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EFFICACY OF WILD PLANT IN COMBINATION WITH MICROBIAL ANTAGONISTS FOR THE CONTROL OF ROOT ROT FUNGI ON MUNGBEAN AND COWPEA

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Abstract

Present work was carried out to investigate the efficacy of *Aerva javanica* in combination with different microbial antagonists namely *Rhizobium meliloti, Pseudomonas aeruginosa, Trichoderma harzianum* and *Aspergillus niger*. Soil amended with *A. javanica* stem, leaves, flower powder @1% w/w and seeds of cowpea (*Vigna unguiculata* L.) and mungbean (*Vigna radiata* L.) were coated with microbial antagonists for the control of root infecting fungi like *Macrophomina phaseolina* (Tassi) Goid, *Fusarium* spp. and *Rhizoctonia solani* Kühn. Infection of *M. phaseolina* and *R. solani* were completely suppressed when seeds were coated with *P. aeruginosa, T. harzianum, A. niger, R. meliloti* and *A. javanica* leaves powder mixed in soil @1% w/w. All antagonists showed reduction in combination with *A. javanica* leaves powder @1% but *T. harzianum* and *P. aeruginosa* in combination with *A. javanica* leaves showed promising results in complete reduction of *R. solani* and *M. phaseolina* on both crops. All growth parameters were maximum when soil was amended with *A. javanica* leaves powder @1% w/w and seeds were coated with *T. harzianum* and *P. aeruginosa.*

Key words: Organic amendment; Microbial antagonists; *Aerva javanica, Vigna unguiculata, Vigna radiate*.

Introduction

Microbial antagonists and organic amendment minimized hazardous effect on root infecting pathogen as compared to chemical pesticides (Perveen & Ghaffar, 1991). Mixing of ecologically friendly material in soil enhance the yield of edible plants and decrease the risk of infection in roots (Stone *et al*., 2003; Chandra *et al*., 1981). Antifungal compounds in higher plants plays an important role in suppression of disease causing agents (Mahadevan, 1982; Singh & Dwivedi, 1987). Dressing of seeds enhance yield and minimize the losses due to many fungal pathogen and pesticides. Seed dressing suppress infection of pathogenic fungi superficially found on the surface of seed or penetrate inside the seed (Martha *et al.*, 2003). When living microorganisms apply on seeds surface they enhance the yield, alternative of biofertilizer and providing nutrients to plants. (Lugtenberg *et al*., 2002). Due to the efficiency of nutrient uptake enhancement, more interest develop in the application of biofertilizer (Adesemoye *et al*., 2008; Malusà *et al*., 2007). Antagonistic bacteria suppress the risk of root rot in *Abelmoschus esculentus* and *Vigna radiata* when multiplied on salt tolerant plant and then applied in soil (Tariq *et al*., 2007). Root infecting fungi attacks on several plants during the growing season and causing various diseases such as wilting, root rot, charcoal rot and damping-off. Previous studies showed that the root-rot fungi like *Fusarium moniliforme, F. oxysporum, F. semitectum, Macrophomina phaseolina, Phytophthora nicotianae* var *parasitica, Pythium irregulare, Sclerotinia sclerotiorum* and *S. rolfsii* were pathogenic to germinating seeds and seedlings of sunflower (Sadashivaiah *et al*., 1986; Zazzerini & Tosi 1987; Ahmed *et al*., 1994).

Different compounds from *Aerva javanica* parts suppress the infection of different plant parasitic fungi (Sharif *et al*., 2011). Use of *A. javanica, Abutilon* spp., *Crotalaria burhia, Capparis decidua, Cleome brachycarpa* and *Suaeda fruticosa* showed antimicrobial activities against plant pathogenic microorganisms (Hameed *et al*., 2011). *A. javanica* parts when mixed with soil at 1 % weight by weight soil borne root infecting fungi were significantly suppresed (Ikram & Dawar, 2013). Ikram & Dawar (2012) find out that *A. javanica* leaves at 1 % significantly enhance the yield and reduce the infection of soil borne root infecting fungi. Present work was carried out to observed the efficacy of *A. javanica* plant parts powder in combination with microbial antagonists for the growth and reduction of root rot pathogens.

Materials and Methods

Collection of plant material: Fresh and healthy plants of *A. javanica* were collected from Karachi University campus. Washed all the parts with distilled water to remove dust, air dried and make fine powder of each part using an electric grinder.

Cultures of microbial antagonists: Cultures of antagonists viz., *Rhizobium meliloti* (R5), *Aspergillus niger* (An 20), *Pseudomonas aeruginosa* and *Trichoderma harzianum* (KUCC 65) obtained from Karachi University Culture Collection (KUCC).

Soil used in experiment: Sandy loam soil with pH 8-9, water holding capacity 40% (Keen & Raczkowski, 1922), nitrogen 0.081% (Mackenzie & Wallace, 1954), 8-9 sclerotia of *M. phaseolina* were isolated by wet sieving technique (Sheikh & Ghaffar, 1975), 15% *R. solani* by baiting technique (Wilhelm, 1955) and 2000 cfu/g *Fusarium* spp., isolated from soil by serial dilution technique (Nash & Snyder, 1962). Soil was amended with *Aerva javanica* leaves, stem and flower powder @ 1 % w/w.

Seed treatment with microbial antagonists: *Vigna unguiculata* seeds coated with *R. meliloti* (80×10^7),60 *P. aeruginosa* (60×10^7), *Aspergillus niger* (48×10^3) and *T. harzianum* (75×10^3). *Vigna radiata* seeds coated with *R. meliloti* (71×10^7), *P. aeruginosa* (69×10^7), *A. niger* (40×10^3) and *T. harzianum* (130×10^3).

Experimental set up in green house: Plastic pots filled with 300g soil and five treated seeds were sown in each pot and watered regularly to maintained sufficient moisture required for the growth of plants. The pots were kept in screen house in randomized complete block design with three replicates per treatment. Seeds treated with sterilized distilled water served as control. Growth parameters like shoot, root length and weight, leaf area and number of nodules were recorded after 30 days of seed germination.

Detection of root rot fungi: To determine the infection of fungi in roots, plants were uprooted and after washing in running tap water to remove soil, each root was cut into 5 pieces. These root pieces after surface sterilization with 1% Ca $(OCl)_{2,}$ transferred on potato dextrose agar (PDA) poured plates. Plates were incubated at room temperature (28˚C) and after one week, infection of root infecting fungi was recorded from each root segment.

Data analysis: Data were subjected to analysis of variance (ANOVA) followed by the least significant difference (LSD) test at P = 0.05 (Gomez & Gomez, 1984).

Results

Cowpea: Seed coating with microbial antagonists in combination with plant parts powder enhance the germination of cowpea as compared to *A. javanica* powder using alone (P<0.01). There was significant enhancement in all growth parameters when *A. javanica* parts powder used with antagonists. Length of cowpea plants significantly increased (P<0.001) when seeds of cowpea were coated with *A. niger* and *P. aeruginosa* in combination with *A. javanica* parts powder @1% w/w. Weight of plants enhanced when *A. javanica* parts powder used at 1 % (P<0.001) and seeds were coated with *R. meliloti*. Leaf area and number of nodules significantly (P<0.01) increased when seeds were coated with *P. aeruginosa* and soil was amended with *A. javanica* leaves powder at 1 % (Table 1). Infection of *R. solani* and *M. phaseolina* were completely suppressed when seeds were treated with all microbial antagonists and plant powder amended in soil (Table 3).

Mungbean: Soil borne pathogenic fungi completely suppressed when seeds were coated with microbial antagonists and soil amended with leaves and stem powder (Table 4). Significant increase in mung bean germination (P<0.001) was observed in all treatment as compared to control. Shoot length significantly increased (P<0.01) and maximum shoot length observed when *P. aeruginosa* used with *A. javanica* leaves powder @ 1% w/w. Fresh weight of plant significantly increased (P< 0.001) when *P. aeruginosa* and powder of stem used. Significant increased (P<0.001) in length of roots was recorded when *T. harzianum* and *P. aeruginosa* used with *A. javanica* leaves powder. *T. harzianum* when used with *A. javanica* leaves powder, there was significant increase (P< 0.001) in root weight. *A. javanica* leaves powder when mixed in soil at 1% w/w and seeds were coated with *R. meliloti* showed significant increase in nodules (P<0.5) and area of leaf (P<0.001) (Table 2).

Table 1. Effect of *Aerva javaniva* parts powder in combination with microbial antagonists on growth parameters of cowpea. *Vigna unguiculata* L.

Treatments	Germination percentage	Length of shoot (cm)	Weight of shoot (g)	Length of root (cm)	Weight of root (g)	Leaf area (cm)	Number of nodules
Control	80±20	22.3±1.1	2.53±0.4	7.00±1.5	0.43±0.5	21.0±2.6	5.0±0.5
A. javanica stem @1%	100±0.0	29.6±2.3	3.38±0.2	9.77±0.1	0.80±0.2	24.0±2.0	10±1.5
A. javanica leaves @1%	100±0.0	31.5±1.3	3.45±0.4	10.1±1.7	1.03±0.0	26.5±5.6	7.0±1.5
A. javanica flower @1%	100±0.0	25.6±3.4	3.09±0.2	8.99±3.4	0.79±0.1	22.6±2.4	7.0±1.0
T. harzianum	100±0.0	19.6±2.0	2.88±0.8	9.88±1.5	0.49±0.0	21.3±1.0	7.0±1.0
T. + *A. j.* stem @1%	100±0.0	28.3±0.5	2.99±0.1	10.5±1.1	0.71±0.1	24.1±0.5	10±0.5
T.+ *A. j.*leaves@1%	100±0.0	27.6±1.6	3.33±0.1	11.6±1.5	0.81±0.0	24.8±0.5	11±0.5
T.+ *A. j.* flower@1%	100±0.0	22.8±1.1	2.94±0.8	10.4±2.1	0.59±0.1	23.4±1.0	9.0±1.0
A. niger	100±0.0	33.9±4.3	3.05±3.5	8.99±2.7	0.47±0.0	22.4±1.5	6.0±1.5
A. + *A. j.*stem @1%	100±0.0	33.9±0.8	3.26±3.7	10.5±2.1	0.62±0.0	24.5±0.6	10±3.0
A.+ *A. j.*leaves@1%	100±0.0	35.6±0.7	3.35±1.1	11.4±1.7	0.63±0.1	26.0±1.5	14±0.0
A.+ *A. j.*flower@1%	100±0.0	29.6±1.0	2.92±2.8	8.66±0.5	0.49±0.0	25.2±3.0	12±1.5
R. meliloti	100±0.0	26.6±3.2	3.29±0.3	9.33±1.5	0.79±0.1	22.2±1.0	7.0±1.0
R. + *A. j.*stem @1%	100±0.0	27.3±3.2	3.22±0.3	10.7±0.3	0.89±0.1	25.7±1.5	9.0±1.5
R.+ *A. j.*leaves@1%	100±0.0	28.4±2.6	3.45±0.1	12.1±1.8	0.86±0.1	26.4±0.5	8.0±0.5
R.+*Aerva* flower@1%	100±0.0	27.8±2.3	3.29±0.2	9.44±0.6	0.81±0.2	24.5±0.1	6.0±1.0
P. aeruginosa	100±0.0	33.5±3.5	3.49±0.4	9.88±0.5	0.48±0.0	20.8±0.5	6.0±0.5
P. + *A. j.*stem @1%	100±0.0	35.5±3.7	3.51±0.6	10.5±1.1	0.57±0.1	23.9±1.5	10±1.5
P+ *A. j.* leaves@1%	100±0.0	35.0±1.1	2.86±0.4	11.6±1.5	0.58±0.0	24.7±0.5	13±3.2
P.+ *A. j.* flower@1%	100±0.0	32.5±2.8	3.26±0.9	10.4±4.0	0.53±0.1	23.5±3.2	9.0±0.5
LSD=0.05	7.39	4.04	0.75	3.08	0.21	4.58	3.65

Table 2. Effect of *Aerva javaniva* parts powder in combination with antagonists on growth parameters of mungbean. *Vigna radiata* L.

Treatments	Germination percentage	Length of shoot (cm)	Weight of shoot (g)	Length of root (cm)	Weight of root (g)	Leaf area (cm)	Number of nodules
Control	60±20	20.5±0.5	1.22±0.1	6.00±0.5	0.37±0.0	8.22±0.3	3±0.5
A. javanica stem @1%	100±0.0	26.7±1.5	1.81±0.2	11.2±0.4	0.64±0.2	13.3±1.3	6±1.1
A. javanica leaves @1%	100±0.0	24.8±1.6	1.58±0.2	14.3±1.4	0.74±0.2	11.1±0.3	8±0.5
A. javanica flower @1%	100±0.0	23.6±0.6	1.26±0.1	15.1±3.6	0.52±0.1	11.2±2.1	5±1.0
T. harzianum	100±0.0	23.5±0.7	2.19±0.2	10.4±0.3	0.54±0.1	12.2±0.8	6±1.0
T. + A. j. stem @1%	100±0.0	25.5±3.1	1.81±0.3	13.2±1.2	0.58±0.2	14.8±1.0	8±2.5
*T.+ A. j.*leaves@1%	100±0.0	26.1±2.0	2.08±0.4	11.0±1.0	0.59±0.1	15.9±2.1	8±1.5
T.+ A. j. flower@1%	100±0.0	23.1±1.2	2.06±0.1	10.1±1.1	0.43±0.0	14.5±3.2	7±2.0
A. niger	100±0.0	22.6±0.3	1.86±0.1	10.6±1.9	0.33±0.1	10.9±0.1	5±0.5
*A. + A. j.*stem @1%	100±0.0	23.4±1.0	1.97±0.1	10.2±1.0	0.44±0.0	12.5±0.7	7±0.5
*A.+ A. j.*leaves@1%	100±0.0	25.6±1.1	2.09±0.1	9.77±1.2	0.53±0.1	13.4±1.2	7±2.6
*A.+ A. j.*flower@1%	100±0.0	22.8±0.5	2.05±0.0	8.66±0.8	0.34±0.0	11.2±1.5	6±2.6
R. meliloti	100±0.0	21.7±1.0	1.37±0.0	8.63±0.5	0.24±0.0	10.2±0.9	4±1.0
*R. + A. j.*stem @1%	100±0.0	22.1±2.6	1.70±0.1	10.4±0.8	0.32±0.0	16.0±0.6	7±0.5
*R.+ A. j.*leaves@1%	100±0.0	22.2±1.1	1.75±0.0	9.55±0.7	0.34±0.0	16.3±2.0	7±1.5
R.+Aerva flower@1%	100±0.0	21.8±2.7	1.37±0.1	10.1±1.0	0.37±0.0	10.7±1.0	7±1.5
P. aeruginosa	100±0.0	22.2±1.5	1.26±0.0	9.55±0.3	0.26±0.0	11.3±1.5	5±1.0
*P. + A. j.*stem @1%	100±0.0	24.6±2.3	1.40±0.0	9.86±0.5	0.30±0.0	13.3±1.1	8±1.4
P+ A. j. leaves@1%	100±0.0	24.8±1.3	1.73±0.2	8.88±0.1	0.31±0.0	13.7±0.9	8±1.7
P.+ A. j. flower@1%	100±0.0	23.5±2.1	1.40±0.1	9.10±0.7	0.29±0.0	12.3±0.5	6±0.5
LSD=0.05	4.26	2.575	0.298	21.64	0.191	5.101	2.381

Table 3. Effect of *Aerva javaniva* parts powder in combination with antagonists on root rot fungi of cowpea. *Vigna unguiculata* L.

Treatment	*Fusarium* spp.	*R. solani*	*M. phaseolina*
Control	100.0±1.0	100.0±0.0	100.0±0.0
A. javanica stem @1%	53.00±13.00	40.00±20	24.44±13.87
A. javanica leaves @1%	28.66±10.00	22.22±16.78	26.66±11.54
A. javanica flower @1%	48.66±10.00	40.00±20.00	26.66±17.64
T. harzianum	73.00±7.00	61.66±14.01	44.11±25.28
T. + A. javanica stem @1%	13.33±17.63	15.33±26.55	0.000±0.000
T.+ A. javanica leaves@1%	22.00±3.46	0.000±0.000	0.000±0.000
T.+ A. javanica flower@1%	22.00±20.29	22.00±38.10	0.000±0.000
A. niger	24.44±21.42	13.33±17.63	10.88±13.50
*A. + A. javanica.*stem @1%	8.888±10.18	0.000±0.000	0.000±0.000
A.+ A. javanica leaves@1%	13.33±17.63	0.000±0.000	0.000±0.000
*A.+ A. javanica.*flower@1%	8.666±15.01	11.11±19.24	4.44±7.6S9
R. meliloti	6.644±6.666	2.22±3.84	6.643±6.66
R. + A. javanica stem @1%	17.77±20.36	0.000±0.000	0.000±0.000
R.+ A. javanica leaves@1%	26.44±6.67	0.000±0.000	0.000±0.000
R.+A. javanica flower@1%	11.11±19.24	0.000±0.000	2.222±3.840
P. aeruginosa	37.66±32.80	46.33±17.95	46.44±17.26
P. + A. javanica stem @1%	22.22±20.29	0.000±0.000	0.00±0.00
P+ A. javanica leaves@1%	24.44±21.42	0.000±0.000	0.00±0.00
P.+ A. javanica flower@1%	35.55±3.855	0.000±0.000	0.00±0.00
LSD=0.05	18.22	20.61	18.64

Table 4. Effect of *Aerva javaniva* parts powder in combination with antagonists on root rot fungi of mungbean. *Vigna radiata* L.

Treatment	*Fusarium* spp.	*R. solani*	*M. phaseolina*
Control	100.0±1.0	100.0±0.0	100±0.0
A. javanica stem @1%	44.44±13.87	28.88±19.24	50.77±16.44
A. javanica leaves @1%	37.66±16.62	26.44±6.67	40.77±12.44
A. javanica flower @1%	37.33±10.26	44.11±9.97	41.50±26.16
T. harzianum	6.66±6.66	24.44±21.42	6.66±6.66
T. + A. javanica stem @1%	0.00±0.00	0.00±0.00	0.00±0.00
T.+ A. javanica leaves@1%	0.00±0.00	0.00±0.00	0.00±0.00
T.+ A. javanica flower@1%	2.22±3.84	2.22±3.84	2.22±3.84
A. niger	13.31±6.70	64±17.08	42.00±3.46
A. + A. javanica.stem @1%	0.00±0.00	0.00±0.00	0.00±0.00
A.+ A. javanica leaves@1%	0.00±0.00	0.00±0.00	0.00±0.00
A.+ A. javanica.flower@1%	4.44±7.69	2.2±3.81	4.44±7.69
R. meliloti	48.66±10.26	46.66±11.54	46.66±11.54
R. + A. javanica stem @1%	26.44±6.66	24.44±21.42	15.33±13.61
R.+ A. javanica leaves@1%	26.66±11.54	17.77±16.77	17.55±9.89
R.+A. javanica flower@1%	22.2±20.29	24.44±7.69	28.66±25
P. aeruginosa	28.66±10.26	28.66±25	30.66±13.61
P. + A. javanica stem @1%	0.00±0.00	0.00±0.00	0.00±0.00
P+ A. javanica leaves@1%	0.00±0.00	0.00±0.00	0.00±0.00
P.+ A. javanica flower@1%	2.22±3.84	13.33±23.09	6.66±6.66
LSD=0.05	13.64	20.22	19.43

Discussion

The effect of *A. javanica* parts powder in combination with biocontrol agents enhanced the germination percentage and suppress the infection of root infecting fungi. *A. javanica* alone or in combination with seed treatment with antagonists showed significant suppression of root rot fungi viz., *Fusarium* spp., *R. solani* and *M. phaseolina*. *A. javanica* leaves at 1% w/w inhibited root rot fungi and improved growth parameters (Ikram & Dawar, 2012). *Prosopis juliflora* leaves and stem powder @ 1% was effective for the control of root rot fungi and in the enhancement of all growth parameters in cowpea and mungbean (Ikram & Dawar, 2013). *Avicennia marina* leaves and stem powder pellets significantly controlled root rot diseases caused by pathogenic fungi in cowpea and brinjal (Tariq & Dawar, 2011). *A. javanica* was most effective against many bacteria and other microorganisms (Hameed *et al*., 2011).

In our studies, complete suppression in infection of root rot fungi such as *R. solani* and *M. phaseolina* when seeds were coated with *Trichoderma* and *Pseudomonas* and 1 % leaves powder of *A. javanica* mix in soil. Organic amendment with neem cake, karanj cake, mustard cake, vermicompost, farma yard manure and antagonist like *Trichoderma virens* and *Aspergillus niger* were the most effective and showed complete suppression of *Rhizoctonia solani* (Vibha, 2010). Infection of *Clonostachys rosea* and *Macrophomina phaseolina* was completely suppressed when soil was amended with compost and seeds were treated with biocontrol agent *Clonostachys rosea* in cowpea (Ndiaye *et al*., 2010). Current research exhibited the combined effect of *A. javanica* and antagonists not only helpful in suppressing disease but also enhanced the growth of both crops. Three bacterial species (*Bacillus pumilus*, *Bacillus subtilis*, *Streptomyces lydicus)* as biocontrol agents offered a better approach for plant protection (Janousek *et al*., 2009).

Our results revealed that *T. harzianum* alone or in combination with plant parts significantly suppressed the pathogens of root. *T. harzianum* showed superior effect against various soil borne pathogens as compared to other antagonists such as *Bacillus subtilis*, *Pseudomonas flourescens* and *Sacchromyces cerevisiae* (Abdel–Kader *et al*., 2012).

Present studies showed that *P. aeruginosa* in combination with *A. javanica* was most effective for the control of root rot fungi. Amendment in soil with neem cake, pongamia cake, groundnut cake, eupatorium dried leaves and farm yard manure (FYM) by providing nutrients promotes biological activity of antagonists such as *Trichoderma virens*, *T. viride*, *T. harzianum*, *T. hamatum*, *Bacillus subtilis.* They release some inhibitory substances on decomposition, affecting the population of pathogen which significantly suppressed the population of pathogen and promote the growth and vigor of plant (Mallesh *et al*., 2008). Some essential compounds release from *A. javanica* which suppressed the growth of root infecting fungi.

Use of *A. javanica* with microbial antagonists was better for the productivity of crop plants and suppressed the infection of root rot fungi. Therefore there is need to use plant parts of A. javanica in combination with microbial antagonists for the improvement and production of crop plants on large scale.

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