

Short communication

Antifungal activities of organic preparations, botanicals and non-hazardous chemicals against *Rhizoctonia solani* Kuhn causing sheath blight of rice

S.R. Karthika¹, A. Sajeena*², V.K. Girija¹, Jacob John² and G. Heera¹

¹College of Agriculture, Vellayani,, Kerala Agricultural University, Thiruvananthapuram 695522, Kerala, India ; ²Integrated Farming System Research Station, Karamana, Thiruvananthapuram 695002, Kerala, India.

Received 4 June 2017; received in revised form 16 June 2017; accepted 30 June 2017

Abstract

The study was undertaken to evaluate the antifungal potential of selected organic preparations, botanicals and non-hazardous chemicals under *in vitro* against *Rhizoctonia solani* Kuhn causing sheath blight in rice. A total of twenty treatments were tested for their efficacy in inhibiting the mycelial growth of *R. solani*. Among the treatments, six treatments viz., garlic extract (10%), fermented weed (*Setaria barbata*) extract (100%), fermented egg-lemon juice extract (10%), potassium silicate (1%), lime solution (12.5%) and panchagavya (5%) showed cent percent inhibition of *R. solani* in potato dextrose agar medium. Further, dipping the sclerotia for different time intervals in the most effective six treatments, fermented egg-lemon juice extract (10%), fermented weed extract (100%), lime solution (12.5%) and panchagavya (5%) completely inhibited the mycelial regeneration from sclerotia at 24 h after dipping and garlic extract (10%) could inhibit only 72 hours after dipping and potassium silicate (1%) resulted only in less inhibition (27.28%) even after 72 hours of dip. Thus, the study revealed the possibility of eco-friendly management of rice sheath blight disease using organic preparations or botanicals or non-hazardous chemicals.

Keywords: Fermented egg-lemon juice extract, Fermented weed (*Setaria barbata*) extract, Potassium silicate, Rice sheath blight

Sheath blight disease caused by *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is a major threat in rice cultivation (Khan and Sinha, 2006). The indiscriminate and disproportionate use of pesticides might lead to residues in food chain exerting harmful effects in human and animals (Chahal et al., 2016). Hence, the development of novel and safe plant protectants which interfere with the fungal pathogenicity factors is the need of the hour (Srivastava and Singh, 2011). Zambrowicz et al. (2012) reported that many of the proteins present in egg such as lysozyme, ovotransferin, ovomucin, flavoprotein, avidin etc.

are known to possess antimicrobial properties. The juice of ripe as well as unripe citrus fruits could inhibit *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli* (Sarmah and Kumari, 2013). According to Aderogba et al. (2014), invasive and weedy species may serve as a source of biologically active extracts or compounds with application in plant protection. They reported that the acetone crude extract of *Pseudognaphalium luteoalbum* had strong antifungal activity against *Aspergillus niger*, *Aspergillus parasiticus*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Penicillium expansum*, *Penicillium janthinellium*, *Phytophthora*

*Author for correspondences: Phone: 91-4712343586, Email: sajeenamanjima@gmail.com

nicotiana, *Pythium ultimum* and *Trichoderma harzianum*. Two antifungal compounds, viz., hispidulin-7-*o*-glucopyranoside and stigmastero-3-*o*-beta-glucopyranoside were isolated from the weedy species. Ten per cent clove extract of *Allium sativum* was reported to be the most effective treatment in suppressing the mycelial growth of seed borne fungi of green gram, viz., *Fusarium oxysporum* (62.8%), *Aspergillus niger* (62.1%) and *R. solani* (61.2%) (Swami and Alane, 2013). Anees (2014) reported that panchagavya at 2.5, 5 and 10 per cent concentrations completely inhibited the mycelial growth of *Pythium aphanidermatum*. The botanicals viz., *Chromolaena odorata* and *Ocimum sanctum* as well as the neem based formulation, nimbidine were found to be effective against sheath blight disease (Saifunneesa and Niza, 2001). Cow urine at 100 per cent concentration resulted in 87.53 per cent reduction in mycelial growth of *R. solani* causing black scurf of potato (Sirari et al., 2015). The mycelial growth of *R. solani* causing leaf blight disease of amaranthus was completely inhibited *in vitro* by fermented egg - lemon juice extract as well as lime solution, followed by fermented tapioca - rind extract, fermented weed (*Setaria barbata*) extract, fermented papaya leaf extract, fish amino acid as well as turmeric powder – baking soda mixture (Sajeena et al., 2015). Calcium carbonate (250 μ g ml⁻¹) nano particles showed good antibacterial effect and after 16 hours, the *Agrobacterium tumefaciens* totally diminished (Ataee et al., 2011). Bekker et al. (2009) reported that soluble potassium silicate inhibited the mycelial growth of *Phytophthora cinnamomi* and *P. capsici* at all the concentrations (20–80 ml L⁻¹) tested. The concept of ecofriendly and safe disease management has gained wide importance. The present study is a pioneer attempt to evaluate the antifungal potential of selected commonly available, organic preparations, botanicals and non-hazardous chemicals for the *in vitro* management of rice sheath blight fungus.

Rice plants showing typical symptoms of sheath blight disease were collected from seven rice

growing tracts of Thiruvananthapuram district and isolations were made from the seven sheath blight infected samples (Adipathi et al., 2013). Pathogenicity tests of the seven isolates obtained were conducted. Healthy rice plants were transplanted into UV stabilized 600 gauge, 150 μ grow bags (40 cm x 24 cm x 24 cm) which were filled with sandy clay loam soil (10 kg) having a pH of 5.5 with the water level maintained at five cm. Rice plants at active tillering stage (45 days after sowing (DAS) were artificially inoculated in the leaf sheath by inserting five mm mycelial bits of seven days old culture of each isolate along with sclerotia. The inoculated portion of the sheath was then covered with a thin layer of moist cotton. Rice plants inoculated with PDA culture discs alone (without the pathogen) were maintained as the control for each isolate. The artificially inoculated plants were covered with finely perforated polythene covers to maintain humidity in order to induce sheath blight symptom development. All the inoculated plants were observed daily for the appearance of typical symptoms of sheath blight. The pathogen isolates were re-isolated when the characteristic symptoms of the disease appeared on the inoculated leaf sheaths and the pathogenicity confirmed.

Virulence rating (Banniza et al., 1996) was done to determine the most virulent isolate of the pathogen among seven isolates collected from the different rice growing tracts. Each of the seven fungal isolates was artificially inoculated on potted rice plants at 45 days after transplanting. Rice plants inoculated with PDA culture discs alone (without the pathogen) were maintained as the control for each isolate. The observations on symptom development, relative lesion height (RLH) on the 5th, 7th, 10th and 15th DAI as well as the total number of disease affected tillers produced by each isolate were recorded. The RLH (Sharma et al., 1990) was calculated using the formula;

$$\text{RLH} = (\text{Lesion height in cm} / \text{Plant height in cm}) \times 100$$

Table 1. Organic preparations, botanicals and non-hazardous chemicals used against *R. solani*

Treatment	Description
T ₁	Fermented tapioca (<i>Manihot esculenta</i> L.) leaf and rind (1:1) extract in cow's urine diluted in water (1:5)
T ₂	Fermented papaya (<i>Carica papaya</i> L.) leaf in cow's urine diluted in water (1:1:5)
T ₃	Fermented weed (<i>Setaria barbata</i> L.) extracts (100%)
T ₄	Neem cake (500 kg ha ⁻¹)
T ₅	Fish amino acid (5%)
T ₆	Fermented egg – lemon juice extract (10%)
T ₇	Panchagavya (5%) (KAU, 2009)
T ₈	Cow dung supernatant (10%)
T ₉	Biogas slurry (10%)
T ₁₀	Turmeric (<i>Curcuma longa</i> L.) + baking soda (4:1) mixture (5g L ⁻¹ water)
T ₁₁	Turmeric (<i>Curcuma longa</i> L.) + baking soda (4:1) mixture (5g L ⁻¹ rice gruel water (kanjivellam))
T ₁₂	Turmeric (<i>Curcuma longa</i> L.) + baking soda (4:1) mixture (5g L ⁻¹ cowdung supernatant)
T ₁₃	Potassium silicate (1%) in sterile water
T ₁₄	Lime solution (12.5%)
T ₁₅	Jeevamruth (10%)
T ₁₆	Diluted cow's urine (10%)
T ₁₇	Neem (<i>Azadiracta indica</i> . A. Juss.) oil (2%)
T ₁₈	Garlic (<i>Allium sativum</i> L.) extract (10%)
T ₁₉	Tulsi (<i>Ocimum sanctum</i> L.) extract (10%)
T ₂₀	Control

The morphological characters of the pathogen were studied (Gonzalez et al., 2006) by preparing slides stained with cotton blue and observing under 45X and 100X magnification of Leica DM 750. The cultural characteristics of the pathogen were studied (Debbarma and Dutta, 2015) by growing them on PDA medium and observing the mycelial characters such as nature of mycelial growth, colour of the colony, texture and sclerotial characters such as the days for pinhead formation, the number and average size of sclerotia and the distribution pattern of the sclerotia of each isolate. The most virulent isolate was identified and confirmed at NFCCI (National Fungal Culture Collection of India), Pune.

Selected indigenous organic preparations, botanicals and non-hazardous chemicals were tested for their potential in inhibiting the mycelial growth of *R. solani* under *in vitro* conditions. The treatments selected and the methods of preparation are detailed in table 1. Fermented tapioca leaf (mature leaves) and tapioca rind extract and fermented papaya leaf extract (mature leaves) were prepared in cow's urine

diluted in water (1:5) (Sajeena et al., 2015). The common weed *Setaria barbata* (Mary grass/ Maize grass) was used for the preparation of fermented weed extract (Sajeena et al., 2016). Two and a half kg of the weed *viz.*, *Setaria barbata* (mary grass/ corn grass) was weighed, washed thoroughly to remove soil and dirt and was cut into small pieces. Twenty gram each of salt powder, tamarind (pulp) and powdered jaggery (sugarcane product) were added to the cut bits of the weed taken in a container and the mixture was diluted using 10 L of water. The mixture was stirred once daily and was kept for fermentation for a period of 21 days. The fermented preparation was filtered through muslin cloth and used directly.

Fish amino acid was prepared by adopting the method described by Weinert et al. (2014). Sardine fish and jaggery was used for the preparation of fish amino acid. One kg of fresh sardine fish (*Sardina pilchardus*) was cut into small pieces. One kg of jaggery (sugarcane product) was powdered. The cut pieces of fish and powdered jaggery (1:1

Table 2. Lesion development by *R. solani* isolates on artificial inoculation *in vivo*

Isolate	Days for symptom development	Relative Lesion Height (%)				Days after inoculation (DAI)
		No. of tillers affected				
		5	7	10	15	
I ₁ (Chenkai)	3	2.57 ^c	4.67 ^c	5.97 ^c	7.33 ^c	3.00 ^b
I ₁ (Attingal)	3	9.20 ^a	15.70 ^a	19.03 ^a	20.97 ^a	4.67 ^a
I ₂ (Punchakari)	3	0.93 ^d	1.67 ^{de}	3.30 ^{cd}	5.17 ^c	2.33 ^b
I ₃ (Nagaroor)	3	1.90 ^{cd}	3.37 ^{cd}	4.77 ^{cd}	6.50 ^c	2.33 ^b
I ₄ (Vellayani)	3	2.20 ^c	3.57 ^{cd}	4.33 ^{cd}	5.60 ^c	3.00 ^b
I ₅ (Karode)	7	0.00 ^e	0.23 ^e	4.93 ^{cd}	2.17 ^d	1.33 ^c
I ₆ (Karamana)	3	5.03 ^b	11.13 ^b	13.67 ^b	16.07 ^b	3.00 ^b
Control	0	0.0	0.0	0.0	0.0	0.0
CD (0.05)	-	1.04	2.63	5.08	2.27	0.99
SE _m (±)	-	0.28	0.72	1.38	0.62	0.27

Treatments with same alphabets do not differ significantly. All values are average of 3 replications

ratio on w/w basis) were filled in a container with layers, one above the other. The container was covered tightly using a muslin cloth for provision for air circulation. The mixture was kept undisturbed for 21 days. It was filtered through muslin cloth and used at the rate of five per cent concentration for the inhibition studies.

Fermented egg-lemon juice extract was prepared using hen eggs, lemon juice and powdered jaggery (Sajeena et al., 2016). Twelve raw eggs were taken in a container. Lemon juice was squeezed and poured over the eggs such that the eggs were immersed in the juice. This preparation was kept undisturbed for ten days. On the tenth day, 500 g of powdered jaggery (sugarcane product) was added to the mixture and stirred thoroughly. This was kept undisturbed for ten more days. The preparation was

ready for use after 21 days. It was filtered through muslin cloth and used at the rate of ten per cent concentration for further studies.

Panchagavya, an organic preparation was prepared as per the *ad hoc* package of practices recommendations for organic farming (KAU, 2009). Cow dung supernatant and biogas slurry were prepared as described in the *ad hoc* recommendations of Organic Package of Practices (KAU, 2009). Turmeric powder was mixed with baking soda in 4:1 ratio (w/w basis) in 1 litre of water, rice gruel water (1 litre) and cowdung supernatant (1 litre) (Bhadrasree, 2007). Jeevamruth was prepared by adopting the method described by Chadha et al. (2012). Fresh cow dung (10 kg), fresh cow urine (10 L), powdered jaggery (sugarcane product) (2 kg), pulse (green gram) flour (2 kg) and

Table 3. Morphological and cultural characteristics of *R. solani* isolates

Isolate	Mycelial characteristics	Sclerotial characteristics	Mycelial growth (cm)			Days for growth completion	Days for pinhead formation	Number (DAI)	Average Size (mm)	Distribution pattern
	Colour	Texture	Days after inoculation							
	1	2	3							
I ₁	Dark brown	Fluffy	2.93 ^b	7.23 ^b	9	3	4	26.00 ^{cd}	2.13 ^a	Scattered
I ₂	Light brown	Cottony	3.60 ^a	8.26 ^a	9	3	4	59.33 ^b	2.11 ^a	Scattered
I ₃	Light brown	Cottony	1.83 ^c	5.06 ^d	9	3	4	27.00 ^{cd}	1.99 ^{ab}	Central
I ₄	Light brown	Cottony	2.56 ^b	6.33 ^c	9	3	4	34.66 ^c	1.91 ^{bc}	Scattered
I ₅	Light brown	Fluffy	2.96 ^b	7.33 ^b	9	3	4	24.00 ^d	1.76 ^c	Scattered
I ₆	White	Cottony	3.00 ^b	5.96 ^c	9	3	5	104.66 ^a	0.98 ^d	Scattered
I ₇	Light brown	Cottony	2.96 ^b	8.23 ^a	9	3	4	53.66 ^b	2.01 ^{ab}	Central
CD (0.05)	-	-	0.53	0.61	NS	NS	NS	9.03	0.164	-
SE _m (±)	-	-	0.14	0.16	-	-	-	2.43	0.04	-

a hand full of fertile soil (soil collected from cultivated field without any accumulation of chemical fertilizers/pesticides) were taken in a vessel and mixed with 20 L of water. The mixture was filtered through muslin cloth after one week and used at the rate of ten per cent concentration for the *in vitro* studies. Fresh cow urine was diluted ten times with water (Sajeena et al., 2015) and used directly for the *in vitro* inhibition studies. The commercial neem oil @ 2 per cent concentration was prepared as described in the adhoc recommendations of Organic Package of Practices (KAU, 2009). Garlic (*Allium sativum*) extract was prepared as described by Kumar and Tripathi (2012). Basil/Tulsi (*Ocimum sanctum*) 10 per cent extract was prepared as detailed by Saifunneesa and Niza (2001). Potassium silicate with 25 per cent silica and seven per cent potassium was used at the rate of one per cent for the *in vitro* inhibition studies (Devi and Nayar, 2016). Lime @ 12.5 per cent concentration was used for the *in vitro* inhibition studies against *R. solani* (Sajeena et al., 2016).

In vitro evaluation of different treatments in inhibiting the mycelial growth of *R. solani* was done by poisoned food technique (Nene and Thapliyal, 1979). The per cent inhibition of the mycelial growth of *R. solani* over control was calculated using the formula (Vincent, 1927) as follows

$$I = \frac{C-T}{C} \times 100$$

where I is the percentage inhibition, C is the growth (cm) of the fungus in control plates and T is the growth (cm) of fungus in treatment amended plates. Effect of dipping the sclerotia in selected treatments for different time intervals was tested under *in vitro*. The sclerotia collected from seven days old culture plates of *R. solani* were dipped in the treatments separately for different time intervals viz, 24, 48 and 72 hours (Bhadrasree, 2007). The sclerotia, after dipping for each fixed time interval were inoculated at the centre of the Petri plate with solidified PDA medium using sterile forceps. The mycelial regeneration from the sclerotia as well as the per

cent reduction of the mycelial regeneration from sclerotia over control was measured as mentioned above.

Sheath blight affected rice plants were collected from seven different rice growing tracts of Thiruvananthapuram district viz., Chenkal, Attingal, Punchakari, Nagaroor, Vellayani, Karode and Karamana. Pathogenicity test of each isolate on artificial inoculation revealed that typical sheath blight symptoms were produced by each isolate on the artificially inoculated plants. The symptoms initially appeared as circular, oblong or ellipsoid, greenish grey, water soaked lesions on the leaf sheath near water level. The lesions enlarged, became oblong and irregular in outline. Centre of the lesions later turned greyish white with brown margins.

Table 4. *In vitro* suppression of *R. solani* by organic preparations, botanicals and non-hazardous chemicals

Treatment	Mycelial growth (3DAI) (cm)	Percentage inhibition (%)
T ₁	0.73	91.85 ^b (76.59)
T ₂	1.67	81.48 ^d (64.52)
T ₃	0.03	99.63 ^a (87.34)
T ₄	1.17	87.04 ^c (68.99)
T ₅	3.30	63.33 ^{gh} (52.73)
T ₆	0.00	100.00 ^a (89.04)
T ₇	0.00	100.00 ^a (89.04)
T ₈	3.43	61.85 ^{gh} (51.85)
T ₉	2.97	67.04 ^{fgh} (54.96)
T ₁₀	4.73	47.41 ^j (43.51)
T ₁₁	4.37	51.48 ^{ij} (45.84)
T ₁₂	2.10	76.67 ^{de} (61.12)
T ₁₃	0.00	100.00 ^a (89.04)
T ₁₄	0.00	100.00 ^a (89.04)
T ₁₅	2.30	74.44 ^{def} (59.66)
T ₁₆	2.70	70.00 ^{efg} (56.82)
T ₁₇	3.77	58.15 ^{hi} (49.72)
T ₁₈	0.00	100.00 ^a (89.04)
T ₁₉	4.50	50.00 ^{ij} (45.01)
T ₂₀ (Control)	9.00	
CD (0.05)	-	5.857
SEm (±)	-	1.67

Treatments with same alphabet do not differ significantly
Values in parenthesis are arc sine transformed
Values are average of 3 replications

Table 5. Germination of sclerotia of *R. solani* as affected by soaking in different effective treatments

Treatments

Soaking duration (h) of sclerotia of *R. solani* in selected treatments and germination of sclerotia

Treatments	24		48		72	
	MRS (cm)	S (%)	MRS (cm)	S (%)	MRS (cm)	S (%)
T ₃ (Fermented weed extract)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)
T ₅ (Fermented egg-lemon juice extract)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)
T ₆ (Panchagavya)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)
T ₁₂ (Potassium silicate)	9.00	0.00	7.79	13.34 ^c (19.46)	7.17	21.03 ^b (27.29)
T ₁₃ (Lime solution)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)	0.00	100.00 ^a (89.04)
T ₁₇ (Garlic extract)	7.50	16.66 ^b (23.89)	4.16	53.71 ^b (46.88)	0.00	100.00 ^a (89.04)
Sterile water (Control)	9.00	0.00	9.00	0	9.00	0.00
CD (0.05)	-	3.53	-	16.77	-	0.51
SEm(±)	-	0.91	-	4.44	-	0.13

MRS – mycelial regeneration from sclerotia, S – suppression, Treatments with same alphabet do not differ significantly, Values are average of 3 replications, Values in parenthesis are arc sine transformed

Sheath blight symptom development was observed on the 3rd DAI on the leaf sheaths inoculated with all the isolates except I₆ (Karode isolate) which took seven days for symptom development. The *R. solani* isolate collected from Attingal (I₂) produced the maximum relative lesion height on 5th (9.20%), 7th (15.70%), 10th (19.03%) and 15th (20.70%) DAI. The number of affected tillers was also maximum (4.66) in the case of isolate from Attingal (I₂) (Table 2). Thus, the virulence rating of the seven isolates of *R. solani* revealed that Attingal (I₂) isolate was the most virulent one.

Microscopic studies (100 X) of the mycelium of the fungus revealed the presence of right angled branching, septum at the origin and constriction at the base of branching, which are all the characteristic features of *R. solani* (Parmeter and Whitney, 1970). The sclerotia when crushed and observed under the microscope revealed the presence of moniliod cells which further confirm the fungus to be *R. solani* (Sneh et al., 1991). The mycelial colour of the isolates ranged from light brown to dark brown with the isolate from Karode (I₆) exhibiting white coloured mycelium. The mycelial texture also ranged from fluffy to cottony in the various isolates.

The mycelial growth was found to be the maximum in the case of isolate from Attingal (I₂) on 1st (3.60cm) and 2nd (8.26cm) DAI (Table 3). The sclerotia produced by five isolates (I₁, I₂, I₃, I₄ and I₅) exhibited the maximum average size (2.13, 2.11, 1.99, 1.91 and 2.01mm respectively). The isolate from Karode (I₆) produced the maximum number of minute sclerotia (104.66) which was followed by the isolate from Attingal (I₂) (59.33) and isolate from Karamana (I₇) (53.66). The growth pattern of the sclerotia in the Petri plates ranged from scattered to central. The *in vitro* identification of the isolate based on morphological characters revealed it to be *Rhizoctonia solani* J.G. Kuhn (Parmeter and Whitney, 1970). The identification of the isolate based on the ITS (Internal Transcribed space) region of rDNA showed 100 per cent similarity with *Rhizoctonia solani* AG-1 IA isolate CSU8 (NCBI accession number KX674527).

In vitro evaluation of the treatments in inhibiting the mycelial growth of *R. solani* revealed that among the 19 treatments tested for their potential in inhibiting the mycelial growth of *R. solani*, six treatments namely, fermented egg-lemon juice extract (10%), fermented weed (*Setaria barbata*)

extract (100%), panchagavya (5%), garlic extract (10%), potassium silicate (1%) and lime solution (12.5%) resulted in 100 per cent inhibition of the mycelial growth of *R. solani* (Table 4). The biologically active functional molecules on the egg white, yolk, shell and membrane possessing unique functional properties such as antiviral, antibacterial, enzymatic and immunological activities have been reported by Ibrahim et al. (2002). Cystatin, the affinity purified from the chicken egg white has been reported to have antifungal activity against human fungal pathogen, viz., *Candida* sp. (Kolaczowska et al., 2010). Citrus juice has been reported to have significant antimicrobial activity against six gram positive and eight gram negative bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Proteus spp*, *Moraxella catarrhalis*, *Acinetobacter spp.* and *Candida albicans* (Hindi and Chabuck, 2013). The antifungal potential of this extract might be due to the activity of the microbes produced during its preparation and fermentation or due to the presence of metabolites which need to be further confirmed.

Fermented weed extract also revealed 100 per cent inhibition of the mycelial growth of *R. solani*. The exploration of the antifungal activity of weeds remains an area of interest, but not many reports are available on the exploitation of the antifungal property of weed plants against plant diseases. Srivastava and Singh (2011) reported that the dried leaf powder (20 mg ml⁻¹) of two weeds, *Lantana camara* and *Parthenium hysterophorus* inhibited the mycelial growth (59.5 and 45.9% respectively) of *Alternaria* sp. Pal et al. (2013) studied on the effect of eleven weed plants for their antifungal activity against the seed borne *Alternaria* sp. and found that the extracts of the weeds viz., *Ageratum conyzoides* and *Parthenium hysterophorus* showed the most potential antifungal activity against the fungus. The antifungal potential of *Setaria barbata* need to be

further confirmed by future research works.

Lime solution (12.5%) also resulted in 100 per cent mycelial inhibition of *R. solani*. The results are in consensus with the findings of Stosic et al. (2014) who reported that calcium chloride and calcium hydroxide at 1.5 and 2 per cent concentrations significantly decreased the spore germination and germ tube growth of *Colletotrichum acutatum*, *C. gloeosporioides*, *Alternaria alternata* and *Penicillium expansum*.

Potassium silicate at one per cent also completely inhibited the mycelial growth of *R. solani*. The study is in confirmation with the findings of Epstein (1999) who proposed that potassium silicate increased the host defense system or strengthened the plant cell walls, thereby inhibiting infection. The inhibition of fungal growth by potassium silicate was proposed to be due to pH effect.

Garlic extract at ten per cent concentration also completely inhibited the mycelial growth of *R. solani*. The findings are in confirmation with the works of Kyung and Lee (2001) who proposed that several biologically active compounds are present in garlic extract which affect a wide range of soil borne fungal pathogens. The strong inhibition of *R. solani* by garlic extract may be due to the presence of sulphur compounds and its active antimicrobial component viz., allicin as proposed by Singh and Singh (2005) and Portz et al. (2008).

Panchagavya could result in 100 per cent inhibition of *R. solani* *in vitro*. The finding is in consensus with that of Sugha (2005) who reported that panchagavya resulted in 40 to 100 per cent inhibition of the mycelial growth of *R. solani*, *Sclerotium rolfisii*, *Sclerotinia sclerotiorum*, *Phytophthora colcasiae* and *Fusarium solani*. The antifungal property of panchagavya may be attributed due to the presence of antimicrobial substances in cow dung such as patulodin like compounds, CK2108A and CK2108B produced by *Eupenicillium bovisfimosum* present in cow dung as

proposed by Dorothy and Frisvad (2002). The release of the antimicrobial compounds may be due to the microorganisms like bacteria, fungi and actinomycetes present in panchagavya as reported by Swaminathan et al. (2007).

In the study for the inhibition of mycelial regeneration from sclerotia, fermented egg-lemon juice extract (10%), fermented weed extract (100%), panchagavya (5%) and lime (12.5%) completely inhibited the mycelial regeneration after 24 hours of dipping the sclerotia in them (Table 5). Garlic extract (10%) could result in the complete inhibition (100%) after 72 hours of sclerotial dip in it. The reason whether high pH and calcium were fungistatic or fungicidal to the resting spores have not been resolved. He et al. (2014) proposed that the effect of calcium carbonate on *Ralstonia solanacearum* was mainly related to the role of calcium (Ca²⁺). Jiang et al. (2013) reported that the mechanism of action of calcium may be due to the increased activity of peroxidase and poly phenol oxidase.

Potassium silicate resulted only in less inhibition (21.03%) of the mycelial regeneration from sclerotia. Similar results were proposed by Wainwright (1993) who suggested that nutrient free silica gel supported the fungal growth with the gel itself acting as a nutrient source and stimulating spore germination.

Thus the present study revealed the scope for use of commonly available indigenous organic preparations/ botanicals/ non-hazardous chemicals for eco-friendly disease management. Four treatments viz., fermented weed extract, fermented egg-lemon juice extract, lime and panchagavya were effective in completely inhibiting the mycelial growth of *R. solani* as well as in inhibiting the mycelial regeneration from sclerotia within 24 hours of dip. Potassium silicate could completely inhibit the mycelial growth, but had less effect on the inhibition of sclerotia. Thus, the sheath blight pathogen as well as its perpetuating, resting

structures (sclerotia) can be safely managed using organic preparations/ botanicals/ non-hazardous chemicals effectively.

Replacement of synthetic fungicides by natural products which are non-toxic and specific in their action is of importance in the present context. Hence, organic preparations/botanicals/non-hazardous chemicals can be effectively utilized for the management of various plant pathogens which are environmentally safe and economically viable.

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