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USE OF *EUCALYPTUS* SP., IN THE CONTROL OF ROOT INFECTING FUNGI ON MUNG BEAN AND CHICK-PEA

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Abstract

Use of aqueous extract of leaves, stem, bark and fruit *Eucalyptus* sp., in the control of root rot fungi viz., *Fusarium* sp., *Rhizoctonia solani* and *Macrophomina phaseolina* by paper disc and well methods were examined. *R. solani* was less susceptible than *Fusarium* sp., and *M. phaseolina*. Aqueous extract of leaves, stem, bark and fruit of *Eucalyptus* sp., was more effective @ 5% w/v against *M. phaseolina*, *R. solani* and *Fusarium* sp. Soil amendment with leaves, stem, bark and fruit of *Eucalyptus* sp., @ 5% w/w showed significant increase in germination, shoot length, shoot weight, root length and root weight of chick-pea and mung bean plants. Besides this, the infection by *Fusarium* sp., *M. phaseolina*, *R. solani* was also reduced. All parts of *Eucalyptus* sp., were equally effective in the control of root infecting fungi.

Introduction

Diseases of crop plants adversely affect the agricultural economy of a country (Hafeez, 1986). These plant diseases are due to ubiquitous plant pathogens causing severe damages to root system of plants consequently resulting in the death of plants. *M. phaseolina* a soil-borne root infecting fungus, is known to produce charcoal rot of over 500 species of plants (Sinclair, 1982). Another root infecting fungus *R. solani* exists as active mycelium in the soil and attacks more than 2000 species of plants (Parmeter, 1970) and similarly species of *Fusarium* are known to attack a wide range of plants in different parts of the world (Booth, 1971). The chemical control of plant diseases has proved very effective but majority of these chemicals are highly expensive and exhibit lethal effects. The genus *Eucalyptus* of the family Myrtaceae, is a native to Australia. *Eucalyptus* sp., grow under a wide range of climatic and edaphic conditions in their natural habitat. It is found commonly growing in the Karachi University campus. *Eucalyptus* essential oils have shown to have a marked antiseptic activity against a wide variety of infectious bacteria, viruses and fungi (Inouye *et al*., 2001). Organic amendment is an important method for the control of plant diseases. Organic amendments are generally used for the improvement of crop plants and increasing agricultural productivity. Various organic amendments have a suppressive effect on plant parasitic fungi and nematodes (Alam, 1990). The method involves the amendment of different plant parts for the control of fungal and nematode diseases (Mital & Gowswami, 2001). Of the organic substrates, neem cakes (Alam, 1990, Abid *et al*., 1992) have shown promising results in the control of root infecting fungi. The present study was carried out to investigate the antifungal activity of different parts viz., leaves, stem, bark and fruit of *Eucalyptus* sp., in the control of root infecting fungi on mung bean and chick pea.

Materials and Methods

***In Vitro* studies:** Paper disc and well methods were used to study the interaction of root rot fungi with aqueous extract of leaves, stem, bark and fruit of *Eucalyptus* sp. The cultures of *R. solani*, *M. phaseolina* and *Fusarium* sp., were isolated from soil and grown

on potato dextrose agar containing penicillin @ 20,000 unit/litre and streptomycin @ 200 mg/litre. A 5mm disc of the root infecting fungi viz., *M. phaseolina*, *R. solani* and *Fusarium* sp., was placed in the centre and 5 mm paper discs soaked in aqueous extract of leaves, stem, bark and fruit @ 0.1, 1 and 5% w/v were placed on three corners of the Petri dish. The disc soaked in sterilized water placed at other corner served as control. Each treatment was replicated three times for each root infecting fungus. Inoculated Petri dishes were incubated for 5-6 days and zone of inhibition was measured. Similarly in the well method, the four wells on PDA poured plates containing penicillin @ 20,000 unit/litre and streptomycin @ 200 mg/litre were made by cork borer of 5mm. A 5 mm disc of root infecting fungi viz., *M. phaseolina*, *R. solani* and *Fusarium* sp., was inoculated in the centre of Petri dish. Three wells of 5 mm were poured with leaves, stem, bark and fruit aqueous extract @ 0.1, 1 and 5 % w/v .The well inoculated with sterilized water served as control. Each treatment was replicated three times. Inoculated plates were incubated at 30ºC for 5-6 days and zone of inhibition was measured.

***In Vivo* studies:** Different plant parts viz., leaves, stem, bark and fruit of *Eucalyptus* sp., were collected from Karachi University campus, dried and ground in a grinder. The soil used for the experiment was obtained from the experimental plot of Botany Department, University of Karachi. The soil was sandy loam (sand, silt, clay, 70, 19 and 11%), pH ranged from 7.1- 8.1with moisture holding capacity (MHC) of 50% (Keen & Raczkowski, 1922), total nitrogen 0.077-0.099% (Mackenzie & Wallace, 1954), sclerotia of *M. phaseolina* 3-5 g^{-1} soil as found by wet sieving technique (Sheikh & Ghaffar, 1975), 5-10% of *R. solani* on sorghum seeds used as baits (Wilhem, 1955) and 3000 cfu g^{-1} *Fusarium* spp., as assessed by soil dilution technique (Nash & Synder, 1962). Soil was amended with different parts viz., leaves, stem, bark and fruit of *Eucalyptus* sp., used @ 0.1, 1 and 5% w/w and kept in 8cm diam., pot containing 300gm soil and allowed to decompose for 10 days. After 10 days of decomposition of organic matter, 5 seeds of mung bean and chick-pea were sown in each pot. Non-amended soil served as control. Each treatment was replicated three times. After 30 days of germination, mung bean and chick-pea plants were uprooted and growth parameters were recorded. Plant roots were washed in running tap water and surface sterilized with 1% Ca $(OCl)_2$ for 3 minutes. Five pieces of roots were transferred on PDA plates containing penicillin @ 100,000/litre and streptomycin @ 20mg/l. Petri dishes were incubated for five days at room temperature to confirm infection by root infecting fungi.

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

Results and Discussion

Aqueous extract of leaves, stem, bark and fruit of *Eucalyptus* sp., by paper disc and well method showed inhibition of growth of *Fusarium* sp., *R. solani* and *M. phaseolina* with increasing percentage but *R. solani* was less inhibited as compared with other two fungi by both paper disc and well method (Table 1). Three percentages 0.1, 1 and 5% w/v were not equally inhibitory to test fungi but more inhibition was recorded @ 5% w/v. Present results showed that extract of different parts viz., leaves, stem, bark and fruit of *Eucalyptus* sp., exhibited potential to suppress the *Fusarium* sp., *R. solani* and *M. phaseolina* which have a wide host range and are distributed worldwide (Ehtheshamul Haque & Ghaffar, 1994). This study revealed that aqueous extract was effective in general to cause growth inhibition in three test fungi. But test fungal species were not equally susceptible to aqueous extract of *Eucalyptus* sp.

Table 1. Growth Inhibition of root rot fungi with *Eucalyptus* sp., by paper disc and well method.

Treatments		*Fusarium* spp. (mm)	*Rhizoctonia solani* (mm)	*Macrophomina phaseolina* (mm)
		Paper disc method		
0% (control)		0	0	0
0.1% extract	Leaves	17.0	10.0	12.6
	Stem	19.6	10.0	11.6
	Bark	19.0	1.0	7.6
	Fruit	19.3	19.3	13.0
1% extract	Leaves	21.0	16.6	18.0
	Stem	21.0	10.0	16.6
	Bark	19.6	4.6	8.0
	Fruit	21.0	19.3	17.3
5% extract	Leaves	21.3	19.3	18.6
	Stem	22.0	11.0	21.0
	Bark	20.3	5.0	11.6
	Fruit	22.0	24.3	18.0
		Well method		
0% (control)		0	0	0
0.1% extract	Leaves	20.6	11.3	14.6
	Stem	19.0	11.0	16.0
	Bark	20.3	11.6	11.6
	Fruit	21.0	14.0	17.3
1% extract	Leaves	20.6	12.6	18.3
	Stem	21.0	13.3	19.0
	Bark	23.6	12.0	19.0
	Fruit	21.3	16.0	19.6
5% extract	Leaves	21.0	14.6	19.3
	Stem	24.3	13.6	24.6
	Bark	24.0	12.6	22.6
	Fruit	24.3	16.6	20.3

Significant increase in growth parameters was observed where soil was amended with different parts viz., leaves, stem, bark and fruit of *Eucalyptus* sp., used @ 0.1, 1 and 5% w/w (Table 2). There was significant increase in shoot length ($p<0.001$), shoot weight ($p<0.01$), root length ($p<0.001$) and root weight ($p<0.01$) of mung bean plants whereas shoot length of chick-pea plants showed a significant ($p<0.05$) increase. Significant reduction in infection of *R. solani* and *M. phaseolina* ($p<0.05$), ($p<0.01$) was observed on mung bean plants whereas there was complete suppression of *M. phaseolina* ($p<0.01$) infection followed by *R. solani* and *Fusarium* on chick-pea plants where soil was amended with different parts of *Eucalyptus* sp., used @ 0.1, 1 and 5% w/w (Table 2). All parts viz., leaves, stem, bark and fruit of *Eucalyptus* sp., were more effective when used @ 5% w/w. Present results showed that leaves, stem, bark and fruit powder of *Eucalyptus* sp., has potential to reduce infection caused by root infecting fungi viz., *Fusarium* sp., *R. solani* and *M. phaseolina*. Neem cake has shown promising results in the control of root infecting fungi (Alam, 1990; Abid *et al*., 1992). Similarly seaweeds *Stoechospermum marginatum*, neem cake and cotton cake showed promising results in the control of root

infecting fungi on sunflower (Ehtheshamul-Haque *et al*., (1998). Tariq *et al*., (2006) used different parts of *Avicennia marina* viz., leaves, stem and pneumatophore for the control of root infecting fungi. *Eucalyptus* essential oil is considered to have marked antiseptic action against infectious bacteria, viruses and fungi (Inouye *et al*., 2001). There is need to provide information to farmers on the use different parts of *Eucalyptus* sp., in the control of root rot fungi on a large scale.

Table 2. Effect of different parts of *Eucalyptus* sp., on growth parameters and control of root infecting fungi on mung bean and chick–pea plants.

	Plant growth parameters					Infection %		
Treatments	**Germination %**	**Shoot length**	**Shoot weight**	**Root length**	**Root weight**	***Fusarium* spp.**	***R. solani***	***M. phaseolina***
				Mash bean				
Control	100	16.7	0.24	2.76	0.06	100	77.77	55.55
0.1% Leaf	100	21.4	0.49	4.51	0.09	100	33.33	0
1% leaf	100	22.6	0.53	4.9	0.12	88.88	33.33	0
5% leaf	86.66	25.12	0.78	6.56	0.13	77.77	0	0
0.1% stem	100	22.2	0.69	5.7	0.07	88.88	11.11	11.11
1% stem	93	22.2	0.74	7.1	0.15	88.88	0	11.11
5% stem	93	24.52	0.76	7.2	0.20	77.77	0	0
0.1% bark	100	23.5	0.63	6.05	0.08	77.77	44.44	11.11
1% bark	100	24.4	0.80	8.2	0.12	77.77	11.11	11.11
5% bark	100	24.76	0.85	10.23	0.13	66.66	0	0
0.1% fruit	86.66	20.9	0.31	4.5	0.07	88.88	44.44	22.22
1% fruit	66.66	21.3	0.47	4.8	0.08	77.77	33.33	0
5% fruit	93	23.3	0.66	10.6	0.08	66.66	0	0
LSD.05 =	19.017	3.007	0.269	2.618	0.0583	45.202	44.367	22.484
				Chick pea				
Control	100	20.8	0.54	9.9	0.21	100	66.66	22.22
0.1%Leaves	93	23.4	0.69	16.7	0.29	100	55.55	0
1% leaves	93	24.6	0.70	19.0	0.30	77.77	33.33	0
5% leaves	86.66	28.9	0.80	19.6	0.31	66.66	33.33	0
0.1% stem	100	25.2	0.75	18.1	0.35	88.88	66.66	0
1% stem	100	26.6	0.79	21.1	0.36	77.77	55.55	0
5% stem	100	31.1	0.86	26.9	0.38	66.66	44.44	0
0.1% bark	93	24.8	0.71	17.1	0.34	88.88	33.33	0
1% bark	93	28.5	0.81	17.4	0.37	66.66	22.22	0
5% bark	93	30.1	0.82	17.6	0.39	44.44	11.11	0
0.1% fruit	100	25.4	0.65	15.5	0.31	100	22.22	0
1% fruit	86.66	26.1	0.70	16.6	0.33	88.88	11.11	0
5% fruit	86.66	27.0	0.80	20.9	0.36	66.66	0	0
LSD.05 =	22.949	4.660	0.228	10.531	0.194	48.441	49.058	8.993

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