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Effect of brown seaweeds and pesticides on root rotting fungi and root-knot nematode infecting tomato roots

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Summary

Plants with therapeutic effects have received attention of scientists as an alternate method of disease control which protect the environment from the use of hazardous chemicals. Experiments were conducted in the greenhouse to determine the effect of brown seaweeds *Spatoglossum asperum* and *Sargassum swartzii* on pathogenic fungi and parasitic nematode infecting tomato roots. Application of brown seaweeds as soil amendment showed a significant ($p<0.05$) suppressive effect on root rotting fungi *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* and root-knot nematode, *Meloidogyne javanica* in tomato roots. Use of *Sargassum swartzii* with benomyl and *Spatoglossum asperum* with captan showed a better control of *F. solani* than each used individually. Combined use of *Sargassum swartzii* with benomyl and captan and *Spatoglossum asperum* with benomyl also greatly ($p<0.05$) reduced nematode infection than seaweeds or fungicides used individually. Maximum fresh shoot weight and plant height was obtained by the mixed application of seaweed and benomyl. The utilization of these seaweeds as manure may provide necessary nutrients to tomato plants and may protect them from invasion by root pathogens.

Introduction

Tomatos (*Lycopersicon esculentum* Mill.) are a significant source of nutrition for substantial portion of the world human population. Tomatos valued at approximately US$ 5 billion annually, are widely cultivated and extensively consumed both as a fresh vegetable and processed products (POWELL and BENNETT, 2002). In Pakistan tomato crop is cultivated over an area of 46.2 x 10^3 hectares with annual production of 468.1 x 10^3 tons (ANON., 2006). However, diseases are major limiting factors for tomato production. Root diseases caused by soilborne root rotting fungi *Fusarium oxysporum, F. solani, Rhizoctonia solani* and *Macrophomina phaseolina*, and root-knot nematodes *Meloidogyne* spp., are highly destructive to tomato (JONES et al., 1991). Association of root-knot nematodes with wilt fungi caused greater losses than either alone (FRANCL and WHEELER, 1993). In Pakistan severe infestation of root rotting fungi and root-knot nematodes are causing up to 86% losses in tomato crop.

Most soil borne pathogens are difficult to control by conventional strategies such as the use of resistant cultivars and synthetic fungicides (WELLER et al., 2002). Moreover the effect of synthetic fungicides on the environment and on human health is leading to significant changes in the use of fungicides for the management of plant disease. As a consequence there is an increased emphasis on ways to minimize the use of fungicides.

Plants with therapeutic effects have received attention of scientists as an alternate method of disease control, which would also protect our environment from the use of hazardous chemicals. The direct application of seaweeds in farming is a practice that extends over hundreds of year, and it is often more successful than using chemical fertilizers. Seaweeds contain elaborated secondary metabolites that play a significant role in the defense of the host against predators and parasites (PARACER et al., 1987; SULTANA et al., 2008). In our previous studies, use of brown algae, *Sargassum* spp., showed significant control of root infecting fungi in sunflower (ARA et al., 1996) and root-knot nematode in okra (ARA et al., 1997). Oily fractions from ethanol extract of another brown alga, *Spatoglossum asperum* had shown significant activity against root-knot nematode and root infecting fungi *in vitro* (ARA et al., 2005). The present report describes the effect of *Spatoglossum asperum* and *Sargassum swartzii* individually or with synthetic fungicides (benomyl and captan) and nematicide (carbofuran) in suppressing the root rotting fungi *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* and root-knot nematode *Meloidogyne javanica* infecting tomato roots.

Materials and methods

Seaweeds *Spatoglossum asperum* J. Ag., and *Sargassum swartzii* (Turn.) C. Ag., collected from Buleji Beach, Karachi were used in these experiments individually or with synthetic fungicides benomyl (a systemic fungicide famous for its effect on wide range of diseases of fruits, nuts, vegetables and field crops. Due to toxicity, use of benomyl is now not allowed in many countries), captan (a general purpose fungicide that protect crops against a wide range of fungi) and carbofuran (a systemic nematicide). The experiments were conducted separately for each seaweed. Dry powder of seaweeds was mixed with sandy loam soil (pH 8.1) to give a concentration of 0.5 and 1.0% w/w. The soil was naturally infested with 3-7 sclerotia of *Macrophomina phaseolina* g^{-1} of soil as determined by wet sieving and dilution plating (SHEIKH and GHAFFAR, 1975), 2-6% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (WILHELM, 1955) and 3,000 colony forming units (cfu) g^{-1} of soil of a mixed population of *Fusarium oxysporum* and *F. solani* as determined by soil dilution (NASH and SNYDER, 1962). One kg of amended soil was transferred to clay pots (12 cm diameter). The pots were watered daily to allow the decomposition of the organic substrate. After three weeks, four equal size seedlings of tomato (*Lycopersicon esculentum* Mill.) variety Roma, raised in steam sterilised soil were transplanted after root treatment with aqueous suspension of captan and benomyl (200 ppm) for 15 minutes. A set of carbofuran was also kept for comparison (at 0.05 g/kg soil). A set of seaweeds and pesticides separately was also kept for comparison. The pots without seaweeds and without fungicides served as control. The pots were kept randomized on a screen house bench at 50% water holding capacity (WHC) with four replicates of each treatment. The seedlings were inoculated with *Meloidogyne javanica* eggs/juveniles at 2000 per pot after one week of seedlings establishment.

Plants were uprooted after six weeks of nematode inoculation to determine the efficacy of seaweeds and pesticides on the root pathogens and plant growth. Observations were made on plant height, fresh shoot weight, root length and root weight. Nematode infection was determined by counting the numbers of galls per root system and rated on a 0-5 scale (TAYLER and SASSER, 1978). To examine the incidence of root infecting fungi, roots were washed in running tap water and five one cm long root pieces were cut from tap roots.

The root pieces were surface disinfected with 1% $Ca(OCl)_2$ and were placed onto Potato Dextrose agar plates supplemented with penicillin (100,000 units/L) and streptomycin (0.2 g/L). The dishes were incubated at 27°C for 5 days and the incidence of fungal growth was recoded. The experiment was conducted twice. Data were subjected to analysis of variance (ANOVA) and means were separated using the least significant difference (LSD) according to GOMEZ and GOMEZ (1984).

Results

Use of *Spatoglossum asperum* significantly ($p<0.05$) decreased root-knot nematode infection by reducing the number of galls per root system and incidence of root rotting fungi as compared to untreated control. Maximum reduction in gall formation was found in carbofuran treatment (1.8) as compared to untreated control (4.5). A complete suppression of *Macrophomina phaseolina* infection was observed where benomyl and *Spatoglossum asperum* at 1% were used individually or where *Spatoglossum asperum* at 1% was used with benomyl or carbofuran. *Spatoglossum asperum* at 1% with carbofuran completely prevented *R. solani* infection. *Spatoglossum asperum* at 1% with captan also completely prevented *F. solani* infection (Tab. 1). Maximum plant height and fresh shoot weight was observed where *Spatoglossum asperum* was used with benomyl. *Spatoglossum asperum* at 0.5% with benomyl also resulted in maximum root length. Maximum reduction in root weight was observed when *Spatoglossum asperum* at 1% was used with captan (Tab. 2).

Sargassum swartzii significantly ($p<0.05$) suppressed root-knot nematode and root rotting fungi (Tab. 3). Combined use of *Sargassum swartzii* with benomyl or captan showed better control of nematode infection than either used individually. *Macrophomina phaseolina* infection was not observed in the treatments where *Sargassum swartzii* and benomyl were used individually or where *Sargassum swartzii* was used with benomyl, captan or carbofuran. *Sargassum swartzii* at 1% with captan or carbofuran completely prevented *F. solani* infection. No infection of *R. solani* was observed where *Sargassum swartzii* was used individually or with benomyl or carbofuran (Tab. 3). Greater plant height and fresh shoot weight was produced by *Sargassum swartzii* used with benomyl (Tab. 4).

Tab. 1: Effect of *Spatoglossum asperum* and chemical pesticides on suppressing root-rot and root-knot disease of tomato.

Treatments	*M. phaseolina*	*R. solani*	*F. solani*	RKI (0-5 scale)
Control	25	56.2	75	4.5
Spatoglossum asperum 0.5% (A)	6.2	25.0	43.7	3.1
Spatoglossum asperum 1% (B)	0.0	6.2	43.7	3.1
Benomyl	0.0	18.7	43.7	3.7
Captan	12.5	12.5	31.2	3.7
Carbofuran	6.2	6.2	37.5	1.8
Benomyl + A	6.2	12.5	37.5	3.1
Benomyl + B	0.0	56.2	50.0	2.9
Captan + A	6.2	18.7	50.0	3.7
Captan + B	6.2	12.5	0.0	3.1
Carbofuran + A	12.5	6.2	18.7	3.4
Carbofuran + B	0.0	0.0	56.2	3.1
$LSD_{0.05}$	Treatments for fungi = 26.8[1], Fungal pathogens = 10.4[2]			RKI = 1.1[1]

[1] Mean values for treatments in columns showing differences greater than the LSD (Least significant difference) value are significantly different at $P<0.05$.
[2] Mean values for fungal pathogens in rows showing differences greater than the LSD value are significantly different at $P<0.05$.
RKI = Root-knot index

Tab. 2: Effect of *Spatoglossum asperum* and chemical pesticides on the growth of tomato plants.

Treatments	Plant height (cm)	Fresh shoot weight (g)	Root length (cm)	Root weight (g)
Control	11.6	1.8	10.9	1.9
Spatoglossum asperum 0.5% (A)	16.9	3.0	9.1	1.2
Spatoglossum asperum 1% (B)	16.8	3.2	10.3	0.9
Benomyl	16.6	3.4	10.8	1.4
Captan	16.3	2.6	13.9	0.9
Carbofuran	15.9	1.9	8.5	1.3
Benomyl + A	17.3	3.1	17.2	1.5
Benomyl + B	19.5	4.2	16.8	1.5
Captan + A	18.6	2.6	13.8	1.0
Captan + B	18.9	2.8	10.1	0.5
Carbofuran + A	17.1	2.4	12.3	0.6
Carbofuran + B	18.3	2.8	14.1	0.8
$LSD_{0.05}$	5.0[1]	1.7[1]	6.9[1]	0.8[1]

[1] Mean values for treatments in columns showing differences greater than the LSD value are significantly different at $P<0.05$.

Tab. 3: Effect of *Sargassum swartzii* and chemical pesticides on suppressing root-rot and root-knot disease of tomato.

Treatments	*M. phaseolina*	*R. solani*	*F. solani*	RKI (0-5 scale)
Control	25	56.2	81.5	4.5
Sargassum sawrtzii 0.5% (A)	0.0	0.0	31.2	3.1
Sargassum swartzii 1% (B)	0.0	0.0	12.5	3.0
Benomyl	0.0	18.7	31.2	3.6
Captan	12.5	12.5	37.5	3.6
Carbofuran	6.2	6.2	43.7	1.9
Benomyl + A	12.5	12.5	43.7	2.7
Benomyl + B	0.0	0.0	43.7	2.5
Captan + A	18.7	12.5	12.5	2.5
Captan + B	0.0	12.5	0.0	2.5
Carbofuran + A	0.0	31.2	31.2	2.9
Carbofuran + B	12.5	0.0	0.0	2.5
$LSD_{0.05}$	Treatments for fungi = 9.1[1], Fungal pathogens = 4.5[2]			RKI = 1.1[1]

[1] Mean values for treatments in columns showing differences greater than the LSD value are significantly different at $P<0.05$.
[2] Mean values for fungal pathogens in rows showing differences greater than the LSD value are significantly different at $P<0.05$.
RKI = Root knot index

Discussion

Control of plant parasitic nematodes and pathogenic fungi by using antagonistic plants offers alternate strategies to the prevalent use of

Tab. 4: Effect of *Sargassum swartzii* and chemical pesticides on the growth of tomato plants.

Treatments	Plant Height (cm)	Fresh shoot weight (g)	Root Length (cm)	Root weight (g)
Control	12.6	2.0	11.7	2.1
Sargassum swartzii 0.5% (A)	18.8	3.1	9.5	1.3
Sargassum swartzii 1% (B)	19.2	3.9	10.9	1.0
Benomyl	17.2	3.2	11.1	1.5
Captan	16.4	2.7	12.6	1.0
Carbofuran	15.6	2.3	9.3	1.2
Benomyl + A	16.4	2.7	13.4	1.6
Benomyl + B	19.1	3.8	14.1	1.9
Captan + A	16.5	2.8	13.6	1.2
Captan + B	17.5	2.8	10.6	0.9
Carbofuran + A	17.5	3.3	11.9	1.0
Carbofuran + B	18.4	3.5	13.9	0.8
$LSD_{0.05}$	0.77[1]	0.3[1]	ns	ns

[1] Mean values for treatments in columns showing differences greater than the LSD value are
significantly different at P<0.05.
ns = non-significan

synthetic pesticides (DEVAKUMAR, 1994; MANSOOR et al., 2007). Soil amendments have the potential to provide disease control through a variety of mechanisms, including biological and chemical, such as producing antimicrobial compounds during decomposition (BROWN and MORRA, 1997; MAZZOLA, 2004; TENUTA and LAZAROVITS, 2002). A wide variety of materials when incorporated into the soil as amendments have been found to be effective against plant parasitic nematodes and pathogenic fungi associated with crop plants (MISHRA and MOJUMDER, 1994; SULTANA et al., 2008; EHTESHAMUL-HAQUE et al., 1995a; 1996).

The soil amendment with seaweeds *Spatoglossum asperum* and *Sargassum swartzii* significantly (P< 0.05) reduced infection of root rotting fungi viz., *M. phaseolina*, *R. solani*, *F. solani* and root-knot nematode *M. javanica* infection in tomato. There are reports that describe that extracts derived from *Ascophyllum nodosum* reduced the fecundity of the root-knot nematode on tomato (WHAPHAM et al., 1994) and *Radopholus similis* infection on citrus (TARJAN, 1977). Similarly soil amendment with *Sargassum* species significantly reduced infection of *M. phaseolina*, *R. solani* and *F. solani* on sunflower (ARA et al., 1996) and root-knot nematode on okra (ARA et al., 1997). Concentrated extracts of *Ecklonia maxima,* a brown seaweed significantly reduced the root-knot infestation and increased growth of tomato plant (FEATOMBY-SMITH and VAN STANDEN, 1983).

It was observed in the present study that seaweeds besides reducing infection of root infecting fungi and root-knot nematodes, also enhanced plant growth. There are reports that seaweed concentrate prepared from *Ascophyllum nodosum* showed faster growth and greater root and shoot weight than untreated control (STEVENI et al., 1992). Similarly foliar spray of seaweed concentrate (Kelpak) increased grain yield in wheat (BECKETT and VAN STADEN, 1991). The growth enhancement may be due to the presence of growth regulators, like auxins, gibberellin and precursors of ethylene which have been detected in a number of seaweeds (CROUCH and VAN STANDEN, 1993; JOLIVET et al., 1991; LIJUN, 2006).

A better control of *F. solani* infection was observed when *Sargassum swartzii* was used with benomyl or *Spatoglossum asperum* was used with captan than either one used individually. Combined use of *Sargassum swartzii* with benomyl or captan and *Spatoglossum asperum* with benomyl showed better control of nematode infection than either one used individually. Nematicidal activity of benomyl (EHTESHAMUL-HAQUE et al., 1995b) and *Sargassum swartzii* (ARA et al., 1997) has been reported. Presumably, besides direct adverse effect of seaweeds on soil borne pathogens, seaweeds also provide additional food base for fungicide tolerant antagonistic microbes to colonized rhizosphere. Marine biosphere is an untapped reservoir of agrochemically potent compounds. Each year a huge amount of seaweeds are drifted and their rotting produce serious environmental problem in coastal area. Utilization of these seaweeds as manure will not only provide necessary nutrients to plants but will also protect them from invasion by root pathogens.

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