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Nematicidal effect of *Acacia nilotica* and *Gymnema sylvestris* against second stage juveniles of *Meloidogyne incognita*

Nighat Sultana^{1*}, Musarrat Akhter², Muhammad Saleem³ and Yousaf Ali¹

¹Pharmaceutical Research Center, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Karachi-75280, Pakistan.

²Food and Marine Resources Research Centre, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Karachi-75280, Pakistan.

³Department of Chemistry, the Islamia University of Bahawalpur, Pakistan.

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The nematicidal effect of different extracts of *Acacia nilotica* leaves/seeds and *Gymnema sylvestris* leaves were tested against *Meloidogyne incognita* larvae at the concentrations of 1, 0.5, 0.25 and 0.125%, up to three days. All extracts showed nematicidal mortality. Nematicidal mortality was 100% with the use of 1% concentration of leaves ethyl alcohol extract of *G. sylvestris* and ethyl acetate leaves extracts of *A. nilotica* after 2 days. Qualitative analysis of the phytochemicals of alcohol extracts revealed the presence of carbohydrates, saponins, triterpene saponins belonging to oleanane and dammarene, phytosterols, phenols, flavonoids and tannins in all the plants. Quantitative analysis showed that, the crude saponin was the major phytochemical constituent present in highest percentage followed by crude oleanane and dammarene triterpene acids in both of two plants. It is suggested that both of these two plants possess nematicidal properties that could be developed and used as natural nematicides for nematode control.

Key words: *Acacia nilotica*, nematicidal activity, ellagic acid, oleanolic acid 3-glycosides, quercetin 3-glycosides, 3,19-dihydroxy-12-ursen-28-oic acid.

INTRODUCTION

Plant parasitic nematodes constitute one of the most important pest groups of the economic crops, especially in developed and developing countries of the world. The use of the plants and plant products is one of the promising methods for nematode controls. They are cheap, easy to apply, produce no pollution hazards and have the capacity to structurally and nutritionally improve the soil health. In view of these facts, investigations have been undertaken by various groups of scientists (Decker et al., 1981; Gommers et al., 1981; Qamar et al., 1995; Nogueira et al., 1996) which shows an effective control of root-knot nematodes. In the present article, studies on the nematicidal activity of different extracts, fractions isolated from the air dried aerial parts (leaves and seeds)

of *Acacia nilotica* and *Gymnema sylvestris* (Imoto et al., 1991) are described. *A. nilotica* belongs to Leguminosae and sub-family, Mimosoideae (Brenan JPM et al., 1983). It is an economically and medicinally important plant. Its different parts are used for different purposes. Inner bark contains (18 to 23%) tannin, used for tanning and dyeing leather black. Young parts produce a very pale tint in leather, notable goat hides. Pods were used by the ancient Egyptians. Young bark used as fiber, twigs esteemed for tooth brushes. Trees tapped for gum arabic. Because of its resins, it resist insects and water and trees are harvested for timber for boat making, posts, buildings, water pipes, well planking, plows, cabinet work, wheels, mallets and other implements wood yield excellent firewood and charcoal (Duck, 1981).

Medicinally, *A. nilotica* was used in large number of diseases in different parts of world. For example, Zulu take bark for cough, chipi use root for tuberculosis, Masai are intoxicated by the bark and root decoction, said to

*Corresponding author. E-mail: nighat2001us@hotmail.com. Tel: (92-21) 8141832-35. Fax: (92-21) 8141847.

impart coverage even ephrodisia and root is said to cure impotence. Astringent bark used for diarrhea, dysentery and leprosy. According to hear well the gum or bark is used for cancers and tumors of ear, eye and testicles and in duration liver and spleen. It is also said to be used for cancer, colds, congestion cough, diarrhea, dysentery, fever, gallbladder, hemorrhage, leucorrhoea, ophthalmia, sclerosis, small pox and tuberculosis. Bark, gum leaves and pods are used medicinally in West Africa. Different parts are strongly astringent due to diarrhea. Other preparations are used for cough, gargle, toothache, ophthalmia and syphilitic ulcers. In Tonga, root is used to treat tuberculosis. In Lebanon, the resin is mixed with orange flower infusion for typhoid convalescence. Masai use the bark decoction as a nerve stimulant. In Italian Africa, the wood is used to treat small pox. Egyptian Nubians believe that diabetics may eat unlimited as long as they also consume powdered pods (Duke, 1983). Extracts are inhibitory to at least four species of pathogenic fungi (Umodkar et al., 1977). The aqueous extract of fruit rich in tannin (18 to 23%) has shown algicidal activity against chroococcus, closterium, coelastrum, cosmarium, cyclotella, euglena, microcystis, Pediastrum, rivularia, spirogyra and spiroolina (Ayoub, 1983).

No phytochemical investigation has so far been carried out on this specie. Keeping in view the pharmacological significance of the plant, phytochemical studies were undertaken on the constituents of the aerial parts of the plant in this laboratory two years earlier, which resulted in the isolation and characterization of various sugars, include Androst-5-ene-3,17-diol (Chaubal et al., 2003), 2-O-L-Arabinofuranosyl-L-arabinose (Chalk, 1968), 3-O-L-Arabinopyranosyl-L-arabinose (Verkerk et al., 1998), dehydrodigallic acid (Ishimatsu et al., 1989), 3,5-Dihydroxy-4',7-dimethoxyflavone (da Silva et al., 2000), 3-(3,4-dihydroxyphenyl)-2-propenal (Demin et al., 2004), 3,19-dihydroxy-12-ursen-28-oic acid (Zheng et al., 2004), ellagic acid (Press et al., 1969), 24,25-epoxytirucall-7-ene-3,23-diol, (Liu et al., 2001) 3,3',4',5',5',7-hexahydroxyflavan (Miketova, 1998), N-(4-(4-Hydroxybenzoylamino) butyl)-1,3-dimethylumazine-6-carboxamide (Voerman et al., 2005), 2-hydroxy-9,12,15-octadecatrienoic acid (Bohannon et al., 1975), nilocitin (Lee et al., 1989), 2',3,3',4',5,6,7,8-Octahydroxyflavone (Chauhan et al., 2000), oleanolic acid bisdesmosides (Domon, 1984), oleanolic acid 3-glycosides (Nihei et al., 2005), 3,4,8,9,10-Pentahydroxy-6H-dibenzo(b,d)pyran-6-one (Nawwar et al., 1984), 3,3',4,4',9-Pentahydroxy-7,9'-epoxylignan (Dobner et al., 2003), 3,3',4',5,7-Pentahydroxyflavan (Gao et al., 2004), 1,3,7,11,12-Pentahydroxy-14-meliacen-28-oic acid (Torto et al., 1995), Quercetin 3-glycosides (Aqil et al., 1999), 3,4',5,7-Tetrahydroxy-8-prenylflavone (Shin et al., 2002) and Tirucall-7-ene-3,23,24,25-tetrol (Vieira et al., 1998).

Acacia species is a rich source of gallic and ellagic acid (Nighat et al., 2010). *G. sylvestre* leaves contain triterpene saponins belonging to oleanane and

dammarene classes. Oleanane saponins are gymnemic acids (Kennady et al., 1989) and gymnemasaponins, while dammarene saponins are gymnemasides. Besides this, other plant constituents are flavones, anthraquinones, hentri-acontane, pentatriacontane, α and β - chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β -amyrin related glycosides and stigmasterol. Leaves of this species yield acidic glycosides, anthro-quinones and their derivatives (Mukherjee et al., 1995; Chakravarthi et al., 1981; Glaser et al., 1984; Gupta, 1961; Imoto et al., 1991; Kennedy, 1989; Shanmugasundaram et al., 1983; Stocklin et al., 1969b; Yoshikawa et al., 1989a; Yoshikawa et al., 1989b; Yoshikawa et al., 1992a; Mukherjee et al., 1995; Anil et al., 1994).

MATERIALS AND METHODS

General experimental procedures

Visual examination is not completely reliable for determining mortality; therefore, final viability determinations on tomato plants were carried out according to Feldmesser et al. (1983). The inoculated tomato seedlings were examined after three weeks to determine the viability of the nematode inocula expressed as root infection. Five replicates of each treatment were carried out and the results are reported as an average of the five replicates. Infections were evaluated on an arbitrary basis (the root-knot index) by assigning values of 0 = no infection, 1 = 1 to 25% of the roots galled, 2.0 = 26 to 50% galled, 3.0 = 51 to 75% galled and 4.0 = 76 to 100% root infection.

Plant material

The aerial parts of *A. nilotica* and *G. sylvestris*, (15 kg) were collected from Karachi, in June 2010.

Extraction and isolation

Air-dried aerial parts of *A. nilotica* (15 kg dry weight) and *G. sylvestris* were dried and extracted with EtOH (100 L). The EtOH extract was concentrated to a gum (813 g), dissolved in distilled water and extracted thoroughly with petroleum ether (40 L). The hexane soluble portion was dried (74.96 g). The remaining aqueous layer was acidified with acetic acid to pH 3 and then, extracted with CHCl_3 . The CHCl_3 soluble portion was dried (83.84 g). The remaining aqueous layer was basified with NH_4OH to pH 12 and extracted with CHCl_3 (35 L). The CHCl_3 soluble portion was dried (79.84 g). The acidic chloroform soluble portion was dried as a crude mixture. Extraction of aqueous extract with ethyl acetate (5 L) yielded an impure mixture to afford E.AcS extract.

Nematicidal activity

Experiments were performed under laboratory conditions at 28 + 2°C. Fresh egg masses collected from stock culture maintained on tomato root tissue were kept in water for egg hatching. The larvae emerged after 48 h from egg masses incubated at 30°C and were used as test species for larval mortality studies. The movements of nematodes were checked by touching them with needle.

For the nematicidal activity of leave extract, stock solutions (30 mg/ml) of different fractions of *A. nilotica* in ethyl alcohol (AL-AS.),

ethyl acetate (AL-E.AcS.), methanol (AL-MS.), chloroform (AL-CS.) and n-hexane (AL-HS) were prepared. To determine nematocidal effect of various fractions, 100 freshly hatched second stage juveniles were taken in 5 ml tap water.

Measured amounts of stock solution were added to make dilution of 1, 0.5, 0.25 and 0.125%. Standard nematocidal *Azadirachta indica* (0.05%) was taken for comparison and tap water taken as control. After 24 and 48 h exposure with various *A. nilotica* fractions, the larvae were counted for mortality and non-mortality under stereoscopic microscope. The deaths of nematodes were confirmed by keeping them in tap water for 24 h. The percent mortality was worked out from an average of three replicate. The result of percent mortality in different fraction of *A. nilotica* and *G. sylvestris* after 24 and 48 h of leaf extract were given in Tables 1 to 4.

RESULTS AND DISCUSSION

The nematocidal activity of different fractions of leaves extract of *A. nilotica* and *G. sylvestre* (AL-E.Al., AL-E.Ac., AL-Meth., AL-Chl., AL-Hex.) and different fractions of seeds extract (AS-AS., AS-E.Ac., AS-MS., AS-CS., AS-HS) of *A. nilotica* after 24 and 48 hours were tested against a root-knot nematode (*Meloidogyne incognita*).

The total alcohol soluble extract, MeOH soluble, Et-acetate soluble, chloroform soluble and pet. ether soluble extracts of the aerial parts of *A. nilotica* (AL-AS, AL-CS, AL-E-AcS, and AL-PS) (AL-AS, AL-CS, AL-E-AcS and AL-PS) and *G. sylvestre* (GL-AS, GL-CS, GL-E-AcS and GL-PS) were screened for its nematocidal activity against nematodes freshly hatched second stage juveniles of *M. incognita* (root-knot nematode). Negative results were obtained for the nematocidal activity of pet. ether extract of *A. nilotica* leaves. This is the first reported on the nematocidal activity of these compounds and any part of *A. nilotica* and *G. sylvestre*. Thus, both of these plants extract might be beneficial as a potent nematode inhibitor under specified conditions.

The crude ethyl acetate leaves extract of *A. nilotica* (AL-E-AcS) showed 70% mortality at 1.0% concentration after 24 h, while 90% mortality at 1.0% concentration after 48 h, whereas, the pet. ether soluble fraction (AL-PS) showed 65% mortality and the chloroform soluble fraction AL-CS showed 45% mortality at the same concentration after 48 h. Conventional nematocidal *Azadirachta indica* showed 88% mortality after 48 h. The alcoholic soluble (AL-AS), pet.ether soluble (AL-PS) and chloroform soluble (AL-CS) fractions showed 50, 40 and 38% mortality, respectively after 24 h of *M. incognita* larvae.

From the crude leaf extract, fraction AL-AS shows maximum mortality (70%), while fraction AL-CS, shows minimum mortality (38%) at 1% concentration after 24 h. Similarly, fraction AL-E.AcS shows maximum mortality (90%), while fraction (AL-CS.) shows minimum mortality (45%) at 1% concentration after 48 h. Other fractions that is, AL-AS, AL-MS, AL-HS, have percent mortality in between them at the same concentration. The percent mortality of all fractions gradually decreases with decreasing concentration of fraction from 1 to 0.0125%

after 24 and 48 h. Similarly, the nematocidal activity of the crude alcoholic leaves extract, as well as crude alcoholic leaves extract fractions (GL-AS, GL-CS, GL-E-AcS and GL-PS) of *G. sylvestre* were tested against a root-knot nematode (*M. incognita*).

The crude ethyl acetate extract of *G. sylvestre* (GL-AS) showed 90% mortality at 1.0% concentration after 24 h, while 95% mortality at 1.0% concentration after 48 h, whereas, the pet. ether soluble fraction (GL-PS) showed 10% mortality and the chloroform soluble fraction (GL-CS) showed 18% mortality at the same concentration after 48 h. Conventional nematocidal *A. Indica* showed 88% mortality after 48 h. The alcoholic soluble (GL-AS), pet.ether soluble (GL-PS) and chloroform soluble (GL-CS) fractions showed 90, 2.0 and 15% mortality, respectively after 24 h of *M. incognita* larvae. Negative results were obtained for the nematocidal activity of pet. ether extract of *G. sylvestre* leaves. The direct antinemic action shown by GL-AS and its fractions GL-CS, GL-PS in the *in vitro* investigation against second-stage juveniles of *M. incognita* is presented in Tables 1 to 2.

The nematocidal activity of alcohol extract of *G. sylvestris* leaves showed 99, 91, 75 and 56% of death with the use of 1, 0.5, 0.25 and, 0.125% concentrations, respectively, after 3 days. The third day, 0.5% concentration killed more than 91% of the larvae. However, 100% mortality was observed only in 1% concentration alone (Table 1). Only the highest concentration (1%) of all two plants extract showed 95% mortality (Table 3). Among the two plants, the leaves alcohol extract of *G. sylvestris* was found more lethal than other extracts.

The crude methanol extract of *A. nilotica* seeds (AS-MS) showed 85% mortality at 1.0% concentration after 24 h, while 92% mortality at 1.0% concentration after 48 h, whereas, the pet. ether soluble fraction (AS-PS) showed 50% mortality and the chloroform soluble fraction (AS-CS) showed 70% mortality at the same concentration after 48 h. Conventional nematocidal *A. Indica* showed 88% mortality after 48 h. The alcoholic soluble (AS-AS), pet.ether soluble (AS-PS) and chloroform soluble (AS-CS) fractions showed 80, 30 and 65% mortality, respectively, after 24 h of *M. incognita* larvae. The percent mortality was worked out from an average of three replicate. The result of percent mortality in different fraction of *A. nilotica* after 24 and 48 h of leaf extract were given in Tables 3 and 4.

The direct antinemic action shown by AS-AS and its fractions AS-CS, AS-PS in the *in vitro* investigation against second-stage juveniles of *M. incognita* is presented in Tables 1 and 2. It has also been observed that, different fractions of leaf extract at different fraction concentration show same percent mortality after 24 and 48 h. AL-AS at 0.5% concentration and AL-E.AcS at 0.125% concentration, AL-CS and AL-HS at 0.25 and 0.125% concentration, respectively, AL-HS and AL-MS at 1 and 0.125% concentration, respectively. AL-AS at 1% concentration and AL-MS at 0.5% concentration, have

Table 1. Nematicidal activity of different fractions of leaves extract isolated from *A. nilotica* and *G. sylvestris* on the larval mortality of *M. incognita* (Root knot nematode). Percent mortality/concentration after 24 h.

Concentration ↓ (%)	Fractions	Ethanol		E. Acetate		Methanol		Chloroform		Hexane	
		AL (%)	GL	AL (%)	GL	AL (%)	GL	AL (%)	GL	AL (%)	GL
1		50	90	70	80	68	12	38	15	40	2
0.5		42	88	66	60	50	5	28	5	28	-
0.25		22	70	58	50	46	2	10	4	17	-
0.125		16	50	42	44	40	-	05	-	10	-

Control = 2%. AL-AS= *A. nilotica* leaves alcohol soluble; AL-CS= *A. nilotica* leaves chloroform soluble; AL-E-AcS=*A. nilotica* leaves ethyl acetate soluble; AL-PS= Acacia leaves pet.ether soluble; GL-AS= *G. sylvestris* leaves alcohol soluble; GL-CS= *G. sylvestris* leaves chloroform soluble; GL-E-AcS= *G. sylvestris* leaves ethyl acetate soluble; GL-PS= *G. sylvestris* leaves pet.ether soluble; GL= *G. sylvestris* leaves; AL = *A. nilotica* leaves.

Table 2. Nematicidal activity of different fractions of leaves extract isolated from *A. nilotica* and *G. sylvestris* on the larval mortality of *M. incognita* (Root knot nematode). Percent mortality/concentration after 48 h.

Concentration ↓ (%)	Fractions	Ethanol		E. ac.		Methanol		Chloroform		Hexane	
		AL (%)	GL (%)	AL (%)	GL (%)	AL (%)	GL	AL (%)	GL	AL (%)	GL
1		62	99	90	95	85	15	45	18	65	10
0.5		46	91	78	70	62	7	30	7	30	8
0.25		25	75	70	62	50	2	15	5	20	4
0.125		18	56	50	53	42	2	12	5	15	-

Control = 3%. AL-AS = *A. nilotica* leaves alcohol soluble; AL-CS = *A. nilotica* leaves chloroform soluble; AL-E-AcS = *A. nilotica* leaves ethyl acetate soluble; AL-PS = *A. nilotica* leaves pet.ether soluble; GL-AS= *G. sylvestris* leaves alcohol soluble; GL-CS= *G. sylvestris* leaves chloroform soluble; GL-E-AcS= *G. sylvestris* leaves ethyl acetate soluble; GL-PS= *G. sylvestris* leaves pet.ether soluble; GL= *G. sylvestris* leaves; AL = *A. nilotica* leaves.

Table 3. Nematicidal activity of different fractions of seed extract isolated from *A. nilotica* on the larval mortality of *M. incognita* (Root knot nematode). Percent mortality/concentration after 24 h.

Concentration ↓ (%)	Fractions →	AS-AS. (%)	AS-E.AcS. (%)	AS-MS. (%)	AS-CS. (%)	AS-HS. (%)
1		80	60	85	65	30
0.5		60	50	70	60	26
0.25		58	48	58	56	20
0.125		38	40	38	42	12

Control = 2%. AS-AS = *A. nilotica* seeds alcohol soluble; AS-CS = *A. nilotica* seeds chloroform soluble; AS-E-AcS = *A. nilotica* seeds ethyl acetate soluble; AS-PS = *A. nilotica* seeds pet.ether soluble.

Table 4. Nematicidal activity of different fractions of seed extract isolated from *A. nilotica* on the larval mortality of *M. incognita* (Root knot nematode). Percent mortality/concentration after 48 h.

Concentration ↓ (%)	Fractions →	AS-AS. (%)	AS-E.Acs. (%)	AS-MS. (%)	AS-CS (%)	AS-PS. (%)
1		90	70	92	70	50
0.5		75	57	80	67	-
0.25		48	50	63	60	25
0.125		40	40.2	40	45	15

Control = 5%. AS-AS = *A. nilotica* seeds alcohol soluble; AS-CS = *A. nilotica* seeds chloroform soluble; AS-E-AcS = *A. nilotica* seeds ethyl acetate soluble; AS-PS = *A. nilotica* seeds pet.ether soluble.

same percent mortality after 24 h. Similarly, AL-CS at 0.25% concentration and AL-HS at 0.125% concentration, AL-AS at 1% concentration and AL-MS at 0.5% concentration, have same percent mortality after 48 h.

Different fractions at same concentration also showed same percent mortality. AL-CS and AL-HS at 0.5% concentration showed same percent mortality after 24 and 48 h. Conventional nematicide *A. indica* showed 88%

mortality at the 0.5% concentration used in the present studies. It was noted that, at all the concentrations, all the tested fractions exhibited significant larval mortality against the test nematode but the activity decreases with a decrease in concentration in all the cases (Tables 1 to 4).

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