Int. J. Biol. Res., 7(2): 103-109, 2019.

EFFECTS OF FRUIT PERICARPS AS ORGANIC AMENDMENT FOR THE CONTROL OF ROOT KNOT NEMATODE, *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD AND GROWTH OF COWPEA PLANTS

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ABSTRACT

The objective of present study was to evaluate the efficacy of different fruit pericarps as organic amendment for the control of root-knot nematode and growth of cowpea plants under green house conditions. Dried powder of fruit pericarps were amended in soil at 0.3 and 0.5% w/w in pots. Effects on plant growth parameters, photosynthetic pigments and nematode parasitism were observed. Significant increase in root and shoot length and fresh weight was observed in all the treatments, while decrease in growth parameters was shown by *Capsicum annuum*. Maximum reduction in root-knots formation was shown by *Citrus vitis* and *Punica granatum* fruir peels. Plants treated with *Punica granatum*, *Citrus vitis*, *Cassia fistula*, *Ricinus communis* and *Citrus sinensis* fruit pericarps showed increase in chlorophyll a, b, total chlorophyll and carotenoids, while reduction in chlorophyll a and carotenoids was observed in plant amended with *Manilkara zapota* and *Capsicum annuum*. The above findings indicated that fruit pericarps of *Citrus sinensis, Capsicum annuum* and *Punica granatum* may be explored as natural potential nematicides.

KEY-WORDS: Fruit pericarps, Organic amendment, Root knot nematodes, Plant growth, Cowpea.

INTRODUCTION

The root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood is an important plant parasitic nematodes attacking a large number of vegetables and other host plants in Pakistan (Maqbool, 1992). Various measures have been taken for management of plant parasitic nematodes including resistance varieties, chemical nematicides, biological control and organic amendments but its control relied mainly on chemical nematicides (McSorley and Gallaher, 1995; Jatala, 1986; Hague and Gowen, 1987, Shaukat *et al*., 2002). Due to environmental and health concern many nematicides has been withdrawn from market (Dong and Zhang, 2006).

Different parts of plants have been tested for nematicidal potential including roots (Tsay *et al*., 2004), stem (Tariq *et al*., 2007), leaves (Shaukat *et al*., 2002), petiole (Tsai, 2008), flowers (Perez *et al*., 2003), seeds (Zia *et al*., 2003; Siddiqui and Zaki, 2017) seed cakes (Sumbul *et al.*, 2015), fruit waste and fruit pericarps (Ashraf and Khan, 2008; Korayen *et al.*, 1993).

The fruit industry is one of the major agro-industries. The processing of fruit juices produce tremendous amount of waste, could become an environmental problem. Very little attention has been focused on the use of fruit waste or fruit pericarps against plant

parasitic nematodes (Korayen *et al.*, 1993; Ashraf and Khan, 2008). Our previous study of fruit pericarp extracts showed strong nematicidal activity against egg hatching and larval mortality of root-knot nematode, *Meloidogyne javanica* (Akhtar *et al.*, 2019). The aim of the present study was to evaluate the efficacy of different fruit pericarps as organic amendment for the management of root-knot nematodes under greenhouse conditions.

MATERIALS AND METHODS

Collection of fruit pericarps: Fresh pericarps of different mature fruits were obtained from the market. Theses fruit pericarps were dried in shade and pulverized. The powders of peels obtained for different fruits were stored separately in air tight brown bottles.

Green house experiment: Sandy loam soil was obtained from experimental field of Department of Botany, University of Karachi with pH 8.1 and amended with dried powder of different fruit pericarps @ 0.3 and 0.5% w/w. Plastic pots with 8.1 cm diameter were filled with 300g /pot. There were 3 replicates of each treatment. Un-amended pots served as control. Two weeks after amendment, 5 cowpea (*Vigna unguiculata*) seeds were sown in each pot separately. Each pot was inoculated with approximately 5-10 egg masses after 10 days of seeds germination. The pots were placed in completely randomized block design in a greenhouse and watered daily. Plants were harvested after 45 days of nematode inoculation. Data on fresh weight and length of roots and shoots, number of galls/plant, number of nodules/plant and chlorophyll and carotenoids were recorded. The data were statistically analyzed.

Estimation of chlorophyll content: One g fresh leaf material was extracted in 80% acetone. The extract was centrifuge at 4000 rpm for 10 minutes and the supernatant was collected and made to volume upto 6 mL with acetone. The absorbance of supernatant was recorded at 480, 510, 663 and 645 nm and chlorophyll a, b and carotenoids were estimated in accordance with Maclachlan and Zalik (1963). The amount of chlorophyll was expressed as µg/g fresh weight.

Analysis of data: Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to compare the treatment means (Gomez and Gomez, 1984).

RESULTS

Greenhouse Experiment

1. Growth parameters

a. Root length: Root length of cowpea plants increased in all treatments of soil amendment with fruit pericarps. Maximum increase in root length was observed with 0.5% amendment of pomegranate and castor pericarps followed by Cassia and grapefruit used at 0.5% as compared to control (Table 1).

b. Shoot length: Dried fruit pericarps used in this study were found effective to increase shoot length of cowpea plants. Greater increase in shoot length was noticed in fruit pericarps of castor (20 cm), chili (19.6 cm) and cheeku (18.5 cm) as compared to control (12-13 cm) (Table 1).

Table 1. Effects of soil amendment with fruit pericarps on growth of cowpea plant.

Treatments		Root length (cm)	Shoot length (cm)	Root weight (g)	Shoot weight (g)
Grape fruit	0 %	15.75 ± 0.05	16.0 ± 3.08	0.59 ± 0.13	1.43 ± 0.4
	0.3 %	16.6 ± 1.28	16.2 ± 0.87	0.65 ± 0.64	2.08 ± 0.30
	0.5 %	19.1 ± 0.74	17.2 ± 1.07	0.68 ± 0.10	1.60 ± 0.17
Pomegranate	0 %	13.2 ± 1.98	18.6 ± 1.28	0.77 ± 0.11	1.99 ± 0.34
	0.3 %	15.2 ± 1.88	18.6 ± 3.42	0.85 ± 0.18	2.12 ± 0.54
	0.5 %	21.0 ± 1.67	18.3 ± 1.28	1.55 ± 0.53	6.18 ± 0.26
Castor	0 %	13.7 ± 1.98	13.3 ± 0.90	0.68 ± 0.07	1.26 ± 0.21
	0.3 %	19.3 ± 1.88	15.4 ± 1.83	0.86 ± 0.15	1.46 ± 0.27
	0.5 %	21.2 ± 1.39	20.0 ± 086	1.24 ± 0.15	2.30 ± 0.55
Cassia	0 %	11.8 ± 0.86	12.2 ± 1.90	0.48 ± 0.06	0.96 ± 0.26
	0.3 %	18.6 ± 1.12	13.7 ± 0.30	0.75 ± 0.10	1.44 ± 0.10
	0.5 %	20.0 ± 1.06	15.2 ± 0.66	0.79 ± 0.17	1.67 ± 0.46
Orange	0 %	13.8 ± 1.20	14.5 ± 2.04	0.79 ± 0.10	2.20 ± 0.57
	0.3 %	16.0 ± 0.70	13.8 ± 0.66	0.79 ± 0.07	1.55 ± 0.22
	0.5 %	16.5 ± 1.42	14.3 ± 1.24	0.58 ± 0.13	1.39 ± 0.15
Chilli	0 %	16.7 ± 1.86	11.8 ± 1.45	0.78 ± 0.12	1.79 ± 0.31
	0.3 %	15.8 ± 1.01	15.0 ± 1.69	0.93 ± 0.09	1.51 ± 0.24
	0.5 %	14.6 ± 1.16	19.6 ± 1.72	0.93 ± 0.06	1.00 ± 0.18
Cheeku	0 %	16.7 ± 1.60	13.0 ± 1.24	0.78 ± 0.07	1.34 ± 0.20
	0.3 %	18.0 ± 0.88	14.0 ± 2.04	1. 22 ± 0.43	1.66 ± 0.46
	0.5 %	17.6 ± 1.60	18.5 ± 1.32	1.11 ± 0.06	2.40 ± 0.36
$LSD_{0.05}$	Concentration	1.76	1.75	0.206	0.366
	Treatments	2.69	2.65	0.314	0.559

Mean values ± standard error (SE)

c. Fresh root weight: Most of the fruit pericarps used in this study were found effective to increase root weight except cheeku where decrease in root weight was observed. Maximum increase in root weight was observed in the treatment of pomegranate followed by castor used at 0.5% (Table 1).

d. Fresh shoot weight: Dried fruit pericarps of pomegranate, castor, cassia, grapefruit and cheeku showed an increase in shoot weight whereas orange and chili fruit pericarps decreased shoot weight. Maximum increase in shoot weight was observed in the treatment of pomegranate as compared to the control (Table 1).

e. Photosynthetic pigments: Chlorophyll "a" increased with the use of fruit pericarps of pomegranate, grapefruit, cassia, castor and orange. Similarly Chlorophyll "b" also increased with pomegranate, grapefruit and orange. A significant increase in total chlorophyll was observed in all the used fruit pericarps. Maximum increase in total chlorophyll was observed with pomegranate followed by orange. Fruit pericarps of pomegranate, castor and orange showed an increase in carotenoids whereas other treatments of fruit pericarps did not show any significant change in carotenoids (Table 2).

f. Root knots: A significant reduction in number of knots on roots of cowpea was observed with the amendment of fruit pericarps of grape fruit and pomegranate while other pericarps failed to reduce number of knots on roots (Table 2).

Table 2. Effects of soil amendment with fruit pericarps on photosynthetic pigments and nematode parasitism.

Treatments		Chlorophyll-a µg/g	Chlorophyll-b µg/g	Total chlorophyll µg/g	Carotenoids µg/g	No. of Knots/ plant
Grape fruit	0%	6.85 ± 1.20	3.39 ± 0.44	10.22 ± 0.76	2.84 ± 0.46	76 ± 8.12
	0.3%	7.35 ± 1.53	3.09 ± 0.85	10.44 ± 2.39	2.72 ± 0.45	48 ± 8.60
	0.5%	6.19 ± 0.04	3.63 ± 0.43	9.82 ± 0.44	2.60 ± 0.27	40 ± 8.94
Pomegranate	0%	5.63 ± 2.40	2.19 ± 0.75	7.86 ± 3.15	0.81 ± 0.06	66 ± 10.24
	0.3%	7.79 ± 1.38	6.54 ± 0.94	14.34 ± 0.43	3.70 ± 0.18	42 ± 12.00
	0.5%	4.84 ± 0.49	1.72 ± 0.47	6.56 ± 0.96	1.97 ± 0.04	38 ± 6.63
Castor	0%	3.21 ± 0.89	3.15 ± 0.68	5.30 ± 2.09	1.33 ± 0.10	130 ± 3.14
	0.3%	2.68 ± 1.05	2.30 ± 0.07	4.10 ± 0.88	1.36 ± 0.02	58 ± 8.60
	0.5%	4.78 ± 0.06	3.17 ± 0.29	6.96 ± 0.04	2.14 ± 0.20	46 ± 7.48
Cassia	0%	5.40 ± 0.22	3.15 ± 0.68	8.55 ± 0.90	2.22 ± 0.13	212 ± 14.6
	0.3%	6.22 ± 0.19	2.30 ± 0.09	8.53 ± 0.12	2.12 ± 0.02	92 ± 15.93
	0.5%	5.94 ± 0.08	3.17 ± 0.29	9.12 ± 0.38	2.14 ± 0.04	74 ± 12.49
Orange	0%	5.16 ± 0.04	2.58 ± 0.20	7.76 ± 0.54	0.85 ± 0.30	80 ± 7.07
	0.3%	7.30 ± 0.60	6.62 ± 0.34	13.92 ± 1.14	1.85 ± 0.24	84 ± 15.68
	0.5%	5.62 ± 0.10	2.75 ± 0.17	8.38 ± 0.27	1.82 ± 0.92	70 ± 8.36
Chilli	0%	7.46 ± 1.95	3.71 ± 0.92	11.17 ± 2.87	1.11 ± 0.56	60 ± 15.49
	0.3%	1.96 ± 0.46	14.28 ± 4.32	16.24 ± 4.78	1.09 ± 0.52	54 ± 10.24
	0.5%	1.96 ± 0.46	14.28 ± 4.32	16.24 ± 4.78	1.05 ± 0.04	50 ± 8.36
Cheeku	0%	8.36 ± 0.33	4.25 ± 1.30	12.62 ± 1.63	2.96 ± 0.34	82 ± 12.00
	0.3%	7.88 ± 3.38	5.31 ± 0.17	13.19 ± 3.55	1.69 ± 0.48	80 ± 8.36
	0.5%	4.95 ± 0.65	4.85 ± 1.18	9.81 ± 1.83	1.35 ± 0.17	46 ± 5.09
$LSD_{0.05}$ Concentration		1.34	1.65	2.40	0.39	2.29
Treatments		2.05	2.52	3.70	0.59	3.50

Mean values ± standard error (SE)

DISCUSSION

Many researchers studied on plant based materials as organic amendments for the control of plant-parasitic nematodes (Akhtar, 1993; McSorley and Gallaher, 1996; Nico *et al*., 2004). Studies were also conducted on agro-industrial wastes for nematode control. McSorley and Gallaher (1996) were found that yard waste compost was effective against *Paratrichodorus minor*. Sugarcane trash was also found effective in the control of nematode (Akhtar, 1993). Dry cork reduced population of *Meloidogyne* spp. (88%) in olive in the pots (Nico *et al*., 2004). According to Tiyagi and Alam (1995), the populations of *M. incognita* and *R. reniformis* were significantly reduced by oil-seed cakes of neem, castor and mustard. Tsai, (2008) reported that pulpified peels of lemon, orange, and grapefruit kept in the refrigerator for one week showed strong nematicidal activity as compared to extracts of fresh peels. In other studies fruit pericarps extracts of lemon, orange, grapefruit, pomegranate, red hot chili pepper and *Sapindus mukorossi* showed nematicidal activity (Korayem *et al.*, 1993; Meyer *et al*., 2016; Saha *et al*., 2008; Neves *et al*., 2009; Tsai, 2008; Boonmasawai *et al*., 2013). In our previous studies aqueous extracts of different fruit pericarps like *Punica granatum, Capsicum annuum* and *Ricinus communis* showed nematicidal potential against *Meloidogyne javanica* juveniles at various concentrations i.e.; 25, 50 and 100%. Decrease in hatching was also observed by *Capsicum annuum, Citrus sinensis* and *Manilkara zapota* at all tested concentrations (Akhtar *et al*., 2019).

In the present study dried fruit pericarps powder used as organic soil amendment showed significant increase in root and shoot length and fresh weight in all the treatments, while decrease in growth parameters was shown by *Capsicum annuum*. Maximum reduction in root-knots and egg masses formation was shown by *Citrus vitis* and *Punica granatum*. Plants treated with *Punica granatum*, *Citrus vitis*, *Cassia fistula*, *Ricinus communis* and *Citrus sinensis* showed increase in chlorophyll a, b, total chlorophyll and carotenoids, while reduction in chlorophyll a and carotenoids was observed in plant amended with *Manilkara zapota* and *Capsicum annuum*. Fruit waste or fruit pericarps applied as organic amendments suppressed parasitism of plant parasitic nematodes to host plants and improved growth of crop plants (Ismail, 2015; Ashraf and Khan, 2008; Nico *et al*., 2004). Soil amendment with crushed peels of pomegranate suppressed *Meloidogyne javanica* on tomato (Ismail, 2015). About 124 phytochemicals with polyphenols as the primary components were reported from pomegranate fruit (Viuda-Martos *et al.*, 2010; Akhtar *et al.*, 2015; García-Villalba *et al.*, 2015). Ellagitannins, higher levels of polyphenols, isolated from Pomegranate husk and peel are potential sources of biologically active compounds that might act as biological nematicides (Seeram *et al.*, 2005; Akhtar *et al.*, 2015; García-Villalba *et al.*, 2015). Ellagic acid and gallic acids, isolated from pomegranate showed nematicidal potential against animal-parasitic nematode *Haemonchus contortus* and *Caenorhabditis elegans* (Ndjonka *et al.*, 2013; Mondal *et al.*, 2015). Triterpene saponins isolated from fruit pericarp of *Sapindus mukorossi* exhibited inhibitory effect against *Rotylenchulus reniformis* and *Meloidogyne incognita* (Saha *et al*., 2010). Two products of red hot chili containing capsicin and capsaicinoids found effective against *M. javaica* (Neves *et al*., 2009). Limonene was the main component of the essential oils of *Aloysia triphylla* killed more than 80% juveniles of *Meloidigyne* species (Duschatzky *et al.*, 2004) also found in grapefruit and sweet orange.

The results of the present study suggest that the fruit pericarps of pomegranate, chili, orange, grapefruit could be used as a natural nematicide against root-knot disease. The use of fruit industrial waste or fruit pericarps for nematode control not only provide alternative to chemical nematicides but also help disposing the agro-industrial waste.

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(Received July 2019; Accepted September 2019)