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USE OF *EUCALYPTUS* SP., IN THE CONTROL OF *MELOIDOGYNE JAVANICA* ROOT KNOT NEMATODE

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Abstract

Different parts of *Eucalyptus* sp., viz., leaves, stem, bark and fruit used as aqueous and ethanol extracts showed nematicidal effect against *Meloidogyne javanica* root knot nematode, reduced hatching of eggs, increased mortality of juveniles with an increase in exposure of time and showed efficiency in the control of *Meloidogyne javanica* root knot nematode on mung bean and chick-pea plants. Significant increase in shoot length, shoot weight, root length and root weight was observed where soil was amended with leaves, stem, bark and fruit of *Eucalyptus* sp., used @ 0.1, 1 and 5% w/w. Number of knots was also significantly reduced. All plant parts of *Eucalyptus* spp., were more effective @ 5% w/w.

Introduction

Crop plants are of great importance for a country and when these plants suffer with diseases they cause serious losses and adversely affect the agricultural economy of a country (Hafeez, 1986). Among the disease causing organisms, the plant parasitic nematodes (*Meloidogyne* spp.) reduce the yield of the world's 40 major cash crops by an average of 12.3%. The members of this genus have a wide host range of plant species (Goodey *et al,* 1965). Of total 70 *Meloidogyne* species identified so far (Luc *et al*, 1988), only four species viz., *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood and *M. hapla* Chitwood are widely distributed throughout the agricultural regions of the world and appear to be very adaptable to a wide range of agroecosystems. Especially *Meloidogyne incognita* (Kofoid & White) Chitwood is the most abundant and damaging nematode in Pakistan infecting about 102 plant species (Maqbool & Shahina, 2001). It is impossible to be precise concerning the losses caused by *Meloidogyne* spp. However, some estimates of individual crop losses have been made by various authors. In tomato plants, when *M. incognita* occurs, 50 % of fruit yield is suppressed (Lamberti, 1979). In tropical areas root knot nematodes cause losses in potato crop estimated to be 24 % (Sasser, 1979). In addition, these nematodes have the ability to interact synergistically with other plant pathogens and cause upto 5-34% yield losses in vegetables in tropical climates. The presence of root knot nematode *Meloidogyne* spp., is known to increase the severity of *R. solani* root rot in many plants (Shahzad & Ghaffar, 1992). The control of the diseases caused by the soil-borne fungi and nematodes has been achieved by chemical, cultural, biological and genetic methods.
Use of organic amendment in the control of fungal and nematode diseases has proved effective in disease control. It involves the amendment of organic substrates like mangroves, different plant parts and plant cakes. This amendment of organic matter has suppressive effect on plant parasitic nematodes (Alam, 1976, 1990). Therefore, experiments were carried out to study the efficacy of different parts viz., leaves, stem, bark and fruit of *Eucalyptus* sp., that is considered to have potential of antiseptic action.

Materials and Methods

***In vitro* studies:** After extraction of eggs of root knot nematode from roots of plants, number of eggs per ml of suspension was determined in counting chamber. One ml of egg suspension (30-45eggs) and 1ml of aqueous extract of leaves, stem, bark and fruit of *Eucalyptus* spp., was transferred separately in glass cavity blocks and kept at room temperature. There were three replicates of each treatment and hatching of eggs was recorded after 72 hrs interval. One ml suspension of freshly hatched juvenile (30-45 juveniles) and 1ml aqueous extract of each test plant part was transferred separately in glass cavity blocks and kept at room temperature 25-30ºC. There were three replicates of each treatment and juvenile mortality was recorded at 24 hrs interval after 72 hrs. Similarly eggs hatching and juvenile mortality were recorded in 1000ppm and 500ppm ethanol extract of leaves, stem, bark and fruit of *Eucalyptus* spp., of which 2ml of each test extract was transferred separately and left over night for evaporation of solvent followed by addition of 2ml egg suspension and kept at room temperature to record hatching of eggs at 24 hrs interval after 72 hrs. 2ml ethanol extract of each test plant part was transferred separately in glass cavity blocks, left over night for evaporation of solvent at room temperature 25-30ºC and followed by addition of 2ml juvenile suspension (30-45 juveniles). There were three replicates of each treatment and juvenile mortality was recorded at 24 hrs interval after 72 hrs.

***In vivo* studies:** Soil was amended with different parts viz., leaves, stem, bark and fruit powders of *Eucalyptus* spp., @ 0.1, 1 and 5% w/w and kept in 8 cm diam., plastic pot, each pot containing 300 gm soil. Soil moisture was adjusted and maintained at 50% MHC (Keen & Raczkowski, 1922). Pots with organic matter were left for 10 days for decomposition. Non-amended soil served as control. There were three replicates of each treatment. The pots were arranged in randomized complete block design. After two weeks of plant growth, the plants were inoculated with 1000 larvae/pot. Mung bean and chick-pea were used as test plants. After 60 days of growth, plants were uprooted and number of root knots was determined.

Data were analyzed and subjected to analysis of variance (ANOVA) using procedure given by Gomez & Gomez (1984).

Results and Discussion

Eucalyptus is one of the most widely cultivated native trees of Australia. It is distributed in open forests of Tasmania, Southern Victoria and New South Wales. Common name is Tasmanian blue gum. Essential oil produced by *Eucalyptus* sp., and its leaves have shown antibiotic activity (Inouye *et al*., 2001). Their decoction is used for repelling insects and vermin (Morton, 1981).

The present study with aqueous and ethanol extracts of different parts viz., leaves, stem, bark and fruit of *Eucalyptus* reduced the hatching of eggs of *M. javanica* to a varying degree. The aqueous leaf extract was found more effective and stopped hatching after 72 hrs ($p<0.001$). In case of ethanol extract maximum reduction of hatching was observed at 1000ppm concentration followed by 500 ppm concentration ($p<0.05$). Both aqueous and ethanol extracts of leaves, stem, bark and fruit of *Euclyptus* exerted lethal effects and resulted in mortality of *M. javanica* juveniles which increased with an

increase of exposure period. High concentration of ethanol extract (1000 ppm) showed maximum larval mortality as compared with 500 ppm concentration ($p<0.01$). Aqueous and ethanol extracts of leaves, stem, bark and fruit of *Eucalyptus* showed variation in hatching and mortality of *M. javanica* (Table 1, 2). *Eucalyptus* species are known to have essential oils which are composed of mixture of volatile compounds. Presumably the parts of *Eucalyptus* compounds were lethal to root knot nematode. Similarly in previous studies nematicidal property by a number of plants has been investigated for nematode control in agricultural crops (Hoan & Davide, 1979; Al-Obaedi *et al.*, 1987; Firoza & Maqbool, 1996).

Soil amendment with different parts viz., leaves, stem, bark and fruit of *Eucalyptus* showed efficiency in the control of root knot infection. There was significant increase in germination ($p<0.05$), shoot length ($p<0.01$), shoot weight, root length ($p<0.05$), root weight and significantly reduced the knots ($p< 0.01$) when soil was amended with different parts of *Eucalyptus* used @ 0.1, 1 and 5% w/w in mung bean plants (Table 3). No significant increase was observed in germination but significant increase was observed in shoot length, shoot weight ($p<0.05$), root length ($p<0.001$), root weight ($p<0.01$) and significant reduction was observed in the knots ($p<0.05$) in chick-pea plants (Table 3). Four parts viz., leaves, stem, bark and fruit of *Eucalyptus* showed nematicidal activity against *Meloidogyne* sp. The nematicidal activity of four different parts of *Eucalyptus* varied according to their effectiveness. All four parts were more effective when used @ 5% w/w. Similarly Mehdi *et al.*, (2001) reported that a mangrove *Avicennia marina* and *R. mucronata* with or without *Pseudomonas aeruginosa* when used as organic material, significantly reduced the root knot infection in tomato. There is therefore need to use different parts of *Eucalyptus* on a large scale for the control of root knot diseases of crop plants.

Table 1. Effect of different parts of aqueous extract of *Eucalyptus* on hatching and mortality of *Meloidogyne javanica.*

Treatments	0 Hour	24 Hours	48 Hours	72 Hours
	Hatching %			
Control	21	19	38	38
Leaves	21	24	5	0
Stem	18	28	17	11
Bark	14	50	14	14
Fruit	19	37	16	5
LSD0.05=	-	-	-	3.297
	Mortality %			
Control	23	4	4	13
Leaves	15	7	13	33
Stem	8	13	25	38
Bark	15	7	13	40
Fruit	10	10	20	40
LSD0.05=	-	-	-	9.566

Table 2. Effect of different parts of ethanol extract of *Eucalyptus* on hatching and mortality of *Meloidogyne javanica.*

Treatments	0 Hour	24 Hours	48 Hours	72 Hours
	Hatching %			
Control	27	15	30	30
1000 ppm leaves	24	33	21	13
500 ppm leaves	22	14	14	9
1000 ppm stem	23	35	13	13
500 ppm stem	17	12	18	18
1000 ppm bark	25	16	12	12
500 ppm bark	25	20	10	16
1000 ppm fruit	20	20	10	10
500 ppm fruit	24	17	17	13
LSD0.05=	-	-	-	3.542
	Mortality %			
Control	16	0	6	13
1000 ppm leaves	18	11	11	28
500 ppm leaves	18	11	17	22
1000 ppm stem	16	6	6	25
500 ppm stem	16	13	19	19
1000 ppm bark	12	25	25	33
500 ppm bark	14	21	29	36
1000 ppm fruit	12	25	25	33
500 ppm fruit	14	21	21	29
LSD0.05=	-	-	-	3.441

Table 3. Effect of different parts of *Eucalyptus* sp., in the control of root knot nematodes on mung bean and chick-pea plants.

Treatments	Germination %	Shoot length cm	Shoot weight gm	Root length cm	Root weight gm	Number of knots
	Mung bean					
Control	66	16.8	0.23	3.1	0.04	21
0.1%Leaves	60	22.8	0.48	4.3	0.07	14
1% leaves	66	23.1	0.52	7.2	0.09	13
5% leaves	66	23.6	0.64	10.0	0.10	2
0.1% stem	66	21.5	0.46	4.2	0.09	12
1% stem	60	24.3	0.48	5.4	0.11	19
5% stem	100	26.2	0.68	8.1	0.10	10
0.1% bark	73	18.4	0.38	5.7	0.09	9
1% bark	73	20.1	0.45	6.5	0.12	12
5% bark	93	20.2	0.58	6.9	0.32	9
0.1% fruit	73	17.0	0.36	7.3	0.09	8
1% fruit	60	19.7	0.45	8.4	0.10	6
5% fruit	73	21.3	0.46	9.4	0.20	1
LSD.05 =	22.468	4.347	1.577	3.836	0.155	9.419

Table 3. (Cont'd.).

Treatments	Germination %	Shoot length cm	Shoot weight gm	Root length cm	Root weight gm	Number of knots
			Chick-pea			
Control	80	16.3	0.04	3.3	0.02	38
0.1%Leaves	66	16.7	0.06	4.0	0.03	2
1% leaves	80	18.3	0.11	6.3	0.04	0
5% leaves	60	20.5	0.19	7.9	0.08	0
0.1% stem	80	16.6	0.06	5.4	0.04	0
1% stem	66	19.3	0.11	6.3	0.05	0
5% stem	66	20.0	0.22	10.4	0.08	0
0.1% bark	73	18.2	0.05	4.5	0.03	0
1% bark	60	18.9	0.18	6.4	0.07	1
5% bark	73	18.9	0.31	8.1	0.15	7
0.1% fruit	66	19.1	0.26	6.5	0.08	1
1% fruit	60	20.9	0.33	8.7	0.13	4
5% fruit	86	22.6	0.49	17.7	0.17	1
LSD.05 =	23.420	3.893	0.254	4.609	0.079	5.024

References

Alam, M. 1990. Neem in nematode control. In: *Nematode biocontrol (Aspects and prospects*. (Eds.): M.S. Jairajpuri, M.M.Alam and I. Ahmed. BS Publishers and Distributors (Pvt) Ltd. Delhi-110032, India. 51-55pp.

Alam, M.M. 1976. *Organic amendments in relation to nematodes*. Ph.D. Thesis, Aligarh Muslim University. Aligarh.150pp.

Al-Obaedi, J.W, A.R. Al-Askari and Z.A Stephan. 1987. Some pants extracts for the control of root knot nematode *Meloidogyne javanica. Nematol Medit*., 15: 149-153.

Firoza, K and M. Maqbool. 1996. Effect of plant extracts in the control of *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961.*Pak. J. Nematol*., 14: 61 -66.

Gomez, K.A. and A.A. Gomez. 1984. *Statistical procedures for agriculture research.* 2nd ed Wiley, New York, U.S.A. pp. 680.

Goodey, J.B., M.T. Franklin and D.J. Hooper. 1965.Goodey's the nematode parasites of plant Catalogued under their hosts. Farnham Royal, *Commonwealth Agriculture Bureaux*. 3rd. edition pp. 214.

Hafeez, A. 1986. *Plant diseases.* Pakistan Agriculture Research Centre (PARC). Islamabad. pp 552.

Hoan, L. and R.G. Davide. 1979. Nematicidal properties of root extracts of 17 Plant species on *M. incognita*. Philip. *Agricul*., 62: 285-295.

Inouye, S., T. Takizawa and Yamaguchi. 2001. Antibacterial activity of essential oil and their major constituents against respiratory tract pathogens by gaseous contact. *J. Antimicrobial chemotherapy*, 47: 565-573.

Keen, B.A. and H. Raczkowski. 1921. The kjeldahl determination of nitrogen: A critical study of digestion conditions, temperature, catalyst and oxidizing agent. *Aust. J. Chem*., 7: 55-70.

Lamberti, F.1979. Economic of *Meloidogyne* spp., in subtropical and Mediterranean climates, in Root- knot Nematodes (*Meloidogyne* species). *Systematics, Biology and Control* (Eds.): F. Lamberti and C. E. Taylor, Academic Press, New York, pp. 341-57.

Luc, M., A.R. Mageentic and R. Fortuner. 1988. A reappraisal of Tylenchina (Nematol) 9. The family Hetroderoidae Fillip've and Schuurmans Stekhovem, 1941. *Revue Nematol*., 11: 159-176.

Maqbool, M.A. and F. Shahina. 2001. *Biodiversity of nematode fauna in Pakistan* NNRC, University of Karachi, Karachi-75270, Pakistan. 180. pp.

Mehdi, F.S., I.A. Siddique, T. Zia and N.I. Ali. 2001. Use of mangrove for the control of *Meloidogyne javanica* in tomato. *Nematol. Medit*., 29: 127-129.

Morton, J.F. 1981. *Atlas of Medicinal plants of middle America. Bahamas to Yucatan.* C.C. Thomas, Springfield, IL.

Sasser, J.N. 1979. Economic importance of *Meloidogyne* in tropical countries, In: *Root-knot Nematodes* (*Meloidogyne* species) *systematics, Biology and control* (Eds.): F. Lamberti and C. E. Taylor, Academic Press, New York, pp. 359-74.

Shahzad, S. and A. Ghaffar.1992. Effect of different populations of *Paecilomyces lilacinus* on the biological control of *M. phaseolina* and *M. incognita* infection on mung bean. Expert consultation on plant Nematode problems and their control in the Near East Region (IInd International Meeting on plant Nematology, Karachi. p. 77.

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