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IMPACT OF SEED PRIMING WITH *CARICA PAPAYA* (L.) EXTRACT IN THE CONTROL OF ROOT INFECTING FUNGI AND GROWTH OF MASH BEAN AND CHICK PEA

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ABSTRACT

Carica papaya L., family Caricaceae, is cultivated in many tropical countries. Many commercial products are derived from its different parts of which papin and chymopapain are the most prominent ones, produced from fruit, stem and leaves. An attempt was made to understand the mechanism of seed priming treatments using *C. papaya* extracts (leaves, fruit, seeds) for various periods (5, 10, 20, 40 minutes) on mash bean (*Vigna mungo* L.) and cowpea (*Vigna unguiculata* (L.) Walp for the control of root infecting fungi viz., *Rhizoctonia solani* (Kühn), *Fusarium* spp. and *Macrophomina phaseolina* (Tassi) Goid. The results showed significant suppression of root rot fungi and significant enhancement in the growth parameters like shoot length, shoot weight and number of nodules in mash bean while in cowpea all growth parameters showed significant enhancement. Seed priming with seed extract of *C. papaya* for 40 minutes time interval was found to be effective for the control of root rot fungi on mash bean.

KEYWORDS: *Carica papaya* parts, Growth parameters, Root rot fungi, Seed priming.

INTRODUCTION

According to Garrett (1970) root infecting fungi constitute a substantial and omnipresent threat to the welfare of all crops. *Aphanomyces euteiches, Rhizoctonia solani, Fusarium* spp., *Sclerotium rolfsii* are the root rot causing fungi on different crops like pea, chickpea, lentil, faba bean and lupine (Abou-Zeid *et al.*, 1997; Abdel- Kader *et al*., 2002; Infantin *et al*., 2006). Charcoal rot fungi, *Macrophomina phaseolina* (Tassi) Goid produces seedling blight, root rot, stem rot, pod rot on nearly 500 plant species (Dhingra and Sinclair, 1978). Several *Rhizoctonia* spp., cause root rot, but the most common is *R. solani* which causes wilting and death in several hundred genera of plants. *Rhizoctonia* root rot is favoured by relatively high temperatures and intermediate moisture (Steve, 2001). Insect pest and other fungal diseases like damping –off and stem rot attacks on cowpea (*Vigna unguiculata* [L.] Walp) and reduces its yield (Singh *et al*., 1997; Aveling and Adandonon, 2000). Besides *M. Phaseolina, R. solani, Pythium ultimum, Fusarium solani, Sclerotium rolfsii* which are recocnized from different countries like Southern Africa, Nigeria, Asia, Brazil, which are the most visible threats to food production (Adandonon, 2000; Cook, 1994; Fravel, 2005; French and Kennedy, 1963). There are various ways to prevent from these losses like by the use of fungicides, soil drenching, use of microbial antagonists, crop rotation, resistant varieties, plant extracts, soil amendment, hydropriming, and biopriming (Al-Obaedi *et al*., 1987). Biocontrol agents are important with respect to the seed treatment in root rot control (Jahn and Puls, 1998). Extracts obtained from some plants have been reported to play very important role in crop yield (Farooq *et al*., 2006). Priming of seeds is very helpful in uniform germination, improvement in seed tolerance and make them resistant against adverse environmental conditions (Harris *et al*., 1999). Seed priming stimulates chemical changes, activation of enzymes, dormancy breakage and hydrolyses of seed reserves (Asgedom and Becker, 2001; Çatav *et al.*, 2012; Khan *et al*., 2008; Khalil *et al*., 2010).

Biopriming is very important technique and improve method to treat the seeds by other living material at different concentration and intervals to enhance the growth parameters. Seeds priming with PGPR enhance growth and crop yield (Asghar *et al*., 2002; Bashan *et al*., 2004). Many researchers work on extract of *C. Papaya* L. To observe its activity. According to Kermanshai *et al*. (2001); Adebiyi and Adaikan (2005), aqueous seed extracts of *C. papaya* showed antihelminthic activity against *Caenorhabditis elegans*. Likewise, alcoholic extracts of the epicarp, endocarp, roots and seeds from ripe and unripe papaya fruit have antidiarrheic, antidysenteric and antibacterial properties (Osuna-Torres *et al*., 2005; Doughari *et al*., 2007; Emeruwa, 1982). The present study was carried out to find out the antifungal activity of leaves, seeds and fruit of *C. papaya* against *M. phaseolina* (Tassi) Goid, *R. solani* and *Fusarium* spp., and growth of cowpea and mashbean.

MATERIALS AND METHODS

Collection of plant material: Leaves, seeds and fruit of *C. papaya* were collected from local market and after washing them with tap water, dry under shade. After drying ground in an electric grind, powder of different parts kept in jar and use for further studies.

Soil used: Soil was collected from experimental plot of Department of Botany, University of Karachi. Sandy loam soil contains sand, silt, clay in ratio of 74: 14: 12%), pH (7.2-9.6), moisture holding capacity (49%), total nitrogen (0.077-0.099%) (Keen and Raczkowski, 1922; Mackenzie and Wallace, 1954).Natural amount of soil borne fungi was estimated as *M. phaseolina* (3-7 sclerotia g^{-1}), *R. solani* (5-20%), *Fusarium* spp., (2000 cfu g^{-1}) according to the protocol followed by wet sieving technique (Sheikh and Ghaffar, 1975), baiting technique (Wilhelm, 1955) and soil dilution technique (Nash and Synder, 1962).

Seed priming: Seeds of cowpea and mashbean were surface sterilized with 1% Ca $(OCl)_2$ for 5 minutes. Seeds were treated with the extract of seeds, leaves and fruit of *C. papaya* for 5, 10, 20 and 40 minutes then air dried. Five seeds were sown in each pot having 300 g of soil, plants were regularly watered for 30 days. The pots were kept in randomized complete block design with three replicates per treatment. Control having non treated soil and non treated seeds. All the plants were uprooted after 30 days and observe growth parameters like shoot length, root length, shoot weight and root weight.

Isolation of fungi from roots: Roots of each treatment were surface sterilized in Ca $(OCl)_2$ (1%) and then cut five pieces, plated on PDA poured plates containing antibiotics (penicillin @ 20,000unit/litre and streptomycin @ 200mg/litre). Incubate for 5 days at room temperature for the isolation of root infecting fungi on roots. Colonization percentage was calculated as follows (Short *et al*., 1980).

$$\text{Colonization \%} = \frac{\text{Number of roots pieces infected by pathogen}}{\text{Total number of root pieces}} \times 100$$

Analysis of data: Pots were arrange on green house bench according to completely randomized design.Analysis of variance (ANOVA) was performed on the replicated data. Duncan multiple range test was also determined (Gomez and Gomez, 1984).

RESULTS

Biopriming of seeds with leaves, seeds and fruit extract of *C. papaya* showed improved results. Fruit extract for 40 minutes gave maximum shoot weight and root length of mash bean plants ($p<0.05$) while shoot length and number of nodules were maximum when seeds of mash bean primed with leaves extract for 40 minutes ($p<0.05$). However, seed extract for 40 minutes showed enhanced root weight (Table 1). All treatments were significantly reduced root infecting fungi. *M. phaseolina* was reduced upto maximum level due to priming with seed extract for 40 minutes while complete reduction of *R. solani* and *Fusarium* spp., was observed when mash bean was primed with seed and fruit extract for 40 and 20 minutes ($p<0.05$; 0.001).

In case of cowpea, priming with seed extract for 40 minutes enhances shoot weight, shoot length, root weight and number of nodules while maximum root weight was recorded due to priming with fruit extract for 40 minutes followed by seed extract ($p<0.05$; 0.001; Table 1). Colonization of *Fusarium* spp., was significantly reduced ($p<0.05$) when seed extract was used as priming agent for 40 minutes while priming with fruit extract showed reduced *Fusarium* spp. All extracts primed for 40 minutes gave complete reduction of *R. solani* colonization (Table 2).

DISCUSSION

Vegetal extracts having antifungal properties used as a common practice for many years. Biological fungicides are provide us natural management of phytopathogenic fungi. Baños-Guevara *et al*. (2004) highlighted the importance of secondary metabolites in vegetal extracts with pesticide or fungicide effects. *Carica papaya* L., of Caricaceae family used as antihelminthic and antimoeboic activities. The juice is used for warts, cancers, tumors and indurations of skin. Young leaves and other parts of *C. papaya* cotains alkaloids. Latex of *C. papaya* used as dyspepsia cure and apply externally to burns (Reed, 1976) and antiseptic. Seeds contain bactericidal aglycone of benzyle isothiocyanate. Ethanolic compound in *C. papaya* leaves have antifungal activity against *M. phaseolina* (Tassi) Goid, *R. solani* (Kuhn) and *Fusarium* spp. These pathogens are soil borne and causing root rot disease, which are responsible for greater crop losses Chemical control has been expensive and have negative impact on environment. Biopriming and hydropriming are effective methods to control the root rot diseases and promote plant growth. Biopriming of seed used as alternative method for controlling many seed and soil borne pathogen (Harman and Taylor, 1988).

Table 1. Effect of biopriming with extracts of *C. papaya* on growth of mash bean and cowpea.

Treatments	Mash bean					Cowpea				
	Shoot weight (gm)	Shoot length (cm)	Root weight (gm)	Root length (cm)	No. of nodules	Shoot weight (gm)	Shoot length (cm)	Root weight (gm)	Root length (cm)	No. of nodules
Control	0.25	6.2	0.02	2.66	2.73	1.05	11.63	0.2	2.96	4.86
5 min *C. papaya* seed extract	0.36	13.16	0.06	5.26	7.6	1.91	19.1	0.40	6.99	12.51
10 min *C. papaya* seed extract	0.39	15.4	0.11	4.63	4.96	1.62	20.84	0.33	9.23	13.3
20 min *C. papaya* seed extract	0.42	15.86	0.10	6.06	10.33	2.09	23.63	0.49	10.37	14.22
40 min *C. papaya* seed extract	0.51	17.4	**0.37**	7.4	12.17	**2.26**	**25.06**	**0.65**	11.21	**15.63**
5 min *C. papaya* fruit extract	0.47	14.53	0.05	5.9	9.3	1.08	15.2	0.3	4.77	6.89
10 min *C. papaya* fruit extract	0.38	15.6	0.06	5.53	9.33	1.08	18.2	0.28	7.83	7.55
20 min *C. papaya* fruit extract	0.57	17.5	0.086	5.73	4.2	1.39	21.23	0.25	11.63	8.73
40 min *C. papaya* fruit extract	**0.60**	18.63	0.173	**9.26**	6.63	1.61	23.96	0.58	**12.36**	12.83
5 min *C. papaya* leaf extract	0.26	13.53	0.04	5.76	3.1	1.68	18.53	0.24	4.73	6.1
10 min *C. papaya* leaf extract	0.43	14.63	0.11	5.06	3.53	1.71	20.2	0.30	6.06	7.32
20 min *C. papaya* leaf extract	0.57	14.86	0.05	5.13	4.66	1.89	20.15	0.34	8.93	8.57
40 min *C. papaya* leaf extract	0.59	19.83	0.12	7.23	12.53	1.96	21.73	0.44	8.63	10.5
$LSD_{0.05}$=	0.233	5.711	0.227	3.562	6.666	0.891	6.303	0.259	3.585	8.348

Table 2. Effect of biopriming with extracts of *C. papaya* in the control of root rot fungi on mash bean and cowpea.

Treatments	Mash bean			Cowpea		
	Macrophomina phaseolina	*Fusarium* spp.	*Rhizoctonia solani*	*Macrophomina phaseolina*	*Fusarium* spp.	*Rhizoctonia solani*
Control	51.06	35.5	44.43	46.63	44.43	17.76
5 min *C. papaya* seed extract	37.73	26.6	6.63	24.43	26.63	15.5
10 min *C. papaya* seed extract	37.73	28.86	2.2	17.73	19.96	8.86
20 min *C. papaya* seed extract	11.1	19.9	0	8.86	15.53	4.43
40 min *C. papaya* seed extract	8.86	0	0	2.2	11.1	0
5 min *C. papaya* fruit extract	37.7	26.63	2.2	24.4	33.3	0
10 min *C. papaya* fruit extract	24.43	24.4	4.43	17.73	31.06	0
20 min *C. papaya* fruit extract	26.63	8.86	2.2	11.1	13.3	0
40 min *C. papaya* fruit extract	24.43	0	0	8.85	2.2	0
5 min *C. papaya* leaf extract	46.63	33.3	13.3	17.76	24.4	11.06
10 min *C. papaya* leaf extract	22.16	22.1	6.66	8.86	13.3	11.1
20 min *C. papaya* leaf extract	24.4	22.2	0	6.63	8.86	6.66
40 min *C. papaya* leaf extract	17.76	17.73	0	4.43	8.83	0
$LSD_{0.05}$=	30.19	19.21	11.866	23.554	22.934	16.406

Biopriming with *C. papaya* seed, leaves and fruit extract for 40 minutes hydropriming was observed best to increase shoot weight and length and 10 minutes to promote root length and root weight in mash bean. Similar observation was obtained by Zarei *et al*. (2011) on the base of appropriate time interval that 10 hour hydropriming reduce the number of lateral branches of roots than 6 hour hydropriming so, the appropriate hydropriming time was obvious. Seed extracts reduced infection of *Fusarium* spp., on cowpea, similar result was observed by Chaithra (2009) that six plant extracts tested against *Fusarium solani*, mycelial inhibition was noticed in *Allium sativum* L. (Garlic). Biopriming help in disease control was used as alternative method for control soil borne pathogens (Harman and Taylor, 1988). *Carica papaya* seed extract showed significant reduction of root rot fungi both in cowpea and mashbean, so seed extract of *C.* papaya used as best treatment. According to Emeruwa (1982) seed extracts showed bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *E. coli* and *Shigella flexnari* . Many plant extracts, bioagents and hot water treatments are antimicrobials (Bowers and locke, 2000). Seed treatment with water and plant extract enhances seed metabolic activities before sowing and number of seeds germinate greater than non primed seeds So, increase the number of plants in field which leads ultimately to yield and economy of country. Seed treatment with plant extract enhances seed metabolic activities before sowing and number of seeds germinate greater than non primed seeds. So, increase the number of plants in field which leads ultimately to yield and economy of country and inhibited the *Fusarium*, *R. solani* and *M. phaseolina*, there is a need to apply this treatment at field level.

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