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Inhibition of Food Spoilage Organisms with Neem Seed Oil

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The effect of Neem Seed (*Azadirachta indica*) oil on the growth of microorganisms (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium digitatum*) isolated from deteriorating foods was tested in the laboratory. The minimum inhibitory concentration was 0.06 – 0.18% (v/v) while the minimum bacteriocidal concentration stood at 0.09 % – 0.18% (v/v). Neem seed oil may play a useful role in food preservation strategies.

Keywords: Inhibition, Food Spoilage, Neem Seed Oil, Preservation, Organisms. Deterioration.

Neem (*Azadirachta indica*) is native to India and has grown over centuries in various temperate and tropical zones, Nigeria inclusive. The seeds contain substantial amount of essential oil with historical and scientific validity as medicals (Bidiosa *et al.*, 2002; Almas, 1999; Ramakrishna *et al.*, 1993). Food preservation with chemicals often are limited by possibility of bioaccumulation and the resultant toxicity to man hence the search for natural preservatives of plant origin. Various plant extracts and spices have antioxidant and/or antimicrobial properties which enhance shelf stability and safety of food products (Akujobi *et al.*, 2006; Ejechi and Akpomedaye, 2005; Ibekwe *et al.*, 2001; Almas, 1999; Ejechi and Souzey, 1999; Nwafor, *et al.*, 1998 and Fadeyi and Akpan, 1989). The aim of this study is to extend the antimicrobial knowledge of neem seed oil to focus on its potential use as a food preservative.

MATERIALS AND METHODS

Extraction of Neem Seed Oil

This was carried out by the method of Ramakrishna (1993). Powdered neem seed was extracted with hexane (1:5) for 24h at room temperature with intermittent shaking. The filtrate was concentrated in a waterbath at 80°C to a viscous golden colored oil.

Isolation and Identification of Isolates

Using standard methods reported by Cheesbrough (2002, 1999), microorganisms were isolated from various deteriorating foods (garri, bread and cake). The pure cultures were maintained on Nutrient Agar (bacteria) and Potato Dextrose Agar (fungi) at 4°C till needed.

Determination of Minimum inhibitory concentration (MIC) of Neem seed oil on isolates

Molten Nutrient agar (bacteria) and Potato Dextrose agar (fungi) were incorporated with the neem seed oil to give a concentration of 0.08, 0.14, 0.22 and 0.25 (% v/v). Control plates received no neem seed oil treatment. The plates were inoculated with *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *A. flavus*, and *Penicillium digitatum* and incubated at room temperature (30 ± 2°C) for 48h. The MIC was taken as the minimum concentration that prevented growth for 48h.

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Determination of Minimum Bacteriocidal concentration (MBC) of Neem seed oil on isolates

Another set of plates treated as before were incubated for up to 7 days in order to determine the minimum bacteriocidal or fungicidal concentration. Thereafter the inocula from plates that showed no growth were transferred to fresh, untreated NA or PDA to check for resumption of growth. The MBC/MFC was taken as the minimum concentration where growth failed to resume.

RESULTS AND DISCUSSION

All the tested bacteria had MBC of 0.14% (v/v) and MIC of 0.08% (v/v) while the tested fungi had MBC of 0.09% (v/v) and MIC of 0.06% (v/v). All the tested isolates were inhibited at 0.25% (v/v) of neem seed oil(Table 1).

Neem seed oil extract showed both antibacterial and antifungal effects on the food spoilage isolates. Extracts of higher plants usually contain considerable amounts of growth inhibitory phenolic acids which are important in plants' disease resistance (Akujobi *et al.*, 2006). Neem exhibits antimicrobial activity due to the presence of phenolic compounds and essential oils. Neem seed oil disrupts cell membrane synthesis in little concentrations hence its application in medical, agricultural and household products. Its active components are nimbin, nimbidin and nimbinin (Biswa *et al.*, 2002; Rao *et al*., 1995; Butler *et al.* 1992). The antibacterial and antifungal effects of neem seed oil on the tested microorganisms is in agreement with reports by previous workers on the inhibitory effects of plant extracts on various microorganisms (Akujobi *et al.*, 2006; Ibekwe *et al.*, 2001; Almas, 1999; Ejechi and Akpomedaye, 1999; Ejechi and Souzey, 1998; Nwafor *et al.*, 1998; Fadeyi and Akpan, 1989). The results indicate that at concentration of 0.18 (%v/v), neem seed oil possess antimicrobial effects. It could this be incorporated, either alone or in combination with various hurdles, in food preservation.

Table 1. Antimicrobial activities of neem seed oil extract

Test Bacteria	MBC/MFC (%v/v)	MIC (%v/v)
Bacillus cereus	0.14	0.08
B. subtilis	0.14	0.14
Staphylococcus aureus	0.18	0.14
Escherichia coli	0.18	0.14
Test Fungi		
Aspergillus niger	0.18	0.12
A. flavus	0.09	0.06
Penicillium digitatum	0.09	0.06

REFERENCES

1. Akujobi, C. O., Ogbule, J. N. and Uchegbu, U N: Antibacterial activities and preliminary phytochemical screening of *Veronica amygdalina* and *Citrus aurantifolia*. *Nig. J. Microb*. 2006; **20**(1): 649 – 654.
2. Almas, K. The antimicrobial effects of extract of neem *Azadirachta indica* and *Solvadora persica* chewing sticks. *India J. Dent. Res.,* 1999; **10:** 23-26.
3. Biswa, K., Ishita, C., Ranajit, K. B., Uday, B. Biological activities and medical properties of neem (*Azadirachta indica*). *Current Science*. 2002; **82**(11): 1336 – 1345.
4. Butler, L. G., Hemingway, R. W., Lak, P. E. *Plants polyphenol*. 1992; Plenum Pres, New York. 693.
5. Cheesbrough, M. *District laboratory practice in Tropical Countries.* 1999; Part 1. Cambridge University Press, UK. 350.
6. Cheesbrough, M. *District laboratory practice*

in Tropical Countries. 2002; Part II. Cambridge University Press, UK. 320.

7. Cowan, S. T. *Manual for the identification of medical bacteria.* 1985; (2nd ed.). Cambridge University Press, London. 124.
8. Ejechi, B. O. and Akpomedaye, D. E. Activity of essential oil and phenolic acid extracts of pepper fruit (*Dannetia tripetala G. Barker anonceceae*) against some food borne microorganisms. *Afr. J. Biotech.,* 2005; **4**(2): 258-261.
9. Ejechi, B. O. and Souzey, J. A. Inhibition of biodeterioriation of yam tuber *Dioscorea rotundata* prior to storage with phenolic extract of *Acalyha hispidi* Burm F. leaves. *J. stored products Res.,* 1998; **35:** 127-134.
10. Fadeyi, M. O. and Akpan, U. E. C. Antibacterial activities of leaf extract of *Eugenia unilora*. *Phytotherapy Research,* 1989; **4:** 154-155.
11. Ibekwe, V. I., Nnanyere, N. F. and Akujobi, C. O. Studies on antibacterial activity and phyto-chemical qualities of extracts of orange peels. *Int. J. Environ. Health and Human Dev*., 2001; **2**(1): 41-46.
12. Nwafor, O. E., Okoko, F. J. and Ejechi, B. O. Activities of Elimi (*Piptademiastrium africa*) extract against *Microsporium* sp. A preliminary report. *Nig. J. Envt. Microb.,* 1998; **1**(1): 65-68.
13. Ramarkrishna, G., Prasad, N. B. L. and Azeemoddin, G. Cold processing neem seed. J. N. T. U. Technological Research Institute. *Proceedings of the Academy of Environmental Biology, India* (Unpublished). 1993; 138.
14. Rao, D. R., Ruben, R., Nagasampazi, B. A. Development of combined use of neem (*Azadirachta indica*) and water management for the control of culcine mosquitoes in rice fields. *Med. Vet. Entomol. J.,* 1995; **9**(1): 25-33.
15. Scott, A. C. Laboratory control of antimicrobial therapy. In: Coller, J. G., Gugrid, S. P., Fraser, A. G. and Marmiam, B. P. (editors) *Practical Medical Microbiology*. 1989; 13th ed. Churchill Livingstone, Edinburgh, U. K. 161-181.