EFFECTS OF NFFM (*AZADIRACHTA INDlCA)* EXTRACTS ON MYCELIA PRODUCTION AND SPORULATION OF *RHIZOPUS STOLONIFER* AND *BOTRYODIPLODIA THEOBROMAE*

Oduwobi, O. A.

Abstract

The effects of concentrations of Neem leaf and seed extracts on mycelia production and sporulation of ***Rhizopus Stolonifer*** (lilir.) and ***Botryodiplodia the.ohromae*** (Pat.) were investigated. The concentrations range of 50g/l to 400g/l of the extracts significantly reduced the mycelia production and sporulation rates of the fungi. The higher concentration of 400g/l of the Neem leaf extract inhibited mycelia and spore production of the fungi, while same higher concentration of Neem seed extract significantly retarded mycelia and spores production of the fungi. The difference in action of various Neem extracts could be attributed to higher fungicidal action in the Neem leaf extract than in the Neem seed extract.

Introduction

Neem ***(Azadirachta indica)*** a member of family Meliaceae is commonly grown for providing shade and for its numerous medicinal properties (Dutta, 1979). Neem seeds are sources of margose oil and Neem oil. The aromatic oils from the Neem seeds contain sulphur compounds used as anthelmintics (McGraw-Hill, 1969).

Trease and Evans (1978), and Egmond (1998), reported that, powdered root bark of Neem is astringent and febrifuge. Egunjobi and Afolami (1976), reported an effective control of the nematode, ***Pratylenchus brachyurus*** using Neem leaf extracts in maize field. Medicinal plants such as Neem have been found to possess some therapeutic agents such as tannins, alkaloids, saponins and cardiac glycosides (Sofowora, 1984). These therapeutic agents have antimicrobial properties (Ekpe, Ebana and Madunagu, 1990). With the indications given in the literature (Srivastava and Tandon, 1968; Eckert, 1975; Pandey and Trivedi, 1979), that ***Rhizopus stolonifer and Botyrodiplodia theobromae*** are responsible for post-harvest diseases in many food and cash crops resulting in huge spoilage of the crops and economic loss, it was considered worthy to determine the effects of Neem extracts on growth and sporulation of the two fungi.

Materials and Methods *Sources of Sample*

Fresh and healthy Neem leaves and seeds were collected from growing Neem trees in Ilaro, Ogun State.

Characterisation of Fungi Isolates

The fungi used for the study were isolated from infected yam tubers and pawpaw fruits. The fungi were isolated according to the methods of Booths (1971) and identified using conventional methods, thereafter, their identity was confirmed

by IM1 Mycological Institute, London. Fungal pathogenceity was carried out using the methods of Booths (1971). Stock cultures were maintained on commeal agar slants at room temperature (28-31°C). Isolates were aseptically taken from stock cultures when needed.

Preparation ofNeem Extracts

Fresh Neem leaves were used for preparing aqueous extracts, by cutting fresh Neem leaves into pieces, blended in cold distilled water for 30 seconds in a warning blender and sieved through a Buckner funnel. Each extract was prepared in concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4kg fresh leaves/litre of distilled water. Similar concentrations ofNeem seed extract were prepared from crushed healthy seeds. The extracts were filtered into separate Erlenmeyer's flasks and stored in a refrigerator.

Effect of Neeni Leaf and Seed Extracts on Mycelia Production and Sporulation of Test Fungi

Aqueous Neem leaf and seed extractions of 50gm/l to 400hm/l concentrations were incorporated into sterile petridishes of Potato Dextrose Agar (PDA) separately. Similarly, different concentrations of aqueous Neem leaf and seed extracts were incorporated into basal synthetic medium inErlenmeyer's flasks. The petridishes and the flasks were inoculated separately by aseptically introducing $5mm^2$ of each fungal disc of 10-day old pure cultures of Rhizopus and Bortryodiplodia with a sterile $5mm^2$ cork-borer.

A control was set up, in which the PDA agar media plates and the broth medium in the flask, had no incorporation of Neem leaf and seed extracts of different concentrations, but were inoculated aseptically with 5mm2 of each fungal disc of 10-day old pure cultures of the test fungi. Five replicates were made for each sample. All the inoculated petridishes and Erlenmeyer's flasks were incubated at laboratory temperature of 20 - 31°C for 10 days.

The effects of the Neem leaf and seed extracts concentrations were determined on dry mycelia and spores produced by the test fungi, suing the methods of Levy, Campbell and Blackburn (1973) and Ogundana (1989). The results were statistically analysed at $P = 0.05$ using analysis of variance. Parker's (1979) method was used to calculate standard variation.

Results

Effects of Neem Leaf and Seed Extracts on Mycelia Production of Rhizopus and Bortryodiplodia

Neem From	Extract Application (g/1)	rate Mycelia dry Rhizopus	(g) Weight Bortryodiplodia
Leaf	0 (Control)	3.50	3.50
	50	2.30	2.10 .....
	100	1.80	1.60
	200	1.10	1.00
	300	0.40	0.15
	400	0.00	' 0.00
Seed	0 (Control)	3.50	3.50
	50	3.10	3.00
	100	2.75	2.65
	200	2.45	2.20
	300	2.10	1.90
	400	1.65	1.30

Overall mean ± SEM

The results in table 1, showed the effect of Neem lead and seed extract on mycelia dry weight determined. As the concentration of the Neem leaf and seed extracts increased, the inhibitory effect of the extract also increased. However, the Neem leaf extract showed more inhibitory effect on the fungi than the Neem seed extract. While 400g/l concentration of the Neem leaf extract inhibited mycelia production in the fungi, slight mycelia production occurred at the same concentration of the Neem leaf seed. The fungi produced mycelia favourably in the control media in which no Neem leaf and seed extracts were incorporated.

Table 2: Effect of Neem leaf and Seed Extracts on Sporuiation of Rhizopus and Bortryodiplodia

Neem From	Extract Application (g/1)	rate Spore density (xlO^{-6})	perculture
Leaf	0 (Control)	486.4 ±3.0	486.4 ±3.0
	50	322 ±2.4	272.2 ± 1.6
	100	126 ±2.4	84.2 ± 1.2
	200	75.5 ±3.2	30.5 ±2.1
	300	28.6 ± 1.7	** Nil
	400	** Nil	** Nil

* Spore density ± standard error of mean ** No Sporuiation

The test fungi produced spores favourably in the media containing Neem leaf and seed extract at low concentrations of 50g/l to 200 g/1, however, higher concentration the Neem leaf extracts of 300 to 400 g/1 retarded and finally inhibited sporulation of the test fungi (table 2). The Neem leaf extract exhibited higher inhibitory effect of sporulation than the Neem seed extract. The two fungi sporulation favourably in the control medium in which no Neem extract was incorporated.

Discussion

The results of this investigation has shown that the Neem leaf aqueous extracts of 300g/l concentration and above are effective in inhibiting mycelia and spores production in Rhizopus and Bortryodiplodia. The fungicide effect of the Neem seed extracts at 50 to 400g/l concentrations retarded the mycelia and spores production in the test fungi and did not inhibit totally their production. The more effective fungicide action of the Neem leaf extract may be attributed to high concentration of ***Azadirachtin*** in the leaf than in the seeds (Rgunjobi and Larinde, 1976). The Neem leaf extracts were reported by Egunjobi and Larinde, (1976), to be effective nematicidc in controlling populations of ***Pratylenchus brachyrus*** affecting the growth and yield of maize. The observation on the fungi activity of the Neem extracts is in agreement with those of Egunjobi (1998), when he recommended Neem seed and leaf extracts as effective insecticides powerful enough to protect crops with ***Azadirachtin*** as the active chemical ingredient which is environmental friendly and its concentration in the food chain not posing any danger to man and other animal life.

The findings of this study could assist in reducing post harvest storage diseases of common food crops such as timber, legumes, cereals, fruits and vegetables which are highly susceptible to Rhizopus and Bortryodiplodia attack. Neem leaf extracts had been ascertained to be non-toxic to man as they are consumed in most of West Africa and India as multipurpose curative concotion (Egunjobi and Larinde, 1976; Duta, 1979).

References

Booth, C. (1971). Introduction to general laboratory methods. In ***methods and microbiology.*** Academic Press: London.

Dutta, A. C. (1979). ***Botany for degree students 5th edition.*** Oxford University Press: London.

Eckert, J. W. (1975). ***Postharvest diseases of fresh fruits and vegetables.*** Etilogy and control. Symposium on post-harvest biology and handling of fruits and vegetables. The Avi Publishing Company Inc. Connecticut pp 84 - 98.

Egmond, H. P. (1998). Fungicides and aflatoxins Bl, B2 and G1 and G2 extractions. Food laboratory 18: 48 - 53.

Egunjobi, 0. A. and Larinde, M. A. (1976). Nematodes and maize growths in Nigeria. ***Nematologia Mediterranean*** 3: 65 - 73.

Egunjobi, O. A. and Afolami, S. O. (1976). Effect of Neem (***Azadirachtin Indica)*** leaf extracts on population of ***Pratylenchus brachyurus*** and on the growth and yield of maize 22: 125 132.

Ekpe, E. D.; Ebana, R. U. and Madunagu, B. E. (1990). Antimicrobial activity of four local medicinal plants on pathogenic bacteria and phytopathogenic fungi. ***West African Journal of Biological and Applied Chemistry*** 35: 2 - 5.

Levy, J., Campbell, .1. R. and Blackburn, T. II. (1973). ***Introductory microbiology.*** John Wiley and Sons, Inc: New York.

McGraw Hill Book Company (1969). ***Encyclopaedia of science and technology.*** McGraw Hill Company: New York.

Ogundana, S. K. (1989). ***Introductory microbiology: A laboratory manual.*** Obafemi Awolowo University Press Ltd., lie — Ife.

Pandey, S. N. and Trivedi, T. (1979). ***A textbook of botany.*** Vikas Publishing House Ltd., India.

Parker, R. E. (1979). ***Introductory statistic for biology.*** Edward Arnold London, 122 pp.

Sofowora, E. A. (1984). ***Medicated plants and traditional medicine in Africa.*** John Wiley and Sons: New York.

Srivasta, M. P. and Tandon, R. N. (1968). Influence of temperature on Bortryodiplodia rot of citrus and Sapodilia. India: Phytopathology 21: 195 - 197.

Trease, G. E. and Evans, W. E. (1978). ***Pharmacognosy.*** 11th edition. Bailliers Tindall: London, pp 60 - 75.