Non Chemical Control of *Heterodera goldeni* and *Meloidogyne incognita* on Rice Plants using Residues of Oyster Mushroom Cultivation and Supernatant of *Bacillus thuringiensis* before Transplanting under Field Microplots Conditions

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Abstract

Two trials were carried out under field microplots conditions during April-August 2015, to study the effect of soil treatment (five days prior to planting) with decomposed and non decomposed residues of oyster mushroom cultivation (OMC) at 500g/m^2, supernatant (S) of *Bacillus thuringiensis* (Bt) alone and their combinations at the half dose (250g/m^2) and concentration (S/2), with a comparative treatment by the chemical nematicide Vydate® L. (Oxamyl 24% SL) at the recommended dose (5 liters/feddan) on the management of the cyst (*Heterodera goldeni*) and root-knot (*Meloidogyne incognita*) nematodes infecting rice plants cv. Sakha 101. All treatments greatly (*P*=0.05) reduced the numbers of cysts of *H. goldeni*, root galls and egg masses of *M. incognita* on rice plant roots as compared to the control plants infected with either nematode species alone. Of the tested treatments, Vydate® L. provided the highest reduction (90.02%) of *H. goldeni* cysts, followed by the combination of residues of OMC decomposed for 4 weeks and supernatant (S/2) of Bt (84.11%), while the combined treatment with non decomposed residues of OMC and supernatant (S/2) of Bt gave the lowest one (59.15%). Other treatments recorded 66.1-75.04% reduction of cysts. Similarly, Vydate® L., supernatant (S) of Bt (alone) and residues of OMC decomposed for 4 weeks (alone and their combination with supernatant (S/2) of Bt) showed the maximum reduction of root galls (79.17-88.17%) and of egg masses (80.02-88.01%) of *M. incognita* with no significant differences between them. Other soil applications gave 66.54-72.08% reduction of root galls and 61.1-68.56% of egg masses On the other hand, all treatments significantly increased dry weight of rice plants (25.44-80.96%) and improved spikes yield/plant (44.5-173.0%) as compared to the control ones infected with *H. goldeni* only. Likely, these treatments resulted in valuable increase (28.43-75.02%) of total dry weights and 61.2-163.6% increase of yield of spikes/plant, as comparing to those infected with *M. incognita* only. It is worthy to note that residues of OMC decomposed for 4 weeks (alone and their combinations with supernatant (S/2) of Bt) appeared to be effective as 50.5 – 62.8%

of Vydate® L. in controlling *H. goldeni*, whereas supernatant (S) of Bt, residues of OMC decomposed for 4 weeks (alone and their combination with supernatant (S/2) of Bt) found to be as effective as 60-70% of Vydate® L. in managing *M. incognita* on rice plants.

Key words: Nematode management, Rice, *Meloidogyne incognita*, *Heterodera goldeni*, Oxamyl 24%SL, *Bacillus thuringiensis*, residues of oyster mushroom cultivation.

Introduction

Rice, *Oryza sativa* L. (family Poaceae) is considered one of the most important and economic cereal crops in Egypt (the largest rice producer in the near east region). Rice cultivation takes place in Egyptian Nile delta especially in the northern part of the country, and the Egyptian rice yield is one of the highest in the world estimated as 9.1 tonnes per hectare in 2001 (**Arafat *et al*., 2010**). The importance of rice crop emanates from the fact that it occupies a large area, estimated at 1.77 million feddan representing 31% of the 2008 summer planted area, which yields 579.6 million tons of rice (**Othman *et al*., 2011**).

Previous nematological survey studies showed that rice plants are attacked by many genera and species of plant-parasitic nematodes that can seriously affect growth and yield of the rice crop. The bud and stem (*Aphelenchoides besseyi*), the rice root (*Hirschmanniella* spp.), the cyst (*Heterodera* spp.) and the root-knot (*Meloidogyne* spp.) nematodes are considered among the most damaging pests of rice plants in many parts of the world (**Babatola, 1984; Luc *et al*., 1990; Ibrahim *et* al., 2010; Jain *et al*., 2011; and Kepenekci, 2013**).

Concerning the environmental risks associated with application of synthetic nematicides in chemical control of plant nematodes and their toxic residues accumulate in crop or fruit tissues, use of the biological control agents of microorganism origin and/or soil organic amending with agro industrial/plant crop residues or animal wastes has been suggested as safe alternatives to the chemical nematicides. They are naturally resourced, effective, and easily biodegradable in the soil and environment (**Chitwood, 2002**).

Numerous succeed trials have been made to apply non chemical methods to manage nematodes infecting economic crops by treatment of nematode-infested soil with organic amendments or plant residues prior to planting **(Mashela, 2002; Bailey and Lazarovits, 2003; López-Pérez *et al*., 2005; Buena *et al*., 2007; Hassan *et al*., 2010; López-Pérez *et al*., 2010; Chindo *et al*., 2012; Youssef and Lashein, 2013 and El-Sherbiny and Awd-Allah, 2014)**. Also, several attempts have been supported the effectiveness of *B. thuringiensis* and other nematode-antagonistic bacteria in controlling root-knot and cyst nematodes on some economic

plants (**Mohammed *et* al., 2008; El-Sherif and Ismail, 2009; Ashoub and Amara, 2010 and Ibrahim *et al*., 2013)**.

Therefore, our aim of the current study is to investigate the effect of application of nematode-infested soil (before planting) with residues of oyster mushroom cultivation (OMC), soil drenching with supernatant of *Bacillus thuringiensis* (a potential nematode-antagonistic bacterium) and their combinations, as comparing to soil treatment with the liquid nematicide Vydate® L. (Oxamyl 24% SL) in the management of *Heterodera goldeni* and *Meloidogyne incognita* infecting rice plants cv. Sakha 101 under field microplots conditions.

Materials and Methods

Cultures and inocula of the tested nematodes

The cyst nematode, *Heterodera goldeni* was originally obtained from naturally infected roots of kleingrass (*Panicum coloratum* L.) from El-Maamoura region, Alexandria governorate and cultured on the same weeds grown in clay pots (25 cm diameter) containing nematode-infested soil under outdoor conditions. Cysts were extracted from soil by floatation, gently crushed in water under binocular microscope and their contents (eggs) were collected and suspended in water for used as inocula (**Shepherd, 1986**).

The root-knot nematode, *Meloidogyne incognita* (Cofoid and White), was initially isolated from heavily galled roots of the ornamental shrub (*Justicia adhatoda* L.) growing in El-Montazah park, Alexandria governorate, and reared on tomato plants (*Solanum lycopersicum* L.) cv. Gold Stone hybrid F1 in a greenhouse for 60 days. Nematode eggs as inocula were extracted from galled tomato roots using 0.5% sodium hypochlorite for 2 minutes (**Hussey and Barker, 1973**).

Preparation of the bacterial culture and supernatant

The Egyptian isolate (7N) of *Bacillus thuringiensis* (Bt), used in this study was originally obtained from the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The Bt bacterial isolate was cultured on T3 broth liquid medium for 72 hours at 30°C (**Travers *et al*., 1987**). The suspension of Bt was placed in sterilized Eppendorf tubes and ultra centrifuged at 13.000 rpm for 15 min to obtain cell-free supernatant (S), which was transferred to sterilized Erlenmeyer flasks. Standard solution (S) of the Bt supernatant was used as it is in the single treatment, and diluted by sterilized water to obtain half concentration (S/2) for use in the combination treatment with residues of the oyster mushroom cultivation.

Decomposition of oyster mushroom cultivation (OMC) residues

Residues of the oyster mushroom, *Pleurotus ostreatus* (rice straw substrate + remains of fungal growth) were collected from the OMC unit at the Integrated

Protection Laboratory, Plant Protection Research Station, Sabahiya, Alexandria governorate at the termination of harvest time of mushroom fruits. The collected residues were left to complete air drying and well ground in an electric home mill to obtain the fine powder. Another amount of the collected OMC residues were exposed to decompose in piles under outdoor conditions for two different intervals (2 and 4 weeks) following addition of light clay soil (10%) to the piles (9 OMC residues: 1 clay), gently wetted and well-mixing along time of the decomposition process. Finally, they left for air drying and ground to the fine powder as previously mentioned. It was well documented that adding clay soil is important to provide microorganisms responsible for primary decomposition of plant organic materials (**Barker and Koenning, 1998**). Samples (100 g dry material) of decomposed and non decomposed OMC residues were sent to the Department of Soil and Water Sciences, Faculty of Agriculture, Alexandria University for analysis and determination of the C:N ratio.

Field microplots experiments

These trials were carried out during April-August, 2015 at the Integrated Protection Laboratory, Sabahiya, Alexandria governorate. For each trial, five microplots (blocks) were designed (4 m long X 50 cm width, each) and divided into nine partitions (40 X 50 cm, each) using thick layers of plastic sheets inserted into the soil depth (30-40 cm) in order to avoid cross contamination through the drainage. Each partition (replication) was filled with a solarized and homogenized mixture of clay and sand (2:1) and infested with 20000 eggs of either nematode species by pipetting egg inocula inside several holes made at a soil depth 10-15 cm.

Research plots comprised the following nine treatments assigned in a randomized complete block (RCBD) design:

1. Soil infested with nematode alone (control).
2. Nematode-infested soil and treated with the liquid nematicide Vydate® (Oxamyl 24% SL) as a comparative chemical treatment.
3. Nematode-infested soil and treated with cell-free supernatant (S) of *Bacillus thuringiensis* (Bt) alone.
4. Nematode-infested soil and treated with non decomposed residues of OMC (500 g/m^2), equivalent to 2 tons/feddan) alone.
5. Nematode-infested soil and treated with residues of OMC decomposed for 2 weeks (500 g/m^2) alone.
6. Nematode-infested soil and treated with residues of OMC decomposed for 4 weeks (500 g/m^2) alone.
7. Nematode-infested soil and treated with the combination of non decomposed residues of OMC (250 g/m^2, equivalent to 1 ton/feddan) + supernatant of Bt (S/2).

8. Nematode-infested soil and treated with the combination of residues of OMC decomposed for 2 weeks (250 g/m^2) + supernatant of Bt (S/2).
9. Nematode-infested soil and treated with the combination of residues of OMC decomposed for 4 weeks (250 g/m^2) + supernatant (S/2) of Bt.

Soon, after soil infestation with either nematode species, all OMC residues (at their appropriate doses for each partition (100 g for single treatment and 50 g for the combination with the supernatant of Bt) were incorporated into the soil and watered at the field capacity to provide proper decomposition of organic materials. Also, supernatant (S) of Bt alone and its combinations with OMC residues (at S/2) were applied as soil drenching soon after nematode infestation at 100 ml/soil partition (replication). Soil partitions assigned for treatment with the chemical nematicide Vydate® L. obtained their appropriate dose of the nematicide equivalent to the recommended dose, 5 liters/feddan (**Ismail *et al*., 2005**) added to the irrigation water before soil drenching, and those assigned for the control treatment (nematode alone) received water only.

For nursery, grains of the rice cultivar Sakha 101, highly susceptible to *H. goldeni* (**Ibrahim *et al*., 2012**) and *M. incognita* (**Ibrahim *et al*., 1973**), were planted in foam transplanting trays (84 holes) containing sterilized mixture of clay and sand (2:1) in order to produce rice seedlings.

Five days after soil applications and decomposition of plant materials (**Youssef and Lashein, 2013)**, all partitions (replications) were transplanted at the same time with three uniform rice seedlings (30 days old) with 15 cm planting distance between each other. Plants received their needs of water and fertilizers along time of the experiments. Seventy five days after transplanting, plants were carefully harvested from each plot, kept in labeled clean plastic bags and sent to the laboratory. Roots were gently washed using tap water to remove adhering soil particles and stained with an aqueous solution of Phloxine B (0.15 g/l. tap water) for 15 min and rewashed with a stream of tap water to remove stain residuals in order to show egg masses of *M. incognita* for counting (**Holbrook *et al*., 1983**). On the other hand, cysts of *H. goldeni* present in soil and on the infected roots were counted according to **Krusberg *et al*., (1994)**. Harvested experimental plants were dried in an electric oven at 60°C for 48 hours, and the dry weights of the shoot and root systems were determined. Moreover, spikes of the rice plants were gently removed from each plant and weighted.

Statistical analysis

All experimental data were subjected to the analysis of variance (ANOVA) between treatments using SAS software, and their means were compared by the least significant difference (LSD) at 5% level of probability (**SAS, 1997**).

Results and Discussion

According to results illustrated in Table (1), it was clear that all treatments highly (P = 0.05) decreased number of cysts of *H. goldeni* on the rice roots as compared to the control plants infected with nematode only. Soil application with Vydate® L. gave the highest reduction (90.02%) of cysts, followed by treatment with residues of OMC decomposed for 4 weeks alone (84.11%) and the combination of residues of OMC decomposed for 4 weeks and supernatant (S/2) of Bt (80.24%), while the lowest one (59.15%) was provided by the combination of non decomposed residues of OMC and supernatant (S/2) of Bt. Soil drenching with the supernatant (S) of Bt achieved 75.04% reduction of nematode cysts and other treatments resulted in a considerable reduction ranged from 66.1 to 71.39% (Table 1).

On the other hand, dry weights and yield of rice plants grown in all treatments were significantly increased as comparing to those of the control ones infected with *H. goldeni* alone. Vydate® L., residues of OMC decomposed for 4 weeks (alone) and their combination with supernatant (S/2) of Bt were the best treatments in improving growth performance of rice plants, recording 75.26, 80.96 and 70.33% increase of total dry weight, respectively. Also, they provided great increase (159.8-173.0%) of spikes yield/plant. Other treatments gave only 25.44-54.82% increase of total dry weight and 44.5-110.3% increase of spikes yield/plant (Table 2).

Similarly, all soil applications greatly (P= 0.05) reduced the numbers of root galls and egg masses of *M. incognita* infecting rice roots. Vydate® L., supernatant (S) of Bt (alone) and residues of OMC decomposed for 4 weeks (alone and their combination with supernatant (S/2) of Bt) were the best treatments recording the maximum reduction of root galls (79.17-88.17%) and of egg masses (80.02-88.01%) with no significant differences between them. Other treatments gave a valuable reduction (66.54-72.08%) in root galls and (61.1-68.56%) in egg masses as comparing to the check plants infected with *M. incognita* only (Table 3).

Also, dry weights of rice plants in all treatments were significantly increased (28.43-79.02%) and weight of spikes/plant (61.2-163.6%) as compared to those of check ones. Vydate® L., residues of OMC decomposed for 4 weeks (alone) and their combination with supernatant (S/2) of Bt provided the maximum increase (69.25-79.02%) of total dry weight and (143.4-163.6%) of the yield of spikes/plant (Table 4).

Effectiveness of OMC residues in nematode control in the current study is in agreement with other authors (**D'Addabbo *et al*., 2011; Khattak and Khattak, 2011; Aslam and Saifullah, 2013 and El-Sherbiny and Awd-Allah, 2014**). They found that residues of spent mushroom compost and OMC residues were effective as soil amendments in the management of *Meloidogyne* spp. on tomato plants and improving their growth performance and fruit yield.

Table (1): Effect of soil treatment (5 days before planting) with decomposed and non decomposed residues of oyster mushroom cultivation (OMC), supernatant (S) of the bacterium *Bacillus thuringiensis* (*Bt*) alone and their combinations, as compared to soil application with the liquid nematicide Vydate® L. (Oxamyl 24% SL) on the management of the cyst nematode, *Heterodera goldeni* (*Hg*) on rice plants cv. Sakha 101 (75 days after transplanting under field microplots conditions).

Treatment	No. of cysts /plant	Reduction (%)	Relative nematicidal efficacy to Vydate® L.(%)
Heterodera goldeni, *Hg* alone (Control)	423.0 **a**	-	-
Nematicide (Vydate® L.) at 5 litters/feddan + *Hg*	42.2 **f**	90.02	-
Supernatant (S) of *Bt* alone (100 ml/replication) + *Hg*	105.6 **cd**	75.04	40.0
Residues of non decomposed OMC (500g/m^2 soil) + *Hg*	143.4 **bc**	66.10	29.4
Residues of OMC decomposed for 2 weeks (500g/m^2 soil) + *Hg*	121.0 **cd**	71.39	34.9
Residues of OMC decomposed for 4 weeks (500g/m^2 soil) + *Hg*	67.2 **ef**	84.11	62.8
Residues of non decomposed OMC (250g/m^2 soil) + *Bt* (S/2) + *Hg*	172.8 **b**	59.15	24.4
Residues of OMC decomposed for 2 weeks (250g/m^2 soil) + *Bt* (S/2) + *Hg*	124.4 **c**	70.59	33.9
Residues of OMC decomposed for 4 weeks (250g/m^2 soil) + *Bt* (S/2) + *Hg*	83.6 **de**	80.24	50.5
LSD (*P* = 0.05)	37.924		

- Data are means of five replicates (three plants, each).
- Values within columns followed by the same letter(s) are not significantly different according to Fisher's protected LSD at *P* = 0.05.
- Reduction (%) = (Control – Treatment / Control) × 100.
- Relative nematicidal efficacy (%) to Vydate® L. = [1 – (Treatment – Nematicide / Treatment)] × 100

Table (2): Effect of soil treatment (5 days before planting) with decomposed and non decomposed residues of oyster mushroom cultivation (OMC), supernatant (S) of the bacterium *Bacillus thuringiensis* (*Bt*) alone and their combinations, as compared to soil application with the liquid nematicide Vydate® L. (Oxamyl 24% SL) on the dry weight and yield of rice plants cv. Sakha 101 infected by the cyst nematode, *Heterodera goldeni* (*Hg*), 75 days after transplanting under field microplots conditions.

Treatment	Dry weight (g)			Total increase (%)	Weight of spikes/ plant (g)	Increase (%)
	Shoots	Roots	Total			
Heterodera goldeni, Hg alone (Control)	10.15 f	6.87 e	17.02 f	-	5.15 d	-
Nematicide (Vydate® L.) at 5 litters/feddan + *Hg*	17.91 a	11.92 ab	29.83 ab	75.26	14.06 a	173.0
Supernatant (S) of *Bt* alone (100 ml/replication) + *Hg*	14.84 cde	9.89 cd	24.73 de	45.30	10.68 b	107.4
Residues of non decomposed OMC (500g/m^2 soil) + *Hg*	12.73 e	8.62 d	21.35 e	25.44	8.23 c	59.8
Residues of OMC decomposed for 2 weeks (500g/m^2 soil) + *Hg*	15.61 bc	10.74 bc	26.35 bcd	54.82	10.83 b	110.3
Residues of OMC decomposed for 4 weeks (500g/m^2 soil) + *Hg*	18.09 a	12.71 a	30.80 a	80.96	13.38 a	159.8
Residues of non decomposed OMC(250g/m^2 soil) +*Bt* (S/2) + *Hg*	13.02 de	10.43 bc	23.45 de	37.78	7.44 c	44.5
Residues of OMC decomposed for 2 weeks (250g/m^2 soil)+*Bt* (S/2)+ *Hg*	15.15 bcd	10.17 cd	25.32 cd	48.77	9.18 bc	78.3
Residues of OMC decomposed for 4 weeks (250g/m^2 soil)+*Bt* (S/2)+ *Hg*	17.21 ab	11.78 ab	28.99 abc	70.33	13.53 a	162.7
LSD (P = 0.05)	2.2512	1.5585	3.7969		2.176	

- Data are means of five replicates (three plants, each).
- Values within columns followed by the same letter (s) are not significantly different according to Fisher's protected LSD at P = 0.05.
- Increase (%) = (Treatment – Control / Control) ×100.

Table (3): Effect of soil treatment (5 days before planting) with decomposed and non decomposed residues of oyster mushroom cultivation (OMC), supernatant (S) of the bacterium *Bacillus thuringiensis* (*Bt*) alone and their combinations, compared to soil application with the liquid nematicide Vydate® L (Oxamyl 24% SL) on the management of the root-knot nematode, *Meloidogyne incognita* (*Mi*) on rice plants cv. Sakha 101 (75 days after transplanting under field microplots conditions).

Treatment	No. of root galls /plant	Reduction (%)	No. nematode egg masses/ plant	Reduction (%)	Relative nematicidal efficacy to Vydate®L.(%)	
					Root galls	Nematode Egg masses
Meloidogyne incognita, *Mi* only (Control)	375.4 a	-	225.2 a	-	-	-
Nematicide (Vydate® L.) at 5 liters/feddan + *Mi*	44.4 d	88.17	27.0 c	88.01	-	-
Supernatant (S) of *Bt* alone (100 ml/replication)+ *Mi*	70.0 d	81.35	38.6 c	82.86	63.4	70.0
Residues of non decomposed OMC (500g/m^2 soil)+*Mi*	125.6 b	66.54	86.4 b	61.63	35.4	31.3
Residues of OMC decomposed for 2 weeks (500g/m^2 soil)+*Mi*	115.8 b	69.15	80.6 b	64.21	38.3	33.5
Residues of OMC decomposed for 4 weeks (500g/m^2 soil)+*Mi*	74.4 cd	80.18	45.0 c	80.02	59.7	60.0
Residues of non decomposed OMC (250g/m^2 soil) + Bt (S/2)+*Mi*	117.2 b	68.78	87.6 b	61.10	37.9	30.8
Residues of OMC decomposed for 2 weeks (250g/m^2 soil)+Bt (S/2)+*Mi*	104.8 bc	72.08	70.8 b	68.56	42.4	38.1
Residues of OMC decomposed for 4 weeks (250g/m2 soil)+*Bt* (S/2)+*Mi*	78.2 cd	79.17	44.0 c	80.46	56.8	61.4
LSD (*P* = 0.05)	34.689		20.16			

- Data are means of five replicates (three plants, each).
- Values within columns followed by the same letter (s) are not significantly different according to Fisher's protected LSD at *P* = 0.05.
- Reduction (%) = (Control – Treatment / Control) X 100.
- Relative nematicidal efficacy (%) to Vydate® L. = [1 – (Treatment – Nematicide / Treatment)] × 100.

Table (4): Effect of soil treatment (5 days before planting) with decomposed and non decomposed residues of oyster mushroom cultivation (OMC), supernatant (S) of the bacterium *Bacillus thuringiensis* (*Bt*) alone and their combinations, compared to soil application with the liquid nematicide Vydate® L (Oxamyl 24% SL) on the dry weight and yield of rice plants cv. Sakha 101 infected by the root-knot nematode, *Meloidogyne incognita* (*Mi*), 75 days after transplanting under field microplots conditions.

Treatment	Dry weight (g)			Total Increase (%)	Weight of spikes/plant (g)	Increase (%)
	Shoots	Roots	Total			
Meloidogyne incognita, *MI* only (Control)	11.57 f	8.69 e	20.26 f	-	6.27 f	-
Nematicide (Vydate® L.) at 5 liters/feddan + *MI*	18.85 abc	15.44 ab	34.29 abc	69.25	15.52 ab	147.5
Supernatant (S) of *Bt* alone (100 ml/ replication)+ *MI*	17.41 bc	14.16 abc	31.57 bcd	55.82	12.68 d	102.2
Residues of non decomposed OMC (500g/m^2 soil) + *MI*	14.83 de	12.26 cd	27.09 de	33.71	10.11 e	61.2
Residues of OMC decomposed for 2 weeks (500g/m^2 soil) + *MI*	16.80 cd	13.71 bcd	30.51 cde	50.59	12.82 cd	104.5
Residues of OMC decomposed for 4 weeks (500g/m^2 soil) + *MI*	20.07 a	16.20 a	36.27 a	79.02	15.26 abc	143.4
Residues of non decomposed OMC (250g/m^2 soil) + *Bt* (S/2) + *MI*	14.27 e	11.75 d	26.02 e	28.43	11.38 de	81.5
Residues of OMC decomposed for 2 weeks (250g/m^2 soil)+*Bt* (S/2)+ *MI*	16.64 cde	13.58 bcd	30.22 cde	49.16	13.69 bcd	118.3
Residues of OMC decomposed for 4 weeks (250g/m^2 soil)+*Bt* (S/2)+ *MI*	19.55 ab	16.02 a	35.57 ab	75.57	16.53 a	163.6
LSD ($P = 0.05$)	2.4958	2.0618	4.5532		2.4422	

- Data are means of five replicates (three plants, each).
- Values within columns followed by the same letter (s) are not significantly different according to Fisher's protected LSD at $P = 0.05$.
- Increase (%) = (Treatment – Control / Control) × 100.

In accordance with our data, some researchers indicated that bacterial supernatant of Bt can be able to kill free-living nematode *Caenorhabditis elegans* (**Deviddas and Rehberger 1992**) and freshly hatched second stage juveniles (J_2) of *Meloidogyne javanica* within 24–48 hours (**Carneiro *et al*. 1998**). Our results confirmed the nematicidal potential of Bt supernatant against *H. goldeni* and *M. incognita* and they in agreement with those given by other authors (**Mohammed *et al*., 2008 and Ibrahim *et al*., 2013**). It is well-known that Bt can produce an exotoxin called (Thuringiensin) and a number of other toxins with different structure and mode of action against *Meloidogyne* spp. (**Deviddas and Rehberger 1992 and Mohammed *et al*., 2008**).

The modes of nematode suppression occurred following decomposition of organic plant waste materials incorporated into nematode-infested soil prior to planting are attributed to releasing of certain phytochemical constituents found in plant tissues toxic to nematodes (**Chitwood, 2002**), generation of some nematicidal compounds, such as ammonia and fatty acids during decomposition of plant materials, increase in plant tolerance to nematode infection as a result of enhanced plant nutrition due to increase of soil fertility and changes in soil physiology that are unsuitable for nematode behavior (**Oka, 2010).**

It was noticed that soil treatment with residues of OMC decomposed for 4 weeks having a significant nematicidal activity towards *H. goldeni* and *M. incognita* more than those decomposed for 2 weeks and non decomposed ones (Tables 1-3). Previous review of literature has proposed that potential of nematode suppression by organic soil amendments generally depends on the amount of the amendment used, their C:N ratios, and time of decomposition in the soil. Also, grinding of the dried plant materials may be important to make decomposition process easily done. Moreover, organic materials with C:N ratio less than 20:1 have higher biodegradation rates in soil and often nematicidal activities (**McSorely and Gallaher, 1995; Ritzinger and McSorley, 1998 and Mashela, 2002**). Our findings highly agreed with the previous authors, where we found that the C:N ratio (Table 5) of residues of OMC decomposed for 4 weeks (11.5:1) is less than those decomposed for 2 weeks (16.6:1) and non decomposed ones (24.5:1). These observations may explain their high nematicidal performance in the present study.

Table (5): Carbon-Nitrogen (C:N) ratio of the studied residues of the oyster mushroom cultivation (OMC).

OMC residues	C:N ratio
Non decomposed OMC residues	24.5:1
OMC residues decomposed for 2 weeks	16.6:1
OMC residues decomposed for 4 weeks	11.5:1

Based on the calculated relative nematicidal efficacy of all treatments to the nematicide Vydate® L., it was found that soil treatments with residues of OMC decomposed for 4 weeks (alone) and their combination with Bt supernatant (S/2) appeared to be effective as 50.5 – 62.8% of Vydate® L. in controlling *H. goldeni* (Table 1), whereas Bt supernatant (S) alone, residues of OMC decomposed for 4 weeks (alone) and their combination with supernatant (S/2) of Bt found to be as effective as 70.0, 60.0 and 61.4% of Vydate® L., respectively (Table 3). These results are in good agreement with those earlier given by **Zurreen and Khan (1984).** They found that the latex of the plant *Calotropis procera* (10000 ppm) and the nematicide Temik 10G (4 ppm) suppressed egg hatch of *M. javanica* by 65.47 and 61.82%, respectively. Also, **Al-Rajhi *et al*., (1997)** found that ethanolic extract of the wild plant *Rhazia stricta* at 100 ppm was nearly as effective as the nematicide Fenamiphos (Nemacur 10%) at 50 ppm in suppressing egg hatching of *M. javanica* (68.92 vs. 60.37%, respectively). Moreover, soil treatment with powders of some plants, marine algae and animal wastes showed great efficacy as the nematicide Carbofuran 10G in the management of *M. incognita* on common bean plants (**Ibrahim and Ibrahim, 2000**).

Eventually, it was obvious that application of nematode-infested soil (before rice transplanting) with residues of OMC decomposed for 4 weeks alone and their combination with supernatant (S/2) of Bt may serve as promising non chemical alternatives to Vydate® L. in managing *H. goldeni* and *M. incognita* infecting rice plants. These results encourage conducting further trials under large scale to investigate their possible application as nematode suppressants prior to planting and as an economic alternative to other organic and/or chemical fertilizers traditionally used in rice agriculture.

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الملخص العربي

المكافحة غير الكيميائية لنيماتودا الحوصلات ***Heterodera goldeni*** ونيماتودا تعقد الجذور ***Meloidogyne incognita*** على نباتات الأرز باستخدام متبقيات زراعة عيش الغراب المحاري وراشح البكتيريا ***Bacillus thuringiensis*** قبل الشتل تحت ظروف القطع الحقلية الصغيرة

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أُجريت تجربتين تحت ظروف القطع الحقلية الصغيرة خلال الفترة من أبريل – أغسطس ٢٠١٥م، وذلك لدراسة تأثير معاملة التربة (قبل الزراعة بخمسة أيام) بمساحيق متبقيات زراعة عيش الغراب المحاري (المكمورة وغير المكمورة) بمعدل ٥٠٠ جم/م٢، راشح البكتيريا ***Bacillus thuringiensis*** **(S)**، وذلك كمعاملات منفردة وبمخاليطهما معًا عند نصف الجرعة (٢٥٠ جم/م٢) ونصف التركيز (**S/2**)، مقارنة مع المبيد الكيميائي السائل فايديت (أوكساميل ٢٤٪) عند الجرعة الموصى بها (٥ لترات/الفدان)، على مكافحة نيماتودا الحوصلات ***Heterodera goldeni*** ونيماتودا تعقد الجذور ***Meloidogyne incognita*** اللتان تصيبان نباتات الأرز (صنف سخا ١٠١). أدت جميع المعاملات إلى خفض معنوي كبير في أعداد حوصلات نيماتودا ***H. goldeni*** وأعداد العقد الجذرية وأكياس البيض التي تكونها نيماتودا ***M. incognita*** على جذور نباتات الأرز، بالمقارنة مع النباتات المصابة بالنيماتودا فقط. وقد سجلت المعاملات بمبيد الفايديت أعلى نسبة خفض لأعداد الحوصلات بلغت ٩٠.٠٢٪، تبعتها المعاملة المختلطة من كل من متبقيات زراعة عيش الغراب المحاري المكمورة لمدة ٤ أسابيع (٢٥٠ جم/م٢) مع الراشح البكتيري عند نصف تركيزه (**S/2**) محققة نسبة خفض لأعداد الحوصلات قدرها ٨٤.١١٪، بينما أدت المعاملة بمخلوط متبقيات زراعة عيش الغراب المحاري غير المكمورة (٢٥٠ جم/م٢) والراشح البكتيري (**S/2**) إلى أقل نسبة خفض لأعداد الحوصلات بلغت ٥٩.١٥٪. وقد أدت بقية المعاملات إلى خفض في أعداد الحوصلات تراوح بين ٦٦.١–٧٥.٠٤٪. وبصورة مشابهة، أظهرت المعاملة بكل من مبيد الفايدت، الراشح البكتيري (**S**)، المعاملة المختلطة من كل من متبقيات زراعة عيش الغراب المحاري المكمورة لمدة ٤ أسابيع بمفردها (٥٠٠ جم/م٢) أو بمخلوطها (٢٥٠ جم/م٢) مع الراشح البكتيري (**S/2**)، قدرة كبيرة على خفض أعداد العقد الجذرية بنسبة تراوحت بين ٧٩.١٧–٨٨.١٧٪، وكذا أعداد أكياس بيض نيماتودا ***M. incognita*** بنسبة تراوحت بين ٨٠.٠٢–٨٨.٠١٪ (بدون وجود فروق معنوية بين تلك المعاملات). كما نجحت بقية المعاملات في خفض أعداد العقد الجذرية بنسبة تراوحت بين ٦٦.٥٤–٧٢.٠٨٪، وأعداد أكياس البيض بنسبة تراوحت بين٦١.١–٦٨.٥٦٪. ومن ناحية أخرى، أدت جميع المعاملات إلى زيادة معنوية في كل من الأوزان الجافة لنباتات الأرز تراوحت بين ٢٥.٤٤–٨٠.٩٦٪، ووزن محصول السنابل (٤٤.٥–١٧٣.٠٪)، مقارنة بمثيلتها في النباتات المصابة بنيماتودا ***H. goldeni*** فقط. وبصورة مماثلة، أدت جميع المعاملات إلى زيادة قيمة في كل من الأوزان الجافة للنباتات تباينت بين ٢٨.٤٣–٧٥.٠٢٪، وأوزان السنابل / النبات بنسبة ٦١.٢–١٦٣.٦٪، مقارنة بمثيلتها المصابة بنيماتودا ***M. incognita*** فقط. جدير بالذكر، أن المعاملة بمتبقيات زراعة عيش الغراب المحاري المكمورة لمدة ٤ أسابيع بمفردها (٥٠٠ جم/م٢) أو بمخلوطها (٢٥٠ جم/م٢) مع الراشح البكتيري (**S/2**) قد سجلت كفاءة إبادية لنيماتودا ***H. goldeni*** على نباتات الأرز تراوحت بين ٥٠.٥–٦٢.٨٪ من كفاءة مبيد الفايديت، بينما أظهرت المعاملات بكل من الراشح البكتيري (**S**)، متبقيات زراعة عيش الغراب المحاري المكمورة لمدة ٤ أسابيع (بمفردها) وبمخلوطها مع الراشح البكتيري (**S/2**)، كفاءة معنوية كبيرة في مكافحة نيماتودا ***M. incognita*** على نباتات الأرز تراوحت بين ٦٠ – ٧٠٪ من كفاءة مبيد الفايديت.