**Original Research Article**

**Assessment of Germination, Trap Formation and Nematophagous Activities of Nematode-Trapping Fungi in Response to Soil Fungistasis**

**Abstract**

Soil Fungistasis in is a widespread phenomenon affecting germination of most fungal propagules in soils. This study focused on the isolation and identification of nematode-trapping fungi from soils in Uttar Pradesh, India. Five species of nematode-trapping fungi were isolated and identified: *Arthrobotrys oligospora, A. musiformis, A. conoides, Drechslerella brochopaga*, and *D. dactyloides.* These fungi were found to capture and kill nematodes under laboratory conditions. The study also evaluated the fungistatic effects of rhizospheric soils on spore germination, trap formation and nematode- trapping ability of these fungi. Results showed that the soils had a low fungistatic effect on conidial germination of *Arthrobotrys conoides, A. musiformis,* and *A. oligospora* ranging from 94.6% to 96.61%, whereas the conidia of *D. brochopaga* and *D. dactyloides* frequently formed conidial trapsat a higher frequency 90.09-95.77%, with trapping of nearby nematodes by the conidial traps. These nematode-trapping fungi have the ability to germinate, proliferate and trap the nematodes in soils, making them promising tools for biological control of plant-parasitic nematodes.

**Key words:** Nematode- trapping fungi, conidial trap, soil fungistasis, nematodes.

1. **Introduction:**

“Nematode-trapping fungi (NTFs) are a special group of microbes that play an important role in reduction of nematode population in soil by trapping them into adhesive and mechanical traps” (Yang et al., 2012; Meerupati et al., 2013; Andersson et al., 2014; Kumar, 2021). “Some species of nematode-trapping fungi are also known to parasitize the *Rhizoctonia solani”* (Kumar and Gouda, 2018). “Trap formation on hyphae or spores of NTFs are key morphological features of their lifestyle transition from saprophytic to nematophagous phase” (Kumar et al., 2005a; Kumar and Singh, 2006a; Kumar, 2024a). “Some species of NTFs form traps directly upon conidium or on spore germlings (conidial traps) after their inoculation in close vicinity of cow dung” (Deckman et al., 1992) and natural soil (Kumar et al., 2015, Kumar 2024b;). “The subsequent growth and survival of conidial traps in vicinity of soil depends on the capturing and killing of nematodes. Germination of conidia by formation of conidial traps in soil may provide these fungi an opportunity to capture and parasitize the nematodes for their food requirements. Soil fungistasis which inhibits germination of most fungal propagules can influence efficacy of bio-control fungi in soil infested with plant parasitic nematodes. Unfortunately, majority of bio-control fungi fail to germinate in soil due to soil fungistasis” (Zhou and Mo, 2002; Bae and Knudsen, 2000) “and thus reduce the biocontrol potential as expected. Potential use of nematode-trapping fungi to soil in the form of conidia-based formulation seems to the best effective method for large-scale application of such organisms” (Mankau, 1962), provided, these fungi must have better germination and trap formation in agricultural and horticultural soils. Hence, the germination of nematode-trapping fungi in natural soils need to be investigated to elucidate the fate of conidia applied in diverse situations of soil for bio-control purposes. Therefore, the present investigation was carried out to assess the germination, growth, and proliferation of conidia of nematode-trapping fungiin rhizospheric soils of agricultural and horticultural crops to know the fate of conidial bioinoculants applies into the soil for the biological control of plant parasitic nematodes.

**2. Materials and methods**

**2.1. Isolation and identification of fungal strains**

Isolation of the nematode-trapping fungi was performed as per the method given by Duddington (1955) with slight modifications (Singh et al., 2004; Singh et al., 2007). Soils were collected from various locations of Uttar Pradesh, India. Soil samples were collected and were double sealed to maintain soil moisture and brought to the laboratory for the isolation of nematode-trapping fungi. Soil was thoroughly mixed before use for soil plating. Sterilized corn meal agar medium (Corn-20 g, agar-20g and distilled water-1000 ml) cooled near the solidification was poured into several sterile Petri dishes to cover nearly 2/3rd area of a plate. After solidification of corn meal agar medium, melted, and cooled rabbit dung agar (rabbit dung pellets -50 g, agar-20g and distilled water-1000 ml) medium was poured into these Petri dishes to cover remaining area. One gram of each soil sample was sprinkled over the poured medium into Petri dishes and were incubated at room temperature (25-30 0C). The plates were incubated at 25±1◦C for trapping and appearance of conidial heads of nematode-trapping fungi. The conidia of nematode-trapping fungi produced on top of the conidiophores were picked by a fine sterile needle and transferred to fresh corn meal agar (CMA) medium plates (Kumar and Singh, 2006b). After growth of colony from the single conidia, the small bit of media containing hyphae (2mm) were transferred into the Petri plates containing CMA medium. The plates were incubated for 1-2 weeks for growth and sporulation. For identification of isolated nematode-trapping fungi, size of conidia, conidiophore, hyphae, and traps were measured and compared with the fungal descriptions given by Drechsler (1937), Cook and Godfrey (1964), Haard (1968), Barron (1977), Hyde et al., (2014). The culture of all species of NTFswere grown and conserved on CMA medium at 25 ±1°C.

**2.2. Assessment of fungistastic effect of rhizospheric soil on growth and trap formation of nematode–trapping fungi in soil.**

The fungistatic effects of rhizospheric soils on the spores of five species of nematode-trapping fungi were tested by the method described by Jackson (1958). Soil samples were collected from the rhizosphere of Okra, Wheat, Linseed, Rice, Chickpea, Pigeon pea, Cow pea, Tomato, Brinjal and mustard from various fields of Ghazipur, India. Each soil sample was passed through a 2 mm sieve and 50-60 g of thoroughly mixed soil of each sample was placed in 90 mm Petri dishes. Distilled water was added to the soil till its water holding capacity. Four agar discs (10mm size, 3 mm thickness) were placed directly on the soil surface in a Petri dish at an equal distance for inoculation of spores of *A. conoides*, *A. musiformis, A. oligospora, D. brochopaga,* and *D. dactyloides* on each water agar discs separately. The Petri dishes were incubated at 25 0C for 24 hours to allow the diffusates to reach on the agar discs. Conidial suspension of each species of nematode-trapping fungi was prepared by gently washing of agar surface by distilled water (Kumar et al., 2005b). 10μl spore suspension containing approximately 50-60 spores was inoculated on each water agar discs and inoculated Petri dishes were incubated at room temperature (25-300C) for 24 hours. Spore inoculated water agar discs kept on clean slides in a moist chamber was treated as control. Agar discs were removed from the soil by fine forceps and placed on the clean glass slides for observation. The base of the agar discs was rinsed with distilled water to remove the soil material adhered to the base of agar discs. The number of spores germinated, number of conoidal trap formation and number of trapped and killed nematodes were counted and calculated. Conidia were considered to have germinated if the conidial germ tube was at least half the length of conidia. There were ten replications for each treatment.

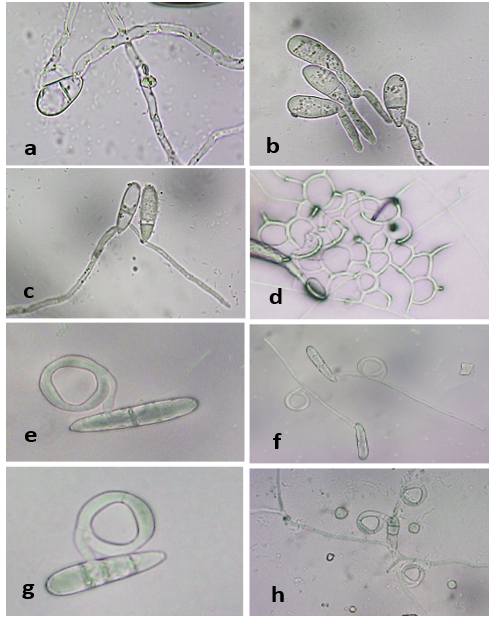
**3. Results**

**3.1. Isolation of nematode-trapping fungi**

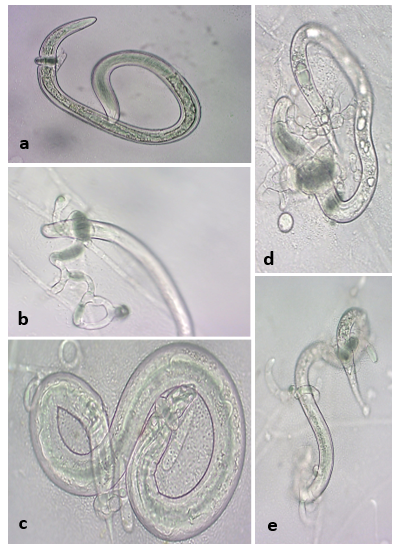
Five species of nematode- trapping fungi namely *Arthrobotrys oligospora*, *A.* *musiformis* and *A. conoidis* forming three-dimensional adhesive traps and *Drechslerella brochopaga* and *Drechslerella dactyloides* forming three-celled constricting rings were identified on the basis of the morphology of hyphae, type of trapping organs, shape and size of conidia and their respective conidiophores of nematode-trapping fungi isolated in the present study were found similar to the species. Hence, the isolated nematode-trapping fungi were identified as *Arthrobotrys musiformis*, *Arthrobotrys conoides*, *A. oligospora,* *D. brochopaga* and *D. dactyloides*. All these fungi were found to capture and kill the nematodes under laboratory conditions which indicates that these fungi are efficient nematode trappers in nature.

**3.2. Evaluation of growth of nematode-trapping fungi in a fungistatic environment of rhizospheric soil**

The conidia of different species of nematode-trapping fungi transferred on to water agar discs in contact with rhizospheric soils of different crops had very low fungistatic effect in comparison to conidia transferred on the agar discs not in contact with soils. Conidia of *A. conoides, A. musiformis* and *A. oligospora* placed on water agar discs had good germination by formation of germ tube (94.6-96.61) in vicinity to rhizospheric soils compared to conidial germination on water agar discs control without soil (98.39-99.30%). Conidia of *A. conoides, A. musiformis* and *A. oligospora* formed very low present of conidial traps in vicinity to rhizospheric soil (0.88-2.28%) of different crops (Table- 1). Contrary, conidia of *D. brochopaga and D. dactyloides* frequently formed conidial trapsin a higher frequency (90.09-95.77%), with good number of free-living nematodes trapped by the conidial traps.The conidial germination of *D. brochopaga and D. dactyloides* was very low (2.71-6.81%).Conidial traps formation by *D. dactyloides* and *D. brochopaga* with trapping of nearby nematodes indicates that this fungus survives in a parasitic phase in soil (Figure - 1 and 2). A higher percentage of spore germination of *A. conoides, A. musiformis* and *A. oligospora* and higher percent of conidial trap formation in *D. brochopaga and D. dactyloides* in response to the fungistatic effect of different soils indicates that these fungi have a better ability to germinate and proliferate and trapped the nematodes in the soils for biocontrol of plant parasitic nematodes (Table \-1). This table also representing the number of total free-living nematode were trapped and killed by the five isolates of nematode trapping fungi. It was observed that the maximum number of free - living nematode was trapped by conidial trap forming NTFs *D. dactyloides* (37.8) followed by *D. brochopaga* (37.1) in rice rhizospheric soil whereas Arthorobotrys *conoides, A. misinforms* and *A. oligospora* trapped very less number of free living soil nematodes.



**Fig-1. a, b, c) Conidial germination of *A. oligospora*, *A. musiformis*, and *A. conoides*, d) 3D network of *A. conoides*, e) Conidial trap of *D. dactyloides*, f) Conidial trap of *D. dactyloides* on germ tube, g) Conidial trap of *D. brochopaga,* h) Conidial trap of *D. brochopaga* on germ tube.**



**Fig-2. a) Soil nematode trapped by *D. brochopaga,* b) Nematode trapped by *A. musiformis,* c) Nematode trapped by *D. dactyloides*. d) *A. oligospora* trapped of nematode by 3D network, e) Nematode trapped by *A. conoides*.**

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| **Table-1. Germination and trap formation of different species of nematode-trapping fungi in vicinity to fungistatic environment of rhizospheric soils of different crops** | | | | | | | | | | | | | | | |
| **Field soil** | 1. ***conoides*** | | | ***A. musiformis*** | | | ***A. oligospora*** | | | ***D. brochopaga*** | | | ***D. dactyloides*** | | |
| **Germ tube**  **(%)** | **CT**  **(%)** | **TKN** | **Germ tube (%)** | **CT**  **(%)** | **TKN** | **Germ tube**  **(%)** | **CT**  **(%)** | **TKN** | **Germ tube**  **(%)** | **CT**  **(%)** | **TKN** | **Germ tube (%)** | **CT**  **(%)** | **TKN** |
| **Okra** | 94.9bc | 0.96b | 0.7**b** | 96.06abcd | 0.94b | 0.5**cd** | 94.82bc | 0.97b | 0.6**bcd** | 2.71c | 95.77a | 29.4cd | 4.37a | 92.57c | 29.2**c** |
| **Wheat** | 96.0ab | 0.95b | 0.9**ab** | 95.84abcd | 0.98b | 0.7**cd** | 95.74abc | 1.01b | 0.8**bc** | 4.17abc | 93.93abc | 29.5cd | 2.91bc | 94.78ab | 27.4**c** |
| **Linseed** | 94.6c | 2.25a | 1.3**a** | 95.38cd | 1.71a | 1.3**a** | 94.61c | 2.28a | 1.4**a** | 2.87c | 94.99ab | 35.6a | 2.54c | 95.26a | 36.9**ab** |
| **Rice** | 96.5a | 0.90b | 0.5**bc** | 96.61a | 0.88b | 0.4**d** | 96.30a | 0.96b | 0.4**b de** | 3.88abc | 92.66cde | 37.1a | 3.33bc | 93.98b | 37.8**a** |
| **Chickpea** | 96.3a | 0.97b | 0.9**ab** | 96.20abc | 0.99b | 0.8**bc** | 96.41a | 0.94b | 0.8**b** | 5.23ab | 90.45e | 30.9bc | 2.95bc | 94.31ab | 35.2**ab** |
| **Pigeon pea** | 95.5bc | 0.96b | 0.1**cd** | 95.94abcd | 0.97b | 0.0**e** | 94.96bc | 0.96b | 0.1**e** | 3.35c | 92.93bcde | 29.6cd | 5.00a | 91.81c | 28.6**c** |
| **Cow pea** | 96.3a | 0.88b | 0.1**cd** | 95.76bcd | 1.00b | 0.0**e** | 96.27a | 0.88b | 0.0**e** | 6.81a | 90.09de | 27.8cd | 3.09bc | 94.61ab | 33.6**b** |
| **Tomato** | 94.6c | 2.28a | 1.2**a** | 95.23d | 1.77a | 1.1**ab** | 94.61c | 2.28a | 1.2**a** | 2.90c | 93.22bcd | 46.8 | 3.18bc | 94.70ab | 35.5**ab** |
| **Brinjal** | 96.5a | 0.91b | 0.2**cd** | 96.50ab | 0.91b | 0.0**e** | 96.48a | 0.91b | 0.1**e** | 3.63bc | 91.48de | 34.2ab | 3.49b | 93.82b | 34.1**ab** |
| **Mustard** | 95.8abc | 1.09b | 0.2**cd** | 96.15abcd | 1.00b | 0.0**e** | 95.83ab | 1.09b | 0.1**e** | 3.05c | 94.41abc | 26.7d | 2.95bc | 94.31ab | 36.1**ab** |
| **Water agar control** | 99.30d | 0.05c | 0.0**d** | 98.53f | 0.91b | 0.0**e** | 98.39d | 0.97b | 0.0**e** | 97.84d | 1.50f | 0.0e | 95.19d | 2.28d | 0.0**d** |
| **C.D.** | 1.00 | 0.33 | 0.39 | 0.73 | 0.34 | 0.33 | 0.95 | 0.34 | 0.37 | 2.51 | 2.66 | 3.56 | 0.78 | 0.97 | 3.27 |
| **SE(m)** | 0.36 | 0.12 | 0.14 | 0.26 | 0.12 | 0.12 | 0.34 | 0.12 | 0.13 | 0.89 | 0.95 | 1.27 | 0.28 | 0.35 | 1.16 |
| **SE(d)** | 0.51 | 0.17 | 0.19 | 0.37 | 0.17 | 0.17 | 0.48 | 0.17 | 0.18 | 1.27 | 1.34 | 1.79 | 0.39 | 0.49 | 1.65 |
| **C.V.** | 1.18 | 33.35 | 79.21 | 0.86 | 35.02 | 84.94 | 1.12 | 31.65 | 82.14 | 22.80 | 3.54 | 13.44 | 7.46 | 1.28 | 12.11 |

CT= Conidial trap formation; TKN= Trapped and killed nematodes; CD= Critical Difference of Means. SE(m): Standard Error of the Mean, SE(d): Standard Error of the Difference; C.V.= Coefficient of Variation,. Data superscript with different letters represents the significant difference of column data by randomized block design at P< 0.05.

**4. Discussion**

“The soil fungistasis is a natural phenomenon occurring in all types of soils” (Dobbs and Gash, 1965; Lockwood, 1977) “and fungistatic intensity varies with the physico-chemical properties of soil” (Handelsman and Stabb, 1996; Mondal and Hyakumachi, 1998; Qian and Johnson, 1987). “It is evident from the results that little fungistatic effect was observed in response to all soils on germination by germ tube formation of *A. musiformis, A. conoidis*, and *A. oligospora* and conidial trap formation of *D. brochopaga* and *D. dactyloides*. Formation of conidial traps by conidia of *D. brochopaga* and *D. dactyloides* in response to rhizospheric soils revealed that the diffusates released from soils reached to the agar discs, resulted in trap formation. Trap formation in nematode-trapping fungi in response to nematode metabolite ‘Nemin’ has been reported by several researchers” (Feder et al.1963; Monoson et al. 1974, Kumar et al., 2015, Kumar 2024b). It appears that soil contains enough ‘Nemin’ due to presence of nematodes which induced trap formation on conidia of *D. brochopaga* and *D. dactyloides* in close vicinity of the soil. The higher percentage of germination of conidia of *A. oligospora, A. conoides* and *A. eudermata* in soil (Table 1) and conidial trap formation of *D. dactyloides* and *D. brochopaga* is attributed to the ability of these fungi to germinate and proliferate in fungistatic responses of soils. The result of the present study indicates that these fungi are adaptive to grow in the fungistatic environment of soils and trapping of nematodes by conidial trap of *D. brochopaga* and *D. dactyloides* is positive attribute of soil fungicides which induce the conidial trap formation in these two fungi for enhanced biocontrol potential against plant parasitic nematodes.

**5. Conclusion**

The findings suggest that the studied nematode-trapping fungi have the potential to grow, proliferate and form traps in soil for trapping and killing of nematodes in soil, however the *D. hrochopaga* and *D. dactyloides* could be used as most potential nematode- trapping fungi in comparison to *A. conoides, A.oligospora* and *A. musiformis* due the formation of higher number of conidial traps by these two constricting ring forming fungi in soil for biological control of plant parasitic nematodes.

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