

**STUDIES ON TOLERANCE AND SENSITIVITY OF  
NEMATODE-TRAPPING FUNGUS *Drechslerella  
brochopaga* TO COMMON FUNGICIDES**

**THESIS**

SUBMITTED TO THE



**Banda University of Agriculture & Technology, Banda-  
210001, Uttar Pradesh, India**

By

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Id. 1372

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
MASTER OF SCIENCE (AGRICULTURE)  
IN  
PLANT PATHOLOGY  
2021**



**Dedicated**  
**To my parents**

**Hariom Dwivedi....✍**

# BANDA UNIVERSITY OF AGRICULTURE & TECHNOLOGY, BANDA

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Dated-

## *Certificate-I*

This is to certify that the thesis entitled “Studies on tolerance and sensitivity of nematode-trapping fungus *Drechslerella brochopaga* to common fungicides ” submitted in partial fulfillment of the requirement for award of the degree of **Master of Science (Agriculture) in Plant Pathology, College of Agriculture**, Banda University of Agriculture & Technology (Banda), is a genuine record of bonafide research work carried out by **Hariom Dwivedi, Id. No. 1372** under my guidance and supervision. The results of the investigation in this thesis have not so far been submitted for any other degree or diploma.

It is further certified that the help or information received during the course of investigation and preparation of the thesis have been duly acknowledged.

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## ***Certificate-II***

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## **LIST OF CONTENT**

<b>SR.NO</b>	<b>DESCRIPTION</b>	<b>PAGE NO.</b>
1.	INTRODUCTION	1-4
2.	REVIEW OF LITERATURE	5-16
3.	MATERIALS AND METHODS	17-21
4.	EXPERIMENTAL FINDINGS	22-29
5.	DISCUSSION	30-33
6.	SUMMARY AND CONCLUSION	34-36
7.	LITERATURE CITED	37-49
	VITA	

## LIST OF TABLES

TABLE NO.	DESCRIPTION	PAGE NO.
1.	Species of nematode- trapping fungi isolated from soil of different location of Banda, Uttar Pradesh.	22
2.	Morphological characteristics of <i>D.brochopaga</i> used in the present study	23
3.	Assessment of <i>in vitro</i> nematode- trapping ability of <i>Drechslrella brochopaga</i> against second stage juvenile of <i>M. incognita</i> in corn meal agar medium (0.4%)	24
4.	Germination, conidial trap formation and trapped nematodes by conidial traps of <i>D. brochopaga</i> in agriculture soils of different location of Banda	27
5.	Details of fungicides used in the present study.	28
6.	Effect of different concentrations of some common fungicides in soil on per cent conidial trap formation of <i>D. brochopaga</i>	29

## LIST OF FIGURES

Fig. No.	Description
1.	Trapping of free living nematode and appearance of <i>Drechslerella brochopaga</i> conidial heads in soil plated on corn meal agar media.
2.	Morphological features of conidia and conidiophores of <i>Drechslerella brochopaga</i> .
3.	Hyphal characteristics of <i>Drechslerella brochopaga</i> conidial head in soil plated on corn meal agar media.
4.	Morphology of conidial population of <i>Drechslerella brochopaga</i> .
5.	Constricting ring of <i>Drechslerella brochopaga</i> and trapping of <i>M. incognita</i>
6.	Formation of conidial traps of <i>Drechslerella brochopaga</i> in vicinity to various soils.
7.	Trapping of soil nematodes by <i>Drechslerella brochopaga</i> in vicinity to agricultural soil.
8.	Trapping of soil nematodes by <i>Drechslerella brochopaga</i> in vicinity to horticultural soil.
9.	Trapping of soil nematodes by <i>Drechslerella brochopaga</i> and growth of fungus in vicinity to soil.

## LIST OF LINE FIGURES

GRAPH NO.	DESCRIPTION
1.	Line figure-2. Assessment of <i>in vitro</i> nematode- trapping ability of <i>D.brochopaga</i> against <i>M. incognita</i> in corn meal agar medium (0.4%). Data are significantly difference in row data at $p < 0.05\%$

## LIST OF ABBREVIATIONS

pH	:	Power of hydrogen
psi	:	Pounds per square inch
Spp.	:	Species
Microbes	:	Microorganisms
100x	:	100 times magnification
%	:	Per cent
Min	:	Minute
CMA	:	Corn meal agar
RPM	:	Revolution per minute
μl	:	Microliter
±	:	Plus or minus
μm	:	Micrometer
°	:	Degree
C	:	Centigrade
<i>et al.</i>	:	and others
Fig.	:	Figure
g	:	Grams
<i>i.e.</i>	:	That is
Hrs.	:	Hours
mm	:	Millimeter
ml	:	Milliliter
no.	:	Number
<i>viz.</i>	:	Namely
l	:	Liter
Sq.	:	Square
RADP	:	Random Amplified Polymorphic DNA

## ABSTRACT

Name: <b>Hariom Dwivedi</b>	Id.No.: 1372
Semester : II <sup>nd</sup>	Degree: M.Sc.(Ag) Plant Pathology
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Major: Plant Pathology	Minor: Entomology
<b>Thesis Title :</b> Studies on tolerance and sensitivity of nematode- trapping fungus <i>Drechslerella brochopaga</i> to common fungicides'	

*Drechslerella brochopaga* Drechsler, a strangulating nematode-trapping fungus was studied for their adoptability in soil and their tolerance against some common fungicides. *D.brochopaga* was isolated from horticultural soil of Baberu, Banda. The fungus was characterized and identified by the use of relevant literature. The fungus was found to effectively capture and kill the 96.4 per cent second stage juveniles of *Meloidogyne incognita* by its three celled constricting ring produced on hyphae within 3 days. The study of adaptability of *D. brochopaga* in various agricultural and horticulture soil of Banda revealed that this fungus is frequently formed conidial trap in vicinity to soil and parasitize nematodes. The study of the tolerance of conidia of *D. brochopaga* with soil amended with different concentrated of common fungicide indicate that this fungus is highly toxic to chlorothalonil was various range of tolerance/sensitivity with common fungicide. Conidia of *D. brochopaga* were found most sensitive to chlorothalonil and propineb at 10  $\mu\text{l ai kg}^{-1}$  concentration followed by carbendazim, mencozeb, copper oxychloride, copper sulphate, thiram which inhibited the conidial trap formation at 25 mg ai  $\text{kg}^{-1}$  ppm. Tebuconazole inhibited completely inhibited the fungus at 100  $\mu\text{l ai kg}^{-1}$  whereas Metalaxyl and Sulphur completely inhibited the fungus at 300 mg ai  $\text{kg}^{-1}$ . *D. brochopaga* showed tolerance to propiconazole up to 300 mg ai  $\text{kg}^{-1}$  but the fungus was completely inhibited at 400 mg ai  $\text{kg}^{-1}$ . The result of the present study indicate that *D. brochopaga* is sensitive to most of the fungicides tested under the present study. The fungus showed tolerance to Metalaxyl, propiconazole and Sulphur below 200 mg/ $\mu\text{l ai kg}^{-1}$  concentration of in soil.

Dr.Dharmendra Kumar	Hariom Dwivedi
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## INTRODUCTION

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Nematophagous fungal species which capture and kill the nematodes by trophic cells belongs to phyla Zygomycota, Basidiomycota and Ascomycota (Li et al. 2000, Kirk et al., 2008). Nematophagous fungi belong to Zygomycota produce adhesive hyphae or hyphal protuberances that passively capture small animals (Saikawa, 2011). Nematophagous fungi in the Basidiomycota produce special adhesive knobs or adhesive spores to capture nematodes (Poloczek and Webster, 1994; Durschnerpelz 1987). More than 90% of the Nematophagous fungi belong to the Orbiliomycetes in the Ascomycota (Li et al., 2000), where they form a monophylogenetic group (Liou and Tzean, 1997 and Li et al., 2010). More than 200 such species from the phyla Ascomycota, Basidiomycota, and Zygomycota have been described, and many scientists hypothesized that their nematophagous lifestyles arose independently via convergent evolution (Yang et al., 2007; Liou, et al., 1997).

Nematophagous fungi of Ascomycota are popularly known as nematode- trapping fungi. These fungi form a loop like structures called traps to capture, kill, parasitize and digest the nematodes (Drechsler, 1937; Drechsler, 1941; Pramer, 1964; Thorn and Barron, 1984; Barron, 1977). For capturing, killing and digestion of nematodes; Nematode- trapping fungi of produce sophisticated trapping structures, including one type of active and non - adhesive mechanical trap (constricting rings) and five types of passive adhesive traps: sessile adhesive knobs, stalked adhesive knobs, adhesive nets, adhesive columns, and non-constricting rings. Traps are initiated in response to a trap-inducing compound released by the nematodes themselves and are formed at intervals along the length of the hyphae. The formation of traps is very important for these fungi to enter in to the parasitic phase. The traps can develop from hyphal branches and these are termed mycelial traps (MT). Alternatively, they can also form directly upon spore germination without an intermediate mycelial phase or on the germination hyphae, forming conidial traps (CT). Mycelial traps can be formed either spontaneously or be induced in response to signals from the environment, including certain amino acids, valyl peptides and nemin that were secreted by host nematodes (Dijksterhuis *et al.*, 1994).

Nematode- trapping fungi have received more attention in the past due to their possible use in biological control of plant-and animal-parasitic nematodes (Kerry, 2000; Larson, 2000). Nematode-trapping fungi occur in all sorts of soil environments where these fungi live both saprophytically and as facultative predators by capturing nematodes (Pramer, 1964. Nordbring-Hertz et al., 2006). To switch over to predacious lifestyle, these fungi forms traps on hyphae on or in which nematodes can be trapped either mechanically or by adhesion. Within a few hours of close contact between nematodes and nematode- trapping fungi, the mycelia of these fungi differentiate spontaneously into functional traps. The traps then capture, penetrate and kill the nematodes. Most of the nematode- trapping fungi produce very few traps constitutively, but they form abundant traps in the presence of live nematodes. Earlier work demonstrated that nematodes secrete a morphogenic substance Nemin that induces trap formation (Pramer and Stoll, 1959). After formation of traps, carnivorous activities precede a series of events including adhesion or trapping, penetration and immobilization of nematodes. At this stage the nematodes become paralyzed (killed), and their internal tissues are rapidly colonized by fungal hyphae (Dijksterhuis et al., 1994). Enzymes released by nematode- trapping fungi during parasitism of nematodes break down the body contents of the nematodes and the nutrients are translocate elsewhere within the hyphal system for growth and reproduction of conidia.

Alternatively, traps may be formed directly upon conidial germination without an intermediate hyphal phase (Persmark and Nordbring Hertz, 1997) or on short spore germlings (Cook, 1964; Kumar, 2003) so-called conidial traps. These conidial traps are induced in response to cow dung (Dackman and Nordbring-Hertz, 1992), rhizosphere soil (Mankau, 1962; Pressmark and Nordbring-Hertz, 1997) and are compound released by soil nematodes (Kumar, 2003). Formation of conidial traps by nematode-trapping fungi may give these fungi an opportunity to capture and extracts the abundant nutrition from the nematode body for growth and development in the soil having intense competition with other fungi for nutrients. Plant-parasitic nematodes are known to cause global agricultural losses amounting to \$157 billion annually (Abad et al., 2008). Formulations of nematode-trapping fungi has been successfully applied in soil to improve plant health by suppressing root-knot nematodes (Stirling and Smith, 1998; Stirling et al., 1998; Kumar and Singh, 2006a; Singh et al., 2007;

Singh et al., 2012), enhancing the accumulation of defense related biomolecules and induce systemic resistance in plants (Singh et al., 2013).

*Drechslerella brochopaga* Drechsler is a nematode-trapping fungus that forms constricting rings to capture and kill the nematodes. Conidial germination of *Drechslerella brochopaga* usually takes place with germ tubes developing into a hypha on which three celled constricting rings forms in presence of nematodes or in specific abiotic environments. When a nematode enters the constricting ring and contacts the inner surface of the ring cells, G protein-coupled receptors activate a down-stream signal pathway that includes cyclic adenosine monophosphate (CAMP), inositol-1, 4, 5-triphosphate (IP3), and Ca<sup>2+</sup>. Subsequently, the ring cells rapidly (within 0.1 s) triple their volume and firmly constrict the nematode. *Drechslerella brochopaga* is also known to form conidial traps in response to soil and soil nematodes. This fungus was found most effective against root-knot disease in of rice (Singh et al., 2007).

Soil fungistasis which inhibits germination of most fungal propagules (Dobbs and Honson, 1953) can influence the efficacy of nematode- trapping fungi. Unfortunately, most of the nematode- trapping fungi fail to germinate in soil due to soil fungistasis (Cook, 1964; Mankau, 1962) and thus reduce the nematode bio-control ability as expected. Similarly, fungicidal contamination of soil adversely affects the efficacy of bio-control fungi in soil (Crump and Kerry, 1986; Kidwai et al., 2006; Fravel et al., 2005.). Fungicides are routinely used in agriculture to control fungal diseases. However, besides the desired effect on fungal pathogens, non-target fungi are also subjected to the fungicide treatment. Applying a fungicide to control pathogens might kill the nematode- trapping fungi in soil.

Introduction of nematode- trapping fungi to soil in the form of spores seems to be most feasible method for large-scale application of such organisms (Mankau, 1962). However, these fungi must have better adoptability in agricultural soils and be compatible with chemical pesticides that are applied in soil or crops to control other pest and diseases. Hence, the sensitivity and tolerance of nematode- trapping fungi against common pesticides need to be investigated to elucidate the fate of these fungi for bio-control purposes. It has been hypothesised that some fungicides suppress nematode- trapping fungi that otherwise would act as competitors against plant parasitic nematodes.

Since, there is no work on sensitivity and tolerance of *Drechslerella brochopaga* to common fungicides, the present investigation was under taken with following objectives.

1. Isolation, characterization and *In vitro* bioefficacy assay of *Drechslerella brochopaga* against nematodes.
2. Study on germination and trap forming ability of *Drechslerella brochopaga* in fungistatic environment of soil .
3. Study of tolerance and sensitivity of *Drechslerella brochopaga* to some common fungicides.

## REVIEW OF LITERATURE

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The history of Nematode-trapping fungi begins after the discovery of well known nematode- trapping fungus *Arthrobotrys oligospora* by Fresenius in 1852. Woronin (1870) reported that germination of *A. oligospora* on old manure forms hyphal nets, but he did not know the function of such nets. Zopf (1888) observed that these hyphal nets captured the moving nematodes. He showed that the fungus penetrated the cuticle of the captured nematode and hyphae grew within the nematode body and consumed the body contents. Zopf was therefore the first scientist to establish the biological relationship between a predacious fungus and a nematode. His descriptions on the process of manifestation of predatism, appeared in ‘Nova acta Leopoldina Carolina Academia’, which remains as one of the greatest classics in mycological research.

Charles Drechsler (1933, 1933a) demonstrated that the hyphal nets of nematode-trapping fungi produced adhesive material, which were responsible for trapping of nematodes. Drechsler was initially interested in *Pythium* and *Phytophthora* parasitic on roots of higher plants. While making observations, he noticed that some fungi attacked nematodes in plates basically plated for the isolation of *Pythium* and *Phytophthora* on a weak nutrient agar medium. The dynamic interaction between fungi and nematodes completely changed Drechsler’s mind for research on nematode- trapping fungi. Drechsler was motivated by the fascinating trapping devices of predacious fungi and dedicated his entire life on the studies of these fungi. His classical publications during 1933 to 1975 are source of evidence and inspirations for many workers interested to pursue research on nematode- trapping fungi.

Duddington (1952-72) concluded that the nematode- trapping fungi are commonly found in natural soils, agricultural soils and all kind of decaying manures. He also described the technique of handling of nematode- trapping fungi (Duddington 1955). His book “The friendly fungi” deals with various aspects of nematode destroying fungi (Duddington 1957). Several other workers from all over the world have also contributed through their studies to this important group of fungi (Juniper 1957; Pramer and Stoll, 1959; Cooke, 1962, 1962b, 1963; Higgins and Pramer, 1967; Barron, 1969, 1977; Mankau and Clark, 1959; Monson

and Ranieri, 1972; Pramer, 1964; Nordbring-Hertz *et al.*, 1977; Stirling *et al.*, 1979; Jansson and Nordbring-Hertz, 1980; Gray, 1983a, 1983 b and Stirling *et al.*, 1998).

Some preliminary works on nematode- trapping fungi were initiated by Das Gupta *et al.* (1964), Sachchidananda (1965), Sachchidananda and Swarup (1966,1967), Shome and Shome (1966), Sachchidananda and Ram Krishnan (1971) and Patil and Pendse (1976, 1981). Nematode trapping fungi have been reported as new records from India (Dayal and Nand, 1973a, 1973b; Dayal and Singh, 1975; Dayal and Gupta, 1975b; Dayal and Srivastava 1978; Srivastava, 1981, Srivastava and Dayal, 1982, 1984; Prasad *et al.*, 1984; Prasad, 1985). The taxonomy of predacious fungi has been a subject of deep concern during the course of studies on these fungi. Drechsler described a large number of new species of nematode- trapping fungi preying on nematodes.

Studies on taxonomy of nematode- trapping fungi have been made by Soprunov (1951); Subramaniam (1963); Cooke and Godfrey (1964); Cooke and Dickinson (1965); Rifai and Cooke (1966) Cooke (1969) and others. The key given by Cooke and Godfrey (1964) is widely used for identification of nematode trapping fungi. Barron (1977) wrote a book entitled “The nematode destroying fungi” as reference book for studies on nematode- trapping fungi.

### **Research on trap formation and swelling of rings cells in Nematode- Trapping fungi**

Couch (1937) reported that abundant rings were formed if some water containing nematodes were added to the pure culture of *D. bembicodes*. Commandon and de Fonbrune (1938) reported that addition of sterile culture filtrates of nematodes in pure culture of nematode- trapping fungus were able to induce the trap formation. Roubaud and Deschiens (1939) showed that trap inducing agent was not restricted to nematode only. Among these, human blood serum and extracts from earthworm were particularly more effective. Pramer and Stoll (1959) proposed the term *nemin* for the substance(s) inducing trap formation. They proved that this chemical molecule was produced by nematode *Neoplectona glaseri*, as triggering molecules for the formation of traps in nematode- trapping fungi.

Tarjan (1960) also reported formation of traps in nematode- trapping fungi by *Penagrellus redivivus*. He noted that the number of nematodes was responsible for trap formation. Feder *et al.* (1960) observed that a single dried nematode induced trap formation

over 1 cm column containing *Dactylella doedycoides*. This clearly indicated that nemin was required in an extremely small quantity for induction of traps. Pramer and Kuyama (1963) suggested that nemin is a peptide of low molecular weight or possibly even a single amino acid of rather common occurrence. Their test of 13 proteins and 49 peptides and 27 amino acids, however, failed to induce morphogenic changes in nematode- trapping fungi.

Feder *et al.* (1963) observed trap formation in nematode- trapping fungi using higher dilution than previously used by Pramer and Stoll (1959). They also suggested that most of the nemin was present in nematode body as endogenous nemin. They studied the effect of endogenous nemin of *P. redivivus* on morphogenesis of five species of *Dactylella* and noted differences in response of these nematode- trapping fungi to nemin. *Dactylella cionopaga* exhibited the morphogenesis even at  $10^{-6}$  dilution, whereas *D. bembicodes* and *Dactylella drechsleri* did not respond to  $10^{-2}$  and  $10^{-3}$  dilutions, respectively. In case of *Dactylella ellipsospora*, they found that this fungus did not respond to endogenous nemin but in contrast it showed morphogenesis in culture in presence of live nematodes. This explains that endogenous nemin and the extract of living nematodes were not identical in chemical composition.

Nordbring-Hertz (1973) reported that amino acid induce trap formation. However, effect of peptides, especially valyl peptide was much more pronounced. She concluded that several peptides were capable of inducing traps in *A. oligospora* and level of nutrition of a medium was of critical importance in the induction of traps. She further concluded that the peptides capable of inducing morphogenesis were produced by biological degradation of proteinaceous material in the soil.

Monoson *et al.* (1974) observed that endogenous nemin extracted from five different species of nematodes induced trap formation. The RNA synthesis inhibitor, 6- methyl purine, when applied at the same time with various nemins, completely inhibited the trap formation by the fungus. Application of 6-methyl purine, one hour after the addition of various nemin had no effect on the number of traps formed. Two compounds that inhibit normal protein synthesis (5-thiouracil and P-fluorophenylalanine) also exhibited a similar trap formation in *Monacrosporium doedycoides* seemed to be at the level of transcription.

Trap formation in nematode- trapping fungi have been shown to be induced by agents

other than nematodes or animal origin. Soprunov (1958) reported that traps were induced if mycelial fragments were added to snow, melted at room temperature or rainwater. He found most abundant and rapid formation of traps when spores of nematode-trapping fungi germinated in rainwater containing 1-2% ethyl alcohol. He observed that combination of CO<sub>2</sub> in rainwater with ammonium compounds formed ammonium carbonate, which induced trap formation. He also noted that due to sterilization of nematode filtrate, morphogenic activity was lost. Nutritional deficiency and water stress are also known to trigger the process of ring formation. Balan and Lechevalier (1972) observed that *A. dactyloides* produced abundant traps in adverse condition (lack of nutrients or lack of water), which remained functional and capable of capturing nematodes. They compared number of traps formed in presence of nematodes with fungus culture in cellophane paper. In double layer cellophane papers, number of ring formation was at par with the ring formation in presence of nematodes *P. redivivus*.

Lawton (1957) observed the spontaneous trap formation on the hyphae of nematode-trapping fungi in culture. He noted that the spontaneous trap formation in *A. dactyloides* was stimulated by contact with glass and was not due to starvation of the fungus.

Bartnicki- Garcia *et al.* (1964) observed that trap formation in presence of nemin in two strains of *A. conoides* was CO<sub>2</sub> dependent. No traps were formed in absence of CO<sub>2</sub>. Though the optimum concentration of CO<sub>2</sub> was not determined, highest concentration (10%) produced maximum number of traps in one of the strains of the fungus.

Commondan and de Fonbrune (1939) using a pneumatic micromanipulator demonstrated that the constricting ring of *D. brochopaga* could be operated if the ring cells were rubbed with a glass micro needle. During inflation of individual ring cells, a number of small vacuoles increased in size and finally condensed to form a single large vacuole.

Muller (1958) observed that the stimulation of the inner wall of the constricting ring cell induced on instantaneous decrease in wall pressure and an increase in membrane permeability in that particular region of the wall. Due to the uptake of water from the surrounding sources, the level of osmotically active substances in the ring cell rose for the hydrolysis of the large molecule present, which maintain inflow of water. This process would continue until the swelling of ring cells was completed. In an alternative hypothesis, he

suggested that osmotic recovery was rather slower. The cell would enable to expand by virtue of its initial osmotic potential, which fell during the expansion from level equivalent to 0.6 M sucrose to one finally equivalent to 0.2 M due to water uptake. The cell would then recover its initial osmotic potential after inflation by relatively slow hydrolysis of polymer present within it, and or by relatively slow transport of solute from the stock cells. He noted that the second version of the hypothesis was less physiologically exacting. It envisaged the stimulation of the cell as inducing a change in wall structure and a change in permeability, but did not require in addition any exceptionally rapid increase in the amount of osmotically active solutes in the cell.

Rudek (1975) reported that the air current caused the constriction of ring cells. He pointed out that rings were in a dry condition and the only available water was by water imbibition through the stock and adjacent ring cell. He suggested that these imbibitions was overshadowed by the tremendous intake of water when the cells were submerged and he believed the complete closure could be explained in part by changes in membrane permeability.

### **Research on parasitic behaviour of nematode- trapping fungi**

Nematode- trapping fungi are poor competitors in the soil. To survive and compete in the diverse situation of soil environment, nematode- trapping fungi depend on the nematodes for their survival. Several experiments on trapping and killing of free living nematodes, fungal feeder nematodes and plant-parasitic nematodes by nematode- trapping fungi have been conducted by many workers to understand the parasitism of these fungi for better interpretation (Cooke and Pramer, 1968; Monoson, 1968; Belder and Jansen, 1994 and Esser, 1995, Kumar et al., 2015).

Cooke and Pramer (1968) found that the presence of mycophagous nematode, *A. avenae*, did not affect the rate of radial growth of nematode trapping fungi. They reported that Population of nematodes was not influenced by the fungal predation. At the initial phase of the experiment nematode population decreased due to the nematode- trapping activity of the fungi but later on predation became in low ebb because of the toxic metabolite produced from the abundant population of the nematode, which eventually contributed to the death of fungi. Due to this reason, nematode-trapping fungi were not visible after 30 days in dual

culture with *A. avenae*.

Effect of temperature on growth and trapping effectiveness of some nematode-trapping fungi were studied by Monoson (1968). Major portion of his experiment dealt with the growth of *A. oligospora*, *A. musiformis*, *A. dactyloides*, *Monacrosporium bembicodes*, *M. cionopagum* in relation to temperature and population of *A. avenae* and *Neotylechus linfordi*. He observed that little fluctuation existed between increased trapping structures with temperature. Furthermore, too many nematodes did not encourage the growth of predacious fungi. His observation also supported the view of Cooke and Pramer (1968) that higher population of nematodes offered smaller amount of growth, which provided a negative correlation. Best predation of *A. oligospora*, *A. musiformis* and *M. bembicodes* was observed at 15-20<sup>0</sup>C against *A. avenae*, whereas *M. cionopagum* was most effective at 20<sup>0</sup>C on V-8 agar medium and *A. dactyloides* at 15<sup>0</sup>C on PDA, 25<sup>0</sup>C on CMA and 30<sup>0</sup>C on V-8 agar medium. However, all the fungi captured *N. linfordi* very effectively at all temperature regardless the media or the number of nematodes present in the inoculated sample. High percentage of trapping was noted in plates containing 100 nematodes.

Heintz (1978) observed that the age of fungus colony, number and type of nematodes added and the ability of the fungus to form traps within the agar affected predation of nematodes. He also calculated predacity index (PI) under different experimental conditions. Smaller was the PI value larger was the capacity of predation. Esser *et al.* (1991) studied interaction of *Dactylella megalospora* with 18 genera of phytonematodes and 9 free living nematodes. He observed that all the nematodes were captured and assimilated by the fungus, however, some nematode exhibited resistance to trapping.

Belder and Jansen (1994) studied the *in vitro* assessment of predacity of different nematode- trapping fungi, viz., *A. oligospora*, *M. cionopagum*, *A. dactyloides*, *A. scaphoides* and *Duddingtonia flagrans* against *Meloidogyne hapla*, *M. incognita*, *Globodera pallida* and *G. rostochinensis*. They observed that an isolate of *A. oligospora* and an isolate of *M. cionopagum* were very much effective in capturing *M. hapla* and *M. incognita*. *Arthrobotrys conoides*, *A. dactyloides* and *A. scaphoides* were intermediate in capturing efficiency. However, capture by some isolate of *Arthrobotrys* and *Duddingtonia* did not occur irrespective of the age of the mycelium. Further, they reported that the isolates, which were

very much effective against *M. hapla* and *M. incognita*, had limited ability to capture the other nematodes e.g. *G. rostochinensis* and *G. pallida*. They concluded that nematode-trapping fungi have specificity in relation to the predation.

Belder and Jansen (1994a) studied the effect of temperature, substrates, light and aging of mycelia on the ability to capture second stage juveniles of *M. hapla* by an isolate of *A. oligospora* (CBS - 29882) in *in vitro* condition. They reported that trapping of nematodes by the mycelia of the fungus occurred within one hour irrespective of temperature and nematode mobility. However, subsequent development of trapping structures was significantly lower at lower temperature (5-10<sup>0</sup>C) than at higher temperature (15-30<sup>0</sup>C). They also observed that nutritional conditions did not correlate to the efficacy in nematode hyphae attachment, but subsequent development of adhesive network was delayed on water agar. Aging of the fungal mycelia did not differ in capturing efficiency up to 70 days. Light, also did not have any influence on nematode capture.

de Gives *et al.* (1994) evaluated the predacity of *A. oligospora* and *A. conoides* against 3<sup>rd</sup> stage and 2<sup>nd</sup> stage larvae of *Haemonchus contortus* and *Nacobbus aberrans*, respectively *in vitro* at different temperatures. *Arthrobotrys oligospora* showed 35.87% and 25.71% trapping at 18<sup>0</sup>C and 25<sup>0</sup>C, respectively. None of the fungi was noted to produce three dimensional adhesive loops at 30<sup>0</sup>C, hence, no capturing was observed. The predacity shown by *A. conoides* was higher than 90% for both nematodes.

Nematode- trapping activity and hatching inhibition by an African isolate of *M. bembicodes* was described by Cayrol and Sawadogo (1991). They observed that three celled constricting ring of the fungus trapped the nematode within one week of inoculation in the Petri dishes. They also observed 80% inhibition in hatching in the presence of fungus.

Galper *et al.* (1995) developed the simple screening methods for assessing the nematode- trapping activity of nematode-trapping fungi and found that these fungi produced traps extensively on agar in response to *Caenorhabditis elegans*. They also noticed that the population of *C. elegans* was reduced by at least 90% within 3 days while the juveniles of *M. javanica* induced fewer traps on agar and most of the nematode- trapping fungi took more than three days to reduce number of nematodes by more than 90%. They also observed that species such as *Dactylella candida* and *A. dactyloides* consistently reduced the number of *M.*

*javanica* juveniles recovered from the soil, whereas network forming species *Monacrosporium* sp., *A. oligospora*, *A. conoides*, *A. musiformis* did not give the same results. They also studied the agar “sandwich” and buried slide technique to monitor trapping activity in soil and concluded that *D. candida* and *A. dactyloides* consistently produced the traps within 5 days of being introduced, whereas network forming species produced fewer traps in the soil and their trapping activity varied from experiment to experiment

Cooke (1977) suggested that nematode- trapping fungi are able to capture and kill nematodes only for a relatively short period after the onset of decomposition of soil amendment. He observed that increasing the amendments above a certain level resulted in a decrease in parasitic activity despite an increase in nematode population. He concluded that nematodes were an alternative rather a sole nutrients source for nematode- trapping fungi. He also suggested that predacious fungi were predaciously active only during competition and the predacious habit was adopted to ease or escape competition for available substrate during decomposition of organic matter in soil. He considered that there was intense competition in the soil for the carbon energy sources but the predacious fungi had the edge because they could utilize nematodes as an additional food source and thus evade some of the stress in the competition for food. He also suggested that the energy from the nematodes could be channeled in to obtaining other nutrients from soil which, when depleted, resulted in a decline in predatory activity.

#### **Fungistatic effect of soil on germination and conidial trap formation of Nematode-Trapping fungi.**

Dobbs and Hinson (1953) observed that the spores of fungi, when placed in contact with soil showed inability to germinate under conditions, which might be considered favourable for spore germination.

Mankau (1962) studied soil fungistasis in relation to *A. arthrobotryoides*, *A. dactyloides* and *Dactylella ellipsospora* by agar disk technique and observed that germinated spores of these predacious fungi on soil were some time lysed or formed trapping organs on spores. He also observed that organic amendments temporarily altered the inhibitory properties of soil. He also reported that nematode- trapping fungi are poor competitors in the soil and their survival in the soil depends on the successful capture of nematodes.

Cooke and Sathchuthananthavale (1968) observed the sensitivity of nematode-trapping fungi to soil fungistasis and found that with the exception of *A. musiformis*, all species showed sensitivity to fungistasis and degree of sensitivity varied from species to species. They concluded that agricultural soils harbour indigenous population of nematode-trapping fungi and it would be difficult to increase the population by adding spores of these fungi.

Cooke (1964) divided the nematode-trapping fungi into two broad groups an insensitive group (network forming) and sensitive groups (branch, knob and ring formers). He concluded that nematode-trapping habit of these fungi was adopted to ease or escape competition for substrates during decomposition of organic matter in the soil.

Cooke (1961) reported that decomposition of sucrose in soil stimulates the population of free living nematodes and activity of nematode-trapping fungi. He observed that decomposition of sucrose in soil reaches a certain stage at which fungi escape to trap the nematode present in the soil. Further increase in sucrose content in the soil stimulates the increased population of free living nematodes but resulted in decreased predacious activity of fungi.

Cooke (1962) observed that nematode-trapping activity of different nematode-trapping fungi introduced into natural soil varies in both intensity and duration. He stated that the nematode-trapping fungi forming constricting rings are very much effective in reducing nematode population in the soil than nematode-trapping fungi forming adhesive traps.

Persmark and Nordbring-Hertz (1997) examined that nematode-trapping fungi which form conidial traps in the vicinity of soil varied among different species, which was greatest in *A. dactyloides* and *M. gephyrophagum* followed by *A. superba* and *A. oligospora*. He also observed that pre incubation of soil or soil extract at room temperature before inoculation of spores increase the conidial trap formation. Reduction in the number of microorganism in soil extracts by sterile filtration, dilution or heating decreased conidial trap formation. The observation suggested that the level of microbial competition for nutrients is necessary for conidial trap formation.

### **Biological control of plant parasitic nematode by nematode-trapping fungi**

Biological control of nematodes by nematode-trapping fungi was reviewed by many

workers (Duddington, 1962; Mankau, 1980 and Siddiqi and Mahmood, 1997). Linford (1937) and Linford *et al.* (1938) carried out classical work on biological control of nematode by adding chopped green pineapple tops to nematode infested soil in pots. They estimated the nematode population and activity of nematode- trapping fungi following the addition of pineapple tops and noted a marked increase in the number of free living nematodes in the soil. The increased population of nematode stimulated the population of predacious fungi that killed nematodes bringing them below the original level.

Linford and Yap (1938, 1939) observed that out of five species of nematode-trapping fungi incorporated in the soil, only few gave minor control of nematodes. However, when nematode- trapping fungi were incorporated with organic matter, they showed better effect. Ali (1990) evaluated the effect of *A. oligospora* and organic amendment by pigeon droppings in green house experiment for the biological control of *M. incognita* on tomato. He observed that when the fungus was inoculated two weeks prior to transplanting and nematode inoculation, it gave 72% reduction in the number of root knots.

Slepetiene *et al.* (1993) observed that preparation of *A. oligospora* was 46.5-81.9% more effective than the use of nematicides against root knot caused by *Meloidogyne* sp. Colombo *et al.* (1995) tested a commercial formulation containing mycelium and viable spores of the nematode- trapping fungi *A. oligospora* and *A. superba* against root knot disease of brinjal in an unheated plastic house prior to planting and at fortnight intervals until harvest. The formulation was effective in reduction of second stage juveniles in the soil during the growing season, but could not reduce the knots on roots as compared to treatment with Fenamiphos. However, both treatments significantly reduced the nematode population.

Arndt (1994) observed that inoculation of the substrate with the *A. oligospora* and *A. dactyloides* reduced the population of *M. incognita* in tomato plant. In another experiment he observed that *M. incognita* was reduced up to 80% by the mite *Sancassonia ultima*. Stirling *et al.* (1998) observed that when granules of *A. dactyloides* and were placed on a glass slide in field soil, hyphae of the fungus proliferated from the granules and always produced traps. They concluded that formulations of the *A. dactyloides* consistently reduced the numbers of *M. javanica* juveniles by more than 90%. They also observed that the field soils which were treated with granules (10g/litre) and planted to tomatoes, the number of galls induced by the

nematode was reduced by 57-96%.

Stirling and Smith (1998) suggested that formulations of *V. chlamydosporium* did not reduce galling while the formulations of *A. dactyloides* applied at 220-440 kg/ha substantially reduced the number of nematodes present in roots 4-8 weeks after planting. Such formulations could be useful in nematode control, particularly if used in conjunction with other control measures.

Singh et al., 2012 reported that co-inoculation of *D. brochopaga* Dp-5 and *M. eudermatum* Mv-1 significantly reduced root-knot disease in tomato (89.63%) and increased the accumulation of total chlorophyll (125.34, 140.53 and 152.67 mg g<sup>-1</sup> fresh wt.), total phenolic compounds (TPC) (37.40, 48.32 and 59.63 µg of gallic acid equivalent), and phenylalanine ammonia lyase (PAL) activity (58.45, 69.05 and 74.57 mM cinnamic acid h<sup>-1</sup> g<sup>-1</sup> fresh wt.) after 10, 20 and 30 days of inoculation, respectively, in the greenhouse. However, in the field experiments, the total chlorophyll content in leaves of *M. incognita* infected tomato plants co-inoculated with *D. brochopaga* Dp-5 and *M. eudermatum* Mv-1 (152.70 mg g<sup>-1</sup> fresh wt.) was higher than the plants treated with *D. brochopaga* Dp-5 or *M. eudermatum* Mv-1 individually (130.87 and 144.73 mg g<sup>-1</sup> fresh wt., respectively) and *M. incognita* treated plants (19.47 mg g<sup>-1</sup> fresh wt.). TPC and PAL activity were found to be higher in tomato leaves co-inoculated with *D. brochopaga* Dp-5 and *M. eudermatum* Mv-1 (62.90 µg of gallic acid equivalent and 79.33 mM cinnamic acid h<sup>-1</sup> g<sup>-1</sup> fresh wt., respectively) than the plants treated with *D. brochopaga* Dp-5 or *M. eudermatum* Mv-1 individually, whereas in *M. incognita* inoculated and control plants, the induction of these two compounds was found to be very low after 30 days of inoculation. The results of this study showed that these fungi reduced root-knot disease and enhanced accumulation of defence related biomolecules in tomato plants and has potential to serve as a biocontrol agents against root-knot nematodes.

#### **Resesrch on effect of Plant Protection chemicals on Nematode- trapping fungi.**

Dibs (1976), Cayrol and Frankowski (1980) and Cayrol, (1983) studied several fungicides and herbicides (including PCNB) at the concentrations similar to be applied on aerial parts of the plants and found that most of the chemicals did not kill *A. irregularis* and *A. oligospora* but slightly retarded the growth. Benomyl was found to suppress the predacious activity of *A. oligospora*.

Crump and Kerry (1986) tested 25 agrochemicals to observe the compatibility with *Verticillium chlamydosporium* and *Cylindrocarpon destructans* at the concentration to be reached in soil and found that the most of the fungicides inhibited growth of these fungi even at lower concentrations except sulphur. They found little effect of insecticides and herbicides on the fungal growth. In nematicides, Oxamyl had major effect on fungal growth decreasing at all concentrations.

Grewal and Sahi (1988) reported that *A. conoides* was less sensitive to different pesticides than *A. oligospora*. Bavistin, BHC, Carbofuron and 60-80 ppm of Diazinon and Dichlorvos inhibited mycelial growth in both species.

Woodward *et al.* (2000) studied the *in vitro* tolerance of the nematophagous fungus *Arthrobotrys oligospora*, to the fungicides chlorothalonil and myclobutanil used to manage diseases on putting greens; the concentration of fungicides obtained from simulated putting green soil; and the ability of the fungus to reduce populations of the ring nematode, *Criconebella ornata*. Both fungicides reduced *in vitro* hyphal growth and germination of conidia above 10 mg kg<sup>-1</sup>. Soil concentrations of chlorothalonil were less than 5 mg kg<sup>-1</sup> and concentrations of myclobutanil were below detection limits. Nematode populations were not affected by *A. oligospora* in simulated greens but nematode populations were lowest in pots inoculated with *A. oligospora* and receiving fungicide treatments. Results of these studies indicate that applications of chlorothalonil and myclobutanil used to manage fungal diseases of bentgrass may not adversely affect *A. oligospora*; however, the fungus may not reduce nematode populations below desired thresholds.

Kumar *et al.* (2015) reported that the conidia of *A. dactyloides* exposed to agricultural soils showed poor germination but formed conidial traps, which captured and killed the soil nematodes. Conidial traps, which trapped the nematodes, grew well in all soils after killing and nutrient absorption from nematode body. Soil amended with 20 mg ai kg<sup>-1</sup> of carbendazim and thiram, 30 mg ai kg<sup>-1</sup> of mancozeb, 50 mg ai kg<sup>-1</sup> of captan, and 100 mg ai kg<sup>-1</sup> of carboxin completely checked the conidial trap formation and nematode capturing. 30, 50 and 100 mg ai kg<sup>-1</sup> of metalaxyl adversely affected the conidial trap formation and nematode capturing in soil. Propiconazole inhibited 15.2% conidial trap formation up to 50 mg ai kg<sup>-1</sup> but caused 93.3% inhibition of conidial traps formation and complete inhibition

of nematode capturing at 100 mg ai kg<sup>-1</sup>. Sulphur, triademefon, and tricyclazole showed least toxic effect on conidial trap formation and nematode capturing activities of *A. dactyloides* in soil up to 100 mg ai kg<sup>-1</sup>.

## MATERIALS AND METHODS

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### **Preparation of Corn Meal Agar:-**

For isolation, purification and maintenance of nematode- trapping fungi, corn meal agar (CMA) medium (Splitted corn grains: 20 g, Agar agar:20 g. and Distilled water: 1000 ml) was used. Twenty grams of broken grains of corn was boiled in distilled water and extract was collected by filtering through muslin cloth. Agar agar powder 20g was dissolved in corn extract and the final volume was made up to 1000 ml. with distilled water. The prepared medium was sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 min and preserved for future use.

### **Preparation of Rabbit Dung Agar:-**

For culture and maintainance of saprophytic nematodes, rabbit dung agar (RDA) medium (Rabbit dung: 100 g., Agar agar:20 g. and Distilled water: 1000 ml) was used. One hundred gram of rabbit dung was soaked in distilled water for 24 hrs and the extract was filtered through muslin cloth. Agar agar powder 20 g was dissolved in the rabbit dung extract which is heated for complete dissolving of agar agrar, and the final volume was made up to 1000 ml. with distilled water. Later, it was sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 min and preserved for future use.

### **Preparation of Corn meal agar (0.4%)**

For interaction of *D. brochopaga* and *Meloidogyne incognita* in *in vitro* conditions, corn meal agar medium of 0.4% (Splitted corn grains: 4 g., Agar agar:4 g. and Distilled water: 1000 ml.) was used. Broken grains of corn was boiled in distilled water and extract was filtered. Agar agar powder 4g was dissolved in extract. Medium was sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 min.

### **Isolation of *Drechslerella brochopaga***

Isolation of *Drechslerella brochopaga* was done by the method described by Duddington (1955). 500 g. of soil samples were collected in separate polyethylene bags from the top profile of soil from different locations of Banda (Table-1). Sterilized corn meal agar medium and Rabitt dung agar medium was poured into sterile Petri dishes to cover nearly 2/3<sup>rd</sup> and remaining area respectively. Each soil sample was thoroughly mixed before use for soil

plating. One gram of each soil sample was sprinkled over the poured medium into Petri dishes. For each soil sample, five Petri dishes were used as replicates. The Petri dishes were incubated at temperature  $25\pm 2^{\circ}\text{C}$ . Incubated Petri dishes were observed for the occurrence of carnivorous fungi under stereoscopic binocular microscope. Nematode trapping was observed and spores formed on the single conidial heads near the trapped nematodes were picked under stereoscopic binocular microscope with a sterilized fine needle and transferred into the corn meal agar medium separately for isolation of single species of carnivorous fungi. The spore inoculated Petri dishes were incubated at  $25\pm 1^{\circ}\text{C}$  for growth and sporulation. After 10 days of incubation, spores of each fungus were again transferred into Petri dishes containing corn meal agar medium. Purification of different *D. brochopaga* was made by single spore isolation (Singh et al., 2004). Pure cultures of *D. brochopaga* were maintained on corn meal agar medium at  $28\pm 1^{\circ}\text{C}$ .

**Calibration of ocular micrometer for different objectives for measurement of fungal structures of *D. brochopaga***

For calibration of ocular micrometer, a bright-field microscope, an ocular micrometer scale, a stage micrometer and oil-immersion was used. Ocular micrometer glass disc was inserted on the metal diaphragm and eye piece was inserted in the microscope. The stage micrometer (one division = 0.01 mm) was placed on the stage. Fine graduations of stage micrometer were observed under a sharp focus under low-power objective, high-power and oil-immersion objectives. The lines of the ocular micrometer parallel with those of the stage micrometer were aligned by fine rotation the stage micrometer and ocular micrometer and ocular micrometer lines coincide with stage micrometer at the left end and another line coincides in right (completely parallel at both ends) was observed. Number of divisions of ocular and stage micrometers between the two coinciding lines was counted. 5 readings were taken with the high-power (20 and 40 $\times$ ) and oil-immersion objectives (100 $\times$ ). These observations are then used to calculate the calibration factor for the objective lens in use.

$$\text{One ocular division (in } \mu\text{m)} = \frac{\text{Number of divisions on ocular micrometer}}{\text{No. of divisions on stage micrometer}} \times 10$$

After calibration, the ocular micrometer was used to measure the size of hyphae, conidiophores, conidia etc. in terms of length, breadth and diameter by following formula.

Size of microorganism ( in micron) = Number of ocular micrometer divisions occupied x  
Calibration factor for one ocular division  
(for the objective lens used)

#### **Identification and characterization of *D. brochopaga***

For identification of *D. brochopaga*, size of conidia, conidiophore, hyphae and trapping structures were measured by a calibrated ocular micrometer at high magnification and compared with the original descriptions of nematode- trapping fungi given by Drechsler (1937), Drechsler (1950), Drechsler (1952), Drechsler (1954) and Cook and Godfrey (1964).

#### **Assay of nematophagous ability of *D. brochopaga* against *Meloidogyne incognita***

Interaction between *D. brochopaga* and soil nematodes was done by the method described by Belder and Jansen (1994). In order to study the interaction between *D. brochopaga* and *Meloidogyne incognita* in dual culture, 5 mm fungal disc of each species was taken from the periphery of 15day old culture and inoculated into each of several Petri dishes containing corn meal agar medium (0.4 % corn and agar) (2mm thickness) in 50 mm Petri dishes. Inoculated Petri dishes were incubated at  $28\pm 1^{\circ}\text{C}$  in dark condition. After 8 days of incubation, fungal discs were removed aseptically. A drop of sterile distilled water containing thoroughly rinsed 500 second stage juveniles of *Meloidogyne incognita* was inoculated with the help of a dropper into full grown cultures of each fungal species. Petri dishes were incubated at  $25\pm 1^{\circ}\text{C}$  for observation. Five replications were maintained for each treatment and the experiments were repeated thrice. Observations on induction of constricting rings in  $2.25\text{ cm}^2$  fungal growth area in response to second stage juveniles of *M. incognita* were noted daily up to 3 days under a research microscope. Data on number of traps in  $2.25\text{ cm}^2$  areas were taken from center, middle and periphery of the fungal growth after nematode inoculation and average number of traps formed on the surface or deep into the medium were also made. Observations on number of captured nematodes were recorded daily for 3- 6 days of each fungal species and percentage of captured nematodes was calculated. Five Petri dishes were used as replicates. The interaction experiment was repeated twice and pooled data were analyzed using two ways factorial C.R.D.

### **Assay of spore germination of *D. brochopaga* in soil**

For assay of germination and nematophagous ability of *D. brochopaga* in diverse condition of agricultural soil, method described by the Jackson (1958) was followed. 500 g soil sample was collected from the top profile of soil from of various farms of Banda University of Agriculture and Technology and various villages of Banda. Each soil sample was passed through a 2 mm sieve and thoroughly mixed before filling in Petri dishes. 50-60 g soil of each sample was placed in 90 mm Petri dishes. The soil was watered near full water holding capacity by addition of distilled water. Water agar blocks (10 mm size, 3 mm thickness) were placed directly on the soil surface. Five agar blocks were placed in a Petri dish at equal distance for inoculation of spores of four species of nematode trapping fungi on each water agar block separately. The Petri dishes were incubated at room temperature (25-30 °C) for 24 hours to allow the diffusates to reach on the agar block. Conidial suspension of *D. brochopaga* was prepared by harvesting the spores of each isolate by a fine needle. A large number of spores of *D. brochopaga* were picked from a pure culture and put into a cavity block containing 0.5 ml of sterilized water. A small drop of spore suspension containing nearly 50-75 spores was placed on each water agar block and inoculated Petri dishes were incubated at room temperature (25-30°C) for 24 hours for observation. Spore inoculated water agar block kept on clean slides in a moist chamber was treated as control. Agar blocks were removed from the soil by fine forceps and placed on the clean glass slides for observation. The base of the agar block was rinsed by distilled water to remove the soil material from the base of agar block. The number of spores, germinated spores was counted. Five replications were taken for each treatment and percentage was calculated.

### **Effect of different concentration of fungicides on germination and conidial trap formation of *D. brochopaga* in close vicinity of soil.**

Germination/ conidial trap formation and trapping of soil nematodes by conidial traps of *D. brochopaga* in vicinity of soil having different concentrations of fungicides were studied by the method described by Jackson (1958). Garden soil free of any fungicides and having good number soil living nematodes was used for this experiment. Soil samples were passed through a 2 mm sieve and thoroughly mixed to homogenize the nematode population. A series of concentrations viz, 1,10,25,50, 100. 200, 300 and 400 mg ai kg<sup>-1</sup> of ten fungicides

(Table-5) namely carbendazim (Bavistin 50 WP), thiram (Thiram 75 DS), mancozeb (Dithan M 45 75 WP), sulphur (Sulfex 80 WP), metalaxyl (Tagron 35 WS), Copper oxychloride (Blitox 50), copper sulphate, sulphur (Sulfex 80 WP), tebuconazole (Folicur 25.9 %), chlorothalonil (Kavach 75%), propineb (Antrocol 70%) were prepared in 15 ml of sterilized distilled water on active ingredient basis and soil in each Petri dishes were drenched by solution of each fungicides. Five agar discs (10 mm size, 2 mm thickness) were placed over the soil containing each concentration of fungicides at equal distance and were incubated at  $25\pm 1^{\circ}\text{C}$  for 24 h to allow soil diffusates and fungicides to reach into agar discs. Conidial suspension of *D. brochopaga* was made as described above. 10 $\mu\text{l}$  suspension containing 50-60 conidia was inoculated on each agar disc and Petri dishes were then incubated at  $25\pm 1^{\circ}\text{C}$  for 48 hours for observations. Conidia inoculated on agar discs placed over the soil without any fungicides served as control. Agar discs placed over the soil were removed after incubation and placed on the clean glass slide for observations. Germination, conidial trap formation and trap formation on small germ tube on water agar discs placed over soil amended with different concentrations of fungicides were observed. Germination of on water agar discs placed over soil without fungicidal treatment (control) was also recorded. Five agar discs placed over soil per Petri dish were considered as one replicate. Ten replications were taken for each treatment.

## EXPERIMENTAL FINDINGS

### Isolation and characterization of nematode – trapping fungi

Sprinkling of pinch of agricultural and horticultural soil over the surface of agar plate containing corn meal agar and rabbit dung agar medium resulted in the occurrence of some fungi that trap and parasitize the nematodes. Four species of nematode- trapping fungi were appeared in agar plates after capturing of nematodes by forming different type of trapping organs (Table-1).

**Table-1 Species of nematode- trapping fungi isolated from soil of different location of Banda, Uttar Pradesh.**

S. No.	Isolated species of Nematode-Trapping fungi	Locations of soil samples collected for isolation of nematode- trapping fungi.
1.	<i>Arthrobotrys oligospora</i>	Horticulture farm BU A T, Banda
2.	<i>Dactylellina gephyropaga</i>	Horticulture farm BU A T, Banda
3.	<i>Arthrobotrys eudermata</i>	Horticulture farm BU A T, Banda
4.	<i>Drechlerella brochopaga</i>	Aonla orchard Baberu, Banda and Mango orchard Banda.

Morphology of spores, conidiophores, hyphae and trapping organs were studied and isolated nematode -trapping fungi were identified as *Dactylellina gephyropaga*, *Drechlerella brochopaga*, *Arthrobotrys oligospora* and *Arthrobotrys eudermata*. These fungi were critically identified by using original description of these fungi given by Drechsler (1937), Drechsler (1950), Drechsler (1952) and Drechsler (1954). The detail morphological characteristics of the *Drechlerella brochopaga* studied during the present investigation are as follows:

#### ***Drechlerella brochopaga***

*Drechlerella brochopaga* was first reported by Charles Dreshsler in 1937 as *Dactylaria brochopaga*. *Drechlerella brochopaga* (Drechsler) is the current name of this fungus. The mycelium of *Drechlerella brochopaga* on corn meal agar medium was spreading, hyphae hyaline, septate, 1.9- 4.6µm wide. Conidiophores hyaline, septate, erect,

70-375µm, more typically 200- 350 µm high, 4- 7µm wide at the base, tapering gradually upward to a width of 2.4- 3.5µm near the tip, there bearing on short blunt sterigmata 3- 12, mostly 3- 8 conidia in beautiful radiating capitata arrangement- or less often and less typically producing up to 14 conidia in more scattered, irregularly racemose arrangement. Conidia hyaline, straight or slightly curved, cylindrical or elongate ellipsoidal, broadly rounded at the apex, usually tapering noticeably toward the somewhat truncate base, 30- 46µm long, 5- 9µm wide and containing 3 cross walls into 4 cells. (Figure-1,2 and 3) In presence of nematodes, the hyphae of the fungus developed constricting ring and captured the nematodes (Table-2, Figure-5). Constricting rings produced on the conidia and on the hyphae were 20 to 34 µm in outside diameter and composed of three arcuate cells.

**Table-2. Morphological characteristics of *D. brochopaga* used in the present study**

<b>Characters</b>	: <i>D. brochopaga</i>
<b>Conidial shape and features</b>	: Hyaline, straight or slightly curved, cylindrical or elongate, broadly rounded at the apex.
<b>Conidial size</b>	: 30-46µm ×5 - 9µm
<b>Conidiophore length</b>	: 70- 375µm (Average-200- 350)
<b>Conidiophore width at base</b>	: 4- 7µm
<b>Conidiophore width at tip</b>	: 2.4- 3.5µm
<b>Trapping devices</b>	: Constricting ring

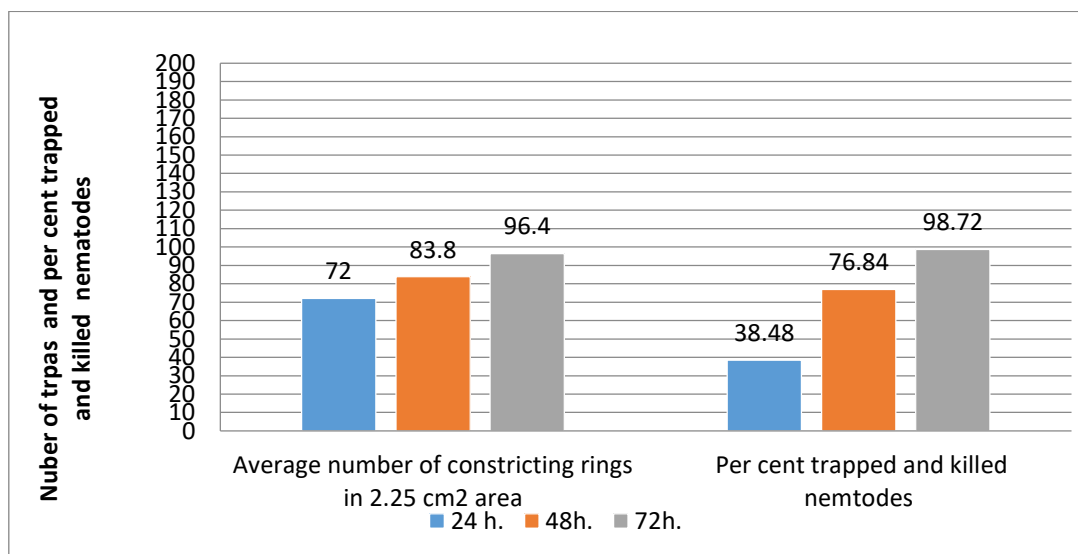
**Assessment of trap formation and carnivorous efficacy of fungi against *M. incognita***

Inoculation of 500 freshly hatched second stage juveniles of *M. incognita* into pure culture of different carnivorous fungi in CMA (0.4%) resulted in induction of constricting rings within 24 h. of nematode addition (Fig. 5 a). Number of constricting rings were increased with the passage of time. Higher number of trap formation was observed in *D. brochopaga* on 3<sup>rd</sup> day of nematode- trapping per 2.25 cm<sup>2</sup> area of fungal growth (98.72). These juvenile were freely moved in the media and captured by constricting rings of *D. brochopaga* (Figure-5). The trapped nematodes were digested completely after 48 hours from trapping. *D. brochopaga* trapped and killed 96.4 % J<sub>2</sub> of *M. incognita* within 72. Nematodes

were trapped at head and tail reason by constricting rings of the *D. brochopaga*. After trapping, three cells of constricting rings swelled and tightly trapped the nematodes. (Table-3 Line figure-1). Results revealed that trap formation and trapping of nematodes had a strong relation for the nematode trapping ability of *D. brochopaga*. The ability of production of higher number of constricting rings in *D. brochopaga* may be attributed to the response of this fungus to the morphogenic substances produced by the nematodes. Other possibility may be the genetic makeup of this fungus which make them able to be more efficient nematode trapper by formation of abundant trap formation and trapping of nematodes. Singh et al. (2007) also reported that constricting ring forming fungi are more carnivorous than adhesive trap forming species.

<b>Table-3 Assessment of <i>in vitro</i> nematode- trapping ability of <i>Drechslerella brochopaga</i> against second stage juvenile of <i>M. incognita</i> in corn meal agar medium (0.4%)</b>			
<b>Observations</b>	<b>Days of observations after inoculation of 500J<sub>2</sub> of <i>M. incognita</i></b>		
	<b>24h.</b>	<b>48h.</b>	<b>96h.</b>
<b>Average number of constricting rings (2.25 cm<sup>2</sup>)</b>	72 ± 4.7 <sup>a</sup>	83.8 ± 3.7 <sup>b</sup>	96.4 ± 2.4 <sup>c</sup>
<b>Per cent trapped juvenile (J<sub>2</sub>) of <i>M. incognita</i></b>	38.48 ± 1.4 <sup>a</sup>	76.84 ± 3.2 <sup>b</sup>	98.72 ± 0.7 <sup>c</sup>

Data are the mean of five replicates. Data superscript with different letters represents the significant difference in row data at p<0.05%



**Line figure-2.** Assessment of *in vitro* nematode- trapping ability of *D. brochopaga* against *M. incognita* in corn meal agar medium (0.4%). Data are significantly difference in row data at  $p < 0.05\%$

### Effect of various agricultural and horticultural soils on conidial trap formation of *D. brochopaga*

Conidia of *D. brochopaga* (Fig.4) placed on to water agar discs not in contact with soil had an average germination of 94.32 % by germ tube whereas conidia exposed to agricultural and horticultural soils of Banda had poor germination (1.57-16.6%) with no further growth. Those conidia, which did not germinate on agar discs placed over soil often, produced traps within 10-12 h. either directly on conidium or on short germ tube with no hyphal extension known as conidial traps. (Fig. 6). Irrespective of various agricultural and horticultural soils, conidial trap formation ranged from 59.03 to 96.31%. (Table-4). No hyphae were formed from conidia bearing traps in the vicinity of soil. Further, no lysis of conidial traps was observed. On water agar discs placed over various soils of Banda, nematodes were usually attracted towards the conidial traps and were captured and paralyzed (Fig.7 to 9). The number of trapped and paralyzed nematodes on the water agar discs placed over each soil sample varied (0.0 to 51.0). The maximum nematode capturing and paralysis was recorded on agar discs placed over Aonla orchard soil, Banda. It was interesting to note that after trapping which resulted in paralysis of nematodes, the cells of a spore produced hyphae (Fig. 8 to 9) on which constricting rings were formed (Fig. 9) which are able to trap and kill other nearby nematodes however, at later stages. The infection hyphae developing from any of the

constricting ring cells penetrated the trapped nematode body through the damaged cuticle. Later on, the fungus grew within the nematode and consumed the whole content of the body. The fungus had developed throughout the bodies of nematodes and also grown on the agar discs with constricting ring formed on the external hyphae. It was also observed that conidial traps, which did not capture the nematodes failed to grow and proliferate in soil due to fungistatic effect of soil. It appears that *D. brochopaga* is a poor saprobe but survive in carnivorous phase by capturing and killing of the nematodes in fungistatic soil environment provided, the soil has good number of nematodes. The formation of hyphae with induction of constricting rings after trapping, paralysis and extraction of nutrients from immobilized nematodes indicates that the *D. brochopaga* can grow and multiply in carnivorous phase until the nematodes in soil are available as a food source for this fungus. Quick formation of conidial traps and trapping of the nematodes in soil by conidial traps indicates that delivery of conidial-based formulation in soil effectively reduce the population of plant - parasitic nematodes.

**Effect of various concentrations of fungicides in soils on conidial trap formation of *D. brochopaga***

The observations on the toxic effect of various fungicides used in soil showed higher sensitivity *D. brochopaga* to chlorothalonil and propineb which inhibited the conidial trap formation by 24.89% and 16.54% respectively and completely inhibited the conidial trap formation at 10 mg ai kg<sup>-1</sup> or greater concentration. Carbendazim, thiram, mancozeb, copper oxychloride and copper sulphate completely inhibited the formation of conidial traps at 25 mg ai kg<sup>-1</sup> or at greater concentrations. Tebuconazole inhibited the 22.75 % conidial trap formation at 50 µl ai kg<sup>-1</sup> but completely inhibited the fungus at 100 µl ai kg<sup>-1</sup>. Metalaxyl and Sulphur caused only 19.37 and 7.80 % inhibition in conidial trap formation respectively at 200 µg ai kg<sup>-1</sup> but completely inhibited the fungus at 300 µg ai kg<sup>-1</sup>. Tolerance of *D. brochopaga* against Propiconazole was found up to 300 mg ai kg<sup>-1</sup> but the fungus was completely inhibited at 400 mg ai kg<sup>-1</sup>. *D. brochopaga* was found to be less sensitive to sulphur, propiconazole and metalaxyl up to 100 mg ai kg<sup>-1</sup> concentration which showed that this fungus can be used with these fungicides at up to 100 µg ai kg<sup>-1</sup> concentrations in integrated pest management programme.

**Table No. 4 Germination, conidial trap formation and trapped nematodes by conidial traps of *D. brochopaga* in agriculture soils of different location of Banda**

<b>Different type of Soil</b>	<b>Conidial traps and traps on germ tube (%)</b>	<b>Germ tube without traps (%)</b>	<b>Non germinated spores (%)</b>	<b>Average number of trapped nematodes on agar block</b>
Hardauli village, Banda	83.55	9.79	6.66	43.00
Pachnehi village, Banda	77.04	10.40	12.56	30.00
Yellow soil palahari, Banda	59.51	16.66	23.42	8.00
Aonla orchard Soil, Banda	93.81	2.53	3.66	51.00
Guava Rhizosphoric Soil, BUAT, Banda	95.82	1.57	2.61	37.00
Fallow Soil BUAT, Banda (Yellow)	59.03	14.06	26.91	0.00
Soil beneath Neem tree, BUAT, Banda	96.31	1.42	2.27	38.00
Mango orchard soil Palahari village , Banda	95.08	2.10	2.81	43.00
BUAT Farm Soil	89.74	3.28	6.98	21.00
Chilehta Village Soil , Banda	89.66	3.46	6.88	39.00
Water Agar Control (without soil )	0.00	94.32	5.68	0.00
C.D. at 5%	1.517	1.651	1.65	-

**Table: 5 Details of fungicides used in the present study**

<b>Trade name</b>	<b>Component</b>	<b>Chemical name</b>	<b>Mode of action</b>
Dithan M 45, 75 WP	Mancozeb	Manganese ethylenebis dithiocarbamate	Inactivates SH groups in amino acids.
Bavistin 50 WP	Carbendazim	Methyl 2 benzimidazole carbamate (MBC)	Inhibiting the biosynthetic process
Thiram 75 DS	Thiram	Tetramethyl thiuram disulphide	Interferes with oxygen uptake and inhibition of sulphur containing enzymes
Blitox 50	Copperoxy chloride (COC)	Dicopper chloride trihydroxide	Kills spores by combining with the sulpha hydryl groups of certain enzymes.
-	Copper Sulphate	N – (2,6 – Dimethyl phenyl)- N-(Methoxyacetyl) alanine methyl ester.	Inhibition of RNA synthesis.
Sulfex 80 WP	Sulphur	Sulphur	Disturb normal hydrogenation and de hydrogenation.
Tilt	Propiconazole	1-(2-(2, 4 dichlorophenyl)- 4 propyl- 1,3 – dioxolan- 2yl methyl)- 1 H -1,2,4 – triazole.	Inhibition of ergosterol biosynthesis.
Ridomet 35% WS	Metalaxyl	N – (2, 6 – Dimethyl phenyl)- N-(Methoxyacetyl) alanine methyl ester.	Inhibition of RNA synthesis.
Folicur 25.9 % w/w	Tebuconazole	(±)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1H, 1,2,4-triazol-1-ylmethyl)pentan-3-ol	Demethylase inhibitors
Kavach	Chlorothalonil	2,4,5,6-tetrachloroisophthalonitrile	Inhibiting a number of enzymes required for metabolic pathway.
Antracol 70% WP	Propineb	Zinc-1-methylethylene isdithiocarbamate	Inhibitory effect on nitrogen metabolism.

**Table No. 6 Effect of different concentrations of some common fungicides in soil on per cent conidial trap formation of *D. brochopaga***

Data in parenthesis in CT is per cent inhibition in conidial trap formation in comparison to conidial trap formation in control

Fungicides	1mg/μl ai kg <sup>-1</sup>	10 mg/μl ai kg <sup>-1</sup>	25 mg/μl ai kg <sup>-1</sup>	50 mg/μl ai kg <sup>-1</sup>	100 mg/μl ai kg <sup>-1</sup>	200 mg/μl ai kg <sup>-1</sup>	300mg/μl ai kg-1	400 mg /μl ai kg-1
<b>Mencozeb</b>	96.06 (1.61)	94.57 (3.54)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>Carbendazim</b>	96.03 (1.40)	91.23 (7.10)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>Thiram</b>	91.53 (3.30)	71.87 (26.23)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>COC</b>	93.53 (4.20)	41.99 (58.29)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>Copper sulphate</b>	95.24 (2.45)	92.71 (5.04)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>Sulphur</b>	95.79 (2.08)	94.64 (3.23)	94.27 (3.44)	92.81 (4.86)	91.87 (4.80)	88.60 (7.80)	0.00 (100)	0.00 (100)
<b>Propiconazole</b>	95.27 (2.61)	93.78 (3.62)	92.48 (4.12)	92.91 (4.23)	86.83 (8.52)	87.87 (9.83)	24.13 (75.63)	0.00 (100)
<b>Metalaxyl</b>	95.71 (2.20)	94.68 (3.66)	93.92 (2.92)	88.36 (8.58)	85.05 (10.72)	76.60 (19..37)	0.00 (100)	0.00 (100)
<b>Tebuconazole</b>	91.76 (6.10)	88.86 (8.48)	83.89 (13.23)	73.10 (22.75)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>Chlorothalonil</b>	70.94 (24.89)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>Propineb</b>	75.50 (16.54)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
<b>Control</b>	96.54	96.54	96.54	96.54	96.54	96.54	96.54	96.54
<b>C.D. at 5%</b>	1.411	1.352	0.892	1.042	0.961	0.743	1.068	0.282

(without fungicides).

## DISCUSSION

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The occurrence of *D. brochopaga* and three other species of carnivorous fungi during isolation from soil of Banda district indicates that these fungi are found in the soil of Bundelkhand. Wide distribution of nematode-trapping fungi have been reported by several workers across the world (Drechsler, 1937; Duddington, 1954; Gray, 1988). The critical examination of the shape and size of conidia and respective conidiophores of fungus used in present study was found similar to *Dactylaria brochopaga* as reported by Drechsler (1937) and Cook and Godfrey (1964). Hence, the nematode-trapping fungus studied during present study were identified as *Drechslerella brochopaga* Drechsler which is the current name of the *Dactylaria brochopaga*. The abundant production of constricting rings on hyphae in response to free living nematodes during isolation suggested that three factors might have worked either alone or concomitantly such as accumulation of morphogenic substance Nemin in higher concentration, temperature and absorption of nutrition by fungus after paralysis of nematodes body (Cruz et al., 2009; de Cruz et al., 2011).

Trapping of the nematodes by these fungi by forming three celled constricting ring also indicates that these fungus having efficient nematode trapping ability in nature (Fig. 5). The slow growths of *D. brochopaga* are attributed to its nature of the growth and the types of nutrients available in the media (Ananko and Teplyakova, 2011). The other media may be useful to increase the growth of this fungus for mass production and development of formulation for bio control purposes.

The formation of average number of constricting rings in 2.25 cm<sup>2</sup> area of hyphal growth of *D. brochopaga* after inoculation of *M. incognita* in corn meal agar medium (0.4%) may be attributed to its trap forming ability of this species. Formation of traps by *D. brochopaga* indicates the higher sensitivity of *D. brochopaga* to the trap inducing substances produced by *M. incognita* in dual culture. Similarly higher per cent of trapping of *M. incognita* by *D. brochopaga* is attributed to the higher number of traps induced within 24 h. of

interface between fungus and nematodes. The absence of traps in nematode free culture of *A. dactyloides* indicated that morphogenic substance 'Nemin' released from nematodes is essential for trap formation (Roubaud and Deschiens, 1939; Pramer and Stoll, 1959). Observation of trapping of nematode by *D. brochopaga* indicates the possibility of the use of this fungus as bio management agent for reduction of plant paraticie nematodes in fields.

Soil fungistasis is a widespread phenomenon occurring in all type of soils (Dobbs and Gash, 1965; Lockwood, 1977) and fungistatic intensity varies with the changing of physico-chemical properties of soil (Handelsman and Stabb, 1996; Mondal and Hyakumachi, 1998; Qian and Johnson, 1987.). In the present study, it was observed that conidia of *D. brochopaga* showed the frequent formation of conidial traps and traps on germ tube with out any further growth (Table-4, Fig.6). Formation of conidial traps more frequently by *D. brochopaga* in response to agricultural and horticulture soils of Banda revealed that the diffusates reached from soils to the agar discs resulted in conidial trap formation. Trap formation in nematode-trapping fungi in response to nematode metabolite 'Nemin' has been reported by several workers (Pramer and Stoll, 1959; Tarjan 1960; Feder *et al.*, 1960; Feder *et al.* 1963; Monoson *et al.*, 1974). It appears that soil contain enough 'Nemin' due to presence of nematodes which induced trap formation directly on the conidia and conidial greslings of *D. brochopaga* in close vicinity of the soil. Formation of conidial traps of *D. brochopaga*, with no further extension without trapping of nematodes is attributed to the strong carnivorous ability of these fungi in soil. Germination of carnivorous fungi in close vicinity to agricultural soil indicates that these fungi are most adoptive in the fungistatic environment of soil. However, Mankau (1962) had reported that the distinct tendency toward the trap- formation by conidia germinating under fungistatic conditions indicates that this activity in the soil may be a response to antagonisms caused by soil microorganisms. Further, Persmark and Nordbring Hertz (1997) reported that formation of conidial traps is a response to competition for nutrients with other microorganisms.

Germination of *D. brochopaga* by conidial traps without further extension of hyphae showed its ability to survive in the soil environment till the capturing of nematodes. Conidial trap formation were also reported in response to cow dung (Dackmam and Nordbring-Hertz, 1992), soil fungistasis (Mankau, 1962; Kumar *et al.*, 2015), rhizosphere soil or soil extracts

(Persmark and Nordbring- Hertz, 1997), and the formation of conidial trap formation was believed to be a response to nutrient deprivation due to strong nutrient competition between soil microorganisms. Barron (1992) also proposed that the parasitic habit by formation of trapping organs give the fungi a competitive advantage over many other fungal saprotrophs growing in soil environments that are characterized by low levels of nitrogen. Persmark and Nordbring-Hertz (1997) reported that *Arthrobotrys dactyloides* and *Monacrosporium gephyropagum* are the fungi most capable of forming a conidial trap. Dackman and Nordbring Hertz (1992) reported the formation of conidial traps as survival structure of nematode-trapping fungi in intense competitions in soil.

The observations of sensitivity and tolerance of *D. brochopaga* with common fungicides indicated that Chlorothalonil, propineb, Copper Oxychloride, Copper Sulphate, carbendazim, thiram and mancozeb were most toxic to *D. brochopaga*. This fungus showed good tolerance to sulphur, metalaxyl and propiconazole even at 200 ai mg kg<sup>-1</sup> concentration (Table-4). This variable toxicity may be attributed to the chemical nature and toxicity of fungicides against *D. brochopaga*. More or less similar observations on the toxic effect of pesticides have been reported on some other nematode-destroying fungi. Crump and Kerry (1986) found benomyl and carbendazim to be most toxic to nematophagous fungi *Verticillium chlamydosporium* and *Cylindrocarpon destructans* at the concentration to be reached in soil even at very low concentration except sulphur. Kapur et al. (1981) tested eight fungicides, including PCNB, Ethirimol and Benomyl, on soil containing nematophagous fungi reported that the equilibrium among various soil biota was altered. *Arthrobotrys oligospora* was little affected by any of the fungicides tested. Other species suffered initial reduction but recovered fully within two months, whereas others recovered partially. Grewal and Sahi (1988) reported that *A. conoides* was less sensitive to different pesticides than *A. oligospora*. Bavistin, BHC and 60-80 ppm of diazinon and dichlorvos inhibited mycelial growth of both the species. Wood ward and Crump (2000) tested tolerance of *A. oligospora* to the fungicide chlorothalonil *in vitro* and found that the growth of the fungus ceased at concentration above 10ppm. Woodward et al. (2005) tested the *in vitro* tolerance of *A. oligospora* against chlorothalonil and myclobutanil used to manage disease on putting greens and observed that both the fungicides reduced hyphal growth and germination of conidia above 10 mg ai kg<sup>-1</sup>.

Kumar et al. (2015) reported that soil amended with 20 mg ai kg<sup>-1</sup> of carbendazim and thiram, 30 mg ai kg<sup>-1</sup> of mancozeb, 50 mg ai kg<sup>-1</sup> of captan, and 100 mg ai kg<sup>-1</sup> of carboxin completely checked the conidial trap formation and nematode capturing. They found that 93.3% inhibition of conidial traps formation and complete inhibition of nematode capturing at 100 mg ai kg<sup>-1</sup> of propiconazole. they also reported that Sulphur, triademefon, and tricyclazole showed least toxic effect on conidial trap formation and nematode capturing activities of *A. dactyloides* in soil up to 100 mg ai kg<sup>-1</sup>.

The environmental side effects associated with plant protection chemicals use in agriculture and ban of many chemicals have spurred research in to nematode control alternatives. Nematode-trapping fungi has been reported to protects the plants against attack of various species of root-knot nematodes (Stirling et. al,1998; Stirling and smith 1998; Kumar and Singh, 2006; Singh et. al, 2007). A feasible method for using nematode-trapping fungi in integrated pest management programme could be the fungal spores, which might germinate and grow after being applied in complex and contaminated soil environment. Compatibility of bio-control fungi with fungicides is necessary to integrate the fungicides and bio-agents for minimization of fungicidal use in feasible management strategies. The result of this study showed the sensitivity of conidia of *D. brochopaga* to various fungicides applied to soil even at very low concentration except sulphur, propiconazole and metalaxyl. These findings were based on experiment in vicinity to soil to predict the similar toxic effect of various fungicides on the growth of *D. brochopaga* in the soil. Hence, fungicides toxic to conidial trap formation, germination and and nematode- trapping activies of *D. brochopaga* can not be integrated with this fungus in integrated pest management programme. Since most of the fungicides had inhibitory effect on *D. brochopaga* even at low concentrations, it shoue be used in fungicide free soil for better effacry of these fungi in soil of management of plant parasitic nematodes.

## SUMMARY AND CONCLUSION

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*Drechslerella brochopaga* Drechsler, a nematode-trapping fungus forming constricting rings for capturing and killing of nematodes were studied with special reference to their *in vitro* carnivorous ability, adoptability in agricultural soil and their sensitivity or tolerance to some common fungicides. *D. brochopaga* was isolated from the Aonla orchard of Baberu, Banda, Uttar Pradesh and Mango orchard of Banda. The fungus was purified, characterized and identified as *D. brochopaga*. Some other fungi nematode trapping fungi were also occurred with *D. brochopaga* which indicates that soil of these region harbours the species of nematode-trapping fungi. *In vitro* nematophagous potential of *D. brochopaga* was studied by inoculation of 500 juveniles of *M. incognita* in pure culture in CMA medium (0.4%). Trap formation and trapping of nematodes were observed within 24 h. of nematode inoculation. High number of trap formation in 2.25 cm<sup>2</sup> area of fungal hyphae and trapping of second stage juveniles was noted in *D. brochopaga*. The number of traps and per cent capturing of nematodes increased with the passage of time. *D. brochopaga* captured almost all (96%) the nematodes within 72 h. of nematode inoculation.

*D. brochopaga* were studied for their potential to grow and parasitize the nematodes in the agricultural and horticultural soil of Banda. It was found that these fungi grow and proliferate well in soil with little of higher fungistatic effect. Conidia of *D. brochopaga* germinated by formation of conidial traps on the conidia or on short germ tube and captured the nearby nematodes. 59.51 to 96.31 per cent of conidia were germinated with formation of conidial traps directly on conidium or on small conidial germ tube in vicinity to soil of Banda which indicates that soil of this region has various ability of fungistatic properties. Formation of traps on the conidia and germ tubes of *D. brochopaga* indicates that these fungus switch over to parasitic phase immediately after germination in close vicinity to agricultural soil. Trapping and killing of soil nematodes by conidial traps of *D. brochopaga* indicates that this fungus parasitize the nematodes and proliferate in the soil for regulation of nematode – population in nature.

Studies on sensitivity and tolerance of *D. brochopaga* with common fungicides was the main focus of this study. Our results indicate that chlorothalonil, propineb, copper oxychloride, copper sulphate, carbendazim, thiram and mancozeb were highly toxic to *D. brochopaga* germination and conidial trap formation of *D. brochopaga*. The tested fungus *D. brochopaga* was found tolerant to sulphur, metalaxyl and propiconazole even at 200 mg ai kg<sup>-1</sup> concentration (Table-4). *D. brochopaga* were found sensitive to all tested fungicides at 400 mg /  $\mu$ l ai kg<sup>-1</sup>

On the basis of present studies on “Studies on tolerance and sensitivity of nematode-trapping fungus *Drechslerella brochopaga* to common fungicides” following conclusions were made:

- Presence of *Drechslerella brochopaga* and some other nematode-trapping fungi in soil of Banda district indicates that these fungi are essential component of soil of Bundelkhand.
- Interface of *M. incognita* and *D. brochopaga* resulted in higher number of constricting rings on mycelium of *D. brochopaga*. As a consequence, the inoculated second stage juveniles of *M. incognita* trapped and killed at faster rate.
- The *D. brochopaga* is adoptive in active phase in agricultural and horticultural soil of Banda by formation of conidial traps or traps on small germ tubes of conidia. This indicated that *D. brochopaga* switch over to parasitic phase by forming conidial trap on the spore or spore germ tube in soil. The conidial traps are fully functional and captured the nearby nematodes for the active parasitic phase in soil.
- Formation of conidial traps by *D. brochopaga*, capturing of soil nematodes and growth of the hyphae on the agar disc placed in close vicinity to soil indicates *D. brochopaga* is better competitors in the diverse situation of soil due to their saprophagous and carnivorous ability.
- Excellent *In vitro* carnivorous efficacy of *D. brochopaga* and its conidial trap forming ability in close vicinity to soil indicates that the conidial trap forming trait of *D. brochopaga* could be harnessed for their potential use in the integrated disease management programme. Growth of the fungus from parasitized nematodes by conidial traps in vicinity to soil indicates that this fungus can be delivered in the

agricultural soil in form of spore based formulations for good control of Plant parasitic nematodes.

- Compatibility and sensitivity test of *D. brochopaga* with eleven common fungicides indicates that chlorothalonil and propineb were found most toxic to *D. brochopaga* at 10 mg ai kg<sup>-1</sup>.
- *D. brochopaga* was found completely inhibitory to 25 mg ai kg<sup>-1</sup> of mancozeb, carbendazim, copper oxychloride, thiram, copper sulfate and 100 mg ai kg<sup>-1</sup> of tebuconazole. *D. brochopaga* showed tolerance to metalaxyl, sulphur and propiconazole up to 200 ppm which indicated that these fungicides can be used with these fungicides below the 200 mg ai kg<sup>-1</sup> concentration.
- *D. brochopaga* is better competitors in the diverse situation of soil due to their conidial trap forming and nematode - trapping ability, however the fungus should be used in organic agriculture or fungicides free soil for better results of biological control of plant-parasitic nematodes.

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