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Effect of Two Organic Amendments, Elemental Sulphur, Bionema and Carbofuran Soil Application to Control Root-Knot Nematode on Growth and Productivity of Thompson Seedless Grapes

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Abstract: Efficacy of two organic amendments as cattle or chicken manure, mineral fertilizer as sulphur and bionema (commercial product containing *Bacillus penetrans*) compared to a chemical nematicide, carbofuran 10% G was investigated under field conditions for controlling root-knot nematode *Meloidogyne incognita*, infesting table grape cv. Thompson Seedless. All the tested materials were differently affected the nematode number in 2004 and 2005 growing seasons. In general, the efficacy on nematode increased at the end of the experiment (June and July 1st). However all treatments enhanced leaves N, P and K content compared with untreated ones. Besides, shoot length, internode length, leaves number and area as well as leaf fresh and dry weight were significantly increased by all treatments. Number of clusters, cluster weight, number of berries/cluster as well as total fruit yield and berries. Physical and chemical properties increased significantly with most treatments as compared to untreated ones.

Key words: Bioagent · carbofuran · *Meloidogyne incognita* · organic amendments

INTRODUCTION

Grape *Vitis vinifera* Linn, is one of the most important economic fruit crop grown in Egypt. This crop was reported to be susceptible to several plant- parasitic nematodes of which *Meloidogyne* occupied the most prominent [1]. Loubser [2] determined the occurrence of *Meloidogyne* spp. in South Africa, *M. javanica* constituted 73.2% of all specics, while *M. incognita* constituted 7.1%. *M. incognita* has 4 generations per year and the eggs exist overwinter in the roots and soil of grape rhizosphere [3]. As control measures, achieved control of *M. incognita* by using certain bioagents and organic amendments [4-6]. At the same trend, this study was designed to evaluate the effect of cattle and chicken organic amendments, elemental sulphur and bionema as a bioagent compared to a nematicide (carbofuran) for controlling *M. incognita* on table grape in newly reclaimed soil and their effect on Thompson grape vine growth and productivity.

MATERIALS AND METHODS

This investigation was carried out all over the growing seasons of 2004 and 2005 in a vineyard of newly reclaimed soil, irrigated by drip irrigation and located in Wadi-El-Natroon, Beheira Governorate, Egypt.The vineyard was grown with grape cv. Thompson Seedless of 8 years-old and naturally infested by root-knot nematode *M. incognita*. Grape seedlings were raised by cuttings and spaced at 1.75 x 2.5 m apart.The seedlings were more or less uniform in their growth and were prunted in winter at the first week of January leaving 68 buds per vine (4 fruiting canes x 14 buds plus 6 renewal spurs x 2 buds). The applied traetments were arranged in a Randomize Complete Block Design with five replicates for each treatment and the treatments were as follows:

- Cattle organic manure at a rate of 16 kg/vine applied in January (2004 & 2005).

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- Chicken organic manure at a rate of 5 kg/vine applied in January (2004 & 2005).
- Elemental sulphur at a rate of 100 g/vine applied in February (2004 & 2005).
- Bionema (containing a strain of *Bacillus penetrans*) as a commercial product at a rate of 15 ml/vine applied in March (2004 & 2005).
- Carbofuran (Nematicide) 10% G at a rate of 40 g/vine applied in March (2004 & 2005).
- Untreated check.

A surfactant namely Triton B 1% was added to all spraying solutions including untreated check.

The tested materials were applied near root zone. Soil and root samples were taken for nematode assay in April, May, June and July in the two seasons (2004 & 2005). An aliquot of 200 g soil per each replicate was processed for nematode extraction by using sieving and decanting methods. Nematode juveniles in 5 g roots from each replicate was extracted according to Young [7]. Horticultural practices including N, P and K fertilization, hoeing, irrigation as well as pest and fungi control were carried out as recommended in vine cultures. Potential effects of treatments were evaluated in terms of the change in growth, vine nutritional status, yield and berries quality. Average of shoot length (cm) was recorded at the middle of June. Average leaf area (cm^2) of the twenty leaves sample from those opposite to the basal clusters was calculated according to Ahmed and Morsy [8] using the formula:

$$LA = 0.56\ (0.79 \times w^2) + 20.01$$

Where; LA = leaf area (cm^2), w = the maximum leaf width (cm). Percentages of N, P, K (on dry weigth basis) were determined in petioles of the selected leaves according to Wilde *et al.* [9]; yield expressed in weight (kg) and number of clusters per vine was recorded. Five clusters were taken at random for determination of average berry weight (g), total soluble solids %, total acidity % (expressed as g tartaric acid/100 ml juice), total soluble solids/total acidity according to A.O.A.C. [10]. The obtained data were tabulated and statistically analyzed according to Gomez and Gomez [11] using L.S.D test.

RESULTS

Effect of the tested materials on root-knot nematodes *M. incognita*: The effect of two organic manure, sulphur and bionema as a bioagent compared to the nematicide, carbofuran were listed in Tables 1 & 2. It was noticed, generally, that the tested materials ware greatly varied in reducing the second stage juveniles of *M. incognita* in soil and roots all over the two seasons of the study. During 2004 growing season, all the tested materials significantly reduced number of the second stage juveniles of *M. incognita* in soil and roots of grape at different degrees. The effect of the tested materials, increased in June and July 1st at the end of the experiment especially by using cattle or chicken organic amendments and the nematicide. In 2005 season, all the tested materials were also, differently reduced the second stage juveniles of *M. incognita* in soil and roots. The bioagent (Bionema) was superior, compared to the other treatments, in reducing nematode juveniles in soil and roots at the end of the experiments.

Effect of the tested materials on growth and nutritional status of grape cv. Thompson Seedless: Data presented in Table 3 indicated that the tested materials significantly affected on N, P and K % in leaves in the two seasons (2004 & 2005). N percentage ranged from 1.02 to 1.31% in the first season (2004) and from 1.11 to 1.83% in the second season (2005). The highest N content was recorded by carbofuran in the first season and bionema, carbofuran and sulphur in the second season without significant differences among them. The lowest N percentage resulted in from cattle manure treament in both seasons. The other tested treatments recorded in between values or failed to give consistent trend through the two seasons.

Leaf P content, failed to indicate any significant differences between the tested materials in the first season, it was significant in the second season. These treatments (caobofuran, sulphur and bionema) resulted in leaf P content from 0.25 to 0.27% in the second season, without significant differences among them. On the other hand, the treatments which indicated consistently low leaf P content in the second season were chicken manure, cattle manure and untreated check. K percentage, generally, it was significantly affected by treatments used and ranged from 1.39 to 1.57% in the first season and from 1.32 to 2.73% in the second season. The highest K percentage resulted in from chicken manure and carbofuran in the first season, while it was gained by carbofuran and bionema in the second season.

Table 4 shows that, shoot length, number of leaves/shoot, internode length, leaf area, leaf fresh

Table 1: Effect of two organic amendments, mineral fertilizer, bionema and a nematicide on *M. incognita* infecting grape under field conditions in 2004 growing season

	Ave. No. second stage juveniles of *M. incognita*							
	Months							
	April 1st				May 1st			
Treatments	Per 1g roots	Red. %	Per 250 g soil	Red. %	Per 1g roots	Red. %	Per 250 g soil	Red. %
Untreated check	102.00	-	567.00	-	142.00	-	600.00	-
Cattle manure	46.00	54.90	128.00	77.43	80.00	43.66	132.00	78.00
Chicken manure	145.00	-42.16	177.00	68.78	19.00	86.62	157.00	73.83
Sulphur	61.00	40.20	526.00	7.23	41.00	71.13	410.00	31.67
Bionema	180.00	-76.47	530.00	6.53	167.00	-17.60	438.00	27.00
Carbofuran	177.00	-73.53	257.00	54.67	170.00	-19.72	189.00	68.50
L.S.D at 5%	43.21	-	43.60	-	23.18	-	78.08	-

	Ave. No. second stage juveniles of *M. incognita*							
	Months							
	June 1st				July 1st			
Treatments	Per 1g roots	Red. %	Per 250 g soil	Red. %	Per 1g roots	Red. %	Per 250 g soil	Red. %
Untreated check	193.00	-	1233.00	-	205.00	-	1567.00	-
Cattle manure	48.00	75.65	137.00	88.89	31.00	84.89	215.00	86.28
Chicken manure	17.00	91.19	128.00	89.62	16.00	92.20	210.00	86.60
Sulphur	35.00	82.29	305.00	75.26	23.00	88.78	153.00	90.24
Bionema	151.00	21.76	380.00	69.18	133.00	35.12	348.00	77.79
Carbofuran	160.00	17.10	182.00	85.40	130.00	36.59	137.00	91.26
L.S.D at 5%	17.59	-	74.45	-	16.71	-	61.23	-

Table 2: Effect of two organic amendments, mineral fertilizer, bionema and a nematicide on *M. incognita* infecting grape under field conditions in 2005 growing season

	Ave. No. second stage juveniles of *M. incognita*							
	Months							
	April 1st				May 1st			
Treatments	Per 1g roots	Red. %	Per 250 g soil	Red. %	Per 1g roots	Red. %	Per 250 g soil	Red. %
Untreated check	54.00	-	243.00	-	41.00	-	117.00	-
Cattle manure	7.00	87.03	355.00	-46.09	14.00	65.85	102.00	12.82
Chicken manure	1.00	98.15	128.00	-47.33	6.00	85.37	43.00	63.23
Sulphur	0.00	100.00	250.00	2.88	24.00	42.50	90.00	23.08
Bionema	0.00	100.00	205.00	15.64	14.00	65.85	77.00	34.19
Carbofuran	7.00	87.04	133.00	45.27	14.00	65.85	97.00	17.09
L.S.D at 5%	13.5	-	43.50	-	7.65	-	24.41	-

	Ave. No. second stage juveniles of *M. incognita*							
	Months							
	June 1st				July 1st			
Treatments	Per 1g roots	Red. %	Per 250 g soil	Red. %	Per 1g roots	Red. %	Per 250 g soil	Red. %
Untreated check	24.00	-	120.00	-	16.00	-	370	-
Cattle manure	14.00	41.67	90.00	25.00	12.00	25.00	87	76.48
Chicken manure	17.00	29.17	113.00	5.83	11.00	31.25	115	68.02
Sulphur	15.00	37.50	73.00	39.17	24.00	-50.00	65	22.43
Bionema	12.00	50.00	67.00	44.17	8.00	50.00	55	85.14
Carbofuran	13.00	45.83	80.00	33.33	13.00	18.75	77	79.19
L.S.D at 5%	4.73	-	17.42	-	6.33	-	28.98	-

Red. %= Reduction percentages

Table 3: Effect of two organic manure, sulphur, bionema and carbofuran on leaf nutrient contents of Thompson Seedless grape grown in sandy soil during the two seasons (2004&2005)

	Characteristics					
	First season (2004)			Second season (2005)		
Treatments	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Untreated check	1.09	0.21	1.40	1.20	0.23	1.61
Cattle manure	1.02	0.20	1.42	1.11	0.21	1.32
Chicken manure	1.13	0.18	1.57	1.27	0.19	2.14
Sulphur	1.21	0.23	1.39	1.72	0.26	2.09
Bionema	1.22	0.19	1.44	1.83	0.27	2.73
Carbofuran	1.31	0.19	1.49	1.76	0.25	2.53
L.S.D at 5%	0.03	NS	0.09	0.22	0.03	0.19

Table 4: Effect of two organic manure, sulphur, bionema and carbofuran on shoot length, internode length, number of leaves, leaf area, leaf fresh weight and leaf dry weight of Thompson Seedless grape grown in sandy soil during the two seasons (2004 & 2005)

	Characteristics											
	First season (2004)						Second season (2005)					
Treatments	Shoot length (cm)	Internode length (cm)	Number of leaves	Leaf area (cm^2)	Leaf fresh weight (g)	Leaf dray weight (g)	Shoot length (cm)	Internode length (cm)	Number of leaves	Leaf area (cm^2)	Leaf fresh weight (g)	Leaf dray weight (g)
Untreated check	111.61	4.91	22.60	132.44	2.95	0.81	115.92	5.10	22.73	139.37	3.07	0.81
Cattle manure	115.21	5.12	22.62	138.11	3.00	0.81	118.91	5.19	22.90	140.23	3.10	0.80
Chicken manure	128.73	5.65	23.13	145.05	3.11	0.83	144.32	6.01	24.01	152.30	3.22	0.82
Sulphur	144.11	5.71	25.11	149.23	3.20	0.85	157.01	6.02	26.08	161.41	3.40	0.92
Bionema	148.16	6.00	25.03	158.36	3.31	0.91	162.00	6.51	25.88	176.37	3.51	0.96
Carbofuran	146.32	6.00	24.81	162.60	3.42	0.91	166.36	6.72	25.76	183.92	3.64	0.99
L.S.D at 5%	4.05	0.41	1.30	3.05	0.10	N.S	6.03	0.37	1.01	5.31	0.09	0.08

weight and leaf dry weight at the different treamentes. Shoot length with those treatments ranged from 144.11 to 148.16 cm in the first season and from 162.00 to 166.36 cm in the second season without significant differences among them. On the other hand, the treatments that consistently produced lower shoot length through the two seasons were attributed to untreated check or cattle manure, it ranged from 111.61 to 115.21 cm in the first season and from 115.92 to 118.91 cm in the second season, without significant differences among them.

The internode length ranged from 5.65 to 6.00 cm in the first season and from 6.51 to 6.72 cm in the second season, without significant differences among them. On the other hand, the lowest internode length through the two season were recorded by untreated check or cattle manure, it ranged from 4.91 to 5.12 cm in the first season and from 5.10 to 5.19 cm in the second season, without significant differences among them. The other tested treatments indicated any consistent trend through the two seasons. The treatments that consistently gave the greatest numbers of leaves/shoot through the two seasons due to: sulphur, bionema and carbofuran and the values ranged from 24.81 to 25.11 cm in the first season and from 25.76 to 26.08 in the second season, without significant differences among them. On the other hand, the lowest number of leaves/shoot through the first season resulted in by untreated check, cattle manure or chicken manure and through the second season due to untreated check and cattle manure. It ranged from 22.60 to 23.13 in the first season and from 22.73 to 22.90 leaves/shoot in the second season, without significant differences among them.

Data also showed that the greatest leaf area through the two seasons as a result of application carbofuran and the values were 162.60 cm^2 in the first season and was 183.92 cm^2 in the second season with significant differences in both seasons.

The highest leaf fresh weights (3.42 and 3.64 g in the first and second seasons, respectively) were obtained by carbofuran. On the other hand, the lowest leaf fresh

Table 5: Effect of two organic manure, sulphur, bionema and carbofuran on number of clusters per vine, cluster weight, yield per vine, cluster length, width and number of berries per cluster of Thompson Seedless grape grown in 2004 and 2005 seasons

	Characteristics											
	First season (2004)						Second season (2005)					
Treatments	No. of clusters/ vine	Cluster weigth (g)	Yield/ vine (kg)	Cluster length (cm)	Cluster width (cm)	No. of berries/ cluster	No. of clusters/ vine	Cluster weight (g)	Yield/ vine (kg)	Cluster length (cm)	Cluster width (cm)	No. of berries/ cluster
Untreated check	15.49	293.00	4.54	16.11	9.70	104.64	16.01	326.00	5.22	16.26	9.41	111.26
Cattle manure	16.00	340.00	5.44	16.21	9.84	106.92	16.83	348.00	5.86	16.92	10.14	104.50
Chicken manure	19.12	360.00	6.88	17.50	10.92	100.00	22.37	365.00	8.72	17.91	11.23	105.98
Sulphur	19.76	382.00	4.54	17.83	11.21	92.72	23.06	422.00	9.73	18.53	12.15	105.50
Bionema	22.30	382.00	8.47	18.74	12.11	92.46	24.00	459.00	11.02	19.72	13.56	116.20
Carbofuran	21.92	380.00	7.82	18.81	12.02	99.44	23.94	452.00	10.82	19.21	13.47	123.16
L.S.D at 5%	1.43	12.03	0.42	1.05	1.11	8.32	1.05	14.90	0.46	1.31	0.92	7.84

Table 6: Effect of two organic manure, sulphur, bionema and carbofuran on average 100 berries weight, average berry weight, berries size, total soluble solids, total acidity and T.S.S/acidity in the juice of Thompson Seedless grape in 2004 and 2005 seasons

	Characteristics											
	First season (2004)						Second season (2005)					
Treatments	Average 100 berries weight (g)	Average berry weight (g)	Berries size (cm)	T.S.S %	Total acidity %	T.S.S/ acidity	Average 100 berries weight (g)	Average berry weight (g)	Berries size (cm)	T.S.S %	Total acidity %	T.S.S/ acidity
Untreated check	193.00	2.80	350.00	16.20	0.626	25.87	180.00	2.93	363.00	16.40	0.629	26.07
Cattle manure	240.00	3.18	420.00	17.20	0.618	27.83	237.00	3.33	400.00	17.60	0.611	28.80
Chicken manure	258.00	3.60	452.00	18.30	0.608	30.09	250.00	3.68	421.00	18.50	0.600	30.83
Sulphur	257.00	3.59	448.00	17.90	0.609	29.39	255.00	3.67	437.00	18.10	0.610	29.24
Bionema	275.00	4.12	460.00	18.80	0.600	31.33	260.00	4.00	452.00	19.00	0.577	32.92
Carbofuran	270.00	4.11	452.00	18.70	0.605	30.90	258.00	3.95	446.00	18.90	0.580	32.58
L.S.D at 5%	12.02	0.40	14.50	0.20	0.012	0.69	13.20	0.33	15.10	0.30	0.020	0.68

weight in the two seasons were recorded with untreated check orcattle manure and the values were 2.95 and 3.00 g in the first season and 3.07 and 3.10 g in the second season, without significant differences among them. Leaf dry weight was only significantly affected in the second season. The highest values were obtained by sulphur, bionema and carbofuran (0.92, 0.96 and 0.99 g, respectively), while the other treatments gave the lowest values without significant differences among them.

Effect of the tested materials on yield characteristics: Data concerning the effect of cattle and chicken manure, sulphur, bionema and carbofuran on the number of clusters per vine, number of berries per clusters, yield per vine, cluster weight, average cluster length and cluster width are shown in Table 5. It is evident that yield expressed in number of clusters per vine (22.30 and 24.0) in 2004 and 2005 seasons and cluster weights (382 and 459 g) were positively affected by spraying bionema at 15 ml/vine (11 liter/feddan) compared to the carbofuran (380 and 452 g) at 40 g/vine and sulphur (357 and 422 g) at 100 g/vine and cattle, chicken manure (340 and 360 g) in 2004 season and (348 and 365 g) in 2005 season compared to the untreated treatment (293 and 326 g). The increase in yield (8.47 and 11.02 kg), cluster length (18.74 and 19.72 cm) and cluster width (12.11 and 13.56 cm) were recorded with bionema compared to the carbofuran, sulphur, cattle and chicken manures and untreated check. The maximum values were obtained when bionema was applied at a rate of (15 ml/vine conc.).

Effect of the tested materials on some physical and chemical properties of the berries: Data in Table 6 obviously reveal that berry weight, average of 100 berries weight (g), berry size (cm), total soluble solids % and total acidity % were changed according to varying rates of

treatments. Soil application of bionema at 15 ml/vine conc. was accompanied with improving quality of the berries in terms of increasing average of 100 berries weight, berry weight (4.12 and 4.00 g), berries size and total soluble solids % (18.80 and 19.00) and in reducing the total acidity % in the two seasons.

DISCUSSION

It was noticed that the juveniles of *M. incognita* infesting table grape cv. Thompson Seedless were suppressed in soil and roots by using the tested materials. Reductions in certain plant parasitic nematodes following addition of some organic amendments have been reported in numerous experiments [6, 12-14]. It has been suggested that during decomposition of organic amendments, the activity of certain organisms antagonistic or predutory to nematodes increases [15, 16], or certain compounds toxic to nematodes are released [17]. Besides, the physical and chemical structures of the soil as well as soil fertility improve resulting increased tolerance of the plants to nematode attack [18]. As for elemental sulphur, it might be oxidized to sulphuric acid by sulphur-oxidizing bacterium (*Thiobacillus* sp.) resulting in lowering the pH value of the soil. This acidification of the soil may increase the solubility of phosphorus and micronutrients originally present in the soil and those applied as fertilizers [19, 20]. The relatively high numbers of nematodes in sulphur-treated plots in the present study could be attributed to improvement in soil properties and nutritional status of plants. These results are agreement with those obtained by Korayem and Osman [21], El-Sonbaty and Korayem [22]. Bionema as a commercial product containing a strain of *Bacillus penetrans*, Sayre [23] reviewed that *B. penetrans* is a candidate agent for biocontrol of nematodes. The suppression effect by certain bacteria may be due to accumulation of unknown nematoxic metabolites in soil. Similar, Ali [24] found that liquid culture of some bacterial species including *Bacillus* sp. or their filterates could inhibit egg hatching and juvenile survival of these plant parasitic nematodes *in vivo* and *in vitro*. The nematicide, carbofuran was reported to inhibit root-knot nematode on grape [25]. Better yield of table grape in treated soil may be due partly to reduction in nematode populaton and/or due to additive effect of nutrients produced by the tested organic amendments [26].

Positive effect of bionema on number of cluster/vine, number of berries/cluster, cluster weight, cluster length and width and cluster size was obtained in the first and second season of study. The present experiment clarified that using bionema at (15 ml/vine) gave satisfactory promotion on yield of Thompson Seedless grape vines. These results were true in both seasons.

The positive action of bionema in enhancing growth and vine nutritional status could reflect in increasing yield, consequantly cluster weight, logically the merits of bionema on improving yield, cluster weight and number of clusters surely reflected on promoting the yield. These results are in the same line with those obtained by Ahmed *et al.* [27], Moustafa [28], Gobara *et al.* [29]. The best results in both seasons were obtained by the addition of bionema at (15 ml/vine). The best results of the quality of the berries were obtained when bionema was applied at 15 ml/vine compared to the carbofuran and sulphur plus cattle manure and chicken manure. These results confirmed the findings of Gobara *et al.* [29], Ahmed *et al.* [30], Wassel *et al.* [31] and Aki *et al.* [32].

On the light of the present results, it can be stated that bionema at 15 ml/vine, carbofuran at 40 g/vine and sulphur at 100 g/vine applied in sandy soils is necessary for Thompson Seedless vines compared with the cattle chicken manures. Bionema at 11 liter/feddan is satisfied for producing an economical yield and fairly good quality of berries.

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