root-knot nematodes in these experiments is due to cumulative effect of the used bioagents along with nematicide, carbofuran and agrispon or in combinations. Data in Table (1) showed that the most effective solitary application whether for root galling reduction or all nematode stages suppression was attributed to carbofuran, *T. viride* and *V. chlamydosporium*. Carbofuran acts directly on the nematodes in the soil thereby, preventing or limiting hatching of eggs and the movement of juveniles into roots. This is in agreement with the works of Di-sanzo (1973), Fadina (1991) and Adegbite and Adesiyan (2001). Also, the two used bioagents may suppress nematodes by different mechanisms led to minimizing the plant damage. *Verticillium* spp. are known to be capable of colonizing the rhizosphere of many crops and this genus has been described as the most important egg parasite of *Heterodera avenae* (Woll.) and *H. schachtii* (Schmidt) (Kerry *et al.*, 1984; De Leij & Kerry, 1991). Also *Trichoderma* spp. are known among the antagonistic fungi against many soil born pathogen, Rao *et al.* (1998).

Contrarily, with the treatment of nematode alone (control) whereas, build up rate reached the maximum (9.8) also, with treatment of agrispon alone (8.1) whereas, all favourable conditions for strapping nematodes build up were available i.e. degree days, susceptible host, relatively longtime of crop maturation (six months) and absence of any control measures. Beside, in the treatment of agrispon alone, agrispon made enhancement for growth of sugar beet plants (Table, 2) even under infestation and this may be prepared plants to sustain and harbored more nematodes (supplementary tolerance). This is coincided with the findings of Shurtleff and Averre (2000) whom stated that the short life cycle of 6 to 8 weeks enables root-knot nematode populations to survive well in the presence of a suitable host and their populations build up to a maximum usually as crops reach maturity. Also, Singh and Khurma (2007) concluded that the adult female of root-knot nematode stays inside the giant cells and continues to feed and produces eggmass in a gelatinous matrix protruding out of the root gall. The eggmasses give rise to infective juveniles (J2) which may infect other uninfected roots of the same plant or migrate and infect the nearby plants.

Table (1): Effect of *Verticillium chlamydosporium, Trichoderma viride* in combination with agrispon/furadan on nematode population, egg masses, juveniles/root, and juvenile in soil, females and build up factor of *Meloidogyne incognita* infesting sugar beet plants (combined analysis of 2006/2007 and 2007/2008 seasons.)

Treatments	No. of galls		No. of females		No. of egg-masses		No. of juveniles				Build up	
	per root	Red. %	per root	Red. %	per root	Red. %	per root	Red. %	200 gm soil	Red. %	Rate	Red. %
Mi (control)	117	0.0	103	0.0	121	0.0	523	0.0	237	0	9.8	0.0
Ag + Mi	88	24.8	93	9.7	79	34.7	443	15.3	191	19.4	8.1	17.3
Tv + Mi	26	77.8	31	69.9	22	81.8	97	81.5	39	83.5	1.9	80.6
Vc + Mi	37	68.4	37	64.1	17	86.0	201	61.6	55	76.8	3.1	70.4
F + Mi	19	83.8	23	77.7	25	79.3	9	98.3	30	87.3	0.9	93.0
Tv + Vc + Mi	5	95.7	7	93.2	3	97.5	3	99.4	48	79.7	0.6	90.8
Tv + Vc + F + Mi	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	.00	100.0
Tv + F + Mi	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0.0	100.0
Ag + Tv + Mi	3	97.4	5	95.1	3	97.5	3	99.4	29	87.8	0.4	95.9
Ag + Tv + Vc + Mi	3	97.4	4	96.1	2	98.3	3	99.4	22	90.7	0.3	96.9
Ag + Tv + Vc + F + Mi	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0.00	100.0
L.S.Dat 0.05	2.06		2.05		1.43		3.86		2.32		0.03	
L.S.Dat 0.01	2.77		2.76		1.92		5.18		3.12		0.40	

☒ **Values are averages of four replicates.**

☒ **Mi** = ***Meloidogyne incognita*** **Ag = agrispon** **Tv** = ***Trichoderma viride*** **Vc** = ***Verticillium chlamydosporium*** **F** = Furadan (carbofuran) **Red.** = Reduction

Effect on plant growth:

Combined analysis of data shown in Table (2) proved that the root length, root diameter, root weight, foliage weight and plant weight of sugar beet significantly $P \leq 0.05$ and ≤ 0.01 increased when sugar beet plants were treated with agrispon alone or combined with the two bioagent, i.e., all above mentioned parameters increased when agrispon was applied alone by percentages, 33.8, 27.6, 36.8, 38.3 and 37.4 %, orderly as in the Table (2). Also, solitary application of bioagent, *T. viride* enhanced plant growth by a percentage around 46

% for all measured plant growth parameters. A remarkable increase was observed in plant growth parameters when *T. viride* or agrispon are combined with each other or with carbofuran. Also, from Table (2), the highest increases in the measured growth parameters i.e., root length, root diameter, root weight, foliage weight and plant weight were achieved in plots received the combination of agrispon along with *T. viride*, *V. chlamydosporium* and carbofuran as they were 66.0, 81.0, 141.3, 134.7 and 138.6, respectively. In this regard, the best following combinations after the previous one as indicated increase percentage in root weight/plant viz., agrispon + *T. viride* + *V. chlamydosporium*, agrispon + *T. viride*, *T. viride* + *V. chlamydosporium* + carbofuran and *T. viride* + *V. chlamydosporium* and *T. viride* + carbofuran which participated in increasing root weight/plant by 138.7, 96.9, 48.6, 38.3 and 38.3 %, respectively.

Table (2): Effect of *Verticillium chlamydosporium, Trichoderma viride* in combination with agrispon/furadan on plant growth of sugar beet plants subjected to infestation with *Meloidogyne incognita* (combined analysis of 2006/2007 and 2007/2008 seasons.)

Treatments	Root length		Root diameter		Root weight		Foliage weight		Plant weight	
	cm	Increase %	cm	Increase. %	g	Increase %	g	Increase %	g	Increase %
Mi (control)	24.25	0	7.25	0	622.35	0	428.29	0	1050.64	0
Ag + Mi	32.45	33.8	9.25	27.6	851.63	36.8	592.33	38.3	1443.96	37.4
Tv + Mi	35.55	46.6	9.6	32.4	908.45	46.0	630.15	47.1	1538.60	46.4
Vc + Mi	25.2	3.9	8.5	17.2	803.89	29.2	555	29.6	1358.89	29.3
F + Mi	28.44	17.3	9.25	27.6	857.25	37.7	596.56	39.3	1453.81	38.4
Tv + Vc + Mi	29.32	20.9	9.1	25.5	851.25	36.8	590.45	37.9	1441.70	37.2
Tv + Vc + F + Mi	30.25	24.7	9.75	34.5	925.09	48.6	645.12	50.6	1570.21	49.5
Tv + F + Mi	38	56.7	9.2	26.9	860.6	38.3	600.2	40.1	1460.80	39.0
Ag + Tv + Mi	39.55	63.1	11.9	64.1	1225.15	96.9	853.65	99.3	2078.80	97.9
Ag + Tv + Vc + Mi	38.9	60.4	12.5	72.4	1485.25	138.7	978.68	128.5	2463.93	134.5
Ag + Tv + Vc + F + Mi	40.25	66.0	13.12	81.0	1501.85	141.3	1005.35	134.7	2507.20	138.6
L.S.D at 0.05	1.33		0.31		15.42		99.40		22.40	
L.S.D at 0.01	1.78		0.42		20.72		133.54		30.09	

- **Values are averages of four replicates.**
- **Mi = *Meloidogyne incognita* Ag = Agrispon Tv = *Trichoderma viride* Vc = *Verticillium chlamydosporium* F = Furadan (carbofuran)**

Effect on main yield components of sugar beet:

Analysis of variance for the combined data of the studied two seasons demonstrated in Table (3), significantly marked out the effect of various treatments on average number of survival plants/fad which directly affected roots and sugar yields per feddan. Whereas, the best single application that maintained relatively high plants population was carbofuran alone (22088 plants/fed. with increase equals to 79.6 % than untreated), followed by *T. viride* alone treatment (18550 plant/fed. With increase equals to 50.8 % than untreated). While, the best combination in this regard was *T. viride* + carbofuran (25450 plants/fed. With increase equals to 106.9 % than untreated) followed by *T. viride* + *V. chlamydosporium* + carbofuran (103.2 % increase than untreated), followed by agrispon + *T. viride* + *V. chlamydosporium* + carbofuran (101.2 % increase than untreated) followed by agrispon + *T. viride* + *V. chlamydosporium* (96.9 % increase) and followed by agrispon + *T. viride* (88.6 % increase than the untreated). Data in Table (3) showed also the estimated highest roots yield in ton/fed. was achieved with the combination of agrispon + *T. viride* + *V. chlamydosporium* + carbofuran (37.171 ton roots/fed with 385.5 % increase than untreated) and the lowest was with the treatment of *V. chlamydosporium* alone (12.107 ton roots/fed.). Notably, all combinations that implied agrispon or/and *T. viride* attained distinguished root yield/fed. In regard to sugar yield /fed., the same trend was noticed, whereas, the highest sugar yield obtained with the combinations that implied agrispon or/and *T. viride* as noticed from the same Table (3), 7.734 ton sugar per fed. had been obtained by treatment of agrispon + *T. viride* + *V. chlamydosporium* + carbofuran.

Effect on the quality of sugar beet plants:

Data showed in Table (4) indicated that there were no significant differences ($P \leq 0.05$ and ≤ 0.01) among the most treatments for percentage total soluble solids T.S.S.% but the highest ones achieved with treatments that comprised agrispon and *T. viride* and were around 22 %. but, sucrose % was significantly influenced by treatments, whereas agrispon combinations achieved the highest sucrose percentage as they were 22.4, 20.8 and 20.2 % for Agrispon + *T. viride* + *V. chlamydosporium* + carbofuran, ag + *T. viride* + *V. chlamydosporium* + carbofuran and agrispon + *T. viride*, respectively.

Also, Purity % was the highest with all combinations of agrispon and *T. viride*, whereas all above 90 % with exception for carbofuran alone (96 %). Sugar content per plant as a character of quality was consequently affected with the same aforementioned treatments that affect sucrose and purity percentages whereas the best sugar yield in gm per plant was obtained with the treatment of agrispon + *T. Viride* + *V. chlamydosporium.*

Table (3): Effect of *Verticillium chlamydosporium, Trichoderma viride* in combination with agrispon/furadan on main yield components of sugar beet that subjected to infestation with *Meloidogyne incognita* by combined analysis between two seasons of 2006/2007 – 2007/2008.

Treatments	No. of survival plants		Roots yield Ton/ Fed.		Sugar yield Ton / Fed.	
	plants/fed.	Increase %	Tonnage	Increase %	Tonnage	Increase %
Mi (control)	12301	0.0	7.656	0.0	0.907	0.0
Ag + Mi	16600	34.9	14.137	84.7	2.065	127.7
Tv + Mi	18550	50.8	16.852	120.1	2.536	179.6
Vc + Mi	15060	22.4	12.107	58.1	1.227	35.3
F + Mi	22088	79.6	18.935	147.3	3.223	255.3
Tv + Vc + Mi	20965	70.4	17.846	133.1	1.927	112.5
Tv + Vc + F + Mi	24996	103.2	23.124	202.0	4.137	356.1
Tv + F + Mi	25450	106.9	21.902	186.1	4.031	344.4
Ag + Tv + Mi	23201	88.6	28.425	271.3	5.178	470.9
Ag + Tv + Vc + Mi	24222	96.9	35.976	369.9	6.952	666.5
Ag + Tv + Vc + F + Mi	24750	101.2	37.171	385.5	7.734	752.7
L. S.D at 0.05	186.52		.0.86		0.40	
L.S.D at 0.01	250.58		1.16		0.50	

☒ **Values are averages of four replicates.**

☒ **Mi = *Meloidogyne incognita* Ag = agrispon Tv = *Trichoderma viride* Vc = *Verticillium chlamydosporium* F = Furadan (carbofuran) .**

Table (4): Effect of *Verticillium chlamydosporium, Trichoderma viride* in combination with agrispon/furadan on the quality of sugar beet roots subjected to infestation with *Meloidogyne incognita* by combined analysis between two seasons of 2006/2007 – 2007/2008.

Treatments	Total Soluble Solids (T.S.S)		Sucrose		Purity		Sugar content g per plant	
	%	Increase %	%	Increase %	%	Increase. %	g	Increase %
Mi (control)	18	0.0	14.6	0.0	81.1	0.0	73.7	0.0
Ag + Mi	18.4	2.2	16.4	12.3	89.1	9.9	124.4	68.8
Tv + Mi	19.2	6.7	17.0	16.4	88.5	9.1	136.7	85.5
Vc + Mi	16.2	-10.0	13.2	-9.6	81.5	0.5	81.5	10.6
F + Mi	18.2	1.1	17.6	20.5	96.7	19.2	145.9	98.0
Tv + Vc + Mi	19.8	10.0	18.2	24.7	91.9	13.3	91.9	24.7
Tv + Vc + F + Mi	20.6	14.4	19.2	31.5	93.2	14.9	165.5	124.6
Tv + F + Mi	19.2	6.7	18.8	28.8	97.9	20.7	158.4	114.9
Ag + Tv + Mi	22.4	24.4	20.2	38.4	90.2	11.2	223.2	202.8
Ag + Tv + Vc + Mi	22.4	24.4	20.8	42.5	92.9	14.5	287	289.4
Ag + Tv + Vc + F + Mi	23.8	32.2	22.4	53.4	92.9	14.5	312.5	324.0
L. S.D at 0.05	1.46		1.16		2.53		4.67	
L.S.D at 0.01	1.96		1.55		3.40		6.27	

☒ **Values are averages of four replicates.**

☒ **Mi = *Meloidogyne incognita* Ag = agrispon Tv = *Trichoderma viride* Vc = *Verticillium chlamydosporium* F = Furadan (carbofuran).**

Also, from Tables (2, 3, and 4), the studied agents acted against nematodes and resulted in maintaining a higher number of survival plants / treated plots as compared to untreated plots, which consequently led to notable enhancement in yield components and quality.

The combination among agrispon and the two bioagents and carbofuran resulted in a cumulative adverse effect on nematode as stated before, and positive returns towards sugar beet plants whereas,

carbofuran stopped further attack by nematode juveniles to root of sugar beet plants, also, the other two bioagents suppressed the development of nematode stages, Besides, agrispon as a bio-stimulant (bioregulator) energizes the soil and makes nutrients more available to plants via enriching soil microorganism's activities as explained by Dubravec *et al.* (1995). That's to say that agrispon contributed in an enhancement of the two bioagents activity as well as sugar beet growth and this reflected as on higher yields and better quality as compared with untreated plots. This trend coincided with that reported by Goswami and Mishra, (1994) and in parallel with Goswam*i et al.* (2006).

CONCLUSIONS

The results obtained from the research allow us to draw the following conclusions:

1. The most effective solitary application whether for root galling reduction or all nematode stages suppression was due to carbofuran, *T. viride* and *V. chlamydosporium* and can be used under low to moderate level of contaminated soils with root-knot nematodes in sugar beet fields without adding any nematicide.
2. The combination among *T. viride*, *V. Chlamydosporium*, agrispon and carbofuran resulted in an accumulative effect making the nematode control measurements more efficient and more safe to environment due to low input of chemical nematicide (carbofuran dosage in the combination was 0.25 or 0.15 a.i kg that's equals to 1/4 or 1/8 the used dosage).
3. Integration of agrispon as bio-stimulant for plants and micro-organisms enhanced sugar beet and bioagents growth and positively reflected on the quantity and quality of sugar beet crop.
4. The present investigation thus, clearly shows the significant performance of the cumulative effect by agrispon and both of the bioagents (one as egg parasitic/opportunistic (*V. chlamydosporium*) and the other as toxic, *T. viride*) as the best opportunity in reducing the nematode population and improving plant health. This introduces an ideal integration of management components against soil borne diseases like root-knot nematode. All the three management components in the study viz. The two bioagents, bioregulator and the nematicide are environment-friendly economic material and easy to apply by farmers.

REFERENCES

Adegbite, A.A. and S.O. Adesiyan(2001). Effect of carbofuran (furadan) on the performance of four nematode susceptible varieties of soybean (*Glycine max* L.) Merr. Tropical Oilseeds J., 6: 11-23.

Altman, JAltman, J. and Thomsom, I. (1971). Nematodes and their control. In : Advances in Sugarbeet production principles and practices. pp. 335-370 (ed. T.J. Russell, T.A. John, E.R. George and R.H. George). Ames , Iowa , U.S.A: The Iowa State Univ. Press.

Annual Report for Sugar Crops (2008). Sugar Crops Council, Ministry of Agriculture and Land Reclamation, Giza, Egypt.

Barker, T. R., (1985). Nematode extraction and bioassays . In: *An Advanced treatise on Meloidogyne vol. II.* Barker, T. R.,Carter, C. C. and Sasser, J. N. (Eds). North Carolina State University, pp. 19-35.

De Leij F.A. A.M. And B.R. Kerry (1991). The nematophagous fungus, *Verticillium chlamydosporium* as a potential biological agent for *Meloidogyne srenaria* Rev. Nematol., 14: 157-164.

Di-Sanzo, C.P., 1973. Nematode Response to Carbofuran. J. Nematol., 5: 22-27.

Dubravec, K.; I. Dubrevec and J. Manitasevic (1995). The effect of the bioregulators Agrispon and Ergostim on the vegetative and reproductive growth of apples. J. Sust. Agric.Vo. 5 (1-2): 73-83.

Duncan, D B. (1955). Multiple range and multiple F tests. Biometrics 11:1-42,

Eisenback, J.D. (1985). Detailed morphology and anatomy of second-stage juveniles, males and females of the genus *Meloidogyne* (root-knot nematodes). In: An advanced treatise on *Meloidogyne*. Eds., Sasser, J.N. and Carter, C.C., Biology and Control, North Carolina State Univ. Graphics. 47-77 pp.

Eisenback, J.D.; H. Hirschmann, and A.C. triantaphyllou(1980). Morphological comparison of *Meloidogyne* females head structures, perineal patterns and style. J. Nematol. 12: 300-313.

Fadina, O.O.(1991). Effect of lambdacyhelothrin (Karate) in the control of root-knot nematode (*Meloidogyne incognita*) on soybean and its toxicological effects on rabbits. Ph.D. Thesis, Univ. Ibadan, Ibadan, Nigeria, pp: 236.

Gohar, I.M.A. and M.F. Maareg (2005). Relationship between crop losses and initial population densities of root-knot nematode, *Meloidogyne* in soil of Sugar beet grown in West Nubariya district. Egypt. J. Agric. Res. 83 (4): 1315-1328.

Goswami B, K; R. K. Pandey K. S. Rathour; C. Bhattacharya, and L. Singh (2006). Integrated application of some compatible biocontrol agents along with mustard oil seed cake and furadan on *Meloidogyne incognita* infecting tomato plants. Zhejiang Univ Sci. 7(11):873-875.

Goswami, B.K. and S.D. Mishra (1994). Comparative efficacy of neem cake and carbofuran on plant parasitic nematodes infecting pea. *Curr. Nematol.*, 3(1):7-12.

Kerry, B.R.; A. Simon and A.O. Rovira (1984). Observation on the introduction of *Verticillium chlamydosporium* and other parasitic fungi into soil for control of the cereal cyst nematode *Heterodera avenae*. *Ann. Appl. Biol.*, 105: 509-516.

Le Docte, A. (1927). Commercial determination of sugar in beet roots using the Sacks Le Docte. Intern. Sugar J., 29: 488 – 492.

Maareg, M.F.; Hassanein, M.A.; Allam, A.I. and Oteifa, B.A. (1998). Susceptibility of twenty-six sugar beet varieties to root-knot nematodes, *Meloidogyne spp.* in the newly reclaimed sandy soils of Al-Bostan region. Egyptian Journal of Agronematology, 2(1): 11-125.

Mai, W.F. and H.H. Lyon (1975). Pictorial key to genera of plant parasitic nematodes. 4th Ed., Cornell Univ. Press, Ithaca, N.Y. 172 p.

Mclean, K.L.; J. Hunt and A. Stewart (2001). Compatibility of the biocontrol agent *Trichoderma harzianum* C52 with selected fungicides. New Zealand Plant Prot. 54:84-88.

Pandey, R.K., B.K. Goswami and S. Singh (2005). Management of root Knot nematode and Fusarium wilt disease complex by fungal bioagents, neem oilseed cake and/or VA-Mycorrhiza on Chickpea. Internat. Chickpea and Pigeonpea Newsl., 12:32-34.

Rao, M.S., P.P. Reddy and M. Nagesh (1998). Evaluation of plant based formulations of *Trichoderma harzianum* for the management of *Meloidogyne incognita* on egg plant. Nematol. Medit. 26:59-62.

Shurtleff, M.C. and C.W. Averre (2000). Diagnosing plant disease caused by plant parasitic nematodes. The American phytopatological society, 187 pp.

Singh, R.S., and K. Sitaramaiah (1966). Incidence of root knot of okra and tomatoes in oil cake amended soil. Plant Dis. Rept., 50:668-672.

Singh, S. and U.R. Khurma (2007). Susceptibility of six tomato cultivars to the root-knot nematode, *Meloidogyne incognita*. The South Pacific Journal of Natural Science, 13: 73-77.

Young, T.W. (1954). An incubation method for collecting endo-migratory nematodes. Plant Dis. Rept 38: 794.

تكامل بعض عوامل المكافحة البيولوجية مع الأجرسبون و الفيوردان (كاربوفيوران) لمكافحة نيماتودا تعقد الجذور التي تصيب محصول بنجر السكر بالنوبارية

إبراهيم محمد عبده جوهر [1]– كمال محمد عجمي[2] – محمد مصطفى عبد الرحمن [3]

[1]- قسم بحوث الأمراض و الآفات. معهد بحوث المحاصيل السكرية – مركز البحوث الزراعية - جيزة
[2]- قسم بحوث المعاملات الزراعية. معهد بحوث المحاصيل السكرية – مركز البحوث الزراعية - جيزة
[3]- قسم بحوث الفسيولوجي و الكيمياء. معهد بحوث المحاصيل السكرية – مركز البحوث الزراعية - جيزة

أجريت تجربتان في موسمي 2006/ 2007 و 2007/ 2008 م في منطقة غرب النوبارية و ذلك لدراسة تأثير نوعين من فطريات المكافحة الحيوية مع الأجرسبون كمنشط حيوي و مبيد الكاربوفيوران كمبيد نيماتودي و ذلك على نيماتودا تعقد الجذور *ميلدوجين إنكوجنيتا* التي تصيب بنجر السكر تحت ظروف الحقل. وجدَ من الدراسة أن عاملي المكافحة الحيوية تريكودرما فيريد و الفيرتيسليوم كلاميدوسبوريوم سواء منفردين أو بالجمع بينها و بين الأجرسبون و كاربوفيوران10% محبب حسنت و نشطت نمو نباتات البنجر و خفضت عدد العقد الجذرية/جذر و كتل البيض/جذر و عدد اليرقات/جذر و كذلك عدد اليرقات/200 جرام تربة, وذلك إلى خفض 100% في بعض المعاملات للمتغيرات و القياسات السابق ذكرها. أيضاً أدى تكامل فطري المكافحة الحيوية مع الأجرسبون و الكاربوفيوران إلي تقليل معدل التضاعف للنيماتودا بالمقارنة بغير المعامل وصل في بعض المعاملات إلى الصفر. أدى استخدام الأجرسبون في المعاملات كمنشط حيوي للنبات و الكائنات الدقيقة إلى تحسن ظروف نمو نباتات بنجر السكر وكذلك نمو و تكاثر عاملي المكافحة الحيوية مما أعطى مردود إيجابي على كمية و جودة محصول بنجر السكر في صورة تحسن محصول البنجر من الجذور و السكر للفدان و كذلك تحسن صفات الجودة متمثلة في محتوى النبات من السكر و نسبة المئوي للنقاوة. أوضحت هذه الدراسة الأداء المعنوي للتأثير التراكمي للأجرسبون مع كلاً من عاملي المكافحة الحيوية (أحدهما على كتل البيض – فيرتيسليوم كلاميدوسبوريوم و الأخر ذو التأثير السام علي اليرقات – تريكودرما فيريدي) و الجرعة المنخفضة من الكاربوفيوران 10% محبب مما أدى لخفض تعداد النيماتودا و تحسين نمو النبات مع المحافظة على البيئة الزراعية و توازناتها البيولوجية.