

Efficacy of bio-agents and plant extracts against *Alternaria* leaf blight of mustard (*Brassica juncea* L.)

Yogita Thakur, Sunil Zacharia, Brijesh Singh Chauhan

Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Deemed-to-be University, Allahabad, Uttar Pradesh, India

Abstract

Field experiment was conducted at the research plot of the Department of Plant Pathology, SHIATS, Allahabad, U. P. during the rabi season of 2015-16 to test, "Efficacy of bio-agents and plant extracts against *Alternaria* blight of mustard (*Brassica juncea* L.)"

By foliar spray of certain fungicides, plant extract and bioagents. Per cent disease intensity on leaves at 45, 60 and 75 DAS were recorded. Results showed that the foliar spray of *Trichoderma harzianum* 2% (fs) significantly reduced *Alternaria* blight, increased yield and cost/benefit ratio and was most effective treatment, followed by foliar spray *Trichoderma harzianum* (fs) 2% , with *Trichoderma viride* (fs) 2%, Neem leaf extract(fs) 10%, Dhatura leaf extract(fs)10%, foliar spray of *Allium sativum* 10%. However foliar spray of *Trichoderma harzianum* most effective treatment have shown results at par with the foliar spray of *Trichoderma harzianum* in increasing the yield. The maximum plant height (cm) was recorded at *Trichoderma harzianum* (foliar spray) @ 2%. As compare to treated and untreated check.

Keywords: alternaria blight, *trichoderma* spp., fungicides, *allium sativum*, neem leaf extract, datura leaf extract

Introduction

Rapeseed mustard is the major rabi oil seed crop of Chambal and Gwalior divisions of Madhya Pradesh. *Alternaria* blight are the most important diseases causing heavy losses throughout the country attacking all Brassica species. Four species of *Alternaria* viz., *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schw.) Wiltsh., *A. raphani* Groves and Skolka and *A. alternata* (Fr.) Keissler have been reported for the cause of *Alternaria* blight. Out of which *A. brassicae* is most widely prevalent in India. Biological screening of plant extracts was carried out throughout the world for the determination of their antifungal activity. Synthetic chemicals used to control plant diseases not only pollute the environment, but are also harmful to human health. Because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly biocompounds for the control of plant diseases using different forms of botanicals.

Alternaria blight of mustard is both soil and seed borne disease. The conidia and mycelium may become attached with seeds and penetrate there upon, or they come into soil via diseased plants debris and survive there in. They serve as the source of primary infection in next growing season. With the growing awareness of harmful effects of pesticides integrated use of bioagent (*Trichoderma viride*, *Trichoderma harzianum*) + phytoextract (*Allium sativum*, *Datura stramonium*, *Azadirachta indica*) + Chemical [mancozeb] are used in this study. The concept of integrated disease management seeks to minimize the advantages in the use of fungicide. In present study different bioagents, plant extracts and fungicides are used as seed treatments and foliar spray against *Alternaria* blight in India mustard to find out effective and economical control (Chattopadhyay, 2008) [17].

Material and Methods

Preparation of plant extract

Plant material are chopped in required quantity of water (1:1.w/v), boiled for 30 min. and then strain through cheese cloth to obtained standard plant extract solution (100%). The extract is centrifuge at 5000 rpm for 5 min. and the clear supernatant was used as stock solution.

Application of bulb extract from garlic (*Allium sativum*)

For preparation of bulb extract from garlic, the cloves of garlic was washed in running tap water followed by washing in distilled water dried. The tissues were homogenized in distilled water(1:1w/v) using a blender. The mixture, air was filtered through a four layer of moistened muslin cloth and washed the filter and centrifuged. The supernatant thus obtained was designated as concentrated bulb extract. Garlic bulb extract was used as seed treatment and foliar spray @ 1%(w/v) (Rathi, 2009) [54].

Application of neem leaf extract (*Azadirachta indica*)

Neem leaf extract was prepared according to Paul and Sharma (2002) [42]. 400g (fresh wt) mature leaves were homogenized in a pre-chilled pestle and mortar using chilled, sterilized distilled water. The extract was filtered through four layers of moisture muslin cloth. The final volume was adjusted to 1000 ml with distilled water. The filtrate was centrifuged at 2000g, 4 degree c for 15 min. the supernatant thus obtained was designated as concentrated leaf extract. Dilution of 1:2 was made from this concentrated extract. (Sanjay and Ashok, 2006) [60].

Application of Datura leaf extract (*Datura stramonium*)

Plant materials (50g each) were extracted with 200 ml of absolute ethanol (99% Merck) using a soxhlet extractor at room temperature for 2 days. The extraction was completed in 3 cycles. The solvent was removed with a rotary evaporator at 55 °C and 200 mm Hg. After filtering through a Buchner funnel, the filtrates were concentrated to dryness by rotary evaporator under low pressure. The extract obtained was a solid composition of Datura seed leaf extract (Sharma *et al.*, 2013) [63].

Application of *Trichoderma viride*

Talcum based formulation of *Trichoderma viride* manufactured by Yash Biotech Pvt. Ltd; Allahabad was used for field experiment. Before applying the talcum based formulation of *Trichoderma viride* in the field the c.f.u was

checked in the laboratory. Seed treatment @ 10g/kg of *Trichoderma viride* was used. Foliar spray of *Trichoderma viride* was at 50 DAS @ 10g/l of water and the subsequent spray was given at 15 days interval as suggested by Rathi and Singh (2009) [54].

Application of *Trichoderma harzianum*:

Talcum based formulation of *Trichoderma harzianum* manufactured by Yash Biotech Pvt. Ltd; Allahabad was used for field experiment. Before applying the talcum based formulation of *Trichoderma harzianum* in the field the c.f.u was checked in the laboratory. Seed treatment @ 10g/kg of *Trichoderma harzianum* was used. Foliar spray of *Trichoderma harzianum* was at 50 DAS @ 10g/l of water and the subsequent spray was given at 15 days interval as suggested by Rathi and Singh (2009) [54].

The scale for disease assessment was as follows

Table 1

S. No	Per cent leaf area covered	Grade(X)	No. of leaf falling in grad (Y)	Disease rating (X,Y)
1.	0	0		
2.	1	1		
3.	2-10	3		
4.	11-25	5		
5.	26-50	7		
6.	>50	9		
7.		Total		

Disease intensity

Disease intensity (%) was calculated by using the following formula:

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of rating} \times \text{Maximum disease grade}} \times 100$$

(McKinney’s, 1923) [35]

Results and Discussion

Effect of treatments on plant height (cm) of Mustard

Plant height (cm) at 30 DAS:

The result presented in table 2 and depicted in figure 1 revealed that all the treatments were statistically significant and increased plant height as compared to untreated control. The maximum plant height T₂ *Trichoderma harzianum* (fs) (22.00), followed by T₁ *Trichoderma viride* (fs) (19.00) T₃ *Allium sativum* (fs) (18.16), T₆ Neem leaf extract (fs) (16.50), T₄ Datura leaf extract (fs) (14.83), as compared to treated (23.83), and untreated check (12.33),

Plant height (cm) at 45 DAS

The result presented in table 2 and depicted in figure 1 revealed that all the treatments were statistically significant and increased plant height as compared to untreated control. The maximum plant height (74.03cm) was recorded at 45 days after Sowing in T₂ *Trichoderma harzianum* (fs) (74.03),

followed by T₁ *Trichoderma viride* (fs) (73.73), T₃ *Allium sativum* (fs) (72.23), T₆ Neem extract (fs) (68.43), T₄ Datura leaf extract (fs) (63.53), as compared to treated (79.06) and untreated check (60.13),

Plant height (cm) at 60 DAS

The result presented in table 2 and depicted in figure 1 revealed that all the treatments were statistically significant and increased plant height as compared to untreated control. The highest plant height (103.66) was recorded at 60 days after Sowing in T₂ *Trichoderma harzianum* (fs) (103.66), followed by T₁ *Trichoderma viride* (fs) (100.96), T₃ *Allium sativum* (fs) (101.56), T₆ Neem leaf extract (fs) (95.46), T₄ Datura leaf extract (fs) (91.33), as compared to treated (104.33cm) and untreated check (91.96).

Plant height (cm) at 75 DAS

The result presented in table 2 and depicted in figure 1 revealed that all the treatments were statistically significant and increased plant height as compared to untreated control. The maximum plant height (146.43cm) was recorded at 75 days after Sowing in T₂ *Trichoderma harzianum* (fs) , followed by T₁ *Trichoderma viride* (fs) (144.13), T₃ *Allium sativum* (fs) (141.43), T₆ Neem extract (fs) (137.46), T₄ Datura leaf extract (fs) (134.86), as compared to treated (147.16cm) and untreated check (132.26).

Table 2: Effect of bio-agents and plant extracts on plant height of mustard

Treatments		30 DAS	45 DAS	60 DAS	75 DAS
T ₀	Untreated control	12.33	60.13	91.96	132.26
T ₁	<i>Trichoderma viride</i>	19.00	73.73	100.96	144.13
T ₂	<i>Trichoderma harzianum</i>	22.00	74.03	103.66	146.43
T ₃	<i>Allium sativum</i>	18.16	72.23	101.56	141.43
T ₄	Dhatura leaf extract	14.83	63.53	91.33	134.86
T ₅	Treated control (Mancozeb)	23.83	79.06	104.33	147.16
T ₆	Neem leaf extract	16.50	68.43	95.46	137.46
Overall Mean		18.09	70.16	98.46	140.53
F- test		S	S	S	S
S. Ed. (±)		1.31	1.64	0.94	2.41
C. D. (P = 0.05)		2.87	3.58	2.05	5.26

Fs= Foliar spray

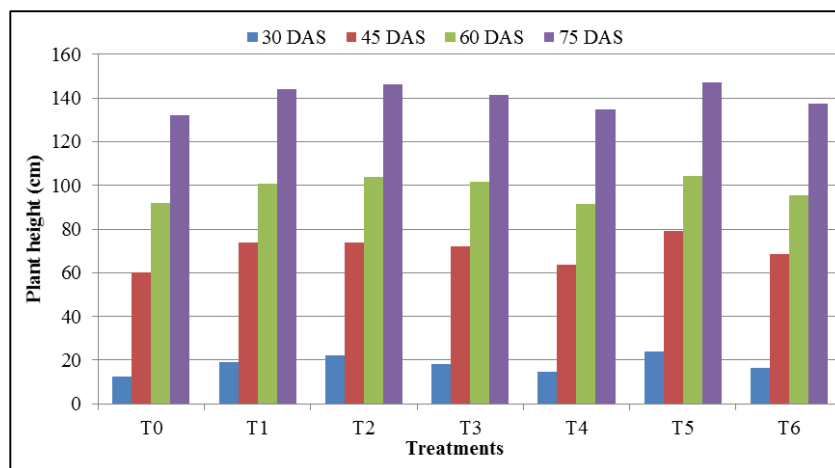


Fig 1: Effect of bio-agents and plant extracts on plant height of mustard.

Effect of treatments on Disease intensity at different days interval

Disease intensity (%) at 45 DAS

The result presented in table 3 and depicted in figure 2 revealed that all the treatments were statistically significant and increased disease intensity as compared to untreated control. The maximum disease intensity (27.81) was recorded at 45 DAS in T₂ *Trichoderma harzianum* (fs), followed by T₁ *Trichoderma viride* (fs) (28.79), T₃ *Allium sativum* (fs) (30.65), T₆ Neem extract (fs) (31.64), T₄ Dhatura leaf extract (fs) (32.41), as compared to treated (26.21%) T₀ untreated check (36.14).

Disease intensity (%) at 60 DAS

The result presented in table 3 and depicted in figure 2 revealed that all the treatments were statistically significant and increased disease intensity as compared to untreated

control. The maximum disease intensity (44.46%) was recorded at 60 DAS in T₂ *Trichoderma harzianum* (fs), followed by T₁ *Trichoderma viride* (fs) (45.15), T₃ *Allium sativum* (fs) (46.71), T₆ Neem leaf extract (fs) (49.14), T₄ Dhatura leaf extract (fs) (50.92), as compared to treated (40.14%) and T₀ untreated check (52.89).

Disease intensity (%) at 75 DAS

The result presented in table 3 and depicted in figure 2 revealed that all the treatments were statistically significant and increased disease intensity as compared to untreated control. The maximum disease intensity (54.52%) was recorded at 90 DAS in T₂ *Trichoderma harzianum* (fs) (54.52), followed by T₁ *Trichoderma viride* (fs) (56.23), T₃ *Allium sativum* (fs) (58.18), T₆ Neem extract (fs) (59.19), T₄ Dhatura leaf extract (fs) (60.62), as compared to treated (53.05%) and T₀ untreated check (62.03).

Table 3: Effect of bio-agents and plant extracts on disease intensity of mustard.

Treatments		45 DAS	60 DAS	75 DAS
T ₀	Untreated control	36.14	52.89	62.03
T ₁	<i>Trichoderma viride</i>	28.79	45.15	56.23
T ₂	<i>Trichoderma harzianum</i>	27.81	44.46	54.52
T ₃	<i>Allium sativum</i>	30.65	46.71	58.18
T ₄	Dhatura leaf extract	32.41	50.92	60.62
T ₅	Treated control (Mancozeb)	26.21	40.14	53.05
T ₆	Neem leaf extract	31.64	49.10	59.19
Overall Mean		30.52	47.05	57.64
F- test		S	S	S
S. Ed. (±)		1.78	1.58	1.18
C. D. (P = 0.05)		3.89	3.44	2.58

Fs=Foliar spray

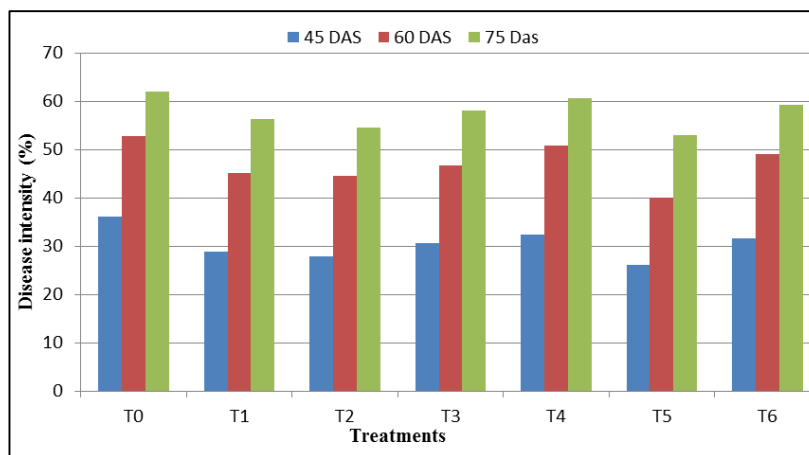


Fig 2: Effect of bio-agents and plant extracts on disease intensity of mustard.

4.5 Yield(q/ha)

Results presented in revealed that the maximum grain yield among the treatment were significant. The highest grain yield (q/ha.) was recorded in T₂ *Trichoderma harzianum* (fs)

(12.70), T₁ *Trichoderma viride* (fs) (12.55), T₃ *Allium sativum* (fs) (11.37), T₆ Neem extract (fs) (10.56), T₄ Datura leaf extract (fs) (9.56), as compare to treated (14.16) and untreated check (7.83).

Table 4: Effect of bio-agents and plant extracts on Yield (q/ha) of mustard.

Treatments		Yield (q/ha)
T ₀	Untreated control	7.83
T ₁	<i>Trichoderma viride</i>	12.55
T ₂	<i>Trichoderma harzianum</i>	12.70
T ₃	<i>Allium sativum</i>	11.37
T ₄	Dhatara leaf extract	9.56
T ₅	Treated control (Mancozeb)	14.16
T ₆	Neem leaf extract	10.56
Overall Mean		11.24
F- test		S
S. Ed. (±)		0.41
C. D. (P = 0.05)		0.91

Fs=Foliar spray

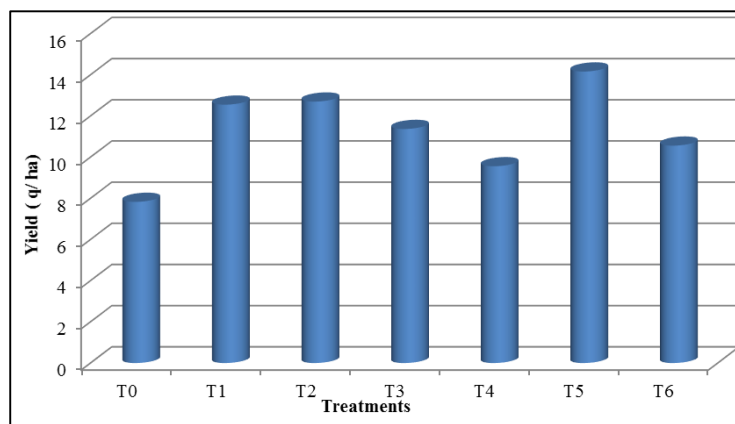


Fig 3: Effect of bio-agents and plant extracts on Yield (q/ha) of mustard

Discussion

Biological screening of plant extracts was carried out throughout the world for the determination of their antifungal activity. Synthetic chemicals used to control plant disease not only pollute the environment, but are also harmful to human health.

Biological control is a promising tool to maintain current level of agricultural production while reducing the release of polluting chemical pesticides to the environment. It is a

complex process made up from several successive steps generally initiated by a remote sensing of host which stimulates directed growth; subsequently contact is made between fungal antagonist and host (pathogen) surface.

The botanicals Garlic bulb, Neem leaf, Datura leaf were evaluated under crude forms against *A. brassicicola* under *in-vitro* condition. All the tested botanicals significantly inhibited the fungal growth. The minimum growth was recorded in *Allium sativum* followed by Neem leaf and Datura leaf. *Allium*

sativum was significantly superior over Neem leaf and Datura leaf. Plant extract *Allium sativum* is confirmed to have antifungal effect on *A. brassicicola* and distinctly reduced the growth of *A. brassicicola*.

The extracts of *Allium sativum*, *Datura stramonium* completely inhibited the spore germination of *Alternaria brassicicola* isolates (Sheikh and Agnihotri, 1972) The Neem leaf extract showed high efficacy to inhibit the radial growth of *Alternaria* spp. (Sharma *et al.* 2007) ^[62].

Results from the present study could be correlated with the studies made by Mughal *et al.* (1996) with leaf extracts from *Allium sativum*, *Datura alba* and *Withaniasomnifera* against *Alternaria alternata*, *A. brassicicola* and *Myrothesiumrodium*; Shinde and Dhale (2011) ^[64] The results proved that Garlic bulb extract in reducing the *Alternaria* leaf blight of mustard. Whereas this experiment contradicts it that Neem leaf extract was best followed by Garlic bulb extract in reducing the *Alternaria* leaf blight of mustard in leaf, silique and also confirmed the results of Chattopadhyay *et al.* (2005) Antagonistic activity of *Trichoderma* species grew much faster than the tested fungi and inhibited the growth of pathogen. But both the species of *Trichoderma* differed with each other in their ability to suppress the growth of the pathogen. Among two, *Trichoderma harzianum* showed high percentage growth inhibition of test pathogen when compared to *Trichoderma viride*. Maximum inhibition 43.81% was recorded from *Trichoderma harzianum*, 30% was recorded from *Trichoderma viride*.

Trichoderma is the most commonly used fungal biological control agent and have long been known as effective antagonists against plant pathogenic fungi (Chet *et al.* (1981) ^[19]; Papavizas, 1985). Some of the isolates of *Trichoderma* spp. included in the present study significantly inhibited several pathogens (Dubey, 2003) ^[20].

The antagonists *Trichoderma harzianum*, *Trichoderma viride*, effectively controlled seed borne pathogen *Alternaria brassicicola* (Vananacci and Harman, 1987)

Effective inhibition of mycelia growth of *Alternaria* spp. Causing leaf blight by *Trichoderma viride* has been reported (Babu *et al.* 2000) ^[6] Use of antagonists, particularly *Trichoderma* species has been reported quite effective against different pathogens (Chattopadhyay *et al.*, 2002) ^[14] particularly as seed treatment followed by fungicidal spray in managing many fungal diseases in various host pathogen combinations (Rashmi Rohila, 2001). *Trichoderma viride* @ 2%, was the most effective bio-agents in managing the disease intensity of leaf blight on mustard caused by *Alternaria brassicicola*. This bio-agent also gave higher yield (kg/ha). Amongst the botanicals Garlic bulb extracts was the most effective and Datura leaf extract, neem leaf extract are also found to be significantly effective in comparison to control as such botanicals lasted in the present experiment have proved their potential and can be used in future for the management of leaf blight of mustard and thus can reduce the indiscriminate use of fungicides by the mustard growers.

Conclusions

In the present investigation, foliar spray with *Trichoderma harzianum* @ 5g/kg followed by *Trichoderma viride* significantly reduced the *Alternaria* blight and increased the seed yield in Indian mustard. *Trichoderma harzianum* was found most effective in reducing *Alternaria* blight, recorded

maximum disease incidence (%), plant height (cm) and increased the seed yield. This present study confirms the *in vitro* antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* towards *Alternaria brassicicola*. Among, *T. harzianum* has shown strong inhibitory effect on the development of *Alternaria brassicicola* compared to *T. viride*.

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