Integrated management of Collar rot of Tomato caused by *Sclerotium rolfsii*

INDRAMANI BHAGAT

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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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INDRAMANI BHAGAT
Department of Botany, Post Graduate Campus,Tribhuvan University, Biratnagar.Nepal

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Twenty-five plant extracts were screened *in vitro* against the *Sclerotium rolfsii*, causing Collar rot of tomato. Among these, *Acorus calamus* dried root showed highest reduction (80%) in mycelial growth and 95.6% inhibition of sclerotial formation. 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 10% concentration.The other best treatments, which showed significant reduction, were leaf extract of *Agave americana* (68%) and bulb of *Allium sativum* (67%).Effective integrated management practices were developed in tomato plants against *S. rolfsii* using neem cake, cow dung, aqueous leaf extract of *Acorus calamus* dried root , bio-control agent like *Trichoderma harzianum* and 0.1% calixin.

Key words: *Selcrotium rolfsii*, plant extract, *Acorus calamus, Agave americana, Allium sativum, Nerium indicum.*

INTRODUCTION

Tomato (*Lycopersicum ecsulentum* Miller) is the world's second largest vegetable crop and known as protective food because of its special nutritive value and its wide spread production. It is a rich source of minerals, vitamins, organic acids and dietary fibers and also regarded as an anticancer food. Lycopene is the main ingredient that helps to prevent cancer. Fungal, bacterial and viral diseases are serious among the important limitations in tomato crop production. Of these, Collar rot caused by *Sclerotium rolfsii* is the most destructive diseases in tomato. Effective and efficient management of crop diseases is generally achieved by use of synthetic pesticides. These are known to pollute the environment, soil and water, besides causing 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). 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 for most effective antifungal activities *in vitro* among various plant extracts against *Sclerotium rolfsii* and development for its integrated management strategies using selected organic additives and fungicide for Collar rot disease of tomato plants

MATERIALS AND METHODS

Plant material

Three tomato varieties F_1 Hybrid Arjuna, Pahuza S-22 and Bahuwa F_1 Hybrid were grown in pots and were used for experimental purpose.

Fungal cultures

Virulent culture of pathogen (*Sclerotium rolfsii*) and potential biocontrol agent (*Trichoderma*

*Correspondence: drimbhagat@yahoo.com

harzianum) were obtained from Immuno-Phytopathology Laboratory, Department of Botany, North Bengal University. After completion of Koch's postulate in tomato plant, the reisolated pathogen was identified as *Sclerotium rolfsii* Sacc. (*Corticium rolfsii* Curzi) by the Global Plant Clinic, Diagnostic and Advisory Service, CABI Bioscience UK which was used in the present investigation.

Inoculum preparation of fungal pathogen

Fungal pathogen (*S. rolfsii*) was grown in sand maize meal (3:1) medium for 7 days at 28^0 C. The inoculum was mixed with sterile soil at the ratio of 1:8. Fungus soil mixture (100 gm) were mixed with the top soil of earthen pots.

Inoculation technique and disease assessment

Tomato seedlings were planted in earthen pots containing 1 kg soil and allowed to be established. Regular watering was done for two weeks and then 100 g of pathogen inoculum was added carefully in the rhizosphere of 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, 10 and 15 days of inoculation. Disease intensity was assessed as rot index, depending on both underground and above ground symptoms on a scale of 0-6 as follows; Rot index: 0- no symptoms; 1 – small roots turn brownish and start rotting; 2 – leaves start withering and 20-40% of roots turn brown; 3 – leaves withered and 50% of roots affected; 4 – shoot tips also start withering, 60-70% roots affected; 5 – shoots withered with defoliation of lower withered leaves, 80% root affected; 6 – whole plants die, with upper withered leaves still remaining attached, roots fully rotted.

Preparation of plant extract

Aqueous extracts of 25 selected plant parts such as rhizomes of *Acorus calamus* and *Curcuma longa* – rhizomes, bulbs of *Allium cepa and Allium sativum* and leaf samples of *Azadirachta indica, Agave americana, Calotopis gigantea, Clerodendron infortunatum, Eucalyptus canalduneasis, Eupatorium odoratum, Lantana camera, Lawsonia inermis, Leucas aspara, Nerium indicum, Ocimum sanctum, Parthenium hysterophorus, Piper betal, Polyalthia longifolia, Ricinus communis, Sesamum indicum, Sida acuta, Solamum melongena, Sorghum vulgare, Tagetes petula, Vinca rosea* 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 2.5, 5.0 and 10.0 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 5 day-old culture of *S. rolfsii* grown on PDA and incubated at 25 ± 2^0C. Radial growth of mycelia and sclerotia formation were recorded after 5 and 15 days respectively. The percent inhibition of mycelial growth and sclerotia formation over control was derived. Besides, cold sterilized crude plant extracts (5 ml) were added and mixed thoroughly with 45 ml sterlized potato dextrose broth (PDB), while 5 ml distilled water was added in control set.The flasks were then inoculated with 4 mm agar blocks with *S. rolfsii* and incubated at 28^0C for 10 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 tomato 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 tomato 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

lb pressure for 1 h for 2 consecutive days, inoculated with 4-6 days old pure culture of *T. harzianum* and incubated at 28 ^{0}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 tomato plants with *Sclerotium rolfsii*.

Organic additives

Cow dung (100 g) was mixed with 1 kg soil and this soil mixture were kept in earthenware pots. Neem cakes were allowed to decompose separately for a week in a clay pot covered with polythene. After decomposition, 100 ml of decomposed neem cake solution was diluted with distilled water and 10 ml of this solution was added in rhizosphere of each tomato seedlings grown in earthenware pots prior to inoculation with the pathogen (*S. rolfsii*).

Fungicide

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

RESULTS

Varietal resistance test of tomato against Sclerotium rolfsii

Pathogen (*Sclerotium rolfsii*) was used for artificial inoculation of three different varieties of tomato plants grown in earthen pots. Twenty plants of each tomato 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 revealed that among three varieties of tomato tested, Pahuza S-22 was found to be highly susceptible and F_1 Hybrid Arjuna was most resistant while Bahuwa F_1 Hybrid of tomato was found to be less susceptible than Pahuza S-22.

In vitro evaluation of plant extracts against Sclerotium rolfsii

Twenty five plant extracts were screened *in vitro* against the pathogen (*S.rolfsii*) to examine the inhibitory effect on mycelial growth and sclerotial germination. Efficacy of these plant extracts at three concentrations (2.5%, 5% and 10%) were evaluated on radial growth of *Sclerotium rolfsii.* Results (Table 2) revealed that highest inhibition (80%) on mycelial growth of *S. rolfsii* was evident on PDA mixed with extract of *Acorus calamus* (rhizome). Other treatments which showed significant reduction, were leaf extract of *Agave americana* (68%) and bulb of *Allium sativum* (67%). Following the mycelial growth of *S. rolfsii* on PDA, sclerotia formation were also recorded and inhibition per cent on number of sclerotia formation were computed for each treatment against medium control and presented in Table 3. Significant inhibition of sclerotia formation was evident in highest concentration(10%) of *Acorus calamus, Curcuma longa, Azadirachta indica* and *Vinca rosea.*

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, Agave americana, Azadirachta indica* and *Vinca rosea* where significant reduction in mycelia dry weight of *S. rolfsii* were noticed.

Evaluation of inducing agents on development of Collar rot of tomato

Effect of application of *Trichoderma harzianum* and plant extract of *Acorus calamus* alone and in combination with organic amendments and fungicide on development of collar rot of tomato (Pahuza S-22) following challenge inoculation with *Sclerotium rolfsii* was evaluated (Fig 1 A-F).

Under pot culture conditions, *Trichoderma harzianum* alone and in combination with neemcake,cow dung and *Acorus calamus* provided effective management practices of collar rot intomato plants (Table 5). Combination with neem cake and cow dung 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% Calixin) , best effective management of tomato plants against *S. rolfsii* was noticed.

Table 1: Varietal resistance test of tomato against *Sclerotium rolfsii*

Tomato Varieties	Disease index [a, b]		
	7	10	15
Pahuza S-22	2.52 ± 0.03	4.91 ± 0.02	5.41 ± 0.01
Bahuwa F_1 Hybrid	1.64 ± 0.02	3.11 ± 0.06	3.48 ± 0.05
F_1 Hybrid Arjuna	0.49 ± 0.05	0.53 ± 0.05	0.67 ± 0.02

[a] Results are an average of 20 inoculated plants
[b] Days after inoculation
± Standard error

Table 2: Efficacy of plant extracts on radial growth of *S. rolfsii* on solid medium

Medium supplemented with plant extracts		Mycelial growth (cm) Concentration (%)		
		2.5	5	10
	Acorus calamus	8.6 (4)	7.2(19)	1.8 (80)
Rhizome	*Curcuma longa*	8.6(4)	8.5(6)	7.3(18)
Bulb	*Allium cepa*	8.7(3)	8.2(9)	7.8(13)
	Allium sativum	3.6(60)	3.06(66)	2.9(67)
Leaf	*Agave americana*	8.7(3)	7.7(14)	2.9(68)
	Azadirachta indica	8.5(5)	7.4(12)	7.3(18)
	Calotopis gigantea	8.5(5)	7.912)	6.9(23)
	Clerodendroninfortunatum	6.9(23)	7.7(14)	7.2(20)
	Eucalyptus canalduneasis	7.0(22)	5.6(38)	4.9(45)
	Eupatorium odoratum	8.1(10)	8.0(11)	6.3(30)
	Lantana camera,	7.2(20)	6.6(26)	5.0(44)
	Lawsoniainermis,	6.8(24)	6.3(30)	5.4(39)
	Leucas aspara	8.1(10)	7.9(12)	7.2(20)
	Nerium indicum	7.9(12)	5.9(34)	4.1(54)
	Ocimum sanctum	8.1(10)	7.2(20)	6.6(26)
	Parthenium hysterophorus	8.5(6)	7.7(14)	7.1(21)
	Piper betal	8.5(6)	8.0(11)	6.7(25)
	Polyalthia longifolia	8.5(6)	7.3(18)	6.3(29)
	Ricinus communis	8.6(4)	8.4(7)	6.6(26)
	Sesamum indicum	7.8(13)	7.6(16)	7.1(21)
	Sida acuta	8.8(2)	8.5(6)	8.2(9)
	Solamum melongena	8.6(4)	8.0(11)	6.8(24)
	Sorghum vulgare	8.4(7)	7.0(22)	6.3(29)
	Tagetes petula	7.8(13)	7.6(16)	5.3(42)
	Vinca rosea	8.6(4)	8.6(4)	8.2(8)
Medium (PDA) Control		9.0		

Values in parenthesis denotes inhibition per cent of mycelia growth of *S. rolfsii* in relation to medium control

Table 3: Effect of plant extracts on sclerotia formation of *S. rolfsii*

Medium supplemented with plant extracts		Number of sclerotia formed		
		Concentration (%)		
		2.5	5	10
	Acorus calamus	72 (21)	14(84)	4 (96)
Rhizome	*Curcuma longa*	18(80)	18(80)	6 (93)
Bulb	*Allium cepa*	23(75)	9(90)	13(86)
	Allium sativum	68(26)	66(28)	32(65)
Leaf	*Agave americana*	70(83)	14.5(84)	12(88)
	Azadirachta indica	20(87)	18(80)	5 (94)
	Calotopis gigantea	9(90)	13(85)	23(75)
	Clerodendron infortunatum	84(4)	19(79)	13(86)
	Eucalyptus canalduneasis	44(47)	45(51)	38(58)
	Eupatorium odoratum	30(67)	25(72)	11(88)
	Lantana camera	54(41)	44(52)	26(71)
	Lawsonia inermis	45(50)	39(57)	30(67)
	Leucas aspara	22(76)	20(78)	12(86)
	Nerium indicum	54(41)	45(50)	34(62)
	Ocimum sanctum	42(54)	26(72)	20(87)
	Parthenium hysterophorus	22(76)	21(77)	14(84)
	Piper betal	31(66)	25(72)	11(88)
	Polyalthia longifolia	48(47)	24(68)	18(80)
	Ricinus communis	72(22)	26(72)	7 (92)
	Sesamum indicum	21(77)	16(83)	12(87)
	Sida acuta	14(85)	9(90)	6(93)
	Solamum melongena	24(74)	21(77)	11(88)
	Sorghum vulgare	29(68)	22(76)	9(90)
	Tagetes petula	41(55)	35(62)	15(84)
	Vinca rosea	62(32)	8(91)	4(96)
Medium (PDA) Control		91		

Values in parenthesis denotes per cent inhibition of sclerotia formation of *S. rolfsii* in relation to medium control

Fig. 1 (A-F). Tomato plants (Pahuza S-22) following treatment with biocontrol agent and organic additives (A) Untreated healthy plant; (B) Untreated inoculated with *S. rolfsii* (C) Neem cake and cow dung amended soil inoculated with *S. rolfsii* (D) Treated with *T. harzianum* (E) Soil amended with cow dung manure, neem cake , treated with *Acrous calamus and T. harzianum* and inoculated with *S. rolfsii* (F) Treated with *T. harzianum* and inoculated with *S. rolfsii*

Table 4: Effect of cold sterilized aqueous leaf extracts on mycelial growth of *S. rolfsii*

Medium (PDB) supplemented with cold sterilized plant extracts		Average mycelial[a] dry weight (mg)
Rhizome	*Acorus calamus*	86.0 ± 1.53
	Curcuma longa	271.4 ± 2.02
Bulb	*Allium cepa*	350.0 ± 1.02
	Allium sativum	243.0 ± 1.81
Leaf	*Agave americana*	88.0 ± 3.03
	Azadirachta indica	85.0 ± 2.04
	Calotopis gigantea	254.8±3.06
	Clerodendroninfortunatum	220.0±2.83
	Eucalyptus canalduneasis	170.0 ±1.54
	Eupatorium odoratum	231.7 ±3.05
	Lantana camera,	185.4 ±1.49
	Lawsoniainermis,	100.0 ± 3.57
	Leucas aspara	264.8 ±2.07
	Nerium indicum	183.0 ± 1.09
	Ocimum sanctum	195.0 ± 3.12
	Parthenium hysterophorus	284.7 ± 3.95
	Piper betal	248.2 ± 2.08
	Polyalthia longifolia	235.0 ± 1.93
	Ricinus communis	240.0 ± 2.34
	Sesamum indicum	110.0 ± 1.09
	Sida acuta	301.2 ± 1.58
	Solamum melongena	290.0 ± 3.07
	Sorghum vulgare	235.0 ± 2.76
	Tagetes petula	290.0 ± 1.88
	Vinca rosea	105.0 ± 1.22
Medium (PDB) Control		361.9 ± 2.09

± Standard Error; [a]Average of three replicates

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 collar rot of tomato (Pahuza S-22) following challenge inoculation with *Sclerotium rolfsii*

Treatment	Disease index[a,b]
Trichoderma harzianum	2.43 ± 0.02
Neem oil cake and cow dung	4.22 ± 0.06
Acorus calamus, root (aqueous extract)	3.25 ± 0.05
T. harzianum with *Acorus calamus, root* (aqueous extract), Neem oil cake and cow dung	1.39 ± 0.01
T. harzianum, Calixin (0.1%), *Acorus calamus, root* (aqueous extract), Neem oil cake and cow dung	0.78 ± 0.06
Untreated Inoculated (UI)	5.98 ± 0.05

[a] Results are an average of 50 inoculated plants
[b] 15 days after inoculation
± Standard error

DISCUSSION

Keeping in view the agroecological systems and the overall situation of 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 study, among twenty-five plant extracts tested, *Acorus calamus* (rhizome) showed highest reduction in mycelial growth, inhibition of sclerotial formation *in vitro*. Next best treatments, which showed significant reduction, were leaf extract of *Agave americana* (68%) and bulb of *Allium sativum* (67%).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 10% concentration. The active chemical compound â - asarone present in the *Acoruscalamus* dried root may be the reason for inhibition of mycelial growth and sclerotial production of *S. rolfsii*. 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 leaf extract of Datura, lemon grass, onion bulb, Zinger rhizome (Bana and Chandra, 2020) 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).

In vivo trials with *Trichoderma harzianum* alone as well as in combination with neem cake, cow dung, aqueous extract of *Acrous 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 treatmenthas also been demonstrated by Tiwari and Mukhopadhyay (2003).They observed that application of carboxymethyl cellulose (CMC) with *G. virens* powder (109 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. Integrated management strategies of seedling blight disease of tea caused by *Sclerotium rolfsii* (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(Bhagat, 2019) have been elucidated. *Azadirachta 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). Foliar application of aqueous leaf extracts of *Azadirachta indica*, *Catharanthus roseus* and *Diplazium esculentum* on tea plants increased level of defense enzymes such as phenylalanine ammonia lyase, chitinase and β-1,3-glucanase following challenge inoculation with *Alternaria alternata,* which support the hypothesis that plant extract may induce defense reactions in tea plants towards the foliar fungal pathogen (Das Biswas and Chakraborty, 2020).The crude leaf extract of *Azadirachta 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). The ability to induce resistance and utilize it optimally in agriculture depends on fundamental knowledge of biochemical changes and on the specificity and compatibility of the signaling systems that regulate their expression.

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