EVALUATION OF PREDACITY AND BIO-EFFICACY OF *Dactylaria brochopaga* FOR ECO-FRIENDLY MANAGEMENT OF ROOT KNOT DISEASE OF RICE (*Oryza sativa* L.*)*

Niranjan Kumar[*1], Kalika Prasad Singh[2]
[1]Department of Crop Production, Faculty of Agriculture, Ibrahim Badamasi Babangida University, Lapai, PMB 11, Niger State, Nigeria
[2]Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India
[*]E-mail: niranjanibbu@gmail.com

Abstract
Experiments were carried out to find out the predacity of Dactylaria brochopaga against plant parasitic nematodes and its bio-efficacy in pots and fields for eco-friendly management of root knot disease of rice. In vitro, the predacity of five isolates of the fungus was tested against plant parasitic nematodes. The biocontrol agents was tested in form of mass culture @ 1% and spore suspension of different concentrations with and without CDM and data were collected on growth parameters of seedlings, root galls and nematode population after 1 month of sowing. In field, the mass culture was applied @ 12 g/1.2 kg CDM per plot and data were collected after 2 months of sowing. Maximum number of nematodes was captured by all the isolates for Meloidogyne spp. followed by T. brassicae and H. dyhestera in dual culture. Application of mass culture of D. brochopaga @ 1% significantly increased plant growth and also reduced the number of galls and nematodes significantly. The application of spore suspension of D. brochopaga also significantly controlled the disease at all the concentrations of the spores as root galls and nematode populations were significantly decreased with significant increase in root and shoot weight. However, shoot length was significantly increased only at higher concentration of spore suspension. In both the situations the bio-efficacy of the fungus was better in combination with CDM. Application of mass culture in rice field heavily infested with M. graminicola significantly control the disease and increased plant growth. Based on the observation, it is concluded that the D. brochopaga found as an effective biocontrol agent for the management of root knot disease of rice in pots and directly sown fields.

Keywords: Meloidogyne graminicola, CDM, soil fungistasis, spore dilution, mass culture

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1. INTRODUCTION

Root knot disease of rice (*Oryza sativa* L.*)* caused by *Meloidogyne graminicola* (Golden and Birchfield, 1965) is serious in nurseries, direct sown crops and transplanted rice in upland and medium low land irrigated fields (Manser, 1968, 1971; Rao and Isrel, 1971, 1973; Bridge et al., 1990; Jairajpuri and Baqri, 1990; Sariano et al., 2000; Sariano and Reversat, 2003), in most of the rice growing countries of the world (Rao et al., 1986; Port et al., 1995; Soriano and Reversat, 2003; Bridge et al., 2005). In India, it has been reported from different States since green revolution period particularly in rice-wheat cropping system (Devi, 2001; Kamalwanshi *et al;* 2002; Kamalwanshi & Kumar, 2004; Dabour and Jain, 2004; Somasekhar and Prasad, 2009; Singh and Singh, 2009). Now the disease has established in almost all the areas when the conditions are favourable under rice-wheat cropping system. This nematode has also been found damaging the wheat crop (Gaur *et al*., 1993, 2003; Pankaj et al., 2010). The disease severity has sown an increasing trend. This disease has been reported to cause extensive damage during condition favourable for the disease development. Root knot disease of rice became so serious in Eastern U.P., India that the expected losses in severely infested fields ranged between 52-80% (Jaishwal, 2007). Since there is no effective control of this disease and the chemical like furadan which had shown some promise, has been found to gradually decrease in its effect in the following years (Fademi, 1884). Thus is next best alternative seems to be the biological control of this disease using predacious fungi. Deiechens (1939 &1942) conducted several experiments

on the biological control of plant parasitic nematodes and reported significant control after of incorporation biocontrol agent. Although not in all experiments but in several, Duddington (1951, 54, 55, 57, 62) reported significant reduction in nematode population by predacious fungi. Linford (1937) and Linford and Yap (1939) reported stimulated activities of the predacious fungi following organic amendment and subsequent reduction in the population of root knot of pineapple after inoculation of predacious fungus. Subsequently, several workers reported that the efficacy of predacious fungi is increased in combination with organic manures (Duddington, 1951, 54, 57, 62; Hoffman and Sikora, 1993; Van Den Boogert, 1994; Singh, 2003; Kumar, 2004; Kumar, 2007; Kumar and Singh, 2011). How organic manures increase efficacy was not known at that time nor is it fully understood today. After the discovery of soil fungistasis by Dobb and Hinson (1953) and its implication on response of the predacious fungi. By few experiments conducted by Mankau (1962) and Cooke (1963) and also negative results obtained by some workers, the prospects of predacious fungi as biocontrol agent became bleak and it was assumed then that biological control by predacious fungi may not turn into reality? However, in last two decades the research on predacious fungi has grown at faster pace and several encouraging results have been obtained (Galper et al., 1995; Stirling and smith, 1998; Stirling et al., 1998; Singh, 2003; Kumar, 2004; Singh et al., 2006; Kumar, 2007; Simon and Anamika, 2011; Kumar and Singh, 2011; Singh et al., 2012). In a pot experiments, Kumar and Singh (2006) reported nearly 80% reduction of root knot of tomato by *Arthrobotrys dactyloides* in pot culture. *Dactylaria brochopaga* is a common predacious fungus commonly found in decaying plant materials, agricultural soil compost, decaying root-galls (Kumar et al, 2011). Five isolates of the fungus collected from different locations were tested for their predacity against some ectoparasite nematodes such as *Xiphinema basiri, Helicotylenchus dihystera,* T*ylenchorynchus brassicae* and *Haplolaimus indicus* and root knot nematodes (endoparasite) *Meloidogyne graminicola* and *M. incognita.* One of the promising isolate of *D. brochopaga* was grown in mass culture on sorghum grain (*Sorghum bicolor*) and tested for the control of root knot disease of rice in pots and fields. In order to avoid the involvement of sorghum grains in pots, the spore of the fungus were collected and used at different concentration for the pot experiments. Further, to understand the effect of organic manures i.e. CDM an experiment on soil fungistasis was conducted which explains the positive role of organic manures in increasing the efficacy of this biocontrol agent. The results of the same are described in the present paper.

2. MATERIALS AND METHODS

2.1 Isolation of Dactylaria brochopaga: Five isolates of *D. brochopaga* were isolated from different substrates and soils of different locations: Bananas Hindu University Varanasi (Isolate A), Chunar, Mirzapur (Isolate B), in U.P State, Pant Nagar (Isolate C), Uttranchal State, Jammu and Kashmir (Isolate D), and Nagaland (Isolate E), India by the method described by Duddington (1955) with slight modification (Bandyopadhyay and Singh, 2000). All the five isolates of *D. brochopaga* were purified by single spore isolation, method given by Singh et al., (2004) and culture of each isolate was maintained at 29± 1^0C on corn meal agar (CMA) medium by regular subculturing at an interval of 30 days. The pure cultures were also maintained in culture tubes containing corn meal agar medium.

2.2 Extraction of Nematodes: Population of second stage juveniles (J_2s) of *Meloidogyne incognita* and *M. graminicola* were obtained from pot cultures of these nematodes regularly maintained on tomato and rice plants respectively in glass house of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University. Sufficient egg masses/root galls of

these nematodes were collected separately from the root galls of these plants and put in cavity block for hatching at room temperature (25^0-30^0 C) for 2 days in order to get required population of J_2s Other plant parasitic nematodes were extracted from soil following the Bayerman's Technique (Southey, 1970). X*iphinema basiri* was obtained from the soil around the roots of croton *(Codiaeum variagatum* var *pictum* (L) Blume) plants and citrus (*citrus aurantiifolia* Swingle), respectively. T*ylenchorynchus brassicae* was obtained from soil around the roots of mustard plants *(Brassica ju*ncea L), grown at Agricultural Farm of Banaras Hindu University. Population of *Haplolaimus indicus* and *Helicotylenchus dihystera* was collected from rhizospheric soil of citrus trees of the garden of Institute of Agricultural Sciences, Banaras Hindu University.

2.3 Predacity Test: Predacity of five isolates of *D. brochopaga* against six plant parasitic nematodes in dual cultures was tested by the method described by den Belder and Jansen (1994). 5 mm fungal discs of each isolate was taken from the periphery of 10 day-old culture and transferred into 50 mm Petri dish containing solidified CMA (1:10) medium (0.2% agar, 2mm thickness). The fungal discs were placed upside down in the centre of the Petri dishes containing CMA. Petri dishes were incubated at 28 ± 1°C in dark. When fungal colony covered almost at the edge of the plate, fungal discs were removed aseptically. In each Petri dish a drop of sterile water containing 50 nematodes (thoroughly rinsed) was poured with the aid of sterilized dropper. Petri dishes were then incubated at 28±1°C for observation. Three replications were maintained for each treatment and the experiment was repeated thrice.

2.4 In order to find out the response of five isolates of *D. brochopaga* to six plant parasitic nematodes, observations were taken on the initiation of constricting rings and their numbers in dual culture. Numbers of constricting rings per microscopic field (1.6 mm2) were noted daily for 6 days under a research microscope at 100X magnification. Several observations on number of constricting rings were made from centre; middle and periphery of the Petri dishes after nematode inoculation and the average number were calculated. Observations on constricting rings formed on the surface or deep into the medium were also made. Similarly data on captured nematodes were recorded at 24 hours interval for 6 days and the percentage of captured nematodes was calculated. For each isolate of *D. brochopaga* and nematode interaction three Petri dishes were used as replicates.

2.5 Mass Culture: To prepare the mass culture of *D. brochopaga* (isolate D), 20 g sorghum (*Sorghum bicolor*) grains were taken separately in 250 ml conical flasks and moisten with 35 ml of water. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 minutes. A 10 mm fungal disc was cut from the periphery of the 10 day old culture of *D. brochopaga* by a sterilized cork borer and inoculated in the centre of a substrate contained in a flask with the help of sterilized inoculation needle. One disc was inoculated into each 250 ml flask. Five replications were maintained for each treatment. The inoculated flasks were incubated at room temperature (30±1°C).

2.6 For evaluation of efficacy of mass culture of *D. brochopaga* (isolate D) to control root knot nematode of rice, nematode infested soil containing 2000 juveniles of *M. graminicola* was used. The experiment was conducted in a glass house. The infested soil was thoroughly mixed to homogenize the nematode inoculum. Mass culture (@ 1% i.e. 10 g mass culture of *D. brochopaga*, containing 4.6 $x10^6$ colony forming unit (CFU) per kg soil, spore suspension of mass culture (undiluted and diluted ten, hundred and thousand times) were amended with or without 5% well decomposed cow dung manure and thoroughly mixed to homogenize the soil separately under various treatments. Nematode-infested soil without fungal inoculum served as a control. Pots were then filled @1 kg soil under various treatments.

Thirty sprouted rice seeds (variety MUT-7029) were sown in each pot on the same day. Data on the plant height, root length, fresh shoot and root weight, number of root knot per plant, number of females and egg per root system were taken after 30 days of pot inoculation.

2.7 For assessment of efficacy of mass culture of *D. brochopaga* (isolate D) against root knot disease of rice in field experiment, the treatments were as follows: mass culture of *D. brochopaga* + CDM, CMD alone, recommended dose of fertilizer and control. The experiment was conducted in nematode infested Agriculture research field, Institute of Agricultural Science B.H.U. Varanasi India. Mass culture at the rate of 1% along with well decomposed cow dung manure at the rate of 1.2 kg in 20 m^2 area, thoroughly mixed to homogenize these treatments. Nematode-infested plots without fungal inoculum served as a control. Plots were sown with rice seeds in rows @ 250 g per plot.
For each treatment three replications were used. Observations on number of root galls, shoot and root length, fresh weight of shoot and root were recorded 45 days after sowing. Also the population of eggs, juveniles and females were recorded. For determination of final population of eggs, J_2 and females/roots were stained. Stained roots were macerated in distilled water and number of eggs, J_2 and females were counted. Data were analyzed using randomized block design (RBD)**.**

2.8 The method described by Jackson (1958) was used for studying the effect of **fungistasis** effect of soil and amended soil on spore germination of *D. brochopaga.* To study the effect of Urea, DAP, Muriate of potash, FYM, Compost and Neem cake on the spore germination of three isolates of *D. brochopaga*, 5 kg soil sample was taken from Agricultural research farm, of B.H.U., Varanasi and passed through a 2 mm mesh sieve. Urea, DAP and Muriate of potash were separately mixed in the soil at the rate of 1%, 0.5% and 0.1% and FYM, Compost and Neem Cake were added in soil @ 5%. 50g of soil at each concentration was taken in 90 mm Petri dishes and soil was watered to full water holding capacity by addition of distilled water. Whatman's filter paper was placed on moist soil at five different locations including centre and periphery of Petri dishes at equal distance. Water agar blocks (10 mm size, 3 mm thickness) were placed on each filter paper and Petri dishes were incubated for 24 hours at room temperature to allow the diffusates in agar blocks. Spore suspension of *D. brochopaga* were made and placed over agar blocks. The spore inoculated Petri dishes were incubated at room temperature (25-30^{0}C) for observation. The observations on spore germinations were taken after every 24 hours and the final observations were recorded after 96 hours of inoculation.

3. RESULTS AND DISCUSSION

3.1 Induction of Rings and Predacity:

Of the six nematodes selected, *M. graminicola* and *M. incognita* were smaller in size, *Tylenchorynchus brassicae* and *Helicotylenchus* were medium size whereas, *Hoploaimus indicus* and *Xiphinema basiri* were larger size. The Predacity studies clearly revealed that the size of nematode was most important factors in capturing of nematodes. Second stage juveniles of *Meloidogyne* species in the present study recorded maximum Predacity followed by *T. brassicae* and *H. dhystera* by all the five isolates of *D. brochopaga*. The longer nematodes i.e. *X. basiri* and *H. indicus* mostly escaped capturing because of the smaller size of constricting rings. *X. basiri* was sporadically captured in tail region mostly at mucro by different isolates of *D. brochopaga*. However, even if the nematodes was captured at mucro, the fungus grew within the body of the nematode and consumed the nematode control in head, tail and even in the middle of the body by apparently larger rings that were formed in duel culture. Observation of the captured nematodes indicated that some of these nematodes died after 12 h of capturing. In general, Irrespective of the nematodes species, the infection hypha

grew from the inflated cells within the nematode. The isolates of *D. brochopaga* varied in their virulence as the percentage of capturing significantly varied. Of all the isolates, Isolates D was found to be most virulent for trapping of most of the nematode species selected.

Observation on the induction of constricting rings in dual culture of *M. incognita* and different isolates of *D. brochopaga* showed that rings were not formed in 24 hours after inoculation (fig. 1).

However, ring induction was recorded in all the isolates after 48h. This indicated that initiation of ring induction occurred between 24 to 48 h after nematode inoculation. The number of constricting rings per unit area increased with passage of time. Maximum numbers of rings were formed in isolate D followed by isolate C which was significantly higher than in isolates E and B.

This again indicated that sensitivity of different isolates of ring induction differed significantly. The 2nd stage juveniles of *M. incognita* were found captured on day 2 in all isolates although fewer in number. The diameter of 2nd stage juveniles being narrower they easily captured by the constricting rings, whose internal diameter were most suitable for the entry of the nematode. The capturing of 2nd stage juveniles of *M. incognita* was frequently at head or tail region. Occasionally constricting rings were also seen in the middle of the nematode body.

Maximum percentage capturing was recorded in isolate D followed by isolate C whereas minimum percentage of capturing was rescored in isolate E (fig. 2).

Careful examination of nematode after ringing for inflation of cells it was noted that the cells did not inflate immediately after the entry of the nematode in a ring. The ring cells required some time i.e. several minutes for inflation. It was also observed that all the three cells of the rings did not inflate at the same time.

The captured nematodes were penetrated by the hyphae produced from the inflated cells at the point of contact. Mycelium of the fungus grew well within the nematode body and consumed the body content of nematode.

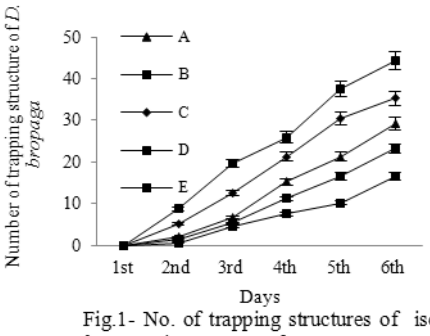

Fig.1- No. of trapping structures of isolates of *D. bropaga* in presence of *M. incognita*

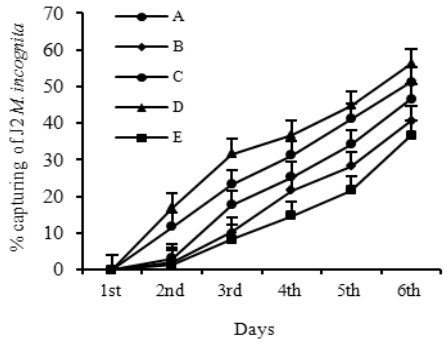

Fig.2- (%) trapping of J2s of *M. incognita* by isolates of *D. brochopaha*

3.2 In dual culture of *M. graminicola* and isolates of *D. brochopaga* the constricting rings were formed in isolate D after 24 h of nematode inoculation, whereas, in other isolates there was no ring formation on day 2 (fig. 3). However constricting rings were formed in all the isolates of *D. brochopaga*. The number of constricting rings per unit area gradually increased with passage of incubation.

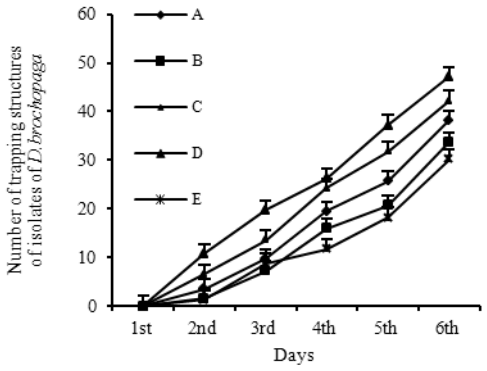

Fig3- No. of trapping structures of isolates of *D. bropaga* in presence of *M. graminicola*

Maximum ring induction on day 6 was recorded in isolate D followed by isolate C, whereas, minimum numbers of induced constricting rings were recorded followed by isolate B. The number of constricting rings in different isolates varied significantly.

2nd stage of juvenile of *M. graminicola* were found captured in dual culture of nematode and isolate D after 24 h of nematode inoculation. After 48h of inoculation the nematodes were captured by constricting rings of all the isolates. The percentage of captured nematode increased with increasing time of incubation. Maximum percentage of nematode capturing was recorded in isolate D followed by isolate C, whereas, minimum capturing was recorded in isolate E (fig.4). Similar to *M. incognita*, (j_2) of *M. graminicola* were also captured frequently at head and tail region.

3.3 The data on interaction between *H. Indicus* and different isolates of *D. brochopaga* are presented in fig (5&6). It was observed that inoculation of *H. indicus* in seven day old cultures of different isolates of *D. brochopaga* did not induce the trap formation up to three days after inoculation. However, on day 4 trapping rings were observed in dual culture. The number of constricting rings increased with increasing period of incubation. In general *H. indicus* was not trapped by the normal size constricting rings produced in culture in response to nematode inoculation. However, few nematodes were found to be trapped in constricting rings with more diameters. This indicated that the size of constricting rings varied greatly. Irrespective of the isolates if the nematodes were captured, the capturing was in the head region. In few instances, the rings were also observed in middle of the captured nematode body. In such cases cells of constricting rings required nearly 30-40 minutes for inflation. The trapped nematodes continuously struggled to escape from the capturing. However, even after great struggle they could not tear off hypha bearing the rings in which the nematode was trapped except for occasional escapes. The inflated cells of such constricting rings were usually very large looking like balloon on side of captured nematode. The swelling of ring size considerably increased when captured nematodes were placed on slides in moist chambers.

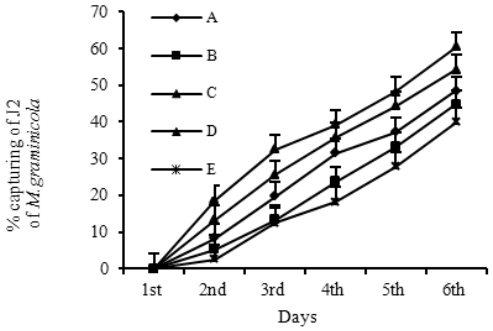

Fig4- (%) trapping of J2s of *M. graminicola* by isolates of *D. brochopaha*

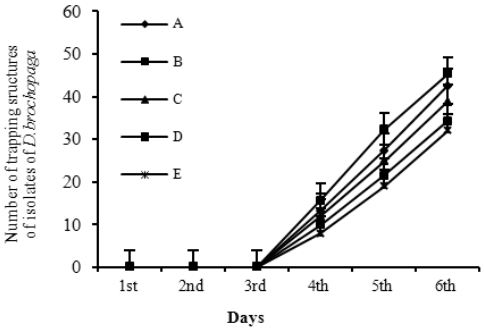

Fig5- No. of trapping structures of isolates of *D. bropaga* in presence of *H. indicus*

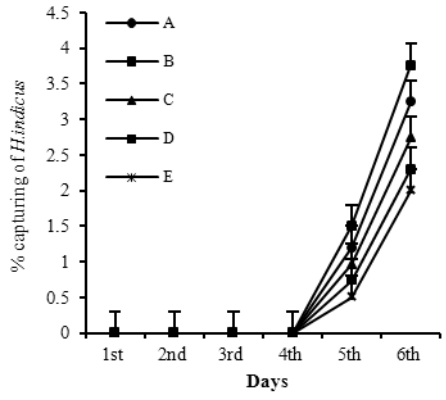

Fig6- (%) trapping of *H. indicus* by isolates of *D. brochopaha*

Such captured nematodes were usually not killed immediately after trapping but they continued their struggle for 15-24 h till they were killed. Although induction of constricting

rings in different isolates of *D. brochopaga* did not differ significantly on day 4. On day 6 significantly higher number of constricting rings was induced by isolate B as compared to isolate E & D. This indicated that the sensitivity of different isolate varies for induction in response to nemin secreted by *H. indicus*. As mentioned above no capturing of *H. indicus* occurred even on day 4 after inoculation, sporadic capturing of this nematode was recorded in dual culture of all the isolates of *D. brochopaga*. Irrespective of the isolates the percentage capturing of *H. indicus* by trapping rings varied between 2-3.7%. The percentage captured nematodes in dual culture of different isolates, however did not differ significantly.

3.4 The data on number of constricting rings and percentage capturing of *T. brassicae* by different isolates of *D. brochopaga* are giving in (fig.7&8). Observations of dual cultures of different isolates of *D. brochopaga* in *T. brassicae* in Petri dishes showed that there was no induction of constricting rings after 24 hours of inoculation. Further fewer rings were induced after 48 h in the isolates of A, B and C, whereas, in isolate D and E there were no ring induction. On day 3 rings were induced in all the culture of the isolates (fig 7).

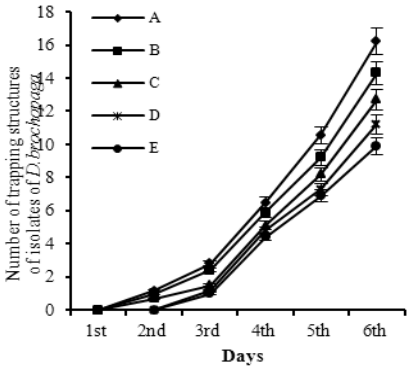

Fig7- No. of trapping structures of isolates of *D. bropaga* in presence of *T. brassicae*

The number of rings having increased with passage of time. On day 6, maximum numbers of rings were induced in isolate A followed by isolate B which were significantly higher than in isolate E, which recorded minimum number of rings per unit area.

The capturing of *T. brassicae* by different isolates of *D. brochopaga* in dual cultures were observed on day 3.

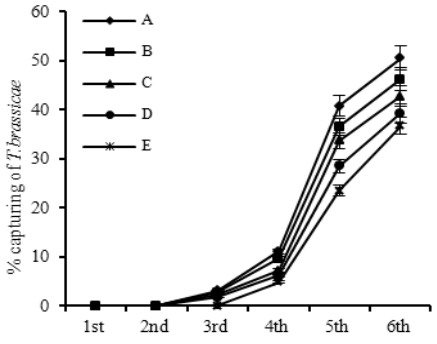

Fig8- (%) trapping of *T. brassicae* by isolates of *D. brochopaha*

However on day 4 and onward capturing of nematode was recorded in culture of all isolates. Maximum percentage of capturing was recorded in dual culture in isolate A followed by isolate B, whereas, minimum percentage trapping of nematode recorded in culture of isolate E (fig. 8). Since the percentage of capturing by isolate C was significantly higher than some of the isolates D and E. Isolate A was found most virulent against *T. brassicae.*

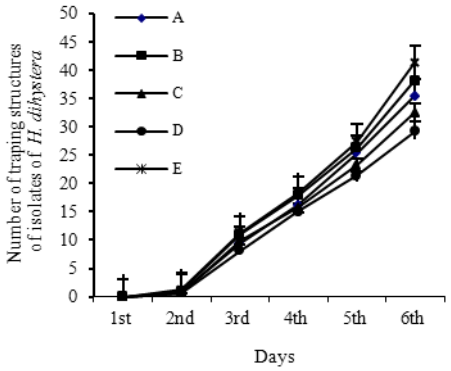

Fig9- No. of trapping structures of isolates of *D. bropaga* in presence of *H. diyhstera*

3.5 The data on induction of constricting rings and capturing of *Helicotylenchus dihystera* by different isolates of *D. brochopaga* are presented in fig (9&10). Observation on induction of constricting rings and capturing in dual culture of different isolates of *D. brochopaga* and *H. dihystera* revealed that

there was no induction of constricting rings after 24 h of inoculation. However, after 48 h few rings were observed in all the isolates of *D. brochopaga*. As noted in other nematodes the number of constricting rings increased with increasing time of incubation. On day 6 of inoculation, although maximum number of constricting rings were recorded in isolate E, the difference in different isolates were non-significant (fig. 9).

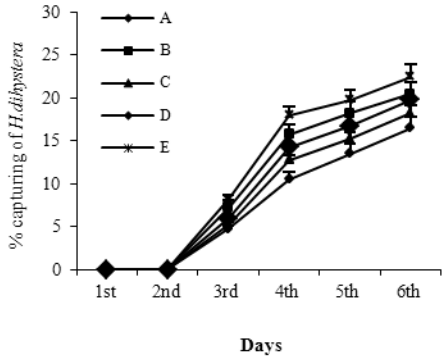

Fig10- (%) trapping of *H. dihstera* by isolates of *D. brochopaha*

The capturing of *H. dihystera* in dual cultures by induced constricting rings were first observed on day 3 after inoculation. The percentage capturing of the nematode increased with passage of time irrespective of the isolates (fig. 10). *H. dihystera* being narrower at head and tail region was captured frequently by the constricting rings of all the isolates.

There was no significant difference in the virulence of different isolates of *D. brochopaga*, as the percentage capturing of the nematode was insignificant. In trapped nematode the hyphae grew from the inflated cells, penetrated and colonized entire body of nematode and utilized the body content of the nematode making the nematode body hollow after 3-4 days of capturing.

3.6 Observation on interaction between *X. basiri* and different isolate of *D. brochopaga* are given in (fig 11 & 12). The inoculation of *X. basiri* in seven day old culture of different isolates of *D. brochopaga* showed that constricting rings were induced on 2nd day of inoculation. The number of constricting rings increased with passage of time in all the dual cultures of all the isolates of *D. brochopaga*. Maximum numbers of constricting rings per unit area were recorded in isolate A followed by isolate B whereas, minimum constricting rings were formed in isolates E followed by isolates D (fig. 11). This observation also indicated that sensitivity of different isolates varied in response to nemin like substances secreted by *X. basiri*, as induction of rings differed significantly.

X. basiri being a lager and wider nematode usually escaped trapping because of the smaller size of rings. Occasionally few nematodes were found trapped at tail tip. The captured nematodes even at the tail tip were finally killed by the fungus as it grew within the nematode body. Since the captured nematode were very sporadic, it was not possible to decide about the degree of virulence of different isolates (fig. 12).

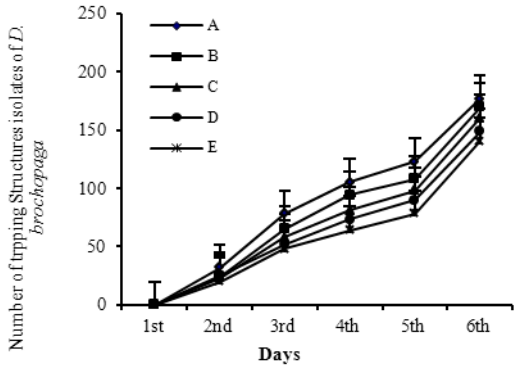

Fig11- No. of trapping structures of isolates of *D. bropaga* in presence of *X. baseri*

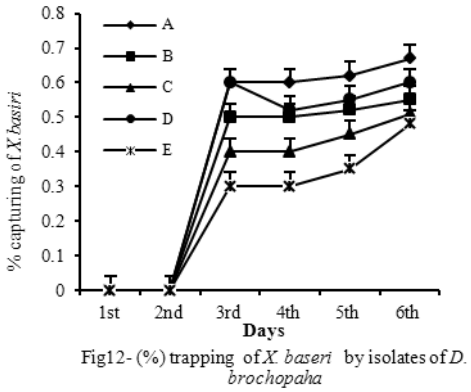

Fig12- (%) trapping of *X. baseri* by isolates of *D. brochopaha*

3.7 The effect of mass culture and spore suspension of *Dactylaria brochopaga* with or without CDM on growth parameters of rice seedling 30 days after sowing and root knot

and population of *Meloidogyne graminicola* are presented in table (1). From observations it is evident that all the growth parameters of rice plants were significantly increased when seedling were raised in infested soil amended with mass culture and spore suspension of different dilutions The growth parameters increased significantly more when mass culture or spore suspensions of *D. brochopaga* were amended in soil in combination with CDM.

Irrespective of the growth parameters, maximum increase in different growth parameters were recorded in pots, treated with mass culture of *D. brochopaga* and CDM closely followed by undiluted spore suspension and CDM.

The observations on effect of different dilution of spore suspension of *D. brochopaga* on number of root knot and nematode population (female, eggs and J_{2s}) (Table 1) clearly indicate that spore suspension of *D. brochopaga* had similar effect as mass culture in reducing the number of root galls and nematode population. Further, all dilution of spore suspension of *D. brochopaga* significantly reduced the number of root knot and nematode population indicating that the biocontrol agent is effective even at lower concentration of spores in amended soil. From this experiment also it was found that spore suspension irrespective of the dilution, or in combination with CDM increases the efficacy of the fungus.

Amendment of soil with mass culture of *D. brochopaga* and its spore suspension of different concentrations significantly increased root length, shoot length and fresh weight of seedlings as compared to control. The growth parameters decreased with increasing dilution of spore suspension, although even highest dilution gave significant increase over uninoculated control. Maximum growth of rice seedlings were also increased after amending the soil with cow dung manure @5%. The mass culture/spore suspension in combination with CDM significantly increased the growth. The rice seedling grown in treated pots with mass culture or spore suspension were very vigorous and therefore the shoot and root weight were nearly double in same treatments.

Table 1: Effect of *Dactylaria brochopaga* on root knot disease of rice caused by *Meloidogyne graminicola*

Treatments	Parameters						
	Galls/ seedling	SL/ seedling (cm)	SW/ seedling (mg)	RL/ seedling	RW/ seedling (mg)	No. of Fem./ seedling	No. of eggs/ seedling
Control	14.1^{a}	14.7^{cd}	67.4^{e}	6.0^{d}	43.1^{j}	85.6^{a}	15535.5^{a}
FYM	13.7^{a}	16.1^{bc}	76.5^{e}	6.8^{cd}	57.8^{i}	79.8^{a}	13237.4^{b}
Db.(mc) +CDM	3.9^{e}	22.3^{a}	185.4^{a}	12.8^{a}	147.3^{a}	34.4^{g}	4987.2^{j}
Db.(SS) + CDM	4.0^{e}	21.4^{a}	178.8^{a}	11.7^{b}	134.2^{b}	36.3^{g}	5123.8^{j}
Db.(10^{1}) +CDM	5.1^{e}	19.1^{b}	162.3^{b}	11.2^{b}	124.6^{c}	41.4^{f}	7258.4^{i}
Db.(10^{2}) +CDM	6.2^{d}	18.9^{b}	157.4^{b}	10.8^{b}	118.6^{d}	46.2^{e}	9875.2^{h}
Db.(10^{3}) +CDM	8.6^{c}	16.4^{bc}	139.6^{c}	8.2^{c}	89.6^{h}	51.8^{d}	11574.6^{h}
Db.(mc)	5.2^{e}	18.3^{b}	154.7^{b}	10.8^{b}	115.6^{d}	41.6^{f}	40876.3^{g}
Db.(SS)	6.6^{d}	17.4^{b}	142.3^{c}	10.0^{b}	110.4^{e}	47.3^{e}	46956.2^{f}
Db.(10^{1})	7.2^{cd}	16.2^{c}	134.5^{c}	9.4^{b}	102.3^{f}	58.6^{c}	82110.8^{e}
Db.(10^{2})	10.2^{b}	15.7^{cd}	118.4^{d}	8.2^{c}	97.5^{g}	56.7^{c}	85650.4^{d}
Db.(10^{3})	11.4^{b}	14.8^{cd}	108.6^{d}	7.6^{c}	59.2^{i}	62.3^{b}	10428.9^{c}

Data represent the mean of three replications; in a column, mean followed by different letters show significant difference in randomized block design test
at $p < 0.05$ by Duncan's multiple range test.

SL = Shoot length, **SW** = Shoot weight, **RL** = Root length, **RW** = Root weight, **Db** =, *Dactylaria brochopaga.* **mc** = mass culture, **ss** = spore suspension

Table 2: Effect of *Dactylaria brochopaga* on root knot disease of rice caused by *Meloidogyne graminicola* (Sprouted seeds of rice)

Parameter	Treatment			
	Control	CDM	*Dactylaria brochopaga*	*Dactylaria brochopaga* + CDM
Galls/seedling	14.2[a]	13.6[a]	6.0[b]	5.2[c]
Shoot length	14.8[c]	15.4[c]	16.8[b]	18.2[a]
Shoot weight/seedling (mg)	77.4[c]	109.2[b]	122.6[b]	137.4[a]
Root length	6.2[c]	8.4[b]	9.4[b]	10.8[a]
Root weight/seedling (mg)	43.2[d]	77.2[c]	97.6[b]	105.2[a]
Number of females/seedling	97.2[a]	91.8[a]	29.6[b]	21.8[b]
Number of eggs + J_2/seedling	24,198.8[a]	21,334.6[a]	8,994.2[b]	5,929.8[c]

Data represent the mean of three replications; in a row, mean followed by different letters show significant difference in randomized block design test at $p < 0.05$ by Duncan's multiple range test.

Table 3: Effect of *Dactylaria brochopaga* on root knot disease of rice caused by *Meloidogyne graminicola* in field Experiment

Parameters	Treatments			
	Control	CDM	Fertilizer	*Dactylaria brochopaga* + CDM
Galls/seedling	13.2[a]	12.3[a]	12.8[a]	2.86[b]
Shoot length	19.4[c]	29.8[b]	28.3[b]	41.5[a]
Shoot weight/seedling (mg)	305.4[c]	870.4[b]	888.3[b]	2864.3[a]
Root length	5.4[c]	8.8[b]	7.4[b]	9.4[a]
Root weight/seedling (mg)	198.0[c]	267.2[b]	236.4[b]	900.8[a]
Number of females/seedling	216[a]	113.5[b]	95.4[c]	27.4[d]
Number of eggs + J_2/seedling	38,808.4[a]	37,018.4[a]	39,041.6[a]	6,362.4[b]

Data represent the mean of three replications; in a row, mean followed by different letters show significant difference in randomized block design test at $p < 0.05$ by Duncan's multiple range test.

3.8 The data on growth parameters of rice seedlings, number of root knots and population of nematodes (females, eggs and J_2) under the influence of various treatments (Table 2) clearly indicated the number of root knot raised from unsprouted seeds recorded significantly lower number of root knot after treating the root knot infested soil with mass culture of *D. brochopaga* in combination with CDM. Similarly total population of females, eggs and juveniles of *M. graminicola* reduced by 80.2 and 85.7% of rice seedlings raised from unsprouted seeds, whereas in case of rice seedlings raised from sprouted seeds were 77.5 and 75.2% respectively.

3.9 Application of mass culture of *D. brochopaga* in combination with CDM @ 5% per hectare significantly reduced the number of root knot and nematode population under field conditions. The root gall number was reduced by 65% whereas total number of nematode was reduced by 84% (Table 3). The application of CDM and fertilizer did not affect the number of root knot or root knot nematode populations, however, growth parameter of rice plant were significantly increased. Mass culture of *D. brochopaga* in combination with CDM not only control nematode but also increase growth parameters of rice plants significantly more than the CDM or fertilizer alone. From these observations it is fully established that *D. brochopaga* is a very effective bio-control agent of root knot nematode of rice.

3.10 The data on effect of fertilizers and organic manure amendments in soil on germination of spore of three isolates of *D.*

brochopaga are presented in table (4). It is evident that all the three fertilizers viz., urea, diammonium phosphate and Muriate of potash inhibited spore germination of selected isolates of *D. brochopaga* at higher concentration. Maximum inhibition of spore germination was recorded in soil amended with urea followed by MOP. At 1% concentration of urea there was no spore germination even after 96 hours. Even at 0.5% concentration of urea the percentage of spore germination was much less as compared to soil indicating that urea had inhibitory effect on spore germination even at this concentration. MOP also had inhibitory effect on spore germination at 1% concentration in observation on 72 and 96 hours after inoculation, whereas DAP recorded significant inhibition of spores at 1% after 72 h of inoculation only.

At 0.1% concentration, all the fertilizers significantly increased spore germination of the *D. brochopaga*. The reduction in spore germination on agar blocks placed on soil as compared to water agar blocks clearly indicated that soil had significant fungistatic effect. It was interesting to note that soil amended with organic manures significantly increased spore germination of all the selected isolates of *D. brochopaga* as compared to unamended soil. This clearly indicated that soil amendments with organic manures nullified the effect of soil fungistasis and thereby increased percentage of spore germination of *D. brochopaga*.

3.11 Observations on number of constricting rings in the presence of six plant parasitic nematodes in dual culture (fig. 1-12) indicated highest number of ring formation in *D. brochopaga* with *X. basiri* which indicates that the quantity of nemin secreted by this nematode was certainly higher than other nematodes. Nemin is a mixture of different peptides (Pramer and Kuyama, 1963), or amino acids.

It is therefore also possible that quality of nemin produced by different nematodes also varied. Nordbring–Hertz (1973) also observed different types of response on morphogenesis with different types of peptides in *A. oligospora* in synthetic media.

In presence of *H. indicus*, ring formation in culture of *D. brochopaga* was observed on forth day which indicates that concentration of nemin was also an important factor for stimulation of ring structures.

Table 4: Effect of fertilizers and organic manures on percentage germination of spores of three isolates of *Dactylaria brochopaga*

Treatment	(%) germination					
	Isolate B		Isolate C		Isolate D	
	72h	96h	72h	96h	72h	96h
Urea (%)						
1.0	-	-	-	-	-	-
0.5	7.9	18.5	9.4	19.8	7.8	15.6
0.1	35.5	72.1	38.2	75.2	33.4	68.2
DAP (%)						
1.0	14.2	48.5	10.5	44.6	12.25	42.8
0.5	22.2	61.2	21.4	57.4	21.22	64.5
0.1	63.2	83.5	58.5	81.2	59.56	79.4
MOP (%)						
1.0	3.5	17.2	5.3	21.6	8.71	21.7
0.5	34.4	94.2	35.4	93.7	42.29	89.6
0.1	90.9	97.4	73.4	95.2	77.7	95.8
Neem Cake (0.5%)	80.8	97.9	78.5	95.3	78.20	97.2
Vermicompost (5%)	83.5	98.8	89.1	98.2	83.95	99.2
F.Y.M. (5%)	73.5	99.4	63.3	97.8	71.1	97.8
Water agar	87.1	99.6	79.5	99.2	81.76	98.4
Soil	39.5	45.4	36.7	47.5	35.71	42.5

The rate of production of nemin was possibly slow. So it indicates that once ring formation is stimulated, process remain inevitable as amino acids act in a simulative way on the initiation of morphogenic process as supported by other works in different fungi (Dicker *et al.*, 1969). In case of plate cultures of *D. brochopaga*, rings were mostly formed in the bottom of culture medium. Formation of rings in the bottom of culture plates suggests that partially anaerobic condition favors ring formation.
This implies that the nematodes crawling mostly in the lower side of the medium may exhibit more capturing than those moving usually on the surface or upper horizon of medium. However, in the present study there was no clear difference in the movement of nematodes in the upper or lower horizons of medium which may be accounted for higher or lower predation. The capturing ring were mostly lying vertically oriented in response of all the nematodes whereas, a horizontal orientation of rings was rare. Vertical orientation may facilitate ringing and capturing of nematodes. Since the orientation of rings was mostly similar, this factor may not be accounted for higher capturing of nematodes like *M. incognita and M. graminicola*. It may be seen that some of the nematodes species that were not captured and killed in induced ringed spore suspension in cavity block, due to their grater body diameter than the internal diameter of rings, were also captured in killed in plate culture. This was obviously due to larger size of the rings produced in presence of nematodes. Majority of the rings formed in the dual cultures were in the lower range of the ring size, so most of the larger nematodes escaped. However, few to many big or medium sizes of rings were produced in culture in presence of nematodes that captured some nematodes. Nematodes in general, taper towards head and tail regions having lower body diameters in these regions and hence capturing was more frequent in such regions. Thus size of the rings formed and the size of nematodes appeared to be most important factor in the predacity of nematodes. Density of rings per 1.6 mm^2 of culture may be another factor responsible for variation in the percentage of capturing. However, this criterion may be only useful if such differences exist while still the nematodes sizes are similar.

3.12 The experiments on performance of mass culture of *D. brochopaga* with and without CDM increased growth parameters of rice seedlings and reduced root galls and nematode population (Table 1-3). The increased growth of rice and reduced root galls and nematode population of *M. graminicola* could be attributed to spores and fungal mycelia of *D. brochopaga* as well as sorghum grains colonized by fungus for mass culture. From this experiment the conditions laid by Sterling (1991) are not satisfied fully who stated that any bio control test should have the following treatments. i. The test organism and any organic amendment should be applied at practical application rates; 0.1 % w/w soil is equivalent to 2.5 tonnes per hectares and should represent a maximum dose. Test should always be performed in a non-sterilized soil with a natural residual soil micro flora. ii. Appropriate treatments as well as an untreated control should be included if the organism added with a substrate. These treatments should include the substrate alone, the organism alone and the autoclaved colonized substrates. Too often untreated controls are compared with only with large application of the organism and substrate and this does not allow separation of the effect of the agent from the effect of the substrate. In several tests reported in the literature, application of the substrate alone has decreased nematode population to the same extent as the substrate colonized by agent, and there is no clear evidence of biological control. iii. Population densities of the agent under test should be monitored to ensure that it has survived in soil through the period that activity against the nematode target is required. Such monitoring may require the development of selective media which can be a difficult and time taking task. iv. Nematode mortality caused by the organism under test should be measured to assess whether the differences between

nematode population densities in treated and untreated soil relate to the level of kill caused by the agent. Infection levels are relatively state forward to estimate for most parasites, but repeated sampling required to determine total kills. The effect of agents which produced toxins or have indirect effects on nematodes through competition, the modification of roots exudates or the colonization of feeding cells can only be measured by assessing their impact on nematode developments. v. The impact of the soil environment, host plant and nematode should the tested as these are likely to affect the efficacy of the biological agent, and could account for the lack of activity of potential agents in specific test conditions.

In view of this, experiment were conducted with the total spore suspension obtained from the same amount of mass culture and their dilutions also to have a clear picture if *D. brochopaga* could increase plant growth and reduce the nematode population. Therefore subsequent experiments on biological control of root knot disease of rice were conducted with mass culture, undiluted and diluted spore suspensions in combination with CDM or alone. In these experiments also the growth parameters of plants increased and number of root knot and nematode population decreased indicating that *D. brochopaga* was effective for the control of root knot disease of rice plants.

In rice it was noted that mass culture or spore suspension used in infested soil were effective when unsoaked and unsprouted appears to the fact that in sprouted seeds radicals were established within soil in less than 24 h after sowing which were exposed to infection by J_2 of *M. graminicola* present in infested soil in active form, to the penetration and establishment of nematode was earlier. Thus the time available for capturing of nematode was little. In contrast, unsprouted seeds usually germinated on or after 4 days of sowing. Under this situation a minimum of four days were available between seed sowing and germination and establishment of roots in soils. During this a good proportion of active J_2 might be captured and killed in the soil resulting in increased growth parameters of seedling and reduced number of root knot and nematode population.

The increased efficacy of *D. brochopaga* in combination with CDM may be attributed to the following reasons one or all working together in the soil: i. organic amendment nullifies the effect of soil fungistasis and therefore spores of nematophagous fungi germinate. ii. organic amendment increases population of saprophytic nematodes several folds which provide good pabulous to nematophagous fungi. iii. organic manure supports colonization and growth of nematophagous fungi. iv. combination of mass culture of nematophagous fungi and organic manure, enhanced biocontrol efficacy for a larger period, shows the biocontrol effect is prolonged in combination with CDM. This study also supports the work of Hoffman and Sikora (1993); Van Den Boogert (1994).

3.13 In general, germination of spores of fungi is inhibited is soil as result of soil fungistasis. In view of this commonly used fertilizers viz., urea, DAP and murate of potash and organic manures were amended separately in the same soil and spore germination in these soils were determined. It was noted that all the fertilizers inhibited spore germination of all the three isolates of *D. brochopaga* at higher concentration i.e., 1%. Maximum reduction in spore germination was caused by urea followed by DAP. On the contrary amendment of soil with organic manures nullified the effect of soil fungistasis resulting in an increase in the spore germination over unamended soil.

4. CONCLUSIONS

The results of this research work conclude that presence of nematodes induces the formation of constricting rings of *Dactylaria brochopaga* a predacious fungus. In turns, the fungus captures nematodes through these induced constricting rings. This work also reveals that predacious fungi are very useful for managing root-knot disease of rice and that *D. brochopaga* is a potent agent for the biocontrol of *M. graminicola*. The cow dung manure

should be applied in the soil for better proliferation of the fungus and effective control of *Meloidogyne graminicola*. Furthermore, synthetic fertilizers inhibits the spore germination of *D. brochopaga* whereas, organic manures nullified the effect of soil fungistasis .

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