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## Management of root diseases of eggplant and watermelon with the application of asafoetida and seaweeds

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### Summary

Eggplant (*Solanum melongena* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) are highly susceptible to root rotting fungi *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and root knot nematode (*Meloidogyne* spp.) causing huge losses each year in Pakistan. In field experiments, application of asafoetida, a medicinal gum from *Ferula assafoetida* and seaweeds *Spatoglossum variabile*, *Stokeyia indica* and *Melanothamnus afaqhusainii* showed significant suppressive effect on root rotting fungi *Fusarium solani*, *Macrophomina phaseolina* and root knot nematode *Meloidogyne incognita* attacking watermelon and eggplant and improved plant growth in soil naturally infested with root rotting fungi and artificially infested with root knot nematode. Length of vine of watermelon, shoot length of eggplant and fresh shoot weight were higher in seaweed and asafoetida treated plants as compared to control or Topsin-M, a fungicide, treated plants. Seaweed and asafoetida treated plants also showed earlier fruiting than control or fungicide treated plants. At farmer's field seaweed showed similar suppressive effect on *F. solani* and *M. phaseolina* and root knot nematode on watermelon in soil naturally infested by these pathogens. Application of seaweed produced healthy plants and number of fruits and weight were significantly higher in seaweed and asafoetida treated plants. Asafoetida and seaweeds offer a non-chemical means of disease management.

### Introduction

Cucurbits and vegetable crops are highly susceptible to a number of root and soilborne diseases causing great losses in yield and quality (SHARMA et al., 2004; CHEHRI et al., 2010). Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) is a cucurbit fruit, grown throughout the world and consumed as fresh fruit. *Fusarium* wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* is among the most important diseases of watermelon (ZHOU and EVERTS, 2004). The disease is well known as mature watermelon vine decline (sudden wilt of watermelon). Infected plants also showed the presence of *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Monosporascus cannonballus* and *Pythium* spp. besides *F. oxysporum* (BOUGHALLEB and EL MAHJOUR, 2006). The disease is best controlled through the use of wilt resistant cultivars and crop rotations for a minimum of 5 to 7 years (MARTYN, 1996). However, resistance in commercial cultivars often no longer is effective due to the presence of the highly aggressive race of *F. oxysporum* (MARTYN, 1987). Similarly, among the various vegetables, eggplant (*Solanum melongena*) is one of the most common and extensively grown all over the world. Eggplant wilt complex caused by a number of fungal genera such as *Fusarium*, *Verticillium*, *Rhizoctonia*, *Sclerotium* and *Phytophthora* take a considerable portion of produce annually (NAJAR et al., 2011). Moreover, eggplant is highly susceptible to root knot nematodes, species of *Meloidogyne* (ZARINA and SHAHINA, 2010). Several nonchemical methods including addition of organic amendments are an effective method for controlling soilborne pathogens

and diseases in various field crops (CHELLEMI, 2002; HUBER, 1980; ZHOU and EVERTS, 2004). Marine bioactive substances extracted from seaweeds have been used for several decades to enhance plant growth and productivity. (RATHORE et al., 2009; STIRK and VAN STADEN, 1997). The application of seaweeds as organic soil amendment, has been increased in the recent years due to raising awareness about the adverse effect of chemical pesticides (SULTANA et al., 2007; 2008; 2009; 2011). Similarly, asafoetida or hing, a dry latex or resinous gum from *Ferula assafoetida* has been widely used in various indigenous systems of medicine in India and Pakistan. It is regarded as an effective remedy for worms and other intestinal parasites. From old ages, farmers in Malir, Karachi area of Sindh, are applying asafoetida for preventing the plants from root diseases particularly nematode attacks and increasing yield. However, scientific reasons are still unclear. In this study, efficacy of some seaweeds and asafoetida were evaluated in field plots on watermelon and eggplants in suppressing the soilborne diseases and their effect on plant growth. The experiment was also conducted at farmer's field on watermelon.

### Materials and methods

Asafoetida (*Ferula assafoetida*) was purchased from local market whereas seaweeds *Melanothamnus afaqhusainii* Shameel, *Spatoglossum variabile* Fig. et De Notar [= *S. lubricum* Fig. et De Notar] and *Stokeyia indica* Thivy et Doshi [= *Cystoseira indica* (Thivy et Doshi) Mairh] were collected at the coastal area of Karachi at low tide. The seaweeds were washed under tap water, dried under shade, ground to powder and stored in polyethylene bags at room temperature until used.

### Field plot experiments

The experiments were conducted at the Crop Diseases Research Institute, Pakistan Agricultural Research Council, Karachi University Campus, Karachi. Dry powder of seaweeds *Melanothamnus afaqhusainii*, *Spatoglossum variabile* and *Stokeyia indica* were mixed in sandy loam soil at 70 g per two meter rows and watered 2-3 days interval to allow the organic matters to decompose, whereas aqueous suspension of asafoetida (200 pp) was applied at 400 ml per 2 meter row. The soil had a natural infestation of 5-16 sclerotia/g of soil of *Macrophomina phaseolina* (SHEIKH and GHAFFAR, 1975), 5-12% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (WILHELM, 1955) and 2500cfu/gm of soil of mixed population of *Fusarium oxysporum* and *F. solani* as determined by soil dilution (NASH and SNYDER, 1962). After two weeks of seaweed decomposition, 12 seeds of watermelon were sown in each row. Whereas in the case of eggplant, three-week-old seedlings of equal size, raised in steam sterilized soil, were transplanted in each row at 12 seedlings per row. After one week of seed germination or seedling transplantation, each row was inoculated with aqueous suspension of *Meloidogyne incognita* eggs/juveniles at 2000 / two meter row. Topsin-M (200 ppm) at 400 ml / 2 meter row and carbofuran (1 g /

2 m row) served as positive control against fungi and nematode respectively. Each treatment was replicated four times and plants were watered 2-3 days intervals depending upon requirement of plants.

To determine the efficacy of seaweeds and asafetida on the root pathogens and plant growth, plants were uprooted after six weeks of nematode inoculation. Observations were made on yield, plant height, fresh shoot weight, root length and root weight. Nematode infection was determined by counting the numbers of galls per root system (TAYLER and SASSER, 1978). To determine nematode penetration and infection by root-infecting fungi, roots from each plant were cut into 1 cm long pieces and five pieces of tap roots from each plant were used for assessment of fungal infection. The remaining roots were mixed thoroughly, and 1 gram sub-sample was wrapped in muslin cloth and dipped in boiling 0.25% acid fuchsin stain for 3-5 minutes. Roots were left in the stain to cool, and then washed under tap water to remove excess stain. Roots were transferred to vials containing glycerol and water (1:1 v:v) with a few drops of lactic acid. Roots were macerated in an electric blender for 45 seconds and the resulting suspension was suspended in 50 ml water. Numbers of juveniles and females in five 5 ml sub samples were counted with the aid of dissecting microscope and numbers of nematode/g root were calculated (SIDDIQUI and EHTESHAMUL-HAQUE, 2001). To determine the incidence of fungal infection, 1 cm long root pieces from tap roots (five pieces from each plant) were surface disinfested with 1% Ca (OCl)<sub>2</sub> solution and plated onto potato dextrose agar amended with penicillin (100,000 units/l) and streptomycin (0.2 g/l). After incubation for 5 days at 28°C, colonies of *Macrophomina phaseolina*, *Rhizoctonia solani* and species of *Fusarium* were recorded. The experiment was repeated 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).

#### Farmer's field experiment

Efficacy of asafetida and seaweeds in protecting the watermelon from root rotting fungi and root knot nematode were also evaluated at farmer's field in Malir, Karachi. Watermelon field about 6 acres was selected before the sowing of seeds. Fifteen meter rows were selected for each treatment at different locations in the field and dry powder of seaweeds *Melanothamnus afaqhusainii*, *Spatoglossum variabile* and *Stokeyia indica* were mixed in soil at 35 g per meter and watered 2-3 days interval to allow the organic matters to decompose, whereas aqueous suspension of asafetida (200 pp) was applied at 200 ml per meter. The soil had variable natural

infestation of root rotting fungi and root knot nematode. After two weeks of amendment, seeds of watermelon were sown in each row. Observations were recorded after 80 days of seed germination.

## Results

### Field plot experiment

#### Eggplant

*Fusarium solani* and *Macrophomina phaseolina* were found in higher frequencies in control plants than *R. solani* or *F. oxysporum*. Plants grown in seaweed or asafetida amended soil showed less infection of *M. phaseolina* than control plants. *Spatoglossum variabile* was also found effective against *F. solani* (Tab. 1). Seaweed and asafetida also showed significant suppressive effect on root knot nematode by reducing gall formation on roots and nematode's penetration in roots as compared to untreated control (Tab. 2). Efficacy of asafetida and seaweed against root knot nematode is comparable with carbofuran, a nematicide. Taller plants and greater fresh shoot weight was produced by *S. indica* (Tab. 2).

#### Watermelon

Application of seaweeds *Spatoglossum variabile*, *Melanothamnus afaqhusainii* and asafetida caused a suppressive effect on root rotting fungi by reducing the infection of *M. phaseolina*, *R. solani* and *F. oxysporum* on watermelon roots. *Stokeyia indica* was effective against *F. solani* (Tab. 3). Seaweeds and asafetida also showed suppressive effect on root knot nematode by reducing the nematode's penetration in roots (Tab. 4). Vine length and fresh weight were significantly higher in watermelon grown in *Spatoglossum variabile* amended soil than other treatments (Tab. 4). Plants grown in seaweed or asafetida amended soils also showed earlier emergence of fruits than control of pesticides treatments.

### Farmer's field experiment

#### Watermelon

Infection of *Fusarium solani* was found in higher frequencies in control or treated watermelon plants. However, plants grown in *S. variabile* amended soil showed less infection of *F. solani* than other treatments, whereas topsin-M treated plants showed no infection of *F. solani* (Tab. 3). *Macrophomina phaseolina* infection was found in control plants only. Nematode penetration in watermelon roots were found less in *S. indica*, *S. variabile*, asafetida amended soil or in topsin-m treated plants (Tab. 4). Number of fruits and fruit weight were higher in plants grown in asafetida or seaweed

**Tab. 1:** Effect of asafetida and seaweed on the infection of root infecting fungi on eggplant in field plot experiment.

No.	Treatments	<i>M. phaseolina</i>	<i>R. solani</i> Infection %	<i>F. solani</i>	<i>F. oxysporum</i>
1.	Control	68.7	6.2	31.2	6.2
2.	Topsin-M	12.5	0	12.5	0
3.	Carbofuran	18.7	31.2	43.7	6.2
4.	Asafetida	6.2	50	43.7	0.0
5.	<i>Stokeyia indica</i>	0.0	0.0	50	0.0
6.	<i>Spatoglossum variabile</i>	0.0	6.2	18.7	0.0
7.	<i>Melanothamnus afaqhusainii</i>	12.5	12.5	25	0.0
LSD <sub>0.05</sub>		Treatments= 10.3 <sup>1</sup>	Pathogens= 7.8 <sup>2</sup>		

<sup>1</sup>Mean values in column showing differences greater than LSD values are significantly different at p< 0.05.

<sup>2</sup>Mean values in rows showing differences greater than LSD values are significantly different at p< 0.05.

**Tab. 2:** Effect of asafotida and seaweed on the infection of root knot nematode and growth of eggplant in field plot experiment.

No.	Treatments	No. of knots	J <sub>2</sub> /females /g roots	Plant height (cm)	Fresh shoot wt. (g)	Root length (cm)	Fresh root wt. (g)
1.	Control	92.5	192	14.2	20.2	7.7	11.7
2.	Topsin-M	23.2	124	21.2	29.7	5.2	4.1
3.	Carbofuran	16	80	26.5	33.7	11	10.2
4.	Asafotida	16.5	80	18.7	32.3	9.7	12.7
5.	<i>Stokeyia indica</i>	7	72.7	38.5	40.1	10.5	11.7
6.	<i>Spatoglossum variabile</i>	12	79.2	24.5	29.5	8.2	4.5
7.	<i>Melanothamnus afaqhusainii</i>	24.2	102.5	17	27.6	6.7	2.6
LSD <sub>0.05</sub>		35.1 <sup>1</sup>	15.3 <sup>1</sup>	9.6 <sup>1</sup>	14.0 <sup>1</sup>	3.1 <sup>1</sup>	3.6 <sup>1</sup>

<sup>1</sup>Mean values in column showing differences greater than LSD values are significantly different at p< 0.05.

**Tab. 3:** Effect of asafotida and seaweeds on the infection of root infecting fungi on watermelon in field plot experiment (after 45 days of nematode inoculation) and at farmer's field (after 80 days of sowing).

No.	Treatments	<i>M. phaseolina</i>	<i>R. solani</i> Infection %	<i>F. solani</i>	<i>F. oxysporum</i>
<b>Field plot experiment</b>					
1.	Control	31.2	25	37.5	18.7
2.	Topsin-M	43.7	25	50	12.5
3.	Carbofuran	12.5	6.2	43.7	18.7
4.	Asafotida	6.2	0	18.7	0
5.	<i>Stokeyia indica</i>	50	12.5	18.7	0
6.	<i>Spatoglossum variabile</i>	6.2	6.2	31.2	6.2
7.	<i>Melanothamnus afaqhusainii</i>	0	0	50	0
<b>Farmer's field experiment</b>					
1.	Control	18.7	6.2	37.5	0
2.	Topsin-M	0	0	0	0
3.	Asafotida	0	0	12.5	0
4.	<i>Stokeyia indica</i>	0	6.2	37.5	0
5.	<i>Spatoglossum variabile</i>	0	0	18.7	0
6.	<i>Melanothamnus afaqhusainii</i>	0	0	31.2	0
LSD <sub>0.05</sub> Field plot experiment		Treatments= 9.07 <sup>1</sup>	Pathogens= 6.86 <sup>2</sup>		
Experiment at farmer's field		Treatments= 7.3 <sup>1</sup>	Pathogens= 5.2 <sup>2</sup>		

<sup>1</sup>Mean values in column showing differences greater than LSD values are significantly different at p< 0.05.

<sup>2</sup>Mean values in rows showing differences greater than LSD values are significantly different at p< 0.05.

amended soil than control plants. Vine length and fresh weight was found significantly higher in plants grown in *M. afaqhusainii* amended soil (Tab. 4).

## Discussion

Protecting the plant roots from soilborne plant diseases with organic amendments and improving plant growth and yield is practiced since long by the farmers throughout the world. The suppressive effect of organic amendments on diseases is primarily associated with a reduction in pathogen inoculum density in amended soil (CHUN and LOCKWOOD, 1985; SUN and HUANG, 1985; SUBBARAO et al., 1999). In this study, soil amendment with seaweed or asafotida in general showed suppressive effect on soilborne pathogens and have a positive effect on growth of watermelon both in field plots and at farmer's field. Plant grown in seaweed or

asafotida amended soil showed earlier fruiting, greater number of fruits and weight than control plants. It was reported that seaweed extracts increased plant resistant to pests and diseases, improve plant growth, yield and quality (RATHORE et al., 2009; STIRK and VAN STADEN, 1997; VERKLEIJ, 1992). Application of seaweed to plants can result in decreased levels of nematode attack (ARA et al., 1997; WU et al., 1997; 1998), due to presence of betaines (WU et al., 1997) and root rotting fungi (SULTANA et al., 2007; 2008; 2009; 2011) due to 1-aminocyclopropane-1-carboxylic acid (ACC), which has antimicrobial activity (NELSON and VAN STANDEN, 1985) or due to volatile compounds and essential oils (KAJIWARA et al., 2006). Seaweed could also affect cell metabolism through the induction of the synthesis of antioxidant molecules which could favor plant growth and plant resistance to stress (ZHANG and SCHMIDT, 2000). TARIQ et al. (2011) has already reported the presence of polyphenols and antioxidant activity of seaweeds from the Karachi

**Tab. 4:** Effect of asafetida and seaweeds on the infection of root knot nematode and growth of watermelon in field plot (after 45 days of nematode inoculation) and at farmer's field (after 80 days of sowing).

No.	Treatments	No. of knots	J <sub>2</sub> /females /g roots	No. of fruits/plant	fruit wt. (g)	Vine length (cm)	Fresh shoot wt. (g)	Root length (cm)	Fresh root wt. (g)
<b>Field plot experiment</b>									
1.	Control	1.25	5.75	0	--	58.7	16.2	14.2	0.85
2.	Topsin-M	0.25	1.5	0	--	63.5	39.0	22	2.41
3.	Carbofuran	0.25	0.75	0	--	66	23.5	15	0.87
4.	Asafetida	0	0.75	0.75	--	70	20.9	12.2	1.13
5.	<i>Stokeyia indica</i>	0	1	0.5	--	67.5	32.2	16.7	0.99
6.	<i>Spatoglossum variabile</i>	0	0.75	1.25	--	92	38.7	15.3	1.87
7.	<i>Melanothamnus afaqhusainii</i>	0	0.75	1.0	--	81.5	36.4	14	1.92
LSD <sub>0.05</sub>		0.64 <sup>1</sup>	1.39 <sup>1</sup>	0.88 <sup>1</sup>	--	27.0 <sup>1</sup>	18.6 <sup>1</sup>	7.8 <sup>1</sup>	0.67 <sup>1</sup>
<b>Farmer's field experiment</b>									
1.	Control	4.5	6.5	09	356	124	86	13.1	3.9
2.	Topsin-M	3.5	1.8	14	1264	164	164	12.6	6.2
3.	Asafetida	2.8	2.5	18	3847	188	156	12.5	3.1
4.	<i>Stokeyia indica</i>	5.5	0.75	17.5	1871	129.7	120	12.7	2.2
5.	<i>Spatoglossum variabile</i>	2.8	0.75	16	2585	204	154	12.7	2.5
6.	<i>Melanothamnus afaqhusainii</i>	3	5.75	19.5	4265	227.5	185	12.2	4.2
LSD <sub>0.05</sub>		1.7 <sup>1</sup>	1.12 <sup>1</sup>	2.1 <sup>1</sup>	46.8 <sup>1</sup>	6.8 <sup>1</sup>	5.0 <sup>1</sup>	ns	0.5 <sup>1</sup>

<sup>1</sup>Mean values in column showing differences greater than LSD values are significantly different at p< 0.05.

coast.

In this study, the potential of asafetida in suppressing the soilborne diseases has been confirmed. Asafetida or hing, is regarded as an effective remedy for worms and other intestinal parasites and possesses antimicrobial and antioxidant activities (IRANSHAHY and IRANSHAHI, 2011). Biological activity of sesquiterpene coumarins from *Ferula* species has also been reported (NAZARI and IRANSHAHI, 2011). Similarly LEE et al. (2009) reported antiviral and cytotoxic agents from *Ferula assafetida*. Growth inhibition of plant pathogenic fungus *Fusarium* by the essential oils extracted from asafetida has also been reported (SITARA et al., 2008).

Due to the damaging influence on crop yields, the root knot nematodes *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are considered economically important pests worldwide (ROBERTSON et al., 2006). The damages are much higher in tropical and subtropical countries where environmental factors favor their survival and dispersal (SIKORA and FERNANDEZ, 2005). In this study, the application of asafetida and seaweeds besides, suppressing the same root rotting fungi also showed significant reduction in gall formation on roots and nematode's penetration into roots of eggplant and improve plant growth. Eggplant is susceptible to several insects and pest, particularly *Fusarium* wilts (*F. oxysporum* f. sp. *melongenae*) and root knot nematodes (*Meloidogyne* spp.) which reduced the yield (PHAP et al., 2010). Due to high susceptibility, eggplant is widely used for maintaining the pure culture of root knot nematode (SIDDIQUI et al., 2001; SIKANDER et al., 2009). In this study, efficacy of asafetida and seaweed is comparable to carbofuran, a commercial nematicide. Nonchemical management of soilborne pests has been practiced for centuries. Only in the last 40 years agricultural producers have come to rely on synthetic chemicals for the control of soilborne pest and diseases (CHELLEMI, 2002). However, due to raising awareness about the adverse effect of chemicals, organic farming is gaining popularity (SULTANA et al., 2011). The ability of seaweeds and asafetida in reducing the

root knot nematode on a highly susceptible crop showed its great potential in future nematode pest management.

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