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Use of *Dactylaria brochopaga*, a Predacious Fungus, for Managing Root-Knot Disease of Wheat (*Triticum aestivum*) Caused by *Meloidogyne graminicola*

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A laboratory experiment was conducted to study the induction of constricting rings and test predation of *Dactylaria brochopaga* isolates against second stage juveniles (J2s) of *Meloidogyne graminicola*. Among the five fungal isolates, isolate D showed the greatest number of predatory rings and, consequently, trapped the maximum number of *M. graminicola* J2s in dual cultures. Another pot experiment was conducted to study the effect of *D. brochopaga* (isolate D) on the management of wheat root-knot disease. Applying a mass culture (10 g/pot) and a spore suspension of the fungus with and without cow dung manure to soil infested with 2,000 *M. graminicola* juveniles significantly improved plant height, root length, weights of shoots, roots, panicles and grains per hill compared to those in the control. Moreover, the fungus significantly reduced the number of root-knots, the number of egg masses, juveniles, and females per hill compared to those in the control. Bioefficacy of the fungus was heightened when the mass culture and a spore suspensions were used in combination with cow dung manure to improve the plant growth parameters and reduce the number of root-knot and reproductive factors. Further investigations should be conducted to identify the impact of this fungus in the field.

KEYWORDS : *Dactylaria brochopaga*, Mass culture, *Meloidogyne graminicola*, Spore suspension, Wheat

Meloidogyne graminicola is one of the most economically important root-knot nematodes and is becoming a serious pest, particularly in rice-wheat cropping systems in the Indo-Gangetic plains of South Asia and rice producing areas of southeast Asia [1, 2]. In India, this disease was first reported by Israel *et al.* [3], and, later, the disease was reported from different states by many scientists viz., Andhra Pradesh [4], Assam [5], Bihar [6], Haryana [7], Tripura [8], Uttar Pradesh [9-11] and from West Bengal [12]. *M. graminicola* is a well-established pest of sandy loam to loamy sand soil [2] and can cause yield losses of 20~80% [13, 14]. However, the reduction in grain yield was up to 98% in pot experiments [15]. Soomro and Hague [16] reported that *M. graminicola* significantly suppresses root growth of graminaceous plants such as, rice, wheat, sorghum, and *Echinochloa colonum* L. and that the amount of damage varies with plant species. Moreover, this nematode can survive long anoxic periods and rapidly reinvade roots when soils drain [13, 17]. Soil flooding and nematicides are two of the most effective control measures. Although nematicides provide immediate and effective management of plant parasitic nematodes, they are too expensive for use in developing countries where their uses are limited to a few cash crops [18]. Concern about these chemicals has led to an increased interest in biological control to achieve eco-friendly methods for reducing nematode damage.

Biological control using nematophagous fungi has been investigated for the effective and eco-friendly management of plant parasitic nematodes [19-31]. Furthermore, applying organic manures in combination with nematophagous fungi stimulates the performance of these fungi and results in reducing the population of root-knot nematodes [21-26, 29]. However, the basic mechanisms behind these observations are unknown.

Dactylaria brochopaga is a predacious fungus that dramatically traps and kills saprophytic and parasitic nematodes *in vivo* and *in vitro* by producing three-celled trapping rings [1, 26, 28, 32, 33]. *D. brochopaga* is a common fungus in agricultural soils, decaying substrates and old-decayed root-knots [23, 24, 28, 29, 32, 33]. The performance of this fungus for reducing the number of root-galls and populations of *M. graminicola* and *M. incognita* has been reported by Singh *et al.* [28] and Kumar and Singh [23], respectively. Five *D. brochopaga* isolates were obtained from agricultural soil, leaf litter, and decaying root-galls

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from different locations in India and were tested for their predation against second-stage juveniles (J2s) of *M. graminicola in vitro*. Among the five isolates, isolate D of *D. brochopaga* was mass cultured on sorghum (*Sorghum bicolor*) grains and tested for its ability to control root-knot disease in wheat (variety Malviya-234) in a pot experiment. The observations of the same are described in the present paper.

Materials and Methods

Isolation and maintenance of *D. brochopaga* and collection of *M. graminicola* J2s. Five *D. brochopaga* isolates were obtained from different agricultural soils, decaying leaf litter, and decaying root-galls from different locations in India by the method described by Duddington [34] with a slight modification by Bandyopadhyay and Singh [35]. All five *D. brochopaga* isolates were purified by single spore isolation [36] and each isolate culture was maintained at 29 ± 1°C on corn meal agar (CMA) medium with regular subculturing at 15 day intervals. J2s populations of *M. graminicola* were obtained from rice and wheat plant pot cultures regularly maintained in the greenhouse of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi. A sufficient number of nematode egg masses were collected from root-knots of rice and wheat plants and placed in a cavity block for hatching at room temperature (25~30°C) for 2 days to obtain the required J2s population.

Predation test. To identify the response of the five *D. brochopaga* isolates to *M. graminicola* J2s, observations and their numbers were taken when constricting rings were initiated in dual cultures. Numbers of constricting rings per microscopic field (1.6 mm^2) were noted daily for 6 days under a microscope at ×10 magnification. Several observations on the number of constricting rings were made from center, middle, and periphery of Petri dishes after nematode inoculation. Observations on the number of constricting rings formed on the surface and deep into the medium were also made at 24 hr intervals for 6 days, and the average number of induced rings was calculated. Three Petri dishes were used as replicates for each fungus isolate-nematode interaction. Predation of the five *D. brochopaga* isolates against the *M. graminicola* J2s in dual cultures was tested by the methods described by den Belder and Jansen [37]. A 5 mm fungal discs of each isolate were taken from the periphery of 10 day old cultures and transferred to 50 mm Petri dishes containing solidified CMA (1 : 10) medium (0.2% agar, 2 mm thickness). The fungal discs were placed upside down in the center of the Petri dishes containing CMA. The Petri dishes were incubated at 28 ± 1°C in the dark. When the fungal colony had covered the plate almost to the edge, the fungal discs were removed aseptically. A drop of sterile water containing 50 nematodes (thoroughly rinsed) was poured into each Petri dish with the aid of a sterilized dropper. Petri dishes were then incubated at 28 ± 1°C for observation. Similarly, data on captured nematodes were recorded at 24 hr intervals for 6 days, and the percentage of captured nematodes was calculated. Three replications were maintained for each treatment, and the experiments were repeated three times.

Mass culture. To prepare the *D. brochopaga* mass culture (isolate D), 20 g of sorghum grains were added separately to a 250 mL conical flask and moistened with 35 mL of water. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 min. A 10 mm fungal disc was cut from the periphery of the 10 day old *D. brochopaga* culture with a sterilized cork borer and inoculated into the center of the substrate contained in the flask with the help of a sterilized inoculation needle. One disc was inoculated into each 250 mL conical flask. The inoculated flaks were incubated at room temperature (25~30°C) for 25 to 30 days.

Performance test of *D. brochopaga* mass culture and spore suspensions against wheat root knot disease. Nematode-infested soil containing 2,000 *M. graminicola* J2s individuals was used to evaluate the efficacy of *Dactylaria brochopaga* mass culture and spore suspensions against the nematode. The experiment was conducted in the wire net house at the Department of Mycology and Plant Pathology, Institute of Agricultural sciences, BHU, Varanasi. Infested soil was thoroughly mixed to homogenize the nematode inoculums. The infested soil was thoroughly mixed by hand to create a uniform population of nematodes before amendment. Mass culture was amended at the rate of 1% (10 g/pot) and *D. brochopaga* containing 4×10^6 colony forming unit (CFU). The spore suspension (undiluted) and diluted spore suspension (10 times) was amended with or without 5% well decomposed cow dung manure (CDM). The mass culture, the spore suspension, and a ten-fold diluted spore suspension with and without CDM was uniformly mixed in the infested soil before filling the pots. Root-knot infested soil and amended soil were added to pots (1,000 g/pot), and 20 wheat seeds (variety Malviya-234) were sown in each pot on the same day. Five replications were used for each treatment. Observations on the number of root-knot shoots, root length, fresh weight of the shoots and roots, panicle length, and grain weight per hill were recorded after the wheat plants matured. Also, the populations of eggs, juveniles, and females were recorded. The number of females, egg masses per root system, and J2s individuals were estimated by the methods described by Kumar and Singh [25]. The total

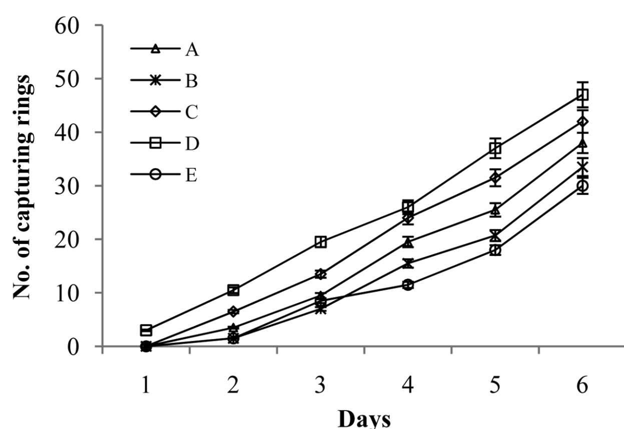

Fig. 1. Average numbr of constricting rings of five isolates of *Dactylaria brochopaga* induced in responce to *Meloidogyne graminicola* in corn meal agar (1 : 10) medium.

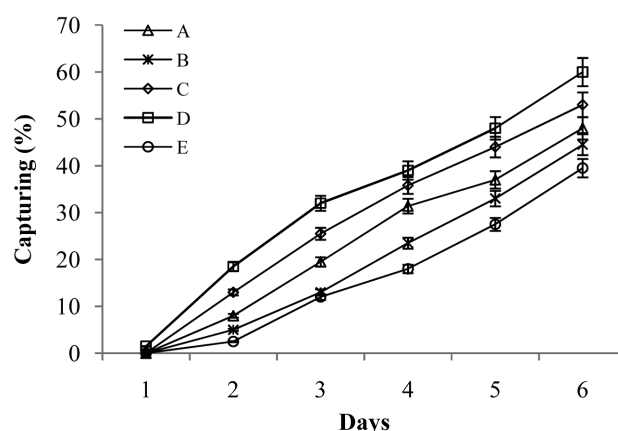

Fig. 2. Percent capturing of second stage juveniles of *Meloidogyne graminicola* by five isolates of *Dactylaria brochopaga* in corn meal agar (1 : 10) agar medium.

number of nematodes present in the soil was added to the root population to obtain the total number of nematodes present in the soil and infected roots. Data were statistically analyzed using an analysis of variance. Treatment means were compared by Duncan's multiple range test [38].

Results and Discussion

The formation of constricting rings and predation of the five *D. brochopaga* isolates against *M. graminicola* J2s in a dual culture were studied in the laboratory (Figs. 1 and 2). The three-celled constricting rings were recorded in isolate D at 24 hr after the nematodes were inoculated. After 48 hr, rings had formed in all isolates. The number of rings per unit area increased with time. Maximum ring induction on day 6 was formed in isolate D followed by isolate C, whereas a minimum number of induced constricting rings was recorded in isolate E followed by isolate B. (Fig. 1). These results indicated significantly different sensitivities of the *D. brochopaga* isolates for ring induction. The significant variation in predation of the five *D. brochopaga* isolates to *M. graminicola* J2s may be attributed to the virulence of the isolates and the number of predatory rings formed in response to J2s. Nematode size is also a major factor for predation variation of predacious fungi [23, 25, 26]. The J2s of *M. graminicola* were captured in dual cultures of isolate D 24 hr after nematode inoculation. After 48 hr, nematodes were captured by the constricting rings of all fungal isolates. The maximum percentage of nematodes that were captured was recorded for isolate D followed by isolate C, whereas the minimum number was recorded for isolate E (Fig. 2). Isolate D captured and killed the highest number *M. graminicola* J2s, which seemed to be related to the maximum induction of rings in response to the nematode. The diameter of

Table 1. Effect of *Dactylaria brochopaga* (isolate D) mass culture and spore suspensions on growth parameters of wheat, the number of root-knots, and reproductive factors of *Meloidogyne graminicola*

Treatment parameter	Control	CDM	Db (10^1)	Db (ss)	Db (mc)	Db (10^1) + CDM	Db (ss) + CDM	Db (mc) + CDM
				Growth parameter of wheat				
Plant height (cm)	21.5 a	26.0 a	37.5 b	43.0 c	51.0 d	48.5 d	57.5 e	61.5 e
Root length (cm)	6.0 a	7.0 a	10.5 b	11.0 b	11.5 b	13.5 c	14.0 c	16.5 d
Shoot weight (mg)	237.5 a	249.5 a	438.0 b	569.0 c	581.5 c	704.5 d	959.0 e	1,232.5 f
Root weight (mg)/hill	101.0 a	142.0 b	177.5 c	189.5 d	213.0 e	209.0 e	271.5 f	312.0 g
Panicle weight/hill (mg)	217.0 a	236.0 a	412.5 b	543.5 c	561.5 c	609.5 d	912.5 e	1,167.5 f
Grain weight (mg)/hill	815.5 a	1,020.0 b	1,243.5 c	1,666.5 d	2,576.0 e	1,704.5 d	3,437.5 e	4,230.0 f
				Reproduction factors of *M. graminicola*				
No. of root-knots/hill	19.5 a	17.5 a	14.5 b	12.0 c	10.5 d	9.5 d	7.0 e	5.5 e
No. of females/hill	114.5 a	108.0 a	74.5 b	58.0 c	47.5 c	56.5 c	34.5 d	30.5 d
No. of eggs/hill	367.5 a	375.0 a	261.5 b	229.0 c	191.5 c	178.0 d	105.5 e	98.5 e
No. of juveniles (J2s)/hill	4,998.0 a	4,451.0 b	3,707.5 c	2,971.0 d	2,167.5 e	2,902.0 d	1,062.5 f	831.5 g

Data with different letters indicate significant difference in the row data ($p \leq 0.05$, Duncan's multiple range test).

CDM, cow dung manure; Db, *Dactylaria brochopaga*; 10^1, ten-fold dilution; ss, spore suspension; mc, mass culture; J2s, second-stage juveniles.

the J2s was narrower, so they were easily captured by the trapping rings, whose internal diameter was suitable for nematode entry. The capturing of *M. graminicola* J2s occurred frequently at the head or tail region. Trapping rings were also occasionally seen in the middle of the nematode body.

Observations on the effect of mass culture and *D. brochopaga* spore suspensions (isolate D) with and without CDM on growth, parameters of the wheat plant and the reproduction factors are presented in Table 1. From these data, it was evident that all growth parameters of the wheat plants were significantly enhanced when seeds were sown in soil infested with 2,000 juveniles of *M. graminicola* per "1,000 g," which was amended with a mass culture or spore suspensions of the fungus. Moreover, even after diluting the spore suspension of the *D. brochopaga* mass culture, the plant growth parameters were enhanced significantly more than those of the control. The maximum increase in growth was recorded in pots treated with the *D. brochopaga* mass culture and CDM, closely followed by undiluted and diluted spore suspensions with CDM. Applying diluted and undiluted spore suspension and mass culture without CDM reduced the number of root-knots of wheat plants by 25.64, 38.46, and 46.15% respectively, whereas diluted and undiluted spore suspensions and the mass culture with CDM reduced root-knots by 51.28, 64.10, and 71.79%, respectively. Similarly, applying diluted and undiluted spore suspensions and the mass culture reduced the number of females by 34.93, 49.34, and 58.51%, and egg masses by 28.84, 37.68, and 47.89% and juveniles by 25.82, 40.55, and 56.64%, respectively. Performance of *D. brochopaga* isolate D as a nematode antagonist was enhanced when diluted and undiluted spore suspensions and the mass culture were applied with CDM, which reduced the number of females by 50.65, 69.86, and 73.36, egg masses by 51.42, 71.29, and 73.19% and juveniles by 41.93, 78.79, and 83.36%, respectively.

The reduction in root-knot galling and reproductive factors in this study was consistent with previously obtained results for this fungus [23]. The enhanced growth of the wheat plants and reduced root-knots and populations of *M. graminicola* could be attributed to the *D. brochopaga* spores and mycelia [23]. Applying *D. brochopaga* in combination with CDM saved plants by capturing and killing a greater number of (J2s) of *M. incognita* and in turn, heightened the growth parameters due to a greater reduction in infection. The results of the present study also showed that the spore suspension of the fungus enhanced plant growth and reduced the root-knots and expression of the reproductive factors.

The well-developed roots duly protected at the initial stage by the capture and killing of nematodes by this fungus supported plant growth and the reduction in the *M. graminicola* population. These findings are more or less similar to Stirling *et al.* [31], who reported that *A. dactyloides* caused a 57~96% reduction in the number of root-knots and a 75~80% reduction in the number of nematodes per tomato plant in pots and field experiments, respectively. The other possibility for the increased effect of *D. brochopaga* may be due to an increase in the population of saprophytic nematodes, which after their capture and killing possibly increased the fungal population. Notably, plant growth increased conspicuously with better root system in pots treated with the fungus and CDM. Recently, Kumar and Singh [23] reported that this fungus reduces the root knots of eggplant by 31.1~57.9%, females by 24.2~49.9%, egg masses by 22.1~42.9%, and juveniles by 30.8~60.7% and further enhanced plant growth in pot experiments. Furthermore, the effect of *D. brochopaga* was enhanced when the fungus was applied in combination with CDM. The results of this study suggest that predacious fungi are very useful for managing root-knot disease of wheat and rice, and that *D. brochopaga* is an efficient agent for the biocontrol of *M. graminicola*. This fungus should be applied in combination with CDM to infested soil so that it can proliferate sufficiently in the soil for better control. Further studies are needed to understand the mode of action and mechanisms of this fungus Moreover, investigations should also be conducted to identify the impact of this fungus in the field.

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