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Evaluation of botanical extracts, animal wastes, organic and inorganic salts, micronutrients and bio-agents against *Sclerotinia sclerotiorum* (Lib) *de Bary* a cause of Sclerotinia rot of rapeseed-mustard under field conditions

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ABSTRACT
Rapeseed-mustard crop occupies a premier position among oilseed crops with 25 per cent of total oil seed production. Among all the diseases affecting this crop, sclerotinia stem rot is emerging as very serious constraint in the production and productivity of rapeseed-mustard across the country. Due to increasing concern for environment and health, need for the replacement of fungicides with safe and eco-friendly management approaches has been arisen. So the present experiment was carried out for the evaluation of botanical extracts, animal wastes products, organic and inorganic salts, micronutrients and bio-agents against sclerotinia rot under field conditions. Botanical extract (garlic bulb, onion bulb) and animal waste products (cow urine, cow dung) were evaluated at 5 and 10 % concentration, organic and inorganic salts eg. sodium bicarbonate, oxalic acid, calcium carbonate and calcium sulphate at 1 and 2% concentration, micronutrients eg. borax, sulphur, zinc oxide and their combinations at 0.5 % concentration, bio agent eg. Trichoderma, Pseudomonas and their combinations at 0.5 % concentration in different formulations as spore suspension, culture filtrate and dust were tested against artificially inoculated pathogen in field. Among all the treatments garlic bulb extract and cow urine depicted maximum reduction in disease incidence (65.9 and 66%) at 5 percent concentration and (66.0 and 66.3%) at 10 percent concentration respectively followed by sodium bicarbonate (55.5% disease reduction) and calcium carbonate (43.9% disease reduction) 2 percent concentration. However, rest of the treatments were not found much effective against the disease with disease reduction ranging between 32.7 to 10.4 percent. Hence, natural or eco-friendly products such as cow urine and garlic bulb extracts can further be utilized to develop Integrated Disease Management (IDM) module for chemical free, safe, eco friendly and affordable management of sclerotinia rot disease in future.
Keywords: *Rapeseed-mustard, Sclerotinia rot, percent disease reduction, management.*

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INTRODUCTION

Oilseed Brassica plays vital role in edible oil production on India. Among them rapeseed mustard cop occupies a premier position accounting for 25 per cent of total oil seed production. This crop is known to be attacked by a number of plant diseases such as Alternaria blight, downey mildew, white rust, Sclerotinia stem rot etc. Among all this, Sclerotinia stem rot is emerging out to be a major constraint to production and productivity of rapeseed mustard in India with 40-72 per cent yield loss under favourable condition [1,2,3]. Sclerotinia rot of Indian mustard (*Brassica juncia*) caused by *Sclerotinia sclerotiorum* (Lib.) de bary has been reported from major rapeseed and mustard growing areas of the world [4,5,6).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 [7,8,1,3]. This pathogen has a wide host range with infecting around 400 plant species (9). Still not much effective and economical control measures for the disease management are known yet.The explosive pathogenicity of the fungi under favourable conditions and the ability of the pathogen to withstand adverse climatic conditions with the help of sclerotia, which is the preferred structure for overwintering and for long term survival in field conditions it has become a potential pathogen for many

crops. Heavily infected crop can produce a large number of sclerotia which ensures the progression of diseases for longer duration. Earlier many researchers have reported fungicides to be an effective management method for Sclerotinia rot [10, 11] and many other scientists reported fungicides and biological control methods to be effective against the disease. Though fungicides have become the integral part of the management of this disease at present but increasing concern for the health and environment due to ill effects of these chemicals the necessity for finding out the safe and eco friendly alternative for fungicides has arisen. So use of such fungicides need to minimized due to their residual toxic effect and wide spectrum activity. The continuous use of these potentially hazardous chemicals is posing an increasing threat to environment as pesticide contamination 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. So bio products such as plant extract, animal waste, bio agents along with non toxic compounds such as, micro nutrients, organic and inorganic salts which have been found effective by other researchers during *in vitro* studies were evaluated against the pathogen under natural/field conditions. As compounds which have been found effective in inhibiting the growth of the pathogen *in vitro*, need to be tested under natural conditions as well for preparing farmer friendly, cost effective IDM module for the management of Sclerotina stem rot of mustard.

MATERIAL AND METHODS

The field experiment was conducted during the Rabi season at Norman E. Borlaug Crop Research Centre of GBUA&T Pantnagar, Uttarakhand, to study the effects of bioproducts (Botanical extracts, animal wastes), organic and inorganic salts, micronutrients and bio agents as seed treatment and/or foliar spray for the management of *Sclerotinia* stem rot of mustard under field conditions. The field experiment was laid out in a randomized block design with 18 treatments under bioproducts (Botanical extracts, animal wastes), organic and inorganic salts, micronutrients and bio agents each with 3 replication with a plot size of 3x1.5 m^2 area. Sowing of *Brassica juncea* cultivar Varuna was done.

Method of application:

Two application methods, seed treatment and foliar spray were adopted with the same treatmentsfor the effective management of the disease.

Seed treatment

To study the effect of plant extracts (garlic bulb) and animal wastes (Cow urin, cow dung), bio-agent (spore suspension and culture filterate) against *S. sclerotiorum*, seeds were dipped in the extracts/formulation for 1 hour before sowing. Organic and inorganic salts (oxalic acid, calcium carbonate, calcium sulphate, sodium bi carbonate), micronutrients (zinc, boron, sulphur) and bio-agents viz. *Trichoderma harzianum* (Pant biocontrol 1), *Pseudomonas flurosence* (Pant biocontrol 2) and *T. harzianum* + *Pseudomonas flurosence* (Pant biocontrol 3) obtained from Biocontrol laboratory, Department, Plant Pathology, GBPUA&T, Pantnagar were used as dust/powder form for seed treatment. Plots with untreated seeds were served as check.

Foliar spray:

Foliar spray of all the treatments was done first at 30 days and second at 45days after sowing by preparing formulations of desired concentration for spray. Spraying was done during evening hours with the help of knapsack sprayer.

Preparation of plant extracts and animal wastes

Plant extracts and animal wastes solutions were prepared by crushing (plant part) or by mixing animal waste in water (1:1 w/v). The mixture was passed through muslin cloth. Desired concentration was made by mixing calculated volume of plant extract/animal waste solution into calculated volume of water.

Preparation of organic-inorganic salts and micronutrients solution:

Organic salt (Oxalic acid), inorganic salts (calcium carbonate, calcium sulphate, sodium bi carbonate), Micronutrients viz. Zn, S and B were applied in the form of Zinc oxide, Zinc sulphate and Borax and used as a spray. The desired concentration of the following was prepared by mixing calculated amount of salt and calculated amount of available forms of micronutrients (a.i.) in the product, into calculated volume of water.

Preparation of bio-agents:

Bio-control agents viz., *Trichoderma herzianum* (Pant biocontrol1), *Pseudomonas flurosence* (Pant biocontrol 2) and *Trichoderma harzianum*+ *Pseudomonas flurosence* (Pant biocontrol3)were used as spore suspension for spray by dissolving calculated amount of bioagent formulation into calculated volume of water.

Preparation of spore suspension of PBT 23 (*Trichoderma harzianum*)

Spore suspension was prepared from *T. harzianum,* PBT 23, culture(obtained from oil seed pathology laboratory) inoculated on sterilized Jhangora grains incubated at 26±1^{0}C for 15 days. The fully colonized grains were suspended into sterilized distilled water was filtered out through muslin cloth several times. The spore concentration (10^8 spores/ml) in the filtrate was adjusted by the aid of haemocytometer.

Preparation of Culture filtrate of PBT 23 (*T.harzianum*)

The Culture filtrate was obtained by filtering out the mycelial mat/cell suspension of the *T.harzianum,* multiplied in potato broth 10 days after incubation at 26±1^{0}C through muslin cloth. Desired concentration was made by mixing calculated volume of culture filtrate into calculated volume of water.

Inoculation of the 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:

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

Where,

DI = Disease incidence (%)

RESULTS AND DISCUSSION

Among all the treatments garlic bulb extract and cow urine depicted maximum reduction in disease incidence (65.9 and 66%) at 5 percent concentration and (66.0 and 66.3%) at 10 percent concentration respectively followed by sodium bicarbonate (55.5% disease reduction) and calcium carbonate (43.9% disease reduction) 2 percent concentration. However, rest of the treatments were not found much effective against the disease with disease reduction ranging between 32.7 to 10.4 percent. Oxalic acid (1%), Zinc oxide, Zinc oxide+ Borax and Pant bio-control 2 and 3 were found at par with the check (Table 1, Fig1).

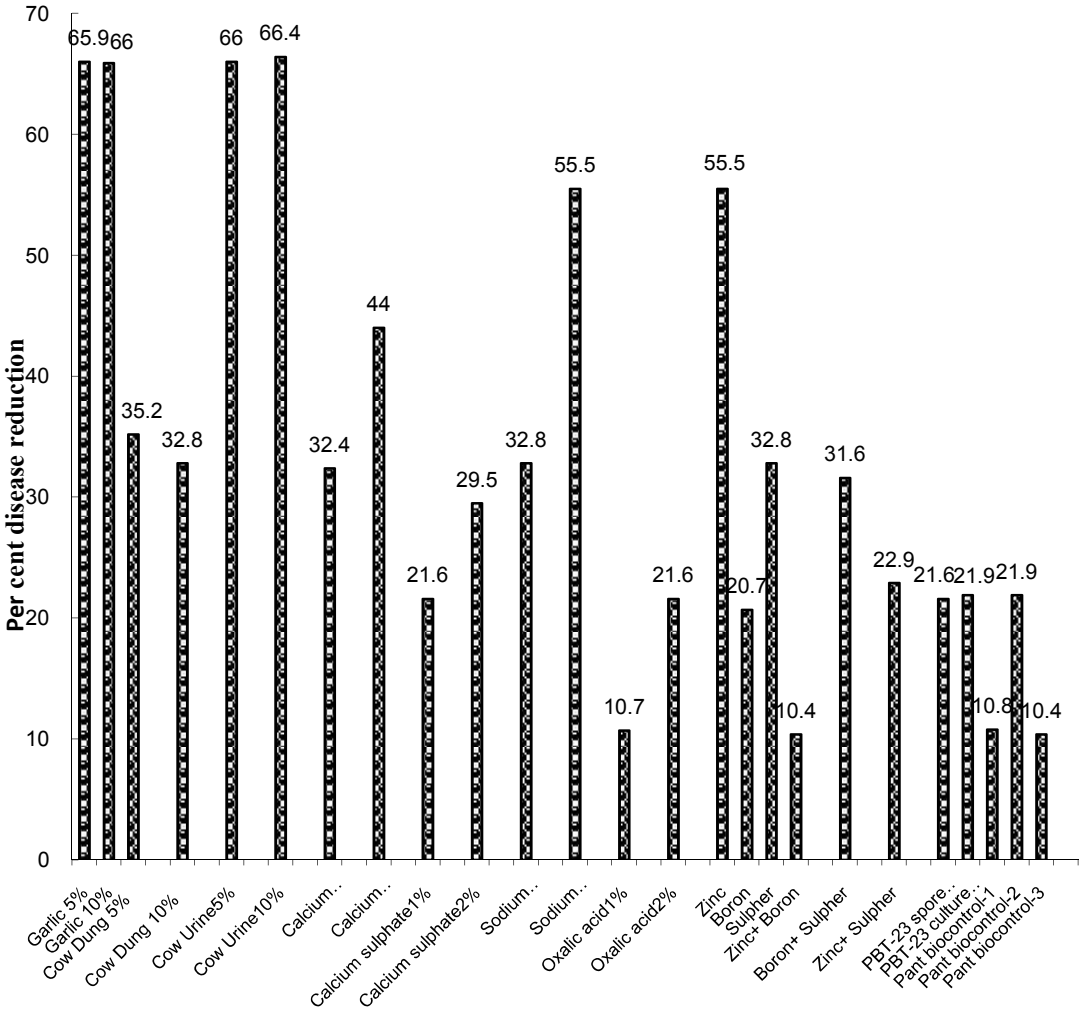
In the present investigation the garlic bulb extracts and cow urine were found significantly superior over other treatments. The earlier reports [12,2,13,14] on eco-friendly management of *S. sclerotiorum* are more or less in favour of the present research who reported effectiveness of garlic bulb extract 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. (15) used cow urine and cow dung for the management of the *Sclerotinia sclerotiorum* in cucumber and strongly supports the belief that it posses antifungal properties. The effectiveness of micronutrients against the pathogen under field was reported by [16]Hallock and Porter 1981 (Zinc @ 0.1-0.2% in peanut, calcium sulphate @ 2.0% in bean); (17) (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* [18,19,20] and *Pseudomonas* [21,22] 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.

Table 1: Evaluation of botanical extracts, animal wastes, organic and inorganic salts, micronutrients and bio-agents against *Sclerotinia sclerotiorum* (Lib) *de Bary*

Treatments	*Inoculated plants (no)	*Infected plants (no)	*Disease incidence (%)	*Disease reduction (%)
Garlic 5%	10.0	3.0	30.0	65.9 (54.5)
Garlic 10%	10.0	3.0	30.0	66.0 (54.6)
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	66.0 (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

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

Fig. Evaluation of bio-products, organic-inorganic salts, micro-nutrients and bio agents against Sclerotinia rot (*S. sclerotionum*) of mustard in field



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