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EFFICACY OF SOME ALGAL SPECIES, AZOLLA AND COMPOST EXTRACT IN CONTROLLING ROOT KNOT NEMATODE AND ITS REFLECTION ON CUCUMBER GROWTH

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By

S. M. Shawky , S. S. M. Mostafa* and A. A. M. Abd El-All *

Nematode Research Department, Plant Pathology Research. Institute Agricultural Research Center, Giza, Egypt

**Microbiol. Department, Soils, Water & Environment. Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt*

ABSTRACT

This study aimed to use nine culture filtrates of algal strains (*Nostoc muscorum*, *Anabaena flos aquae*, *Anabaena oryzae*, *Chlorella vulgaris*, *Wollea saccata*, *Phormedium fragile*, *Oscillatoria* sp., *Nostoc humifusum* and *Spirulina platensis*), *Azolla pinnata* aqueous extract filtrate (1:2 w/v) and compost watery extract filtrate (1:5 w/v) in controlling the population of the root knot nematode, *Meloidogyne incognita* in cucumber under both laboratory and greenhouse conditions. Laboratory experiment revealed that high juvenile mortality percentage occurred during all the exposure periods of all treatments, the best results were after 72 hr exposure. Only five cyanobacterial strains, namely, *Spirulina platensis*, *Oscillatoria* sp., *Anabaena oryzae*, *Nostoc muscorum* and *Phormedium fragile*, followed by compost watery extract, significantly increased juveniles mortality over 70% at the highest concentration of 1:10 (84.3, 80.4, 78.9, 75.4, 72.5 and 70.1%, respectively). A*zolla pinnata* aqueous extract filtrate achieved 69.8% at the same concentration while, *Anabaena flos aquae* and *Chlorella vulgaris* recorded the lowest effect on mortality percentage (52.1 and 40.1%, respectively) at the concentration of 1:10. In the greenhouse experiment, the combination of mixing five algal culture filtrates of *S. platensis*, *Oscillatoria* sp., *A. oryzae*, *N. muscorum* and *P. fragile* with *A. pinnata* aqueous extract filtrate and compost extract achieved the highest reduction in the number of the 2^{nd} stage juveniles in soil, the numbers of galls, developmental stages, females, egg masses, egg numbers/egg mass in roots of cucumber plants comparing with the individual treatment and the non treated control. In addition, all combinations significantly improved fresh weight of roots and shoots and increased the yield of cucumber plants. The combined treatment of mixed algal culture filtrates + *A. pinnata* aqueous extract filtrate + compost extract significantly enhanced the soil biological activity in terms of total bacterial count, total cyanobacterial count, CO_2 evolution, dehydrogenase and nitrogenase activities as well as the soil available nitrogen forms (NH_4^+, NO_3^- and NO_2^-), available phosphorus and potassium over the control and resulting in soil electrical conductivity (EC) and soil pH reduction. It could be recommended that application of bio-organic agents as nematicidal against root knot nematode in cucumber is preferable in order to reduce the soil and plant polluting chemical nematicides.

Key words: *algal culture filtrates, azolla pinnata aqueous extract filtrate, compost watery extract, cucumber.*

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a favorite commodity export for markets and local consumption and represents one of the most important and economic vegetables in Egypt. It is grown in Egypt in the open fields from March to November and under plastic houses from September to May (Bayoumi and Hafez, 2006). Root-knot nematodes, *Meloidogyne* spp., are among the most damaging nematodes in agriculture, causing an estimated US$ 100 billion loss/year worldwide (Oka *et al.*, 2000). Root-knot nematode is a serious malady and causes significant losses in cucumber yield if not treated with nematicides. After hatching from eggs, second-stage juveniles invade roots of host plants and migrate intercellularly to differentiating vascular regions. The symptoms of nematode infection are the formation of root galls which result in growth reduction, nutrients and water uptake reduction, wilting increase and mineral deficiency, resulting in weak and poor yielding plants (Abad *et al.*, 2003). The application of chemical nematicides has been found as an effective measure for controlling nematodes but they have toxic residual effect on the environment

particularly on non-target organisms and human health. In addition, the use of chemical nematicides is prohibited in organic farming. Therefore, there is an urgent need to develop alternative environmental safe strategies for controlling nematodes (Anastasiadis *et al*., 2008). During the last decades, research on nematode control was focused on proposing strategies for the inhibition of egg hatch (Westcott and Kluepfel, 1993), degradation of hatching factor (Oostendrop and Sikora, 1989) or production of metabolites (Meadows *et al.*, 1989). Recently, one of the biological control practices attempted is the study of the nematicidal potential of cyanobacterial culture filtrates that parasitize plant-parasitic nematodes (Khan *et al.*, 2005).

Blue-green algae (Cyanobacteria) are distributed world-wide and contribute to the fertility of many agricultural ecosystems, either as free-living organisms or in symbiotic association with the water-fern *Azolla* (Fay, 1983). *Azolla*, a dichotomously branched free floating aquatic fern, is naturally available mostly in the tropical belt of India. The dorsal lobe which remains exposed to air has a specific cavity containing its symbiotic partner, a blue green algae *Anabaena azollae*. Abundant growth of *Azolla* not only makes a useful addition of combined nitrogen to the ecosystem but can also provide a 'green manure'.

Cyanobacteria that excrete a great number of substances have been reported to benefit plants by producing growth-promoting regulators (PGPR), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol, and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity (Zaccaro *et al*., 2001).

Nematicidal potential of Cyanobacteria has remained unexplored except for a few reports, which suggest that endospores of *Microcoleus* and *Oscillatoria* spp. killed nematodes (Dhanam *et al*., 1994). Culture filtrates of *Microcoleus vaginatus* inhibited hatching of *Meloidogyne incognita* eggs and killed second stage juveniles (Khan *et al.*, 1997). Microalgal metabolites have attracted attention, because they are a resource for toxins, and potential new drugs (Shimizu, 2003).

Compost watery extract, generally defined as a liquid fertilizer, has gained acceptance by homeowners and commercial growers around the world for its ability to stimulate soil biological activity, improve soil structure, and enhance overall plant health and vigor. Reported benefits of using compost extract include enhanced disease suppression, reduced fungicide and fertilizer requirements and associated cost savings. Research efforts to validate these benefits are expanding. Compost extract affect soils, crops and the organic farming system, and have an important role in decreasing nematode population (El Gendy and Shawky, 2006).

This investigation aimed to evaluate the efficacy of algal culture filtrates, *Azolla pinnata* fresh biomass aqueous extract and compost watery extract on the root knot nematode *Meloidogyne incognita* activity *in vitro* under laboratory conditions to select the most promising results to be applied in controlling *M. incognita* in cucumber plants in greenhouses.

2. MATERIALS AND METHODS

2.1. Cyanobacteria and *Azolla pinnata* source and growth conditions

Cyanobacteria strains (*Anabaena flos aquae, Anabaena oryza*e, *Nostoc humifusum, Nostoc muscorum, Oscillatoria* sp., *Spirulina platensis, Phormedium fragile* and *Wollea saccata*) and the green alga strains *Chlorella vulgaris* and *Azolla pinnata* were obtained from the Microbiology Department, Soils Water and Environment Res. Inst., ARC, Giza, Egypt. Cyanobacteria strains were maintained in BG11 medium (Rippka *et al.*, 1979) except *Spirulina platensis* which was cultured in Zarrouk medium (Zarrouk, 1966), while Bold medium (Nichols and Bold, 1965) was used for the green alga *Chlorella vulgaris*. Cultures were incubated in a growth chamber under continuous illumination (2000 lux) and tempe- rature of 25 ˚C ± 2 ˚C for all strains except the mesophilic alga *Spirulina platensis* (32 ˚C ± 2 ˚C). *Azolla pinnata* was grown on modified Yoshida medium (Yoshida *et al.*, 1976).

2.2. Preparation of algal culture filtrates and *Azolla pinnata* aqueous extract

After 30 days of incubation, each algal biomass was separated from its culture medium by filtration. The growth parameters of algal cultures and chemical analyses of their filtrates were determined (Table 1). *Azolla pinnata* was harvested from the culture medium and mixed well with distilled water (1:2 w/v) using an electric mixer, then filtered to obtain the fresh biomass aqueous extract. The chemical composition of *Azolla pinnata* fresh biomass and aqueous solution is presented in Table (2). The algal culture filtrates and *Azolla* fresh biomass aqueous extract (50%) were kept at 4^{o}C till used in laboratory and greenhouse experiments.

Table (1): Algal growth parameters and chemical analyses of algal culture filtrates

Treatments	*Nostoc muscorum*	*Anabaena flous aquae*	*Chlorella vulgaris*	*Oscillatoria* sp.	*Spirulina platensis*	*Anabaena oryzae*	*Wollea saccata*	*Nostoc humifusum*	*Phormedium fragile*
	Cultures growth parameters								
pH	**7.63**	**6.61**	**8.11**	**5.82**	**10.48**	**6.27**	**6.11**	**8.70**	**9.33**
OD	**1.16**	**1.16**	**1.611**	**0.22**	**2.50**	**1.76**	**1.98**	**1.20**	**1.66**
Ch-a (mgl^{-1})	**4.34**	**1.78**	**5.88**	**4.80**	**23.45**	**5.87**	**14.25**	**5.23**	**2.86**
Dry weight	**744.32**	**727.65**	**1052.16**	**140.16**	**2622.40**	**1123.20**	**1267.84**	**760.80**	**1062.40**
	Culture filtrates chemical analyses								
pH	**7.05**	**7.21**	**8.15**	**6.63**	**9.9**	**7.11**	**7.39**	**7.42**	**8.55**
Ec (dSm^{-1})	**0.20**	**0.60**	**1.09**	**32.00**	**19.29**	**0.72**	**0.47**	**0.24**	**0.18**
	Available nutrients (ppm)								
NH_4^+-N	**14.63**	**16.21**	**9.87**	**10.99**	**1.71**	**2.22**	**12.44**	**13.49**	**2.71**
NO_3^{--}-N	**24.40**	**41.56**	**57.57**	**28.21**	**149.50**	**21.73**	**47.28**	**52.61**	**35.08**
NO_2^{--}-N	**1.09**	**0.84**	**1.26**	**2.10**	**5.05**	**2.95**	**2.10**	**4.21**	**0.84**
P	**44.90**	**0.75**	**38.42**	**9.73**	**300.00**	**0.50**	**3.49**	**19.21**	**4.99**
K	**11.03**		**77.55**	**13.23**	**652.90**	**23.16**	**22.05**	**12.86**	**11.76**

Table (2): Chemical composition of *Azolla pinnata* **fresh biomass and aqueous extract**

Azolla pinnata **fresh biomass**		***Azolla pinnata*** **aqueous extract filtrate (50%)**	
Moisture %	93.6	pH	7.84
Total nitrogen %	2.90	Ec ds/m	2.75
Total phosphorus %	0.80	Potassium (ppm)	148.49
Total potassium%	1.16	Phosphorus (ppm)	26.76
Organic carbon %	16.88	Organic carbon (ppm)	1628.68
Organic matter %	29.11	Organic matter (ppm)	2808.00
Ash %	70.89	NH_4^+-N (ppm)	4.432
C/N ratio	5.83	NO_3^{--}-N (ppm)	35.08
		NO_2^{--}-N (ppm)	3.79

2.3. Compost watery extract preparation

Compost watery extract was prepared from compost suspended in water (1:5, v:v) for 7 days and supplied with aquarium pump for continuous aeration (Al-Dahmani *et al.*, 2003). The complete biochemical and biological analyses for compost and its watery extract filtrate are presented in Table (3).

Table (3): Compost and compost watery extract (1:5) biochemical and biological analvses

Characters	Compost	Compost extract
Moisture %	21.00	-
pH (1:10)	8.57	8.42
Ec ds.m^{-1} (1:10)	5.43	18.19
N-NH_4^+ (ppm)	480.00	35.0
N-NO_3^{--} (ppm)	80.00	22.0
Total nitrogen %	1.53	0.012
Organic matter %	37.60	0.052
Organic carbon %	21.80	0.03
Ash%	62.40	-
C/N ratio	14.2	2.5
Total phosphorus%	0.29	0.04
Total potassium%	0.79	0.006
Coliform group (cfu)	nd	nd
Salmonella and Shigella (cfu)	nd	nd
Nematode	nd	nd

2.4. Efficacy of algal culture filtrates, *Azolla pinnata* **aqueous extract filtrate and compost extract on the activity of** *Meloidogyne incognita* **juveniles under laboratory conditions**

For studying the efficacy of the algal culture filtrates, *Azolla pinnata* aqueous extract and compost extract on *Meloidogyne incognita* juveniles *in vitro*, 1 ml of each treatment at three concentrations (1:10 ,1:25, 1:50) of the prepared stock filtrates was added separately to 1 ml of nematode suspension containing 100 juveniles in glass vials. The numbers of active and non-active juveniles were examined and counted microscopically after 24, 48 and 72 hours.

The following treatments were allocated:

1- *Anabaena flos aquae* 2-*Anabaena oryzae*
3- *Chlorella vulgaris* 4- *Nostoc humifusum*
5-*Nostoc muscorum* 6-*Phormedium fragile*
7-*Oscillatoria* sp. 8- *Spirulina platensis*
9-*Wollea saccata*
10- *Azolla pinnata* aqueous extract filtrate
11-Compost extract 12-Vydate (nematicide)

2.5. Efficacy of algal culture filtrates, *Azolla pinnata* **aqueous extract filtrate and compost extract individually and/or in combination on cucumber infection by** *Meloidogyne incognita* **under greenhouse conditions**

This experiment was conducted under greenhouse conditions at Giza Governorate (Gezerat El Dahab) during the summer season of 2008**.** The soil texture was clay loamy, soil pH in water suspension (l:2.5) was 7.68 and EC of soil paste at 25 ^{o} C was 1.53 ds.m^{-1} (AOAC, 1980).

One month-old cucumber seedlings, cv. DP163, were transplanted in nine greenhouses soil naturally infested with *Meloidogyne incognita*. Each greenhouse was divided into 5 ridges. Seedlings were transplanted on the two sides of the ridge (40 meters in length and 1 meter in width) at a spacing of 30 cm between plants within the row. Surface irrigation was used. The treatments were thirteen, each applied to three rows and the last six rows were left without treatment (nematode only) to serve as control. All treatments including control received the same agricultural practices. All treatments were applied at the rate of 25 ml.m^{-2} ,simulating application of 105 l.fad^{-1}, as soil drench added twice, after 7 and 15 days from transplanting. Plants were carefully uprooted and fresh shoot, root and fruits were weighed after 60 day from transplanting. Thereafter, the number of juveniles per 250 g soil and nematode populations in roots were counted according to Franklin and Goodey (1957).

The treatments under greenhouse conditions were as follows:

1- *Anabaena oryzae*. 2- *Nostoc muscorum.*
3- *Oscillatoria* sp. 4- *Phormedium fragile.*
5- *Spirulina platensis*.
6- *Azolla pinnata* aqueous extract filtrate.
7- Compost watery extract filtrate.
8- Mixture of five algae: *Anabaena oryzae, Nostoc muscorum, Oscillatoria* sp, *Phormedium fragile* and *Spirulina platensis* (Mix).
9- Mix + *Azolla pinnata* aqueous extract filtrate.
10-Mix + Compost watery extract filtrate.
11- *Azolla pinnata* extract + Compost watery extract filtrate.
12- Mix + Compost watery extract filtrate + *Azolla pinnata* aqueous extract filtrate.
13- Vydate as chemical nematicide at the recommended concentration (24% Ec).

14- Control with nematodes only (without any treatments).

Data were analyzed in the Central Laboratory for Design and Statistical Analyses in the Agricultural Research Center, Egypt**.** Data were subjected to statistical analyses "ANOVA" as described by Sendecor and Cochran (1980).

2.6. Soil biological and chemical analyses

2.6.1. Soil biological activity: CO_2 evolution was determined according to Gaur *et al.* (1971), total bacterial count was performed on nutrient agar using the spread plate method (APHA, 1985), total cyanobacterial counts by plating ten-fold serial soil suspension-dilutions in triplicate onto agarized BG_{11} medium (Stanier *et al.,* 1971). Soil enzymes, *i.e*., dehydrogenase activity (DHA), was estimated according to Casida *et al.* (1964), while nitrogenase activity was measured by acetylene reduction assay as described by Dart *et al.* (1972).

2.6.2. Soil chemical analyses: Soluble nitrogen forms (NH_4^+, NO_2^- and NO_3^-N) were determined in the soil extract which was prepared according to Allam (1951). NH_4^+-N was determined by the method described by Fawcett and Scott (1960), while NO_2^- and NO_3^--N were determined according to Deutsche Einheitsverfahren (1960). Available phosphorus was extracted using the method described by Soltanpour (1985) and determined spectrophotometrically as mentioned by Olsen and Watanabe, (1965). Available potassium was extracted using the method described by Soltanpour (1985) and determined using flame-photometric method (APHA, 1992). Soil reaction (pH) was measured in 1:2.5 soil water extract using glass electrode pH meter Model (955), and electric conductivity (EC) was measured in 1:5 soil water extract using glass electrode conductivity meter Model Jenway 4310.

3. RESULTS AND DISCUSSION

3.1. Efficacy of algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost watery extract filtrate on the activity of** *M. incognita* **juveniles under laboratory conditions**

Data in Table (4) illustrate that all treatment filtrates had various degrees of effectiveness toward the mortality % of nematode juveniles. Moreover, the percentage of mortality increased with increasing the concentration and exposure period. *Spirulina platensis* (1:10) recorded the highest mortality percentages of nematode juveniles of 79.2, 81.3 and 84.3 after 24, 48 and 72 hrs. exposure time, respectively. *Oscillatoria* sp, *A. oryzae, N. muscorum, P. fragile*, compost watery extract and *Azolla pinnata aqueous extract* ranked in the second level in nematode juveniles mortality Data % at 1:10 during all exposure periods. These treatments showed a lethal effect on nematode juveniles and the mortality percentages were over 70 after 24 hours exposure, except compost watery extract and *A. pinnata* aqueous extract which achieved 66.9 and 65.4% respectively. By increasing the exposure period to 72 hrs. the mortality percentages raised to 80.4, 78.9, 75.4, 72.5, 70.1 and 69.8% with *Oscillatoria* sp, *A. oryzae, N. muscorum, P. fragile*, compost watery extract filtrate and *Azolla pinnata* aqueous extract filtrate; respectively. On the other hand, C*hlorella vulgaris* had the lowest effect on nematode juveniles percentage (44.2) by the end of the exposure period (72 hrs.).

The obtained results indicated that blue green algae such as *Spirulina platensis*, *Oscillatoria* sp., *Anabaena oryzae*, *Nostoc* muscorum and *Phormedium fragile* produce a great variety of secondary metabolites (Gervick *et al.*, 2001), such as nitrogen-containing compounds, polyketides, lipopeptides, cyclic peptides and many others. The efficacies of these algal culture filtrates decreased with their dilution, that may be due to the differences in toxic substances present in the culture filtrates. Similarly, Khan *et al.* (1997) reported that the efficacy of culture filtrates of the cyanobacterium *Microcoleus vaginatus* against egg hatching and mortality of *Meloidogyne incognita* was dependent on its concentration and period of exposure.

The high mortality percentages recorded for nematode juveniles exposed to the algal culture filtrates may be due to the presence of some phenolic compounds and mineral salts that facilitate and accelerate the rate of penetration of algal byproducts through snail's skin, hence increasing their harmful effects (Bakry *et al.,* 1999 and Mahmoud, 2001).

The presence of high quantities of acrylic acid in *Spirulina* was substantiated at the end of the seventies. This substance shows anti-microbial activity, at a 2 mg/l of biomass concentration. Propionic, benzoic and mandelic organic acids were also found (Lee, 2004). Abd El-Baky *et al.* (2009) indicated that *S. platensis* secretes organic substances or metabolic products such as phycobiline, phenols, terpenoids, steroids, polysaccharides and saponins. Low concentrations of saponin fractions increased mortality of *B. alexandrina* snails, miracidia, cercariae and adult worms of *Schistosoma mansoni*, and decrease atchability and egg production (Tadros *et al.*, 2008).

Table (4): Effect of algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost watery extract filtrate on the activity of** *M. incognita* **juveniles after different exposure periods under laboratory conditions.**

	Conc.	Exposure periods (in hours)		
		24	48	72
		Mortality%		
Control (Nematode only)		**0.9**	**1.2**	**1.8**
Anabaena flos aquae	**1:50**	**34.7**	**38.1**	**40.4**
	1:25	**41.5**	**43.5**	**47.5**
	1:10	**45.4**	**50.2**	**52.1**
Anabaena oryzae	**1:50**	**60.4**	**64.5**	**67.2**
	1:25	**68.3**	**70.2**	**73.7**
	1:10	**73.4**	**75.6**	**78.9**
Chlorella vulgaris	**1:50**	**32.2**	**35.2**	**37.5**
	1:25	**35.9**	**38.8**	**40.1**
	1:10	**40.1**	**42.4**	**44.2**
Nostoc humifusum	**1:50**	**50.4**	**53.2**	**57.6**
	1:25	**57.8**	**60.4**	**64.7**
	1:10	**63.4**	**65.5**	**67.8**
Nostoc muscorum	**1:50**	**58.7**	**60.7**	**64.5**
	1:25	**66.8**	**68.1**	**71.9**
	1:10	**70.6**	**72.9**	**75.4**
***Oscillatoria* sp.**	**1:50**	**66.4**	**68.2**	**70.2**
	1:25	**71.3**	**74.1**	**76.9**
	1:10	**76.9**	**78.2**	**80.4**
Phormedium fragile	**1:50**	**55.6**	**58.4**	**62.1**
	1:25	**64.8**	**67.5**	**70.4**
	1:10	**68.1**	**70.7**	**72.5**
Spirulina platensis	**1:50**	**70.2**	**74.8**	**79.1**
	1:25	**75.8**	**78.3**	**82.5**
	1:10	**79.2**	**81.3**	**84.3**
Wollea saccata	**1:50**	**49.5**	**50.5**	**54.9**
	1:25	**55.6**	**57.4**	**60.7**
	1:10	**61.3**	**64.7**	**65.1**
***Azolla pinnata* (aqueous extract filtrate)**	**1:50**	**51.8**	**54.4**	**58.9**
	1:25	**60.9**	**63.7**	**65.4**
	1:10	**65.4**	**67.3**	**69.8**
Compost extract	**1:50**	**53.8**	**56.1**	**60.7**
	1:25	**62.7**	**66.8**	**68.5**
	1:10	**66.9**	**68.4**	**70.1**
Vydate (recommended concentration)		**84.5**	**87.4**	**90.7**

3.2. Efficacy of algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost watery extract filtrate in combination or single application on cucumber infection by** *M. incognita* **under greenhouse conditions**

All tested algal culture filtrates, *A. pinnata* aqueous extract filtrate and compost watery extract filtrate in single and/or in combined applications caused a remarkable decrease in the number of root galls under greenhouse conditions. Fig. (1) shows that the combination treatment of Mix (five algae of *A. oryzae, N. muscorum, Oscillatoria* sp. *P. fragile* and *S. platensis*) with *A. pinnata* aqueous extract filtrate and compost watery extract filtrate resulted in the lowest number of root galls, whereas , the individual treatment of *A. pinnata* showed the highest number of root galls compared to the other treatments. The same trend was obtained with number of developmental stages, females, egg masses and eggs numbers/root. Data in Table (5) reveal that the combination of Mix (five algae of *A. oryzae, N. muscorum, Oscillatoria* sp. *P. fragile* and *S. platensis*) with *A. pinnata* extract and compost extract resulted in the lowest final population numbers of *M. incognita* (PF) compared with the other treatments, whereas the highest final population of *M. incognita* was associated with the individual treatment of *A. pinnata* only after two months from treatment application. The present results indicate that the combination of the algal culture filtrates, compost extract and *A. pinnata* extract induced resistance in cucumber roots against *M. incognita*. The same results were achieved by Khan *et al.* (2005), who suggested that the application of algal filtrate might have helped the plant to resist nematode attack or may have played a direct role in the plant defense mechanism. Microorganisms and compounds of microbial origin have been found to induce defense responses and/or resistance in plants towards pathogens.

Fig. (2) illustrates that the combination treatment of Mix (five algae) with *A. pinnata* and compost caused the highest reduction in % of *M. incognita* (which reached 85.3) ,compared to the other treatments, while the individual treatment of *A. pinnata* showed the lowest reduction % of *M.incognita*, being 56.8 after two months from treatments.

Fig. (3) shows shoots and roots fresh weights of the cucumber plants infected with nematode. The results are expressed as percentage of increase over the control. The data indicated that the increase % of fresh weight of the whole plant was greatly improved with the combination treatment of the five algal culture filtrates with *A. pinnata* and compost water extract filtrates where the percentage of increase reached 84.5%,. The individual treatment of *A. pinnata* recorded the lowest value in the percentage of increase of fresh weight of the whole plant (50.4%).

Yield of cucumber plants infected with nematode in the greenhouse is shown in Fig. (4). The data indicate that the yield was the highest with the combined treatment of mix of algal culture filtrates with *A. pinnata* and compost water extracts (3400 g plant^{-1}), while, the individual treatment of *A. pinnata* aqueous extract recorded the lowest value of 2300 g plant^{-1}. Liu *et al.* (2008) found that the stimulation of plant growth by using compost + compost tea or seaweed extracts may be attributed to the combined effect of compost, compost tea (which contains humic acids, vitamins, amino acids and both macro and micro nutrients which enhanced cucumber growth) and seaweed extracts which contain some growth regulators such as cytokinins, auxin and gibberellins. Actively aerated compost extract, which is supplied with active aeration of high oxygen concentration, stimulates population growth of aerobic microbes, which helps disease prevention, nutrient cycling, retention of micro-nutrients, soil structure, and decomposition of plant-toxic materials. Moreover compost extract mode of action could be attributed to release of compounds toxic to nematodes, like ammonia (NH_3), nitrites, hydrogen sulphide, polyphenols and tannins (Rivera and Aballay, 2007). Soil organic amendments have been used as an alternative dual method in the control of nematodes and improving plant growth and its yield (Hasabo, 2006). Organic matter affects nematode populations in two different ways, directly by possessing nematicidal properties during its degradation (Sitaramaiah and Singh, 1978), or indirectly by enhancing the development of nematode natural enemies (Hasabo, 2006). Also, Rotenberg *et al.* (2005) reported that additions of organic amendments (composts) to agricultural soils can lead to improved soil quality and reduced severity of crop diseases as well as increased cucumber yield.

Khan *et al.* (2005) studied the nematicidal potential of culture filtrates of the blue-green alga, *Microcoleus vaginatus* (Cyanobacterium). against *Meloidogyne incognita* on tomato under greenhouse conditions. Prior to the transplantation

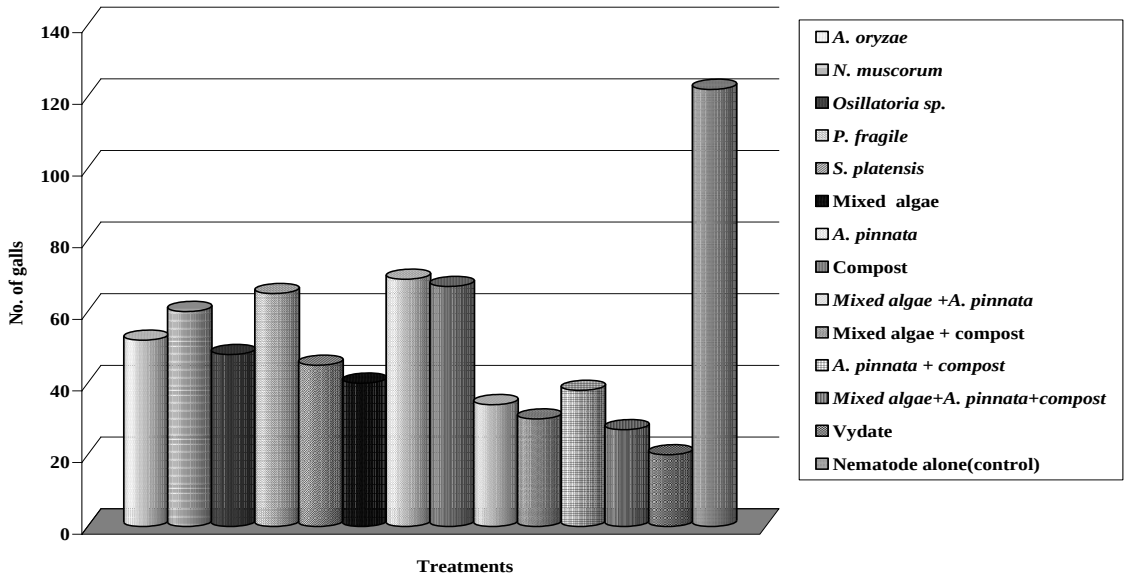

Fig (1): Effect of algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost watery extract filtrate, individually and/or in combination on the number of galls of** *M. incognita* **on cucumber plants.**

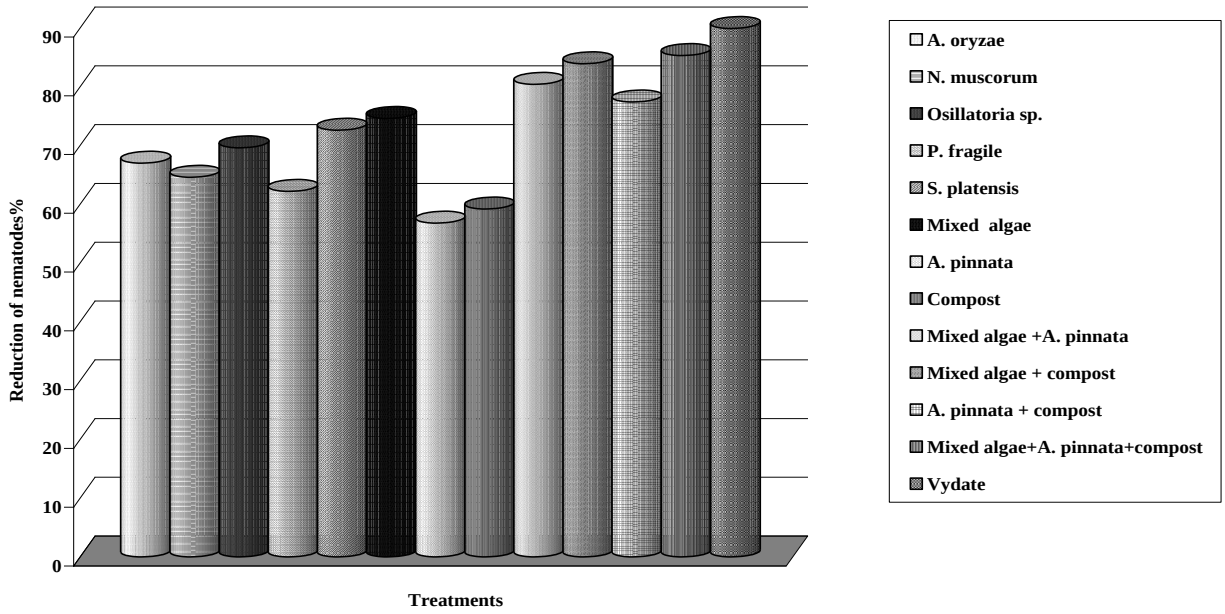

Fig. (2): Effect of a algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost extract individually and/or in combined application on the reduction of nematodes% on cucumber plants.**

Table (5): Efficacy of algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost extract, individully and/or in combined Application, on the reproduction of** *M. incognita* **infecting cucumber plants under greenhouse conditions.**

Treatments	Nematode population					
	No. of juveniles in soil/250g	**No. of developmental stages/root**	**No. of females/ root**	**No. of egg-masses/ root**	**No. of eggs/ egg-mass/root**	**Final nematode population (PF)**
Control with nematode	2200	108	122	118	410	50810
Anabaena oryza	420	62	52	48	338	16758
Nostoc muscorum	480	65	60	51	340	17945
Oscillatoria sp.	380	58	48	45	332	15426
Phormidium fragile	540	67	65	54	343	19194
Spirulina platensis	340	55	45	41	328	13888
Mixed algae	300	52	40	39	320	12872
Azolla pinnata	660	73	69	60	352	21922
Compost	600	71	67	57	350	20688
Mixed algae+ *A. pinnata*	320	48	34	31	308	9950
Mixed algae+Compost	220	45	30	27	291	8152
A. pinnata +Compost	260	50	38	35	317	11443
Mixed algae+*A. pinnata*+Compost	180	43	27	25	288	7450
Vydate	100	28	20	19	260	5088
L.S.D. (5 %)	43	3	3	2	2.6	113

Table (6):The biological activity of *M. incognita* infected soil remaining after cucumber harvesting.

Treatments	Dehydrogenase activity (µg TPFg^{-1} dry soil Day^{-1})	Nitrogenase activity (µ mole C_2H_4 g $soil^{-1}hr.^{-1}$)	CO_2 evolution (mg100g $soil^{-1}$ day^{-1})	Total bacterial count (10^5cfu g $soil^{-1}$)	Total cyano-bacterial count (10^2cfu g $soil^{-1}$)
Control with nematode	19.53	13.95	5.41	37	2.0
Anabaena oryzae	29.23	25.51	10.82	114	5.6
Nostoc muscorum	34.84	122.59	12.27	140	10.0
Oscillatoria sp.	30.10	131.35	11.13	204	13.4
Phormidium fragile	23.00	123.66	13.08	179	10.7
Spirulina platensis	30.24	149.65	13.24	210	15.8
Mixed-algae	35.83	175.50	16.08	270	18.4
Azolla pinnata	27.13	127.36	14.21	192	11.8
Compost	35.38	156.54	15.55	220	16.1
Mix+ *Azolla pinnata*	37.66	258.86	18.81	388	22.2
Mix+Compost	57.46	262.69	19.28	516	30.0
A. pinnata +Compost	39.16	243.12	17.45	310	21.0
Mix+*A. pinnata* + Compost	58.75	291.33	19.73	720	33.5
Vydate	60.13	294.21	20.25	810	35.0
L.S.D. (5 %)	0.44	1.12	0.46	5.32	0.26

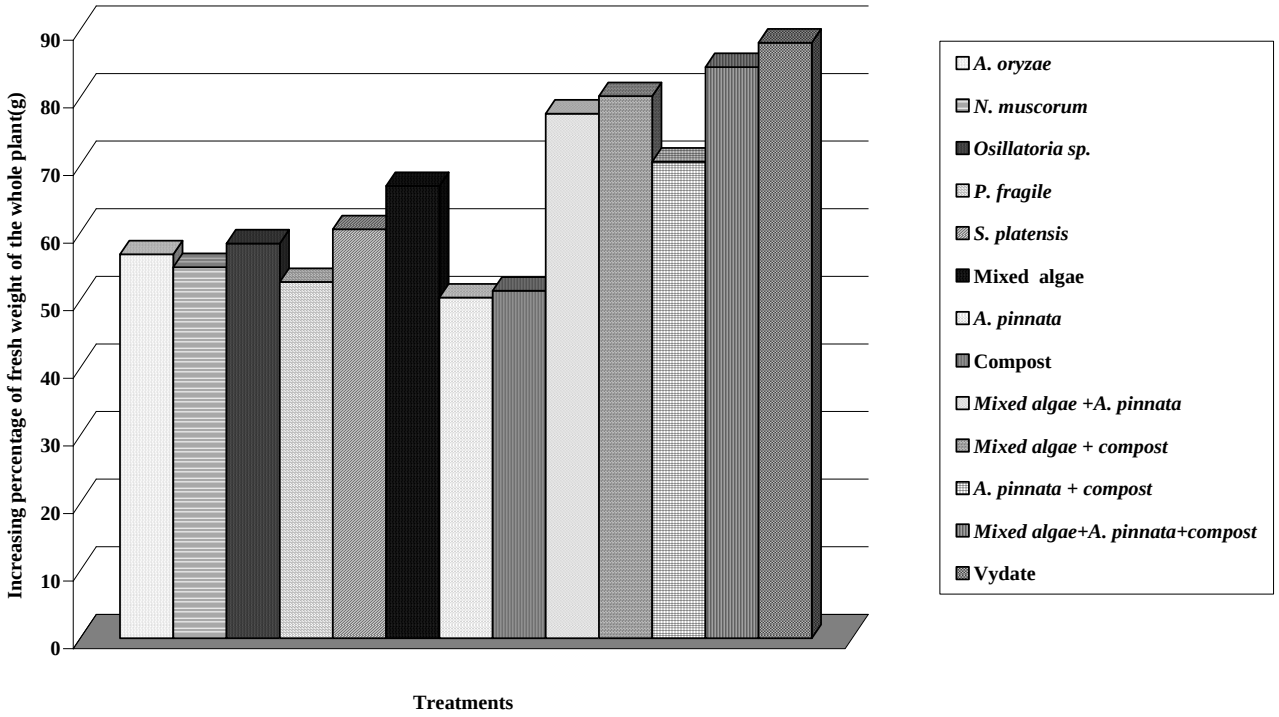

Fig. (3): Effect of algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost extract, individually and/or in combined application on the fresh weight of shoots and roots increasing % over the control of cucumber infected by** *M. incognita***.**

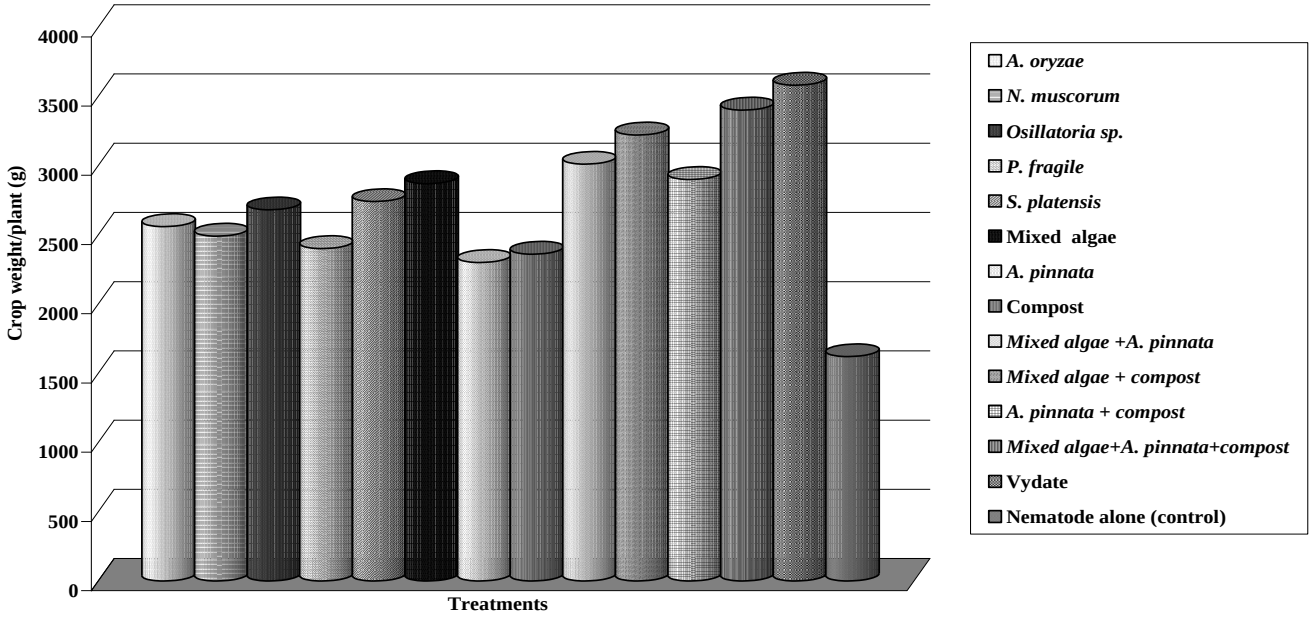

Fig. (4): Effect of algal culture filtrates, *A. pinnata* **aqueous extract filtrate and compost extract, individually and/or in combined application on the yield of cucumber plants infected by** *M. incognita* **.**

of tomato seedlings, roots were dipped in different concentrations (0.2, 0.5, 1, 2, 10, 50 and 100%) of culture filtrate of *M.vaginatus* for 30 min. Root dip treatment reduced the root galling and final population of *M. incognita* and increased vegetative growth of plants and root-mass production compared to the control. The beneficial effect of root-dip treatment increased with increasing culture filtrate concentration. Root galling and final nematode populations were reduced by 65.9 and 97.5%, respectively, when treated at the highest concentration. On the other hand, three species of blue green algae, *Anabena oryzae*, *Nostoc calcicola* and *Spirulina* sp., were tested versus *Meloidogyne incognita* infecting cowpea cv. Baladi in single and/or in combined treatments. It was found that *N. calcicola* alga was superior to all the other treatments followed by *Spirulina* sp., in reducing the number of nematode galls and egg masses as compared to the untreated treatment. In combined treatments, the 3 algae together achieved the highest significant reduction in the number of galls and egg masses. All the treatments significantly improved plant growth criteria as measured by fresh and dry weight of shoots and roots and length of shoots and increased the number of nodules (Youssef and Ali, 1998).

3.3. The biological and chemical analyses of *M. incognita* **infected soil remaining after cucumber harvesting.** The remaining soil after cucumber harvesting was analyzed to spot the changes in soil biological and chemical conditions due to the different treatments in the greenhouse experiment. Generally, all the combined treatments enhanced soil biological activity than the individual ones (Table 6).

However, the superiority of biological activity of the soil was due to the synthetic nematicide (Vydate). The combined treatment of mixed algal culture filtrates + *A. pinnata* aqueous extract filtrate + compost extract revealed the highest soil biological activity compared with other remain treatments and achieved values of dehydrogenase (58.75 µg TPF g^{-1}dry soil day^{-1}), nitrogenase (291.33 µmole C_2H_4 g dry $soil^{-1}$ $hr.^{-1}$), CO_2 evolution (19.73 mg 100 g $soil^{-1}$ day^{-1}), total bacterial count (720 x 10^5g cfu g $soil^{-1}$) and cyano- bacterial count (33.5 x 10^2 cfu g $soil^{-1}$). These results are in agreement with those obtained by Mahmoud *et al.* (2007), who reported that cyanobacteria combined with organic amendments significantly enhanced the soil biological activity in terms of increasing the total bacterial, total dehydrogenase, nitrogenase and phosphatase activities. The data in Table (7) show the chemical properties of the soil after cucumber harvest. The results indicate that the combined treatment of mix of algal culture filtrates, *A. pinnata* aqueous extract filtrate and compost extract increased significantly the soil available nitrogen forms (NH_4^+, NO_3^{--} and NO_2^{--}), as well as K and P over the control. The corresponding K and P values were 80.33 and 17.76 ppm. The soluble nitrogen forms were investigated in all treatments. The highest values were 10.0, 76.12 and 0.51 ppm in the treatment of the combination between mix of algal culture filtrates, *A. pinnata* aqueous extract filtrate and compost extract for ammoniac, nitrate and nitrite-nitrogen, respectively**.**

Generally, the combined treatments revealed higher ammoniacal levels in the soil than the remaining treatments. The high rates of N required for consistent nematicidal activity from ammoniacal fertilizers can be expected to result in significant phytotoxicity through the accumulation of metabolic byproducts in the soil (Rodriguez-Kabana, 1986). These results agree with Omar and Shawky (2006) who stated that the increase of ammonium levels around tomato rhizosphere caused a high reduction of nematode population.

The action of soil microorganisms on an organic material during the decomposition process can produce a wide range of chemicals like ammonia, nitrites, hydrogen sulfide, organic acids and enzymes (Stirling, 1991). These chemicals are known to possess nematicidal properties that affect egg hatch and/or motility of juveniles (Korayem, 2003).

The effectiveness of organic amendments to control nematodes is a function of the C:N ratio and time of microbial decomposition of organic matter and subsequent release of nitrogen for utilization by higher plants. With organic matter of a C:N ratio greater than 20:1, N will temporarily be immobilized in microbial tissue, creating a nitrogen deficiency. The nematode management potential of an organic soil amendment is directly related to N-content or inversely related to the C:N ratio. Amendments with narrow C:N ratios result in better nematode control than those with wide ratios (Agbenin, 2004). However, it is possible that nematicidal activity of nitrogenous by–products should be most evident when C:N ratio of the compost is less than 20: 1 (Stirling, 1991). The pH of the soil (Table 7) was affected by the organic and biological treatments. However, its nematicide

Table (7): Some chemical properties of *M. incognita* infected soil remaining after cucumber harvesting.

Treatments	Available – K (ppm)	Available – P (ppm)	Available-N			pH	Ec
			NH_4^+	NO_3^{--}	NO_2^{--}		
Control with nematode	22.11	9.46	2.45	31.82	0.04	8.57	**0.76**
Anabaena oryzae	51.50	11.04	3.02	60.23	0.54	7.77	**0.54**
Nostoc muscorum	47.05	11.44	0.82	71.59	0.57	8.00	**0.55**
Oscillatoria Sp.	51.34	12.27	2.80	44.25	0.61	8.09	**0.48**
Phormidium fragile	65.37	12.95	2.16	45.45	0.57	7.50	**0.55**
Spirulina platensis	54.09	13.79	1.32	52.27	0.94	8.17	**0.67**
Mixed-algae	61.75	16.44	3.50	72.18	0.57	8.10	**0.49**
Azolla pinnata	57.25	11.89	3.30	73.18	0.81	7.95	**0.53**
Compost	66.37	14.80	2.80	69.32	0.54	8.10	**0.46**
Mix+ *A. pinnata*	76.95	15.35	7.05	79.55	0.41	8.00	**0.48**
Mix+Compost	77.45	16.57	5.93	75.00	0.54	8.30	**0.47**
A. pinnata +Compost	75.04	15.05	4.25	73.52	1.01	8.20	**0.47**
Mix+*A. pinnata* + Compost	80.33	17.76	10.00	76.12	0.51	8.25	**0.45**
Vydate	85.40	18.22	11.18	77.27	0.61	8.62	**0.45**
L.S.D. (5 %)	0.21	0.19	0.14	1.27	0.05	0.01	**0.02**

cyanobacterial counts, CO_2 evolution, effects would be due mainly to the release of ammonia in a slightly alkaline pH and a C/N proportion within the optimal range, according to Stirling (1991). The combined treatments led to a slight reduction in soil electrical conductivity (Table, 7). Biological inoculation of soil led to a reduction in soil pH and electrical conductivity (El-Gaml, 2006).

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كفاءة استخدام بعض أنواع الطحالب والازولا ومستخلص الكمبوست في مكافحة نيماتودا تعقد الجذور وانعكاس ذلك على نمو الخيار

سماء محمود شوقي عبد المنعم و سها سيد محمد مصطفى * و عزة أحمد محمد عبد العال *

قسم بحوث الأمراض النيماتودية - معهد بحوث أمراض النباتات
- قسم الميكروبيولوجيا- معهد بحوث الأراضي والمياه والبيئة
- مركز البحوث الزراعية – الجيزة-مصر

ملخص

تهدف هذه الدراسة إلى إستخدام راشح تسع مزارع سلالات طحلبية وهي: *Anabaena oryzae* ، *Anabaena flos aquae* ، *Oscillatoria* sp.، *Chlorella vulgaris*، *Nostoc muscorum* ، *Nostoc humifusum* ، *Spirulina platensis* ، *Phormedium fragile*، *Wollea saccata* بالإضافة إلى راشح المستخلص المائي لنبات *Azolla pinnata* (50%) وراشح المستخلص المائي للكمبوست (5:1) لمقاومة نيماتودا تعقد الجذور *Meloidogyne incognita* والتي تصيب الخيار تحت ظروف كلا من المعمل والصوبة.

أظهرت التجارب المعملية موت يرقات النيماتودا بنسبة عالية أثناء فتره التعرض لمدة 72 ساعه في كل المعاملات. كما أوضحت النتائج أن خمسة أنواع من الطحالب تنتمي إلى الطحالب الخضراء المزرقة أعطت أفضل النتائج المعملية وهي : *Spirulina platensis* ، *Oscillatoria* sp.، *Anabaena oryzae*، *Nostoc muscorum* و *Phormedium fragile* بالإضافة إلى راشح المستخلص المائي للكمبوست (84.3 ، 80.4 ، 78.9 ، 75.4 ،72.5 و 70.1% على التوالي) حيث زادت نسب موت يرقات نيماتودا تعقد الجذور عن 70% عند أعلى تركيز (10:1).

أدى استخدام راشح المستخلص المائي للـ *Azolla pinnata* إلى نسبة موت لليرقات وصلت إلى 69.8% عند نفس التركيز. بينما أظهر استخدام *Anabaena flos aquae* و *Chlorella vulgaris* أقل تأثير في نسب موت اليرقات (52.1 و 40.1 % على التوالي).

أوضحت تجارب الصوبة الإنتاجية أن جميع المعاملات أدت إلي تقليل تعداد النيماتودا في كل من التربة و الجذور. حيث أظهرت المعاملة المختلطة من مخلوط راشح مزارع خمسة طحالب (*Spirulina platensis*، *Oscillatoria* sp.، *Anabaena flos aquae* ، *Nostoc muscorum* و*Phormedium fragile*) مع راشح المستخلص المائي لكلا من *Azolla pinnata* والكمبوست أعلى كفاءة في خفض تعداد الطور اليرقى الثاني في التربة وكذلك أعداد كلا من العقد النيماتودية والأطوار غير المكتملة والإناث وأكياس البيض وعدد البيض/كيس بيض علي جذور نباتات الخيار . بينما أدى استخدام المعاملة المنفردة لمعاملة *Azolla pinnata* إلي اقل تأثير علي تعداد النيماتودا.

أدى استخدام جميع المعاملات خاصة المختلطة إلى حدوث زيادة في الوزن الغض للمجموع الخضري والجذور وكذلك زيادة محصول الخيار.

يمكن من هذه الدراسة التوصية باستخدام المعاملات البيو-عضوية لمكافحة نيماتودا تعقد الجذور في الخيار وخصوصاً تحت ظروف زراعة الصوب الإنتاجية و تقليل الاعتماد على المبيدات الكيميائية الملوثة لكل من النبات والتربة، كما نوصي بتكرار التجربة على المحاصيل الأخرى و بخاصة محاصيل الخضر.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (60) العدد الرابع (أكتوبر 2009) : 443-459 .