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New Approach for Biocontrolling Root-Knot Nematode, *Meloidogyne incognita* on Cowpea by Commercial Fresh Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

For biocontrolling root-knot nematode, *Meloidogyne* incognita on cowpea**.** antagonistic fungi which considered among the main biological agents used are Basidiomycetes (Mushrooms). These are abundantly producing natural antibiotics in their secondary metabolites possess antimicrobial,nematicidal, antitumor, and antioxidant properties. In the laboratory, three aqueous extract concentrations of commercial fresh oyster mushroom (*Pleurotus ostreatus*) fruits at 5,10 and 15g /100ml distilled water were tested for their effect on the second stage juveniles (J_2) of root-knot nematode, *M. incognita*. They proved that there is positive relation between the tested concentrations and percentages net mortality of juveniles. Under screen house conditions, three rates of mashed fresh mushroom fruit residue at 5,10 and 15 g each per pot were applied for controlling *M. incognita* on cowpea. Using oyster mushroom at the highest rate (15g) achieved the highest percentages of reduction of nematode reproduction by 86.4% and galls by 92.4% compared to the control. The tested rates increased number of bacterial nodules. Also, they increased and improved plant growth and yield (pods and seeds) criteria coinciding with these rates. It could be concluded that commercial fresh mushroon as organic amendment could effectively affect *M. incognita* root-knot nematode and improve cowpea plant vegetative growth and yield parameters.

Keywords: Biocontrol, cowpea, oyster mushroom, root-knot nematode, *M.incognita*.

1. Introduction

As cited by Edwin and Jacob (2017), cowpea, *Vigna unguiculata* (L.) is considered to be an important cash and food crop with high nutritional value for poor farmers in some parts in the world. When there is shortage in animal proteins, cowpea becomes a source of dietary proteins. Infection by root-knot nematodes all over the world takes place to about 2000 plants exhibiting poor growth, a loss in quality and yield of the crop and breaking the resistance of host plant. Also, a high infection by root-knot nematode greatly affects utilization of water and fertilizers, resulting in additional losses for the farmers (Back *et al*., 2002; Castello *et al*., 2003; Manzanilla-Lopez and Bridge, 2004).

For evaluating alternative control strategies, biological control of root-knot nematodes is essential. In this context, biocontrolling phytoparasitic nematodes by the fungi are among the main used biological agents (Li *et al*., 2007)), including *Meloidogyne* species (Swe *et al*., 2011; Degenkolb and Vilcinskas, 2016a,b) due to that they capture and parasitize nematodes (Goswami *et al*., 2006; Haseeb and Kumar 2006; Swe *et al*., 2011), through producing several antagonistic substances (Tranier *et al*.,2014; Degenkolb and Vilcinskas, 2016a). Basidiomycetes(Mushrooms) greatly produce natural antibiotics which possess antimicrobial, antitumor, and antioxidant properties (Sivanandhan *et al*., 2017). Among them, species of *Pleurotus* produce many substances with nematicidal properties including different fatty acids (Li *et al*., 2007); these fungi reduced galls on tomato plants as demonstrated by Putzke *et al.* (2007).In Egypt,there is one study recorded on the effect of commercial mushroom fungi on root-knot nematode,*M. incognita* (El-Sherbiny and Awd Allah, 2014).

Based on the previous information, this study aimed to evaluate the *in vitro* potential of different aqueous extract concentrations of commercial fresh oyster mushroom (*Pleurotus ostreatus*) fruits on *M. incognita* mortality and to evaluate the efficiency of its dfferent rates for *M. incognita* biocontrol on cowpea,and consequently on plant growth and yield under screenhouse conditions.

2. Materials and Methods

2.1. Aqueous extract of commercial fresh oyster mushroom fruits

In order to prepare aqueous extract concentrations, commercial mashed oyster mushroom of fruiting bodies was mixed in proportions of 5,10 and 15g/100ml distilled water.The mixtures were kept for 72 hours. Then, they were filtered through Whatman filter paper No.1.

2.2. Identification and preparing of root-knot nematode pure culture

The tested species of root-knot nematode, *M. incognita* was identified by using protocol described by Taylor and Sasser (1978) by using nematode adult female based on its

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perineal pattern morphological characteristics. Pure culture of *M. incognita* was reared on tomato by a single egg-mass of this nematode inoculated to susceptible tomato cultivar in a screen house at 30 ± 5°C. Newly hatched second stage juveniles (J_2s) of nematode were used as inocula.

2.3. In vitro test

A population of the second stage juveniles(J_2) of *M. incognita* that was reared as pure culture on suseptible tomato plant was used. The population of J_2 from soil was extracted by using method referred to Barker (1985). For extraction of J_2 of *M.* nematode in roots, galled tomato roots bearing egg masses were washed thoroughly with tap water to avoid soil aggregation and debris and teased into small pieces. Then, they were incubated in plastic capsule filled with sufficient water to help egg hatching present in egg masses on roots. To prevent water evaporation, these capsules were covered and J2s collected every 24hrs (Young, 1954**).** *In vitro* test was applied by adding water filtrate at concentrations of 5**,** 10 and 15g fresh mushroom/100ml distilled water. One ml distilled water that contained 300 individuals in plastic capsule was added to 9ml of each mushroom filtrate. Mushroom filtrate was made by soaking each rate in distilled water for three days and filtered by using Whatman filter paper no.1.Equal number of juveniles was also added to equal number of plastic capsules filled with 9 ml distilled water served as control. There were 5 replicates for each treatment.

Under light microscope, numbers of dead and alive juveniles per each treatment were counted 24, 48 and 72 hrs after treatment. The J_2s were considered dead when they did not move when touched with a fine needle. The percentages of nematode mortality were calculated according to Abbott's Formula (1925) as follows:

$$\text{Juvenile mortality (\%)} = (m - n)/ (100 - n) \times 100$$

The percentages of mortality of juveniles in the treatment and control were represented by m and n, respectively. Each capsule containing nematodes after 72hr was filled with distilled water at the same volume replacing mushroom suspension concentration. % nematode recovery in distilled water was determined in which was subtracted from % total mortality after 72hr to obtain % nematode net mortality.

2.4. Effect of fresh mushroom fruit residue on the biocontrol of M. incognita in cowpea plant

To assess effect of different rates of commercial fresh mashed mushroom fruit residue on root-knot nematode,*M. incognita*, 5,10 and 15 g each were added to pots in screen house of Plant Pthology Department, National Research Centre (NRC).Three to four seeds of cowpea (*Vigna unguiculata* (L.) Walp.) cv. Baladi were sown in each pot (20-cm diameter) that contained 2 kg of solarized sandy loamy soil(1:1). One week after seed emergence, each pot was thinned to two plantlets and then, inoculated with 3,000 newly hatched second stage juveniles (J_2) added in four holes made around the plant. At the same time, cowpea plants were treated with the tested three mushroom filtrate concentrations and nematode only with distilled water served as untreated control. The compound, Okadean (containing nitrogen-fixing bacterium namely, *Bradyrhizobium* spp.) at recommended rate was added to pots in all treatments.A completely randomized design was used to arrange pots with 5 replicates for each treatment and control put on a bench and maintained at 30 ± 5 °C. Then, the plants were irrigated as needed.

Three months after the date of nematode inoculation (harvest stage of cowpea), plants of cowpea were carefully uprooted and roots were washed thoroughly as described previously. Then, roots were divided into two portions. Egg mass as well as gall numbers per plant were counted in one portion of roots. Then, the second portion of roots was processed as mentioned previously to extract second stage juveniles from egg masses. Also, number of J_2 in the soil per pot was extracted as mentioned previously. Number of bacterial nodules was recorded for each treatment.

The vegetative parameters of cowpea viz., shoot length (cm), fresh and dry weights of shoots and roots (g) were measured. Number of pods and seeds, and weights of pods and100 seeds (g) were recorded.

Means of total percentages of nematode reduction, plant growth and yield increases were calculated for each treatment as follows:

Mean of total percentages of each treatment (%) = Sum of the percentages of nematode reduction or plant growth and yield increases for each treatment/ number of these parameters ×100. This parameter was used to compare among treatments.

2.5. Statistical analysis

In this experiment, analysis of variance (ANOVA) procedures was performed for significance at 5% level of probability. For comparing among treatments, Duncan's Multiple Range Test by Snedecor and Cochran (1989) was used. This was done by Computer Statistical (COSTAT) software.

3. Results

3.1. Effect of the tested fresh oyster mushroom fruit residue filtrate on nematode mortality

As for mortality of nematode juveniles by using the tested filtrate concentrations of commercial fresh fruits of mushroom, it was noticed that this filtrate caused the nematode mortality depending on its concentration and exposure period, as the percentage of nematode mortality increased with increasing the mushroom filtrate concentration and time of exposure and vice versa. The highest concentration of 15g/100 ml induced the highest percentage net mortality (58%) after exposure period of 72 hrs followed by those of moderate (50%) and the lowest concentrations (40%) (Table 1).

Table 1. % mortality of second-stage juveniles (J_2s) of *Meloidogyne incognita* as affected by fresh oyster mushroom water filtrate concentrations under *in vitro* test.

Treatments	Concentration (g/100ml distilled water)	Nematode(J_2) mortality (%)			Nematode recovery(%)	Nematode net mortality (%)
		Exposure period				
		24h	48h	72h		
Mushroom	5	62	68	70	30	40
	10	70	72	78	28	50
	15	70	78	81	23	58
Distilled water	-	0	0	0	0	0

3.2. Effect of the tested fresh oyster mushroom fruit residue on nematode parameters

Fresh oyster mushroom fruit residue potential for biocontrolling root-knot nematode was proved in *the* present study in which it significantly($p \leq 0.05$) reduced the reproduction and galls of *M. incognita* and improved cowpea plant growth, bacterial nodulation and yield. Nematode biocontrol using mushroom fresh oyster fruit residue at the highest rate(15g) achieved the highest mean of total percentage of reduction of nematode parameters (86.4%) compared to the control.This was followed by 79.2 and 79.3% achieved by 10 and 5g fresh fruits of mushroom, respectively. The highest percentage of gall reduction(92.4%)was obtained by the higest rate of mushroom residue. However, the highest percentage bacterial nodule increase was achieved by the lowest rate of mushroom followed by the highest and moderate rates(Table 2).

Table 2.Effect of mushroom oyster fruit residue rates on root-knot nematode, *Meloidogyne incognita* infecting cowpea

treatments	Number and % reduction of nematode productive parameters and galls/pot or root system									No. of nodules/root system	% Inc.
	J_2 in soil/ Pot	% Red.	J_2 in roots	% Red.	Egg masses	% Red.	% mean of percentages of nematode red.	galls	% Red***.		
Control	8000a**	-	225a	-	95a	-	-	158a	-	15c	-
5g	4200b	47.5	63b	72.0	11b	88.4	69.3	15b	90.5	27a	35.0
10g	2800c	65.0	40c	82.2	9b	90.5	79.2	13b	91.8	18bc	20.0
15g	2380c	70.3	30d	96.3	7b	92.6	86.4	12b	92.4	20b	33.3

*-Each value is mean of 5 replicates. **-The same letter (s) following means of each column indicated that the treatments are not significantly different according to Duncan`s multiple range test at level ($p \leq 0.05$). ***-Red. = Reduction and Inc. =Increase.

3.3. Effect of the tested mushroom fruit residue on cowpea growth and yield

As for vegetative growth and yield criteria of cowpea plant, the means of total percentages of increases of the different plant growth and yield (pods and seeds) criteria were positively related the different rates, as higher mushroom residue rate, higher increase in the different criteria of plant growth and yields and vice versa. On this basis, the highest mean of increase (60.2%) was achieved by using the highest rate of mushroom residue followed by 24.8 and 5.6% increases occurred by moderate and the lowest rates, respectively (Tables 3 and 4).

Table 3. Effect of fresh oyster mushroom fruit residue rates on growth and yield criteria of cowpea as affected by root-knot nematode, *Meloidogyne incognita* infection.

Treatments	Shoot parameters			Root parameters		Pod parameters		Seed parameters		
	Length (cm)	Fresh w. (g)	Dry w. (g)	Fresh w. (g)	Dry w. (g)	N.***	W.*** (g)	N.of seeds/ pod	W. of seeds/ pod (g)	W. of 100 seeds (g)
control	51b**	40.0c	11.1b	7.6b	1.8b	4a	2.5b	6	0.63b	11.4a
5g	51b	41.7c	12.4b	6.8c	2.1b	4a	2.7b	6	0.72b	11.5a
10g	56ab	63.8b	13.9b	7.1c	2.5a	5a	3.0b	7	0.95a	11.6a
15g	60a	89.0a	15.6a	8.6a	2.8a	6a	4.1a	7	1.01a	11.8a

*-Each value is mean of 5 replicates. **-The same letter (s) following means in each column indicate that the treatments are not significantly different according to Duncan`s multiple range test at level ($p \leq 0.05$). ***-W. =weight and N= Number.

Table 4. % Increases of growth and yield parameters of cowpea infected by *Meloidogyne incognita* as affected by fresh oyster mushroom rates.

Treatments	increases (%)							Seed parameter increases (%)			
	Shoot parameters			Root parameters		Pod parameters					
	Length	Fresh	Dry w.	Fresh w.	Dry w.	N.*	W.**	N. of seeds/ pod	Seed W. / pod	W. of 100 seeds	Mean of increases (%)
control	-	-	-	-	-	-	-	-	-	-	-
5g	0.0	4.3	11.7	-	16.7	-	8.0	-	14.3	0.9	5.6
10g	9.8	59.5	25.2	-	38.9	25.0	20.0	16.7	50.8	1.8	24.8
15g	17.6	122.5	40.5	13.2	55.6	50.0	64.0	16.7	60.3	3.5	60.2

*-N =Number, **W=Weight.

4. Discussion

Aqueous filtrate of fruit fresh oyster mushroom in the present study reduced numbers of root-knot nematode,*M. incognita* by affecting juveniles motility under *in vitro* conditions. This resulted in the mortality and reducing of this parasitic nematode (Kulkarni and Sanget, 2000; Luo *et al.*, 2007; Wille *et al.*,2019). Nematodes became immobilized, paralyzed and straightened bodies as soon when they approached to the fungal colony, as reported by Palizi *et al.* (2009) on their work on oyster mushrooms.

In previous studies, Basidiomycete species proved to be effective in the control of root-knot nematode, *M. javanica* by using formulations produced from vegetative phase of fungi (mycelium) (Heydari *et al.*, 2006). However, fruiting bodies of fungi have higher activity, concentration and diversity of compounds in comparison with those found in mycelium (Tidke and Rai 2006; Ganeshpurkai and Jain, 2010). Besides, Barron and Thorne (1987)indicated that mushroom fungi can infect nematodes by secretory hyphal cells releasing toxins as droplets. This was confirmed by tiny droplets secreted on water agar by all tested strains of *Pleurotus* species (Barron and Thorne, 1987; Chitwood, 2002). Several compounds as polysaccharides were found in medicinal mushrooms which cause therapeutic activities of many fungal genera. These compounds have antoxidant, anticancer, antimicrobial and antiviral activities as shown by Elkhateeb *et al.* (2019). On this basis, mushroom oyster fruit residue used in this study was effective on *M. incognita* which corresponded with the results of El-Sherbiny and Awd Allah (2014). Also, our results are in agreement with those achieved by Goswami *et al.* (2006), Putzke *et al*. (2007) and Wille *et al.* (2019) on the effect of oyster mushroom on root-knot nematodes. Other investigators proved that the degree of decomposition of organic materials and consequently their suppressive effect against root knot nematode may be influenced by some factors, from which a very complete mixing of these materials with soil, enough soil moisture (Morra and Kierkegaard, 2002) and suitable soil temperature (Ploeg and Stapleton, 2001; López-Pérez *et al*., 2005).

From the present study, it was noticed that there is corresponding increase of cowpea plant growth and yield parameters with the percentage of nematode reduction at all cases, which may be due to that the improvement of plants exhibited by increases of shoot and root growth and consequently cowpea yield was favored by the reduction in nematode as shown by Wille *et al.* (2019). The consistent relation between population density of root-knot nematode and plant growth and yield in this study indicated its validity.

5. Conclusion

It could be concluded, based on the present study, that commercial mushroon as organic amendment could effectively reduce *M. incognita* root-knot nematode, and improve cowpea plant vegetative and yield. Also, these criteria were found to be positively related with the aqueous concentrations and rates of commercial oyster mushroom. Further studies are needed to investigate different kinds of mushrooms and factors affecting their decomposition in soil to biocontrol root-knot nematode and other nematodes.

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