Effect of Plant Extracts as Seed Treatments on Growth Parameters, Seedlings Mortality and Biochemical Changes in Tomato

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Abstract

The extract of plant parts like bark of *Eucalyptus lanceolotus*, *Terminalia arjuna*, tubers of *Cyperus rotundus*, leaves of *Withamia sominifera, Azadirachta indica, Datura stromonium, Acacia arabica, Cymbopogon flexosus and Parthenium hysterophorus*, cloves of *Allium sativum,* bulb of *Allium cepa*, fruits of *Emblica officinalis* and rhizome of *Zingiber officinale* as seed treatment was found that all the plant extracts significantly increased seed germination and growth of tomato plant both on glass house and laboratory condition by Blotter method. Biochemical analysis of plant extract treated seedlings showed that there was increase in the level of total soluble protein and total phenols content as compared to healthy and diseased plant. The mortality % of tomato seedlings was also reduced in all plant extract treated seedling. The minimum mortality per cent with 5.62 was found in garlic extract treated plant against 80.92% in case of control 2.

1. Introduction

Tomato (*Lycopersicon esculentum* L. Krust) is one of the most valued vegetable crops grown throughout the world owing to its high nutritive value as well as its antioxidant and curative properties. It is attacked by a number of pest and diseases. Fusarium wilt caused by F*usarium oxysporum* f. sp. *lycopersici* (Sacc). Synder and Hansen (1940) is one of the most prevalent and damaging diseases of tomato causing considerable losses in India. The pathogen is a soil inhabitant and survives in infected plant debris as mycelium and in all its spore forms (micro and macro conidia) but commonly in cooler temperate regions as chlamydospores. The pathogen enters into the plant by penetrating through root tips directly or through wounds. The mycelium advances through the root cortex intercellularly and reaches xylem vessels. A combination of different processes like vessel clogging by mycelia, spores, gels, gums and tyloses and crushing of vessels by proliferating adjacent parenchyma cells results in break down of water economy of infected plant and there is increased transpiration. The fungus invades all tissues of the plant extensively; reaches surface of dead plants and sporulate there profusely. Various cultural practices, chemical and biological control measures and resistant varieties are used to manage the disease. (Singh and Singh, 2000; Katan, 1989; Chauhan et al. 1988; Raj et al., 1994; Larkin and Fravel, 1998; Howell, 2003). But providing protection of seed as initial stage is the best strategies for management of disease which can only be done through seed treatment. Biochemical changes are associated with the effect of seed treatment. Plants and plants product are being used as inducers to induce resistance in plant against pathogens (Baysal et al., 2002). Hence the strategies have been taken in the present investigation.

2. Materials and Methods

2.1. Collection of diseased plant sample

The present investigation was undertaken during 2008-2010 at Department of Plant Pathology, CSA University of Agriculture and Technology, Kanpur, Uttar Pradesh, India. The diseased plant samples showing typical wilt symptoms were collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture & Tecnology, Kanpur.

2.2. Isolation and purification of the pathogen

The diseased plant's stem showing typical wilt symptom was washed thoroughly with distilled water and a part of it was cut with sterilized knife into small pieces. The small pieces were further sterilized by dipping in 0.1% $HgCl_2$ solution and then

washed with distilled water thrice. The surface sterilized stem pieces were then placed over Petri plates which were previous poured with sterilized 2% PDA. The plates were then sealed and incubated at 27±1°C. The fungus was then purified by hyphal tip method and the purified fungus was identified as authentic description given by Synder and Hansen (1940) Sacc. .

2.3. Collection of plant products

The plant products listed in Table 1 were collected from Students Research, Farm C.S. Azad University of Agriculture and Technology, Kanpur and nearby places. The extracts of such plants were used for present investigation.

2.4. Preparation of plant extracts

Exactly 2 g of each plant part was weighed and crushed in a mortar and pestle along with 12 ml distilled water. The prepared products were filtered with muslin cloth to obtain pure extracts. The extracts were then stored in vials for further study.

2.5. Seed treatment

Truly labeled tomato seeds of variety Azad T-6 were used to conduct the experiment. About 2 g of tomato seeds were placed in each Petri plate containing different plant extracts separately and kept it for overnight. Similarly, 2 g of seeds were soaked overnight in distilled water and 2 g in conidial suspension of *Fusarium oxysporum* f. sp. *lycopersici.* After 24 hrs, the seeds were taken out from the treatments and dried in shade at room temperature. The treated seeds were used for testing germination percentage and growth parameters of tomato seedling under laboratory (blotter method) and glass house condition.

2.5.1. Blotter method

Table: 1. List of plant parts used for preparing plant extracts

	Common name	Botanical name
i)	Bark of *Eucalyptus*	*Eucalyptus lanceolotus*
ii)	Bark of Arjun	*Terminalia arjuna*
iii)	Tubers of motha	*Cyperus rotundus*
iv)	Ashwagandha leaves	*Withamia sominifera*
v)	Neem leaves	*Azadirachta indica*
vi)	Onion bulb	*Allium cepa*
vii)	Datura leaves	*Datura stromonium*
viii)	Garlic cloves	*Allium sativum*
ix)	Babool leaves	*Acacia arabica*
x)	Lemongrass leaves	*Cymbopogon flexosus*
xi)	Anola fruit	*Emblica officinalis*
xii)	Ginger rhizome	*Zingiber officinale*
xiii)	*Parthenium* leaves	*Parthenium hysterophorus*

The Blotter paper method was employed with slight modification for germination test and also to determine the growth of seedling. Petri dishes of 90 mm diameter were taken to conduct the experiment. On each lower half of Petri plates some cotton was placed followed by thick layer of sterile Whiteman blotter paper reaching halfway up to the side walls. Similarly, thick layer of sterile blotter paper were placed on upper half of each Petri plates. Each lower half and upper cover of Petri plates was moistened with sterile distilled water. Exactly 30 treated seeds with each plant extract were uniformly placed on blotter paper of lower half of Petri plates. Three replications were kept for each treatment. Exactly 30 untreated seeds soaked overnight in distilled water and another 30 seeds in conidial suspension of the pathogen were also placed on Petri plates separately, which served as control-1 and control-2, respectively. All these plates were placed in a growth chamber at 20±1°C. The data were recorded on germination and growth parameter of seedling.

2.5.1.1. Germination percentage

Observations on germination of seeds were taken at every 24hrs up to 10 days of seedling. The germination percentage was calculated by use of following formula-:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 100$$

2.5.1.2. Root and shoot length

To evaluate the growth parameters, observations on growth of seedling were taken by measuring root and shoot length of seedling at every 24 hrs for up to ten days of seedling.

2.5.2. Glasshouse condition

The experiment was conducted in the glasshouse complex, Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur. Earthen pots were taken and filled with mixture of sterilized soil and compost as ratio of 2:1 and moistened properly. The pots were then inoculated with spore suspension of *Fusarium oxysporum* f. sp. *lycopersici* @ 1 ml per pot. All the plant extract treated seeds were sown in pots separately. About twenty seeds were placed in each pot. Seeds soaked overnight in distilled water and conidial suspensions of the fungus were also sown in separate pots to serve as control-1 and control-2, respectively. Three replications were kept for each treatment.

2.5.2.1. Germination percentage

As the seedling began to emerge, the germination percentage was calculated by recording the number of emerged seedling in 100 and the germination of seed was calculated at every 24 hrs intervals for up to one month.

2.5.2.2. Shoot length

Observations pertaining to the effect of different treatments with plant extract on growth of plants were recorded by measuring shoot length of emerged seedling at every 24 hrs interval for up to one month with the help of a scale.

2.5.2.3. Root length

Prior to measuring of root length of tomato plants, pots were irrigated and the seedlings were uprooted carefully. Roots of the seedling were separated from shoots and washed with water to remove soil particles. Root length was then measured with a scale and represented as cm in length.

2.5.2.4. Seedling mortality

The mortality % of seedlings seedling were observed at 5 & 10 days age of seedlings.

2.6. Biochemical analysis of tomato seedling

Analysis of biochemical changes in tomato seedling due to seed treatment with different plant extracts was carried out to determine the effect of different treatments on the contents of soluble protein and phenol in the seedling.

2.6.1. Estimation of total phenols

The accumulation of phenols in tomato seedlings grown in glass house condition was estimated following the procedure developed by Bray and Thorpe (1954) with slight modification. In this method, the total phenols estimation was carried out with Folin-Ciocalteu reagent (FCR), which was measured at 650 nm calorimetrically.

Exactly, 1.0 g of tomato seedlings were ground in a pestle and mortar along with 80% ethanol (1:10 w/v). It was then centrifuged at 10,000 rpm for 30 minutes at room temperature in order to homogenate the suspension. Supernatant was separated and re-extracted with 5 times volume of 80% ethanol, centrifuged and the supernatant were pooled. It was then evaporated near to dryness and residues were dissolved in 5 ml of distilled water. Different aliquots were pipette out into test tubes and the volume in each tube was made to 3 ml with distilled water. A test tube with 3 ml distilled water served as blank. Subsequently, 0.5 ml of FCR was added and after three minutes, 2 ml of 20% Na_2CO_3 solution was thoroughly mixed in each tube. After that the tubes were placed in boiling water for 1 min and then cooled at room temperature. Then absorbance at 650 nm against blank was measured using Ultra Violet Visible (UV-VIS) spectrophotometer and the standard curve using different concentration of catechol was prepared. From the standard curve the concentration of phenols in the test sample was determined and expressed as mg g^{-1} of sample materials.

2.6.2. Estimation of total soluble protein

2.6.2.1. Protein extraction

Tomato seedlings were harvested from plants sprayed with different treatments and then washed with distilled water several times and blotter dried. A quantity of 1.0 g of each sample was cut into small pieces and ground in pestle and mortar using alkaline copper as extraction buffer. The concentration was kept 1: 5 (w/ v). Alkaline copper solution was prepared by mixing 20% sodium carbonate in 0.1 N NaOH and 0.5% copper sulphate in sodium potassium tartrate. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4 ºC. The supernatant was collected and used for quantification of protein.

2.6.2.2. Quantification of protein

The method developed by Lowry et al. (1951) was used with slight modification for quantification of the total soluble protein content. Different aliquots of working standard solution of Bovine Serum Albumin were pipette out into a series of test tubes. Similarly, same volumes of sample extracts were also pipette out and kept in other test tubes separately. Then volume in all the tubes was made up to 1 ml with distilled water. A tube with 1 ml of distilled water served as a blank. Later on, 5 ml of alkaline copper solution was added in each test tube and incubated at room temperature for 10 min. Thereafter, 0.5 ml of FCR was mixed well and incubated at room temperature for 30 min in dark place. The absorbance at 660 nm against the blank was read. The standard graph of BSA was drawn to calculate the amount of soluble protein in different samples. Protein estimated was represented as mg g^{-1} of fresh leaf samples.

3. Results and Discussion

3.1. Germination and growth parameters of tomato seedling (blotter method)

3.1.1. Germination percentage

The result presented in Table 2 indicate that the seed treatment with plant extracts significantly increased the germination percentage of tomato seed over control-1(healthy) as well as over control-2 (seed treated with pathogen). The maximum germination was recorded in treatment with extracts of garlic (95.56%) followed by ginger (94.45%), onion (94.45%), *Cyprus rotundus* (93.34%), neem (88.79%) and *Withamia sominifera* (92.23%). From the Table, it is also cleared that all treatments showed a significant increase in germination percentage over control-1 and control-2. Enikuomehin (2005) reported increased germination in sesamum seeds treated with different plant extracts along with reduced disease incidence of *Cercospora* leaf spot in treated plots. In present investigation also, all the plant extract resulted in increased germination percentage and vigour of plants.

3.1.2. Shoot length

The effects of seed treatment with various plant extracts on shoot length of germinating tomato seedling were studied under laboratory condition by Blotter method. The observations of shoot length were taken at every 24 hrs interval for 10 days. The data presented in Table 2 shows that the shoot length of tomato seedlings was maximum in garlic extract treated seedling (8.51 cm) which was 18.74% and 23.79% higher than control-1 and control-2. The seed treatment with neem extracts registered second highest in case of shoot length with the value of 8.46cm which is also statistically at par with garlic extract. The treatments like ginger, onion and motha also enhanced seedling vigor showing 21.31%, 20.458% and 17.97% increased, respectively over control-1 and 24.5%, 22.87% and 20.09% increased over control-2. Riaz et al. (2010) found that aqueous bulb extracts of *Allium cepa* and *A. sativum* and rhizome extracts of *Zingibar officinale* enhanced shoot growth to variable extents which also able to control Fusarium corm rot disease in gladiolus.

3.1.3. Root length

Similarly, root length was also increased in case of treated plants than healthy (control-1) and diseased (control-2) plants. The result presented in Table 2 shows that after ten days, the root length of tomato seedling under garlic extract treatment was the best (6.56 cm) followed by neem (6.36 cm), ginger (6.04 cm) and onion (5.75 cm). The percent increase in root length over control-1 were 85.84%, 80.17%,71.10% and 62.37% respectively and over control-2, the percent increase were 92.94%, 86.80%, 76.99% and 68.09% respectively.

3.2. Germination and growth parameters of tomato plant (glass house condition)

3.2.1. Germination percentage

The result in Table 3 indicated that highest germination percentage is noted in garlic extract treatment (86.67%) followed by neem, ginger and onion treatment representing 83.34% germination in each case against 68.33% in control-1 and 63.34% in control-2 The other treatments like motha, ashwagandha, babool and lemongrass also showed significantly better germination as compared to untreated healthy and diseased control.

3.2.1.1. Shoot length

The result presented in Table 3 indicates that seed treatment with various plant extract significantly increases the growth and the vigour of seedlings. Shoot length of garlic treated plants (20.29cm) was significantly higher than both controls showing 39.64% increase over control-1 and 71.28% over control-2. Other significantly better treatments were neem (19.66 cm), ginger (18.68 cm), motha (17.59 cm) and onion (17.55 cm) representing 35.31%, 28.56%, 21.06% and 20.78% respectively higher than control-1 and 63.49%, 51.36%, 37.87%

Table 2: Effect of seed treatment with plant extracts on germination, shoot length and root length (cm) of tomato (Blotter method)

S.No.	Treatment	Germination (%)	Shoot length (cm)	% increase		Root length (cm)	% increase	
				Over control-1	Over control-2		Over control-1	Over control-2
1.	*Eucalyptus lonceolotus*	91.11	7.34	6.95	7.77	4.52	28.05	30.37
2.	*Terminalia arjuna*	90.01	7.10	3.41	3.81	4.08	15.58	16.87
3.	*Cyperus rotundus*	93.34	8.10	17.97	20.09	5.43	53.82	58.28
4.	*Withamia somninifera*	92.23	7.88	14.77	16.51	5.16	46.18	50.00
5.	*Azadirachta indica*	88.79	8.46	23.22	25.96	6.36	80.17	86.80
6.	*Allium cepa*	94.45	8.27	20.45	22.87	5.75	62.37	68.09
7.	*Datura stromonium*	90.01	7.32	6.61	7.39	4.32	22.38	24.23
8.	*Allium sativum*	95.56	8.51	23.94	26.78	6.56	85.84	92.94
9.	*Acacia arabica*	88.89	7.87	14.62	16.35	4.95	40.23	43.56
10.	*Cymbopogon flexosus*	92.24	7.75	12.88	14.4	4.73	33.99	36.81
11.	*Emblica officinalis*	93.05	7.55	9.96	11.14	4.61	30.59	33.13
12.	*Zingiber officinale*	94.45	8.37	21.91	24.5	6.04	71.10	76.99
13.	*Parthenium hysterophorus*	86.67	7.10	3.41	3.81	3.95	11.98	12.97
14.	Control-1 (Healthy)	80.48	6.87			3.53		
15.	Control-2 (Diseased)	64.47	6.14			3.26		
	SEd	1.1829	0.0615			0.0601		
	CD (p=0.05)	2.416	0.1256			0.1228		

and 37.37%, respectively over control-2. The treatments like eucalyptus, arjun, ashwagandha, datura, babool, lemongrass, aonla and parthenium also showed significantly increased vigour of seedlings

3.2.1.2. Root length

Among the various treatments, garlic extract followed by neem, ginger, onion and ashwagandha showed significant increase in root length of seedling which were 11.87 cm, 11.66 cm, 11.37 cm, 10.97 cm and 10.46 cm, respectively against 8.55 cm in case of control-1 and 6.35 cm in control-2. From the Table it is also cleared that all the treatments had significantly higher root length than control-1 and control-2 (Table 3). Garlic, neem, ginger, onion and ashwagandha treatments showed 38.83%, 36.37%, 32.98%, 28.30% and 22.34% increase over control-1 and 52.31%, 49.00%, 44.43%, 38.13% and 30.09% increase over control-2. Zarina et al. (2003) found that soil amendment with leaf extract of neem as well as datura increased the growth of brinjal plants while controlling root knot nematode, *Meloidogyne javanica.*

3.3. Biochemical changes associated with seed treatment

To evaluate the biochemical changes associated with seed treatment, the 10 days old seedling raised from phytoextract treated seeds were used. The total soluble protein and total phenol content of the seedling were estimated. The results obtained are shown in Table 4.

3.3.1. Soluble proteins

The resulted presented in the Table 4 and shows that all treatment lead to significant increase in total soluble protein content in seedling as compared to control-1 and control-2. The highest soluble protein content is observed in garlic extract treated seedlings (23.22 mg g^{-1}) followed by neem (22.97 mg g^{-1}), ginger (21.86 mg g^{-1}) and onion (20.90 mg g^{-1}) treatments which are 52.86%, 51.22%, 43.91% and 37.60%, respectively higher than control-1 (healthy untreated seedlings) and 80.62%, 78.11%, 66.97% and 57.32% higher than control-2 (diseased seedling). Chandrasekaran and Rajappan, (2001) found the alteration in protein and sugar content of soyabean plants as induced by plant extract, antagonists and chemicals.

3.3.2. Total phenol

Similarly, the total phenol content (Table 4) is found significantly higher in case of garlic extract treated seedling (2.0 mg g^{-1}) as compared to control-1 (1.08 mg g^{-1}) and control-2 (1.03 mg g^{-1}). It is also significantly higher than other treatments. Seeds treated with neem, ginger, onion and motha have total phenol contents as 1.79 , 1.77, 1.65 and 1.63 mg g^{-1} of fresh leaves respectively where as, in case of control-1 and control-2, the values are 1.08 mg g^{-1} and 1.03 mg g^{-1}, respectively. According

Table 3: Effect of seed treatment with plant extract on germination, shoot and root length of tomato plants (Glass house experiment)

S. No.	Treatment	Germination (%)	% increase		Shoot length (cm)	% increase		Root length (cm)	% increase	
			Over control-1	Over control-2		Over control-1	Over control-2		Over control-1	Over control-2
1.	*Eucalyptus lonceolotus*	73.34	7.33	7.91	16.48	13.42	24.13	9.65	12.87	17.33
2.	*Terminalia arjuna*	71.67	4.89	5.27	15.44	6.26	11.26	9.36	9.47	12.76
3.	*Cyperus rotundus*	80.00	17.08	18.42	17.59	21.06	37.87	9.63	12.63	17.02
4.	*Withamia sominifera*	77.00	12.69	13.69	17.30	19.06	34.28	10.46	22.34	30.09
5.	*Azadirachta indica*	83.34	21.97	23.69	19.66	35.31	63.49	11.66	36.37	49.00
6.	*Allium cepa*	83.34	21.97	23.69	17.55	20.78	37.37	10.97	28.30	38.13
7.	*Datura strononium*	73.34	7.33	7.91	16.13	11.01	19.80	9.64	12.74	17.17
8.	*Allium sativum*	86.67	26.84	28.95	20.29	39.64	71.28	11.87	38.83	52.31
9.	*Acacia Arabica*	76.67	12.21	13.17	17.05	17.34	31.12	10.09	18.01	24.26
10.	*Cymbopogon flexosus*	76.67	12.21	13.17	16.97	16.79	30.19	9.84	18.08	20.32
11.	*Emblica officinalis*	73.34	7.33	7.91	16.8	15.62	28.09	9.78	14.39	19.38
12.	*Zingiber officinale*	83.34	21.97	23.70	18.68	28.56	51.36	11.37	32.98	44.43
13.	*Parthenium hysterophorus*	70.34	2.94	3.17	15.05	3.57	6.435	9.13	6.78	9.13
14.	Control-1 (Healthy)	68.33			14.53			8.55		
15.	Control-2 (Diseased)	63.34			8.08			6.346		
	SEd	3.47			0.077			0.0262		
	CD (p=0.05)	7.078			0.157			0.0535		

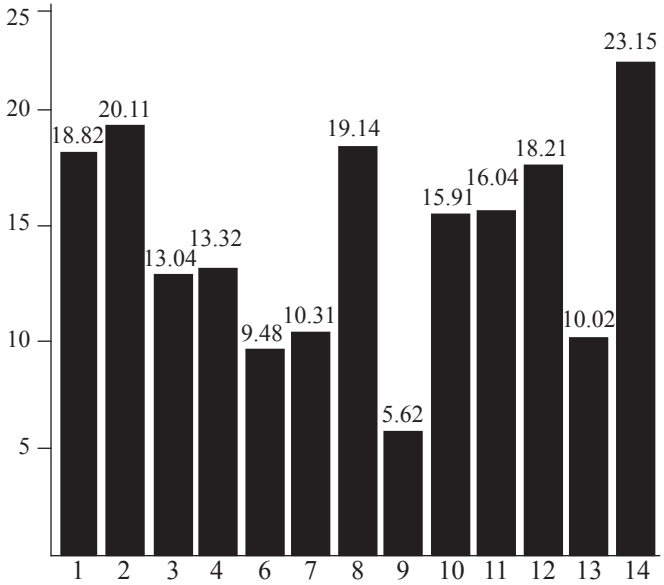
Table 4: Effect of seed treatment with plant extracts on total soluble protein and total phenol content of tomato seedlings

S.No.	Treatment	Total soluble protein content mg g^{-1} seedling	% increase		Total phenol content mg g^{-1} seedling	% increase	
			Over control-1	Over control-2		Over control-1	Over control-2
1.	*Eucalyptus lanceolotus*	18.46	21.52	32.83	1.15	6.48	6.79
2.	*Terminalia arjuna*	17.53	15.40	23.49	1.13	4.63	4.85
3.	*Cyperus rotundus*	20.79	36.87	56.22	1.63	50.93	53.39
4.	*Withamia somnifera*	19.79	30.28	46.18	1.34	24.07	25.24
5.	*Azadirachta indica*	22.97	51.22	78.11	1.79	65.74	73.78
6.	*Allium cepa*	20.90	37.60	57.32	1.65	52.78	55.34
7.	*Datura stromonium*	17.89	17.77	27.11	1.14	5.56	5.82
8.	*Allium sativum*	23.22	52.86	80.62	2.003	85.46	89.61
9.	*Acacia arabica*	19.26	26.79	40.86	1.29	19.44	20.38
10.	*Cymbopogon flexosus*	18.63	22.64	34.53	1.27	17.59	18.45
11.	*Emblica officinalis*	18.57	22.25	33.93	1.19	10.18	10.68
12.	*Zingiber officinales*	21.86	43.91	66.97	1.77	63.88	71.18
13.	*Parthenium hysterophorus*	15.96	5.06	7.73	1.11	2.77	2.91
14.	Control-1 (Healthy)	15.19			1.08		
15.	Control-2 (Diseased)	9.96	-0.344		1.03		
	SEd	0.7246			0.014802		
	CD (p=0.05)	0.147964			0.030227		

to Kuc (1995) induced resistant prior to challenge infection elevates the levels of some defense compounds and sensitize the plants to rapidly produce some compounds after infection and thereby provide protection against the disease. Daayf et al. (2000) reported induction of phenolic compounds in two cultivars of cucumber by treatment with extract of *Renoutria sachalinensis*.

3.3.3. Effect of treatment with plant extracts on seedling mortality

The effect of seed treatment with plant extracts on seedlings mortality against Fusarium wilt revealed that there is a decline in seedling mortality due to various treatments under glasshouse condition (Figure 1). The susceptible variety Azad T-6 of tomato showed 80.92% mortality in case of *Fusarium oxysporum* f. sp. *lycopersici* treatment. The minimum seedling mortality was recorded in garlic extract treated plants which was 5.62% followed by neem, ginger, onion and motha treated plants showing 9.48%, 10.02%, 10.31% and 13.04% respectively. The decrease in seedling mortality might be due to the activities of plant extracts which induces resistance in plant against *Fusarium oxysporum* f. sp. *lycopersici*. Riaz et al. (2010) found that aqueous bulb extracts of *Allium cepa* and *A. sativum* and rhizome extracts of *Zingibar officinale* and able to control Fusarium corm rot disease in gladiolus.



1: *Eucalyptus lanceolotus;* 2: *Terminalia arjuna;* 3: *Cyperus rotundus;* 4: *Withamia sominifera;* 5: *Azadirachta indica* 6: *Datura stromonium*; 7: *Acacia arabica;* 8: *Cymbopogon flexosus*; 9: *Parthenium hysterophorus;* 10: *Allium sativum;* 11: *Allium cepa*; 12: *Emblica officinalis;* 13: *Zingiber officinale*

Figure 1: Effect of plant extract on seedling mortality in tomato against fusarium wilt

4. Conclusion

The extract of cloves of *Allium sativum* proved best in recording highest germination%, shoot and root length both in glass house experiment and blotter method along with lowest seedling mortality, also maximum total soluble protein and phenol content. Further evaluation may be done before commercialization

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