NIGER. J. PL. PROT. 26 (1) 142 - 146 (2012)

IN-VITRO EVALUATION OF DIFFERENT GROWTH MEDIA FOR SPORULATION OF SOME NEMATOPHAGOUS FUNGI IN ZARIA, NIGERIA.

[1]Bello, L. Y, [2]P.S Chindo, [2]N.O. Agbenin, and [2]M.D. Alegbejo

[1] Department of Crop Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State, Nigeria.
[2] Department of Crop Protection, Institute for Agricultural Research / Faculty of Agriculture, Ahmadu Bello University, Samaru Zaria, Kaduna State, Nigeria.
e-mail – alyunusbello@yahoo.com

SUMMARY

Biological control is based on the use of micro-organisms to combat population of a specific plant pathogen. Nematophagous fungi have shown some promise as agents of bio-control of plant parasitic nematodes. These organisms produce special structures along the hyphae, named traps, which are responsible for capturing and killing the nematode pre-parasite stages. The growth of *Arthrobotrys* species isolated from some orchard plants were subjected to sporulation test for 21 days in Yeast glucose agar, Czapek's agar, Sabouraud's dextrose agar, Emerson YPSs agar and Potato dextrose agar media. Mycelial growths were taken at three days interval and spores produced in each medium were assessed using haemocytometer after 21days of inoculation. Nine days after inoculation, significant response was observed in the mycelial sporulation (Pd"0.01) of the fungi in all the growth media except in Emerson YPSs and Sabouraud's dextrose agar media in which there was no significant difference in mycelial growth when compared with the control.. All the media were found to support mycelia growth within 15 days of inoculation, since the 9cm Petri-dishes were completely covered between 15-18 days of observation but with variation in their spore production. Czapek's and Yeast glucose agar median supported production of spores more than Sabouraud's, Emerson YPSs and Potato dextrose agar media.

***Key words:* Arthrobotrys species, growth media, mycelia growth, fungi sporulation.**

Nematode populations can be controlled by several measures. First and foremost is the use of nematicides. But all these compounds are toxic to human beings and cause environmental hazards. They require extra care in handling. The use of resistant cultivars is effective but eventually may fail due to outbreak of virulent nematodes. Hence there is the need to control these nematodes using some eco-friendly techniques. 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, three-dimensional adhesive networks along the length of the hyphae, responsible for immobilization and penetration of the nematode cuticle (2 and 10). Nematophagous fungi comprising a diverse range of species which are able to infect nematodes can be divided into four categories: endoparasitic fungi, nematode-trapping fungi, fungi which parasitize eggs and sedentary females as well as toxin-producing fungi (3 and 4). There are various ways for soil-borne fungi to suppress nematode multiplication, which are either direct or indirect. In direct mechanism, the fungi feed on nematodes, while the indirect approach kill nematodes by mycotoxin (3), through the destruction of the feeding sites of sedentary nematodes in roots (5). 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 endoparasitic fungi e.g. *Dactylella*, *Arthrobotrys*, *Paecilomyces lilacinus*, *Verticillium chlamydosporium* etc. (6). Information on mycelia sporulation capacity which may lead to mass or biomass production is necessary to determine the potential of nematophagous fungi as efficient agents in programmes of biological control. Maintenance of cultures for prolonged periods maybe obtained by means of different culture media that may depend on different characteristics of the fungal species, on the facility of carrying out the method and as well as the economic costs. The objective of this study was to evaluate different growth media for sporulation of some isolated predacious fungi from decayed plant debris in Zaria.

MATERIALS AND METHODS

The radial growth and sporulation of the isolated nematophagous fungi (*Arthobotrys* sp. and *Dactylella* sp.), were studied on yeast glucose medium (YGM), potato dextrose agar (PDA), Czapek's agar (CZP), Emerson YPSs agar (EMS) and Sabouraud's dextrose agar media (SBR). The experiment was carried out in the Department of Crop Protection, Faculty of Agriculture/Institute for Agricultural Research, Ahmadu Bello University Samaru, Zaria. Each medium was separately prepared in 1000ml distilled water, poured into Petri-dishes and inoculated with 5mm disc of 15days old culture of the isolated predacious fungi. Each fungus was 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. After 21 days, each Petri-dish was flooded with 50ml 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 hemocytometer and the number of spore per ml was calculated.

RESULTS

Radial growth of *Arthrobotrys* and *Dactylella* species.

After 3days, there was no significant difference in radial growth in EMS and PDA media (Table 1).

However, there was a significant difference between radial growth in YGM and all other media except at 9 and 12 days where there was no significant difference between YGM and CZP. The *Arthrobotrys* sp tend to respond better to radial growth at 9 and 12 days, because there were no significant differences between EMS and PDA as well as between YGM and CZP, but there was a significant difference between SBR and other media. All the media supported mycelial growth at 15 and 21 days after inoculation since the 9 cm Petri-dishes were completely covered. There were no significant differences among all the media between 15 and 21 days (Table 1).

Table 2 indicated that, there were no significant differences among EMS and CZP, and between PDA, YGM and SBR. Similar trend was observed at 6 days for EMS and CZP, but not for PDA and other media. In *Dactylella* species (Table 2), there was a significant difference between EMS and all other media. However, after 18 and 21 days, there were no significant differences among the media.

Spore count of *Arthrobotrys* and *Dactylella* species after 21 days of radial growth

There was a significant difference in the number of spores produced per mil after 21 days for all the media except for CZP in which there was no significant difference between the number of spores produced by *Arthrobotrys* and *Dactylella* sp (54 and 54.). YGM gave the highest number of spore count (161) and least count in SBR (50). In *Dactylella* species, CZP media produced high number of spores (54), however, there was no significant difference between the number of spores produced in EMS and TGM after 21 days (Table 2).

DISCUSSION

All the growth media supported the two fungi radial growth after 21 days. All the 9 cm Petri-dishes were completely covered between 15 and 18 days of inoculation. The mycelial growth spread at the rate of about 1.5 cm per 72 hours to attain 9 cm after 21 days. *Arthrobotrys yunnanensis* attained 6cm diameter radial growth in corn meal agar (CMA) medium in 5 days at 28°C mycelia spread and at 0.5 cm per 24 hours that their conidia and conidiophores are produced after 4 days. (9). Similarly, (1) reported that the mean growth rate of the fungus *Verticillium chlamydosporium* varied from 0.34 to 0.47 cm/day in different culture media with the maximum being in CMA. Their study indicated that sporulation is independent of mycelia growth which clearly proved that among the three synthetic chemical media evaluated for growing the fungi, Czapek Dox Agar (CDA) was the best. (7) also obtained and reported similar results on CDA.

Isolate of *A. robusta* and *Monacosporium thaumasium* were reported to produce spores in YPSSA (4g yeast extract; 1g k_2HPO_4; 0.5g $MgSO_4.7H_2O$; 20g soluble amide, 20g agar; 1 l distilled water) in 9 cm Petri-dishes and that there was no significant difference at Pd" 0.05 among the fungi. However after 10 days of culture at 25°C all the samples produced spores on the surface of YPSSA stored at 4°C (8).

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

Treatment(media)	Period per 3days						
	3	6	9	12	15	18	21
EMS	5.02a	6.41a	7.58a	8.71a	9.00a	9.00a	9.00a
PDA	4.33a	6.17a	7.58a	8.75a	9.00a	9.00a	9.00a
YGM	3.31b	4.85b	6.36	7.69b	9.00a	9.00a	9.00a
CZP	2.27c	4.08c	5.98b	7.65b	9.00a	9.00a	9.00a
SBR	2.02c	3.45c	4.78c	6.15c	9.00a	9.00a	9.00a
SE±	0.12	0.11	0.08	0.05	0.02	0.02	0.00

Means in column followed by different letter are significantly different ($P \leq 0.05$)

Table 2: Radial growth of *Dactylella* species in different media

Treatment(media)	Period per 3days						
	3	6	9	12	15	18	21
EMS	1.69^{b}	2.84^{c}	4.27^{b}	5.77^{b}	7.35^{b}	9.00^{a}	9.00^{a}
PDA	3.31^{a}	4.48^{a}	5.65^{a}	6.85^{a}	8.39^{a}	9.00^{a}	9.00^{a}
YGM	2.90^{a}	3.98^{ab}	5.44^{a}	6.68^{a}	8.01^{a}	9.00^{a}	9.00^{a}
CZP	2.10^{b}	3.35^{b}	5.16^{a}	6.35^{a}	8.01^{a}	9.00^{a}	9.00^{a}
SBR	3.10^{a}	3.80^{ab}	5.28^{a}	6.61^{a}	8.18^{a}	9.00^{a}	9.00^{a}
SE±	0.06	0.09	0.08	0.07	0.05	0.00	0.00

Means in column followed by different letter are significantly different ($P \leq 0.05$)

Table 3: Interactive effect of nematophagous fungi spore count in different Media after 21 days

Treatment(media)	Nematophagous fungi species (spore per mil x 10^{6})	
	Arthrobotrys spp.	*Dactylella spp.*
EMS	29.90^{f}	60.02^{c}
PDA	6.13^{h}	79.09^{b}
YGM	27.71^{f}	161.16^{a}
CZP	54.04^{d}	54.13^{d}
SBR	18.81^{g}	50.22^{d}
SE±	0.32	

Means in column followed by different letter are significantly different ($P \leq 0.05$)

LITERATURE CITED

1. **Anands, R.,Sundaramoorthi.C., Saritha, E., Divya Krishna and Bhuvaneswari, K. 2009.** Mass multiplication of Verticillium chlamydosporium on plant extracts and plant based solid substrates. *Advanced Bioech* p. 16-20
2. **Barron, G.L. 1977 .** The nematode-destroying fungi. Canadian Biological Publications, Guelph,140p
3. **Barron, G.L and Thorne,R.G. 1987.** Destruction of nematodes by species of *Pleurotus.Canadian Journal of Botany* 65: 774-778.
4. **Dackman, C., Janson, H.B. and Nordbring -Hertz B. 1992.** Nematophagous fungi and their activities in soil. In: *Soil Biochemistry*. (eds. G. Stotzky and J. M., Bollag).Marcel Daekker, New York: 95-103.
5. **Glawe, D. A., and C. M. Stiles. 1989.** Colonization of soybean roots by fungi isolated from cysts of *Heterodera glycines*. Mycologia 81: 797-799.
6. **Goswami, B.K., and Uma Rao.1995** Fungi for biocontrol of plant parasitic nematodes-Nematode pest management an appraisal of Eco-friendly approaches-Chapter 6.p.50-52.
7. **Jissa, N.V. 2002.** Standardization of optimum conditions for mass multiplication of *V ..chlamydosp -orium* a potential nematode biological control agent. M.sc Thesis. Kurinji College of Arts and Science, Thappakkulam,Trichy (Bharathidasan University).
8. **Marcelo de, A.M, Artur, K.C, and Jackson, V.A. 2003.** Sporulation, radial growth and biomass production of *Arthrobotrys robusta* and *Monacrosporium thaumasium* subjected to different methods of preservation. *Brazilian Journal of Microbiology*.34. (2). Print version ISSN 1517- 8382.
9. **Mo, M.H., Huang, X.W., Zhou, W., Huang, Y., Hao, Y.E and Zhang, K.Q. 2005.** *Arthrobotrys yunnanensis* sp. nov., the fourth anamorph of *Orbilia auricolor*. *Fungi Diversity* 18: 107-115.
10. **Nordbring-Hertz, B. 2004.** Morphogenesis in the nematode-trapping fungus*Arthrobotrys oligospora* – an extensive plasticity of infection structures. *Mycologist* 18:125-133.