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*Full Length Research Paper*

# *Azadirachta indica* extracts influenced some pathogenic fungi

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**Active components of neem leaves and seeds were extracted with different methods in order to study the effect of different extract concentrations on the inhibition of some pathogenic fungi. High-performance liquid chromatography (HPLC) was used to identify the active components of neem extract. Highest inhibition percentage of ethanolic neem leaf extract was recorded with *Rhizoctonia solani*, while the lowest was recorded with *Alternaria solani.* A complete inhibition percentage was recorded with 40% ethanolic neem leaf extract of *R. solani* and *Fusarium oxysporum*. The highest inhibition percentages were recorded with *F. oxysporum* (10, 20, 30 and 40%) concentrations of hexane neem leaf extract, while the lowest was recorded with *A. solani*. The highest inhibition percentages were recorded with *R. solani* (10, 20 and 30%) concentrations of methanolic neem leaf extract, while the lowest was recorded with the same mentioned concentration of *Sclerotinia sclerotiorum*. A complete inhibition percentage was recorded with 40% methanolic neem leaf extract of *F. oxysporum* and *R. solani*, while the lowest was recorded with *S. sclerotiorum*. The highest inhibition percentage was recorded with *R. solani* (10 and 20%) concentrations of ethanolic neem leaf extract and the lowest was recorded with *A. solani*. The highest inhibition percentage was recorded with (10, 20, 30 and 40%) hexane neem seed extract of *F. oxysporum.* The lowest inhibition percentages with the same mentioned concentrations were recorded with *A. solani*. The highest inhibition percentage was recorded with (10 and 20%) methanolic neem seed extract of *R. solani.* The lowest inhibition percentage was recorded with *S. sclerotiorum*. The inhibition percentage of tested fungi increased by increasing neem leaf and seed extract by different rates. Also, neem seed organic extracts had higher inhibition percentage than that of neem leaf organic extracts. HPLC chromatogram of neem organic extract showed that nimonol (82%) is the major active component of neem organic extract.**

**Key words:** Neem, extraction, pathogenic fungi, High-performance liquid chromatography (HPLC).

## INTRODUCTION

The neem tree, *Azadirachta indica* A. Juss (Meliaceae), is indigenous to India, and now it is cultivated widely in tropical areas of the world. Various parts of the neem tree have been used for food, medicine and insecticides since ancient times. Many bioactive constituents have been isolated and identified from various parts of neem tree (Koul et al., 1990).

Azadirachtin and other limonoids occurring in the seeds of neem tree show potent insecticidal effects against a wide variety of insect pests and low toxicity to humans. The flower of the neem tree (locally called “dok sadao” in

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Thai) is commonly consumed as a vegetable in Thailand, and it is also known to have some medicinal effects. There are some reports suggesting health uses of the neem tree. Okpanyi and Ezeukwu (1981). reported that the methanol extract of the leaves of the neem tree e-xerts anti-inflammatory, antifungal and antipyretic effects (Trakoontivakorn et al., 1999).

Neem is one of th most important multipurpose tree species, among its uses are prevention of soil erosion and improvement of soil microclimate as well as it is good for fuel and timber wood (Berjak et al., 1995).

Every part of the tree has a great commercial value where the oil extracted from the seeds goes into soap, waxes, lubricants and fuels for lighting and heating. Also, the seed cake is employed as a fertilizer and soil amender. The bark is tapped for gum and extracted for tannins and dental care products. On the other hand, the leaves are sometimes used for emergency livestock feed and the flowers are a prized source of honey (Kumar and Bangarwa, 1996; Gamene et al., 1996).

Triterpenoids from neem leaves and seeds which are obtained by using high-performance liquid chromato-graphy (HPLC) are an important group of constitutive defense substances present at sufficient concentrations to ward off potential plant pathogenic fungi (Grayer and Harborne, 1994).

Extracts of neem leaf, neem oil and seed kernels are effective against certain human fungi including *Trichophyton*, *Epidermophyton*, *Microsporum, Trichosporon*, *Geotricum* and *Candida*. High antimycotic activity with extracts of different parts of neem has already been reported (Schmutterer, 1995).

Soil borne diseases are important biotic constraints in sustainable crop production systems because the com-plexity of the soil environment makes their control with chemical fungicides difficult. *Fusarium* vascular wilt is a soil borne disease caused by *Fusarium oxysporum.* Preplant soil fumigants such as methyl bromide (bromomethane) that have a broad spectrum of activity have been used extensively to protect high-value crops from soil borne pathogens (Biswas et al., 2002).

Subsequent to the isolation of azadirachtin from need seed kernels in 1968, extensive work has been done on the chemistry and pesticidal properties of compounds from the neem tree, *A. indica* A. Juss using HPLC. Infor-mation relating to the antifungal activities of compounds from neem is limited (Parveen and Alam, 1993).

Neem leaves have been shown to possess antifungal activity either by direct soil amendment or as extracts of them, active against a number of phytopathogens (Ryo et al., 2005). They reduced radial growth and spore germination of *Curvularia lunata*, successfully controlled fruit rots of cucurbitaceous plants caused by *F. equiseti* and *F. semitectum*, and significantly reduced fruit rot of tomatoes caused by *Aspergillus flavus* and *A. niger*. Aqueous neem leaf extracts controlled foliar diseases of groundnut, viz., *Puccinia arachidis* and *Mycosphaerella Berkeleyi* (Suresh et al., 1997).

## MATERIALS AND METHODS

### Neem leaves and seeds

Neem leaves *Azadirachta indica* A. Juss were obtained from Arafat area, Saudi Arabia.

### Plant materials

Plant parts were cleaned with deionized water and dried at 50℃ for 24 h. The dried plant was ground and then sieved with 80 mesh.

### Extraction

The method of Phasuda and Varipat (2004) was adopted for extraction with little modification. Briefly, 20 g portions of the powdered plant were soaked separately in solvents (80 ml ethanol, hexane and petroleum ether) at ambient temperature for 24 h under shaking condition at 130 rpm. The extract was then filtered using Whatman filter paper No. 1 and re-filtered using 0.22 µ (Sartorius, Germany). The filtrate was kept in the freezer at -20°C.

### Microorganisms

The microorganisms used included: *F. oxysporum*, *R. solani*, *A. solani* and *S. sclerotiorum*, respectively, which were obtained from Microbiological Resource Center "MIRCIN", Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

### Assay of the antifungal effects of the neem leaves and seeds organic extracts

To assay the antifungal effects of the organic extracts of neem leaves and seeds using tested microorganisms, measurement of radial growth of the used organisms were made following the technique of Phasuda and Varipat (2007).

### Isolation and identification of neem compounds

Neem oil obtained by using a cold mechanical expeller was partitioned between *n*-hexane and 0% methanol (MeOH) and the MeOH extract was concentrated to dryness *in vacuo* at 45℃ (62.8 g). The extract was subjected to preparative HPLC for the isolation of triterpenoids. Details of the isolation and purification of major compounds from neem oil, that is, deacetylnimbin, azadiradione, nimbin, salannin and epoxyazadiradione, were described as discussed previously. Pure compounds were identified by HPLC analysis. Standard pure compounds were routinely purified in our laboratory through preparative HPLC which forms the source according to the method described by Govindachari et al. (1995).

## RESULTS AND DISCUSSION

The data presented in Table 1 shows the inhibition percentages of different concentrations of ethanolic neem leaf extract on growth of pathogenic fungi. It can be noticed that the highest inhibition percentage was recorded with *R. solani* (10, 20 and 30%) concentrations of ethanolic neem leaf extract and the values were 55.10,

**Table 1.** Inhibition percentages of different concentrations of ethanolic neem leaf extract on growth of pathogenic fungi.

| Fungi | Concentrations of neem leaf ethanolic extract | | | |
|---|---|---|---|---|
| | 10% | 20% | 30% | 40% |
| *F. oxysporum* | 36.25 | 69.15 | 87.90 | 100.0 |
| *R. solani* | 55.10 | 71.10 | 90.88 | 100.0 |
| *A. solani* | 32.26 | 54.85 | 83.05 | 84.00 |
| *S. sclerotiorum* | 45.70 | 55.95 | 83.90 | 86.15 |

**Table 2.** Inhibition percentages of different concentrations of hexane neem leaf extract on growth of pathogenic fungi.

| Fungi | Concentrations of neem leaf hexane extract | | | |
|---|---|---|---|---|
| | 10% | 20% | 30% | 40% |
| *F. oxysporum* | 43.90 | 47.56 | 68.50 | 89.35 |
| *R. solani* | 28.28 | 46.18 | 54.15 | 77.20 |
| *A. solani* | 28.21 | 37.00 | 43.26 | 73.12 |
| *S. sclerotiorum* | 36.33 | 40.34 | 61.58 | 76.46 |

**Table 3.** Inhibition percentages of different concentrations of methanolic neem leaf extract on growth of pathogenic fungi.

| Fungi | Concentrations of neem leaf methanolic extract | | | |
|---|---|---|---|---|
| | 10% | 20% | 30 % | 40% |
| *F. oxysporum* | 30.95 | 36.24 | 64.15 | 100.0 |
| *R. solani* | 40.10 | 45.42 | 72.28 | 100.0 |
| *A. solani* | 26.25 | 35.00 | 50.77 | 78.35 |
| *S. sclerotiorum* | 21.40 | 27.34 | 44.13 | 67.11 |

71.10 and 90.88%, respectively; while the lowest recorded was found with the same mentioned concentration of *A. solani* and the values were 32.26, 54.85 and 83.05%, respectively. On the other hand, a complete inhibition percentage (100%) was recorded with 40% ethanolic neem leaf extract of *R. solani* and *F. oxysporum*, while the lowest recorded was with *A. solani*. These results are in agreement with the finding of Sanjeet et al. (2005).

The data given in Table 2 shows the inhibition percentages of different concentrations of hexane neem leaf extract on growth of pathogenic fungi. The obtained results indicated that the highest inhibition percentages were recorded with F. oxysporum (10, 20, 30 and 40%) concentrations of hexane neem leaf extract and the values were 43.90, 47.56, 68.50 and 89.35% respectively; while the lowest were recorded with *A. solani* and the values were 28.21, 37.00, 43.26 and 73.12% respectively. Similar results were reported by Mossini et al. (2004).

The data presented in Table 3 shows the inhibition percentages of different concentrations of methanolic neem leaf extract on growth of pathogenic fungi. It can be mentioned that the highest inhibition percentage was recorded with *R. solani* (10, 20 and 30%) concentrations of methanolic neem leaf extract and the values were 40.10, 45.42 and 72.28%, respectively; while the lowest recorded was found with the same mentioned concentration of *S. sclerotiorum* and the values were 21.40, 27.34 and 44.13%, respectively. On the other hand, a complete inhibition percentage (100%) was recorded with 40% methanolic neem leaf extract of *F. oxysporum* and *R. solani*, while the lowest was recorded with *S. sclerotiorum* (67.11%). These results are in agreement with the finding of Gupta and Bansal (2003).

The data given in Table 4 shows the inhibition percentages of different concentrations of ethanolic neem seed extract on growth of pathogenic fungi. It can be noticed that the highest inhibition percentage was recorded with *R. solani* (10 and 20%) concentrations of ethanolic neem leaf extract and the values were 58.96 and 75.34%, respectively; while the lowest recorded was

**Table 4.** Inhibition percentages of different concentrations of ethanolic neem seed extract on growth pathogenic fungi.

| Fungi | Concentrations of neem leaf methanolic extract | | | |
|---|---|---|---|---|
| | 10% | 20% | 30% | 40% |
| *F. oxysporum* | 49.85 | 73.66 | 100.0 | 100.0 |
| *R. solani* | 58.96 | 75.34 | 100.0 | 100.0 |
| *A. solani* | 36.10 | 57.18 | 86.69 | 89.50 |
| *S. sclerotiorum* | 49.25 | 59.68 | 87.80 | 92.55 |

**Table 5.** Inhibition percentages of different concentrations of hexane neem seed extract on growth of pathogenic fungi.

| Fungi | Concentrations of neem seed hexane extract | | | |
|---|---|---|---|---|
| | 10% | 20% | 30% | 40% |
| *F. oxysporum* | 52.25 | 57.62 | 68.10 | 97.91 |
| *R. solani* | 46.83 | 49.55 | 64.85 | 82.40 |
| *A. solani* | 31.68 | 40.85 | 53.46 | 80.14 |
| *S. sclerotiorum* | 39.67 | 44.10 | 48.75 | 80.54 |

**Table 6.** Inhibition percentages of different concentrations of methanolic neem seed extract on growth of pathogenic fungi.

| Fungi | Concentrations of neem seed methanolic extract | | | |
|---|---|---|---|---|
| | 10% | 20% | 30% | 40% |
| *F. oxysporum* | 43.50 | 64.15 | 100.0 | 100.0 |
| *R. solani* | 48.77 | 69.22 | 100.0 | 100.0 |
| *A. solani* | 42.95 | 47.85 | 57.97 | 80.68 |
| *S. sclerotiorum* | 24.60 | 29.91 | 52.25 | 71.23 |

found with the same mentioned concentration of *A. solani* and the values were 36.10 and 57.18%, respectively.

The data presented in Table 5 shows the inhibition percentages of different concentrations of hexane neem seed extract on growth of pathogenic fungi. It can be noticed that the highest inhibition percentage was recorded with (10, 20, 30 and 40%) hexane neem seed extract of *F. oxysporum* and the values were 52.25, 57.62, 68.10 and 97.91%, respectively; while the lowest inhibition percentage with the same mentioned concentrations was recorded with *A. solani* and the values were 31.68, 40.85, 53.46 and 80.14% respectively. These results are in agreement with the findings of Amadioha (2004).

The data given in Table 6 shows the inhibition percentage of different concentrations of methanolic neem seed extract on growth of pathogenic fungi. It can be mentioned that the highest inhibition percentage was recorded with (10 and 20%) methanolic neem seed extract of *R. solani* and the values were 48.77 and 69.22%, respectively; while the lowest inhibition percentage was recorded with *S. sclerotiorum* and the values were 24.60 and 29.91% respectively. On the other hand, a complete inhibition (100%) was recorded with 30 and 40% methanolic neem seed extract of *F. oxysporum* and *R. solani*; while the lowest was recorded with *S. sclerotiorum* and the values were 52.25 and 71.23% respectively. Similar results were reported by Korunic (2004). From the obtained results, it could be concluded that the inhibition percentage of tested fungi increased by increasing neem leaf and seed extract by different rates. Also, neem seed organic extracts had higher inhibition percentage than that of neem leaf organic extracts.

The high performance liquid chromatographic pattern of neem organic extract is shown in Table 7. It can be noticed that neem organic extract contained 10 bands, of which band 1 contained azadirachtins A (7%), B (5%) and C (8%), while band 3 contained azadirachtins A (11%), B (10%), D (4%), H (9%) and 6 De-acetyl nimbin (39%). Band 5 contained azadiradione (51%), while nimonol (82%) was detected in band 6. Bands 7 and 8 contained epoxyazdiradione (8 and 43%, respectively).

**Table 7.** High performance liquid chromatographic pattern of neem organic extract.

| Band no eluted | $R_t$ *(min.)* | Total peak area detected (%) | ID |
|---|---|---|---|
| Band 1 | 8 | 69.8 | Azadirachtins, A (7%), Bv (5%), C (8%) |
| Band 2* | 15 | 21.6 | n.d. |
| Band 3 | 21 | 56.4 | Azadirachtins, A (11%), B (10%), D (4%), H (9%), 6 De-acetyl nimbin (39%) |
| Band 4 | 33 | 32.7 | n.d. |
| Band 5* | 37 | 40.0 | Azadiradione (51%) |
| Band 6 | 52 | 66.0 | Nimonol (82%), |
| Band 7 | 61 | 74.0 | Epoxyazadiradione (8%) |
| Band 8 | 68.4 | 30.0 | Epoxyazadiradione (43%) |
| Band 9* | 75 | 32.0 | n.d. |
| Band10* | 88 | 25.0 | n.d. |

On the other hand, no active component of neem organic extract was detected with these conditions at bands 2, 4, 9 and 10, respectively. Finally, it could be concluded that nimonol (82%) is a major active component of neem organic extract.

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### REFERENCES

Berjak P, Campbell G K, Farrant JM, Omondi–Oloo W, Pammenter NW (1995). Responses of seeds of *Azadirachta indica* (neem) to short- term storage under ambient or chilled conditions. Seed Sci. Technol. 23, 779-792.

Biswas I, Chattopadhyay R, Banerjee K, Bandyopadhyay U (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). Curr. Sci. 82(11):1336 – 1345.

Gamene CS, Kraak HL, Van Pijlen JG, De Vos CH (1996). Storage behavior of neem (*Azadirachta indica*) seeds from Burkina Faso. Seed Sci. Technol. 24: 441- 448.

Govindachari TR, Suresh G, Gopalakrishnan G (1995). A direct preparative high performance liquid chromatography procedure for the isolation of major triterpenoids and their quantitative determination in neem oil. J. Liq. Chromatogr. 18:3465-3471.

Grayer RJ, Harborne JB (1994). A survey of antifungal compounds from higher plants 1982-1993. Phytochemistry 7:19-42.

Gupta RK, Bansal RK (2003). Comparative efficacy of plant leaf extracts and fungicides against *F. oxysporum* Schlecht inducing fenugreek wilt under pot house condition. Ann. Biol. 19(1): 35-37.

Koul O, Isman MB, Ketkar CM (1990). Properties and uses of neem, *Azadirachta indica*. Can. J. Bot. 68:1-11.

Kumar R, Bangarwa K (1996). Seed storability in *Azadirachta indica* A. Juss. Ann. Biol. 12(1):62- 66.

Mossini SA, Oliveira KP, Kemmelmeier C (2004). Inhibition of patulin production by *Penicillium expansum* cultured with neem (*Azadirachta indica*) leaf extracts. J. Basic Microbiol. 44(2):106-113.

Okpanyi SN, Ezeukwu GC (1981). Anti-inflammatory and antipyretic activities of *Azadirachta indica.* Planta Media 4(1):34-39.

Parveen G, Alam MM (1993). Bioactivity against plant pathogens. pp.144-153. *in:* Randhawa, N.S. and Parmar, B.S. [Eds.] Neem Research and Development. Society of Pesticide Science, New Delhi, India.

Phasuda J, Varipat A (2004). Antimicrobial Activity in Some Indigenous Plant Extracts. J. Ethnopharm. 94:49-54.

Phasuda J, Varipat A (2007). Antimicrobial Activity in Some Indigenous Plant Extracts. J. Ethnopharm. 97: 81-87.

Ryo I, Kentaro S, Hideo N, Han-Young L, Takayuki M (2005). Foliar spray of validamycin A or validoxylamine A controls tomato *Fusarium* wilt. Phytopathology 95(10):1209-1216.

Sanjeet K, Upadhyay JP, Sanjeev-Kumar (2005). Evaluation of plant extracts for control of Alternaria leaf spot of *Vicia faba* . Ann. Plant Protect. Sci. 13(1):258-259.

Schmutterer H (1995). The Neem Tree Azadirachta indica A. Juss . and Other Meliaceous Plants. VCH Verlagsellchaft Pub. Weinheim, New York, Cambridge, Tokyo, pp. 74-92.

Suresh G, Narasimhan NS, Palani N (1997). Structure of nimonol from fresh whole green leaves of *Azadirachta indica. Phytochemistry* (in press).

Trakoontivakorn G, Nakahara K, Shinmoto H, Tsushida T (1999). Identification of Antimutagenic Substances (Ames Test) from *Boesenbergia pandurata* Schl. (fingerroot) and *Languas galangal* (galanga). JIRCAS J. 7:105-116.