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Eco-friendly management of root knot nematode *M. graminicola* (Golden and Brichfield, 1965) with two indigenous fungal bio-agents in Wheat

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Abstract:

Pot experiments conducted for the evaluation of efficacy of biocontrol agent viz., Arthrobotrys oligospora and Bauveria bassiana against root knot nematode Meloidogyne graminicola in wheat revealed that soil application of Arthrobotrys oligospora and Bauveria bassiana alone or in combination was able to control the nematode population and improve the yield. Combined soil application of A. oligospora (@ 5ml/pot) + B. bassiana (@ 5ml/pot) as soil application was effective to check the root knot nematode in wheat.

Key words:

Introduction

Wheat (*Triticum aestivum* L.) is a member of Poaceae family, which includes major cereals crops such as sorghum, maize, wheat, rice, millet and barley (Briggle and Reitz, 1963). Wheat is one of the eight food sources which provides 70-90% of all calories and 66-90% of the protein consumed in developing countries. Globally, wheat provides nearly 55% of the

carbohydrate and 20% calories consumed globally (Breiman and Graur, 1995). The wheat is cultivated in wide range of climatic condition. *Meloidogyne graminicola* is known to infect and cause serious damage to cereals, especially rice and wheat in many countries (Arayarungsarit, 1987; Bridge, 1990; Plowright and Bridge, 1990; Prot and Matias, 1995; Padgham *et al.,* 2004; Pokharel *et al*., 2007). *Meloidogyne graminicola* Golden and Birchfield, is the most common RKN species infecting wheat. In India, it is reported to cause 17-30% yield loss due to poorly filled kernels (MacGowan, 1989; Jain *et al.,* 2007). Second stage juvenile of *M. graminicola* is considered to be the infective stage, making their point of entry at the zone of elongation just behind the root cap and occasionally through the root cap, damaged root tip or at the juncture of developing rootlets, at 4 h after inoculation. After penetration, the juveniles enter into the cortex, orienting their bodies parallel to the stele, and move in the cortical layers of cells in the direction of the apical meristem within 20-24 h after invasion and commence feeding. Juveniles become sedentary themselves in the region of the apical meristem 24-42 h after inoculation. Within 42-72 h after inoculation extreme hypertrophy of cortical cells accompanied by hyperplasia may occur. At 3 days, a root knot/gall is formed around the site of establishment of the nematode. Giant cells are formed in the stellar region around the head of nematodes in the nearest metaxylem vessel. Mechanical disruption of metaxylem vessels interfere with the uptake of water and nutrients (Patnaik & Padhi, 1987).

Materials and Methods

The experiment was laid out in the research plot of the department of Plant Protection, Sam Higginbottom Institute of Agriculture Technology and Sciences, during the *Rabi* season of 2013. Allahabad district of Uttar Pradesh, India is situated at 25.27^0 north and 81.50^0 east latitude with an altitude of 98 m

above the mean sea level. The climate is typically semi-arid and sub-tropical. The maximum temperature reaches up to 47.5 ^{0}C in the summers and drops down to 1.5 ^{0}C in the winters. The inoculums of *Meloidogyne graminicola* was collected from 5 months infected root of wheat from central field of Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, U.P., India.

Preparation of pots and inoculation of bio-agents: The pots (18 nos.) having capacity of 1 kg filled with sterilized soil at 15/lb. pressure for 3 hrs at 121^{0}c and further the filled pots were treated/ inoculated with 5 ml bio-agents suspension formulation before one week to inoculation of nematode. The bio-agents were isolate and purified in different specific media. The C.F.U. of bio-agents was taken before inoculation to soil. The two weeks old wheat seedlings were inoculated with 2000 J_2 of *Meloidogyne graminicola* were obtained from the infested wheat plants root by sieve method. Pots were divided into six groups, each with three replicates and were arranged in a randomized block design (RBD). There should be well management with the normal agricultural practices. The pots were inoculated with 2000 J_2 larvae of *M. graminicola* per pot to each treatments *viz.* T_1, T_2, T_3, T_4, T_6 except control T_5 (Pang, 2009).

Preparation of formulation of bio-agents

The liquid formulation of *Arthrobotrys oligospora* (2x 10^5cfu/ml) and *Bauveria bassiana* (2x 10^6cfu/ml) were obtained from NFCCI, Pune, multiplied into the PDA for soil treatments. The bio-agent *A. oligospora* and *Bauveria bassiana* were grown on PDA and SDA respectively at 28 ^{o}C for three weeks, until sporulation was completed. The conidiospores were then obtained by gently washing the culture plates with a small quantity of sterile distilled water and thoroughly mixed with the soil one week after sowing into the pots (Chandranathan *et*

al., 1998). The numbers of conidia were counted using a haemocytometer.

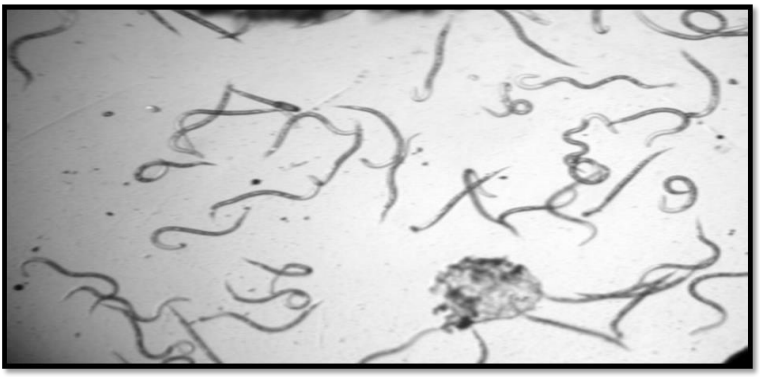


Fig: Microscopic observation of third stage larvae of *M. graminicoal* (40x).

The observations were recorded for the shoot length, shoot weight, root length, root weight, number of nematode galls formation and number of larvae/root system. The plants in each pot were uprooted and gently separated from soil, washed with tap water and dried by pressing lightly between blotting paper. Observations were taken on following plant growth parameters *viz*., Shoot Length (cm) – 30, 60 and 90 DAS, Root Length (cm) - 90 DAS, Shoot Weight (gm) –90 DAS, Root Weight (gm) –90 DAS, No. of root knot at root system - 90 DAS, No. of Juveniles at root system – 90 DAS.

Results and Discussion

In this study two fungi *viz. Arthrobotrys oligospora, Bauveria bassiana* were used as bio-control agents and Carbofuran 3G against *Meloidogyne graminicola* in pot experiment condition.The selected bio-control agents were evaluated to observe the effect on *M. graminicola* root knot development and growth parameters and reduce the population of *M.*

graminicola compared to the untreated control **(Table; 1, 2, 3 & Fig; 1a, 2a, 3a).**

Table: 1. Effect of bio-agents on Shoot Length at 30, 60 and 90 DAS.

Treatments	Avg. Shoot Length (cm) at 30 DAS	Avg. Shoot Length (cm) at 60 DAS	Avg. Shoot Length (cm) at 90 DAS
T1- *A. oligospora* @5ml	11.67	21.14	37.93
T2- *B. bassiana* @5ml	12.00	20.76	33.53
T3- *A.oligospora* + *B. bassiana* @5ml	12.33	19.30	39.27
T4- Carbofuran 3G @ 2g/pot	10.83	20.76	37.87
T5- Control without nematode	12.67	19.77	32.90
T6- Control With nematode only.	9.33	17.57	29.90
CD (p=0.05%)	**2.73**	**3.79**	**7.55**

Fig: 1a. Effect of bio-agents on Shoot Length at 30, 60 and 90 DAS.

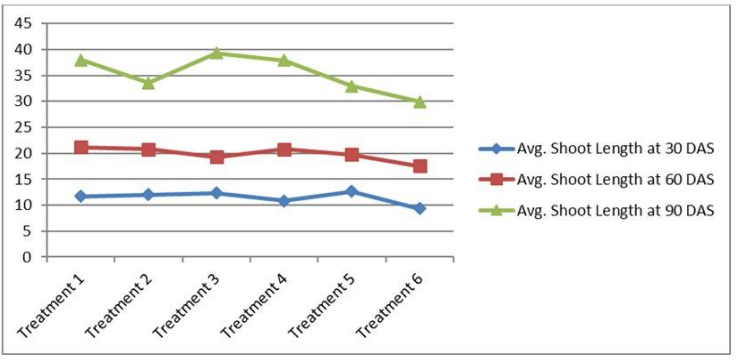


Table: 2. Effect of bio-agents on growth parameters after 90 DAS.

Treatments	Avg. Shoot Weight (gm)	Avg. Root Length (cm)	Avg. Root Weight (gm)
T1- *A. oligospora* @5ml	2.33	8.83	1.08
T2- *B. bassiana* @5ml	2.11	6.13	0.71
T3- *A.oligospora* + *B. bassiana* @5ml	1.50	7.10	0.96
T4- Carbofuran 3G @ 2g/pot	1.20	9.40	0.73
T5- Control without nematode	2.20	6.47	0.93
T6- Control With nematode only.	1.10	3.53	0.64
CD (p=0.05%)	**1.47**	**2.79**	**0.41**

Fig: 2a. Effect of bio-agents on growth parameters of wheat at 90 DAS.

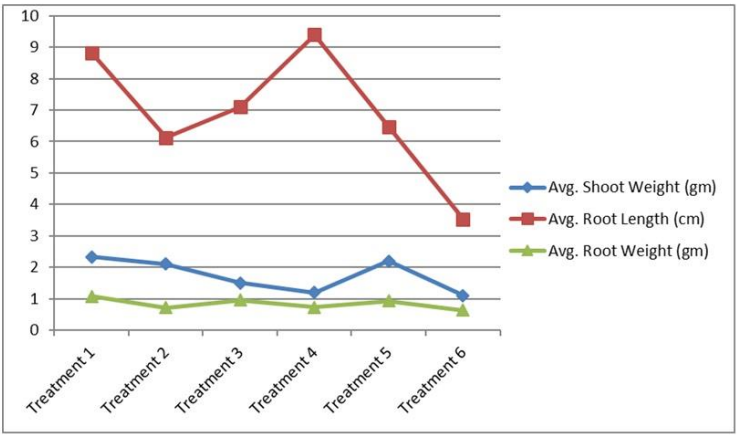
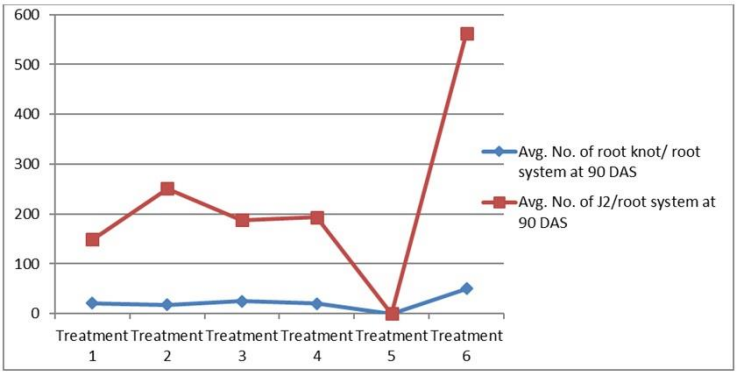


Table: 3. Effect of bio-agents on No. of galls formation and No. of J_2 at 90 DAS.

Treatments	Avg. No. of root knot/ root system at 90 DAS	Avg. No. of J_2 / root system at 90 DAS
A. oligospora @5ml	21.00	148.67
B.bassiana @5ml	18.00	251.00
A.oligospora + *B.bassiana* @5ml	25.33	188.00
Carbofuran 3G @ 2g/pot	20.00	193.67
Control without nematode	0.00	0.00

Control With nematode only.	50.00	563.00
CD (p=0.05%)	**10.89**	**138.92**

Fig: 3a. Effect of bio-agents on No. of galls formation and No. of J_2 at 90 DAS.



Our results suggest that the dose of 5ml/pot each of *A. oligospora*, *B. bassiana* alone and combined could provide significant reduction in *M. graminicola* population and effective to growth of wheat crop. Data on shoot length, root length, shoot weight, root weight and nematode multiplication and development were recorded after 90 DAS. This finding suggested that application of biological agents is significant and safer for wheat cultivation. Present results showed that soil application of *A.oligospora*, *B.bassiana* alone and combined gave significant reduction in *M. graminicola* infestation and also to increase the growth of wheat crop compared with Carbofuran 3G.

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