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EFFECTS OF NUTRITIONAL AND ENVIRONMENTAL FACTORS ON GROWTH AND ANTAGONISTIC ACTIVITIES OF THREE *Aspergillus* SPECIES AGAINST COCOA BLACK POD ORGANISM - *Phytophthora palmivora*

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ABSTRACT

In vitro screening was undertaken to assess the effects of nutritional and environmental factors on the potential of three isolates of Aspergillus namely Aspergillus fumigatus, A. repens and A. niger as biological control agents against the cocoa black pod pathogen, Phytophthora palmivora. Nitrogen and carbon sources were the nutritional factors tested while temperature and pH were the environmental factors evaluated. This study was carried out in the Department of Plant Biology of the University of Ilorin, Nigeria between 2009 and 2010. The test antagonists were incubated under different conditions and their activities against the pathogen recorded. Results revealed that the nutritional factors were essential for inoculum biomass production as well as for sustaining biological activities of the test antagonists. Antagonistic activities of the test organisms were expressed over a range of temperatures (15-35°C) but were best expressed at 30°C. Antagonistic activities dropped outside this range under the conditions of this study. Strong inhibition of the cocoa pathogen by the test organisms was observed between pH 5 and 6.5. It can be

concluded that the efficiency of any organism to function as a biocontrol agent is affected by several factors including nutrition and the environmental conditions.

INTRODUCTION

In all countries where cocoa is grown, the tree is prone to numerous constraints including disease. Among the diseases is black pod disease caused by a fungus, *Phytophthora palmivora* which is one of the most destructive fungal genera in temperate and tropical regions. Losses due to this fungus are in billions of dollars (1 - 4). The interactions between fungi and some host plants can be manipulated to reduce the damage caused by such fungi on plants. Competition for resources is one of such interactions that can be manipulated. Competition is the active requirement for resources in excess of those immediately available to two or more organisms. Organic energy is the resource considered to be most important for fungi. Thus, most competition exists for access to energy for growth and maintenance.

Plant diseases need to be controlled to maintain the quality and abundance of food, feed and fibres produced by growers around the world. Different methods used to control plant diseases include agronomic and horticultural practices, chemical fertilizer and pesticide application. All these had contributed significantly to improvement in crop productivity and quality. However, the side effects of the excessive use of chemicals have led to the strict regulations on the use of chemicals and there is political pressure to remove the most hazardous chemicals from the market. Following from this, the best alternative is biological control (5).

Nutritional and environmental conditions have been reported to affect the growth and antagonistic activity of certain fungi (6). Laboratory assays for antagonism using the dual culture method indicated that antagonism differed among organisms with respect to media used (7). Adebola and Amadi (5) had also reported that *Aspergillus fumigatus, A. repens and A. niger* successfully checked the growth of *Phytophthora palmivora,* the

cocoa black pod pathogen. The aim of this study was to investigate the effect of environmental and nutritional factors on the potential of *Aspergillus fumigatus, A. repens and A. niger* to suppress the growth of *Phytophthora palmivora*.

MATERIAL AND METHODS

Media

Influence of media on antagonism was evaluated on five solid media namely carrot meal agar, malt extract agar, cassava dextrose agar, corn meal agar and potato dextrose agar (PDA). Mycelial plugs (5mm) were cut from the edge of each of the actively growing test *Aspergillus* species and inoculated at the periphery of sterile culture plates of the test media for 2 days at 28 ± 2°C. A mycelial plug (5mm) of *P. palmivora* was inoculated 5cm away from the inoculum of the test antagonist. The doubly-inoculated plates were incubated for an additional 9 days at 28 ± 2°C. Mycelial spread of the pathogen and the test antagonists was measured and recorded. The experiment was replicated three times. A control test was also set up using sterile agar plugs in place of the test antagonists. Percentage inhibition of growth was determined and analyzed using ANOVA and DMRT (8)

Effect of nutrition

This was assessed on M9 medium composed as follows: NaH_2PO_4 (6g); KH_2PO_4 (3g); NaCl (0.5g); NH_4Cl (1g); and distilled water (1 litre) (6). Agar was added to solidify the medium and then supplemented with 1M $MgSO_4.7H_2O$ (2 ml); 20% glucose (10ml); 1M $CaCl_2$ (0.1ml) and 1% vitamin B (thiamine-HCl) 0.5ml. Seven carbon sources namely lactose, pectin, sucrose, starch, fructose, maltose and galactose were added in turn to the M9 medium at a final concentration of 0.2% (w/v) to substitute glucose. Also, seven nitrogen sources methionine, lysine, urea, Ca $(NO_3)_2$, $NH_4H_2PO_4$, KNO_3, and $NaNO_3$ were incorporated into the medium at a final concentration of 0.2% (w/v) to substitute NH_4Cl. The carbon and nitrogen sources were added separately after

autoclaving. The pathogen and the antagonists were inoculated on the medium 5cm apart and incubated at 28 ± 2°C for 7 days. Each test factor was replicated three times. Antagonism was assessed using the dual culture method (5 and 7). The percentage inhibition of growth of the pathogen was calculated and the results analyzed using ANOVA and DMRT.

Effect of temperature and pH

The M9 medium as described above was also used for this study. Doubly-inoculated culture plates were incubated at 15, 20, 25, 30 and 35°C. The same medium was adjusted with sodium hydroxide (NaOH) or hydrochloric acid (HCl) to obtain the different pH values tested (pH 5.0 to 8.0). Each test was replicated three times. Results were analyzed using ANOVA and DMRT.

RESULTS

Effect of media on antagonism

Results of this study showed that all the five media tested had significant effects ($P < 0.05$) on the potential of the test antagonists to hinder the growth of the black pod pathogen, *P. palmivora*. Malt extract agar (MEA) supported the activities of the test antagonists best with a record seventy percent (70%) mean reduction in the growth of the pathogen followed by PDA (68.1%) and corn meal agar (67.4%) (Table I). No significant difference was observed in the activities of the three *Aspergillus* species tested under the conditions of this study.

Table I. Effect of media on inhibition of *Phytophthora palmivora* by *Aspargillus* species

Medium	*Growth inhibition (%)
Carrot meal agar	66.1a
Cassava dextrose agar	67.1a
Corn meal agar	67.4a
Potato dextrose agar	68.1a
Malt extract agar	70.0a

*Results are means of three replicates

Means followed by the same letters are not significantly different (P=0.05) (DMRT)

Nutritional factors

The three *Aspergillus* species tested in this study displayed various levels of antagonism against the cocoa pod pathogen with respect to different carbon sources tested. *A. flavus* and *A. fumigatus* utilized pectin best in the M9 medium while *A. repens* utilized glucose best (Fig. 1). Though the *Aspergillus* species showed preference for one carbon source over another, there was no significant difference ($P < 0.05$) in their antagonistic activities against the cocoa pod pathogen. The eight nitrogen sources tested also affected the antagonistic activities of the three *Aspergillus* species. The test organisms showed strongest antagonism when $NH_4H_2PO_4$ was used as the source of nitrogen, followed by $Ca(NO_3)_2$, NH_4Cl and urea in that order (Fig. 2). *A. niger* displayed the strongest antagonism against *P. palmivora* in each of the nitrogen sources tested. The differential utilization of the nitrogen sources did not, however, amount to any significantly different level of antagonism of the pathogen by the test organisms.

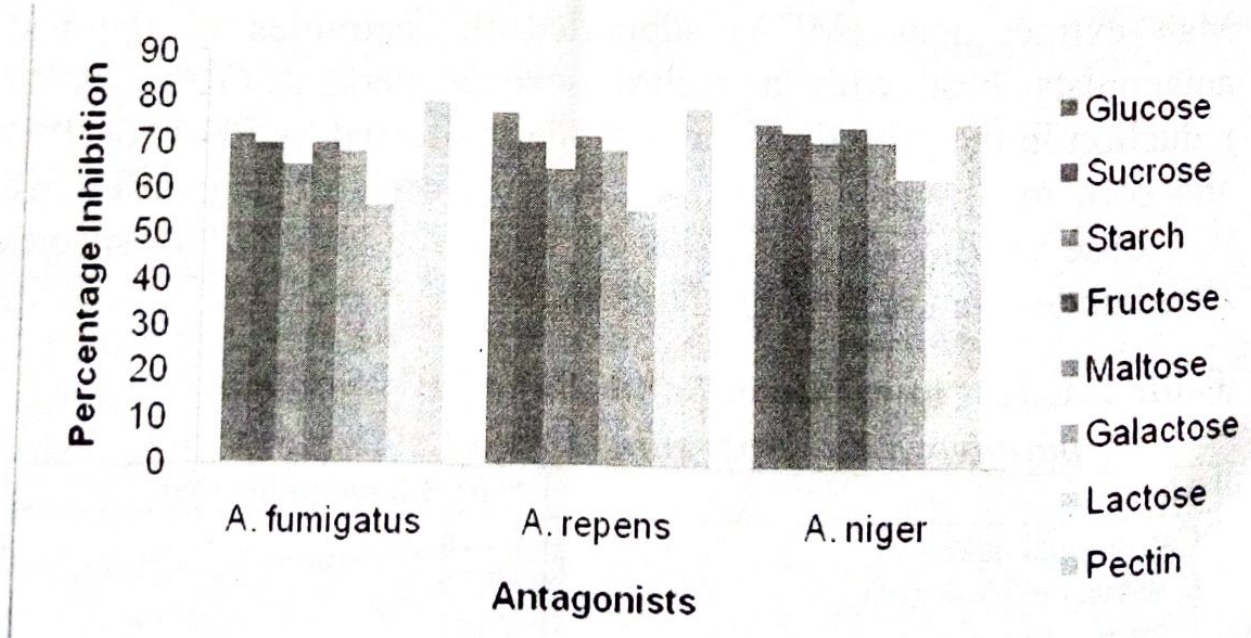

Fig. 1. Effect of carbon sources on inhibition of *Phytophthora palmivora* by three *Aspergillus* species in dual culture

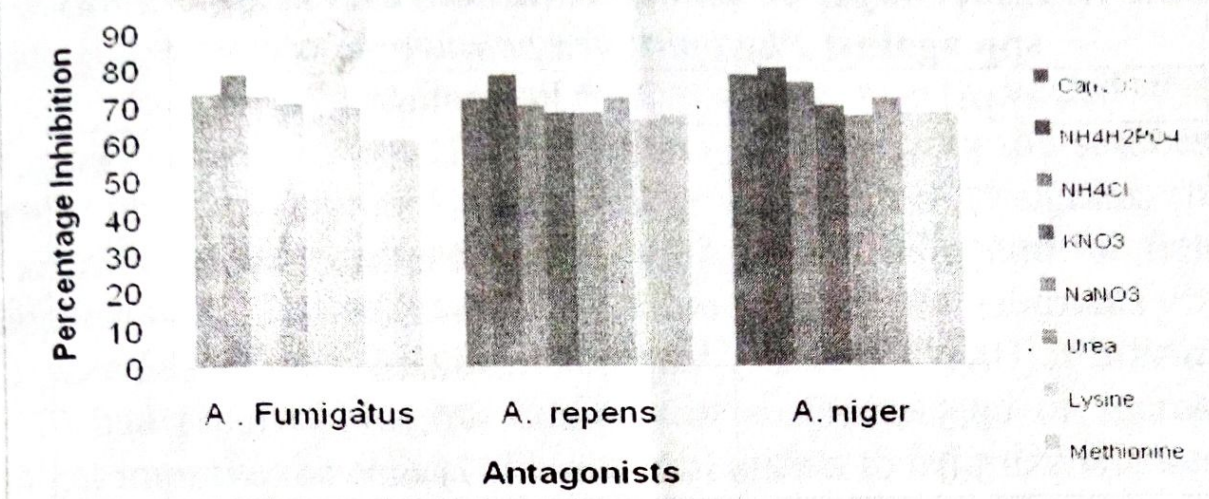

Fig. 2. Effect of nitrogen sources on inhibition of *Phytophthora palmivora* by three *Aspergillus* species in dual culture

Environmental factors

The antagonistic activities of *Aspergillus* species against *P. palmivora* was found to be affected by the pH of the medium used. The effect of pH of the medium produced significant difference in the level of antagonism displayed by the *Aspergillus* species ($P < 0.05$) (Table II). All the test organisms caused very strong inhibition of the pathogen in acidic medium. The observed antagonism decreased with increase in pH. Also, *A. niger* produced the strongest inhibition followed closely by *A. fumigatus*. Result is the mean of the three *Aspergillus* species.

Temperature was also observed to significantly affect ($P < 0.05$) the antagonistic activities of the three *Aspergillus* species against *P. palmivora* (Table III). Inhibition of the pathogen was observed at all the temperature levels tested (15, 20, 25, 30 and 35°C). Pathogen inhibition was most effective at 30°C and dropped above this level. There was no significant difference in pathogen inhibition between 20° and 30°C.

Table II. Effect of pH on antagonistic activities of *Aspergillus* spp against *Phytophthora palmivora*

pH	* Percentage Inhibition
8	41.5a
7.5	43.9ab
7.0	48.9b
6.5	54.9c
6.0	62.4d
5.5	70.1e
5.0	71.6e

*Mean of three replicates

Means followed by different letters differ significantly (P=0.05) (DMRT)

Table III. Effect of temperature on antagonistic activities of *Aspergillus* species against *Phytophthora palmivora*

Temp (^{0}C)	* Percentage inhibition
15	43.3b
20	64.6c
25	68.1c
30	68.6c
35	35.9a

*Mean of three replicates

Means followed by different letters differ significantly (P=0.05) (DMRT)

DISCUSSION

The results of this study showed that the media tested namely malt extract agar, potato dextrose agar, cassava dextrose agar, carrot meal agar and corn meal agar all supported good growth of both the pathogen and the antagonists. This suggests that the media may be handy when formulating media for biocontrol of *P. palmivora.* According to Drenth and Sendall (9), *P. palmivora* grew very well on nutritionally rich media. These media may have assisted the

establishment of the antagonists and thereby hindered the establishment of the pathogen in this study.

Results of the carbon and nitrogen tests revealed that *Aspergillus* species had some preference for some of the sources. This observation can prove important in the designing of experiments for mass production of biocontrol inoculum for field application. The utilization of some of these nutrients by *Aspergillus* species had been reported (10 and 11). Hjorth *et al.* (12) had reported that the addition of surfactant and or sucrose might improve the ability of biocontrol agents to colonize witches' broom fungus and promote sporulation in the field. The effect of nutrition on fungi in addition to inoculum biomass production also includes sustaining biological activity (6 and 13). Among the nitrogen sources which enhanced growth of the antagonists are ammonium hydrogen phosphate, calcium nitrate, ammonium chloride and urea. These compounds are common ingredients of commercial fertilizers. Simon and Sivasithamparam (14) reported that the ammonium fertilizer used in the Western Australian wheat belt had suppressive effect on the take-all wheat fungus. Since urea is commonly used as nitrogen fertilizer in agricultural practices, the utilization of the fertilizer might be useful in the control of the sterile red fungus (15).

Environmental factors such as pH and temperature were found in this study to affect the inhibitory potentials of *Aspergillus* species against *P. palmivora.* Strong inhibition was observed in the acidic medium. This result was in agreement with the report that the optimum pH for inhibitory activity of biocontrol agents was approximately pH 5 (16 and 17). The effect of pH on nutrient utilization might be due to the changes in the bonding potentials of various compounds and differential permeability of cell membrane at different pH levels (6). For instance, the cells of *Zygorrhynchus moelleri* were reported to be permeable to glucose and acetate only at pH 6.8 whereas at pH 3.4 the cells were permeable to all the tricarboxylic acid cycle intermediates but not to acetate. The antifungal activities of all the three *Aspergillus* species were

observed between 15 - 35°C. The optimum antifungal activity varied from 25 - 30°C but dropped below or above 15 and 35°C, respectively (18). It was observed that the broad temperature range (15 -30°C) for antifungal activity of the red sterile take-all wheat fungus enhanced its potential to be used as a biocontrol agent in the Western Australian wheat field. It is very important that the factors that may affect the activities of all biocontrol agents be understood with a view to maximizing the application of this technique for plant disease control. In this way the hazards of environmental and ecosystem disruption will be minimized.

CONCLUSION

- Biological control of diseases in plants can be regarded as one of the safest plant disease control measure.
- It is known to be environmentally friendly and economically viable.
- The findings of this study support the use of three *Aspergillus* species namely *Aspergillus fumigatus, A. repens and A. niger* as biological control agents against the cocoa black pod pathogen, *Phytophthora palmivora*.
- For maximum production of inoculum biomass it is important to ensure that all the necessary factors are provided as reported in this study including nutritional and environmental factors.

REFERENCES

1. Appiah, A.A., Flood, J., Bridge, P.D., Archer, S.A. Inter- and intra- specific morphometric variation and characterization of *Phytophthora* isolates from cocoa. Plant Pathology. 52 : 168 - 180. 2003.
2. Erwin, D.C., Ribeiro, O.K. *Phytophthora* Diseases Worldwide. American Phytopathological Society Press. St Paul, USA. 1996.

3. Iwaro, A.D., Screenivasan, T.N., Umaharan, P. *Phytophthora* on cocoa: Influence of pod morphologyical characteristics. Plant Pathology. 46 : 557 - 565. 1997.
4. McMahon, P., Purwantara, A. *Phytophthora* on cocoa. *In*: Diversity and Management of *Phytophthora* in Southern Asia. Andre Brenth, David. I. Guest (eds.) ACIAR Monograph. P. 114. 2004.
5. Adebola, M.O., Amadi, J.E. Screening three *Aspergillus spp.* for antagonistic activities against the cocoa black pod organism (*Phytophthora palmivora)* Agric. Biol. J.N. America. 1 (3) : 362 - 365. 2010.
6. Shankar, M., Kurtboke, D.I., Sivasithamparam, K. Nutritional and environmental factors affecting growth and antifungal activity of a sterile red fungus against *Gaeumanomyces graminis* var. *tritici.* Can. J. Microbiol. 33 : 515 - 519. 1994.
7. Upadhyay, R.S., Rai, B. Studies on antagonism between *Fusarium udum* Butler and root region microflora of pigeon pea. Plant Soil. 101 : 76 - 93. 1987.
8. Whipps, J.M. Effect of media on growth and interactions between a range of soil-borne glass house pathogens and antagonistic fungi. New Phytol. 107 : 127 - 142. 1987.
9. Drenth, A., Sendall, B. Isolation of *Phytophthora palmivora* from infected Plant Tissue and soil, and Principles of species identification. Diversity and Management of *Phytophthora* in South East Asia . A. Drenth, D. Guest (eds.) ACIAR monograph. P. 114. 2004.
10. Ghisalberti, E. L., Sivasithamparam, K. Antifungal antibiotics produced by *Trichoderma* spp. Soil Biol. Biochem. 23 : 1011 - 1020. 1991.
11. Urquhart, E.J., Menzies, J.G., Punja, Z.K. Growth and Biological activity of *Tilletiopsis* species against powdery mildew on green house cucumber. Phytopatho. 84 : 341 - 351. 1994.

12. Hjorth, S., Pomella, A.W.V., Hockenhull, J., Hebbar, P.K. Biological control of Witches` Broom disease, *Crinipellis perniciosa*, with the co-evolved fungus, *Trichoderma stromaticum*: Testing different delivery regimes. Fourteenth Int. Cocoa Res.Conf. PP. 691 - 697. 2003.
13. Maplestone, P.A., Whipps, J.M., Lynch, J.M. The effect of peat-bran inoculum of *Trichoderma* species on biological control of *Rhizoctonia solani* in lettuce. Plant Soil. 136 : 257 - 363. 1991.
14. Simon, A., Sivasithamparam, K. Effect of crop rotation, nitrogen fertilizer and lime on biological suppression of the take-all fungus. *In*: Biological Control of Soil-borne Plant Pathogens. D. Horn (Ed.) Commonwealth Agricultural Bureau International, Wallingford, U.K. PP. 215 - 226. 1990.
15. Wilson, J. R. Advances in Nitrogen Cycling in Agricultural Ecosystem. Proceedings of the symposium on advances of Nitrogen cycling in agricultural Ecosystem. Commonwealth Agricultural Bureau International, Wallingford, U.K. 1988.
16. Reddy, M.C., Hynes, R.K. Relationship between *in vitro* growth inhibition of pathogens and suppression of pre-emergence damping-off and post emergence root rot of white bean seedlings in the green house by bacteria. Can J. Microbiol. 40 : 113 - 199. 1993.
17. Sudirman, L.I., Iraqi, A.I.H., Febure, G.L.E., Kiffer, E., Botton, B. Screening of some basidiomycetes for biocontrol of rubber tree parasites. Mycol. Res. 96 (8) : 621 - 625. 1992.
18. Sutton, J.C., Peng, G. Biocontrol of *Botrytis cinerea* in strawberry leaves. The American Phyto.Soc. 83 (6) : 615. 1993.

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