



ANTIFUNGAL ACTIVITIES OF SEED OIL OF NEEM (*Azadirachta indica* A. Juss.)

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Abstract

This study was carried out to investigate the effects of Neem (*Azadirachta indica* A. Juss.) seed oil on four fungi, namely: *Fusarium* sp. *Rhizopus* sp. *Curvularia* sp. and *Aspergillus* sp. which are pathogenic in nature. The crude extract of neem seed was obtained using petroleum ether. The extract inhibited the growth of all the fungi tested. The extent to which the extract inhibited the growth of the fungi was observed to be different for each of the fungi. Growth inhibition was highest in *Curvularia* sp. (which did not grow beyond the initial point of its radial growth before introduction of the extract), while the lowest effect was observed in *Rhizopus* sp.

Keywords: *Azadirachta indica*, Antifungal, Neem seed oil, *Curvularia* sp.

1.0 Introduction

Fungi are the major cause of plant diseases and are responsible for large scale harvest failures in crops like maize and other cereals all over the world (Suleiman and Omafè, 2013). The fungi genera typically found in stored grains are *Aspergillus*, *Penicillium*, *Fusarium* and some xerophytic species, several of them with capabilities of producing toxins (Castellari *et al.*, 2010). Seed fungi especially species of *Aspergillus*, *Diplodia*, *Penicillium*, *Fusarium*, *Trichoderma* and a number of phycmycetes affect the seed of all forest species. Previous studies by Pacin *et al.* (2009) identified *Aspergillus* and *Fusarium* as mycotoxigenic species in stored grains. So also, their mycotoxins and fumonisins were reported in different concentrations by Moreno *et al.* (2009) in stored grains.

Chemical control of fungal pathogens has been of help in the increase of crop yield. However, usage of these chemical products is being discouraged due to the resultant environmental pollution which leaves toxic residues in soil, water and food. Some chemicals are also harmful to non-target organisms and this leads to ecological imbalance and development of fungicidal resistant strains. All these limitations call for an alternative plant disease management strategy such as biological control (Gardener and Fravel, 2002; Lokeshia and Benagi, 2007). Biological control method is preferred because it is selective with no side effects, and is relatively cheap. Moreover, resistance to biological control is rare and biological control agents are self-propagating and self-perpetuating.

Neem (*Azadirachta indica*) is a widely prevalent tree, mainly cultivated in India subcontinent (Karl, 1997). Various parts of the tree have been used as traditional Ayurvedic medicine in India (Brahmachari, 2004). Neem oil in particular was widely used as a traditional medicine in India, Sri Lanka, Burma, Thailand, Malaysia and Indonesia and already has more than 2000 years history. Neem oil was often administered orally, for deworming and constipation, and is applied topically to relieve rheumatism, ulcer, itching and cure chronic skin diseases (Aggarwal and Dhawan, 1995). There is evidence that neem oil has acaricidal, antibacterial, antifungal, antimalarial, antiparasitic, anti-inflammatory as well as immunomodulatory properties in different animal species (Mulla and Su, 1999; Biswas *et al.*, 2002; Brahmachari, 2004; Gossé *et al.*, 2005; Du *et al.*, 2007, 2008, 2009; Xu *et al.*, 2010; Zhang *et al.*, 2010). Due to its efficacy, biodegradability and minimum side effects, azadirachtin, a tetranortriterpenoid obtained from neem seeds, has emerged as a natural biopesticide (Locke, 1995; Martinez, 2002).

The objective of this study was to evaluate the efficacy of neem seed oil against selected pathogenic fungi species that constitute major threats to agricultural products in Nigeria.

2.0 Materials and Methods

2.1 Media Preparation and Culture

Potato Dextrose Agar (PDA) combined with Chloramphenicol was used for fungi isolation and enumeration. Plates were incubated at room temperature for 3–5 days. For identification purpose, the microscopic and macroscopic features of the hypha mass, morphology of cells and spores, nature of the fruiting bodies, among other criteria were used according to the method of Uzma and Shahida (2007).

2.2 Preparation of Neem Seed Oil

Seed oil extraction was carried out using the method of Eksteen *et al.* (2001) with slight modifications. Seeds from neem trees were obtained from the campus of Kwara State College of Education, Ilorin, Nigeria. The shells were separated from the kernel and were sun-dried. The shells were blended, air-dried and later oven-dried, in order to remove every moisture trace that might remain. The powder of the neem seed kernel (250g) obtained was soaked in one litre of petroleum ether and placed on a shaker for about 72 hours. Using a muslin cloth, the mixture was filtered and the cake was kept. The filtrate obtained was made to undergo distillation to separate the oil obtained from the neem seed powder from the solvent.

2.3 Tests for Sterility of Extract

About 1ml of the oil obtained was inoculated onto 4 sterile petri dishes. About 9ml of agar medium were then poured into the plates and mixed and then allowed to set. Subsequent incubation showed no growth on the mixture thus confirming the sterility of the extract, the environment and the apparatus used.

2.4 Determination of Effectiveness of Neeem Seed Oil on the Test Organisms

The neem seed oil was used directly in its pure form without varying its concentration. The use of 100% concentration of the test oil was informed by the outcome of a preliminary survey of its efficacy at different concentrations which led to the exclusion of lower concentrations on account of their ineffectiveness. Application of extracts against the test organisms was carried out using the pour plate and the cork-boring methods, both of which were undertaken according to the method of Suleiman and Omafè (2013). Two control experiments were also set up using distilled water on the one hand and petroleum ether on the other hand in place of the test oil.

2.5 Determination of Percentage Inhibition

The diameter of radial growth of each test organism on the control plates was measured at regular intervals of 24 hours for 5 days and the mean was calculated and designated as **X**. The radial growth on each experimental plate was also measured and the mean calculated and designated as **Y**. The percentage inhibition of each organism by the test oil was calculated using the conventional formula as follows:

$$\frac{(X-Y)}{X} \times 100\%$$

[X= mean radial growth on control plates. Y= mean radial growth on experimental plates]

3.0 Results

3.1 The Effects of the Neem Seed Oil on the Test Fungi

From the results of the experiment, neem seed oil can be observed to have completely inhibited the growth of *Curvularia* sp. and substantially retarded the growth of *Aspergillus* and *Fusarium* species. However, the treatment had no notable effect on *Rhizopus stolonifer* (Tables 1 and 2).

Table I: 5-Day radial growth of some fungi species following treatment with neem seed oil

Organisms	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	MEAN (mm)
<i>Aspergillus sp</i>	2.05	3.00	3.00	3.05	3.10	2.84
<i>Curvularia sp</i>	1.50	1.50	1.50	1.50	1.50	1.50
<i>Fusarium sp</i>	2.05	2.60	3.60	3.80	3.85	3.18
<i>Rhizopus sp</i>	6.00	8.70	11.00	13.50	16.00	11.00

Table 2: 5-Day radial growth of some fungi species in the control set up*

Organisms	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	MEAN (mm)
<i>Aspergillus sp</i>	2.50	3.00	3.50	3.90	4.90	3.56
<i>Curvularia sp</i>	1.80	2.10	2.40	2.70	3.05	2.41
<i>Fusarium sp</i>	2.50	3.00	3.40	3.90	4.40	3.44
<i>Rhizopus sp</i>	6.00	8.70	11.40	14.10	15.80	11.20

*Measurements indicate observations in both distilled water and petroleum ether.

A comparison of the results in Tables 1 and 2 shows that neem seed oil contains anti-fungal properties which made it possible for it to successfully retard the growth of the four test organisms used in this experiment. However, the effectiveness of the oil varied across the organisms as indicated by their % inhibition (Table 3). Table 3 shows that the lowest percentage inhibition was recorded in both *Rhizopus* and *Fusarium* species i.e. 1.79 and 7.56 respectively. In contrary, the percentage inhibition observed in *Aspergillus* and *Curvularia* sp were substantial, being 20.22 and 37.76 respectively. Thus, the neem seed oil had the highest inhibitory activity on the growth of *Curvularia* sp and the lowest on *Rhizopus* sp.

Table 3: Comparison of the mean radial growths of organisms in the treatment and control experiments

Organisms	Mean radial growth (mm)			Percentage inhibition (%)
	Treatment	Control	Difference	
<i>Aspergillus sp</i>	2.84	3.56	0.72	20.22
<i>Curvularia sp</i>	1.50	2.41	0.91	37.76
<i>Fusarium sp</i>	3.18	3.44	0.26	7.56
<i>Rhizopus sp</i>	11.00	11.20	0.20	1.79

4.0 Discussion

The results of this study show that petroleum ether extract of neem seed oil had antimicrobial properties against three of the four fungi species studied, *Rhizopus sp* being the only exception. Many workers have reported the use of plant extracts in the control of fungal diseases (e.g. Dubey *et al.*, 2009; Satish *et al.*, 2008). Many phytofungicides have been obtained from a number of plant extracts. These include “Fitoekols-IF” from *Pinus sylvestris* and *Picea abies* greens extract, “Fitosativum” from *Allium sativum* extract, “Fitocapsicum” from *Capsicum annum* extract, “Fitokrisanthemium” from *Chrysanthemum sp.* leaf extract, “Fitoarmoracium” from *Armoracia rusticana* root and leaf extract, “Fitotabacum” from *Nicotiana tabacum* and *N. rustica* extracts, “Fitopelargonium” from *Pelargonium sp.* leaf extract and “Fitosinepium”-from white mustard (*Sinapis alba*) plant and seed extract (Zarins *et al.*, 2009). Citrus fruits have also been acknowledged by Munoz and Marcos (2006) to possess a variety of phytofungicides that help to inhibit fungal growth and development.

Afzal *et al.* (2010) reported *Allium sativum* to have a wide antifungal spectrum that effected 60-82% inhibition in the growth of seed borne *Aspergillus* and *Penicillium* fungi. This was attributed to phytochemical properties of garlic plant, allicin which could decompose into several effective antimicrobial compounds such as diallyl sulphide, diallyl disulphide, diallyl trisulphide, allyl methyl trisulphide, dithiols and ajoene (Salim 2011; Tagoe, 2011).

According to Mulla and Su (1999) and Biswas *et al.* (2002), neem oil, extracted from the seeds of *Azadirachta indica*, has versatile medicinal properties, including antifertility, antifungal, antibacterial, immunostimulant, antipyretic and acaricidal activities. Chloroform extracts and petroleum ether extracts of neem oil have also been found to exhibit potent acaricidal activity against *Sarcoptes scabiei* var. *cuniculi* larvae (Du *et al.*, 2008, 2009). Neem extract was also found by Da-Costa *et al.* (2010) to have inhibited the fungal growth (*i.e.* mycelia dry weight, diameter of colony and growth rate) of *Aspergillus flavus* on solid media at concentrations from 0.5 to 5.0% v/v, although it significantly increased sporulation in the same conditions. Bhutta *et al.* (2001) tested 32 different seed diffusates against *Aspergillus alternata* and *Fusarium solani* and found that the diffusates from *Corriander sativum* and *Memoranda charata* exhibited inhibitory effects at 0.5% and 1% concentrations. Eksteen *et al.* (2001) also tested 11 plant extracts against different pathogenic fungi including *F. oxysporum* and *Rhizopus solani* by the agar dilution method and obtained encouraging results comparable inhibitory effects on mycelial growth with reference to those obtained using Carbendazim and Difenconazole. Similar observations were recorded against *Alternaria solani* by using *Allium cepa* extract (Khallial, 2001).

Locke (1995), Martinez (2002) and Da-Costa *et al.* (2010) all reported that due to the antifungal efficacy of neem seed extract, its biodegradability and minimum side effects, azadirachtin, a tetranortriterpenoid obtained from the seed has emerged as a natural biopesticide. In addition, the percentage inhibition against the tested fungi were found to increase at different rates by increasing the concentration of neem leaf and seed extract with the result that neem seed organic extracts had higher inhibition percentage than that of neem leaf organic extracts.

5.0 Conclusion

From the results of this study, it can be concluded that the antifungal effects of neem seed extract was highest against *Curvularia sp.* (37.76% inhibition) followed by that of *Aspergillus sp.* (20.22% inhibition) and *Fusarium sp.* (7.56% inhibition). The extract had no significant inhibitory effect on *Rhizopus sp.*

6.0 References

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