Efficacy of Bioagents, Plant extracts and chemical fungicides against root rot disease in Zea mays caused by Sclerotium rolfsii

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Abstract *– Zea mays is a nutritional crop and heavy source of carbohydrate. The root rot disease in Zea mays caused by Sclerotium rolfsii. This study was evaluated for management of S. rolfsii due to it is more dangerous soil borne fungus. It was controlled by two antagonists, two metabolites of bioagents, two chemical fungicides and three plants extracts at different concentration using poisoned food technique. Histopathological study the root rot disease infected part of plant cut section to show with SEM. The T.S. of root and stem was observed by SEM image showed the mycelia of S. rolfsii was scattered in root and stem of infected Zea mays plant. The culture filtrate of Penicillium citrinum and P. italicum was inhibited by 100% growth of S. rolfsii at 75% concentration. Carbendazim was more effective compared to conika at different concentration. In vitro efficacy, the extracts of plants viz. Azadirachta indica, Syzygium aromaticum, Trachyspermum ammi having antifungal activity and checked mycelial expansion of S. rolfsii under in vitro condition. Syzygium aromaticum and Trachyspermum ammi were inhibited by 100% at 400 ppm and 50% concentration respectively. The extract of medicinal plants Syzygium aromaticum and Trachyspermum ammi were most significantly effective compared to A. indica on 3rd day. Amongst all treatments were significantly and ecofriendly effective against mycelial expansion of S. rolfsii under in vitro study.*

Keywords *– Bioagents, Chemical fungicides, Plant extracts, Sclerotium rolfsii, SEM, Zea mays.*

INTRODUCTION

Zea mays seeds are nutritious and edible that serves as good source of carbohydrates, protein fat and vitamin. Betacarotene and selenium also found in *Zea mays* that helps in treatment of thyroid gland [1]. The antioxidant property found in corn seeds removed free radicals which is responsible for cellular damage and cancer [2]. Corn seeds are used for alcohol production and stem fibres for manufacturing of paper [3]. *Zea mays* crop is infected by bacteria, fungi and pest but fungal disease like root rot more dangerous. The root rot disease is caused by *Sclerotium rolfsii*. *Sclerotium rolfsii* is a Soil-borne fungus [4], [5], [6], [7], [8]. The infected *Zea mays* crop showed reduced growth and floppy and finally crop died. *Sclerotium rolfsii* is infects many economically important plants at different stages of growth and development. Pathogen firstly infect lower portion of plant and after then it infect whole parts of the plant [12]. The cutinolytic enzymes viz., Pectinase and cellulase which degrade cuticle of plant were produced by *S. rolfsii* [13]. The vascular bundles of infected part of plant became brownish [14].

Sclerotium rolfsii is also known as white mold and a dreaded soil-borne plant pathogen [11], symbiotic, parasite and saprophyte fungus which is responsible for causing various diseases on crops like collar-rot, sclerotium wilt, seedling blight, stem canker, bulb rot, crown blight, tuber rot, fruit rot, crown rot, systemic wilt or blight of whole plant, stem-rot, charcoal rot, foot-rot, stem blight and root-rot in more than 500 plants species including tomato, chilli, , maize, groundnut, sunflower, cucumber, brinjal, soybean, bean, watermelon etc[4], [5], [6], [7], [8]. It was first observed by Peter Henry Rolfs in the year 1892 on tomato plants with 70% losses [17]. Fungus produced numerous tiny round white *sclerotia* of the same size when immature and dark brown on mature stage [6], [9] and sclerotia diameters ranged 4-8 mm in size which is characteristic feature of *S. rolfsii*. *Sclerotium rolfsii* develops at soil moisture level (70%)

of field capacity and temperature ranged between 25^0C to 30^0C [10].

Trichoderma viride, Aspergillus niger and *Penicillium* species were most effective against *S. rolfsii* causing stem rot in groundnut under *in vitro* condition [24], [25]. *In vitro* efficacy *Trichoderma harzianum* contained antagonistic activity against mycelial growth of *S. rolfsii* [32]. The percent inhibition of mycelial expansion of *S. rolfsii* by five *Trichoderma* species viz *T. hamanatum*, *T koningii*, *T. harzianum*, *T. ressei* and *T viride*, indicated as enhanced antagonists in sustainable agricultural and environmental protection [35]. *Trichoderma viride* contained antagonistic activity against mycelial expansion of *S. rolfsii* under *in vitro* condition [36].

Hexaconazole was most effective compared to Carbendazim, difenoconazole, mencozeb, chlorothalonil at 125 ppm, 250 ppm, 500 ppm concentration against *S. rolfsii* under *in vitro* condition [31]. Taj et al. [30] studied seven fungicides viz. Captan, Carbendazim, Chlorothalonil, Mancozeb, Tebuconazole, Tricyclazole and Trifloxystoburin against *Sclerotium rolfsii* and inhibited effectively mycelial expansion of *Sclerotium rolfsii* at 1000 ppm concentration.

Mahato et al. [33] studied about eight medicinal plant extracts *viz. Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Andrographis paniculata*, *Curcuma longa*, *Catharanthus roseus*, *Ocimum sanctum* and *Zingiber officinale* against *S. rolfsii* at 5, 10 and 20% concentration under *in vitro* condition. *Aspergillus* spp., *Rhizopus* sp., *Penicillium* spp. and chemical fungicides including tolclofos methyl, thiram, carboxin, fludioxonil, azoxystrobin metalaxyl-M were inhibited mycelial expansion of *S. rolfsii* under *in vitro* condition at different levels [15]. The leaf extracts of plants viz. *Annona squamosa* Linn, *Brassica campestris* Linn and *Ocimum sanctum* Linn were more effective against *S. rolfsii* under *in vitro* condition at difference concentration [16].

The present study evaluated that the bioagents, plant extracts and chemical fungicides were most significantly effective at different concentration against *S. rolfsii*. This experiment may be effective in field condition and control the root rot disease in maize and other beneficial crop.

OBJECTIVES OF THE STUDY

Study on bioagents, plant extracts and chemical fungicides against root rot disease caused by *Sclerotium rolfsii* in *Zea mays* crop. The use of above treatments may be ecofriendly effective in the field condition.

MATERIALS AND METHODS

Sample collection

The samples were collected from an infected agriculture field of maize from district Barabanki. The infected parts of maize crop and their rhizospheric and non-rhizospheric soil were carried out in sterilized polythene bags separately. Some medicinal plants were collected from the BBAU campus and other areas of Lucknow. Some selected bioagents were isolated from collected soil samples. Chemical fungicides were procured from the local market of Lucknow.

Separation and recognition of Pathogen:

Sclerotium rolfsii was isolated from infected root of *Zea mays*. The infected root of plant was washed and cut into small pieces (2-4 mm length) and sterilized with 0.5% mercuric chloride ($HgCl_2$) solution for 30 seconds followed by washing in sterilized distilled water and left at room temperature for 5 minutes. Small sections were placed on PDA Petriplates and incubated at 27 ± 1^0 C in an incubator for seven days. After seven days, the culture was maintained on PDA slant for further studied [17]. The fungus recognized by morphological study. Morphologically the pathogen bear hyphae grow state forward on the surface of the infected plant stem with white mycelium and scattered inside and outside of the infected stem nearby soil surface. The old hyphae produce many tiny rounds, white *sclerotia* of the same size (Fig.1) when immature and dark brown sclerotia on mature stage like mustard seed [6]. The sclerotia diameters are 4-8 mm which is characteristic feature of *S. rolfsii* [1].

Antagonistic activity of some fungal species against *Sclerotium rolfsii*:

Antagonistic activity of *Penicilium citrinum* and *P. italicum* were evaluated against the expansion of *S. rolfsii* using dual culture technique under *in vitro* condition [18]. In dual culture technique, 5 mm diameter block of *S. rolfsii* was inoculated one side and antagonist on opposite side of the same PDA containing Petriplate as a treatment and one block of *S. rolfsii* inoculated in the center of another Petriplate as control. The treatments and control plate was incubated at 27 ± 1^0C temperature in an incubator. All treatments and control plates were set in triplicates. On 1st, 2nd and 3rd day of incubation, the mycelial expansion of *S. rolfsii* in control as well as treatments plate was measured. Inhibition of *S. rolfsii* was calculated by following formula.

$$\text{Percent Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Preparation of metabolite

The metabolite was extracted in PD broth. In this method firstly three blocks (5mm) of *Penicilium citrinum* and *Penicilium italicum* cut with 5mm diameter cork borer from the actively growing margin of 5 days old culture was inoculated separately in 500 ml conical flask containing 300 ml of potato dextrose broth [19]. After 15 days, cultures were filtered firstly by Whatman filter paper no 42 and then filtered by Vacuum Seitz filter. Finally the filtrates were stored in sterile conical flask at 4^0C for further study.

Management of *Sclerotium rolfsii*

The culture filtrates of *Penicilium citrinum* and *Penicilium italicum*, chemical fungicides Carbendazim 50% WP and Conika 50% WP, plant extract *Azadirachta indica*, *Syzygium aromaticum* and *Trachyspermum ammi* were tested against mycelial expansion of *S. rolfsii* using poisoned food technique [20]. According to this method, the *Penicilium citrinum*, *Penicilium italicum*, Carbendazim 50% WP, Conika 50% WP, *Azadirachta indica*, *Syzygium aromaticum* and *Trachyspermum ammi* at different concentration were added separately in PDA medium and poured into sterilized Petri plates in laminar flow. After solidification, cut 5 mm block of fresh culture of *S. rolfsii* placed in centre of each Petri plates and incubated at 27 ± 1^0 C. The mycelial expansion of *S. rolfsii* was recorded on 1st, 2nd and 3rd day. Inhibition of mycelial expansion of *S. rolfsii* was calculated by following formula [21].

$$\text{Inhibition Percent} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Histopathological study

The root rot disease incidence in *Zea mays* plant caused by *Sclerotium rolfsii* and histological study by using SEM analysis. The samples were analyzed from USIC BBAU Lucknow. In this method, the infected part of *Zea mays* was washed under running water followed by distilled water and then cut T.S. of root & stem. Thse sectioned were fixed with 2.5% glutaraldehyde for 2-6 hours. The fixed sectioned were washed with phosphate buffer for 3-4 times followed by series of dehydration process in 30-100% ethanol and then kept in dry acetone. Samples were mounted on aluminium stubs with double sided carbon conductive tape and were coated with thin layer of palladium under a vacuum. The images of samples were observed under Scanning Electron Microscope (Jeol JSM-6490 LV).

Data analysis

The data were expressed as Mean and analyzed statistically using one way ANOVA (analysis of variance), coefficient variance, standard error, standard deviation, DMRT and Least significant difference test ($p \leq 0.05$) were applied.

RESULTS AND DISCUSSIONS

Root rot disease causing soil borne fungus *Sclerotium rolfsii* was controlled significantly ($p \leq 0.05$) by using *Penicilium citrinum*, *Penicilium italicum*, Carbendazim 50% WP, Conika 50% WP, *Azadirachta indica*, *Syzygium aromaticum* and *Trachyspermum ammi* at different concentration on 3rd day incubation period under *in vitro* condittion. *Penicillium citrinum* (20.39%) and *P. italicum* (17.14%) inhibited expansion of *S. rolfsii* using dual culture method under *in vitro* condition (Table 1). Whereas culture filtrate *Penicillium citrinum* and *P. italicum* were inhibited 100% mycelial growth of *S. rolfsii* at 75% v/v concentration while 84.40%, 82.38%, 84.51% and 71.52%, 70.94%, 83.35% growth inhibition was recorded at 10%, 25%, 50% v/v concentration respectively on 3rd day (Table 2&3).

At 600 ppm concentration, Conika 50% WP inhibited the growth by 70.57% compared to 100 ppm (1.99%) 200 ppm (7.99%) 400 ppm (57.86%) concentration (v/v) against mycelial expansion of *S. rolfsii* on 3rd day of incubation period (Table 4). Carbendazim 50% WP was most effective and inhibited the expansion of *S. rolfsii* by 84.19% at 6 ppm (v/v) concentration whereas at 1 ppm, 2 ppm, 4 ppm (v/v) concentration the expansion of *S. rolfsii* inhibited by 17.08%, 75.20%, 81.38% respectively on 3rd day of incubation under *in vitro* condition (Table 5). Amongst these chemical fungicides, the Carbendazim 50% WP was most effective ($p \leq 0.05$) compared to Conika 50% WP against mycelial growth of *S. rolfsii* at different concentration on 1st, 2nd and 3rd day of incubation period.

Azadirachta indica inhibited maximum (79.65%) at 75% v/v concentration followed by 18.59%, 32.10%, 71.05% at 10%, 25%, 50% v/v concentration on 3rd day against *S. rolfsii* (Table 6). On another hand, *Syzygium aromaticum* oil inhibited 100% growth of *S. rolfsii* at 400 ppm v/v concentration while at 100 ppm, 200ppm v/v concentration it inhibited the growth by 13.23%, 79.65% of *S. rolfsii* on 3rd day respectively (Table 7). *Trachyspermum ammi* seeds powder inhibited the growth by 100% at 50% v/v concentration followed by 22.11%, 31.73% at 10%, 25% v/v concentration against *S. rolfsii* on 3rd day of incubation period under *in vitro* condition (Table 8). Among all tested medicinal plants,

Syzygium aromaticum and *Trachyspermum ammi* inhibited the growth by 100% at 400 ppm (v/v) and 50% (v/v) concentration respectively on 3rd day. *Syzygium aromaticum* oil and *Trachyspermum ammi* were significantly ($p \leq 0.05$) effective compared to *Azadirachta indica* at different concentration on 1st, 2nd and 3rd day under *in vitro* condition. Histopathological study the T. S. of root & stem of infected *Zea mays* was analyzed with SEM which images showed the xylem vessels of root and stem were ruptured from *S. rolfsii* (Fig.2).

All antagonists contain inhibitory activity and to bind actively growing mycelial tip of the pathogen and inhibits the mycelial growth of *S. rolfsii* [22]. Madhavi and Bhattiprolu [29] tested two *Trichoderma* sp. viz. *T. viride, T. harzianum* against *S. rolfsii* using dual culture technique and reported the growth inhibition by 55.8%, 57.5% respectively. In a study nine antagonistic microorganisms were selected for their efficacy against *S. rolfsii* using dual culture technique under *in-vitro* condition, out of these, the maximum mycelial expansion of *S. rolfsii* reduced (63.33%) by *Trichoderma viride* [23]. Karthikeyan et al. [37] isolated three bioagents viz; *T. viride*, *T. harzianum* and *Pseudomonas fluorescens* and studied their effectiveness against growth of *S. rolfsii*. Out of these three bioagents, only *T. viride* inhibited 69.40% mycelial development and 62.50% sclerotial germination of *S. rolfs Trichoderma viride, Aspergillus niger* and *Penicillium* species were most effective against *S. rolfsii* causing stem rot in groundnut under *in vitro* condition [24], [25]. Ekundayo et al. [17] observed similar result in root-rot disease in *Zea mays* when treated with *Aspergillus flavus, A. niger, T. viride* and *Penicillium italicum* against mycelium expansion of *S. rolfsii* under *in vitro* condition. Curtis et al. [26] found that *R. solni* and *S. rolfsii* causing crown and root-rot in tomato plants significantly checked by biocontrol agent as eco-compatible farming. The metabolites of *Trichoderma viride* and *T. harzianum* inhibited collar rot disease incidence in sunflower caused by *S. rolfsii* and improved the crop yielding [27].

Chaurasia et al. [28] tested nine chemical fungicides viz., Bavistin, Brassicol, Captan, Dithane M-45, DM-145, Fytolan, Parasan, Manzate, Sulfex against *S. rolfsii* under *in vitro* condition and reported that all fungicides had adverse effect on the expansion of *S. rolfsii* and inhibited the growth by 100% at 0.1% concentration. In a study, carbendazim inhibited maximum growth of *S. rolfsii* by 93.7% at 0.2% concentration under *in vitro* condition [29]. Taj et al. [30] studied seven fungicides viz. Captan, Carbendazim, Chlorothalonil, Mancozeb, Tebuconazole, Tricyclazole and Trifloxystoburin against *Sclerotium rolfsii* and inhibited effectively mycelial expansion of *Sclerotium rolfsii* at 1000 ppm concentration. Hexaconazole was most effective compared to Carbendazim, difenoconazole, mencozeb, chlorothalonil at 125 ppm, 250 ppm, 500 ppm concentration against *S. rolfsii* as a similar result to be found *in vitro* condition [31]. Wavare et al. [32] evaluated that chemical fungicide Carbendazim checked mycelial expansion of *S. rolfsii* by 100% as a similar result to be found.

Mahato et al. [33] studied about eight medicinal plant extracts *viz. Azadirachta indica, Allium cepa, Allium sativum, Andrographis paniculata, Catharanthus roseus, Curcuma longa, Ocimum sanctum* and *Zingiber officinale* against *S. rolfsii* at 5, 10 and 20% concentration under *in vitro* condition. They reported that *Allium sativum* showed maximum inhibition (84.89%) of *S. rolfsii* at 20% concentration followed by *Azadirachta indica* (80.86%), while *Ocimum sanctum* inhibited the growth by 53.47%. Gupta, et al.[34] evaluated the efficacy of some plants extracts like ashok (*Polyalthia longifolia*), bhang (*Cannabis sativa*), clerodendron (*Clerodendrum inerme*), eucalyptus (*Eucalyptus globulus*), garlic, ginger, lantana (*Lantana camara*), karanj (*Pongamia pinnata*), madar (*Calotropis gigantea*),marigold (*Tagetes erecta*), mehandi (*Lawsonia inermis*), neem (*Azadirachta indica*), onion, parthenium (*Parthenium hysterophorus*), sadabahar (*Catharanthus roseus*), tulsi (*Ocimum tenuiflorum*) against *S. rolfsii* causing collar rot of chickpea at 5 and 10% concentration under *in vitro* and found that garlic extract inhibited growth of *S. rolfsii* by 100% at 10% concentration on 42 hours compared to neem (97.0%), ginger (95.8%), marigold (95.6%), lantana (87.1%), madar (79.9%), ashok (77.4%), parthenium (70.8%), clerodendron (66.3%), eucalyptus (61.9%) and onion (55.9%).

Table 1. Antagonistic activity of some bio-agents against mycelial growth (in mm) of *Sclerotium rolfsii* using dual culture technique under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
Control	32.58±**1.24** (0.00)	63.08±0.79 (0.00)	84.58±0.67 (0.00)	36.03
P. citrinum	31.33±1.07 (3.83)	58.33±3.20 (7.63)	67.33±7.64 (20.39)	30.95
P. italicum	32.17±1.03 (1.26)	60.25±4.05 (4.49)	70.08±9.43 (17.14)	31.9
F-cal	7.608*	3.148*	6.499*	-

Mean±SD, Bracket data represent % inhibition, CV% = Coefficient of variation F-cal= F-calculated value at 5% level
**=Significant at p=0.05*

Table 2. *In vitro* evaluation of Toxicity of culture filtrate of *Penicillium citrinum* at different concentration and incubation period against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
Control	35.66±**0.88** (0.00)	63.75±0.96 (0.00)	86.58±1.56 (0.00)	34.09
10%	13.5±1.44 (62.14)	13.5±1.44 (78.82)	13.5±1.44 (84.40)	10.37
25%	15.25±1.28 (57.23)	15.25±1.28 (76.07)	15.25±1.28 82.38	8.19
50%	13.41±1.31 (62.39)	13.41±1.31 (76.96)	13.41±1.31 (84.51)	9.47
75%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
F-cal	1570.875**	5659.317**	9049.605**	-

Mean±SD, Bracket data represent % inhibition, CV% = Coefficient of variation F-cal= F-calculated value at 5% level
***=Highly Significant at p=0.05*

Table 3. The culture filtrate toxicity of *Penicillium italicum* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
Control	35.66±**0.88** (0.00)	63.75±0.96 (0.00)	86.58±1.56 (0.00)	34.09
10%	18.66±1.23 (47.67)	24.66±1.37 (61.32)	24.66±1.37 (71.52)	13.85
25%	20.75±1.42 (41.)	25.16±1.58 (60.53)	25.16±1.58 (70.94)	10.89
50%	14.41±1.37 (59.59)	14.41±1.37 (77.39)	14.41±1.37 (83.35)	9.22
75%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
F-cal	1582.333**	4642.639**	7542.84**	-

Mean±SD, Bracket data represent % inhibition, CV% = Coefficient of variation F-cal= F-calculated value at 5% level
***=Highly Significant at p=0.05*

Table 4. Effect of Conika 50%WP against mycelial growth (in mm) of *Sclerotium rolfsii* at different concentration and incubation period under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
Control	30.08±**0.90** (0.00)	65.42±0.51 (0.00)	91.75±0.62 (0.00)	41.06
100ppm	29.58±0.51 (1.66)	65.33±0.49 (0.14)	89.92±0.51 (1.99)	40.77
200ppm	27.92±0.51 (7.18)	60.83±2.69 (7.02)	84.42±0.90 (7.99)	40.8
400ppm	22.5±0.67 (25.19)	30.58±0.90 (53.25)	38.66±1.07 (57.86)	22.07
600ppm	19.16±1.40 (36.30)	27±0.60 (58.73)	27±0.60 (70.57)	15.79
F-cal	366.596**	2514.522**	19355.34**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation
F-cal= F-calculated value at 5% level
**=Highly Significant at p=0.05

Table 5. Effect of Carbendazim 50%WP against mycelial growth (in mm) of *Sclerotium rolfsii* at different concentration and incubation period under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
Control	30.08±**0.90** (0.00)	65.42±0.51 (0.00)	91.75±0.62 (0.00)	41.06
1ppm	26.42±0.79 (**12.17**)	51.58±0.67 (21.15)	76.08±0.79 (17.08)	40.05
2ppm	18.92±0.67 (37.10)	22.75±0.75 (65.22)	22.75±0.75 (75.20)	9.12
4ppm	15.25±0.62 (49.30)	17.08±0.66 (73.89)	17.08±0.67 (81.38)	6.55
6ppm	9.75±0.62 (67.59)	14.5±0.79 (77.83)	14.5±0.79 (84.19)	18.43
F-cal	1532.165**	13255.92**	30120.66**	-

Mean±SD, Bracket data represent % inhibition, CV% = Coefficient of variation F-cal= F-calculated value at 5% level
***=Highly Significant at p=0.05*

Table 6. Effect of *Azadirachta indica* leaf extract at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±**1.11** (0.00)	70.5±1.88 (0.00)	90.08±0.99 (0.00)	40.14
10%	28.25±0.96 (5.29)	56.91±1.44 (19.27)	73.33±1.07 (18.59)	35.81
25%	22±0.95 (24.25)	45.16±0.93 (35.94)	61.16±1.11 (32.10)	38.18
50%	14.58±0.90 (51.12)	22.33±1.30 (68.32)	26.08±0.90 (71.05)	23.61
75%	11±0.95 (63.12)	14.91±0.79 (78.85)	18.33±0.88 (79.65)	21.35
F-cal	851.571**	3676.523**	11369.65**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level
**=Highly Significant at p=0.05

Table 7. Effect of *Syzygium aromaticum* oil at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±**1.11** (0.00)	70.5±1.88 (0.00)	90.08±0.99 (0.00)	40.14
100ppm	20.25±3.51 (32.11)	49.58±2.53 (29.67)	78.16±1.74 (13.23)	48.89
200ppm	11.33±1.23 (62.02)	15.08±0.79 (78.61)	18.33±2.01 (79.65)	21.52
400ppm	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
600ppm	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
F-cal	667.725**	5652.262**	14047.61**	-

Mean±SD, Bracket data represent % inhibition, CV% = Coefficient of variation F-cal= F-calculated value at 5% level
***=Highly Significant at p=0.05*

Table 8. Effect of *Trachyspermum ammi* seeds powder at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±**1.11** (0.00)	70.5±1.88 (0.00)	90.08±0.99 (0.00)	40.14
10%	23.41±1.16 (21.52)	49.75±1.60 (29.43)	70.16±3.58 (22.11)	40.9
25%	20.5±1.44 (31.27)	41.33±3.14 (41.37)	61.5±1.67 (31.73)	41.62
50%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
75%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
F-cal	2465.581**	3687.923**	6278.842**	-

Mean ±SD, Bracket data represent % inhibition, CV% = Coefficient of variation F-cal = F-calculated value at 5% level
***=Highly Significant at p=0.05*

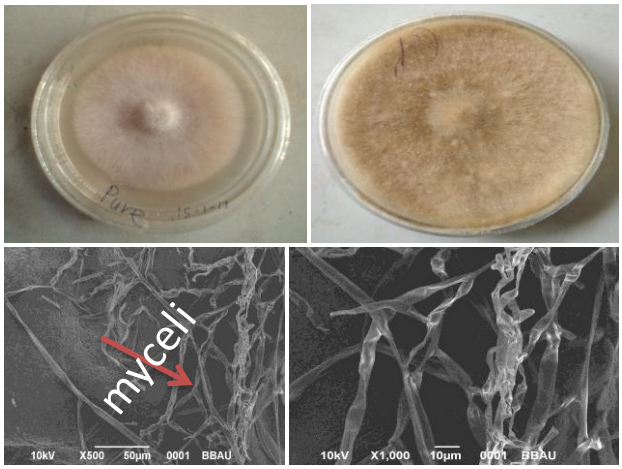

Fig.1: Petriplates culture and Scanning Electron Microscope (SEM) Images of *Sclerotium rolfsii*

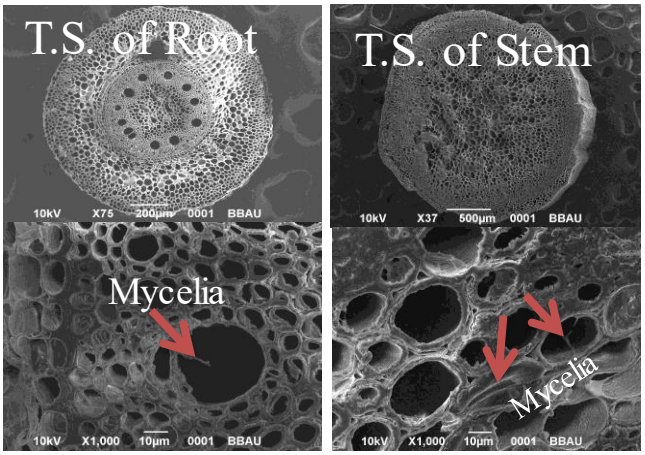

Fig.2: SEM Images of T.S. of Root and Stem of root rot disease incidence in *Zea mays* caused by *Sclerotium rolfsii*

CONCLUSIONS AND RECOMMENDATIONS

Sclerotium rolfsii was controlled through bioagents, plant extracts and chemical fungicides at different concentration under *in vitro* condition. The bioagents *Penicillium citrinum* and *P. italicum* were inhibited 20.39% and 17.14% respectively in dual culture method while culture filtrate of *Penicillium citrinum* and *P. italicum* checked mycelial expansion of *S. rolfsii* by 100% on 3rd day under *in vitro* condition using poisoned food technique. Carbendazim 50% WP and Conika 50% WP were inhibited by 84.19% and 70.57% at 6ppm and 600 ppm concentration respectively against expansion of *S. rolfsii* on 3rd day. *Syzygium aromaticum* and *Trachyspermum ammi* were inhibited by 100% at 400 ppm and 50% concentration respectively compared to *Azadirachta indica* against *S. rolfsii* on 3rd day. The selected above bioagents, plant extracts and chemical fungicides having anti-pathogenic activity which inhibited significantly against *Sclerotium rolfsii*. The SEM image showed that hyphae of *S. rolfsii* was entering in the xylem vessels of the maize and blocked their vessels. The authors recommended that the *Penicillium citrinum, P. italicum*, Carbendazim 50%, *Syzygium aromaticum, Trachyspermum ammi* were effectively inhibited mycelial growth of *S. rolfsii* and also it may be effectiveness in the field.

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