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Management of White Mold Fungus *Sclerotinia sclerotiorum* (Lib) De Bary Causes Disease in Tomato under *In vitro* Conditions

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A B S T R A C T

Tomato crop affected by different fungal diseases in which Fruit rot known as target spot disease incited by *Sclerotinia sclerotiorum* is one of the most destructive disease. Disease severity varied (30.50 to 18.60%) in different locations of Kanpur. Highest disease severity was (30.50%) and the lowest disease severity (18.60%) was noticed at the farmer's field of Billhore, Kanpur. *Sclerotinia sclerotiorum* is a soil borne pathogen which causes symptoms on ripe fruits. Pathogen isolated from infected fruits. Copper oxychloride, Kavach, Tebucanazol, Andrachite, Dithane M-45, Metalaxyl, Roko and Bavistin completely suspected the growth of pathogen*in vitro*. Among eight fungicides, Tebuconazol, Andrachite, Metalaxyl, Roko, and Bavistin were found effective against pathogen under *in vitro* and *in vivo* conditions. However, seven bio-agents (*Trichoderma* spp.), *T. koningii*, *T. virens*are found effective in bothconditions. *In vitro* eight fungicides were tested against pathogen in which Metalaxyl, Roko, Tebucanazol, Andrachite and Bavistin showed 100% growth inhibition. Seven bio-agents (*Trichoderma*spp.) were tested *in vitro* while, among them *T. koningii* showed maximum inhibition followed by *T. virens*, *T. longibrachiatum*, *T. atroviridae*, *T. asperallum*, *T. viridae* and *T. reesei*, respectively. Eight treatments were applied *in vivo* for disease management however, among them T_6 (*Bacillus subtilis* + bavistin) recorded the minimum disease incidence and maximum fruit yield.

Introduction

Sclerotinia sclerotiorum (Lib.) is a serious fungus affecting yield and product quality of many susceptible hosts. It is a widespread soil borne plant pathogen with an extremely wide host range of more than 400 plant species including many of economic importance (Gao *et al.*, 2014). *S. sclerotiorum* is responsible for more than 60 plant diseases (Purdy, 1979). The pathogen produces sclerotia, which survive for long periods and attackroots of growing and mature plants, resulting in root rot, basal stemcanker, and wilt (Duncan *et al.*,

2006). Sclerotinia Stem Rot (also known as white mold or Sclerotinia Stem and Root Rot) is one of the most important tomato soil borne diseases. Plant infection occurs either by myceliogenic germination of sclerotia or by ascospores released from apothecia during carpogenic germination of sclerotia. The myceliogenically germinating sclerotia are the main source of infection on processing tomato crops leading to rotting of aerial parts of the plant in contact with soil (Gao *et al.,* 2014; Purdy, 1979). Crop rotation and cultural methods are not sufficiently effective in controlling Sclerotinia Stem Rot disease because of pathogen's large host range including weeds, its ability to survive as sclerotia, and possible plant infection by airborne ascospores released from germinating sclerotia in nearby infected fields (Elkahoui *et al.,* 2014). Thus, the biological, plant extracts and fungicidal management may be effective in controlling disease.

Tomato is also well known as productive food. In India production of tomato during 2016-17 was 196.96lakh tones and total area under tomato production was 809.0 ha. This constitutes 10.8 per cent of total vegetable area and 12.2% of total vegetable production. The productivity of tomato in India is 24.4 MT/ha during 2016-17 which is very low as compare to other countries of the world like USA, 84.0 tones /ha (NHB, 2017).

One of main reason of low productivity of tomato in India is diseases which are caused by fungi, bacteria, virus, nematode and abiotic factors (Naema *et al.,* 2016). It was well known that the pathogen of white mould could survive on the infected seeds for several days. The study was undertaken with entitled "management of white mould fungus *Sclerotinia sclerotiorum* (Lib.) de Bary causes disease in tomato under *in vitro*" to find out and development of suitable strategies for disease management.

Materials and Methods

Survey for ascertaining the incidence of fruit rot of tomato was carried out at regular interval during *Rabi* crop season 2015-16 at vegetable research farm, Kalyanpur, Chandra Shekhar Azad University of agriculture and technology, Kanpur and its adjacent areas from where diseased samples were collected for further studies.

The diseased specimens were brought to the laboratory and critically examined for the presence of causal organism. These specimens were used for isolation of the pathogen in culture, preserved, labelled and kept in dry and wet forms for further investigations and record.

Pathogenicity test

The pathogenicity of the isolated fungus was conducted on Healthy stems of host plant in order to establish the pathogenic nature of the fungus. The pathogenicity of the fungus was tested according to Koch's Postulate's (1882). The sterilized healthy seeds of tomato were grown in earthen pots of 25 cm diameter containing sterilized soil. Mycelial discs of 5.0 mm diameter were used from the margin of 7 days old culture grown on 20per cent potato dextrose agar medium, and were placed at the base of one month old injured and uninjured healthy tomato plants, already washed with sterilized water.

The homogenized 200 ml mycelial suspension was prepared in sterilized water with 7 days old culture. The inoculated plants were covered with polythene bags for 48 hrs to create the humidity for infection. In this method the pots were shifted to the glass house just after inoculation, where they were watered periodically to maintain sufficient moisture to proper growth of plants for disease development.

Screening of chemical fungicide against the pathogen *in vitro*

Eight chemical fungicides *viz*., Copper oxychloride, Kavach, Tebucanazole, Andrachite, Dithane M-45, Metalaxyl, Roko, and Bavistin belonging to different groups were evaluated against the pathogen under laboratory condition to find out their relative efficacy in inhibiting the growth of the pathogen in culture by the Food Poison Technique (Schmitz, 1930). Requisite quantity of each fungicide was incorporated in two per cent potato dextrose agar medium and thoroughly mixed by shaking prior to pouring in sterilized Petri plates. The medium was allowed to solidify and then inoculated with 5 mm disc of inoculums from seven days old culture of the pathogen. The fungal discs were reversed so that the pathogen could come in contact with the medium, directly. Three replications were made for each treatment. The Petri plates were incubated at 20 ± 1^0C with one set of control in which the medium was not mixed with any fungicide but simply inoculated with pathogen. The data on radial growth of fungal colony measured till the control plates were not filled up. The per cent inhibition over control was calculated by the following formula (Bliss, 1934).

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of fungus in control (mm)
T = Growth of fungus in treatment (mm)

Screening of bio-agents (*Trichoderma* spp.) against the pathogen *in vitro*

This experiment was done with seven isolates of different bio-agents *viz*., *Trichoderma viridae, T. longibrachiatum, T. reesei, T. koningii, T. asperallum, T. atroviridae, T. virens* were evaluated by dual culture techniques to find out the efficacy of bio-agents against the pathogen. Discs of 5mm diameter were taken from the actively growing colonies of the test pathogen and antagonists with the help of sterilized cork borer. The discs of the pathogen and antagonists were placed on the other side in agar plates aseptically. The discs of antagonists were placed on the other side at about 30-40 mm distance of pathogen in same plates. The plates were incubated at 20±1 ºC, after 6 days of incubation the mechanism of interaction was observed and the data were expressed as per cent inhibition by following formula.

$$\text{Per cent inhibition (P.I)} = \frac{\text{Growth in control (mm)} - \text{Growth in treatment (mm)}}{\text{Growth in control (mm)}} \times 100$$

Effect of chemical fungicides and bio-agents *in vivo*

This parameter of treatments was applied to find out the efficacy of those chemical fungicides and bio-agents which showed highly effective against the pathogen *in vivo.* A field trial was conducted in Glass house compound, Department of Plant Pathology C. S. Azad University of Agriculture and Technology, Kanpur in 2015-16. The experiment was conducted with 8 treatments *viz*., T_1= Foliar application with *Bacillus subtillis*, T_2= Seed treatment with *Trichoderma* spp., T_3= Foliar application with Roko (Thiophanate methyl), T_4= Seed treatment with Bavistin, T_5= *Bacillus subtillis* + Roko, T_6= *Bacillus subtillis* + Bavistin, T_7= *Trichoderma* + Roko and T_8= *Trichoderma* + Bavistin in 3 replications and plot size was 2.50 × 1.50 square meters. The observation on total fruit per plant was count in each treatment. The disease incidence was calculated by formula given below:

Disease incidence = $\frac{\text{NO of infected plant per plot}}{\text{Total plant per plot}} \times 100$

Results and Discussion

For ascertaining disease incidence, survey was conducted at different localities in Kanpur during crop season in the month of January 2016. The diseased plants were collected for isolation for further studies. It is obvious from the data presented in table 1 that the disease severity varied from 30.50 to 18.60per cent in different locations. However, it was highest (30.50) at vegetable research farm, Kalyanpur, Kanpur. The lowest disease severity was noticed at the farmer fields at Billhore, Kanpur.

Pathogenicity test of the pathogen

To ascertain the pathogenicity of the isolated fungus, one month old plants of tomato cultivar (T-4) was preferred as test plant and the result revealed that the infection of plants with mycelia suspension proved to be the best method of infection whereas inoculation with sclerotia showed poor infection among the methods tried. Reisolation of the fungus was done from inoculated plants. In each case, it was found that infection per cent was more in injured fruit than the uninjured ones, which suggested that injury on the fruit facilitate the pathogen for easy and quick infection. After three days of inoculation of pathogen, symptoms produced in the form of small, brown, water soaked lesions on the fruit which finally turned into brown in colour.

The symptoms observed were similar to those as observed under natural conditions in all the respect. Reisolation from artificially inoculated plant was done and the yield of same fungus *Sclerotinia sclerotiorum* was found like previously isolated from naturally infected tomato plants. In this way isolation, inoculation and re-isolations proved the Koch's postulates, as this pathogen is capable of attacking both the injured and uninjured, it proved that *Sclerotinia sclerotiorum* is a potential pathogen of tomato.

Fig. 1. Effect of bio-agents against *S. sclerotiorum in vitro*

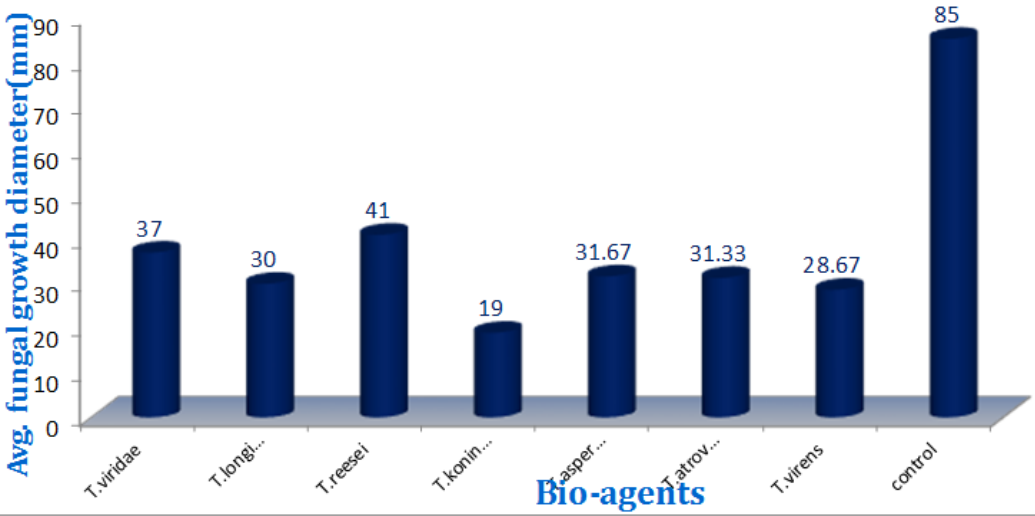

Fig. 2. Effect of chemical fungicides against *S. sclerotiorum in vitro*

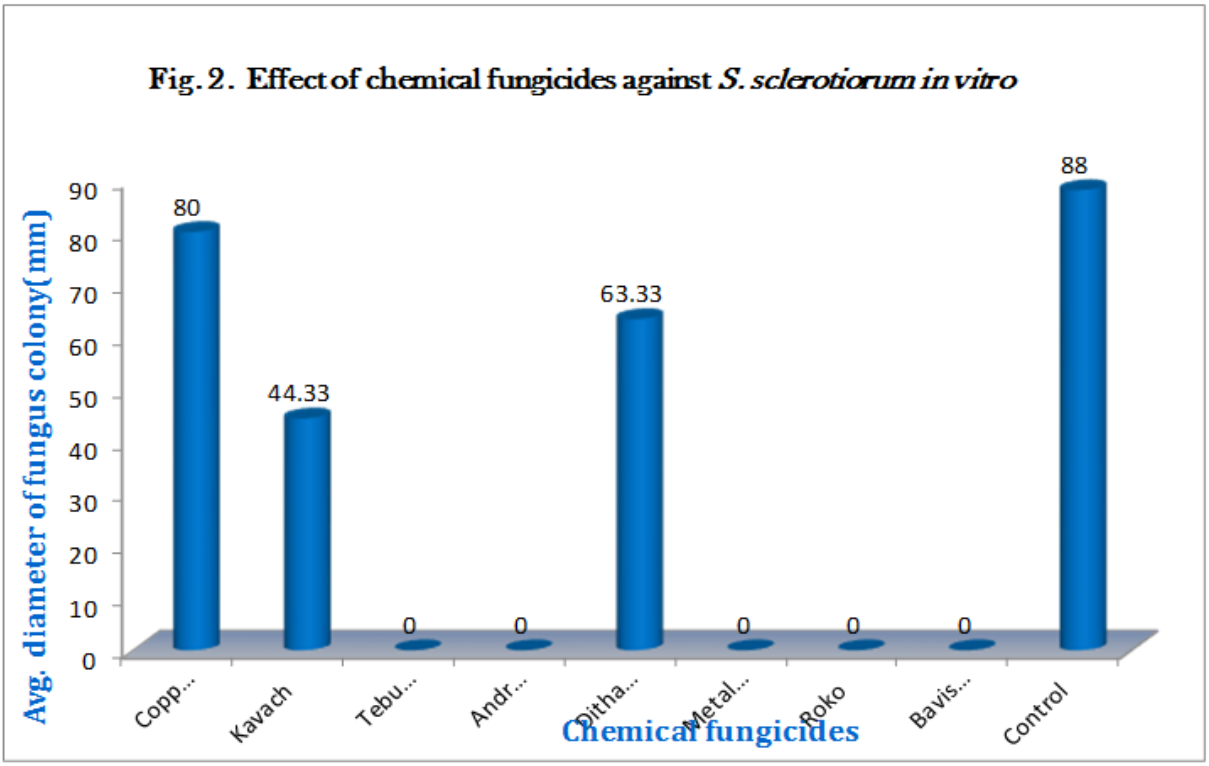

Fig. 3. Effect of incidence and yield of *S. sclerotiorum in vivo*

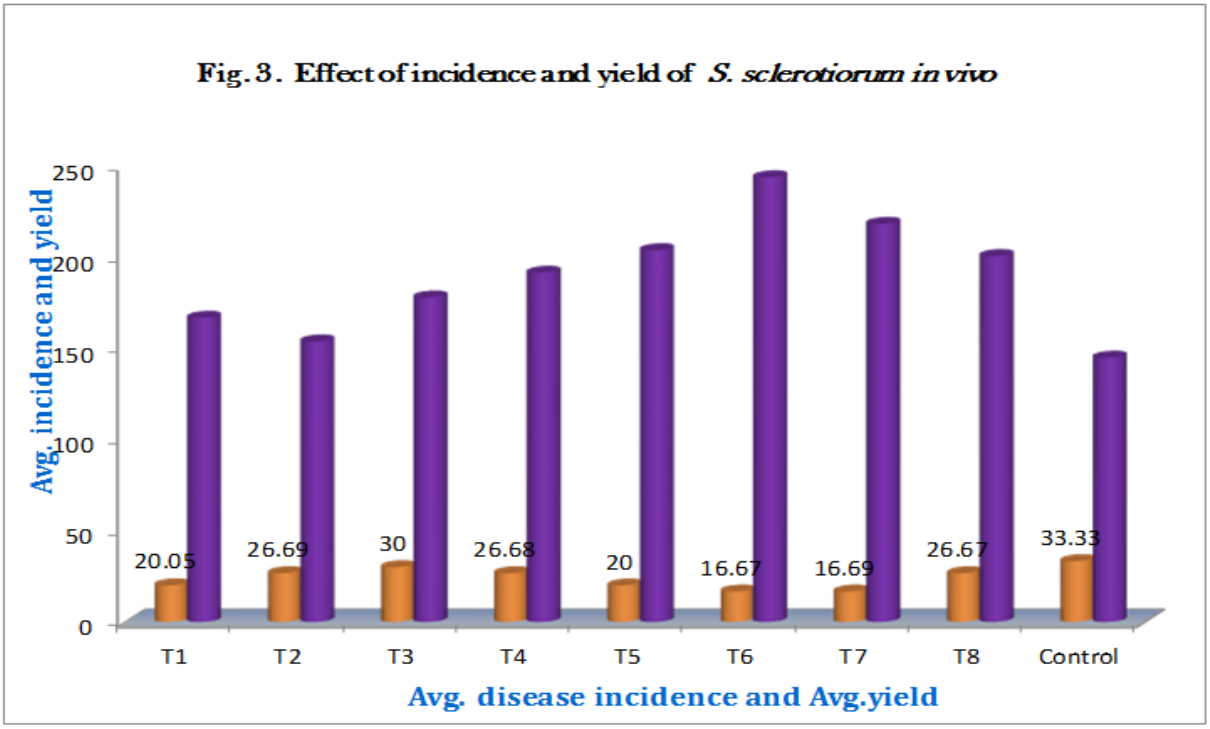

Plate 1. Pathogenicity test of the pathogen

A. Inoculated
B. Uninoculated

Plate 2. Identification of the pathogen

A. Growth of *S. sclerotiorum* on PDA medium

B. Sclerotia of *S. sclerotiorum*

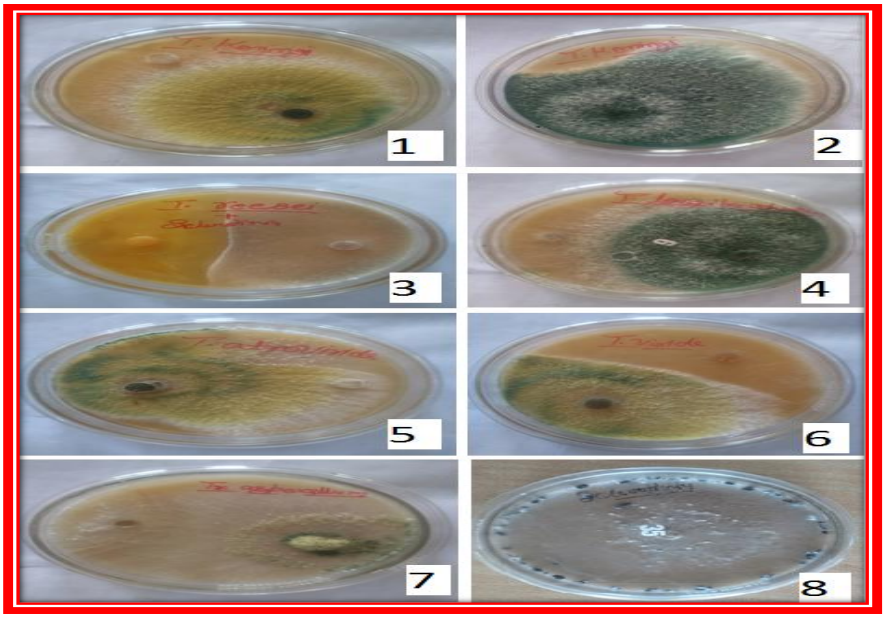

Plate.3 Effect of bio-agents against *S. sclerotiorum in vitro*

T_1- *Trichoderma koningii*, T_2- *T. virens*, T_3- *T. ressei*, T_4- *T. longibrachiatum*

T_5- *T. atroviridae*, T_6- *T. viridae*, T_7- *T. asperallum*, T_8- *Control*

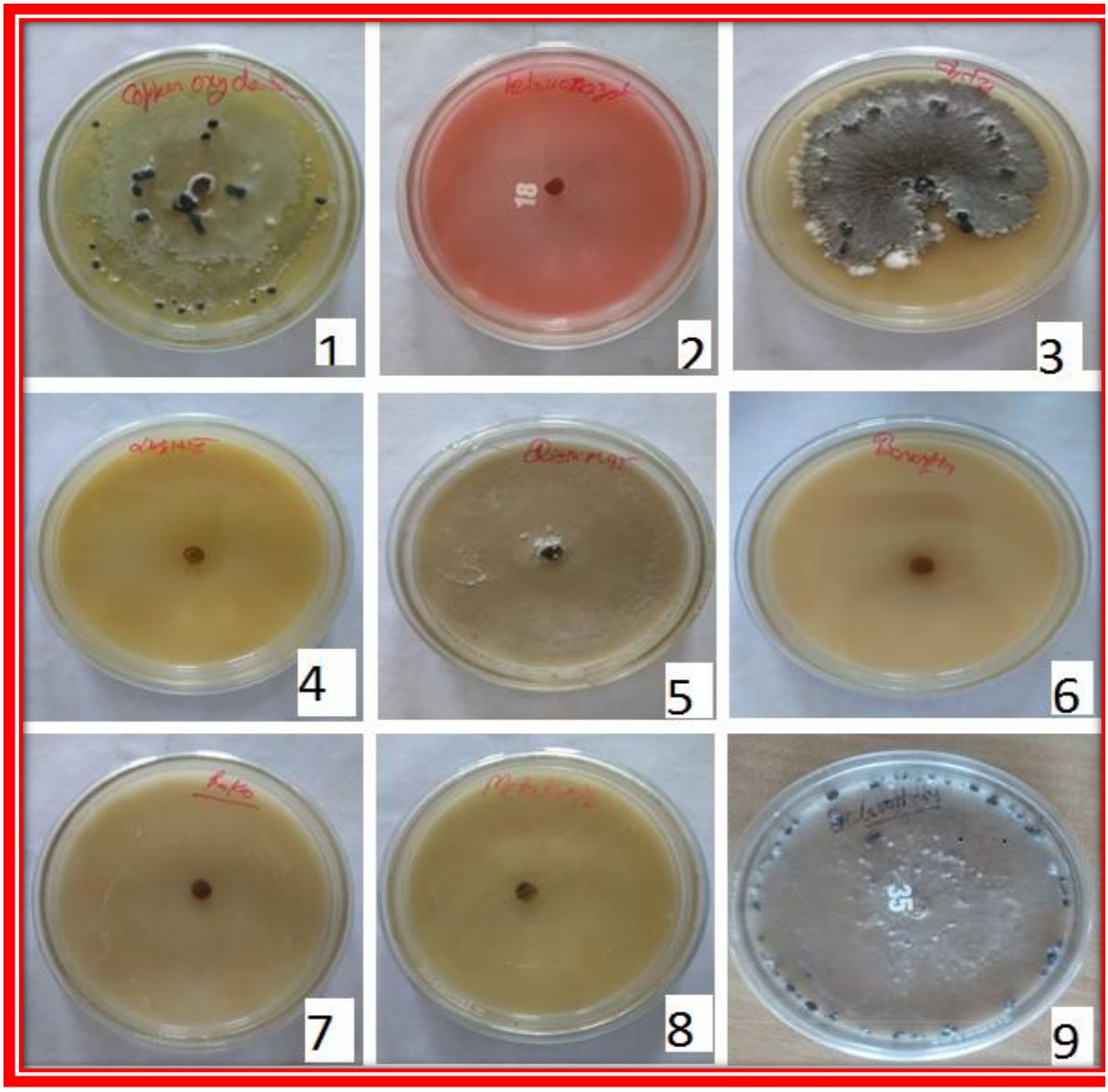

Plate 4. Effect of chemical fungicides against *S. sclerotiorum* *in vitro*.

T_1- Copper oxychloride,T_2- Tebucanazole,T_3- Kavach, T_4- Andrachite
T_5 - Dithane M-45 T_6- Bavistin, T_7- Roko, T_8- Metalaxyl, T_9- Control

Table.1 Incidence of white mold of Tomato at different localities of Kanpur

Sr. No.	Locality	Average disease incidence (per cent)
1.	Vegetable Research Farm Kalyanpur, Kanpur	30.50
2.	Student Instructional Farm, C S A UNIV. Kanpur	22.30
3.	Farmers field, Billhore, Kanpur (city)	18.60

Table.2 Pathogenicity of the fungus exhibited through different method of inoculation

Sr. No.	Treatment	No. of plant subjected to infection	No. of plant showing disease symptoms	Per cent infection
1.	Inoculation with disc of the fungus (i) infected (ii) Uninfected	 25 25	 20 13	 80 52
2.	Inoculation with mycelial suspension (i) infected (ii) Uninfected	 25 25	 23 21	 92 84
3.	Inoculation with sclerotia (i) infected (ii) Uninfected	 25 25	 16 10	 64 40

Table.3 Effect of bio-agent on the growth of *S. sclerotiorum in vitro*

Sr. No.	Bio-agents	Avg. radial growth of the pathogen (mm)	Percent inhibition over control
1.	*T. viridae*	37.00	56.47
2.	*T. longibrachiatum*	30.00	64.70
3.	*T. reesei*	41.00	51.76
4.	*T. koningii*	19.00	77.64
5.	*T. asperallum*	31.60	62.82
6.	*T. atroviridae*	31.33	63.14
7.	*T. virens*	28.66	66.28
8.	Control	85.00	—
	CD at 5%	**3.85**	

Table.4 Inhibitory effect of chemical fungicides on the growth of *S. sclerotiorum in vitro*

Sr. No.	Fungicides	Dose per cent	Av. diameter of fungal colony in (mm)	Inhibition over control (per cm.)
1.	Copper oxychloride	0.10	80.00	9.09
2.	Kavach	0.10	44.33	49.62
3.	Tebucanazol	0.10	00	100.00
4.	Andrachite	0.10	00	100.00
5.	Dithane M-45	0.10	63.33	23.03
6.	Metalaxyl	0.10	00	100.00
7.	Roko	0.10	00	100.00
8.	Bavistin	0.10	00	100.00
9.	Control	—	88	—
	CD at 5%		2.53	

Table.5 Per cent disease incidence and yield *in vivo*

Sr. No.	Treatment	Avg. per cent disease incidence	Avg. yield (in quintal per ha.)
1.	Foliar appli. with *Bacillus subtilis*	20.05	166.66
2.	Seed tr. with*Trichoderma spp.*	26.69	153.70
3.	Foliar appli. With rook	30.00	177.77
4.	Seed tr. withbavistin	26.68	191.35
5.	*Bacillus subtilis +roko*	20.00	203.70
6.	*Bacillus subtilis* +bavistin	16.67	243.70
7.	*Trichoderma*+roko	16.69	218.14
8.	*Trichoderma*+bavistin	26.67	200.49
	Control	33.33	144.81
	CD at 5%	**13.45**	**11.85**

Screening of bio-agents against *S. sclerotiorum*

Seven different Bio-control agents *viz.*; *T. viridae, T. longibrachiatum, T. reesei, T. koningii, T. asperallum, T. atroviridae,* and *T. virens* were evaluated *in vitro* by dual culture technique for the biological management of the disease. The results are presented in Table 3, plate 1 and its corresponding graph (fig.1) indicated that all the bio-agents suppressed the colony growth of *Sclerotinia sclerotiorum.* The suppression of growth of pathogen was maximum with *Trochoderma koningii* (77.64%)followed by *T. Virens* (66.28%), *T. longibrachiatum* (64.70%), *T. Atroviridae* (63.14%), *T. asperallum* (62.82%), *T. viridae* (56.47%) and the least effective bio-agent was *T. reesei*(51.76%). These findings are in accordance of Naema *et al.,* (2016); Abdel-Kader *et al.,* (2012); Abo rehab *et al.,* (2013).

Evaluation of chemical fungicides against *S. sclerotiorum*

A preliminary screening of 8 chemicals belonging different groups were done.

Thedose of fungicides was used based on the preparatory formulation of each fungicide. Growth of the pathogen was measured and average diameter of colony in each petri dish was recorded. Per cent inhibition over control was calculated separately for each treatment and results are presented in Table 4, plate 4 and its corresponding graph (fig. 2) indicated that all tested fungicides showed there better response in minimizing the radial growth of pathogen over control. Hundred per cent radial growth of fungus was checked with Tebucanazole, Andrachite, Metalaxyl, Roko and Bavistin followed by Kavach (49.62%) and Dithane M-45 (23.03%). However, the maximum radial growth was observed with copper oxychloride (80 mm). These results are in collaboration with Abdel-Kader *et al.*, (2013) found that, combination of(compost + *T. harzianum*+ thyme) and (compost + *T. harzianum*+ lemongrass) reduced the peanut crownrot disease incidence at both pre- and post-emergence growth stages, respectively compared with Vitavax-Captan at 3 g/kg and untreated control.

Effect of chemical fungicides and bio-agents *in vivo*

In order to know impartial treatment on per cent disease incidence on field and yield per plant in tomato (T-4) was observed and efficacy of different treatment against test pathogen was also recorded and summarised in table 5 with its corresponding graph (Fig. 3). All treatments showed better response in minimizing the disease incidence as well as increasing the yield over control. The minimum disease incidence above as 16.67% in T_6 (*Bacillus subtilis* + bavistin) which gave maximum yield (243.70 q/ha) was recorded followed by T_7 (*Trichoderma* + roko) showed the disease incidence 16.69% and found the yield 218.14 q/ha. However, the maximum disease incidence (30%) was in T_3 (Foliar application with roko) and minimum yield was recorded 153.70 q/ha in T_2 (seed treatment with *Trichoderma*). Eisa *et al.*, (2013) recorded that, underfield conditions combining the fungicide Folicur with compost has enhanced the control of white rot ofonion and bulb yield compared with using alone. These findings are in collaboration with the findings of other workers (Pane *et al.*, 2013; Elkahoui *et al.*, 2014; Naema *et al.*, 2016) reported similar response in management of white mould fungus i*n vitro* Conditions.

References

Abdel-Kader M.M., Abdel-Kareem F., El-Mougy N.S. and El-Mohamady R.S. 2013. Integration between compost, *Trichoderma harzianum* and essential oils for controlling Peanut crown rot under field conditions. *Journal of Mycology*, pp.1-7.

Abdel-Kader M.M., El-Mougy N.S., Embaby E.I. and Lashin S.M. 2012. Occurrence of Sclerotiniafoliage blight disease of cucumber and pepper plants under protected cultivation system in Egypt. Chemical and Biological Control Measures *in vitro*. *Advances in Life Sciences*, 2(1): 20-27.

Abo Rehab M.E.A., Korra A.K.M., Kamhawy M.A.M. and Youssef K.Y.A. 2013. Fungal speciesassociated with graft union on grapevine, its impact on graft failure process and attempted solutions in Egypt. *International Journal of Agriculture and Forestry*, 3(2): 52-59.

Bliss C.L. 1934 The Methods of Probits. *Science*, 79: 38.

Duncan R.W., Dilantha Fernandoa W.G. and Rashidb K.Y. 2006. Time and burial depth influencing the viability and bacterial colonization of *Sclerotinia sclerotiorum*. *Soil Biol Bioche.*, 38: 275-284.

Eisa N.A., Hafez M.A., Khalifa M.M.A., Khan E.E. and Mahdy H.A.M.M. 2013.

Effect of different types of compost in combination with some biological agents and folicur fungicide on onion white rot disease. *Journal of Applied Sciences Research,* 9(4): 2803-2810.

Elkahoui S., Djébali N., Karkouch I., Hadj Ibrahim A. and Kalai L. 2014. Mass spectrometry identifcation of antifungal lipopeptides from *Bacillus* sp. BCLRB2 against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. *Appl. Biochem. Microbiol.*, 50: 161-165.

Gao X., Han Q., Chen Y., Qin H. and Huang L. 2014. Biological control of oilseed rape Sclerotinia stem rot by *Bacillus subtilis* strain Em7. *Biocontrol Sci. Technol.*, 24: 39-52.

N.H.B. 2017. Handbook of Indian horticulture database. *National Horticulture Board, Gurgaon*., 177-185.

Naema A.G., Mahdy A.M.M., Fawzy R.N. and Ahmed G.A. 2016. Integrated Management of Tomato White Mold Disease Caused by *Sclerotinia sclerotiorum* using the Combined Treatments of Compost, Chemical Inducers and Fungicides. *Middle East J. Agric. Res.*, 5(4): 479-486.

Pane C., Piccolo A., Spaccini R., Celano G. and Villecco D. 2013. Agricultural waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-borne fungi *Rhizoctonia solani* and *Sclerotinia minor*. *Appl Soil Ecol.*, 65: 43-51.

Purdy L.H. 1979. *Sclerotinia sclerotiorum*: History, diseases, symptomatology, host range, geographic distribution, and impact. *Phytopathology,* 69: 875-880.

Schmitaz H. 1930. A suggested toximetric method for wood preservative. *Indus. and Engg. Chem. Analyst.*, 4: 361-363.

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