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ORGANIC AND INORGANIC FERTILIZERS ALONG WITH *THUJA ORIENTALIS* L. FOR THE CONTROL OF ROOT INFECTING FUNGI

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Abstract

Macrophomina phaseolina (Tassi) Goid, *Rhizoctonia solani* Kuhn and *Fusarium* species Schlecht are reported to be associated with wilting and root rot diseases in different crop plants all over the world. To improve crop production, fertilizers like cow dung, goat dung, urea and Di Ammonium Phosphate (DAP) with doses of 0.01 and 0.1 % w/w were mixed in soil in combination with seed treatment with stock solution of *Thuja orientalis* L. plant at 100% (w/v). Results revealed that maximum improvement in growth and reduction in colonization of root infecting fungi like *M. phaseolina, R. solani* and *Fusarium* spp., were recorded when 0.1 and 0.01% goat dung was incorporated in soil in combination with seed treatment with *T. orientalis* extract on cowpea and mash bean plants.

Key words: Fertilizers with different doses, Cowpea and mash bean plants, Aqueous extract of *T. orientalis,* Root infecting fungi.

Introduction

Thuja orientalis L. belongs to the family cupressaceae, commonly known as morpankhi. Their leaves contain essential oil like carnphor, fenchone, isothujone, thujone, mono and sesquiterpenes which includes carbohydrate, phenol, alcohols, ether, aldehyde and ketone are used in different activities like treatment of fungal infection, cancer, moles, as antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic (Sokovic *et al.,* 2010; Yeung, 1985). The chemical constituents of *T.orientalis* leaves contained rhodoxanthin, amentoflavone, hinokiflavone, quercetin, myricetin, carotene, xanthophylls, ascorbic acid and thujone which is found naturally in two diastereomeric forms: α-thujone and β- thujone (Perry *et al.,* 1999). Nowadays, plant extracts with their phytochemical constituents are getting interest due to have potential sources against microbial, viral inhibitors and for human health (Jasuja *et al*., 2012). Great biological activity against microorganisms have been reported due to antioxidant compounds and bioactive constituents of plants like tannins, flavonoids, saponins, terpenoids and alkaloids which provide protection against diseases of free- induced oxidative stress (Sulaiman *et al*., 2011; Mandalari *et al*., 2007; Avato *et al*., 2006; Funatogawa *et al.,* 2004; Navarro & Delgado, 1999).

The most common root infecting fungi like *Fusarium* species, *Macrophomina phaseolina, Rhizoctonia solani* causes various complex root rot and wilt diseases on many leguminous and non-leguminous crops which results in the death of plants (Hassan *et al.,* 2014; Nancy *et al.,* 1997). *Fusarium* spp., causing stem rot, root rot and wilt diseases produced economic losses on a wide variety of crop plants (Ploetz *et al.,* 2007; Larkin & Fravel, 1998). *F. oxyporum* have association with endo-mycorrhizal organisms causes wilt in banana, cotton, sweet potatoes, tomato, asparagus, muskmelon, cantaloupe and ginger (Stover, 1962). *M. phaseolina* produces infection in all stages of plant growth initiated from soil, seed and infected plant debris (Reuveni *et al.,* 1983). *M. phaseolina* found in soil produced sclerotia which remain dormant and under suitable environmental condition produce hyphae which cause infection in the roots of host plant (Clare *et al.,* 2010; Ammon *et al.,* 1974). Charcoal rot, seedling blight, pod rot, root rot and stem rot diseases are caused by *M. phaseolina* resulting in the death of seedling due to the blockage of xylem tissues (Srivastava *et al.,* 2001). *R. solani* Kuhn is also a root rot, facultative plant pathogen which causes seed rot, wilting, root rot, damping off of seedling, black scurf of potatoes, bare patch of cereals, root rot of sugar beet, belly rot of cucumber and sheath blight of rice (Bolton *et al.,* 2010; Godoy-Lutz *et al.,* 2008).

Organic manures are cheap and ecofriendly source for the improvement of crop production in low-input agriculture. Use of plant along with organic and inorganic fertilizers has gained importance in sustainable crop production (Abdullahi & Sheriff, 2013). Mixing of organic manures in soil gave positive effects on physical and biochemical properties of soil. Many research work has confirmed efficacy of organic and inorganic fertilizers mixed in soil shows promising result in improvement of plant growth and effectively suppress certain soil borne plant pathogens (Tariq *et al.,* 2008; Abdullahi *et al.,* 2014; Ikram & Dawar, 2015).

The objectives of this study was to investigate the efficacy of *T. orientalis* as seed treatment, along with different fertilizers for the control of diseases caused by *M. phaseolina, R. solani* and *Fusarium* spp., on different crop plants.

Materials and Methods

Plant material and fertilizers collection: *Thuja orientalis* L. plant was collected from Department of Botany, University of Karachi. Plant was washed with tap water followed by sterilized distilled water to avoid dusts and other unwanted materials from the natural environment. These dust free plant was allowed to dried in shade for 2 weeks and then powdered using an electric grinder. This plant powder (10 g) dissolved in 100 mL sterilized distilled water, left overnight to allow constituents to get dissolved in water. Extract was filtered using Whatman's No.1 filter paper and the residue obtained was centrifuge at 500 rpm for 15 minutes and the supernatant was taken as stock solution. In our previous work seed treatment with 100, 75 and 50% of *T. orientalis* was used, of which 100%

concentration for 10 minutes gave better result in improvement of crop plants so selected for further studies (Sarfaraz, 2016). Inorganic and organic fertilizers like Di Ammonium Phosphate (DAP), urea was purchased from local market while cow dung and goat dung obtained from cattle farm of Karachi.

Screen house experiment: Pot experiment was set in a screen house of Botany Department, University of Karachi where sandy loam soil (300 g) was placed in pots having pH ranged from 7-7.6 with moisture holding capacity (MWHC) of 23.12% (Keen & Raczkowski, 1922), total nitrogen 1.5% (Mackenzie and Wallace, 1954). Natural soil contained 4-6 sclerotia/g of *M. phaseolina* (Sheikh & Ghaffar, 1975), 6-9% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3700 cfu g^{-1}*Fusarium*spp., (Nash & Snyder, 1962). Seeds of mash bean and cowpea treated with stock solution of *T. orientalis* for 10 minutes and dried on blotter paper. Soil was mixed with fertilizers (DAP, urea, cow dung and goat dung) at rate of 0.01 and 0.1 % w/w separately, transferred in pots containing 300 g/pot and 4 treated seeds of mash bean and cowpea were sown in each pot separately. Concentrations of fertilizers were selected on basis of previous work by different research which showed that highest dose of fertilizers gave fatal effect on plant growth (Kumar *et al.,* 2005; Sikander *et al.,* 2009). A set of seeds treated with sterilized distilled water was also kept as control. The experiment was performed on screen house bench in randomized complete block designed with three replicates of each treatment. For comparison, control set was also kept in which untreated seeds and soil without fertilizer were used. After thirty days of germination of seeds, plant length weight, number of nodules and number of leaves were observed. Colonization of root rot fungi was recorded by washing roots with tap water and surface sterilized with 1% sodium hypochloride and roots were placed on Petri plates containing Potato Dextrose Agar supplemented with antibiotics (Kanwal *et al.,* 2017; Rafi *et al.,* 2016).

Analysis of data: Data of growth parameters and colonization % of root infecting fungi were analyzed using ANOVA and means were compared by least significance difference at 5 % probability level (Sokal & Rohlf, 1995).

Results

All fertilizers used in this research gave maximum enhancement in growth parameters like shoot length, shoot weight, root length, root weight, number of nodules and leaves, while root infecting fungi were significantly reduced on mash bean plant. Shoot length was enhanced when soil was mixed with cow dung at 0.1% (w/w) in combination with seeds treatment by *T. orientalis* stock solution. Similarly, shoot and root weight were increased significantly ($p<0.001$) when soil was amended with DAP at 0.01% (w/w) along with seeds treated with *T. orientalis* extract. There was improvement in root length ($p<0.01$) and number of nodules when goat dung applied in soil in combination with seeds treatment with *T. orientalis* extract ($p<0.01$). Increased number of leaves were recorded when soil was amended with cow dung @ 0.01% (w/w) in combination with seed treatment with *T. orientalis* extract. Complete reduction of *R. solani* ($p<0.05$) was observed when cow dung at 0.1 % w/w was used along with seeds treated with *T. orientalis* extract while significant reduction of *Fusarium* spp., ($p<0.05$) and *M. phaseolina* were recorded when goat dung at 0.1 % w/w was used in combination with seed treatment with *T. orientalis* (Table 1).

In case of cowpea, shoot length and root length ($p<0.001$) were increased significantly ($p<0.05$) when soil was mixed with goat dung at 0.01% w/w in combination with seeds treated with *T. orientalis* extract. Shoot weight was significantly ($p<0.01$) increased when soil was mixed with DAP at 0.1% w/w and seeds treated with extract in contrast to control. Similarly, root weight was significantly ($p<0.05$) increased when seeds were treated with *T. orientalis* extract in combination with soil amendment with urea at 0.01% (w/w). Maximum number of nodules ($p<0.01$) and leaves were recorded when soil was mixed with cow dung 0.1% (w/w) in addition with treated seeds as compared to control. Goat dung and cow dung at 0.01 and 0.1% w/w was observed to best in reduction of *Fusarium* spp., *R. solani* ($p<0.001$) and *M. phaseolina* ($p<0.001$) colonization on cowpea roots (Table 2).

Cow dung and goat dung at 0.1 %, 0.01 % gave much better results in improvement of mash bean and cowpea plants when used in combination with *T. orientalis* extract used for seed treatment followed by DAP and urea as well as reduced the colonization of root infecting fungi particularly *R. solani.*

Discussion

Goat and cow dung considered as valuable fertilizer used for centuries in providing necessary nutrients like micronutrients for crop growth. They are extensively used by growers for the production of different crops due to presence of medicinal properties according to unani or ayurvediic medicine. Presently cow dung at 0.1% and goat dung at 0.01% w/w in combination with seeds treatment with stock solution of *T. orientalis* extract were found to enhanced the plant growth and showed inhibition of root infecting fungi like *Fusarium* spp., *R. solani* and *M. phaseolina* followed by DAP and urea. In cowpea, when soil was amended with goat dung and cow dung @ 0.01% (w/w) and urea 0.01% (w/w) in combination with 100% (w/v) extract of *T. orientalis,* significant increment in growth parameters and reduction in the incidence of root infecting fungi were recorded. Previous observations showed improvement in growth of *Zea mays*, *Abelmoschus esculentus*, *Oryza sativa*, *Luffa cylindrical* and *Lycopersicon esculentum* and reduced pathogenic fungi like *Colletotrichum capsici*, *Sclerotium rolfsii*, *Alternaria alternata*, *Penicillium* species, *R. solani*, *Phytophthora palmivora*, *Helminthosporium* species by the use of cow dung and cow urine (Nautiyal *et al.,* 2006). Cow dung showed much effectiveness against conidial germination and mycelial growth of *Fusarium oxysporum* and *F. solani* on cucumber plants (Basak & Lee, 2001; Basak *et al.,* 2002). Cow dung coated seeds showed more survival, improved shoot length, dry weight and number of nodules as compared to non cow dung treated plants (Nautiyal *et al.,* 2013). Present results showed that root pathogens were successfully reduced by the addition of cattle manure while *R. solani* colonization was completely inhibited in mash bean when used in combination with extract of *T. orientalis.* Seeds coated with cow dung and sown in the presence of mixture of wilt complex fungi treated soil could reduce cell wall degrading enzymes activities produced by plant roots against pathogens (Nautiyal *et al.,* 2013).

Table1. Combined effect of different fertilizers along with *T. orientalis* aqueous extract on growth and in the control of root rot fungi of mash bean plants.

Treatments			Growth parameters						Colonization % of root infecting fungi		
Fertilizers	**Concentrations (%)**	**Methods**	**Shoot length (cm)**	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**	**No. of nodules**	**No. of leaves**	***F. oxysporum***	***R. solani***	***M. phaseolina***
-	0	-	21.64	0.44	11.68	0.05	2.33	5.66	90.66	30	30
Cow dung	0.01	Treated seeds with *T. orientalis*	25.14	0.34	18.66	0.04	5.22	8.22	43.33	26.67	20
		Non treated seeds	24.16	0.38	16.75	0.14	6.55	4.44	76.66	6.67	13.33
	0.1	Treated seeds with *T. orientalis*	25.21	0.17	12.17	0.02	5.55	6.33	46.67	0	26.67
		Non treated seeds	22.5	0.47	21.97	0.18	8.66	6.22	86.77	23.33	20
Goat dung	0.01	Treated seeds with *T. orientalis*	23.11	0.45	14.51	0.06	1.88	5.77	50	30	16.67
		Non treated seeds	24.28	0.56	19.14	0.21	6.88	6.33	80	26.67	46.67
	0.1	Treated seeds with *T. orientalis*	23.56	0.72	16.91	0.51	9.22	6.55	33.33	16.67	10
		Non treated seeds	21.41	0.32	10.24	0.09	2.44	5.44	83.33	18.33	13.33
Urea	0.01	Treated seeds with *T. orientalis*	21.78	0.46	14.61	0.08	0.33	5.44	46.67	6.67	16.67
		Non treated seeds	20.58	0.52	11.07	0.31	0.77	5.38	63.33	20	20
	0.1	Treated seeds with *T. orientalis*	23.7	0.22	23.84	0.05	3.88	5.77	60	43.33	20
		Non treated seeds	19.1	0.41	6.03	0.14	0	5.88	66.67	6.67	16.67
DAP	0.01	Treated seeds with *T. orientalis*	22.44	0.17	13.77	0.01	1.66	6.22	53.33	53.33	13.33
		Non treated seeds	22.61	0.60	15.64	0.12	4.11	7.22	73.33	36.67	33.33
	0.1	Treated seeds with *T. orientalis*	26.73	0.22	10.84	0.03	1.66	6.55	50	40	3.33
		Non treated seeds	20.0	0.57	6.48	0.17	0.11	6.77	53.33	20	20
$LSD_{0.05}$	Concentrations		1.60	0.10	2.77	0.68	1.74	0.86	9.62	11.72	7.14
	Methods		1.85	0.12	3.20	0.78	2.01	0.99	11.11	13.54	8.24
	Fertilizers		1.30	0.08	2.26	0.55	1.42	0.70	7.85	9.57	5.83

Table 2. Combined effect of different fertilizers along with *T. orientalis* aqueous extract on growth parameters and in the control of root rot fungi of cowpea plants.

Treatments			Growth parameters						Colonization % of root infecting fungi		
Fertilizers	**Concentrations (%)**	**Methods**	**Shoot length (cm)**	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**	**No. of nodules**	**No. of leaves**	***F. oxysporum***	***R. solani***	***M. phaseolina***
-	0	-	31.91	1.31	9.3	0.19	0.88	5.88	96.66	53.33	43.33
Cow dung	0.01	Treated seeds with *T. orientalis*	31.78	2.19	8.77	0.44	1.11	6.44	70	10	13.33
		Non treated seeds	34.73	1.80	7.61	0.45	0.88	5.11	86.66	20	19.63
	0.1	Treated seeds with *T. orientalis*	37.84	2.15	10.46	0.26	2.22	6.22	73.33	3.33	6.67
		Non treated seeds	32.36	2.08	11.54	0.64	2.66	5.44	83.33	22.67	16.66
Goat dung	0.01	Treated seeds with *T. orientalis*	37.91	2.07	16.14	0.26	5.22	7.22	50	13.33	3.33
		Non treated seeds	36.5	2.27	14.17	0.62	2.00	6.00	66.66	23.33	13.36
	0.1	Treated seeds with *T. orientalis*	37.53	2.18	13.45	0.29	1.77	7.00	80	20	30
		Non treated seeds	32.74	2.36	15.97	0.61	0.44	6.36	83.33	10	13.33
Urea	0.01	Treated seeds with *T. orientalis*	32.95	1.97	12.62	1.51	0.77	6.44	70	3.33	16.67
		Non treated seeds	32.77	2.63	13.77	0.30	0	4.55	72.66	13.33	16.66
	0.1	Treated seeds with *T. orientalis*	32.25	1.95	12.74	0.35	0.44	6.22	76.67	23.33	3.33
		Non treated seeds	29.62	1.54	10.84	0.37	0.55	6.22	70	20	20
DAP	0.01	Treated seeds with *T. orientalis*	36.76	2.10	13.88	0.33	1.00	7.01	66.67	10	33.33
		Non treated seeds	33.71	2.27	14.83	0.82	1.33	6	50	10	10
	0.1	Treated seeds with *T. orientalis*	29.58	2.38	8.37	0.97	0.22	6.11	66.60	10.67	13.33
		Non treated seeds	28.62	1.98	8.61	0.56	0	5.11	50	16.67	26.66
$LSD_{0.05}$	Concentrations		1.81	1.01	1.45	1.43	0.60	0.69	36.21	6.26	5.68
	Methods		2.09	1.16	1.68	1.65	0.69	0.80	41.81	7.23	6.56
	Fertilizers		1.48	0.82	1.18	1.17	0.49	0.56	29.56	5.11	4.63

Cattle manure is made up of digested grass, grain and rich contents of nitrogen, phosphorus and potassium which is helpful in eliminating harmful ammonia gas and pathogens. By use of manure not only moisture holding capacity improved but also helpful in addition of generous amount of organic matter in soil (Phipps, 2016; Srivastava *et al.,* 2010). Necessary nutrients and pest repellent properties are obtained from cow dung while urine contain micronutrients and increase fertility helpful in providing food which was free from hazards of chemical fertilizers and pesticides (Chauhan, 2000). According of Ebenezer *et al.,* (2012) goat dung extract with concentrations (1.0 and 5.0%) was helpful in completely reduction of *Sclerotium rolfsii* growth on oil palm plantation.

Results of the present study clearly suggest that organic fertilizers like cow and goat dung at 0.1 and 0.01% inhibited the growth of root rot pathogens. DAP and urea also reduced the fungal pathogens on roots but less activity was recorded as compared to cow and goat dung. It can be therefore be suggested that both dungs in combination with seed treatment with *T. orientalis* aqueous solution could be useful in the growth and development of crop plants with the same time enriching soil and provide valuable nutrients in growth promotion.

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