Egypt. J. Agronematol., Vol. 13, No.2, PP. 23–39(2014)

Comparative Efficacy of some Algal Species, Azolla, Pleurotus and Olive Mill in Controlling Root Knot Nematode on Banana

Samaa. M. Shawky*; Azza. A. M. Abd El-All ** and Ahlam M. Al-Ghonaimy ***

* 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

***Plant Protection Department, Nematology Unite- Desert Research Center, Cairo, Egypt

Abstract

The study aimed to use two culture algal *Spirulina platensis, Anabaena azollae*, *Azolla pinnata* and *Pleurotus columbinus* besides olive mill waste in controlling root knot nematode, *Meloidogyne javanica* in banana was monitored under both laboratory and commercial 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. *Spirulina platensis* followed by *Anabaena azollae, Azolla pinnata, Pleurotus columbinus* and olive watery extract significantly increased juveniles mortality up to 70%after 72h at the highest concentration of 1:10 (85.2, 81.4, 79.9, 73.5, 71.7 and 70.1%, respectively).

In the productivity greenhouse experiment, the combination of culture filtrates of *Spirulina platensis*, *Anabaena azollae, Azolla pinnata, Pleurotus columbinus* and olive mill waste water achieved the highest reduction in the number of total nematodes in both soil and roots, also in numbers of galls. In addition, all combinations significantly increased the crop yield of banana plants comparing with the individual treatment and the control.

The combined treatments significantly enhanced the CO_2 evolution, dehydrogenase and nitrogenase activities over the control. It could be recommended that application of biological control agents against root knot nematode in banana is preferable to reduce the chemical nematicides inputs.

Key words: Banana, *Meloidogyne javanica, Spirulina platensis*, *Anabaena azollae, Azolla pinnata, Pleurotus columbinus,* olive mill waste water.

Introduction

Banana is a favorite commodity export for markets and local consumption and represents one of the most important and economic fruits in Egypt. Banana; Grand Naine' cultivar was studied under both field and in the high tunnel conditions.

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 banana 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).** Root-knot nematodes *Meloidogyne incognita* and *Meloidogyne javanica* occur on banana and plantain roots wherever this crop is grown.

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'. *Azolla pinnata* was found as a very effective agent for normal growth of root and shoot with half-standard dose of mustard oil cake. Both of these agents together created resistance to plants and toxic materials might suppress the nematode activity of galling by *M. javanica* **Tahmid *et al*. (2002).**

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)**. In productivity 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 2nd 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 plant yield **Shawky *et al*. (2009).**

This investigation aimed to evaluate the efficacy of *Spirulina platensis*, *Anabaena azollae, Azolla pinnata, Pleurotus columbinus* and olive mill waste water on root knot nematode, *Meloidogyne incognita* activity *in vitro* under laboratory conditions to select the most promising results to be applied in controlling *M. javanica* in banana plants under productivity greenhouse conditions.

Materials and Methods

Table (1): Physic- chemical properties of the experimental soil.

Coarse sand %	Fine sand%		Silt%	Clay%	$CaCO_3$ %		Texture	O.M%
14.7	26.5		31.5	27.3	1.15		Sand clay loam	0.32
Anions (meq L^{-1})			**Cations (meq L^{-1})**				**pH 1:2.5**	**EC(dS m^{-1})**
HCO_3^-	Cl^-	SO_4^{--}	K^+	Na^+	Mg^{++}	Ca^{++}	7.5	1.3
1.0	10.7	1.3	0.7	7.5	1.5	3.3		
Available nutrients (mg kg^{-1} soil)								
Cu	Zn		Mn	Fe	K		P	N
0.04	1.7		1.9	2.3	393.7		18.5	59.6

1. Microorganisms and *Azolla pinnata* source and growth conditions

The cyanobacteria, *Anabaena azolla* which isolated from *Azolla pinnata* **Abd el all (2013)** was grown on BG11 medium **Rippka *et al.*(1979)**.The culture was incubated in growth chamber under continuous illumination (2000 lux) and temperature of 25˚C± 2˚, while *Spirulina platensis* was grown on Zarrouk medium **Zarrouk (1966**) at 35˚C± 2*. Azolla pinnata* was grown on modified Yoshida medium **Yoshida *et al*. (1976)** and strain of white rot fungi *Pleurotus columbinus* was grown on Potato Dextrose Agar medium, PDA **Martin (1950)**.Microorganisms and *Azolla pinnata* were obtained from Agricultural Microbiology Research Department, Soils, Water and Environment Res. Inst. (SWERI), Agric. Res., Center (ARC). One strain of white rot fungi (*Pleurotus columbinus*) was obtained from Unit of Mushroom Production, Faculty of Agriculture, Ain Shams University

2. Preparation of algal culture filtrates, *Azolla pinnata*, *Pleurotus columbines* and olive mill waste water

After 30 days of incubation, each algal biomass was separated from its culture medium by filtration. The growth parameters of algal cultures filtrates were determined **Table 2**. *Pleurotus columbinus* was mixed in an electric mixer and *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 growth parameters of *Azolla pinnata* fresh biomass and aqueous solution are presented in **Table 2.** The algal culture filtrates, *pleurotus* and *Azolla* fresh biomass aqueous extract (50%), 10 % concentration of olive mill waste water were kept at 4°C.

3. Soil biological activities

CO_2 evolution was determined according to **Gaur *et al.* (1971)**, Dehydrogenase activity (DHA) was estimated according to **Casida *et al.* (1964),** nitrogenase activity was measured by acetylene reduction assay as described by **Dart *et al.* (1972).**

4. Determination of culture growth parameters

Culture growth parameters, pH values and algal dry weight were estimated according to **Vonshak (1986).** Culture concentrations were determined as optical density (OD) by spectrophotometer at 560 nm **Leduy and Therien (1977).** Electric conductivity (EC) of algal culture filtrates was measured using glass electrode conductivity meter Model Jenway 4310.

Table (2): Algal culture growth parameter.

Treatments	EC	pH	(OD) at 560nm	D.W/ 100ml
Anabaena azollae	0.20	6.39	1.42	0.40
Spirulina platensis	17.76	9.48	0.97	1.76
Azolla pinnata	1.68	7.50	4.3	0.32
Olive mill	3.34	6.90	1.05	0.45
Pleurotus columbinus	1.28	6.49	1.15	0.27
***Anabaena azollae* + *Spirulina platensis*.**	8.92	9.48	0.76	0.49
Anabaena azollae* +*Azolla pinnata	1.30	6.95	6.03	0.27
***Anabaena azollae* + olive mill**	1.85	6.64	1.39	0.20
Spirulina platensis* +*Azolla pinnata	10.04	8.90	1.76	0.75
***Azolla pinnata* + olive mill**	2.23	6.20	4.53	0.50
***Spirulina platensis* + olive mill**	10.91	8.42	1.02	4.09
***Anabaena azollae* + *Spirulina platensis* +*Azolla pinnata* + olive mill**	6.53	7.91	0.92	0.10
Anabaena azollae* + *Spirulina platensis* +*Azolla pinnata* + olive mill + *Pleurotus columbinus	5.40	7.17	4.03	0.53

5. Efficacy of *S. platensis*, *A. azollae*, *A. pinnata*, olive mill and *P. columbinus* on the activity of *M. javanica* juveniles under laboratory conditions

For studying the efficacy of *Spirulina platensis* culture filtrates, *Azolla pinnata*, *Anabaena azollae,* olive mill and *Pleurotus columbinus* on *M. javanica* 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. New blue R (0.05% aqueous solution), potassium permanganate (0.062-0.50% KMnO4 in aqueous solution) and chrysoidin were used to stain dead nematodes **Barker *et al.* (1985).**

The treatments were as follows:

1. *Spirulina platensis*.
2. *Azolla pinnata*e.
3. Olive mill waste water.
4. *Anabaena azollae*.
5. *Pleurotus columbinus*.
6. Oxamyl (vydate).

6. Efficacy of *S. platensis*, *A. azollae, A. pinnata*, olive mill and *P. columbinus* individually and/or in combination on banana infected by *M. javanica* under productivity greenhouse conditions

This experiment was conducted under productivity greenhouse conditions of (Gezerat El Dahab) Giza Governorate, during the summer season of 2013**.** The soil texture was clay loamy, soil pH in water suspension (I:2.5) was 7.5 and EC of soil paste at 25 ^{o} C was 1.3 ds.m^{-1} **AOAC (1980).**

Banana plants, cv. Grand Naine', were cultivated in nine productivity greenhouses in soil naturally infested with *M. javanica*. Each productivity greenhouse was divided into 5 ridges (40 meters in length and 1 meter in width) at a spacing of 45 cm between plants within the row. Drip irrigation was used. The treatments were fourteen, each applied to three rows and the last three rows were left without treatment (nematode only) to serve as control. All treatments were applied at the rate of 25ml.m^{-2}, as soil drench added twice, after 7 and 21 days. Plants were carefully uprooted fruits were weighed after four months. Thereafter, the number of juveniles per 250 g soil and nematode populations in roots were counted according to **Franklin and Goodey (1957).**

The treatments were as follows:

1. *Spirulina platensis*.
2. *Anabaena azolla*e.
3. *Azolla pinnata*.
4. *Pleurotus columbinus*.
5. Olive mill waste water.
6. *Anabaena azollae* + *Spirulina platensis.*
7. *Anabaena azollae* +*Azolla pinnata*.

8. *Anabaena azolla*e + olive mill waste water.

9. *Spirulina platensis* +*Azolla pinnata.* 10. *Azolla pinnata* + olive mill waste water.

11. *Spirulina platensis* + olive mill waste water.

12. *Anabaena azolla*e + *Spirulina platensis* +*Azolla pinnata* + olive mill waste water.

13. *Anabaena azolla*e + *Spirulina platensis* +*Azolla pinnata* + olive mill waste water + *Pleurotus columbinus*

14. Oxamyl (vydate) as nematicide at the recommended concentration (24% EC), and

15. Nematodes (N) alone (control).

7. Statistical analysis procedure

All obtained data were subjected to analysis of variance (ANOVA) **Gomez and Gomez (1984)** and means were compared by using L.S.D. at 5 % level of significance.

Results and Discussion

1. Efficacy of *S. platensis*, *A. azollae, A. pinnata*, olive mill and *P. columbinus* on the activity of *M. javanica* juveniles under laboratory conditions

Data in **Table 3** illustrate that treatment filtrates had various degrees of effectiveness toward the mortality percentages 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 79.5, 82.3 and 85.2 after 24, 48 and 72 hrs exposure time, respectively. *Anabaena azolla*e, *Azolla pinnata* and *Pleurotus columbinus* ranked in the second level in nematode juveniles mortality % at 1:10 during all exposure periods. Treatments showed lethal effect on nematode juveniles and the mortality percentages were over 70 after 72 hours except olive extract which achieved 71.7%. By increasing the exposure period to 72 hrs. the mortality percentages raised to 85.2, 81.4, 79.9, 73.5, 71.7 and 70.1%, respectively with *Spirulina platensis*, *Anabaena azollae, Azolla pinnata, Pleurotus columbinus* and olive mill. On the other hand, olive extract had the lowest effect on nematode juveniles percentage (71.7) at 1:10 by the end of the exposure period (72 hrs.).

Obtained results indicated that blue green algae, *Spirulina platensis* produced a great variety of secondary metabolites **Gervick *et al.* (2001)**, such as nitrogen-containing compounds, polyketides, lipopeptides, cyclic peptides and others. Efficacies of 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.

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 **Mahmoud (2001) and Shawky *et al*. (2009).**

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 the egg production **Tadros *et al* (2008).**

Table (3): Effect of *S. platensis*, *A. azolla*e, *A. pinnata*, *P. columbines* and olive mill on the activity of *M. javanica* juveniles under laboratory conditions.

Treatments	Conc.	Exposure periods (in hours)		
		24	48	72
		Mortality %		
	1:50	71.4	75.6	79.1
Spirulina platensis	1:25	76.1	78.4	83.7
	1:10	79.5	82.3	85.2
	1:50	61.2	65.8	68.3
Azolla pinnata	1:25	69.4	71.9	74.5
	1:10	74.3	76.7	79.9
	1:50	67.9	69.7	71.5
***Anabaena azolla*e**	1:25	73.5	75.1	77.9
	1:10	78.9	79.2	81.4
	1:50	56.6	59.4	63.1
Pleurotus columbinus	1:25	65.8	68.5	71.4
	1:10	69.1	71.7	73.5
	1:50	54.8	57.1	61.7
Olive mill	1:25	63.7	67.8	69.5
	1:10	67.9	69.4	71.7
Oxamyl		**81.7**	**85.4**	**89.6**
Nematode alone		**0.7**	**1.1**	**1.9**

2. Efficacy of *S. platensis*, *A. azollae*, *A. pinnata*, *P. columbines* and olive mill on banana infection by *M. javanica* under productivity greenhouse conditions

Data in **Table 4** reveal that the combination of *S. platensis*, *A. azollae, A. pinnata*, *P. columbines* and olive mill resulted in the lowest final population of *M. javanica* (PF) compared with the other treatments, whereas the highest final population of *M. javanica* was associated with the individual treatment of olive mill only after two, three months of application. The combination of *S. platensis*, *A. azollae, A. pinnata*, *P. columbines* and olive mill induced resistance in banana roots against *M. javanica*. Results were achieved by **Khan *et al.* (2005)**, who suggested that the application of algal filtrate helped the plant to resist nematode attack or may 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. Also, **Shawky *et al*. (2009)** reported that in the productivity greenhouse experiment, the combination of five algal culture filtrates of *S. platensis*, *Oscillatoria* sp., *A. oryzae*, *N. muscorum* and *P. fragile* with *A. pinnata* and compost extract achieved the highest reduction in the number of the 2nd 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

The efficacy of *S. platensis*, *A. azollae, A. pinnata*, *P. columbines* and olive mill in comparison with oxamyl were tested under productivity greenhouse conditions in an experiment lasted four months. Data in **Table 4** showed that all tested treatments were effective in decreasing the final nematode population and rate of build-up of root knot nematode in both soil and roots. Total nematode population in both soil and root samples revealed the suppressive effect of all materials on the nematode counts. In general, the nematode counts decreased gradually in both soil and root of treated plants. All treatments decreased in the total nematode population ranging between 320 and 1680 one month after treatment in comparison to the control that reached 2400. Then, remarkable suppression in nematode counts obtained after three months in both soil and root samples. The combination of *S. platensis*, *A. azollae, A. pinnata*, *P. columbines* and olive mill resulted in the highest decrease in the total number of nematodes in both soil and root samples as soil application and the highest efficacy treatments reached to 86 % after the three months in comparison with the other treatments after the oxamyl treatment that reached 89 % while, olive mill was the lowest efficacy treatment that reached 63% after the three months. These results agree with **Shawky *et al.* (2009)**, **Shawky *et al.* (2010)** and **Soliman *et al*. (2011)**.

Table (4): Efficacy of *S. platensis*, *A. azollae*, *A. pinnata*, *P. columbines* and olive mill in combination or single application on banana infection by *M. javanica* under productivity greenhouse conditions.

Treatments	Initial	After one month		After two months		After three months		After four months	
	Total population in soil/ 250 g	Total population in soil/ 250g + in root/g	PF/PI	Total population in soil/ 250g + in root/ g	PF/PI	Total population in soil/250g + in root/g	PF/PI	Total population in soil/250g + in root/ g	PF/PI
A. azollae	1820	1560 *(33)	0.85	1440 *(54)	0.79	1300 *(66)	0.71	1420 *(65)	0.78
S. platensis	1940	1600 *(35)	0.82	1470 *(56)	0.75	1320 *(67)	0.68	1500 *(66)	0.77
A. pinnata	1860	1640 *(31)	0.88	1480 *(53)	0.79	1320 *(66)	0.70	1490 *(64)	0.80
Olive mill	1820	1680 *(27)	0.92	1540 *(50)	0.84	1400 *(63)	0.76	1560 *(62)	0.85
P. columbinus	1800	1600 *(30)	0.88	1480 *(52)	0.82	1360 *(64)	0.75	1500 *(63)	0.83
A. azollae + *S. platensis.*	1820	960 *(59)	0.52	820 *(74)	0.45	740 *(81)	0.40	840 *(80)	0.43
A. azollae+*A. pinnata*	1800	1320 *(42)	0.73	1260 *(59)	0.70	1180 *(69)	0.65	1280 *(68)	0.67
A. azollae +olive mill	1940	1460 *(41)	0.75	1400 *(58)	0.72	1300 *(68)	0.67	1420 *(67)	0.73
S. platensis+*A. pinnata*	1880	1240 *(48)	0.65	1180 *(63)	0.62	960 *(76)	0.51	1040 *(75)	0.55
A. pinnata + olive mill	1960	1500 *(40)	0.76	1440 *(57)	0.73	1360 *(67)	0.69	1480 *(66)	0.70
S. platensis+ olive mill	1900	1280 *(47)	0.67	1150 *(65)	0.60	980 *(75)	0.51	1180 *(72)	0.57
A.azollae + *S. platensis* +*A. pinnata* +olive mill	1920	640 *(74)	0.33	720 *(78)	0.37	800 *(80)	0.41	920 *(79)	0.47
A. azollae + *S. platensis* +*A.pinnata* + olive mill +*P. columbinus*	1940	570 *(77)	0.29	500 *(85)	0.25	560 *(86)	0.28	720 *(84)	0.37
Oxamyl	1900	500 *(80)	0.26	460 *(86)	0.24	440 *(89)	0.23	560 *(87)	0.29
Nematodes alone	1900	2400	1.26	3200	1.68	3900	2.05	4200	2.21
L. S. D. at 0.05%	19.1	16,7	0.11	10.9	0.24	14.6	0.29	11.2	0.17

Figures in parentheses indicate percentage of nematode reduction in soil according to Henderson and Tillton, (1966).

* Efficacy %= 100 x [1- (Total nematode population of treated plots after application x Total nematode population of check plots before application) / (Total nematode population of treated plots before application x Total nematode population of check plots after application

Treatments of *S. platensis*, *A. azollae, A. pinnata* aqueous extract filtrate, *P. columbines* and olive mill waste water in single and/or in combined applications caused remarkable decreases in the number of root galls under greenhouse conditions. **Fig. 1** shows that the combination treatment of *S. platensis*, *A. azollae, A. pinnata* aqueous extract filtrate, *P. columbines* and olive mill waste water resulted in the lowest number of root galls, whereas, the individual treatment of olive extract 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.

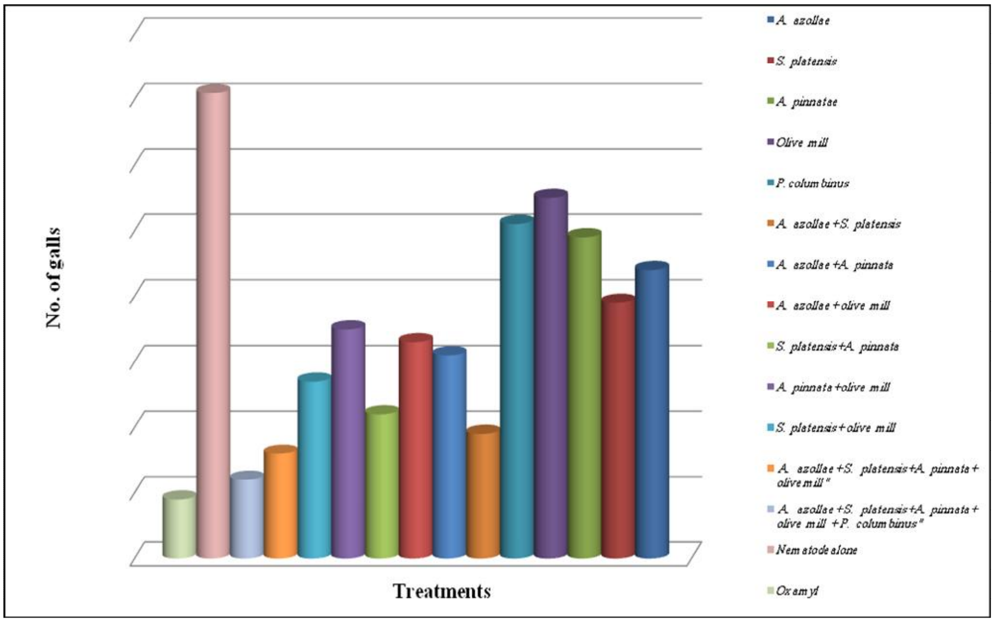

Fig. (1): Effect of *S. platensis*, *A. azolla*e*, A. pinnata*, *P. columbines* and olive mill in combination or single application on the number of galls of *M. javanica* on banana plants under productivity greenhouse conditions.

Yield of banana plants infected with *M. javanica* in the productivity greenhouse is shown in **Fig. 2**. The yield was the highest with the combination of *S. platensis*, *A. azollae, A. pinnata* aqueous extract filtrate, *P. columbines* and olive mill waste water 47 % over control, while, the individual treatment of olive mill waste water recorded the lowest percentage increase 10. **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.

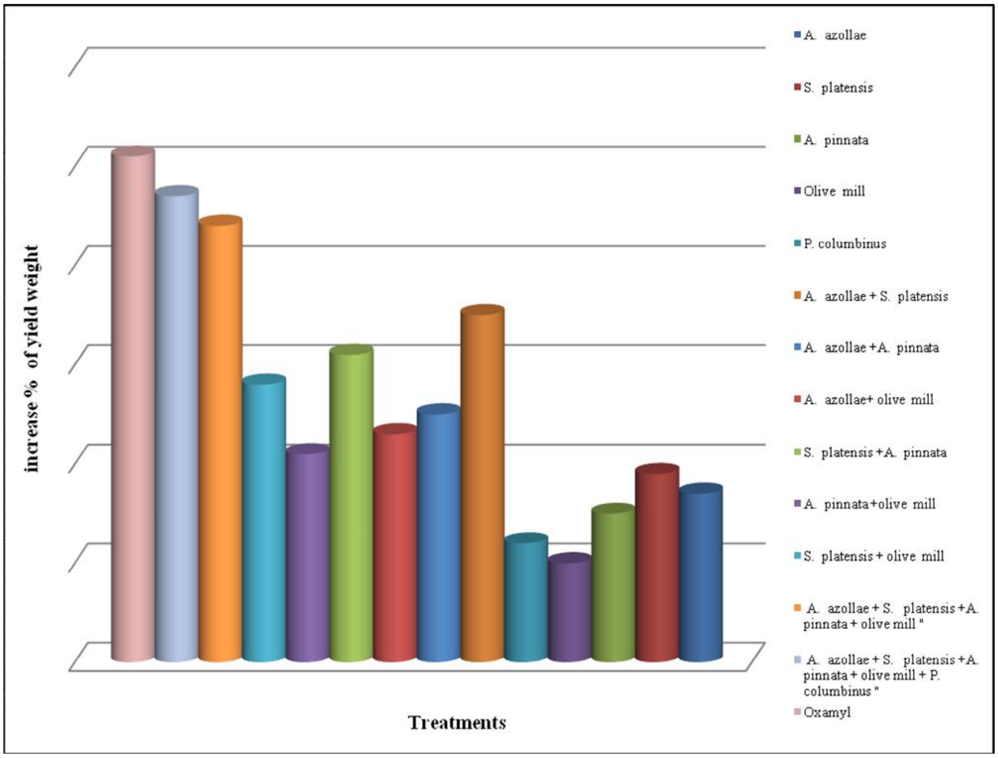

Fig. (2): Effect of *S. platensis*, *A. azollae, A. pinnata*, *P. columbines* and olive mill in combination or single application on increase % of yield weight of banana plants infected with *M. javanica* under productivity greenhouse conditions.

Shawky *et al.* (2009) mentioned that, in the productivity greenhouse experiment, the combination of 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 nematodes and 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.

3. Biological and chemical analyses of *M. javanica* infected soil remaining after banana harvesting

The remaining soil after banana harvesting was analyzed to record the changes in soil biological and chemical conditions due to the different treatments in the productivity greenhouse experiment. Generally, all the combined treatments enhanced soil biological activity than the individual ones **Table 5.**

Table (5): Biological activity of *M. javanica* infected soil remaining after banana harvesting.

Treatments	CO_2 evolution (mg100g soil^{-1})	Dehydrogenase activity (µg TPFg^{-1} dry soil Day^{-1})	Nitrogenase activity (µ mole C_2H_4 g soil^{-1}hr.$^{-1}$)
*A. azolla*e	44	24.23	226.67
S. platensis	50.6	25.46	73
A. pinnata	45	24.51	182.59
Olive mill	30.8	21.9	55
P. columbinus	33	23.82	62
A.azollae + *S. platensis*	110.0	28.07	583.47
*A. azolla*e +*A. pinnata*	66	27.39	489.97
A. azollae + olive mill	51.7	26.43	245.64
S. platensis +*A.pinnata*	220.0	28.76	644.69
A. pinnata +olive mill	61	26.56	430.02
S. platensis + olive mill	67	27.39	562.29
*A. azolla*e + *S. platensis* +*A. pinnata* + olive mil	146.3	39.99	872.41
*A. azolla*e + *S. platensis* +*A. pinnata* + olive mill + *P. columbinus*	220	44.24	1015.09
Oxamyl	270.0	46.16	3012.57
Nematode only	16.5	14.23	42.11
L. S. D. at 0.05%	5.4	3.7	78.5

The superiority of biological activity of the soil was due to the synthetic nematicide; (oxamyl). The combined treatment of *A. azolla*e + *S. platensis* +*A. pinnata* + *P. columbinus* + olive mill revealed the highest soil biological activity compared with other treatments. All treatments affected significantly the biological activity of the soil, **Table 5.**

It could be noticed that dehydrogenase and nitrogenase activities, as well as CO2 production, in all treatments were higher compared to control. The maximum microbial activity was achieved by the chemical pesticide followed by combined effect of biofertilizer with olive mille as soil organic amendment. While, the changes of biological activity in root rhizosphere were greatly fluctuated among the other treatments, Moreover, the application of both Azolla and cyanobacteria enhanced the soil microbial activity. Obtained results are in line with those found by **Waseem *et al.* (2012)** who reported biological health of the soil due to application of Azolla has resulted in improving mineralization and consequent increase microbial status of the soil. Also, cyanobacteria promoted establishment of microbial population, increased organic matter and nutrient content and soil stability **Acea *et al.* (2003).** The blue green algae positively affected plant characteristics which led to improve in yield and fruit quality of banana trees. 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 represented by dehydrogenase, nitrogenase and phosphatase activities. Also, **Shawky *et al.*(2009)** said that in the productivity greenhouse experiment, 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.

It could be recommended that application of bio-control agents of mixed *A. azolla*e + *S. platensis*+*A. pinnata* + olive mill waste water + *P. columbinus* as plant growth promoting substances to increase plant nutrients availability and has nematicidal effect to control root knot nematode in banana.

References

Abad P., Favery B., Rosso M. and Castagnone-Sereno P. (2003). Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Mol. Pl. Path., 4: 217-224.

Abd El-All Azza (2013). Characterization of Anabaena azollae isolated from *Azolla Pinnat.* Egypt.J.Agric.Res., 91 (3): 801-807.

Abd El-Baky H.H., El Baz F.K. and El-Baroty G.S. (2009). Phenolics from *Spirulina maxima*: Over-production and *in vitro* protective effect of its phenolics on CCl4 induced hepatotoxicity. Journal of Medicinal Plants Research, 3(1): 24-30.

Acea, M.J.; Prieto.F, A.; and Diz,C. N. (2003). "Cyanobacterial inoculation of heated soils: effect on microorganisms of C and N cycles and chemical transformation. Plant and Soil, 34:17- 28.

Anastasiadis I.A., Giannakou I.O., Prophetou-Athanasiadou D.A. and Gowen S.R. (2008). The combined effect of the application of a biocontrol agent *Paecilomyces lilacinus*, with various practices for the control of root-knot nematodes. Crop Prot., 27: 352-361.

AOAC (1980). Official Methods of Analysis of the Association of Official Analytical Chemist. 12^{th} ed. Washington, D.C., USA.

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

Casida L. E., Klein D.A. and Santoro T. (1964). Soil dehydrogenase activity. Soil Sci., 98: 371-376.

Dart P.J., Day J.M. and Harris D. (1972). Assay of nitrogenase activity by acetylene reduction. In: Use of isotopes for study of fertilizer utilization by legume crops. FAO/IAEA Technical Report Series, 149: 85-97.

Fay P. (1983). The Blue-Greens. Edward Arnold, London.

Franklin M.T. and Goodey J.B. (1957). A cotton-blue lactophenol technique for mounting plant parasitic nemato des. J. Helminthological Abstracts, 23: 175- 178.

Gaur A.C., Sadasivan K.V., Vimal O.P. and Mathur R.S. (1971). A study of decomposition of organic matter in an alluvial soil. CO_2 evolution, microbiological and chemical transformation. Plant and Soil, 34:17-28.

Gervick W.H., Tan L.T. and Siachitta N. (2001). In: Cordell, G. (Ed.), The Alkaloids, Vol. 57. Academic Press, San Diego, p. 75–184.

Gomez K. W. and Gomez A. A. (1984). Statistical procedures for agricultural research 2 nd Ed. p. 680 John Wiley and Sons inc, New York.

Henderson, G.E. and E.W. Tillton. (1955). Test with acaricides against the brown wheat mite. J. Econ. Entomol.,48:157-161.

Khan Z., Jairajpuri M.S. and Khan M.W. (1997). Effect of culture filtrate of a blue-green alga *Microcoleus vaginatus* on mortality and hatching of root-knot nematode *Meloidogyne incognita*. International Journal of Nematology, 7: 100–102.

Khan Z., Park S.D., Shin S.Y., Bae S.G., Yeon I.K. and Seo Y.J. (2005). Management of *Meloidogyne incognita* on tomato by root-dip treatment in culture filtrate of the blue-green alga *Microcoleus vaginatus*. Bioresource Technology, 96: 1338-1341.

Leduy, A. and N. Therien (1977). An improved method for optical density measurement of semimicro blue-green alga *Spirulina maxima*. Biotechnol Bioeng. 19:1219-1222

Lee Y. (2004). Microalgal mass culture system and methods: their limitation and potential. J. Appl. Phycology,13(4): 1573-1576.

Liu T., Wang L., Duan Y.X. and Wang X. (2008). Nematicidal activity of culture filtrate of *Beauveria bassiana* against *Meloidogyne hapla*. World J. Microbiol. Biotechnol., 24: 113–118.

Mahmoud A.A., Mostafa, S.S.M., Abd El-All,A. A.M. and Hegazi A.Z. (2007). Effect of cyanobacterial inoculation in presence of organic and inorganic amendments on carrot yield and sandy soil properties under drip irrigation regime. Egypt. J. of Appl. Sci., 22(12B):716-733.

Mahmoud M. B. (2001). Physiological Studies on *Biomphalaria alexandrina* Snails and Their Susceptibility to *Schistosoma mansoni* Infection. Ph.D. Thesis, Fac. Science, Ain Shams Univ., Cairo, Egypt 118 pp.

Martin, J.D. (1950). Use of acid rose Bengal and Streptomycien in the plate method for estimating soil fungi.Soil Sci., 69: 215.

Meadows J., Gill S.S. and Bone L.W. (1989). Factors influencing lethality of *Bacillus thuringiensis kurstaki* toxin for eggs and larvae of *Trichostrongylus colubriformus* (Nematoda). Journal of Parasitology, 75:191–194.

Oka Y., Koltai H., Bar-Eyal M., Mor M., Sharon E., Chet I. and Spiegel Y. (2000). New strategies for the control of plant parasitic nematodes. Pest Manage. Sci., 56:983-988.

Oostendrop M. and Sikora R. A. (1989). Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar-beet. Revue Nematologie, 12:77-83.

Rippka, R., J. Deruelles, J. B. Waterburg, M. Herdman and R.Y. Stanier (1979). Generic assignments,strain histories and properties of pure cultures of cyanobacteira. J. of General Microbiol, 111: 1-16.

Shawky, Samaa M., A.E. Khalil and Manal M. Soliman (2010). Non chemical control of root-knot nematode; *Meloidogyne javanica* on peanut in Egypt. Zagazig J. Agric. Res., 37 (1): 185-206.

Shawky, Samaa M. ; Soha S. M. Mostafa and Azza A. M. (2009). Efficacy of some algal, Azolla and compost extract in controlling root-knot nematode and its reflection on cucumber. Bull. Fac. Agric., Cairo Univ. 60:443-459

Soliman Amira Sh., Shawky Samaa M. and Omar M. N. A. (2011). Efficency of bioagents in controlling root knot-nematode on *Acacia* plants in Egypt. American-Eurasian J. Agric. & Environ. Sci., 10(2):223-229.

Tadros M.M., Ghaly N.S. and Moharib M.N. (2008). Molluscicidal and schistosomicidal activities of a steroidal saponins-containing fraction from *Dracaena fragrans* (L.). J. Egypt. Soc. Parasitol., 38 (2): 585-598.

Tahmid, H. Ansari, M. A. Malek, M.U. Ahmed, Sota Tanak and Tetsushi Yoshida (2002). Influence of *Azolla pinnata* in combination with mustard oil cake on galling of *Meloidogyne javanica* and growth of eggplant. Pakistan Journal of Biological Sciences 5(6): 665-668.

Waseem, R.; Preeti, R.; Suchit, A. J., and Pramod, W. R. (2012). Azolla-Anabaena Association and Its Significance in Supportable Agriculture. J. Biol. & Chem., 40 (1): 1–6.

Westcott III S.W. and Kluepfel D.A. (1993). Inhibition of *Criconemella xenoplax* egg hatch by *Pseudomonas aureofaciens*. Phytopathology, 83: 1245–1249.

Vonshak A. (1986). Laboratory techniques for the cultivation of microalgae. In Handbook of microalgal mass culture. (ed. A. Richmond). Boca Raton:CRC Press.

Yoshida, S.; Forno, D. A.; Cock, J. H. and Gomez, K. A.(1976). Laboratory manual for physiological studies for rice. I.R.R.I., The Philippines.

Zaccaro M.C., Salazar C., Zulpa de G., Caire Stroni de M.M., Cans and Stella A.M. (2001). Lead toxicity in cyanobacterial prophyrin metabolism. Environ. Toxocol.and water Quality, 16: 61-67.

Zarrouk C. (1966). Contribution á l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthése de *Spirulina maxima* (Setch. Et Gardner) Geitler. Ph. D. Thesis, University of Paris, France 87 pp.

الملخص العربي

مقارنة كفاءة استخدام بعض أنواع الطحالب والازولا وعيش الغراب ومخلفات الزيتون في مكافحة نيماتودا تعقد الجذور على الموز

سماء محمود شوقي عبد المنعم*، عزة أحمد محمد عبد العال، أحلام محمد الغنيمى***

* قسم بحوث الأمراض النيماتودية – معهد بحوث أمراض النباتات– مركز البحوث الزراعية – الجيزة– مصر.

** قسم الميكروبيولوجيا– معهد بحوث الأراضي والمياه والبيئة – مركز البحوث الزراعية – الجيزة– مصر.

*** قسم وقاية النبات– وحدة النيماتودا – مركز بحوث الصحراء – القاهرة – مصر.**

تهدف هذه الدراسة إلى استخدام اثنين من المزارع الطحلبية وهي: *Spirulina platensis, Anabaena azollae* بالإضافة إلى المستخلص المائي لسرخس *Azolla pinnata* وفطر عيش الغراب والمستخلص المائي لمخلفات عصر الزيتون لمقاومة نيماتودا تعقد الجذور *Meloidogyne javanica* والتي تصيب الموز تحت ظروف المعمل والصوبة الإنتاجية.

أظهرت التجارب المعملية موت يرقات النيماتودا بنسبة عالية في كل المعاملات وعند كل فترات التعريض حيث تحققت أفضل النتائج عند فترة تعريض ٧٢ ساعة. كما أوضحت النتائج أن الطحلب *Spirulina platensis* بالإضافة إلى *Anabaena azollae, Azolla pinnata, Pleurotus columbinus* والمستخلص المائي لمخلفات عصر الزيتون أعطي أفضل النتائج المعملية (٨٥.٢ ، ٨١.٤ ، ٧٩.٩ ، ٧٣.٥، ٧١.٧ و ٧٠.١% على التوالي) حيث ارتفعت نسب موت يرقات نيماتودا تعقد الجذور عن ٧٠% عند التركيز الأعلى (١:١٠).

أوضحت تجارب الصوبة الإنتاجية أن المعاملة المختلطة من مخلوط راشح مزارع *Spirulina platensis*, *Anabaena azollae Azolla pinnata, Pleurotus columbinus* والمستخلص المائي لمخلفات عصر الزيتون أدت إلى لأعلى نسبة انخفاض في تعداد النيماتودا في كل من التربة والجذور وكذلك في عدد العقد النيماتودية كما حققت زيادة ملحوظة في محصول الموز بالمقارنة بالمعاملات المنفردة والكنترول. كما أدى استخدام جميع المعاملات المختلطة إلى زيادة معنوية في النشاط البيولوجي للتربة (انطلاق ثاني أكسيد الكربون وإنزيمات الديهيدروجينيز والنيتروجينيز) عن الكنترول. كذلك يمكن من هذه الدراسة التوصية باستخدام المكافحة الحيوية لنيماتودا تعقد الجذور على الموز لتفادي الآثار السيئة المترتبة عن استخدام المبيدات النيماتودية.