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ECO-FRIENDLY MANAGEMENT OF SCLEROTINIA ROT OF RAPESEED- MUSTARD CAUSED BY *SCLEROTINIA SCLEROTIORUM* (LIB) *DE BARY*

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Abstract

Sclerotinia rot caused by *Sclerotinia sclerotiorum* has become a serious constraint in production and productivity of rapeseed-mustard in India. The present experiment was carried out on evaluation of botanicals, animal wastes, non toxic chemicals, bio-agents and micro nutrients for management of pathogen in both in-vitro and field conditions to develop Integrated Disease Management (IDM) module. Animal waste products (cow urine, cow dung, vermiwash), botanicals (garlic bulb, onion bulb, neem kernel, eucalyptus leaves) were evaluated in vitro at different levels of concentration (1, 2.5 and 10 %) by mycelial inhibition test while non toxic chemicals (sodium bicarbonate, oxalic acid, calcium carbonate and calcium sulphate) were evaluated at three levels (0.2, 0.5, 1 and 2%) concentration by same method. Treatments which were found successful under in-vitro were evaluated under field condition against artificially inoculated pathogen along with formulations of bio agents (*Trichoderma*, *Pseudomonas* and their combinations) and micronutrients (Borax, oxalic acid, zinc oxide and their combinations). Among them cow urine at 5%, garlic bulb extract at 10% and sodium bi carbonate at 1% found effective with 100% inhibition of mycelial growth in vitro. Under field conditions among all treatments garlic bulb extract and cow urine depicted maximum reduction in disease incidence of 30%, at 5 and 10 percent respectively followed by sodium bicarbonate and calcium bi carbonate at 1 and 2 percent respectively with 50 percent disease incidence. However, rest of the treatments were not found much effective under field conditions as they showed disease incidence ranging between 56.6 to 80 percent. Hence natural or eco-friendly products were found most effective in managing sclerotinia rot disease in lab as well as under field conditions. So it can be a potential and eco-friendly alternative for chemicals in managing this disease for future use.

Key words: Rape seed, Mustard, Sclerotinia rot, *S. sclerotiorum*

Introduction

Rapeseed-mustard crop occupies a premier position accounting for 25 per cent of total oil seed production In India. Rapeseed-mustard is the second largest oilseed crops after the groundnut and grown mainly in North-Western and Central part of India in different ecosystem and cropping sequences.Rapeseed and Mustard are the most important sources of edible oil. The oil content of the seeds of different forms ranges from 30 to 48 percent. The oil is mainly used as a cooking in northern India and cannot be easily replaced by other edible oils.

Sclerotinia rot of Indian mustard (*Brassica juncia*) caused by *Sclerotiniasclerotiorum* (Lib.) de bary has been reported from major rapeseed and mustard growing areas of the world (Morrall*et al.*,1976; Horning, 1983; Regnault and Pierre, 1984; Kang and Chahal, 2000).In India, disease has been reported from Assam, Uttar Pradesh, Haryana, Punjab, Rajasthan and Madhya Pradesh. In Rajasthan it has been observed in almost all the districts where its incidence varied up to 72 per cent (Lodha*et al*., 1992; Krishnia*et al*., 2000; Shivpuri*et* al., 2000 and Ghasolia *et al*., 2004). In severe infection, it caused seed yield losses up to 74 per cent in the country (Chauhan *et al*., 1992; Singh, 1998; Kang and Chahal, 2000).The pathogen is reported to have a wide host range, known to infect about 400 plant species (Kolte, 1985). Still disease has not been controlled consistently and economically. The explosive pathogenicity of the fungi under favourable conditions and the ability of the sclerotia to withstand adverse conditions allow them to be successful pathogen on many crops. Sclerotia are the preferred structures for overwintering and for long term

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survival in field conditions. Large number of sclerotia can be produced in heavily infected crops.

Earliar many workers reported management of *Sclerotinia* rot of mustard by fungicides (Rajinder Singh *et al*., 1994). Chattopadhyay*et al*. (2002) and many other scientists reported fungicides and biological treatment to be effective against the disease. Though the use of fungicides is necessary at present, but their use can be minimized, as a long term solution to the crop health problem. However, chemical control measures alone are not economical and eco-friendly because of their residual toxic effect and wide spectrum activity. The continuous use of these potentially hazardous chemicals is posing an increasing threat to environment. The pesticides contamination of the environment is harmful to wild life and to other non-target beneficial micro-organism. Thus in recent years, an increasing consciousness about environmental pollution due to pesticides and development of fungicide resistant strains in plant pathogens has challenged plant pathologists to search for eco-friendly tools for disease management. Botanical pesticides and animal waste products are one of the vital components of integrated pest management programme and have determined to be environment friendly because of their low persistence, biodegradability and low mammalian toxicity. Hence, use of botanicals and animal waste products for management of plant diseases is valuable as they are eco-friendly and cost effective for stabilizing the production in the country.

Materials and Methods

Plant extracts (Botanicals)

In order to acertain the bio-efficacy of botanicals with antifungal compounds and their distribution in different plant parts. The plant extracts viz., *Allium sativum* (bulb), *Allium cepa* (bulb), *Azadirachtaindica* (kernel) and Eucalyptus (leaves) were evaluated against the pathogen.

Plant extracts were prepared with the help of mortar and pestle by crushing plant parts and adding sterilized distilled water (1:1 w/v). The extract was filtered through four layer of muslin cloth and was sterilized by passing it through sintered glass filters G1, G3 and G5 under aseptic conditions.The required concentration (1, 2.5 and 10%) of each sterilized plant extract was prepared in test tubes. Each concentration of plant extract was mixed thoroughly in sterilized Potato Dextrose Agar (PDA) medium flasks before plating. The poured plates were kept for few hours to solidify. Three replications were maintained in each treatment. The test fungus was evaluated by poisoned food technique on PDA medium. Percent mycelial inhibition was calculated by using the following formula:

$$\text{Per cent inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where,

C = growth of fungus in control

T = growth of fungus in treatment

Animal wastes

The animal waste viz., fresh cow dung, fresh cow urine and vermiwash were taken for the study. Fresh cow dung was suspended in sterilized distilled water (1:1 w/v). The fresh cow dung suspension, cow urine and vermiwash were passed through G1, G3 and G5 sintered glass filters for sterilization. The final desired concentration of 1, 2, 5 and 10 per cent of these animal wastes were prepared and evaluated against the test pathogen, using Poisoned food technique, observations were recorded and per cent mycelial inhibition was calculated as earlier.

Non toxic chemicals

The effect of different non-toxic chemicals viz., sodium bicarbonate, oxalic acid, calcium carbonate and calcium sulphate at different concentrations (0.2, 0.5, 1 and 2%) on the radial growth of the test fungus was evaluated by poisoned food technique PDA medium as earlier and per cent mycelial inhibition was calculated.

Ecofriendlychemicals, bioproducts (bioagents, plant extracts and animal wastes) and micronutrients against the Sclerotinia rot (best n*S.sclerotiorum*) of mustard under field conditions

Treatments which were found best *in vitro* were evaluated under field condition as seed treatment and foliar spray first 45 days after sowing (24 hrs before inoculation of the test pathogen, *S. Sclerotiorum* and second at 15 days after first spray. Plant extracts and animal wastes solutions were prepared by crushing (plant part) or by mixing in water (animal wastes) in 1:1 w/v ratio. Desired concentration was made by mixing calculated volume of plant extract/animal waste solution into calculated volume of water. Micronutrients viz., Zn, S and B were applied in the form of Zinc oxide, Zinc sulphate and Borax as spray. The desired concentration was prepared by mixing calculated amount of available forms of micronutrients (a.i.) in the product into calculated volume of water. Application of biocontrol agents was done by preparing spore suspension and culture filterate. Desired spore suspension of bio-control agents viz., *Trichoderma harzianum* (Pant biocontrol1), *Pseudomonas fluorescence* (Pant

biocontrol2) and *Trichoderma harzianum* + *Pseudomonas fluorescence* (Pant biocontrol3) were prepared by dissolving calculated amount of bio-agent formulation into calculated volume of sterilized distilled water. Culture filtrate was obtained by filtering out the mycelial mat/cell suspension of *Trichoderma harzianum* (PBT 23) through muslin cloth 10 days after incubation at 26±1^{0}C multiplied in potato broth. Desired concentration was prepared by mixing calculated volume of culture filtrate into calculated volume of water.

Inoculation of test pathogen: The plants were inoculated with mycelial disc of *S. sclerotiorum* just before flowering (45 DAS). Ten plants were inoculated in each replication with 3 days old actively growing mycelium culture (disc of 5mm dia. 2 in nos.) of *S. sclerotiorum* grown on PDA. The inoculum was placed at the joint portion of main and sub-branch of the plant 24 hrs after application of treatments. Inoculated portion of the stem was covered with moist cotton and wrapped with plastic tape. The inoculated plants without any treatment were served as check.

Observations:

Inoculated plants were examined periodically for the disease symptoms and final data were recorded 45 days after inoculation with test pathogen. Reduction in disease incidence was calculated by following formula:

$$\text{Reduction in DI (\%)} = \frac{\text{DI in check (\%) - DI in treatment (\%)}}{\text{DI in check (\%)}} \times 100$$

Where,

DI = Disease incidence (%)

Results

Evaluation of plant extracts:

The data (**Table 1, Fig 1**) revealed that among all the plant extracts tested, garlic completely inhibited (100%) mycelial growth of the test pathogen followed by onion (20%) and neem (17.7%) at 10 per cent concentration. However, at 5 percent concentration only garlic was found effective (74.2% inhibition). All the treatments significantly reduced the mycelial growth of the pathogen only at 10 percent concentration. The findings of the earlier workers (Singh,1979; Shivpuri and Gupta,2001 and Dar *et al.,* 2007) were also in accordance with the present findings as they reported that garlic bulb extract is the most effective plant extracts in inhibition of mycleial growth of the *S. sclerotiorum*. However, Pinto *et al.,* (2008) observed that leaf extract of *Eucalyptus citriodora* completely inhibited mycelial growth of the pathogen at 0.1 per cent. The information obtained in the present investigation supports the effectiveness of garlic in reducing the fungal growth, whereas other botanicals did not show any marked reduction in the growth of the pathogen.

Table 1: Effect of plant extract on the growth of test pathogen

Plant extract	Concentration (%)							
	1		2		5		10	
	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)
Allium sativum (Garlic)	28.3	64 (53.59)	22.3	72 (58.12)	18	77.5 (61.70)	0.00	100 (90)
Allium cepa (Onion)	67	16.25 (23.73)	64	20 (26.50)	59	26.25 (30.78)	56	30 (33.20)
Azadirachta indica (Neem)	63	21.25 (27.420	57	28.75 (32.41)	57	28.75 (32.39)	54	32.5 (34.73)
Eucalyptus globules (Eucalyptus)	80	0.00 (0.00)	80	0.00 (0.00)	68	15 (22.75)	65	18.75 (25.60)
Check	80	_	80	_	80	_	80	_
	Pathogen (A)			Concentration(B)			AXB	
Per cent inhibition								
CD (0.05)	1.88			1.88			3.76	
CV (%)	6.54							
Radial growth								
CD (0.05)	2.33			2.33			4.66	
CV (%)	5.34							

*Mean of three replication; Values in parenthesis are angular transformed

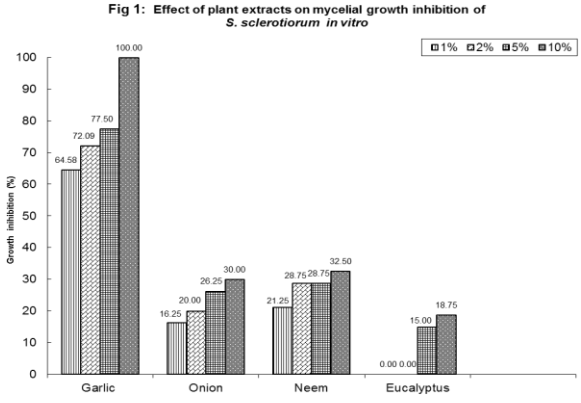


Fig 1: Effect of plant extracts on mycelial growth inhibition of *S. sclerotiorum in vitro*

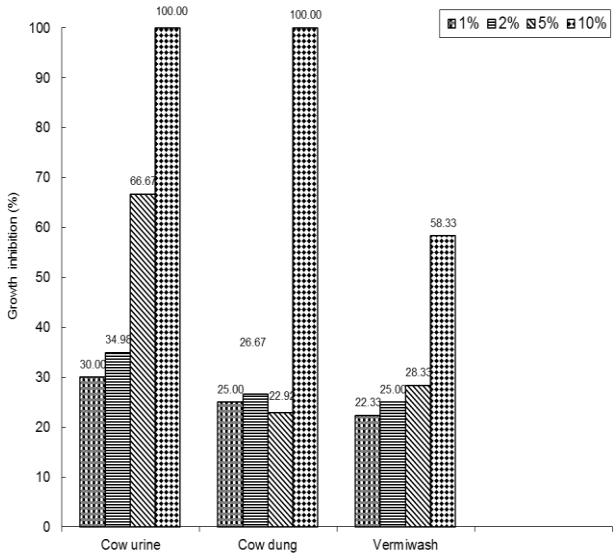
Evaluation of animal wastes

Among all the treatments viz., cow urine, cow dung and vermiwash tested in vitro the best results were obtained with the cow urine (100% inhibition) at 5 per cent concentration followed by cow dung (100% inhibition) and vermiwash (51.4% inhibition) at 10 per cent concentration (**Table 2, Fig 2**). All the treatments significantly reduced the mycelial growth of the pathogen at all the concentrations except vermiwash (at 1%). Banik*et al.* (2002) also observed antifungal activity of cow dung and cow urine against *S .sclerotiorum* in vitro. Complete mycelial inhibition of the pathogen was observed with cow urine, while 75.9 percent inhibition by cow dung which is more or less similar to the present findings.

Animal waste	Concentration (%)							
	1		2		5		10	
	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)
Cow urine	56	27.08 (31.35)	52	35 (36.20)	56	100 (90)	0.00	100 (90)
Cow dung	60	25 (29.9)	60	26.6 (31.08)	53	33.75 (35.51)	0.00	100 (90)
Vermiwash	62	17.75 (24.9)	60	25 (29.9)	58	27.5 (31.62)	33.3	58.3 (49.80)
Check	80	_	80	_	80	_	80	_
	Animal waste (A)			Concentration(B)			AXB	
Per cent inhibition								
CD (0.05)	1.59			1.84			3.19	
CV (%)	3.98							
Radial growth								
CD (0.05)	2.58			2.98			5.16	
CV (%)	6.38							

*Mean of three replication; Values in parenthesis are angular transformed

Fig 2: Effect of animal wastes on mycelial growth inhibition of *S.sclerotiorum in vitro*



Evaluation of eco-friendly nontoxic chemicals

Among all the treatments, complete mycelial inhibition (100%) was observed with sodium bicarbonate and calcium carbonate at 1 and 2 per cent concentration respectively (**Table 3, Fig 3**). However, rest of the non toxic chemicals was found ineffective at all their concentrations except oxalic acid (2%). These non toxic chemicals can be utilized as a better option for the management of *S. sclerotiorum*.

Fig 3: Effect of nontoxic chemicals on mycelial growth inhibition of *S. sclerotiorum in vitro*

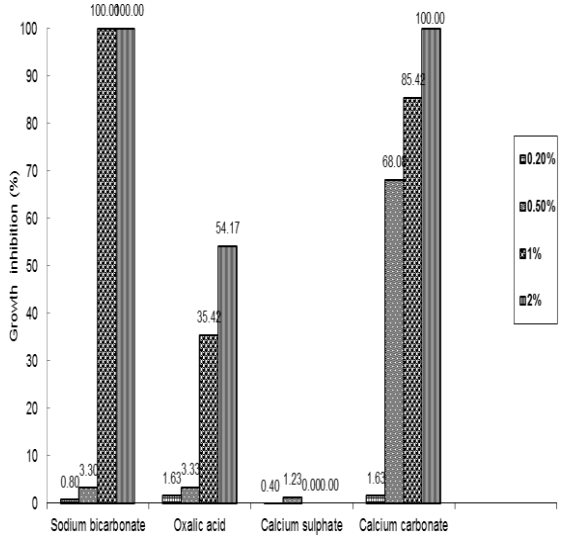


Table 3: Effect of non toxic chemicals on the growth of test pathogen under in-vitro conditions

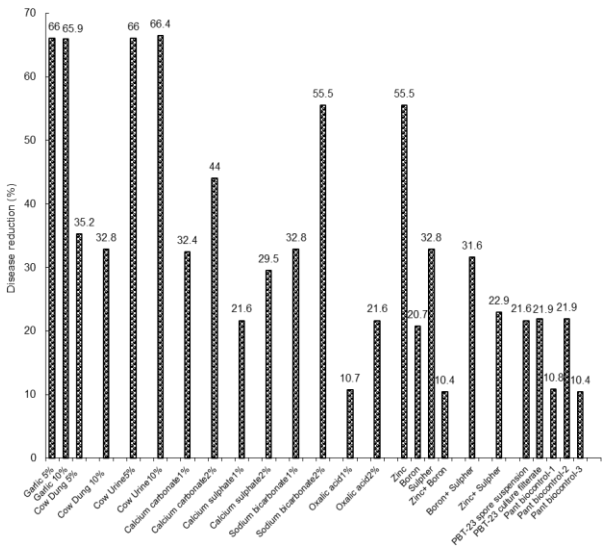
	0.2		0.5		1		2	
	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)	*Radial growth (mm)	*Inhibition (%)
Sodium bicarbonate	79.3	0.8 (4.19)	77.3	3.3 (10.42)	0.00	100 (90)	0.00	100 (90)
Oxalic acid	78.6	1.63 (7.22)	77.3	3.33 (10.37)	51.6	35.41 (36.39)	36.6	54.1 (47.39)
Calcium sulphate	79.6	0.4 (2.09)	79	1.23 (5.12)	80	0.00 (0.00)	80	0.00 (0.00)
Calcium carbonate	78.6	1.63 (5.79)	25	68.08 (55.64)	26.6	85.41 (68.06)	0.00	100 (90)
Check	80	_	80	_	80	_	80	_
	Chemical (A)			Concentration(B)			AXB	
Per cent inhibition								
CD (0.05)	2.77			2.77			5.55	
CV (%)	10.21							
Radial growth								
CD (0.05)	4.67			4.67			9.35	
CV (%)	10.58							

*Mean of three replication; Values in parenthesis are angular transformed

Evaluation of eco-friendly chemicals, bioproducts (bioagents, plant extracts and animal wastes) and micronutrients against Sclerotinia rot of mustard in field

Maximum significantly disease reduction was observed with garlic and cow urine at 5 and 10 percent with a disease incidence of 30 per cent followed by sodium bicarbonate (2%) and calcium carbonate (1 and 2%) with a disease reduction of 50 per cent. However, rest of the treatments were not much effective as they reduced the disease incidence ranged from 56.6-80 percent. All the treatments significantly reduced the disease incidence except Oxalic acid (1%), Zinc oxide, Zinc oxide+ Borax and Pant bio-control 2 and 3 which were at par with the check (**Table 4, Fig 4**).

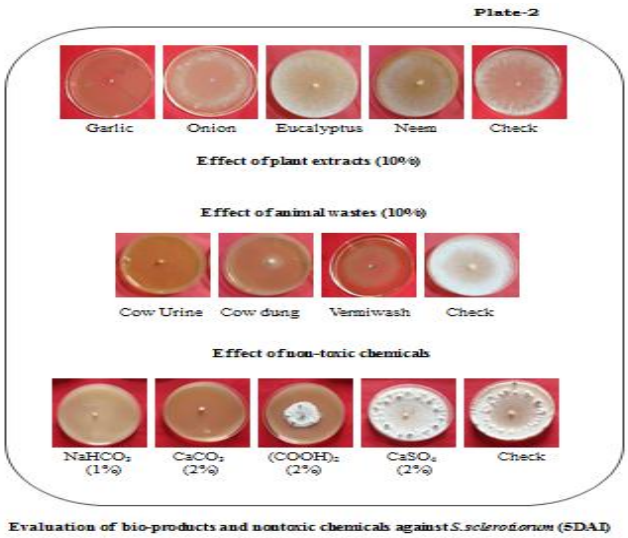
Fig 4 : Evaluation of bio-products, non toxic chemicals and micro-nutrients against Sclerotinia rot (*S. sclerotionum*) of mustard in field



*Mean of three replications; Values in parenthesis are angular transformed

Table 4: Evaluation of eco-friendly chemicals against the *S. sclerotiorum* under field condition

Treatments	*Inoculated plants (no)	*Infected plants (no)	*Disease incidence (%)	*Disease reduction (%)
Garlic 5%	10.0	3.0	30.0	66.0 (54.6)
Garlic 10%	10.0	3.0	30.0	65.9 (54.5)
Cow Dung 5%	10.0	6.0	60.0	32.7 (31.05)
Cow Dung 10%	10.0	5.6	56.0	35.1 (34.8)
Cow Urine5%	10.0	3.0	30.0	66 (54.6)
Cow Urine10%	10.0	3.0	30.0	66.3 (54.5)
Calcium carbonate1%	10.0	6.0	50.0	32.4 (34.3)
Calcium carbonate2%	10.0	5.0	50.0	43.9 (41.5)
Calcium sulphate1%	10.0	7.0	70.0	17.9 (27.3)
Calcium sulphate2%	10.0	6.3	63.0	29.5 (32.15)
Sodium bicarbonate1%	10.0	6.0	60.0	32.7 (34.8)
Sodium bicarbonate2%	10.0	4.0	40.0	55.5 (48.2)
Oxalic acid1%	10.0	8.0	80.0	10.7 (15.5)
Oxalic acid2%	10.0	7.0	70.0	21.5 (27.3)
Zinc 0.5%	10.0	8.0	80.0	10.5 (15.2)
Boron 0.5%	10.0	7.0	70.0	20.7 (22.44)
Sulpher 0.5%	10.0	6.0	60.0	32.7 (34.8)
Zinc+ Boron 0.5%	10.0	8.0	80.0	10.36 (15.34)
Boron+ Sulpher 0.5%	10.0	6.0	60.0	31.5 (33.19)
Zinc+ Sulpher 0.5%	10	7.0	70.0	22.8 (27.92)
Th. spore suspension	10	7.0	70.0	21.5 (27.34)
Th. culture filterate	10	7.0	70.0	21.9 (27.5)
T. herzianum (Pant biocontrol1)	10	7.0	70.0	21.9 (27.5)
P. flurosence (Pant biocontrol2	10	8.0	80.0	10.8 (15.7)
Th.+ *P. flurosence* mixture (Pant biocontrol3	10	8.0	80.0	10.36 (15.34)
Check	10	9.0	90.0	0.00 (0.00)
CD (0.05)		1.6	16.8	14.74
CV (%)		16.6	16.6	27.80



Evaluation of bio-products and nontoxic chemicals against *S.sclerotiorum* (5DAI)

In the present investigation the garlic bulb extracts and cow urine were found significantly superior over other treatments. The earlier reports (Singh, 1979;Chattopadhyay, 2004; Meena, 2009) on eco-friendly management of *S. sclerotiorum* are more or less in favour of the present research who reported effectiveness of garlic bulb extract against Sclerotiniarot on many crops. However, inhibitory use of cow urine is in preliminary stage against the tested pathogen (*S.sclerotiorum*) and not much of the reports are available. Banik et al. (2002) used cow urine and cow dung for the management of the pathogen and strongly supports the belief that it posses antifungal properties.

The effectiveness of micronutrients against the pathogen under field was reported by Hallock and Porter 1981 (Zinc @ 0.1-0.2% in peanut) ;Venette, 1998 (calcium sulphate @ 2.0% in bean); SangitaSahni*et al.*, 2007 and Sharma *et al.*,2007 (ZnSO4 and oxalic acid in chickpea). However in the present study these nontoxic chemical and micronutrients were found ineffective significantly in reducing the disease.

Distinct ability of bio-control agents in successful management of Sclerotinia rot of oilseed Brassica has been reported by many scientists, indicating efficacy of *T.harzianum* (Inbar, 1996; Menendez et al., 1998; Sharma *et al.*, 1999; Abdullah, 2008) and *Pseudomonas* (Savchuk*et al.*, 2002; Fernando *et al.*, 2007) isolates as a potential biological control agents against *S. sclerotiorum*. However, the results obtained in the present investigation disagree with the competency of bio-control agents as none of the bio-control agents showed any marked effect on disease reduction in the inoculated plants.

Discussion:

In this study among the bio products which were evaluated against the pathogen cow urine at 5%, garlic bulb extract at 10% and sodium bi carbonate at 1% of concentration were found effective with 100% inhibition of mycelial growth in vitro. Under field conditions among all treatments garlic bulb extract and cow urine depicted maximum reduction in disease incidence of 30%, at 5 and 10 percent respectively followed by sodium bicarbonate and calcium bi carbonate at 1 and 2 percent respectively with 50 percent disease incidence. However, rest of the treatments were not found much effective under field conditions as they showed disease incidence ranging between 56.6 to 80 percent. Hence natural or eco-friendly products were found most effective in managing sclerotinia rot disease in lab as well as under field conditions can be a potential substitute of chemicals in managing scletotinia rot. It will not only help in reducing hazardous effects of chemicals on environment and human health but will also ensure sustainable development of agriculture in long run .So it can be a potential and eco-friendly alternative for chemicals in managing this disease for future use.

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