GROWTH RESPONSE OF FOUR NEMATOPHAGOUS FUNGI IN DIFFERENT GRAIN AND BRAN MEDIA

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ABSTRACT

Nematophagous fungi such as Arthrobotrys, Dactylaria, Dactylella and Monacrosporium species were isolated and identified from leaf debris of mango, cashew and orange based on their morphological characteristics in Zaria, Kaduna State, Nigeria. Four different cereal grain and bran media were screened to determine their ability to support sporulation and growth of the fungi. After 21 days of inoculation, there was complete colonization of the Petri dishes diameter, all the fungi spreading at the rate of about 0.26 to 0.46 cm per day. Arthrobotrys and Monacrosporium species in grain (sorghum) medium produced about (17.54 x 10^4) spores per milliliter, respectively. The four isolated fungi genera had good mycelia growth in wheat, millet, sorghum and rice grain media, while Arthrobotrys and Monacrosporium spread better in sorghum medium. Dactylella and Monacrosporium species gave the highest spore count in sorghum and millet bran. The experiments revealed that all the four isolated nematophagous fungi can be culture in these grain media for mass production of these fungi at minimum cost.

KEYWORDS: Nematophagous fungi, leaf debris, media and spore count

INTRODUCTION

Predatory fungi are recognized as having potential for the biological control of plant parasitic nematodes. These fungi produce ring-shaped structures that maybe constrictors or non-constrictors, threedimensional adhesive networks along the length of the hyphae, responsible for immobilization and penetration of the nematode cuticle (Barron, 1977, Nordbring-Hertz, 2004). The nematophagous fungi have a significant contact with their prey especially nematodes in their vicinity and thus, can constantly capture and destroy them (Li *et al*., 2000; Liu and Zhang, 2003; Dong *et al*., 2004; Mo *et al*., 2005; Li *et al*., 2005; Bello *et al.,* 2012). There are various ways for soil-borne fungi to suppress nematode multiplication, which are either direct or indirect manner. In direct mechanism, the fungi feed on nematodes, while the indirect manner fungi kill nematodes by mycotoxin (Barron and Throne, 1987), through the destruction of the feeding sites of sedentary nematodes in root (Glawe and Stiles, 1989). Some bacteria and fungi produce metabolic by products which interfere with nematode behaviour, and many soil organisms parasitize or prey on nematodes. The fungi used for biocontrol of nematodes include predaceous fungi and endoparasites fungi e.g. *Dactylella*, *Arthrobotrys*, *Paecilomyces lilacinus*, *Verticillium chlamydosporium* etc. (Goswami and Uma, 1995).

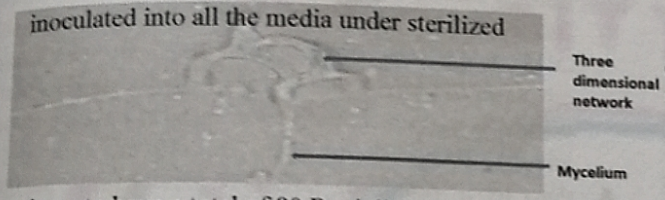
Knowledge aspect such as mycelia sporulation capacity which may lead to mass or biomass production of spores is necessary to determine the potential of nematophagous fungi as efficient agents in programmes of biological control. Maintenance of cultures for prolonged periods in a suitable medium may be obtained by means of different culture medium that may depend on different characteristics of the fungal species, availability of the facilities for carrying out the method(s) and as well as the economic costs (Bello *et al*.,2012). Management of plant parasitic nematodes with high doses of some nematicides did not receive much popularity and have been withdrawn from world market due to their harmful effects on the environment. In view of these, researchers tend to diversify and look inward into developing alternative control measure such as biopesticides (Nordbring-Hertz, 2004). Biological control which is an alternative to nematicides has been gaining ground and becoming important in

recent years, viz: using natural enemies within the same rhizosphere to control nematodes. One of such natural enemies of nematodes that their population is abundant in all types of soils is the predacious fungi (Bello *et al*., 2012). However, these fungi produce study to evaluate different grain and bran growth media for sporulation of some isolated predacious fungi from decayed plant debris in Zaria, Nigeria.

ring-shaped that maybe constrictors or nonconstrictors (Barron, 1977). Plates 1-5 are some constricting organs and nematodes captured from our previous work. This formed the basis of the research

conical flask was allowed some few minutes to cool; the medium was dispensed gently into sterilized 9 cm Petri-dishes to covered about 2/3rd area of each plate and inoculated with 5 mm disc of 15 days old culture of the isolated predacious fungi. Each fungus

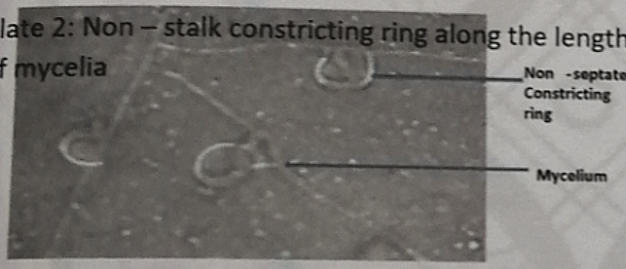
inoculated into all the media under sterilized



condition and replicated four times. The plates were randomly arranged on the laboratory bench and radial growth measured at 3 days interval for 21 days. Four different nematophagous fungi isolated were inoculated into each medium and replicated four times to have a total of 80 Petri dishes in a complete randomize design (CRD). After 21 days, each Petridish flooded with 50 ml sterile water and agitated for separation of spore. Spores from each Petri-dish were collected into a separate beaker. All the collected spores were counted using Neubauer chamber

Plate 1: A typical three dimensional networkring of *Arthrobotrys* species

Plate 2: Non – stalk constricting ring along the length of mycelia



hemocytometer and the number of spore per ml was analyzed and calculated as follows.

Spore count of the isolated fungi using Neubauer improved haemocytometer. After 21 days of incubation, each Petri dish was flooded with 50 ml distilled water, agitated for separation of spore using an electric mixer for about 60 seconds. The

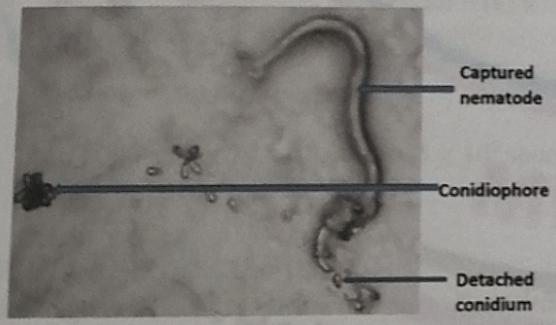


Plate 3: Nematophagous fungus , bunch of conidia, detached conidium and nematode captured at both ends

METHODOLOGY

The radial growth and sporulation of the isolated nematophagous fungi (*Arthrobotrys* sp., *Dactylaria* sp., *Dactylella* sp. and *Monacrosporium* spp. were studied on Bean and grain of Wheat (*Triticum aestivum*), Rice (*Oryza sativa*), Sorghum (*Sorghum bicolor*) and Millet (*Pennisetum glaucum* (L.). The experiment was carried out in the Department of Crop Protection, Faculty of Agriculture/Institute for Agricultural Research, Ahmadu Bello University Samaru, Zaria. Each bran and grain media contain 20g bran, 20 g agar and 1000 ml of distilled water, autoclaved for 15 minutes at 15 psi pressure at 121°C in a separate labelled conical flask. After 15 mins, the homogenized mixture was filtered through a Muslin cloth and the filtrates made up to 50 ml. All the filtrate spores were collected separately from each Petri dish for each isolated fungus. The spores were counted using Neubauer haemocytometer and the number of spores per ml of water was calculated as shown below.

(a) All the total spores in the four corner squares were counted

(b) The spore count were calculated using the equation: spores/ml = (n) x 10^4, where: n = the average cell count per square of the four corner squares counted and those outside the four corner squares were neglected.
Example: if the calculated average (n) of spores in the four 1 mm corner squares of the haemocytometer is 70: Cell/ml = (n) x 10^4 or spores/ml = 70 x

10,000 = 700,000 spores/ml

In 50ml =50 x 70 x 10,000 = 35 x 10^6 spores (Janice, 2010)

RESULTS AND DISCUSSION

Radial growth of *Arthrobotrys*, *Dactylella*, *Dactylaria* and *Monacrosporium* species in different grain media at three days intervals for a period of twenty days of incubation

Radial growth of the isolated nematophagous fungi was carried out in different whole grain media of millet, rice, sorghum and wheat. The mycelia growth were observed and recorded for a period of 21 days. The *Arthrobotrys* sp. responded to radial growth in all the media in which there was no significant difference between rice and wheat grain media at each interval for a period of 21 days of incubation. Similar observation was recorded for millet and sorghum grain media except at day 3 (3.98, 4.99) in which significant difference was recorded respectively. Millet and sorghum grain media responded to radial growth of *Arthrobotrys* sp. (Fig. I) than rice and wheat grain media, all the Petri dishes were covered with mycelia growth and there was a significant difference between millet and sorghum (9.00, 9.00), rice and wheat (7.91, 8.04) at 18 days of incubation respectively. All the media were completely covered after 21 days of incubation.

Radial growth of *Dactylella* species in different grain media at three days intervals for a period of twenty one days of incubation.

After 3 days of incubation, response of *Dactylella* sp in radial growth was more than *Arthrobotrys* sp. (Fig. 11) and there was no significant difference between millet and wheat grain media (4.58, 4.04), but high significant differences were recorded between rice and sorghum grain media; similar observation was recorded after 6 days of incubation. There was a significant difference between all the media after 9 days of incubation with sorghum grain (8.99) recording the highest radial growth and the least in wheat grain (6.40). Between 12 and 21 days of incubation of this fungus, the mycelia growth increased, the Petri dishes were completely covered and there was no significant difference between the grain media at 15 and 21 days of incubation.

Radial growth of *Dactylaria* species in different grain media at three days intervals for a period of twenty one days of incubation.

Dactylaria sp responded faster in radial growth than all the isolated nematophagous fungi in these grain media (Fig.III) after 3 days of incubation with no significant difference between rice, sorghum and wheat grain media. However, after 6 days of incubation of this fungus, significant difference was recorded between millet, rice and sorghum as well as between millet, wheat and sorghum respectively, but no significant difference between rice and wheat grain media (8.23, 7.95). After 9 days of incubation, there was no significant difference ($P \leq 0.05$) between millet, rice and wheat grain with the exception of sorghum grain media (8.38) in which significant difference was recorded. All the grain media responded to mycelia growth of this fungus, Petri dishes were completely covered between 9 and 21 days of incubation except

sorghum grain media in which 8.38 cm was recorded at 9 days of sporulation.

Radial growth of *Monacrosporium* species in different grain media at three days intervals for a period of twenty one days of incubation. Significant difference was recorded between all the grain media ($P \leq 0.05$) after 3, 6, and 9 days of incubation of *Monacrosporium* sp. with sorghum grain media having the highest radial growth of (6.83, 7.91, 8.95) and least in millet grain media of (4.75, 5.98, 7.09) respectively. After 12 days of incubation, there was no significant difference between sorghum and wheat grain media; however significant difference was recorded between millet and rice grain media. Complete radial growth was recorded for this fungus between 15 and 21 days and there was no significant difference between all the grain media after 21 days of incubation (Fig. IV).

Sporulation of the isolated nematophagous fungi in different grain media after 21 days of incubation.

The spore count of the isolated nematophagous fungi was carried out after 21 days radial growth in all the grain media, for *Arthrobotrys*, *Dactylaria* and *Dactylella* species, significant difference was recorded in the numbers of spores produced after 21 days of incubation in all the grain media (Figure V), however, there was no significant difference between millet and sorghum media (16.63, 16.53) in the numbers of spores produced for *Monacrosporium* sp.

Mycelia radial Growth of *Arthrobotrys, Dactylaria, Dactylella* and *Monacrosporium* species in Different Bran Media at three days Intervals for a Period of Twenty one days of Incubation.

All the bran media tested supported the growth of the isolated nematophagous fungi after 21 days of incubation. The radial growth of *Arthrobotrys* spp.

increased significantly ($P \leq 0.05$) with increase in incubation period up to 9 days in all the media except rice bran in which there was no significant increase. Rice bran medium supported mycelia radial growth best when compared with others after 9 days of incubation (Table 1).

Significant increase was observed in mycelia radial growth of *Dactylaria* spp. after 3 days of incubation in rice bran medium when compared with others. Rice bran medium was observed to be the best in mycelia radial growth of *Dactylaria* spp. There was no significant increase in mycelia radial growth of this nematophagous fungus after 12 days of incubation except in millet bran in which slight mycelia radial growth was observed from 8.46 cm to 9.00 cm. In all the media tested, there was no significant increase in mycelia radial growth of *Dactylaria* sp. between 15 – 21 days after incubation (Table 2).

Significant increase was observed in mycelia radial growth of *Dactylella* spp. between 3 - 9 days of incubation in all the bran media in which millet bran was the best at 9 days (8.56 cm) after incubation and the least mycelia radial growth was observed in sorghum bran medium throughout the period of incubation (Table 3). Significant increase in mycelia radial growth of *Dactylella* spp. was observe between 12 – 18 days in all the media, except in millet and wheat bran media in which there was no significant increase in mycelia radial growth after 18 days of incubation. There was no significant increase in mycelia radial growth of *Dactylella* spp. in all the tested media after 21 days of incubation (Table 3). All the bran media tested supported the growth of *Monacrosporium* spp. (Table 4). Mycelia radial growth of this fungus increase significantly ($P \leq 0.05$) in rice bran media with increased in incubation period up to 6 days of incubation. There was no further increase of mycelia radial growth for *Monacrosporium* sp. between 9 – 21 days of incubation in rice bran medium. Rice bran medium supported mycelia radial growth best after 9 days of incubation when compared with other media. Similarly, as observed in *Arthrobotrys* (Table 1) and *Dactylaria* (Table 2) species in rice medium, there was no significant increase in mycelia radial growth of *Monacrosporium* spp. between 18 – 21 days of incubation (Table 4). It was observed that, rice bran medium was the best medium that supported mycelia radial growth of *Arthrobotrys*, *Dactylaria* and *Monacrosporium* species, while *Dactylella* spp. (Table 3) gave the best result in millet bran medium after 12 days of incubation.

Sporulation of Four Nematophagous Fungi to Different Bran Media

All the bran media significantly produced high number of spores after 21 days of culture. Rice bran medium was the best for *Arthrobotrys* spp. and millet bran medium was the best for *Dactylella* and

Monacrosporium species when compared with others. However, there was no significant increase in the number of spore produced for *Dactylaria* spp. in millet and sorghum bran media, but the highest number of spore produced was obtained in sorghum bran medium after 21 days of culturing (Fig. VI).

CONCLUSION AND RECOMMENDATIONS

All the bran media supported the four fungi radial growth after 21 days of incubation; however mycelia growth spread faster in all the media for *Arthrobotrys* than *Dactylella* species. Tables 1 & 2 also indicated that *Arthrobotrys* and *Dactylella* species attained full mycelia growth at 12 and 21 days of incubation respectively. This observation is in agreement with (Mo and Zhang, 2005) in which it was reported that *Arthrobotrys yunnanensis* attained 6 cm diameter radial growth in corn meal agar medium in 5 days at 28°c and mycelia spreading at 0.5 cm per 24 hours. Tables 2 indicated that rate of spread of *Dactylella* sp is slow compared to Arthrobotrys sp (Table 1) One of the main aims of going into the use of biocontrol to control nematodes is to have cheap means of producing the control agent's materials for the end users, the farmers. For addition of the nematophagous fungi in nematode infested soil, there is an urgent need for quick and mass production of these fungi in suitable locally available substrates that are cheap all over the country. In all the cereals tested for mycelia radial growth and sporulation of the four isolated fungi, complete growth was recorded in all the grain media prepared from rice, sorghum, wheat and millet between 15 and 21 days of incubation, however. *Dactylaria* spp. grew better in millet and rice grain media at 6 and 9 days, respectively. In all the media, average radial growth of the mycelia spread at the rate of about 0.26 to 0.46 cm per day was achieved. In view of these findings, for in-vitro mass sporulation of *Arthrobotrys, Dactylella, Dactylaria and Monacrosporium* species, rice and millet bran are recommended respectively. This is because; mycelia growth of *Arthrobotrys* and *Dactylella* species completely covered all the Petri dishes of these bran media between 6 and 9 days, and between 9 and 12 days respectively.

Table 1: Mycelia Radial Growth of *Arthrobotrys* species in Different Bran Media.

	Radial growth (cm) of *Arthrobotrys* species (days)						
Treatment	3	6	9	12	15	18	21
Media							
	4.74[b]	6.04[b]	7.66[b]	9.00	9.00	9.00	
Millet bran							9.00

	4.13[b]	8.06[a]	9.00[a]	9.00	9.00	9.00	9;00
Soy bean	5.60[a]	6.86[b]	8.15[b]	9.00	9.00	9.00	9.00
Soybean bran	5.76[a]	6.90[a]	8.14[b]	9.00	9.00	9.00	9.00
Wheat bran	0.798	0.878	0.464	NS	NS	NS	NS
LSD							

Means within a column followed by the same letter(s) are not significantly different at 5% level of probability *

NS = Not significant

Table 2: Mycelia Radial Growth of *Dactylaria* species in Different Bran Media.

	Radial growth (cm) of *Dactylaria* species (days)					
3	6	9	12	15	18	21
4.23[c]	5.38[d]	7.24[c]	8.46[b]	9.00	9.00	9.00
7.25[a]	9.00[a]	9.00[a]	9.00[a]	9.00	9.00	9.00
4.81[b]	6.09[c]	7.49[b]	9.00[a]	9.00	9.00	9.00
6.90[a]	7.85[b]	9.00[a]	9.00[a]	9.00	9.00	9.00
0.325	0.257	0.104	0.118	NS	NS	NS

Means within a column followed by the same letter(s) are not significantly different at 5% level of probability *

NS = Not significant

Table 3: Mycelia Radial Growth of *Dactylella* species in Different Bran Media.

	Radial growth (cm) of *Dactylella* species (days)						
Treatment	3	6	9	12	15	18	21
Media	6.01[a]	7.13[a]	8.56[d]	9.00[a]	9.00[a]	9.00[a]	9.00
Millet bran	4.51[b]	5.30[b]	6.41[b]	7.36[b]	8.48[b]	8.93[a]	9.00

Sorghum bran	1.01[d]	1.96[d]	4.80[d]	6.11[d]	7.48[c]	8.84[a]	9.00
Wheat bran	2.98[c]	4.20[c]	5.54[c]	6.75[c]	8.06[b]	9.00	9.00
LSD	0.398	0.403	0.427	0.491	0.415	0.130	NS

Means within a column followed by the same letter(s) are not significantly different at 5% level of probability *

NS = Not significant

Table 4: Mycelia Radial Growth of *Monacrosporium* species in Different Bran Media.

Treatment Media	Radial growth (cm) of *Monacrosporium* species (days)						
	3	6	9	12	15	18	21
Millet bran	4.58[a]	5.96[a]	7.59[b]	9.00[a]	9.00[a]	9.00	9.00
Rice bran	4.48[a]	7.01[a]	9.00[a]	9.00[a]	9.00[a]	9.00	9.00
Sorghum bran	3.65[b]	5.08[b]	6.46[c]	7.61[b]	8.68[b]	9.00	9.00
Wheat bran	5.21[a]	6.61[a]	7.78[b]	9.00[a]	9.00[a]	9.00	9.00
LSD	0.617	0.817	0.578	0.327	0.215	NS	NS

Means within a column followed by the same letter(s) are not significantly different at 5% level of probability *

NS = Not significant

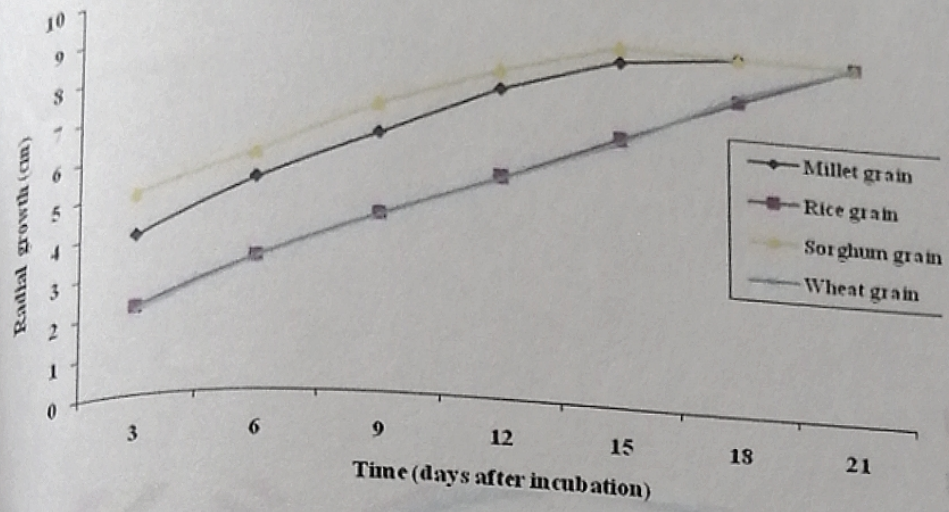


Fig. I: Radial growth of *Arthrobotrys* species in different grain media at three days intervals for a period of twenty one days of incubation

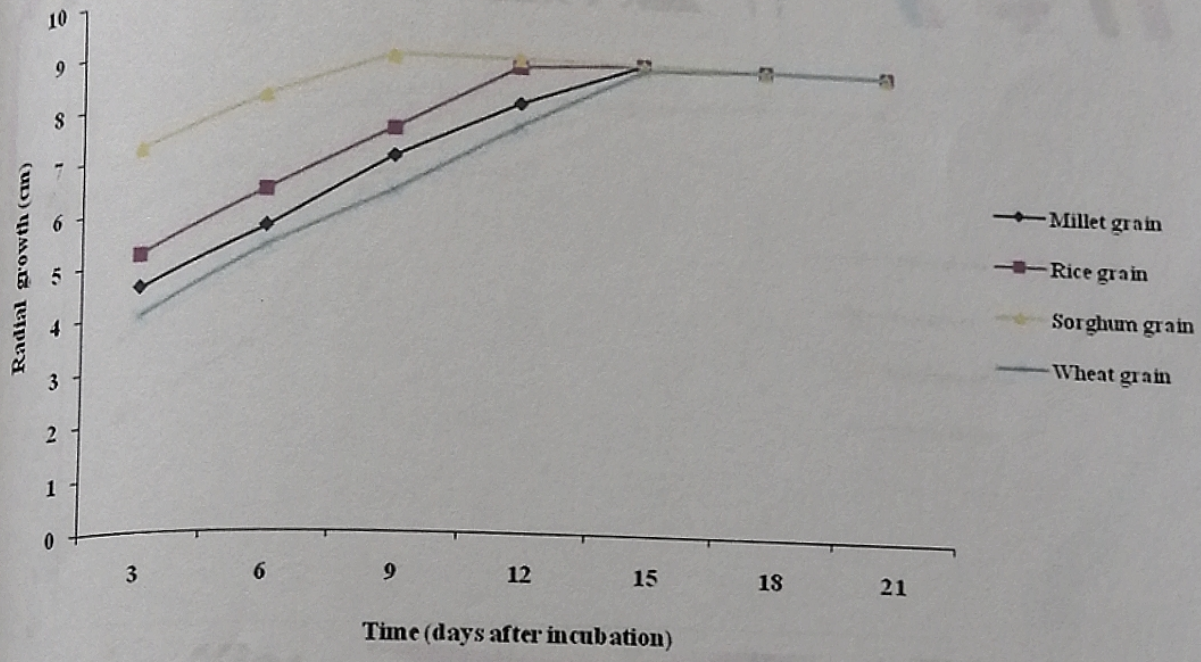


Fig. II: Radial growth of *Dactylella* species in different grain media at three days intervals for a period of twenty one days of incubation

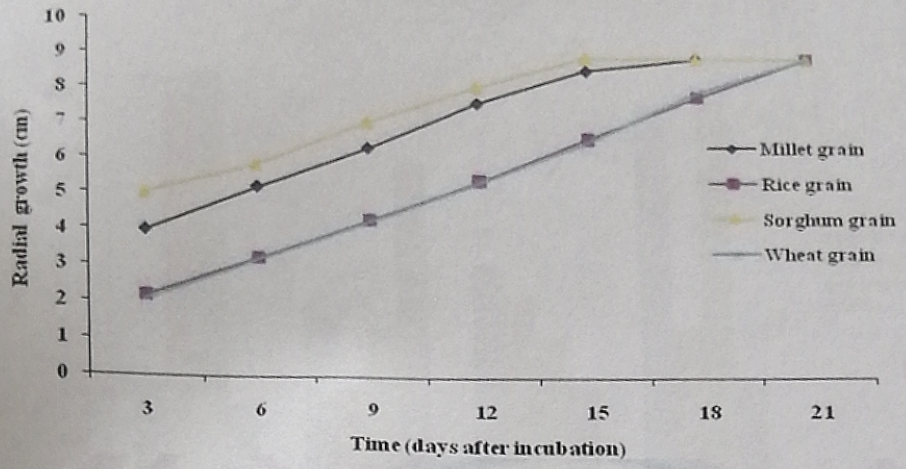


Fig. III: Radial growth of *Dactylaria* species in different grain media at three days intervals for a period of twenty one days of incubation

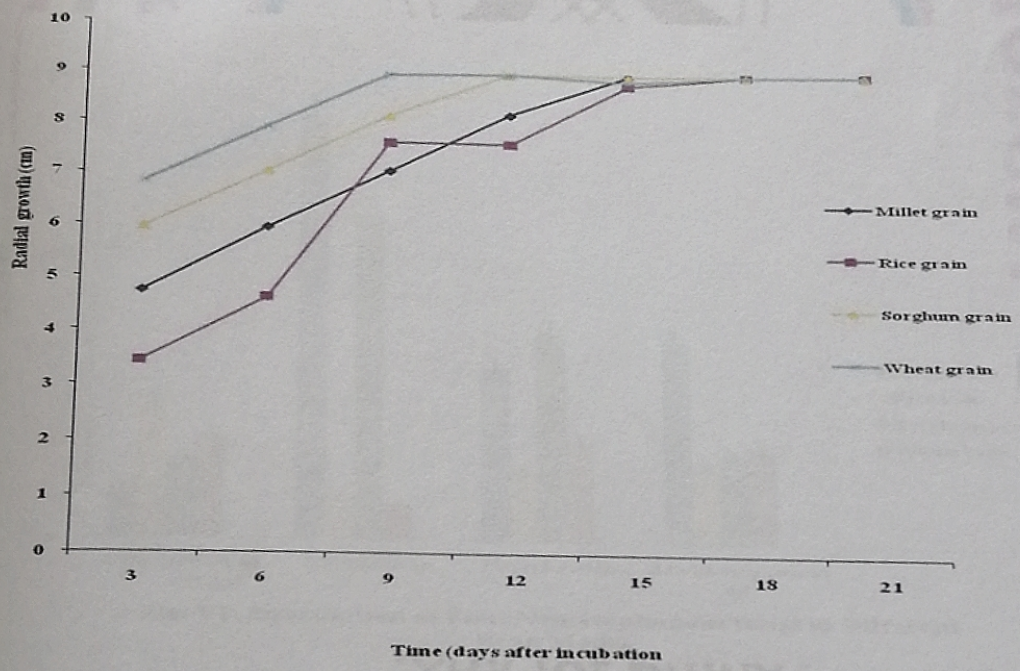


Fig. IV: Radial growth of *Monacrosporium* species in different grain media at three days intervals for a period of twenty one days of incubation

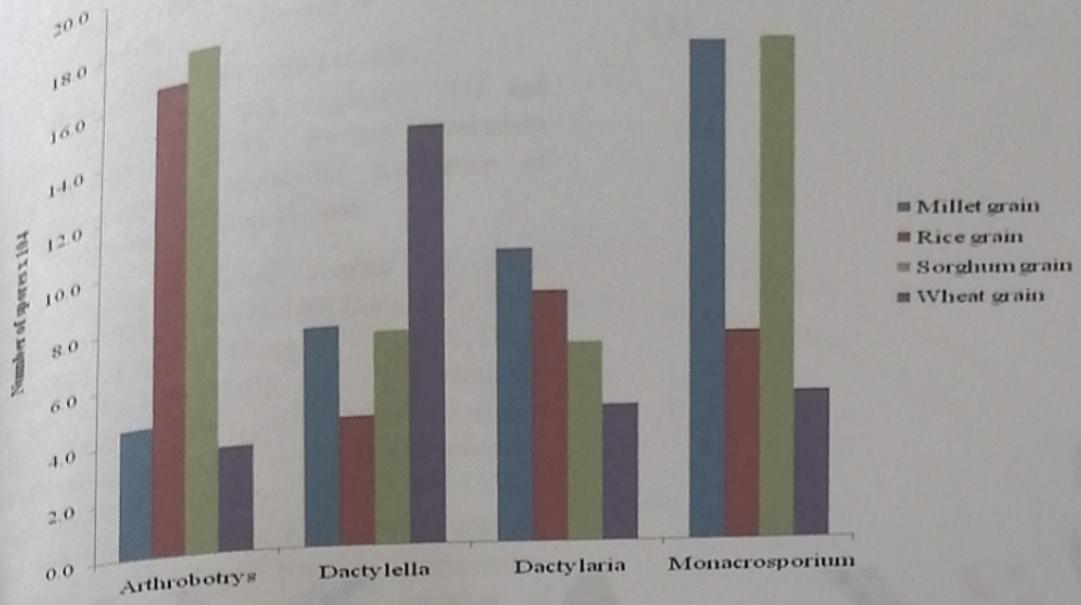


Fig. V: Sporulation of the isolated nematophagous fungi in different grain media after 21 days of incubation

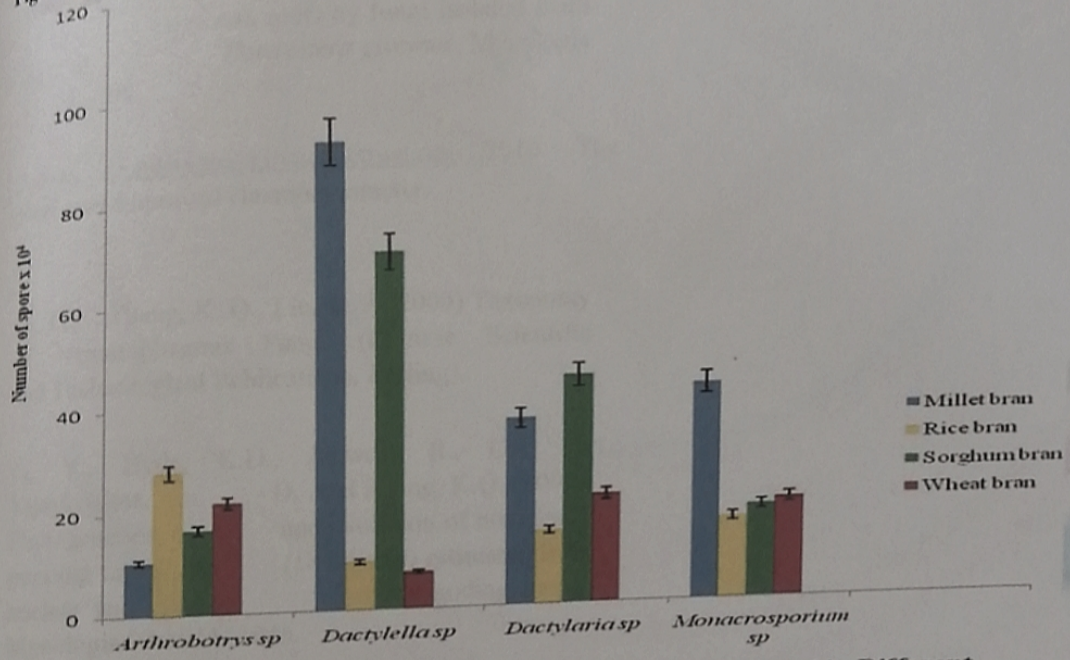


Fig. VI: Sporulation of Four Nematophagous fungi in Different Bran Media

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