

# Integrated management of Leaf Spot of Brinjal caused by *Alternaria alternata*

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## **Integrated management of Leaf Spot of Brinjal caused by *Alternaria alternata***

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Plant extracts, biocontrol agent, and fungicides were screened *in vitro* against *Alternaria alternata*, causing leaf spot on brinjal. Among these, *Acorus calamus* dried root showed highest reduction (82.22%) in mycelial growth and 91.7% inhibition of spore germination. It was also observed that as the concentration of extracts increased in the medium the effectiveness of extracts also increased and maximum growth inhibition was recorded at 15% concentration. The other best plants extract against the pathogen were treatments, which showed significant reduction, were *Allium sativum*, bulb (73.33%), *Azadirachta indica*, leaf (63.4%), *Allium cepa*, bulb (57.8%) and *Datura stramonium*, leaf (56.7%). Effective integrated management practices were developed in brinjal plants against *Alternaria alternata* using neem cake, cow dung, aqueous leaf extract of *Acorus calamus* dried root, bio-control agent like *Trichoderma harzianum* and 0.1% calixin.

**Keywords:** *Acorus calamus*, *Allium sativum*, *Alternaria alternata*, *Catharanthus roseus*, plant extract,

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### **INTRODUCTION**

Brinjal (*Solanum melongena* L.) is one of the important species cum vegetable crops of Nepal and India. It is also called Bhanta and is grown for its fruits. About 25 pathogens have been reported to occur on brinjal. *Alternaria alternata* (Fries) Keissler is one of the important fungal pathogens. It is the major foliar leaf spot disease of Biratnagar brinjal growing area of Nepal, which results in substantial yield losses. *Alternaria alternata* is one of the important seed-borne pathogen causing leaf spot on brinjal. *Alternaria* leaf spot is the most serious and destructive disease causing in brinjal growing areas of the world. Due to extensive cultivation of brinjal in non-traditional areas, the pathogen has emerged as a major constraint in its successful cultivation. Effective and efficient management of crop diseases is generally achieved by the use of synthetic pesticides. These cause deleterious effects on human health and biosphere. A search for an environmentally safe and economically viable strategy for the control of diseases had led to an increased use of plant-based products

in agriculture (Chakraborty and Bhagat, 2017; Das Biswas and Chakraborty, 2020; Bhagat, 2022). It is now widely recognized that biological control of plant pathogen is a distinct possibility for the future and can be successfully exploited in modern agriculture, especially within the framework of integrated disease management systems (Chowdhury *et al.* 2019). In the present investigation attempts have been made to screen resistant varieties, the most effective antifungal activities *in vitro* among various plant extracts against *Alternaria alternata* and development for its integrated management strategies using selected plant extracts, organic additives, biocontrol agent and fungicide for leaf spot disease of brinjal.

### **MATERIALS AND METHODS**

#### ***Plant material***

Three brinjal varieties which include F<sub>1</sub> hybrid long purple, local Lalgulaband hybrid- Sumo green long grown in pots as well as fields and were used for experimental purpose.

#### ***Fungal cultures***

Virulent culture of pathogen (*Alternaria alternata*) and potential biocontrol agent (*Trichoderma*

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*harzianum*) were obtained from Immuno-Phytopathology Laboratory, Department of Botany, North Bengal University. After completion of Koch's postulate in brinjal plant, the re-isolated pathogen was identified as *Alternaria alternata*.

### **Inoculum preparation of fungal pathogen**

Fungal pathogen (*A. alternata*) was grown in sand maize meal (3:1) medium for 7 days at 28°C. The inoculum was mixed with sterile soil at the ratio of 1:8. 50 ml of fungus suspension containing 10g mycelia was added to each pot containing 1kg sterilized soil and incubated for 48 hr in shade. Fungus soil mixture (10 g) were mixed with the top soil of earthen pots.

### **Inoculation technique and disease assessment**

Brinjal seedlings were planted in earthen pots containing 1 kg soil and allowed to be established. Regular watering was done for two weeks and then 10g of pathogen inoculum was added carefully in the rhizosphere as well spray on each plant. Disease assessment was done on the basis of visual observation of symptoms and disease index was calculated from 0-6 scale as described by Chakraborty *et al.* (2016) and was calculated after 7, 15 and 30 days of inoculation. Disease intensity was assessed as spot index, depending on both underground and above ground symptoms on a scale of 0-6 as follows; spot index: 0- no symptoms; 1 – small lesions appear at lower leafy region; 2 – spot appear on middle leaves and 10-20 % of the leaves turn having brown spot; 3 – leaves blighted and 20-40% leaves became dry with browning of shoot; 4 – extensive spotting at the leafy region, 60- 70% leaves affected; 5 – leaves withered and shoot became brown, 80% leaves affected; 6 – whole plants die, with upper withered leaves still remaining attached, leaves fully spotted.

### **Preparation of plant extract**

Aqueous extracts of 11 selected plant parts such as dried root of *Acorus calamus*, bulbs of *Allium cepa* and *Allium sativum* and leaf samples of *Adhatoda indica*, *Argemone maxicana*, *Lantana camera*, *Azadirachta indica*, *Catharanthus roseus*

were prepared separately following the method of Paul and Sharma (2002). These were homogenized in an electric blender using sterilized distilled water. The extracts were filtered through two layers of moistened muslin cloth. The final volume was adjusted to 1000 ml with distilled water and the filtrate was centrifuged at 10,000 rpm for 20 min. The clear supernatant was collected and cold sterilized by vacuum filtration through G-S filter before use for in vitro test as well as for foliar application.

### **In vitro evaluation and foliar application of plant extract**

For *in vitro* evaluation of antifungal activities of the extracts, desired concentrations of 5.0, 10.0 and 15.0 per cent were obtained by adding appropriate amount of standard basic stock of plant extracts to PDA (Potato Dextrose Agar) in petri plates replicated thrice for each treatment. PDA without plant extract served as control. Each plate was inoculated with a 6 mm diameter mycelial disc taken from 6 day-old culture of *A.alternata* grown on PDA and incubated at 25 ±3°C. Radial growth of mycelia and spore formation were recorded after 5 and 15 days respectively. The percent inhibition of mycelial growth and spore formation over control was derived. Besides, cold sterilized crude plant extracts (5 ml) were added and mixed thoroughly with 45 ml sterilized Potato Dextrose Broth (PDB), while 5 ml distilled water was added in control set. The flasks were then inoculated with 5mm agar blocks with *A.alternata* and incubated at 28°C for 15 days. At the end of the incubation the mycelia were harvested and their dry weights were determined. Plant extracts were supplemented with Tween 80 prior to spraying on brinjal plants which was done using a hand driven sprayer. Control plants were sprayed with distilled water and Tween 80.

### **Inducing agents and their application on brinjal plant**

#### **Biocontrol agent**

*Trichoderma harzianum* (Biocontrol agent) was mass multiplied on carrier medium comprising of wheat bran and sand (1:1). This medium (500

g) was filled in each polythene bags, sterilized at 15 lbs pressure for 1 h for 2 consecutive days, inoculated with 4-6 days old pure culture of *T. harzianum* and incubated at 28°C for 10 days. During incubation, these bags were gently hand shaken to promote uniform sporulation over the carrier medium and avoid clusters. Addition of biocontrol agent in soil was done 10 days prior to inoculation of brinjal plants with *A. alternata*.

### Organic additives

Cow dung (100 g), chicken manure (100 g), goat manure (100 g) were mixed with 1 kg soil and this soil mixture were kept separately in earthenware pots. Neemcakes and oil cake were allowed to decompose separately for a week in a clay pot covered with polythene. After decomposition, 100 ml of decomposed neem cake and oil cake solution were diluted with distilled water and 10 ml of these solution were added in rhizosphere of each brinjal seedlings grown in earthenware pots prior to inoculation with the pathogen (*A.alternata* ).

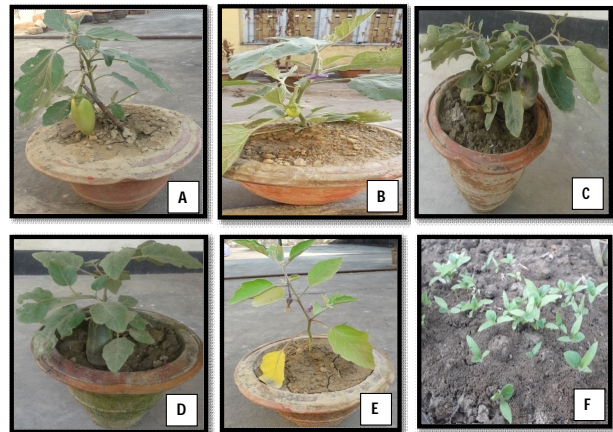
### Fungicide

Calixin (0.1%) mixed with Tween 80 in distilled water was sprayed four times at 7 day intervals on brinjal plants. The control plants were sprayed with distilled water mixed with Tween 80. Both treated and untreated plants were inoculated with *A.alternata* and disease assessment was made.

## RESULTS

### Varietal resistance test of brinjal against *A.alternata*

Pathogen (*A.alternata*) was used for artificial inoculation of three different varieties of brinjal plants grown in earthen pots. Twenty plants of each brinjal varieties were used for inoculation. Disease assessment was done on the basis of visual observation of symptoms and disease index was calculated and presented in Table 1. It showed that among three varieties of brinjal tested, local Lal gulab was found to be highly susceptible and hybrid-Sumo green long was most resistant while F1 Hybrid-long purple varieties of brinjal was found to be less susceptible than local Lal gulab.



**Fig.1 : A-F.**Brinjal plants following treatment with organic amendments.(A) Amended with cow dung manure.(B&E) Untreated healthy.(C) Amended with chicken manure.(D) Amended with goat manure.(F) Seedlings of hybrid- sumo green long grown in pot.

### In vitro evaluation of plant extracts against *Alternaria alternata*

Eleven plant extracts were screened *in vitro* against the pathogen (*A. alternata*) to examine the inhibitory effect on mycelial growth and spore formation. Efficacy of these plant extracts at three concentrations (5.0%, 10% and 15%) were evaluated on radial growth of *Altrnaria alternata*. Results (Table 2) revealed that highest inhibition (82.22%) on mycelial growth of *A. alternata* was evident on PDA mixed with extract of dried root of *Acorus calamus*. The other best treatments which showed significant reduction, were bulb of *Allium sativum* (73.33%) and leaves of *Azadirachta indica* (63.4%). Following the mycelial growth of *A.alternata* on PDA, spore formation were also recorded and inhibition per cent on number of spore formation were computed for each treatment against medium control and presented in Table 3. Significant inhibition of spore formation was evident in highest concentration (15%) of *Acorus calamus*, *Allium sativum*, *Azadirachta indica* and *Allium cepa*. Mycelial growth of the test fungus was inhibited by all the plant extracts (cold sterilized) supplemented in potato dextrose broth medium (Table 4). Activity of these extracts were evident in *Acorus calamus*, *Allium sativum*, *Allium cepa* and *Azadirachta indica* where significant reduction in mycelial dry weight of *A. alternata* were noticed.

**Table 1:** Varietal resistance test of *Alternaria alternata* on different Brinjal varieties

Brinjal Varieties	Disease index <sup>a</sup>		
	Days 7	after 15	inoculation 30
F <sub>1</sub> hybrid long purple	2.41 ± 0.01	3.03 ± 0.02	3.52 ± 0.06
Local Lalgulab	1.55 ± 0.05	3.32 ± 0.03	4.08 ± 0.04
Hybrid- Sumo green long	0.25 ± 0.04	1.06 ± 0.04	1.33 ± 0.05

<sup>a</sup> Results are an average of 25 inoculated plants

± Standard error

**Table 2:** Efficacy of plant extracts against of *Alternaria alternata*

Medium supplemented with Plant extracts	Concentration of plant extracts					
	5% Mycelial Growth (cm)	Inhibition (%)	10% Mycelial Growth (cm)	Inhibition (%)	15% Mycelial Growth (cm)	Inhibition (%)
Control	9.0	-	-	-	-	-
<i>Acorus calamus</i> ,dried root	4.9	45.56	2.8	68.89	1.6	82.22
<i>Allium cepa</i> bulb	4.9	45.0	4.6	48.2	3.8	57.8
<i>Datura stramonium</i> leaf	5.5	38.9	3.89	53.3	3.8	56.7
<i>Allium sativum</i> ,bulb	4.9	45.56	3.7	58.87	2.4	73.33
<i>Adhatoda indica</i>	5.7	35.67	4.7	47.78	4.4	51.1
<i>Argemone maxicana</i>	6.7	25.56	5.9	34.44	5.2	42.22
<i>Lantana camera</i> ,leaf	5.1	43.33	4.2	53.44	4.1	54.44
<i>Leucas aspara</i>	5.9	34.4	4.9	45.56	4.6	48.2
<i>Zinger officinale</i>	6.7	25.56	5.8	35.56	5.1	43.33
<i>Catharanthus roseus</i>	5.0	44.4	4.8	46.67	4.5	50.0
<i>Azadirachta indica</i> leaf	4.9	45.0	4.6	48.2	3.3	63.4

**Table 3:** Efficacy of plant extracts against spore germination of *A. alternata*

Plant extracts*	Spore germination (%)	Inhibition (%)
Control	91.7	-
<i>Acorus calamus</i> ,dried root	14.6	84.07
<i>Allium cepa</i> ,bulb	40.0	56.37
<i>Datura stramonium</i> leaf	44.0	52.01
<i>Allium sativum</i> , bulb	26.1	71.5
<i>Adhatoda indica</i>	46.0	49.8
<i>Argemone maxicana</i>	50.0	45.4
<i>Lantana camera</i> ,leaf	45.0	50.9
<i>Leucas aspara</i>	48.0	47.7
<i>Zinger officinale</i>	49.5	46.0
<i>Catharanthus roseus</i>	47.0	48.74
<i>Azadirachta indica</i> ,leaf	33.4	63.6

\*All the extracts were tested at 15%.

**Table 4:** Effect of cold sterilized aqueous leaf extracts on mycelial growth of *A. alternata*

Medium (PDB) supplemented with cold sterilized plant extracts	Average mycelial dry weight (mg)
<i>Acorus calamus</i> , dried root	73.0±0.03
<i>Allium cepa</i> bulb	98.0±0.02
<i>Datura stramonium</i> , leaf	10.01±0.04
<i>Allium sativum</i> , bulb	74.0±0.04
<i>Adhatoda indica</i>	150.4±0.04
<i>Argemone maxicana</i>	126.0±0.03
<i>Lantana camara</i> , leaf	120.0±0.02
<i>Leucas aspara</i>	140.4±0.06
<i>Zinger officinale</i>	155.0±0.05
<i>Vinca rosea</i>	201.0±0.02
<i>Azadirachta indica</i> , leaf	96.0±0.05
Medium (PDB) Control	262.0±0.07

± Standard Error; <sup>a</sup>Average of three replicate**Table 5:** Effect of application of *Trichoderma harzianum* and plant extract of *Acorus calamus* alone and in combination with organic amendments and fungicide on development of leaf spot of brinjal (long hybrid sumo-green) following challenge inoculation with *A. alternata*

Treatment	Disease index <sup>a,b</sup>
<i>Trichoderma harzianum</i>	2.53 + 0.04
Oil cake and chicken manure	4.32 + 0.05
<i>Acorus calamus</i> , root (aqueous extract)	3.4+ 0.04
<i>T. harzianum</i> with <i>Acorus calamus</i> roots (aqueous extract), oilcake and chicken manure	1.45+ 0.02
<i>T. harzianum</i> , Calixin (0.1%), <i>Acorus calamus</i> , root (aqueous extract) oil cake and chicken manure	0.84±0.04
Untreated Inoculated (UI)	6.25 ± 0.05

<sup>a</sup>Results are an average of 50 inoculated plants<sup>b</sup> 15 days after inoculation

± Standard error

**Table 6:** Growth promotion in Brinjal seedlings following soil amendment with neem cake and oil cake in *Alternaria alternata*

Brinjal variety	One month				Two months			
	Healthy		Infected		Healthy		Infected	
	Increase in height(cm)	Increase no. of leaves	Increase in height (cm)	Increase no. of leaves	Increase in height (cm)	Increase no. of leaves	Increase in height cm	Increase no. of Leaves
Hybrid-Sumo green long Untreated	5.0±0.4	4.0±0.7	2.0±0.8	7.0±0.6	5.0±0.4	5.0±0.5	4.0±0.6	3.0±0.4
Treated Neemcake	7.0±0.8	5.0±0.7	11.0±0.6	7.0±0.7	16.0±0.5	12.0±0.8	17.0±0.4	14.0±0.5
Oil cake	10.0±0.3	7.0±0.9	12.0±0.7	8.0±0.5	19.5±0.6	20.0±0.4	21.0±0.5	22.0±0.7
F <sub>1</sub> hybrid long purple Untreated	4.5±0.8	3.0±0.4	3.5±0.7	2.0±0.8	7.0±0.6	4.0±0.4	5.0±0.9	3.0±0.6
Treated Neem cake	8.0±0.5	4.0±0.4	9.0±0.3	14.0±0.5	8.0±0.7	12.0±0.4	12.0±0.3	7.0±0.6
Oil cake	10.0±0.6	7.0±0.4	11.0±0.6	8.0±0.7	20.5±0.4	20.±0.5	17.0±0.5	22.0±0.4

± Standard deviation, average of three replicates

**Table 7:** Growth promotion in brinjal seedlings by different organic components after inoculation with *Alternaria alternata*

Brinjal variety	One month				Two months			
	Healthy		Infected		Healthy		Infected	
Hybrid-Sumo green long	Increase in height (cm)	Increase no. of leaves	Increase in height (cm)	Increase no. of leaves	Increase in height (cm)	Increase no. of leaves	Increase in height (cm)	Increase no. of leaves
Untreated	4.0±0.8	5±0.4	3.5±0.8	3±0.5	8±.6	7.0±.04	4.5±.08	6±0
Treated Cow dung	11.5±.8	6±.09	9.0±.06	5±0.07	18±.04	16±.07	12.5±.05	10±.08
Goat manure	18±.09	12±.06	13±.08	10±.07	24±.04	50±.08	17±.05	19±.04
Chicken manure	16±.06	25±.09	15.5±.6	23±.08	40±.07	50±.05	22±.05	24±.09
F <sub>1</sub> hybrid Long purple Untreated	5±.04	4±.09	3±.06	3±.08	10±.05	8±.04	7±.09	5±.04
Treated Cow dung	9±.05	5±.06	8±.05	4±.07	20±.04	20±.08	18±.07	12±.08
Goat manure	20±.09	20±.06	18.5±.7	12±.04	26±.05	23±.08	23±.05	20±.08
Chicken manure	15±.07	10±.06	13.5±.8	8±.09	35±.04	28±.09	23.5±.05	25±.06

±Standard deviation, average of three replicates

### **Evaluation of inducing agents on development of leaf spot of brinjal**

Effect of application of *Trichoderma harzianum* and plant extract of *Acorus calamus* alone and in combination with organic amendments and fungicide on development of leaf spot of brinjal variety (hybrid-sumo green long) following challenge inoculation with *Altrnaria alternata* was evaluated (Fig 1 A-H). Under pot culture conditions, *Trichoderma harzianum* alone and in combination with oil cake, chicken manure and dried root of *Acorus calamus* provided effective management practices of leaf spot disease of in brinjal plants (Table 5). Combination with oil cake, chicken manure as organic additives showed highest disease index than biocontrol agent and plant extract alone. However, when all three combinations were integrated with fungicide (0.1% Kalinin), best effective management of brinjal plants against *A. alternaria* was noticed.

### **Growth promotion in brinjal seedlings**

Brinjal seedlings of two varieties (hybrid- Sumo green long and local Lalgulab) were grown in soil

amended with neem cake and oil cake separately. Each treatment consisted of 10 plants, in triplicate and the values are an average of 30 plants. Results were recorded after one-month interval and up to two months following the treatment of neem cake and oil cake and after inoculation with *Alternaria alternata*. Results (Table 6) revealed that the growth of brinjal seedlings had been increased following amendment with neem and oil cakes than those treated plants inoculated with *Alternaria alternata* in relation to untreated uninoculated brinjal seedlings as recorded after two months following treatment.

Similarly, seedlings of two brinjal varieties (F<sub>1</sub> hybrid long purple & hybrid- sumo green long) were grown in soil amended separately with cow dung, goat manure and chicken manure. Each treatment consisted of 10 plants, in triplicate and the values are an average of 30 plants. Results were recorded after one month interval up to two months following the treatment of organic components and after inoculation with *Alternaria alternata* has been observed that the growth of brinjal seedlings had been increased in treated

uninoculated than treated inoculated brinjal seedlings (Table 7). Among the three treatments with chicken manure gave very good and healthy growth of brinjal seedlings than cow dung and goat manure.

## DISCUSSION

Using an agricultural production, integrated plant disease management has been considered as a holistic approach which includes the rotational application of cultural, biological and chemical control methods, as well as the coordination and integration of various procedures for the purpose of controlling the damage due to disease (Chowdhury *et al.* 2019). In the present investigation, among eleven plant extracts tested, *Acorus calamus* (dried root) showed highest reduction in mycelial growth, inhibition of spore formation *in vitro*. Other best treatments, which showed significant reduction, were bulb extract of *Allium sativum* (73.33%) and leaf extract of *Azadiracchta indica* (63.4%). Maximum effectiveness of plant extracts was recorded at 15% concentration. The active chemical compound - asarone present in the *Acorus calamus* dried root may be the reason for inhibition of mycelial growth and spore production of *A.alternaria*. Mungkotnasawakul *et al.*(2002) have discussed the efficacy of *Acorus calamus* rhizome extract against the mycelial growth of *Alternaria* spp. Growth inhibition of *S. rolfsii* using leaf extract of *Azadiracta indica* and *Catharanthus roseus* (Bhagat, 2013) and root extract of *Acorus calamus*, leaf extract of *Agave americana* and bulb of *Allium sativum* (Bhagat, 2022) has also been described. Several other scientists have documented that plant extracts significantly inhibit the fungal growth because of their antifungal activity (Sab *et al.* 2014; Sana *et al.* 2016).

*Azadiracchta indica* leaf extract induces resistance in barley against leaf stripe disease (Paul and Sharma, 2002). Role of botanical plant extracts to control plant pathogens have been discussed by Choudhury *et al.* (2018). *C. roseus*, *D. esculentum* and salicylic acid were supplied in the field grown tea plants in order to manage of *Alternaria* Blight disease. These extracts may induce defence reactions in tea plants towards the foliar fungal pathogen (Das Biswas and

Chakraborty, 2020). The crude leaf extract of *Azadiracchta indica* exhibited better reduction in disease incidence and severity than organophosphate and strobilurin fungicides under field conditions against leaf blight of onion caused by *Stemphylium vesicarium* (Sharma *et al.* 2022)

*In vivo* trials with *Trichoderma harzianum* alone as well as in combination with neem cake, cow dung, aqueous extract of *Acorus calamus*, root and calixin (0.1%) provided marked reduction in collar rot disease of tomato and there by exhibiting integrated disease management (IDM) practices. Similar results were obtained by Sonali and Gupta (2004) when *T. viride* alone and in combination with neem oil, neem cake and deodar needles used in radial growth of *S. rolfsii* resulted in a total control of the disease. But repeated application of neem cake, oil cake with various combinations of cow dung, rabbit manure and chicken manure were found to be less significant. Management of chickpea root rot and collar rot against *S. rolfsii* by integration of biological and chemical seed treatment has also been demonstrated by Tiwari and Mukhopadhyay (2003). They observed that application of carboxymethyl cellulose (CMC) with *G. virens* powder (10<sup>9</sup> spores per g) in combination with vitavax provided maximum (81.9%) protection to the crop against chickpea root rot and collar rot pathogens in glasshouse. Chickpea seeds treated with GV powder + CMC + vitavax significantly increased seedling emergence (47.9%); final plant stand (85.8%) and grain yield (79.7%) which was statistically at par with the treatment GV powder + vitavax and GV suspension + vitavax in a sick plot. Upamanyu *et al.* (2002) reported the management of root rot and web blight caused by *Rhizoctonia solani*. They observed that *T. viride* showed the maximum tolerance to carboxin, tebuconazole and carbendazim followed by *T.virens*, *T. harzianum* when used in integrated disease management along with fungicides and oil cakes both under glass house and field conditions. Harish *et al.* (2004) working on control of brown spot of rice caused by *Helminthosporium oryzae* with 15 seed extracts under laboratory condition found that 10% rhizome extract of turmeric (*Curcuma longa*) showed maximum inhibition of mycelial growth and spore germination.



Integrated management strategies of seedling blight disease of tea caused by *Sclerotium rolsii* ( Bhagat and Chakraborty, 2015) and management of Fusarial wilt of tomato caused by *Fusarium oxysporum* f sp. *lycopersici* by integration of bio-control, fungicide, organic amendments and plant extract have been elucidated (Bhagat, 2019).

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