

Induction of Systemic Resistance in Sugar- Beet against Root-Knot Nematode with Commercial Products

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Abstract

The potentials of Bio-arc (a commercial formulation of the *Bacillus megaterium*) at the rate of 5, 10, 15 and 20 ml and Nemastrol (a commercial formulation of active ingredients) at the rate of 0.25 ml, for induction of systemic resistance to sugar-beet var. Negma infected with *M. incognita* were conducted in two soil types. Results revealed that all treatments with tested rates were found to have nematocidal activity against nematode infection and improved plant growth parameters of sugar-beet with various levels of success. The dual application of Bio-arc+Nemastrol at the rate of 20 ml +0.25 ml proved to be the best and showed significant improvement in plant growth parameters in terms of shoot length (92.6,127.5%) and total plant fresh weight (91.7, 370.4) of sugar-beet grown either in clayey or sandy soil, respectively. Among all treatments Nemastrol ranked next to oxamyl and performed the best and significantly suppressed total nematode population (Rf=1.9, 2.2), root galling (RGI=3.0, 3.0), number of egg masses (EI=3.0, 3.0) and number of eggs / egg mass (Red. %=76.5, 74.5) in clayey and sandy soil, respectively. However, concomitant treatment showed better results than did Bio-arc alone at four tested rates. The greatest suppression in total nematode population was recorded with clayey and sandy soil receiving the dual application of Nemastrol (0.25 ml) and Bio-arc (20 ml) with reproduction factor 2.2, 2.6 and reduction percentages reached 92.8, 92.6% respectively. Leaves of sugar-beet were assayed for their biochemical profiles with respect to NPK, total chlorophyll, total carbohydrates, proteins, and phenols. Moreover, remarkable induction in such chemical constituents except phenol content was recorded with the application of Bio- arc+Nemastrol (20 ml+0.25 ml). On the other hand, activities of related enzymes i.e. Peroxidase (PO) and Polyphenol Oxidase (PPO) were evaluated in roots of sugar-beet infected with *M. incognita*. The enzymes accumulation was much greatest in Bio-arc+Nemastrol (20+0.25 ml) treated plants compared to control as they reached their peak at day 9th from nematode inoculation.

Keywords: Induced resistance; Enzyme activity; *Meloidogyne incognita*; Sugar-beet; Biochemical activities; Soil type

Introduction

Sugar-beet (*Beta vulgaris* L.) is considered an important root crop, which is ranked second to sugar-cane for supporting the expansion of Egyptian sugar industry. Root-knot nematodes (RKNs) *Meloidogyne* spp. are among the most deleterious plant pathogens since these organisms play a detectable role in limiting the productivity of such economic agriculture crop. The root -knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood is among the most important nematode infesting sugar-beet. Many efforts to protect such crop from root-knot nematodes infestation are crucial. Because of the lack of plant resistance to most species of root-knot nematode as well as the environmental restrictions on nematicidal use for controlling plant parasitic nematodes, biological control and other eco-friendly disease control measures have gained recently increasing interest. The activation of the plant's own defense system through biotic and abiotic agents, called elicitors, has been considered as a focus of research only in recent years for the control of plant pathogens. The resulting elevated resistance due to an inducing agent upon infection by a pathogen is called induced systemic resistance (ISR) or systemic acquired resistance (SAR) [1].

However, induced resistance to plant parasitic nematodes has not been as extensively studied as that of fungi and bacteria. Ibrahim, et al. [2] recorded the capability of humic acid as well as thiamine at the concentration of 2000 ppm to induce resistance in sugar-beet against *M.incognita* and increase the activity of polyphenol oxidase (PPO) and peroxide oxidase (PO) enzymes compared with non-infected plants. On the other hand, plant growth promoting rhizobacterium (PGPR) belonging to *Bacillus* spp. are being exploited commercially

for plant protection to induce systemic resistance against various pests and pathogens. PGPR mediated rhizobacteria is often associated with the onset of defense mechanisms by expression of various defense related enzymes such a glucanase, chitinase, phenylalanine ammonia lyase (PAL), peroxidase (PO), and polyphenol oxidase (PPO) and accumulation of phenols [3]. In this point of view, the present work was carried out in order to study the impact of promoting growth rhizobacterium (PGR), *Bacillus megaterium*, Nemastrol active ingredients extract as resistance inducers to sugar-beet plant infected with *M. incognita* under greenhouse conditions.

Materials and Methods

Two greenhouse experiments were conducted at Nematological Research Unit (NERU), using sandy and clayey soil in order to evaluate the nematicidal properties of the commercial formulation of rhizobacterium, *Bacillus megaterium* (Bio-arc), the commercial biocide, Nemastrol against the root-knot nematode, *M. incognita*

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and the resulting effect on plant growth parameters of sugar-beet var. Negma. Induced resistance (IR) of such bio-agents was assayed through chemical composition and enzyme activities.

Tested bio-agents

Bio-arc: A native commercial formulation of phosphorus soluble bacterium, *Bacillus megaterium* (25×10^6 cfu/g) @ 2.5 g/L of distilled water, was obtained from Agricultural Research Institute, Giza, Egypt and enrolled by the Ministry of Egyptian Agriculture under No. 1087.

Nemastrol: A native commercial formulation of active ingredients containing glycosinolates (12%), chitinase (12×10^5 IU), cytokinins (200 ppm), flavonoids (5%) and β 1-3, Glucanase (2×10^5 IU) @ the rate of 5 L/ feddan, was obtained from Royal Company, Egypt.

Tested bio-agents rates: The tested bio- agent, Nemastrol was applied @ the rate of 0.25 ml/pot. However, Bio-arc was added using four different rates of 5, 10, 15 and 20 ml/pot in single application.

The chemical nematicide: Oxamyl 10%G; S-methyl -1(dimethylcarbamoyl)-N-[(methylcarbamoyl) oxyl] thioforminidate, was applied as a standard nematicide @ the rate of 0.3 g/pot

Experimental design: For each soil type, forty eight plastic pots (15-cm-d) containing 800 g steam-sterilized soil were planted with 3-5 seeds/pot of sugar-beet var. Negma, irrigated with water as needed then thinned to one seedling/pot after one month from germination. Fifteen days later, plant seedlings were inoculated with 2000 viable eggs of root-knot nematode, *M. incognita*. One week later, plants were treated with the selective materials as soil drench at the previous mentioned rates. For each soil type, four pots were treated with oxamyl @ the rate of 0.3 g/pot. However, four pots were left free of nematode infection and any treatment to serve as control (Ck1). Another four pots were received nematode alone and served as control (Ck2). Pots were then arranged in a randomized complete block design in a greenhouse @ $27 \pm 3^\circ\text{C}$, with four replicates and received water as needed. Therefore, treatments for each soil type were as follows:- 1-Bio-arc @ 5 ml/pot; 2- Bio-arc @ 10 ml/pot; 3- Bio-arc @ 15 ml/pot; 4- Bio-arc @ 20 ml/pot; 5- Nemastrol @ 0.25 ml/ pot; 6- Bio-arc @ 5 ml/pot +Nemastrol @ 0.25 ml/ pot ; 7- Bio-arc @ 10 ml/pot+Nemastrol @ 0.25 ml/ pot; 8- Bio-arc @ 15 ml/ pot +Nemastrol @ 0.25 ml/ pot; 9- Bio-arc @ 20 ml/pot+Nemastrol @ 0.25 ml/ pot; 10- Oxamyl (O), 11- Untreated Uninoculated plants (Ck1) and 12- Nematode alone (Ck2). Plants were harvested 45 days after nematode inoculation and roots were washed free from adhering soil. Data dealing with fresh shoot and root weight, dry shoot weight, shoot and root length, were recorded. Nematodes were extracted from soil using sieving and modified Baermann technique [4]. Roots were stained in 0.01 acid fuchsin [5] and examined for the developmental stages, females, galls and egg masses under stereomicroscope. Root galling or egg masses were rated on a scale of 0-5 where 0=no galls or egg masses, 1=1-2 galls or egg masses, 2=3-10 galls or egg masses, 3=11-30 galls or egg masses, 4=31-100 galls or egg masses, 5=more than 100 galls or egg masses per root system [6].

For each treatment, dry weight of shoot (1 g) was subjected to chemical analysis in order to evaluate total nitrogen, crude protein, total carbohydrate and total phenol. Samples of dried leaves were ground, wet digested and nitrogen (N), phosphorus (P), potassium (K) contents were determined according to kjeldahl methods [7] A.O.A.C. (1980) described by number of researchers (Pregl, Jackson, John) [8-10].

Determination of enzymatic activities: Sugar-beet plants treated with Bio-arc (20 ml/pot) and Nemastrol (0.25 ml/pot) singly and concomitantly were inoculated with 2000 second juveniles of *M.*

incognita and tested for enzymatic activities. The same protocol as outlined before was repeated. Roots were collected at different intervals (0, 3, 9 and 15 days after treatment and nematode inoculation) and assayed for activities of Peroxidase (PO) and Polyphenol Oxidase (PPO).

Preparation of enzyme extract: Enzyme extracts were prepared following the method described by [3] Maxwell and Bateman (1967). Dry root tissues (0.5 g) of each treatment were ground in 3 ml Naphosphate buffer at pH 6.8 in a mortar and then centrifuged at 1.500 g/20 min at 6°C . The resultant supernatant fluids were processed for enzyme assays.

Peroxidase activity (PO): Peroxidase was assayed using photochemical method as described by [11] Amako et al. The reaction mixture was added as the following sequences, 1500 ml phosphate buffer., 480 ml hydrogen peroxidase., 1000 ml pyrogallol, 20 ml sample extract. The increasing in the absorbance at 430 nm was recorded against blank with phosphate buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme, which changing the optical density at 430 nm per min. at 25°C under standard assay conditions. Specific activity was expressed in units by dividing it to mg protein.

Polyphenol oxidase (PPO): Polyphenol oxidase was assayed using photochemical method as described by Coseteng and Lee [12]. The reaction mixture was added as the following sequences: 2.7 ml potassium phosphate buffer 90.05 M, pH 6.2, 0.25 ml of 0.25 M catechol, 0.05 ml of enzyme extract. The increasing in absorbance at 420 nm was measured. One unit of enzyme activity is defined as the amount of the enzyme that causes an increase of 0.001 absorbance unit per minute at 25°C .

Data analysis: Statistically, the obtained data were subjected to analysis of variance (ANOVA) [13] (Gomez and Gomez) followed by Duncan's multiple range tests to compare means [14].

Results

The influence of the two bio-control agents namely Bio-arc (a commercial formulation of *B. megaterium*) at four tested rates (5, 10, 15, and 20 ml) and Nemastrol @ 0.25 ml singly and concomitantly on plant growth response of sugar-beet plant var. Negma infected with *M. incognita* and grown in two soil types i.e. clayey and sandy is summarized Table 1. (Results revealed that *M. incognita* infection caused a significant reduction in plant growth parameters (shoot and root length, shoot weight) with reduction percentage in total plant fresh weight reached 35.0 and 64.0% in clayey and sandy soil respectively. Irrespective to soil type and tested rates, all treatments showed remarkable increase in plant growth parameters in terms of shoot length, shoot and root weight with various degrees. In single application, it was evident that the effectiveness of bio-arc to enhance plant growth parameters increased with the increase of addition in the two soil types. Plant growth response of sugar-beet infected with *M. incognita* was pronounced in sandy soil more than clayey soil. In sandy soil, a significant improvement in shoot length (85.8%), plant fresh weight (174.1%) and shoot dry weight (350.0%) was recorded with Bio-arc @ the rate of 20 ml/plant. Similar trend was noticed with sugar-beet grown in clayey soil with percentage of increase in shoot length, total plant fresh weight and dry shoot weight reached 70.4, 41.7 and 180.0%, respectively. However, Nemastrol at the rate of 0.25 ml resulted a pronounced improvement in plant growth parameters in terms of shoot length (100.0, 10.8%), total plant fresh weight (70.5, 44.4%) and shoot dry weight (200.0, 150.0%) of sugar-beet grown in clayey and sandy

Treatments	Rates/ml	*Plant growth response					Shoot Dry wt.(g)	Inc. %	
		Shoot Length (cm)	Inc.%	Shoot fresh wt. (g.)	Root fresh wt. (g.)	Total Plant fresh wt.(g)			
Clayey Soil									
Bio-arc	5	18.3 ^a	35.6	10.9 ^{de}	5.8 ^d	16.7	26.5	1.7 ^{ef}	70.0
	10	21.0 ^d	55.6	11.3 ^{c-e}	5.8 ^d	17.1	29.5	1.7 ^{ef}	70.0
	15	22.0 ^{cd}	63.0	11.7 ^{c-e}	6.2 ^d	17.9	35.6	2.2 ^{de}	120.0
	20	23.0 ^c	70.4	12.0 ^{cd}	6.7 ^{cd}	18.7	41.7	2.8 ^{cd}	180.0
Nemastrol	0.25	27.0 ^a	100.0	13.7 ^{a-c}	8.8 ^a	22.5	70.5	3.0 ^{b-d}	200.0
Bio-arc+Nemastrol	5+0.25	25.0 ^b	85.2	12.4 ^{b-d}	8.5 ^{ab}	20.9	58.3	3.2 ^{a-c}	220.0
	10+0.25	25.3 ^{ab}	87.4	13.6 ^{a-c}	8.7 ^a	22.3	68.9	3.5 ^{a-c}	250.0
	15+0.25	25.5 ^{ab}	88.9	14.9 ^{ab}	8.5 ^{ab}	23.4	77.3	3.7 ^{ab}	270.0
	20+0.25	26.0 ^{ab}	92.6	15.9 ^a	9.4 ^a	25.3	91.7	3.9 ^a	290.0
Oxamyl		18.8 ^e	39.3	11.9 ^{cd}	7.6 ^{bc}	19.5	47.7	1.3 ^f	30.0
Plant free of N		13.8 ^f	2.2	11.1 ^{de}	9.2 ^a	20.3	53.8	1.2 ^f	20.0
N alone		13.5 ^f	0.0	9.3 ^e	3.9 ^e	13.2	0.0	1.0 ^f	0.0
Sandy Soil									
Bio-arc	5	12.9 ^{ef}	7.5	2.1 ^f	2.5 ^{ef}	4.6	70.4	0.6 ^{g-i}	50.0
	10	14.5 ^e	20.8	2.1 ^{ef}	2.9 ^{d-f}	5.0	85.2	0.9 ^{f-h}	125.0
	15	17.3 ^d	44.2	2.9 ^{de}	2.5 ^{e-g}	5.4	100.0	1.5 ^d	275.0
	20	22.3 ^c	85.8	4.4 ^c	3.0 ^{de}	7.4	174.1	1.8 ^{cd}	350.0
Nemastrol	0.25	13.3 ^{ef}	10.8	2.1 ^f	1.8 ^{gh}	3.9	44.4	1.0 ^{fg}	150.0
Bio-arc+Nemastrol	5+0.25	18.0 ^d	50.0	3.1 ^d	2.2 ^{f-h}	5.3	96.3	1.0 ^{ef}	150.0
	10+0.25	22.5 ^c	87.5	5.4 ^b	4.5 ^c	9.9	266.7	2.2 ^{ab}	450.0
	15+0.25	25.5 ^{ab}	112.5	5.7 ^b	5.3 ^b	11.0	307.4	2.1 ^{bc}	425.0
	20+0.25	27.3 ^a	127.5	6.7 ^a	6.0 ^a	12.7	370.4	2.5 ^a	525.0
Oxamyl		18.5 ^d	54.2	1.3 ^g	1.6 ^h	2.9	7.4	0.6 ^{hi}	50.0
Plant free of N		24.0 ^{bc}	100.0	4.2 ^c	3.3 ^d	7.5	177.8	1.5 ^{de}	275.0
N alone		12.0 ^f	0.0	1.0 ^g	1.7 ^h	2.7	0.0	0.4 ⁱ	0.0

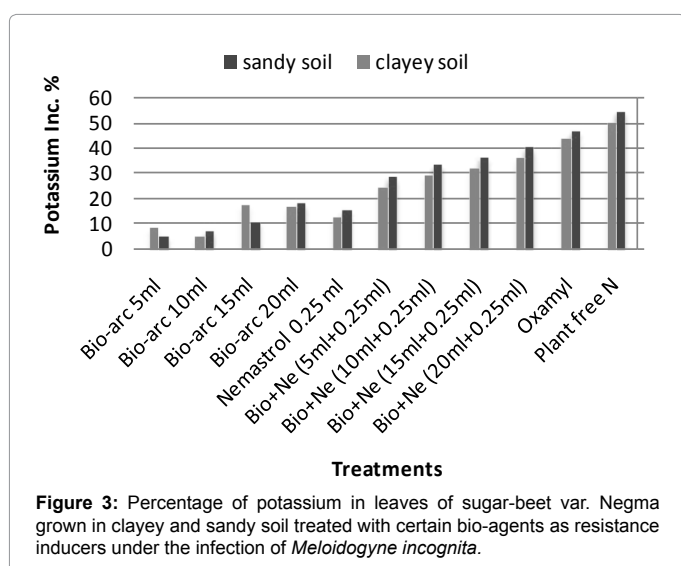
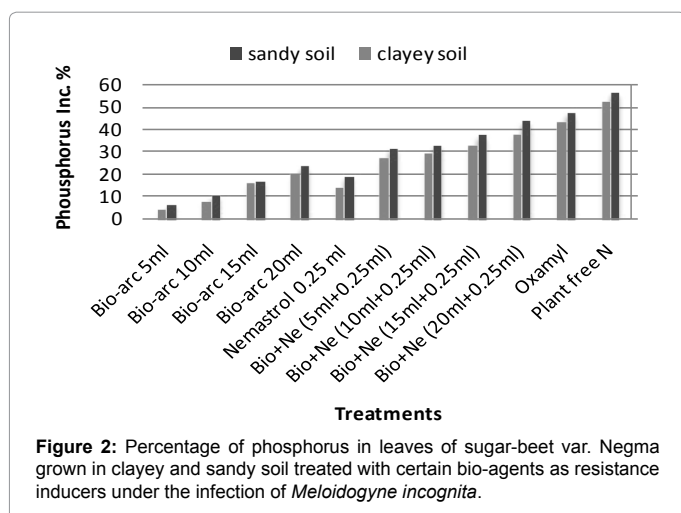
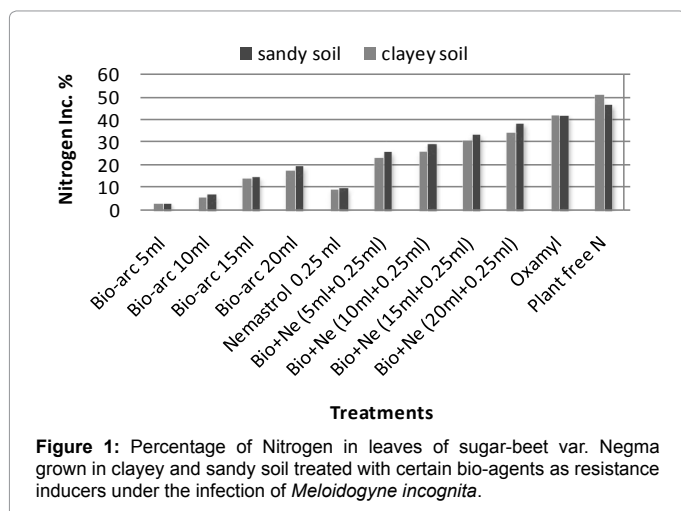
Means in each column followed by the same letter(s) did not differ at P≤0.05 according to Duncan's multiple range test. Each value presented the mean of four replicates N = *M. incognita* (2000 eggs/ plant)

Table 1 : Impact of Bio-arc (*Bacillus megaterium*) and Nemastrol (a mixture of active ingredients) on plant growth response of sugar-beet var. Negma grown in two soil types infected with *M. incognita* under greenhouse conditions (27 ± 3°C).

Treatments	Rates/ ml	No. of galls*	RGI**	No. of egg masses	EI*	No. of eggs/ eggmass	Nematode population in Soil	Females population in Root	Final population (Pf)	Rf***
Clayey soil										
Bio-arc	5	32.0 ^b	4.0	22.3 ^b	3.0	1009.95 ^b	584.5 ^b	34.5 ^b	23141.4	11.6
	10	29.8 ^b	3.0	20.8 ^b	3.0	874.95 ^c	439.5 ^c	33.5 ^b	18672.5	9.3
	15	20.5 ^c	3.0	17.5 ^c	3.0	689.95 ^d	399.5 ^d	22.5 ^c	12496.4	6.2
	20	20.0 ^{cd}	3.0	14.5 ^{cd}	3.0	587.45 ^e	388.5 ^e	21.3 ^{cd}	8927.8	4.5
Nemastrol	0.25	13.8 ^e	3.0	11.3 ^e	3.0	312.45 ^f	299.5 ^k	15.8 ^e	3846.0	1.9
Bio-arc +Nemastrol	5+ 0.25	19.3 ^{cd}	3.0	14.3 ^{cd}	3.0	489.95 ^f	372.5 ^f	20.5 ^{cd}	7399.3	3.7
	10+ 0.25	18.0 ^{c-e}	3.0	13.0 ^{de}	3.0	417.45 ^g	361.2 ^g	19.5 ^{c-e}	5807.6	2.9
	15+ 0.25	16.0 ^{de}	3.0	11.8 ^{de}	3.0	392.45 ^h	359.5 ^h	18.3 ^{c-e}	5008.7	2.5
	20+ 0.25	15.8 ^{de}	3.0	11.3 ^e	3.0	349.95 ⁱ	337.5 ⁱ	17.5 ^{de}	4309.4	2.2
Oxamyl		7.3 ^f	2.0	10.0 ^f	2.0	249.95 ^k	300.5 ^j	10.3 ^f	2810.3	1.4
N alone		53.5 ^a	4.0	43.8 ^a	4.0	1339.95 ^a	1399.5 ^a	54.8 ^a	60145.6	30.0
Sandy soil										
Bio-arc	5	41.5 ^b	4.0	33.3 ^b	4.0	1074.95 ^b	1443.8 ^{ab}	45.5 ^b	37285.9	18.6
	10	41.3 ^b	4.0	30.5 ^c	3.0	964.95 ^c	1000.5 ^{a-c}	44.0 ^b	30476.0	15.2
	15	17.3 ^d	4.0	30.3 ^c	3.0	862.45 ^d	810.0 ^{bc}	37.0 ^c	26979.7	13.5
	20	24.8 ^d	3.0	22.5 ^d	3.0	736.25 ^e	507.6 ^{bc}	26.3 ^d	17099.5	8.5
Nemastrol	0.25	14.3 ^{fg}	3.0	11.5 ^{fg}	3.0	351.25 ^j	279.5 ^c	15.0 ^f	4333.9	2.2
Bio-arc +Nemastrol	5 + 0.25	23.8 ^d	3.0	22.3 ^d	3.0	574.95 ^j	400.5 ^c	24.8 ^{de}	13246.7	6.6
	10+ 0.25	18.5 ^e	3.0	14.5 ^e	3.0	509.95 ^g	384.5 ^c	21.8 ^{de}	7800.6	3.9
	15+ 0.25	18.3 ^{ef}	3.0	12.8 ^{ef}	3.0	449.95 ^h	362.5 ^c	21.3 ^e	6143.2	3.0
	20+ 0.25	16.3 ^{ef}	3.0	11.8 ^{ef}	3.0	417.45 ⁱ	347.5 ^c	21.0 ^e	5294.4	2.6
Oxamyl		11.5 ^g	3.0	11.0 ^g	3.0	117.45 ^k	360.5 ^j	12.5 ^f	1665.0	0.8
N alone		58.5 ^a	4.0	50.5 ^a	4.0	1379.95 ^a	2069.5 ^a	61.0 ^a	71819.8	35.9

*Each value presented the mean of four replicates N = *M. incognita* (2000 eggs/ plant) . Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan's multiple range test. ** Root gall index (RGI) or egg masses index (EI) was determined according to the scale given by Taylor and Sasser as follows : 0= no galls or egg masses, 1= 1-2 galls or egg masses , 2= 3-10 galls of egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses and 5= more than 100 galls or egg masses ** Reproduction factor (Rf) = No. of eggs per root + Nematode population in soil + No. of developmental stages + No. of females/ No. of eggs inocula

Table 2: Impact of Bio-arc and Nemastrol (a mixture of active ingredients) singly and concomitantly on the development and reproduction of *M. incognita* infecting sugar-beet var. Negma grown in two soil types under greenhouse conditions at 27 ± 3°C.



soil respectively. In concomitant treatment, Bio- arc (20 ml)+Nemastron (0.25 ml) was the best and showed significant improvement in plant growth parameters in terms of shoot length (92.6, 127.5%) and total

plant fresh weight (91.7, 370.4) of sugar-beet grown either in clayey or sandy soil, respectively. Oxamyl as a standard nematicide showed moderate improvement in previous criteria of sugar-beet grown in clayey soil with increase percentages 39.3, and 47.4 respectively. Similar trend was noticed with shoot length (54.2%) and dry shoot weight (50%) of sugar-beet grown in sandy soil treated with oxamyl.

Regarding the impact of Bio-arc and Nemastron singly and concomitantly on the development and reproduction of the root-knot nematode, *M. incognita* infecting sugar-beet grown in clayey and sandy soil is documented (Table 2). Irrespective to soil type and tested rates results revealed that total nematode population was significantly suppressed with all tested treatments with reproduction factor ranged from 1.4 to 11.6 in clayey soil and from 0.8 to 18.6 in sandy soil compared to inoculated plants (Rf=30.0, 35.9) respectively. Among tested treatments, Nemastron significantly suppressed total nematode population (RF=1.9, 2.2), root galling (RGI=3.0, 3.0), number of egg masses (EI=3.0, 3.0) and number of eggs /10 egg masses (Red. %=76.5, 74.6) in clayey and sandy soil, respectively. However, concomitant treatment showed better results than did bio-arc alone at four tested rates. Among the concomitant treatment the greatest reduction in total nematode population was recorded in clayey and sandy soil which received the dual application of Bio-arc (20 ml) and Nemastron (0.25 ml) with reproduction factor 2.2, 2.6 and reduction percentages reached 92.8, 92.6%. Meanwhile, total nematode population was significantly suppressed with oxamyl introduced to clayey soil (Rf=1.4) and sandy soil (Rf=0.8) relative to control plants where Rf=30.0 and 35.9, respectively. Nevertheless, number of eggs/ egg mass were significantly suppressed with oxamyl application with percentage of reduction amounted to 76.7 and 74.5% in clayey and sandy soil respectively.

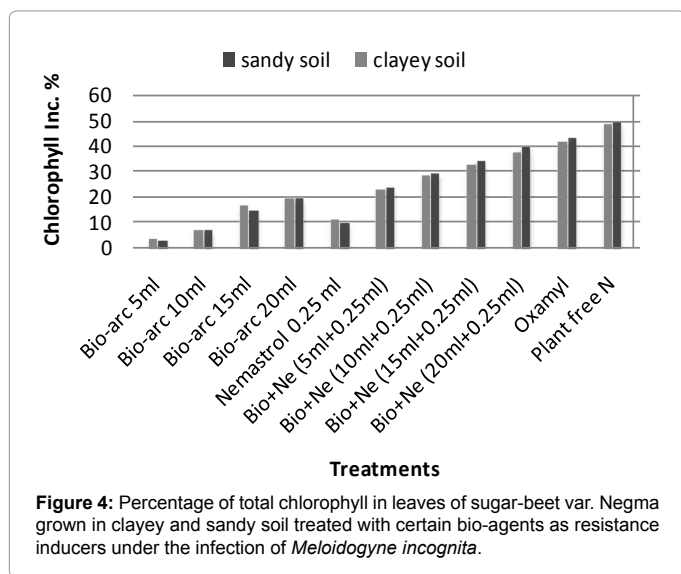
Biochemicals activities

Nitrogen, phosphorus and potassium contents: NPK contents were significantly suppressed due to nematode infection with reduction percentages 34.1, 35.5 and 39.2% in clayey soil and 32.2, 37.5 and 39.1% in sandy soil. However, a remarkable induction in NPK content was recorded with the application of Bio- arc + Nemastron (20 ml+0.25 ml) with % of increase amounted to 34.94, 41.40, 48.43 and 39.55, 45.0, 48.99 in clayey and sandy soil respectively (Figures 1-3).

Total chlorophyll content: Chlorophyll *a* and *b* were moderately affected due to nematode infection with reduction % in total chlorophyll reached 32.5 and 32.3% in clayey and sandy soil respectively. Application of such treatments revealed a considerable induction with the dual application of Bio + Nemastron (20 ml+ 0.25 ml) and oxamyl as well with % of increased reached 37.0, 42.6 and 38.6, 40.9% in clayey and sandy soil consecutively (Figure 4).

Total carbohydrates: Total carbohydrates were significantly suppressed due to nematode infection with reduction percentage 20.38 and 16.9% in clayey and sandy soil respectively. The highest increase was recorded in leaves of sugar-beet treated with Bio-arc+Nemastron (20 ml+0.25 ml) with values 13.4 and 15.3% in clayey and sandy soil respectively (Figure 5).

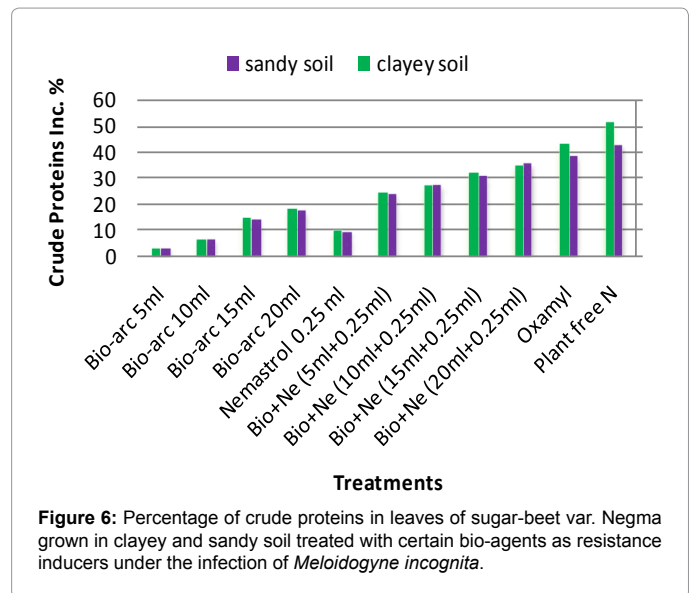
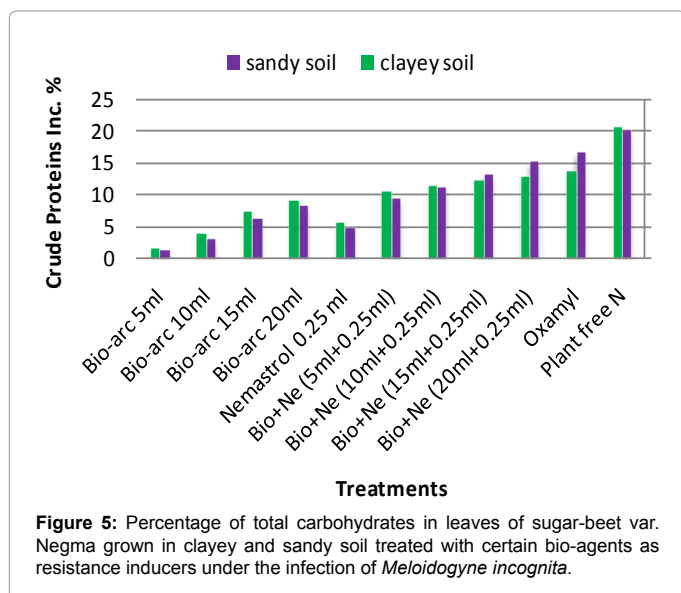
Crude proteins: Untreated sugar-beet infected with *M. incognita* exhibited significant reduction in total proteins as compared with untreated uninoculated plants with percentage of reduction reached 34.1 and 32.1% in clayey and sandy soil respectively. Among all tested compounds, the highest increase percentage in crude proteins was obtained with oxamyl (43.4 and 42.2%) in clayey and sandy soil, respectively (Figure 6) followed by Bio-arc+Nemastron (20 ml+0.25 ml) with values averaged 35.0 and 39.2% in clayey and sandy soil,



then Nemastronl compared to untreated inoculated plants. In such treatments, the dual application Bio-arc + Nemastronl performed the best since PO& PPO activities were increased and reached their peak at the 3rd day after nematode inoculation then declined at 9th day followed by slightly increment at 15th day . Meanwhile, the increased activity of PO &PPO remained higher in plants treated with oxamyl and reached their peak at 9th day after nematode inoculation.

Discussion

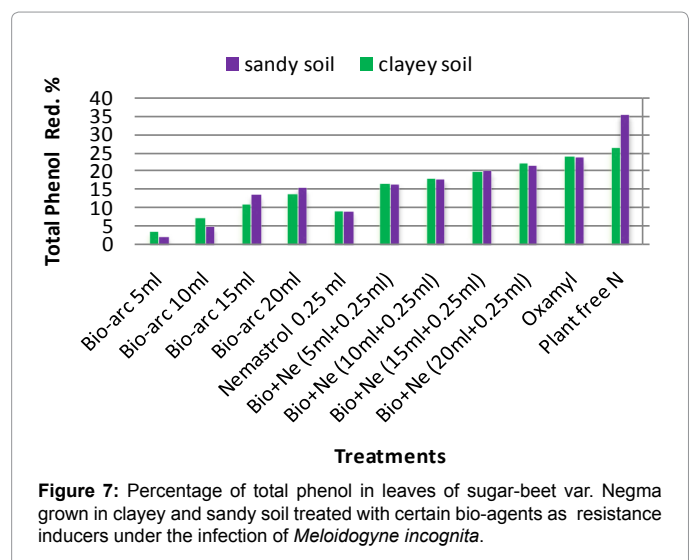
Induction of systemic resistance (ISR) of plants against pathogens is a widespread phenomenon that has been intensively investigated in fungi and bacteria with respect to its potential use in plant protection. However, little attention has been given to nematode pests. Acquired or induced resistance can be achieved by inoculating a plant with incompatible or weak pathogens or by applying biotic or abiotic inducers [15]. The root-knot nematode, *M. incognita* caused a significant reduction in plant growth parameters (shoot and root length, shoot weight) with reduction percentage in total plant fresh weight reached 35.0 and 64.0% in clayey and sandy soil respectively. Application of



respectively.

Phenol content: The total phenol evaluated in leaves of sugar-beet infected with *M. incognita* revealed a moderate enhancement compared to control plants. However, phenol content showed different degrees of reduction in all treatments compared to untreated uninoculated plants grown in clayey and sandy soil. (Figure 7)

Defense related proteins: The tested materials viz. Bio-arc (20 ml), Nemastronl (0.25 ml), Bio-arc+Nemastronl (20 ml+0.25 ml) and oxamyl as well differed in their ability to stimulate Peroxidase (PO) and Polyphenol Oxidase (PPO) activities in sugar-beet plant inoculated with *M.incognita* (Figure 8). In untreated uninoculated plants, the activities of PO and PPO remained higher and attained their peak at the 9th day and thereafter a decline was noticed at 15th day. On the other hand, the least induction of PO and PPO was recorded with plants untreated and inoculated with nematodes and showed slight decline 3 days after nematode inoculation then increased and reached their peak at 9th day. However, increased PO and PPO activities were more pronounced in Bio-arc+Nemastronl (20 ml+0.25 ml) followed by oxamyl



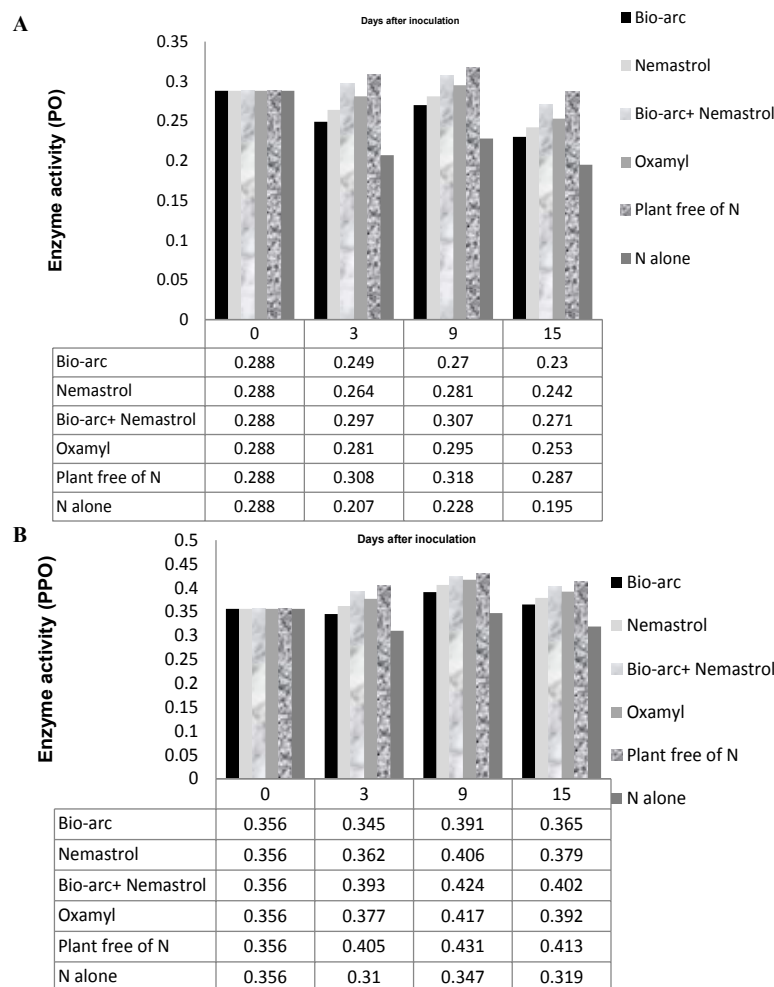


Figure 8: Impact of Bio-arc and Nemastrol as biotic resistance inducers on peroxidase (PO) and polyphenol oxidase (PPO) activities in roots of sugar-beet var. Negma after 0, 3, 9 and 15 days of *Meloidogyne incognita* inoculation. A= peroxidase activity, B= polyphenol oxidase activity.

phosphorus solubilizing bacterium (PSB), *B. megaterium* singly or concomitantly with Nemastrol has potential as a promising biocontrol candidate against root-knot nematode, *M. incognita* infecting sugar-beet var. Negma. As for single application, the effectiveness of Bio-arc to enhance plant growth parameters increased with rates increase in the two soil types.

Plant growth response of sugar-beet infected with *M. incognita* was more pronounced in sandy soil than clayey soil. In sandy soil, a significant improvement in shoot length, plant fresh weight and shoot dry weight was recorded with Bio-arc @ the rate of 20 ml/plant. This result support the findings of El-Deriny and Ibrahim [16,17]. However, in concomitant treatment, Bio- arc+Nemastrol (20 ml+0.25 ml) performed the best and showed significant improvement in plant growth parameters in terms of shoot length (92.6; 127.5%) and total plant fresh weight (91.7; 370.4%) of sugar- beet grown either in clayey or sandy soil. The presence of cytokinins in Nemastrol suggests a dynamic role for lateral root development. Irrespective to soil type and rates of application, total nematode population, root galling, number of egg masses and number of eggs/ egg mass were significantly suppressed with all treatments of Bio-arc and/or Nemastrol. The phosphate solubilizing bacterium (PSB) *B. megaterium* is considered a microorganism capable of dissolving the

unavailable phosphorus compounds in soil rendering them available for growing crops [18]. Increased phosphorus concentration may lead to reduction in root-knot nematodes. *B. megaterium* has been evaluated for their effects on a variety of root-knot nematodes [16,17,19,20] reported that *B. megaterium* greatly reduced numbers of galls, females and egg masses of *M. incognita* in the roots of sugar-beet followed by *B.subtilis*, *Paecilomyces lilacinus*, *P. fumosoreus* and *Trichoderma album* respectively. Furthermore, *B. megaterium* can extensively colonize the rhizosphere and reduce the sugar-beet cyst nematode infection under greenhouse trials [21]. *B. megaterium* produce antibiotic compounds [22] although no compounds from *B. megaterium* have been reported with activity against nematodes.

Nevertheless, Nemastrol performed the best and significantly suppressed total nematode population; root galling, number of egg masses and number of eggs /egg mass in clayey and sandy soil. The suppressive effect of such product could be attributed to the presence of mixture of enzymes i.e. chitinase and glucanase that dissolve chitin of nematode egg shell. However, concomitant treatment using Nemastrol+ Bio-arc showed better results than did Bio-arc alone at four tested rates. The greatest reduction in total nematode population was recorded with clayey and sandy soil receiving the dual application of Bio-arc (20

ml) and Nemastrol (0.25 ml) with reproduction factor and reduction percentage reached 2.2; 2.6 and 92.8; 92.6% respectively.

The impact of screened treatments on chemical components viz. NPK, chlorophyll, total carbohydrates, crude protein and total phenol in sugar-beet leaves infected with *M. incognita* revealed a remarkable induction in chemical constituents with the application of Bio-arc+Nemastrol (20 ml+0.25 ml). Conversely, the highest increase in total phenol percentage was recorded with untreated inoculated plants as a hypersensitive reaction (HR) to nematode infection. Plants are endowed with defense genes which are quiescent in healthy plants. When these genes are activated with various factors they induce systemic resistance against disease. Rhizobacteria induce systemic resistance by activation of various defense-related enzymes viz. PO, PPO and PAL. Recently, research work has demonstrated that the bio-agent *Pseudomonas fluorescens* might stimulate the production of biochemical compounds associated with the host defense [23]. Of these, early induction of peroxidase is more important as it is the first enzyme in the phenylpropanoid pathway, which leads to production of phytoalexin and phenolic substances leading the formation of lignin [24]. Conspicuously, the current investigation recorded the higher activity of Peroxidase in plants treated with Bio-arc+Nemastrol (20 ml+0.25 ml) and reached its peak at 9 days generating the speculation of induced defense responses in sugar-beet infected with *M. incognita*. Peroxidase activity in roots is important in the reinforcement of cell walls at the border of infection in resistant plants and that are considered as important components of active defense response of nematode invaded tissue [25]. The trend of increasing PPO activity was similar to that of PO in all treatments. Increased activity of Peroxidase (PO) or Polyphenol Oxidase (PPO) has been elicited by biocontrol agent strains in different plants [26,27]. Finally it can be concluded that use of such inducers viz. Bio-arc and Nemastrol singly and concomitantly represent a promising new approach for the control of the target root-knot nematode, *M. incognita* infecting sugar- beet within an environmental friendly integrated pest management via enhancing the resistance of plant to nematode.

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