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MANAGEMENT OF WHITE MOLD DISEASE OF CABBAGE (*BRASSICA OLERACEA* VAR. *CAPITATA*) THROUGH DIFFERENT PLANT EXTRACTS AND CHEMICALS

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ABSTRACT

The cabbage (*Brassica oleracea* var. *capitata*) is an herbaceous annual or biennial vegetable in the family Brassicaceae grown for its edible head. The cabbage white mold is one of the destructive diseases prevalent worldwide. The present study reports the finding of four fungicides and botanicals for the management of the pathogen. The efficacy of four fungicides was evaluated against the pathogen *Sclerotiorum sclerotiorum* (Lib) de Bary on PDA medium through the poisoned food technique. The recorded data advocated that the percentage inhibition of the fungal growth increased as the concentration of the fungicides increased. According to the results, fungal mycelial growth inhibition ranged from 92.91% to 34.69% after 96 hours. Similarly, the efficacy of three plant extracts was examined under laboratory conditions at four concentration levels viz. S, S/25, S/50 and S/75 by poisoned food technique on PDA. The radial growth of the fungus was recorded after 48 hours, 72 hours, and 96 hours of incubation. The inhibition percentage was measured and the results revealed that the garlic extract among all the three tested plant extracts was the most effective to inhibit the mycelial growth (49.54, 58.67, and 73.90%) of *S. sclerotiorum* at S/50, S/25, and S respectively after seven days followed by neem leaf extract (32.77, 44.97 and 61.73%). Leaf extract of safeda gave the least inhibition percentage of fungal growth of *S. sclerotiorum*.

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INTRODUCTION

The vegetables are one of the most important and cheap components of a balanced diet, which people now realize due to their high nutritious values necessary for the body. The increasing demands of brassicaceous vegetables in the market reveal its importance. Economically *Brassica* is the most primary genus in the Brassicaceae family (Srivastava et al., 2010). Different genera of cabbage, broccoli, cauliflower, Brussels sprouts, and kale are included in *Brassica* vegetables, which are consumed all over the world (Podsędek, 2007). Moreover, these vegetables also possess both antioxidant and anti-carcinogenic properties (Coe, 2002). Cabbage (*Brassica capitata* L.) is an important vegetable crop of *Brassicaceae* family cultivated in both tropical and temperate areas of the globe. Cabbage is a delicate crop and in Pakistan it is grown in winter season. Unfavorable environmental conditions damage the crop mostly at vegetative and reproductive stages. Their cultivation is badly affected by many factors in which diseases have significant role (Kashyap and Dhiman, 2010). The total area covered by cabbage cultivation in 2017 was 4.72 thousand hectares; total production was 69.08 thousand tons while the average yield was 14.69 tons per hectare. Brassica crops are heavily challenged by numerous pathogens and insects,

whereas microorganisms and infective agents have very little impact on their yield. The crop mainly suffers from fungal and bacterial diseases such as leaf spot (*Alternaria alternata*), alternaria blight (*A. brassicae* and *A. brassicicola*), black leg (*Phoma lingam*), mould disease (*Botrytis cinerea*), ring spot (*Mycosphaerella brassicicola*), downey mildew (*Peronospora parasitica*), storage rot (*Fusarium avenaceum*), soft rot (*Sclerotinia sclerotiorum*), stalk rot (*Rizoctonia soloni*), damping-off (*Pythinium aphanidermatum*) throughout the world (Bains et al., 1981; Chakrabarty et al., 1989; Gupta and Shyam, 1995; Sharma and Sain, 2005).

From all of these, Sclerotinia stem rot (*Sclerotinia sclerotiorum*) is a severe disease of various cruciferous crops and often causes a serious damage to the commercial production of these crops worldwide (Uloth et al., 2014).The fungus is a high threat to crop yield and has ability to minimize the seed yields by 88-90% (Sharma, 1979). Present investigation showed that the disease also affect the seed production of cabbage in many states. Optimum temperature and wet conditions allow the pathogen to attack the plants. *S. sclerotiorum* comprises a widespread host range and may remain active in soil under harsh climatic situations. Many fungicides were evaluated successfully against the pathogen however their prolonged use had several health issues in addition to contaminating the soil and spring water. As this pathogen has gigantic host range and remain active in soil for long period, crop rotation did not solve the problem completely (Nelson, 1998). So there is need to adopt such strategies which minimize the threat and cause of this disease on crop yield. White mold disease (also called as watery soft rot, cottony rot) affects more than 370 species of plants covering 64 plant families. It infects plants grown in fields and in greenhouses all over the world and is more prevailing in the cool, wet regions of the world. Depending on the host (crop as well as weed host) it can cause a blasting or rotting of any above or below-ground parts of the plant. In the beginning, disease epidemics are generally sporadic and discontinuous. But availability of suitable temperature and moisture conditions during the emergent period, the occurrence of the disease can be high and its progress can be widespread. Reducing disease incidence can be challenging. Crop rotation is used as a management strategy in field production. Reducing humidity beneath the foliage, sanitation as well as weed management are effective cultural practices used to limit spread of disease attack. Chemical control through fungicides is a rapid and effective tool when used prior to epidemic condition. Keeping in view the destructive potential of this disease, the present study was carried out to determine the pathogen (s) responsible for white mold disease as well as to find out effective fungicides against it.

MATERIALS AND METHODS

Collection of samples

Diseased samples were collected from Ayub Agricultural Research Institute (AARI), Faisalabad. Collected diseased samples were placed in alkathene bags and taken to the laboratory. The samples were stored at low temperature i.e. 5 °C. Disease severity was also noted on different parts of plants.

Isolation and Identification

To isolates the fungus on Potato Dextrose Agar from the diseased samples, small size leaves and stalks were used. All the bits were cut at junction of affected and healthy plant parts. These small size bits were sterilized with 0.1 % mercuric chloride solution and used for this purpose. The bits firstly placed in this solution for 10-15 seconds after that they were washed three times with sterilized water. To remove excess water they were placed on filter paper. Finally, they were placed in petri plates as well as on slants containing PDA. The petri plates having 3 to 4 layers of filter paper were used. All this work was done under the aseptic condition. The plates and slants were incubated at a temperature of 25±1 °C. After 4 to 5 days, the cultures were obtained. After the identification of fungus under the microscope, purification was done by using single hyphal tip method. After 4-5 days of incubation, the growth was observed. The culture was maintained on potato dextrose agar medium at 4±1 °C.

Pathogenicity test through Koch's postulates

To verify the link between the isolated fungus and disease, an experiment was conducted by using Koch's postulates. Developed culture on PDA was used on cabbage plants to prove the pathogenicity of the fungus.

In vitro assessment of fungicides against *S. sclerotiorum*

The effectiveness of four fungicides (Raydar, Crest, Curzate and Mancozeb) was assessed against *S. sclerotiorum* at three different concentrations i.e. 50, 100 and 200 ppm by using poisoned food technique. PDA medium with different concentration of fungicides was transferred to petri plates. The colony diameter was calculated by using Schimmer method.

In vitro* assessment of plant extracts against *S. sclerotiorum

To check the antifungal activity of different plant extracts belonging to different plant species against *S. sclerotiorum*, poisoned food technique was used. The technique was given by Vega *et al* (2003). Plant extracts of neem (*Azadirachta indica),* garlic *(Allium sativum)* and safeda (*Eucalyptus cameldulensis*) were tested at four concentrations viz. S, S/25, S/50, S/75.

Preparation of plant extracts

To get the extract of above-mentioned plant species, their tissues were macerated with distilled water. For this purpose, pestle and mortar was used. About 200 g plant material was washed with tap water thoroughly and then placed in pestle and mortar. Small quantity of distilled water was added and macerated for 5 minutes. The same procedure was done in each case. With the help of electrical blender, the extract was homogenized and made a known volume (100ml) of the distilled water. Filtration of the homogenate was done by using Whatman No. 1 filter paper. The solutions of desired concentrations were prepared by adding distilled water.

RESULTS

In vitro* assessment of fungicides against *S. sclerotiorum

The efficacy of four fungicides was evaluated against the pathogen (*S. sclerotiorum*) on PDA medium through poisoned food technique. The recorded data advocated that the percentage inhibition of the fungal growth increased as the concentration of the fungicides increased. According to the results fungal mycelial growth inhibition ranged from 92.91% to 34.69% after 96 hours (Figure 1, Table 1).

Table 1. Comparison of inhibition (%) of fungal growth by fungicides.

Treatment	Inhibition (%) after		
	48 hours	72 hours	96 hours
Crest 100	26.680 d	50.226 d	66.690 d
Crest 200	29.254 c	55.066 c	73.114 c
Crest 50	20.680 f	38.930 f	51.690 f
Curzate 100	19.370 f	38.026 f	50.668 f
Curzate 200	25.104 de	49.286 de	65.668 de
Curzate 50	16.694 h	32.776 g	43.668 g
Mancozeb100	14.410 i	28.288 h	37.690 h
Mancozeb 200	18.392 fe	36.512 fe	48.114 fe
Mancozeb 50	13.262 i	26.036 h	34.690 h
Raydar 100	32.672 b	61.504 b	81.668 b
Raydar 200	36.856 a	69.376 a	92.914 a
Raydar 50	24.048 e	45.276 e	60.114 e
HSD-Value	2.2692	4.3356	5.7502

Means sharing common letters in each column do not differ significantly.

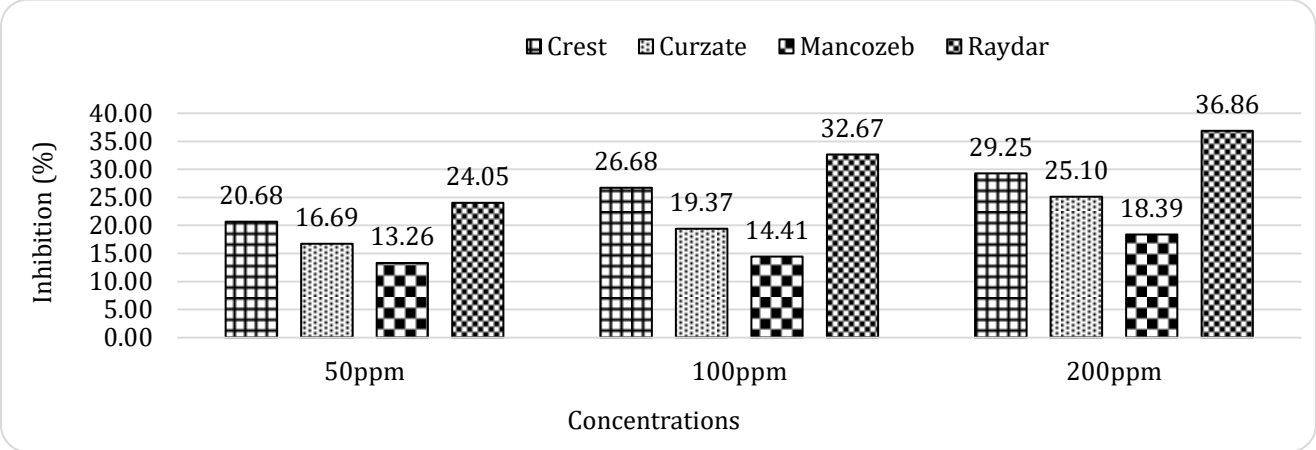

Figure 1. Efficacy of chemicals against the growth of *S. sclerotiorum.*

In vitro* assessment of plant extracts against *S. sclerotiorum

The efficacy of three plant extracts were examined under laboratory conditions at four concentration levels *viz*. S, S/25, S/50 and S/75 by poisoned food technique on PDA. The radial growth of the fungus was recorded after 48 hours, 72 hours and 96 hours of incubation. The inhibition percentage was measured and the results revealed that the garlic extract amongst all the three tested plant extracts was the most effective to inhibit the mycelial growth (49.54, 58.67 and 73.90%) of *S. sclerotiorum* at S/50, S/25 and S respectively after seven days followed by neem leaf extract (32.77, 44.97 and 61.73%). Leaf extract of safeda gave the least inhibition percentage of fungal growth of *S. sclerotiorum* (Figure 2, Table 2).

Table 2. Comparison of inhibition (%) of plant extracts against after 48, 72 and 96 hours.

Treatment	Inhibition (%) after		
	48 hours	72 hours	96 hours
Garlic @ S	38.940 a	53.820 a	70.580 a
Garlic @ S/25	34.220 b	47.320 b	62.060 b
Garlic @ S/50	28.660 c	39.640 cd	52.820 c
Garlic @ S/75	21.540 e	29.780 e	39.680 e
Neem @ S	29.340 c	42.000 c	55.060 c
Neem @ S/25	25.920 d	37.120 d	48.660 d
Neem @ S/50	18.340 f	26.240 f	34.420 f
Neem @ S/75	11.300 h	16.160 h	21.220 g
Sufeda @ S	15.460 g	23.180 g	30.860 f
Sufeda @ S/25	12.020 f	18.020 h	24.020 g
Sufeda @ S/50	8.2400 i	12.320 i	15.960 h
Sufeda @ S/75	4.2600 j	6.3800 j	8.2400 i
HSD-Value	2.1365	3.0069	3.9778

Means sharing common letters in each column do not differ significantly.

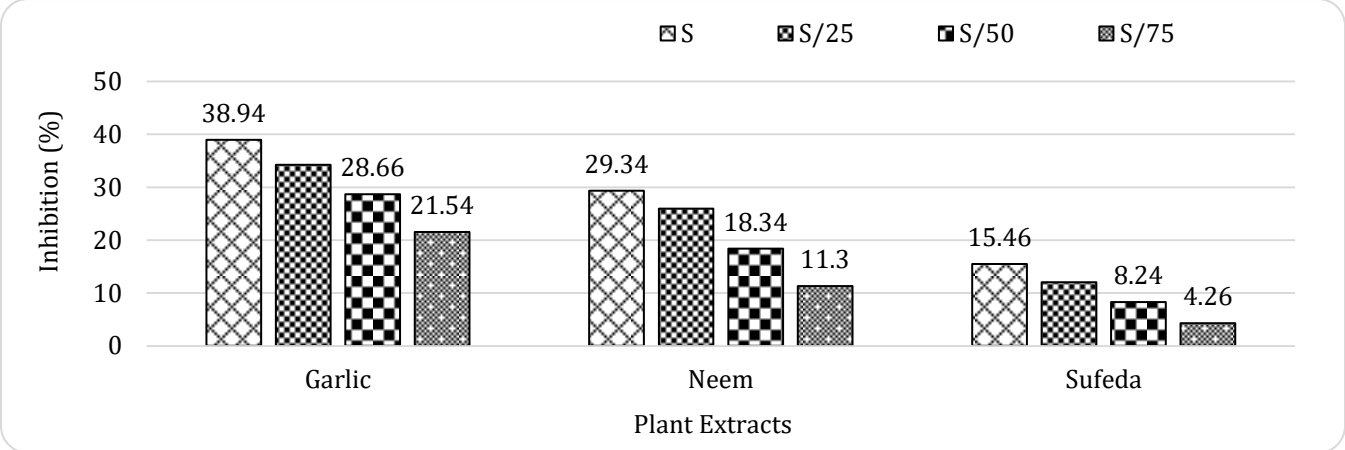

Figure 2. Efficacy of plant extracts against growth of S. sclerotiorum.

DISSCUSSION

Sclerotinia sclerotiorum is the most devastating fungi and an important causal organism of white rot of cabbage. In the current studies the pathogen was isolated from infected portions of cabbage on PDA medium. Elgorban et al. (2013) also proved that Potato Dextrose broth medium is good for growth of plant pathogenic fungi including *S. sclerotiorum*. The cause of the white rot was ascertained and the fungus associated with the infected leaves, stems and curds was isolated in pure culture. The fungus produced aerial mycelium, which was hyaline, well developed and appeared cottony. Sclerotia were produced at growing margins or center of colony at 5 days of incubation and formed concentric rings, became pigmented and were black in color with age. The fungus was identified as *Sclerotinia*

sclerotiorum (Lib.) de Bary and the characters were in accordance with the keys given by earlier workers (Garg et al., 2010; Gill et al., 2014; Goswami et al., 2012; Pathak et al., 2001; Wang and Rong, 2013).

The efficacy of four fungicides was evaluated against the pathogen (*S. sclerotiorum*) on PDA medium through poisoned food technique. Four fungicides viz. Raydar, Crest, Curzate and Mancozeb were evaluated against *S. sclerotiorum* at three different concentrations i.e. 50, 100 and 200 ppm. According to the results fungal mycelial growth inhibition ranged from 92.91% to 34.69% after 96 hours. The results of current studies showed that the chemicals and fungicides performed better in relation to control fungal growth. The results of current studies were in line with research of a numbers of researchers.

Application of chemical fungicides are often cost prohibitive, impractical and hazardous to environment and human health. Keeping this in view, the need was felt for an alternative method to manage this disease in eco-friendly manner by using microbial bioagents and plant extracts. After 96 hours the comparison of treatments with different concentrations levels exhibited that garlic with concentration performed best amongst all the treatments applied garlic with S concentration inhibited the fungal growth up to 70.58% followed by garlic S/25 whilst sufeda leaf extracts showed very poor response for all the concentrations and exhibited minimum inhibition. These results are in agreement with the results of Chattopadhyay et al. (2004), Chattopadhyay et al. (2007), Prasad and Kumar (2012) and Meena et al. (2013). They reported effectiveness of garlic clove extract in disease control against *S. sclerotiorum*.

CONCLUSION

This experiment concluded that curzate chemical manage the disease severity more affectively. Curzate chemical inhibit the mycelial growth of Sclerotinia fungus 92.91% as compared to raydar 36.85%. Application of garlic plant extract showed maximum mycelial inhibition 38.94%as compared to sufeda 4.26%. Further study will be need for the alternate use of chemicals and more focus on the uses of different plant extracts.

AUTHORS' CONTRIBUTION

All the authors equally designed the study, conducted surveys, performed the experiments, collected and analyzed the data, wrote the manuscript and proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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