

Original Research Article

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***In-vitro* Effect Evaluation of Botanicals against *Sclerotinia sclerotiorum* (Lib.) De Bary, Caused Stem Rot Disease in Rapeseed-Mustard**

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ABSTRACT

Mustard (*Brassica juncea* (L.) de Bary) is one of the most important oilseed crops in India. It's cultivated in various states of India and around the world. It is grown as a traditionally crop (Pure crop) as well as intercrop (mixed crop) in marginal and sub-marginal soils in the some part of eastern, northern and north western region of the Indian states. Its favourable climate is cool and moist weather situation during the period of winter season, for its better growth and as well as its productivity. Many biotic and abiotic factors are involved to decrease the yield of rapeseed- mustard in every year. Among them, Stem rot (*Sclerotinia sclerotiorum*) disease is the most destructive fungal disease that causes maximum damage in rapeseed-mustard. The first symptom of *Sclerotinia* stem rot was seen as water soaked patches on leaves and on root of the plant at its flowering stage. Madhya Pradesh is an important rapeseed mustard producing state of India contributing more than 10 per cent of the total production in the country.

Keywords

Botanicals, *In-vitro*,
Sclerotinia sclerotiorum and
Stem rot

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Introduction

In India mustard group of crops are the most important oilseed crops during Rabi season. These crops are grown both in subtropical and tropical countries. India occupies the first position with regards to average and production of rapeseed-mustard in the world.

In India, rapeseed-mustard crop are cultivated on an area of 6.45 million ha with a production of 7.28 million tones and with an average yield 1128 kg/ha (Anonymous, 2015). In Madhya Pradesh rapeseed-mustard crops are cultivated in an area about 7.12 lac hectares with the production of 7.36 lac metric tons and productivity 1054 kg/ha.

(Anonymous, 2009), out of this Morena, Bhind and Gwalior jointly contribute >60% production of these crops in the state.

The first record of its occurrence on rapeseed-mustard was made from India. White rust, *alternaria* blight, white blight, downy mildew and powdery mildew are the important diseases of rapeseed-mustard in India. Among all diseases *Sclerotinia* stem rot (white blight) was earlier considered as a minor disease but during the last two decades its problem has been increased in several rapeseed and mustard growing areas of country. The disease has also been reported from Canada, China, Denmark, Finland, France, Sweden, U.K., Brazil and U.S.A. (Kolte,1988). *Sclerotinia* stem rot of mustard (*Brassica juncea*) is becoming a problem in some parts India viz. Madhya Pradesh, Uttar Pradesh, Rajasthan, Bihar, Haryana and Himachal Pradesh. In Madhya Pradesh the disease has become an important biotic constraint in several locations of Chambal and Gwalior division. Sharma and Kapoor (1997) reported the inhibition of mycelium growth of *Sclerotinia sclerotiorum* by the extract of *Lantana camera*. The extract of neem (*Azadirachta indica*) inhibited *Sclerotinia sclerotiorum* under *in-vitro* condition (Sharma and Basandrai, 1999). Kapil and Kapoor (2005) evaluated six neem-based bio-pesticides at two concentrations (0.5 and 0.1%) against *S. sclerotiorum* was completely inhibited the mycelial growth at both the tested concentrations while neem-gold completely inhibited the growth at higher concentration (0.5%). Bajoriya (2007) reported that eucalyptus oil @ 5% concentrations as the most effective for the growth inhibition of *Sclerotinia sclerotiorum* under *in-vitro* condition, followed by calotropis leaf, eucalyptus leaf, tobacco leaf, neem leaf, neem bark, *Trichoderma viride*, garlic bulb, neem oil, *lantana* leaf and *Bacillus sp.*

The fungus survives in soil for a long duration in the form of sclerotia. Therefore the chemical control may not be amuch more effective pattern for the management of that disease then, theuse of some botanicals extracts, these are partially inhibit the germination of Sclerotia, hence the botanicals may be evaluated and standardized in search of a component management strategy.

Materials and Methods

Experimental site

The present studies were conducted in the Department of Plant Pathology, College of Agriculture, Gwalior and Zonal Agricultural Research Station, Morena during Rabi season of 2009-2010.

Test organism

Sclerotinia sclerotiorum (Lib) De Bary, the causal organism of the sclerotinia stem rot of rapeseed-mustard was isolated from the infected plant samples, which was collected from the farmers and experimental fields during the investigation. These samples were brought in the laboratory and after its surface sterilization; the pathogen was isolated and inoculated in to potato dextrose agar slant under aseptic condition. After inoculation, these slants were incubated at 25°C for 48 hours and there after the culture was purified and used in the present study

Survey

The cultivator fields of Morena, Bhind and Gwalior were surveyed to find out the incidence of *Sclerotinia* stem rot on rapeseed-mustard. For such survey six villages from each district were randomly selected and from each village three fields were randomly selected.

In each field the incidence of *Sclerotinia* stem rot was recorded on five 1m² patches by counting the infected plants and total number of plants then the incidence per cent was calculated as given formula.

Disease incidence (%) = Infected plants/ Total number of plants x 100

Symptomatology

The symptoms of white blight of mustard were studied on experimental and farmers' fields. The infected plant was closely examined for external and internal symptoms.

Botanicals

The botanicals viz., Neem (*Azadirachta indica*), Nilgiri (*Eucalyptus isobus*), Tobacco (*Nicotinatabacum*), Oak (*Calotropisprocera*), Datura (*Daturastramonium*), Tessu (*Lantana camera*), Beshram (*Ipomeabatata*), Tulsi (*Ocimum* sp.), Garlic (*Allium sativum*), Mint (*Menthacitrata*) and Turmeric (*Curcuma longa*), were evaluated against the pathogen by using poisoned food technique.

Evaluation of inoculation methods

Following inoculation methods were evaluated to find out the most effective method for artificial inoculation of the pathogen.

Tooth pick method: A mycelial disc of 5 cm diameter was taken from 7 c with the help of sterilized cork borer grown on PDA medium, placed in road hole made at the base of the plant and covered with absorbent, cotton inoculum placement method: In this method the stem of 45 days old mustard plant was scratched at the height of 10-12" from soil level. On the scratched portion the inoculum was placed with the help of cotton and it was covered with cello tape so that it remains in the contact with the stem.

Inoculation with Sclerotia: In this method the stem of 45 days old mustard plant was scratched at the height of 10-12" from soil level. On the scratched portion the sclerotia was placed with the help of cotton and it was covered with cello tape so that it remain in the contact with the stem.

Paraffin wax film: In this particular method surface of stem was rubbed and 7 days old culture was applied on rubbed surface. This was later covered by paraffin wax film and left for the appearance of symptoms.

Control (without inoculation)

At the time of inoculation some of disease free plants were tagged and left un-inoculated for comparison with inoculation methods.

The experimental details are as follows,

Treatment (Inoculation methods)	— 5
Replications	— 4
Design	— RBD
No. of plants selected / treatment/ replication	— 20

The per cent disease incidence was calculated as per the above given formulae.

Results and Discussion

Survey

The data summarized in Table-1 reveals that the incidence of sclerotiniastem rot in Morena, Bhind and Gwalior districts was 14.64, 3.17 and 2.76 per cent, respectively and an average 6.86% incidence of sclerotinia stem rot was recorded in northern Madhya Pradesh comprising Morena, Bhind and Gwalior districts.

The disease incidence was higher in Morena district and it was varied form 11.20 % to 21.61 % in the surveyed locations/ villages of Morena district, The maximum incidence was recorded in Dimni (21.61%) followed by

Ranpur (19.17%), Mudikheda (12.63%), Jeegni (11.32%), Khusgaon (11.42%) and Khedamebda (11.20%). The two locations of Bhindviz, Cheerol and Gorai were free from the disease, while it's maximum incidence (6.94%) was recorded in Cheemak and Lotanpur followed by 2.58% in Madiayan and Checchai. Maharajpura and Khurari locations of Gwalior district were free from the disease while its maximum incidence was recorded in Jalalpur (6.48%) followed by Badagaon (4.17%), Tekanpur (3.33%) and Jujharpur (2.58%).

Symptomatology

The symptoms of white blight of mustard caused by *Sclerotinia sclerotiorum* were observed from pre flowering stage to harvesting stage in Gird Zone. Two types of symptoms viz. external and internal symptoms were observed.

External symptoms

The first symptom of sclerotinia stem rot was noticed as water soaked patches on the main stem of plant at flowering stage of the crop, which soon turned into whitish brown large patches on the main stem, branches and inflorescence under severe infection the stem and branches of the crop shows brownish appearance along with defoliation of lames and the crop fell down.

The portion above the affected parts gets ultimately wilted or broken down. The surface of affected parts including stem, leaf, branches and pods were covered with white creeping strands of the fungal mycelium and under severe infection the black sclerotia were also observed on the mycelia mat. The Sclerotia were also observed on the root of severely infected plant.

Internal symptoms

The internal symptom was observed by spitting tine diseased part parts at different stages of 'infection. The stem becomes hollow and they were having irregular sized sclerotia were observed. The sclerotia were pink in the early stages of development but later turned black and become smooth. The small sized sclerotia were usually rounded but large sized sclerotia were flatter irregular in shape.

The affected tissues when examined microscopically showed a hyaline mycelium in the intra and intercellular spaces. The middle lamella was destroyed and the tissues looked very much disintegrated due to maceration of the cell walls. Roots were not affected internally. When the roots of the diseased plants were examined, the mycelium and sclerotia were not found in the pith region.

***In-vitro* evaluation of botanicals against test pathogen (*Sclerotinia sclerotiorum*)**

The botanicals viz., Neem, Nilgiri, Tobacco, Oak, Datura, Tessu, Beshram, Tulsi, Garlic, Mint, and Turmeric were evaluated in the form of powdered extract @ 10% against *S. sclerotiorum* under *in-vitro* condition. The fungal growth was recorded at 2, 3, 4, 5 and 6 days after inoculation and the data are summarized in (Table-2), which indicate that all the treatments were significantly superior over control. At 6 days after inoculation Oak was found most effective (17 mm growth) followed by Nilgiri (20 mm), Tobacco (26 mm), Besharm (27 mm), Neem (30 mm), Mint (52 mm), Garlic (60 mm), Turmeric (61 mm), Datura (65 mm), Tessu (72 mm) and Tulsi (75 mm) while a maximum of 90 mm growth was recorded in control.

Table.1 Incidence of *Sclerotinia* stem rot of mustard in Morena, Bhind and Gwalior during 2009-10

Sl.No.	Districts	Villages (Locations)	Incidence (%)
1	Morena	Mudikheda	12.63
		Jeegni	11.82
		Khedamebda	11.20
		Khusgaon	11.42
		Ranpur	19.17
		Dimini	21.61
		Mean	14.64
2	Bhind	Madiayan	2.58
		Cheerol	0.00
		Cheemak	6.94
		Lotanpur	6.94
		Gorai	0.00
		Checchai	2.58
		Mean	3.17
3	Gwalior	Jalalpur	6.48
		Maharajpura	0.00
		Jujharpur	2.58
		Tekanpur	3.33
		Badagaon	4.17
		Khurari	0.00
		Mean	2.76
		Over all mean	6.86

Table.2 *In-vitro* evaluation of botanicals against *Sclerotinia sclerotiorum*

Sl.No.	Treatments *	Radial growth of fungal mycelium in mm at DAI				
		2 DAI	3 DAI	4 DAI	5 DAI	6 DAI
1	Neem	5	11	19	25	30
2	Nilgiri	0	8	13	15	20
3	Tobacco	0	5	7	15	26
4	Oak	0	0	8	13	17
5	Datura	8	20	39	53	65
6	Tessu	0	8	12	21	27
7	Beshram	0	18	32	55	72
8	Tulsi	14	32	46	58	75
9	Garlic	18	28	37	46	60
10	Mint	0	15	25	41	52
11	Turmeric	20	30	38	47	61
12	Control	35	68	85	90	90
	SE(m)±					1.847
	CD at 5%					5.453

Botanicals were used in the form of powdered extract @ 10gr/0
DAI = Days after inoculation

Oak (*Calotropis procera*) was significantly superior over all from other botanicals except Nilgiri. Nilgiri (*Eucalyptus indica*) was found significantly superior to Beshram, Neem, Mint, Garlic, Turmeric, Datura, Tessu and Tulsileaf extract, and at par with Oak and Tobacco.

Survey

In present study the cultivator's field of Morena, Bhind and Gwalior were surveyed to find out the incidence of Sclerotinia stem rot on rapeseed-Mustard. The incidence of Sclerotinia stem rot in Morena, Bhind and Gwalior districts was 14.64, 3.17 and 2.76% respectively with the mean of 6.86% incidence of disease in northern Madhya Pradesh comprising Morena, Bhind and Gwalior Districts. The disease incidence was higher in Morena district where it was in the range of 11.20% (Khedamebda) to 21.61% (Dimni). The two locations of Bhind viz., Cheerol and Gorai were free from the disease, while its maximum incidence (6.94%) was recorded in Cheemak and Lotanpur followed by 2.58% in Madiayan and Checchai. Maharajpura and Khurari locations of Gwalior district were free from the disease while its maximum incidence was recorded in Jalalpur (6.48%) followed by Badagaon (4.17%), Tekanpur (3.33%) and Jujharpur (2.58%).

Dhakad (2006) also surveyed mustard fields of northern Madhya Pradesh and reported 3.34, 0.36 and 0.39% incidence of sclerotinia stem rot in Morena, Bhind and Gwalior districts respectively with the overall mean of 1.07% in northern Madhya Pradesh. This indicate that incidence of the disease was low earlier which has been sharply increased. The pathogen survived in soil for long duration and monoculture practice along with the application of high doses of nitrogenous fertilizers may rise in this sharp increase in the incidence of the disease.

Symptomatology

The first symptom of sclerotinia stem rot was noticed as water soaked patches on the main stem of plant at flowering stage of the crop, which soon turned into whitish brown large patches on the main stem, branches and inflorescence. Under severe infection the stem and branches of the crop showed brownish appearance along with defoliation of leaves. The severely infected tissues of the plant become soft and they easily peeled off into shreds. The lesions finally girdled the stem completely and the crop fell down. The portion above the affected parts gets ultimately wilted or broken down. The sclerotia were also observed on the root of severely infected plant. The internal symptom was observed by splitting the diseased plant parts under different stages of infection. The pith of the stem becomes hollow in which irregular sized sclerotia were observed. The sclerotia were pink in the early stages of development but later turned black and become smooth. The small sized Sclerotia were usually rounded but sclerotia were flatter irregular in shape.

The present findings have a similar results just like of the finding of Sikarwar (1987) reported elongated water soaked spots of the disease the stem of plant, which later get covered by cottony mycelial growth of the fungus. The affected stem becomes soft and easily peeled off into shreds. The lesions finally girdle the stem from where it breaks and the plant dries.

In-vitro evaluation of botanicals against *Sclerotinia sclerotiorum*

The result of present study reveals that the botanicals viz., Neem, Nilgiri, Tobacco, Oak, Datura, Beshram, tessu, Tulsi, Garlic, Mint, and Turmeric in the form of powdered extract @ 10% significantly inhibited the growth of

S. sclerotiorum under *in-vitro* condition. Oak was found most effective followed by Nilgiri, Tobacco, Beshram, Neem, Mint, Garlic, Turmeric, Datura, Tessu and Tulsi. Oak was significantly superior over all other treatments except eucalyptus. Nilgiri was found significantly superior over Beshram, Neem, Mint, Garlic, Turmeric, Datura, Tessu and Tulsi extract, but at par with Tobacco.

The present finding is supported by Shivpuri *et al.*, (1997) who reported fungi-toxic effect of plant extracts from 10 plant species (*Allium cepa*, *A. sativum*, *Azadirachtain*, *Calotropis procera*, *Datura stramonium*, *Ocimum sanctum*, *Polyalthia longifolia*, *Tagetes erecta*, *Vince rosea* and *Glihadlasomnifera*) againsts five pathogenic fungi viz., *Alternaria brassicicola*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

Summary and conclusion is as follows:

Rapeseed-mustard group of crops play a vital role in Indian economy. It ranks second among the oilseed crops after groundnut. These groups of crops are vulnerable to major diseases such as Alternaria blight, Sclerotinia blight (White blight), White rust, Downy mildew and Powdery mildew and cause severe losses in yield. Continuous cultivation of mustard crop over the years in different parts of the country lead to the problem of sclerotinia stem rot.

Sclerotinia blight (*Sclerotinia sclerotiorum*) has become a potentially important disease in northern Madhya Pradesh comprising Morena, Bhind and Gwalior. Since the fungus survives in soil for long period in the form sclerotia, hence the chemical control may not be very effective for disease management. Plant extracts, buttermilk and Cow urine etc. may inhibit the growth of pathogen. In view of the above facts, the present study, was

carried out in the Department of Plant Pathology, College of Agriculture, Gwalior and Z.A.R.S., Morena during rabi season of 2009-10 and the results are summarized as follows:-

The incidence of sclerotinia stem rot in Morena, Bhind and Gwalior districts was 14.64, 3.17 and 2.76% respectively with the mean of 6.86% incidence of disease in northern Madhya Pradesh. The disease incidence was higher in Morena district where it was in the range of 11.20% (Khedamebda) to 21.61% (Dimni). In Bhind district the maximum disease incidence was recorded in Cheemak and Lotanpur 6.94% while its maximum incidence in Gwalior was recorded in Jalalpur (6.48%).

The first symptom of sclerotinia stem rot was noticed as water soaked patches on the main stem of plant at flowering stage of the crop, which soon turned into whitish brown large patches on the main stem, branches and inflorescence. Under severe infection the stem and branches of the crop shows brownish appearance along with defoliation of leaves. The severely infected tissues of the plant become soft and they easily peeled off into shreds. The lesions finally girdled the stem completely and the crop fell down. The sclerotia were also observed on the root of severely infected plant. The internal symptom was observed by splitting the diseased plant parts under different stages of infection. The pith of the stem becomes hollow in which irregular sized sclerotia were observed. The sclerotia were pink in the early stages of development but later turned black and become smooth. The small sized sclerotia were usually rounded but large sized sclerotia were flatter irregular in shape.

All the tested botanicals in the form of powdered extract @ 10% significantly inhibited the growth of *S. sclerotiorum* under

in-vitro condition. Oak was found most effective followed by Nilgiri, Tobacco, Beshram, Neem, Mint, Garlic, Turmeric, Datura, Tessu and Tulsi. Oak was significantly superior over all other treatments except eucalyptus. Nilgiri was significantly superior over Beshram, Neem, Mint, Garlic, Turmeric, Datura, Tessu and Tulsi extract, but at par with Tobacco.

Leaf extracts of Neem, Nilgiri, Tobacco, Oak and Beshram significantly inhibited the growth of *S. sclerotiorum* in all four tested concentrations (5, 10, 15 and 20%) but none of them could absolutely inhibited the growth although their effectively gradually increased with the increase in concentration . The maximum inhibition was recorded under the treatment Oak leaf extract followed by Tobacco, Nilgiri, Beshram and Neem, while maximum growth was recorded in control. Oak leaf extracts significantly superior over Neem, Beshram and Nilgiri but was at par with Tobacco leaf extract.

In conclusions, the entire investigation carried out was successful completed and reflected in the following conclusions drawn there from:

Sclerotinia stem rot (white blight) is an important disease of mustard in northern Madhya Pradesh. It incidence in Morena, Bhind and Gwalior districts was 14.64, 3.17 and 2.76% respectively.

The first symptom of the disease is the appearance of water soaked patches on the main stem of plant at flowering stage, which soon turned into whitish brown large patches. The pith of the stem becomes hollow containing irregular sized sclerotic which were pink in colour but later turned to brown black in colour.

Leaf extract of botanicals (Neem, Nilgiri, Tobacco, Oak and Beshram) @ 5, 10, 15, and

20% significantly inhibited the growth of *S. sclerotiorum* under *in-vitro* condition and their effectively has been increased with the increase in the concentration.

Among the botanicals the Oak (*Calotropes procera*) leaf extract was found most effective.

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