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In Vitro and *In Vivo* Management of *Alternaria* Leaf Spot of *Brassica campestris* L.

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Abstract

Alternaria black leaf spot caused by *Alternaria brassicae* is one of the most destructive disease of brassicaceae crops and causes 30 to 45% overall yield loss in the world.

Plant susceptibility toward this saprophytic and necrotrophic pathogen is greatly influenced by extreme weather conditions e.g. temperature and humidity.

Six plant extracts, six Biological agents and six fungicides were evaluated both *in vitro* and *in vivo* experiment for their effectiveness to manage Alternaria leaf spot of *Brassica campestris*. In cause of *in vitro* pathogenic fungus was applied in the field at 2 g colonized mustard seeds kg-1 soil. plant extract, Biological agents and six fungicides were evaluated for their efficacy at various concentrations 5%, 10%, 15% and were sprayed in the field at 0.2% a.i. l-1. Out of all treatments, *Allium sativum, Parthenium hysterophorus, Trichoderma harzianum, Trichoderma viride,* Wisdom (50% WP) and Proctor (60% WP) were screen out in laboratory at 15% concentration. The maximum growth inhibition (in laboratory 57.83%, in field 6.07% and in greenhouse 26.32%) was recorded by *Allium sativum* followed by *Parthenium hysterophorus* (in laboratory 53.01%, in field 17.05%, in green and house 29.08%). Out all biological agents, the maximum growth inhibition (in laboratory 61.44%, in field 27.34% and in greenhouse 38.45%) by *Trichoderma harzianum* followed by *Trichoderma viride* (in laboratory 55.42%, in field 29.63%, in green and house 29.08%). Out of all fungicides, the maximum growth inhibition (in laboratory 98.79%, in field 56.08% and in greenhouse 63%) by Wisdom (50% WP) and followed by Proctor (60% WP) (in laboratory 100%, in field 51.76% and in greenhouse 55.16%). It was worth noting that the fungicides, Wisdom (50% WP) and Proctor (60% WP) have highest net value as compare to other treatments but the biological agents also show off their importance.

Keywords: Alternaria leaf spot; *In vitro* and *in vivo* management of *Alternaria* leaf spot by plant extracts; Biological agents and chemicals

Introduction

The origin of mustard (*Brassica compestris* L.) lies in south-east Asia [1]. Mustard is one of most important and oldest known oil seed crop of subcontinent with global contribution of 28.3% acreage and 19% of production [2]. Its oils contain low erusic acid and glucosinolates contents. The percentage of poly-unsaturated fatty acid and linolenic acid of the total fatty acid increase from 15-0% and from 8-12%, respectively [3].

Among the biotic stress of *Alternaria* leaf of mustard and the causal agent is *Alternaria brassicae*. It has been reported from all the continents of the world and is one among the important diseases of mustard causing up to 47% yield losses [4]. Different species of *Alternaria* on *Brassica* spp. vary in host specificity. *Alternaria brassicae* also depending on host susceptibility and environmental factors [5]. *Alternaria brassicae* infected the plant at all growth stages. Fungus infect all parts of plant as leaves, pods, branches, pods and stem but the special target point of fungus are leaves and pods. Often lesions are produced on green leaves and during sever attack in pods seeds become shrivel and early ripening or shattering [6]. Conventionally plant diseases are controlled by applying fungicides, but this practice increase input cost on the crop on one hand and on the other hand cause environmental pollution [7]. So this situation compels to focus on disease management by utilizing biological agents, plant extracts and fungicides in lowest concentration. Application of biological agents and extract is eco-friendly and a sustainable approach apart from being a promising alternative to fungicide application. In the absence of resistant cultivars, chemical fungicides provide the most reliable means of disease control. The present study was aimed at determining a cost-effective management of Alternaria leaf spot.

Material and Method

Study area and sampling

In 2012, eight *brassicae* fields were visited, randomly diseased samples were chosen. A survey was conducted at eight different locations in district Lahore for prevalence, severity and mortality of *Alternaria* leaf spot of mustard at maturity of mustard crop during cropping season. To assess disease prevalence, severity and mortality, and ten plants were selected in each quadrate in a diagonal configuration depending on the geometry of the field. The following formula was calculated percentage prevalence.

$$\text{Prevalence}\ (\%) = \frac{\text{Locations with disease symptoms}}{\text{Total Locations}} \times 100$$

$$\text{Severity}\ (\%) = \frac{\text{Sum of rating scale}}{\text{Total number of leaves observed}} \times 100$$

$$\text{Mortality}\ (\%) = \frac{\text{Sum of dead plants}}{\text{Total plants}} \times 100$$

Alternaria leaf spot disease was collected randomly, with at least five lesions in the leaf blade. From each of the five lesions per leaf,

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fragments of tissues containing fungi structures were taken out and microscope preparations on glass slides containing a drop of blue Aman were made. The slides were, afterwards, observed in optical microscope at 400X magnification. Identification was made according to literature [8-10]. After identification, pure cultures were submitted in Pakistan First Fungal Culture Bank Institute of Agriculture Science, University of the Punjab, Lahore.

In vitro screening of treatments

Alternaria brassicae inhibitory effect was checked against different plants extract in laboratory under food poisoning technique. The food poisoning technique was adopted for invitro testing of biological agents and fungicides. For this purpose, six plants were selected *Coronopus didymus* (Leaves), *Medicago sativa* (Leaves), *Zingiber officinale (Bulb), Chenopodium hirsutum* (Leaves), *Allium sativum* (Bulb) and *Parthenium hysterophorus* (Leaves). Ten grams of plants relevant part were grinded with help of pistol and mortal by adding equal amount of distal water (1: 1 w/v). At last extract was filter with the help of muslin cloth. Aqueous solution (100%) was obtained. Further, the extract was diluted by adding sterilized water to get 10 percent concentration. Future plants extract were need to heat at 50°C to avoid contamination. 2 ml plant extract was poured in 20 ml MEA petriplate and gently shake both for mixing of plants extract in media. When MEA and plants extract solidified then 8 mm disc of 10 days old pathogen was placed in center of every petriplate. All pertiplates were incubated at 23°C for 10 days. Growth inhibition of pathogen, inoculated and uninoculated was calculated according to the formula given by Vincent.

$$\% \text{ Inhibition over control: } \frac{C\text{-}T}{C} \times 100$$

Where;

I=Percent inhibition

C=Growth in control

T=Growth in treatments

Biological agents (*Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum, Trichoderma koningii*, *Trichoderma reesei* and *Trichoderma aureoviride)* were obtained from Fungal Culture Bank of Pakistan Institute of Agriculture Science, University of the Punjab, Lahore. These biological agents were screened in laboratory condition against *Alternaria brassice*. Culture of both pathogen and biological agents (8 mm) were collected from margin of actively growing mycelium and transfer to MEA medium on opposite site of about at 1 cm from wall of the plate. The petriplates were subsequently incubated at 25 ± 1°C. After 5 days fungal colonies were observed and recorded.

Fungicides inhibitory assessment against *A. brassicae* was performed in laboratory by food poisoning technique. Seven fungicides Ridomil (20% WP), Diesomil (30% WP), Topsin-M (45% WP), Thiram (35% WP), Dolomile (30% WP), wisdom (50% WP) and Proctor (60% WP) were used for confirmation of efficacy against *A.brassicae* under invitro conditions. All selected fungicides were tested at 0.1% concentration. Two ml of each fungicide were incorporated in sterilized petriplates and gently mixed it. After solidifying MEA media, 8 mm disc of ten days old sporulating culture of *Alternaria brassice* was inoculated in center of every petriplate. Controls were maintained. Inoculated petriplates were incubated at room temperature 28°C in the laboratory. The colony diameters were measured after 10 days when the control plates were full of fungal growth.

Green house experiment

Pots having 25 cm depth and 20 cm diameter were used. Each treatment was replicated thrice. Sandy loam soil was used and each pot was filled with 10 Kg sterilized soil. Seed were sown in pots at the depth of 2 cm in January 16, 2016. Three seeds per pot were sown. Pots were irrigated twice in a week. Green house plants become 2 to 3 leaf stage then pathogen inoculated in form of suspension in February, 7 2016. Inoculum was obtained from culture of *Alternaria brassicae* grown on malt extract agar. Inoculum was prepared in suspension form, 10 ml sterile water added in petriplates and shake well. The numbers of spores were counted with heamocytometer and spores were adjusted to 32×10^7 sporesml^{-1}.

About 500 ml of inoculums suspension is used with sprayers that run off from top of leaf.

After two weeks leaves established disease symptoms. Older leaves were more severely infected from *Alternaria* leaf spot as compare to younger leaves. Initially leaves beared light brown lesion which gradually become dark brown and at last dark spot on whole leaf. In severe condition, gradually it spread to other parts of plants such as pods, stem and branches.

Disease management

The fresh plants extracts (*Allium sativum,* bulb and *Parthenium hysterophorus,* leaves) were gently washed under tap water and finally in sterile distilled water. They were separately grind in sterile water at the rate of 1 mlg^{-1} of plant material in pistal and mortal. Then it stained through double layer of muslin cloth and finally through sterilized whatman no. 1 filter paper. This formed 100% standard plant extract solution. Further its dilution performed of required concentration with sterilized water [11]. Plants extract application at 5%, 10% and 15% concentrations.

One week old culture of *Trichoderma harzianum* and *Trichoderma viride* were obtained from FCBP. The spore's concentration was adjusted to 32×10^6 spores ml^{-1} by hemocytometer. Biological agent's was applied at 5%, 10% and 15% concentrations. Fungicides with recommended dozes were used that are available in market and Spray in field at 5%, 10% and 15% concentrations.

Field experiment

The experiment was conducted by randomized block design (RBD) with three replications and the sowing was done om 10 m×15 m plots, with a spacing of 90 cm×60 m on 2015 and 2016.

Plants were inoculated with a suspension of pathogen (*Alternaria brassicae*) at February 7 2016. The spore suspensions of pathogens performed as in green house experiment and application according to section about 2000 ml of inoculums suspension is used with hand sprayers that run off from top of leaf.

Disease management

Plant extract preparation and application was as discussed in green house experiment. Biological agents preparation and application was as discussed in green house experiment. Fungicides preparation and application was as discussed in section greenhouse experiment.

Statistical analysis

Treatment mean and standard error were calculated from the data obtained for various parameters using package Costat version 3.03.

Results

Disease survey

After peripatetic survey prevalence, severity and mortality of

Alternaria black spot disease was recorded. The disease prevalence percentage ranged between 20 to 60 at different locations. The maximum prevalence was (60%) recorded at P.U campus and minimum (20%) recorded at Multan road (Sunder) and G.T road (Rana town). Disease severity percentage was range between 30 to 70 at different locations. The maximum severity (70%) was recorded at P.U campus and minimum (30%) was recorded at G.T road (Rana town) and Raiwind road (Bubtiya chowk). Mortality was ranged between 8 to 25 percent at different locations. The maximum mortality was (25%) recorded at P.U campus and minimum was 8% recorded at G.T road (Rana town) (Figure 1).

In vitro screening of treatment against *Alternaria brassicae*

Six plants extract (*Parthenium hysterophorus, Coronopus didymus, Medicago sativa, Chenopodium hirsutum, Zingiber officinale and Allium sativum*) were tested against *Alternaria brassicae* growth. According to result shown in Figure 2, fungal growth inhibition ranged between 53.01% to 57.83%. The maximum growth inhibition (57.83%) was recorded by *Parthenium hysterophorus,* followed by *Allium sativum* (53.01%). *Chenopodium hirsutum inhibited (39.75%) followed by Medicago sativa* (38.55%). The minimum growth inhibition (30.12%) was recorded by *Zingiber officinale followed by Coronopus didymus* (24.93%).

Six biological agents (*Trichoderma harzianum, Trichoderma reesei, Trichoderma viride, Trichoderma aureoviride, Trichoderma konngii* and *Trichoderma hamatum*) were tested for their antifungal activity against *Alternaria brassicae*. According to results shown in Figure 3, fungal growth inhibition ranged recorded between 15.23 to 61.44%. The maximum growth inhibition (61.44%) was recorded by *Trichoderma harzianum* followed by *Trichoderma viride* (55.42%) and *Trichoderma konngii* (40.96%). Meanwhile, *Trichoderma reesei* inhibited (33.01%) followed by *Trichoderma hamatum* (31.32%). The minimum growth inhibition (15.90%) was recorded by *Trichoderma aureoviride.*

Seven fungicides (Ridomil, Diesomil, Topsin-M, Thiram, Dolomile, Wisdom and Proctor) were tested against *Alternaria brassicae* growth. According to results shown in Figure 4, fungal growth inhibition ranged between 63.85 to 100 percent. The maximum growth inhibition (100%) was recorded by proctor. Wisdom inhibited (98.79%) growth at 0.1% concentration followed by Topim (81.52%). Dolomile inhibited 75.90% followed by Ridomil 72.28%. The minimum growth inhibition (69.87%) was recorded by Diesomil followed by Thirm (63.85%).

Field and green house studies for prevalence, severity and mortality of *Brasscia compestris*

From data observation, in field mean disease prevalence, severity and mortality were recorded (40%, 55% and 15% respectively) followed

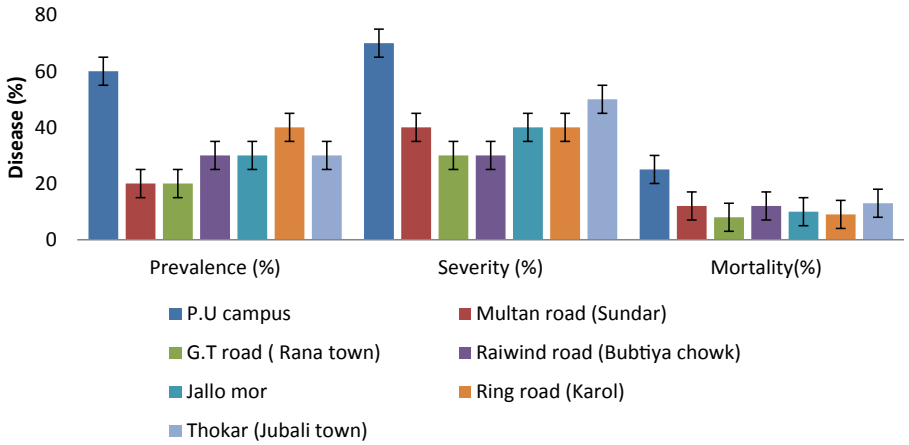

Figure 1: Prevalence (%), severity (%) and mortality (%) of *Alternaria* leaf spot of mustered at seven different survey location.

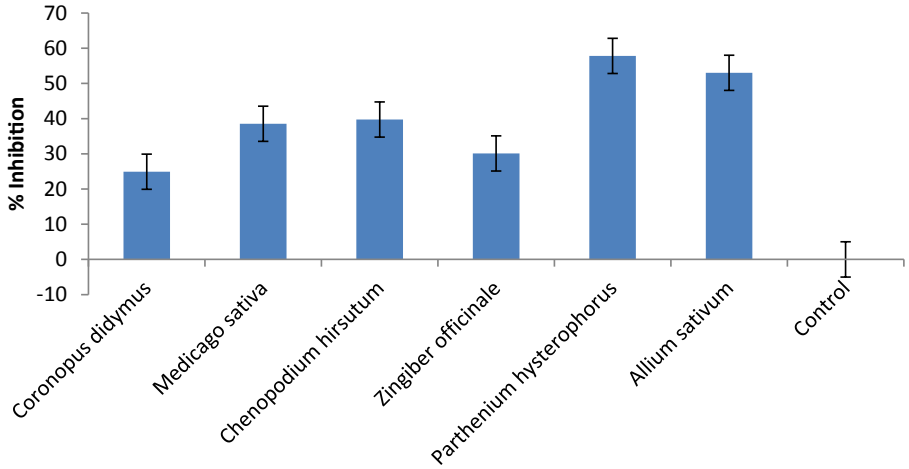

Figure 2: *In vitro* screening of plant extracts.

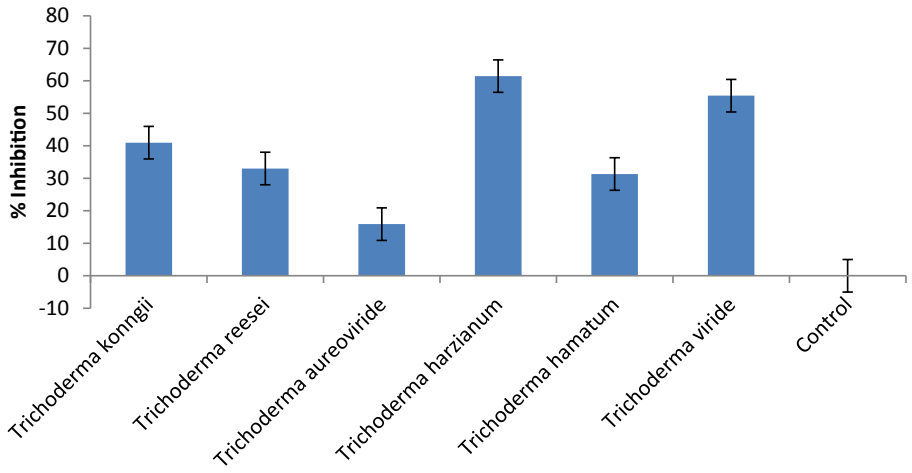

Figure 3: *In vitro* screening of Biological agents.

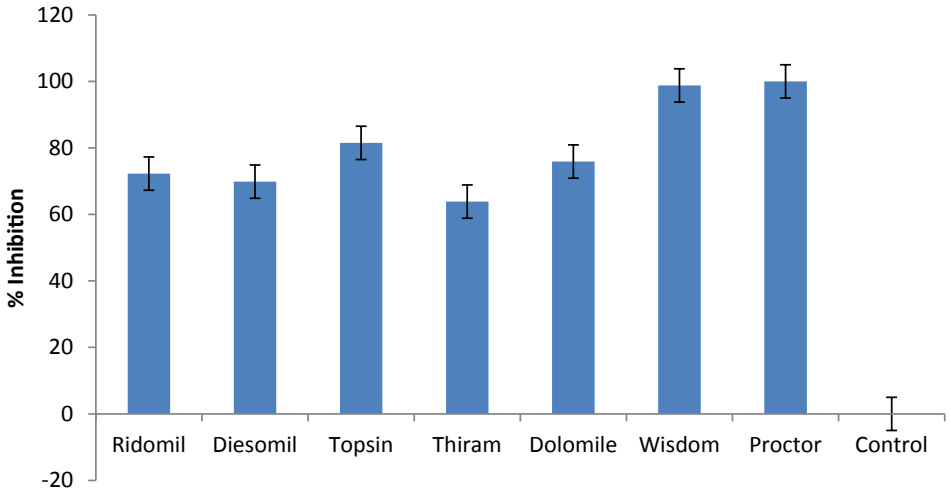

Figure 4: *In vitro* screening of Fungicide.

by control (4%, 2% and 0% respectively). In green house mean disease prevalence, severity and mortality were recorded (50%, 50% and 10% respectively) followed by control (3%, 1% and 0% respectively) (Figure 5). Field symptoms categories according to disease rating scale (Plate. 4) and plant disease index was calculated by the following formula;

$$\text{Plant Disease index } (\%) = \frac{\text{Sum of numerical rating}}{\text{Number of leaves infected}} \times 100$$

Disease management in field

Field experiment for management of *Alternaria* leaf spot of *Brasscia compestris:* After disease management according to result shown that maximum PDI was (88.02%) recorded in plot of control followed by *Allium sativum* (81.43%), *Parthenium hysterophorus* (70.7%), *Trichoderma harzianum* (60.86%), *Trichoderma viride* (58.57%), Proctor (36.44%) and minimum PDI was recorded by Wisdom (32.12%).

The maximum leaf spot disease (56.08%) was reduces in plot treated with 5%, 10% and 15% concentration of Wisdom, followed by Proctor (51.76%), *Trichoderma viride* (29.63%), *Trichoderma harzianum* (27.34%), and *Parthenium hysterophorus* (17.5%) *Allium sativum* (6.77%); whereas no any disease reduction was recorded at untreated plot (Table 1).

Green house disease management: After disease management according to result shown that maximum PDI was (86.66%) recorded in plot of control followed by *Allium sativum* (60.34%), *Parthenium hysterophorus* (56.86%), *Trichoderma harzianum* (48.5%), *Trichoderma viride* (48.14%), Proctor (31.5%) and minimum PDI was recorded by Wisdom (23.66%).

The maximum leaf spot disease (63%) was reduces in plot treated with Wisdom, followed by Proctor (55.16%), *Trichoderma viride* (38.16%), *Trichoderma harzianum* (38.45%), and *Parthenium hysterophorus* (38.45%) *Allium sativum* (26.32%); whereas no any disease reduced was recorded in plants not sprayed with fungicides (Table 2).

Discussion

Disease prevalence ranged between 20 to 60% followed by severity 30 to 70% and mortality 25 to 8% at different locations. Maximum disease prevalence, severity and mortality (60, 70 and 25% respectively) were recorded at P.U campus followed by Thokar (30, 50 and 12%

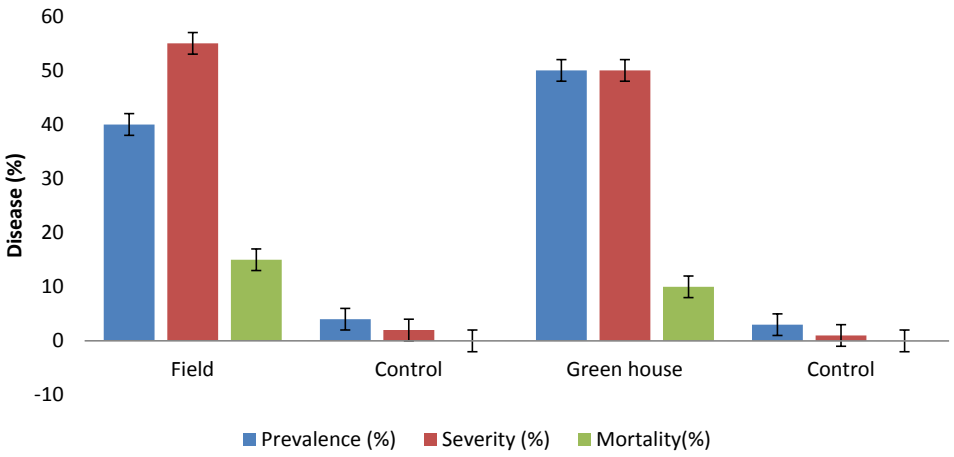

Figure 5: *Alternaria* leaf spot disease in field and green house.

Nature of Treatments	Alternaria Leaf spot													
	Replicate 1				Replicate 2				Replicate 3				%PDI	% Decrease
	5%	10%	15%	Mean	5%	10%	15%	Mean	5%	10%	15%	Mean		
Allium sativum	80	72	64	70 ± 2.42	81	73	65	73 ± 3.97	79	71	62	70.66 ± 3.10	81.43 ± 5.02	6.77 ± 0.02
Parthenium hysterophorus	74	64	49	60.4 ± 2.04	75	65	50	63.4 ± 3.31	73	62	49	61.33 ± 2.21	70.7 ± 4.41	17.5 ± 0.09
Trichoderma harzianum	63	54	44	51.7 ± 1.54	64	55	45	54.7 ± 2.30	62	52	44	52.67 ± 1.90	60.86 ± 3.92	27.34 ± 0.91
Trichoderma viride	64	49	43	50 ± 1.52	65	50	44	53 ± 2.21	61	48	42	50.34 ± 1.12	58.57 ± 2.41	29.63 ± 2.08
Wisdom	41	34	09	26 ± 0.01	42	35	10	29 ± 0.04	40	33	10	27.67 ± 0.03	32.12 ± 0.02	56.08 ± 3.41
Proctor	39	44	19	28.6 ± 0.08	40	45	20	31.6 ± 0.00	41	45	23	36.33 ± 0.41	36.44 ± 0.05	51.76 ± 2.52
Untreated Control	91	84	90	87.4 ± 3.95	92	85	90	88.4 ± 5.04	90	86	88	88 ± 5.41	88.2 ± 5.92	0 ± 0.00

± Standard error

Table 1: Field studies for *Alternaria* leaf spot of *Brassica compestris* by using plant extract, biological agents and fungicides.

Nature of Treatments	Alternaria Leaf spot													
	Replicate 1				Replicate 2				Replicate 3				%PDI	% Decrease
	5%	10%	15%	Mean	5%	10%	15%	Mean	5%	10%	15%	Mean		
Allium sativum	69	61	53	61 ± 1.98	71	63	55	63 ± 3.49	66	57	50	57.67 ± 3.14	60.34 ± 3.90	26.32 ± 0.02
Parthenium hysterophorus	63	57	45	55 ± 1.92	65	55	45	55 ± 2.59	70	60	46	58.67 ± 3.31	56.86 ± 3.38	29.8 ± 0.08
Trichoderma harzianum	52	47	41	46.66 ± 2.02	54	45	40	46.34 ± 3.25	60	50	40	50 ± 2.52	48.21 ± 3.0	38.45 ± 1.12
Trichoderma viride	53	46	40	47.34 ± 2.08	55	46	41	47.34 ± 3.25	61	47	41	49.66 ± 2.21	48.5 ± 2.9	38.16 ± 1.42
Wisdom	34	26	11	23.67 ± 0.02	32	26	10	22.67 ± 1.35	37	27	10	24.66 ± 1.91	23.66 ± 1.95	63 ± 3.23
Proctor	32	37	19	29.33 ± 0.03	30	35	17	27.33 ± 2.05	40	45	22	35.67 ± 2.97	31.5 ± 2.06	55.16 ± 2.51
Untreated Control	89	81	88	86 ± 4.95	90	80	88	86 ± 9.82	87	85	90	87.33 ± 5.07	86.66 ± 6.56	0 ± 0.00

±Standard error

Table 2: Green house studies for *Alternaria* leaf spot of *Brassica compestris* by using plant extract, biological agents and fungicides.

respectively), Multan road (20, 40 and 8% respectively), Ring road (40, 40 and 12% respectively), Jallo mor (30, 40 and 10% respectively), Raiwind raod (30, 30 and 9% respectively) and minimum disease was (20, 30 and 13% respectively) recorded at G.T road (Rana town). Similar results were reported by Maltoni et al. [12].

The fungus, *Alternaria brassicae* was isolated and identified by its morphological characteristics as described by Lelivet. It was confirmed as cause of *Alternaria* leaf spot disease of *Brassica compestris*. The findings were conformed from Fungal Culture Bank of Pakistan. *Allium sativum* and *Parthenium hysterophorus* was screen out by food poisoning technique. According to data they inhibited maximum (53.01% and 57.83% respectively) fungal growth followed by *Trichoderma harzianum* and *Trichoderma veridie* (61.44% and 55.42% respectively) and wisdom and Proctor (98.79% and 100% respectively).

Chemical control is ultimate and easy solution of disease. But biological management is more acceptable to environment and human being. Lot of literature is available regarded to justify biological management of *Alternaria* leaf spot of *B. compestris*. Spray of soil isolates of *Trichoderma viride* at 45 and 75 days after sowing could manage *Alternaria* blight of Indian mustard (*Brassica juncea*) as effectively as mancozeb and other fungicides, which have been incorrigible later in multiplication trials (AICRP-RM, 2007). Botanicals viz., bulb extract of *Allium sativum* has been reported to effectively manage *Alternaria* blight of Indian mustard [13].

The maximum leaf spot reduction was (63%) recorded of Wisdom followed by Proctor (55.16%), *Trichoderma viride,* (38.16%), *Trichoderma harzianum* (38.45%) and minimum disease reduction was recorded from *Parthenium hysterophorus* (29.8%) followed by *Allium sativum* (26.16%).

Chemical management is most favorable and widely used method against disease. One sided it completely control disease but other hand it has many unseen sides' effects which directly or indirectly transfer to human beings. Meanwhile, it also has hazardous effect in our environment. Although fungicide remains more effective in reducing diseases in plants, increasing public concern about environmental health is proving to be major hindrance in use of chemical pesticides including fungicides.

References

1. Vavilov NI, Dorofeev VF (1992) Origin and Geography of Cultivated Plants. Transl.by Doris Love. Cambridge University Press p: 498.
2. Bandopandopadyay L, Basue D, Sikdar SR (2013) Identification of genes involved in wild Crucifer *Rorippa indica* resistance response on mustard aphid *Lipaphis erysimi* challenge. PLoS One 8: e73632.
3. Tayo TO, Morgan DG (1979) Factors influencing flower and pod development in oil-seed rape (*Brassica napus*). J Agric Sci Cambridge 92: 363-373.
4. Kolte SJ (1985) Diseases of Annual Edible Oilseed Crops, Vol. II, Rapeseed-Mustard and Sesame Diseases. CRC Press Inc. Boca Raton, Florida p: 135.
5. Paul VH, Rawlinson CJ (1992) Diseases and Pests of Rape. Gelsenkirchen-Buer, Germany: Verlag Th. Mann.
6. Conn KL, Tewari JP, Awasthi RP (1990) A Disease Assessment Key for Alternaria Black Spot in Mustard. Can Plant Dis Sur 70: 19-22.
7. Chattopadhyay AK, Bagchi BN (1994) Relationship of disease severity and yield due to leaf blight of mustard and spray schedule ofmancozeb for higher benefit. J Mycopathol Res 32: 83-87.
8. Gilman JC (1945) A manual of Soil Fungi. 2nd ed. The Lowa State University Press, Ames, Lowa, USA.
9. Domsch KH, Gams W, Andreson TH (1980) Compendium of soil fungi. Volume 1. Academic press. A subsidiary of Harcourt braceJovanovich, publishers. London, New York, Toronto, Sydney, SanFrancisco.
10. Barent HL, Hunter BB (2003) Illustrated Genera of Imperfect Fungi. APS Press, The American Phyto pathological society St. Paul, Minnesota.
11. Shekhawat PS, Prasada R (1971) Antifungal properties of some plant extract and inhibition of spores germination. Indain phytopathol 24: 800-802.
12. Maltoni ML, Magnani S, Baruzzi G (2000) Screening forAlternaria black spot resistance in strawberry plants 62: 95-97.
13. Patni CS, Kolte SJ (2006) Effect of some botanicals in management of Alternaria blight ofrapeseed-mustard. Ann Pl Prot Sci 14: 151-156.

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