



## SURVEY AND BIO-EFFICACY OF BIO-AGENTS AGAINST ALTERNARIA BRASSICAE CAUSING BLIGHT DISEASE OF MUSTARD

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### ABSTRACT

Rapeseed-mustard are the major rabi oilseed crops of the country. A number of diseases of different origins attack rapeseed-mustard in which, *Alternaria blight* caused by *Alternaria brassicae* (Berk.) Sacc. is taking heavy toll every year by decreasing the quantity and quality of produced. In the present investigation, a survey was conducted during December to March 2013-14 and 2014-15 at Oilseed farm of University of Agriculture and Technology, Kanpur (U.P.) and four areas adjoining it viz. Research farm Nawabganj, Vegetable farm Kalyanpur, Shivrajpur, Billhaur, Bagha and eastern U.P. comprising N.D. Univesrity Kumarganj, B.H.U Varanasi, Rampur Jaunpur and Mariahu Jaunpur to get precise information on efficacy of bio control agents in controlling blight disease in mustard. *Trichoderma harzianum* and *Trichoderma viride* were isolated from mustard leaf. In dual culture test *Trichoderma harzianum* caused maximum inhibition of radial growth of *Alternaria brassicae*.

Keywords : Bio-efficacy, bio-agents, alternaria, mustard

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is the second largest oilseed crop in India after Groundnut. Among rapeseed-mustard, Indian mustard is one of the most important oilseed crops which contribute about 85 per cent of total rapeseed-mustard produced in India (Kumar and Chauhan, 2005). Rajasthan, U.P.,

M.P., Haryana, West Bengal, Gujarat, Bihar and Punjab are the major mustard growing states in India. *Alternaria blight* of mustard group of crops is most important devastating and widespread disease throughout the world (Kolte, 1985, Khan *et al.*, 2007a, Khan, 2011) on Indian mustard. *Alternaria blight* causes average yield loss of 46.5% (Kolte *et al.*, 1987). The control of *Alternaria blight* is exclusively based on chemical pesticidal application. Several recommended pesticides are effective for the pathogen but not considered as long term solution. Due to hazardous fungicidal residue and environmental imbalance they are not considered to be long term solution. Some alternative methods of control have been adopted to

modify this solution. Researchers have focussed on long lasting environment friendly safe and effective bio-control methods for the plant disease management. Bio control agents were used for control of soil borne, foliar and post harvest disease in various crops. Fungi in the genus *Trichoderma* are promising bio-control agents against wide range of plant pathogens (Howell, 2003). They act as bio-control agents against plant pathogens directly by mycoparasitism production and indirectly by nutrient and space competition, environmental conditions or plant growth modification and defensive promotion (Benítez *et al.*, 2004).

### MATERIALS AND METHOD

#### Survey for disease intensity of *Alternaria blight* of Indian mustard

A survey was conducted at Oilseed farm of University of Agriculture & Technology, Kanpur and four areas adjoining it viz. Research farm Nawabganj, Vegetable farm Kalyanpur, Shivrajpur, Billhaur, Bagha and eastern U.P. comprising N.D. Univesrity Kumarganj, B.H.U Varanasi, Rampur,

Jaunpur and Mariahu, Jaunpur to get precise information on disease intensity. Observation on disease severity will be recorded on the basis of leaf area affected by using 0-5 scale. Three plants were randomly selected from one sq meter plot at three places of same field during the course of survey. The diseased and healthy leaf count was made of three

selected plants. These leaves were arranged into six groups from zero to five on the basis of the percentage leaf area affected. Disease intensity was recorded at the maturity stage as per the scale. The percentage disease index was calculated by the following formula.

$$\text{Disease severity (\%)} = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves examined} \times 5} \times 100$$

### Dual culture test of bio-control agents in-vitro

A dual culture test was conducted in-vitro to assay the biocontrol agents viz. *Trichoderma viride* and *Trichoderma harzianum* to inhibit hyphal growth of *Alternaria brassicae* to observe interaction between colonies. The antagonistic potential of indigenous isolates of *Trichoderma* was evaluated against *Alternaria brassicae* using dual culture technique with slight modifications. Five millimeter diameter mycelial disc of each test antagonist (*Trichoderma* isolates) taken from 7 day old culture was used against same sized mycelia disc of *Alternaria brassicae* at opposite end on PDA contained in 90 mm diameter Petri- plates. The pathogen and antagonist disc were placed at equal distances from

the periphery of the petri-plate. The PDA plates inoculated only with antagonists served as control. The plates were incubated at  $25 \pm 2^\circ\text{C}$ . The experiment was conducted under Completely Randomized Design (CRD). The growth of the pathogen in both test and control experiments were recorded. Data were obtained for percent inhibition of radial growth (PIRG) =  $(R1 - R2) / R1 \times 100$ . Where R1 = radial growth of pathogen in control and R2 = radial growth of pathogen in dual culture experiments with antagonists. The numbers of days taken by *Trichoderma* isolates to completely overlap the pathogen colony were recorded. The isolate taken the shortest number of days was counted for signifying good antagonistic properties.

### Results And Discussion

The observations presented in the survey data revealed that *Alternaria* blight was prevalent in all the areas where this crop was grown. **Among various locations the highest disease intensity was found at Oilseed farm Kalyanpur (53.60 and 55.20%) followed by N.D. Uni. Kumarganj (47.20 and 50.40%) and Mariahu, jaunpur (48.80 and 52.80%) locations during 2013-14 and 2014-15.** Whereas, the lowest disease severity was recorded at Bagha, Kanpur (37.60 and 40.20%) followed by Research farm Kanpur (38.40 and 42.80%) during

2013-14 and 2014-15. Similarly, Average spot size of *Alternaria* blight were largest at Oilseed farm, Kalyanpur (10.4 mm) followed by N.D. Uni. Kumarganj (10.3 mm) and Mariahu, jaunpur (10.3 mm). Lowest average spot size were recorded at Bagha, Kanpur (7.8 mm) followed by Research farm, Kanpur (8.7 mm). **Howlader et al., (1989)**, and **Prasad et al., (2003)** reported that the early sowing reduced the disease severity.

**In biological control, the most important principle of choosing an antagonistic organism is its capacity to grow fast and occupy most of the**

place in the site of probable exists of the pathogen. *Trichoderma viride* and *Trichoderma harzianum* were evaluated against *Alternaria brassicae* using dual culture test. A dual culture test was conducted in vitro to assay the ability of bio-control agents to inhibit hyphal growth of *Alternaria brassicae* as well as to observe interactions between colonies. In the present study, the highest percentage of inhibition in mycelial growth was 55.07 (PDA) for *Trichoderma harzianum* and taken 96 hours to over grow the colony of *A. brassicae*. Dharmaputra, (1994) tested two isolates of *T. harzianum* and one isolates of *T. viride* against three isolates of *Ganoderma* from oil palms, all three *Trichoderma* isolates inhibited the mycelial growth of the pathogen but *T. harzianum* showed the best performance among the three isolates. *Trichoderma harzianum* showed 44.25% inhibition growth at 72 hours and least inhibition growth was 40 % at 48 hours respectively.

The rapid growth gives *Trichoderma* an important advantage in the competition for space and nutrients with plant pathogenic fungi even before deploys arsenal of mycotoxins (Barbosa *et al.*, 2001). One of the key elements of sustainable agriculture is the ecological approach to solving the problems with plant pathogens, by the application of biocontrol agents. The present study showed that *T. viride* caused up to 51.90% inhibition of radial growth at 96 hours followed by 28.50% in 72 hours in *Alternaria brassicae*, only a slight decrease in growth of pathogenic fungus was recorded. Kumar *et al.* (2005) and Abd-El-Kareem (2007) observations on *Alternaria alternata* caused disease in bean plants are supporting to this study. Regular growth of both the fungus was observed in dual culture petri-plates and shown in Table-2 and depicted in Figure-2.

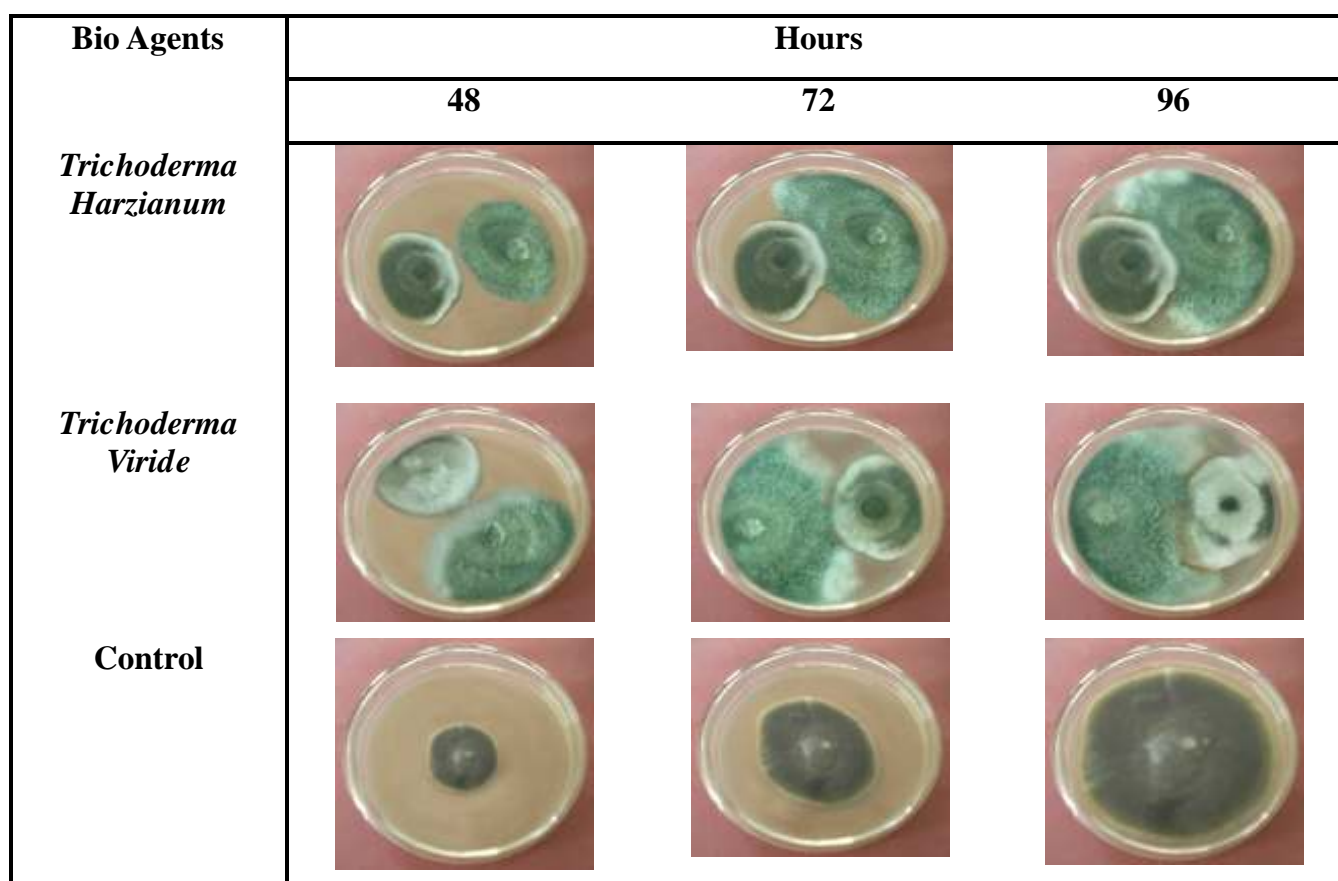
**Table -1: Disease intensity of Alternaria blight of Indian mustard at different locations**

S.No.	Location	Average spot size (mm)	% Disease severity (2013-14)	% Disease severity (2014-15)
1.	Research farm Kalyanpur, Kanpur.	8.7	38.40	42.80
2.	Oilseed farm Kalyanpur, Kanpur.	10.4	53.60	55.20
3.	Vegetable farm Kalyanpur, Kanpur.	9.3	41.60	44.60
4.	Bagha, Kanpur.	7.8	37.60	40.20
5.	Shivrajpur, Kanpur.	9.8	44.00	44.60
6.	Billhaur, Kanpur.	9.6	44.80	47.00
7.	N.D. Uni. Kumarganj.	10.3	47.20	50.40
8.	B.H.U., Varanasi.	9.1	39.20	41.60
9.	Rampur, Jaunpur.	9.4	42.40	45.00
10.	Mariahu, jaunpur.	10.3	48.80	52.80

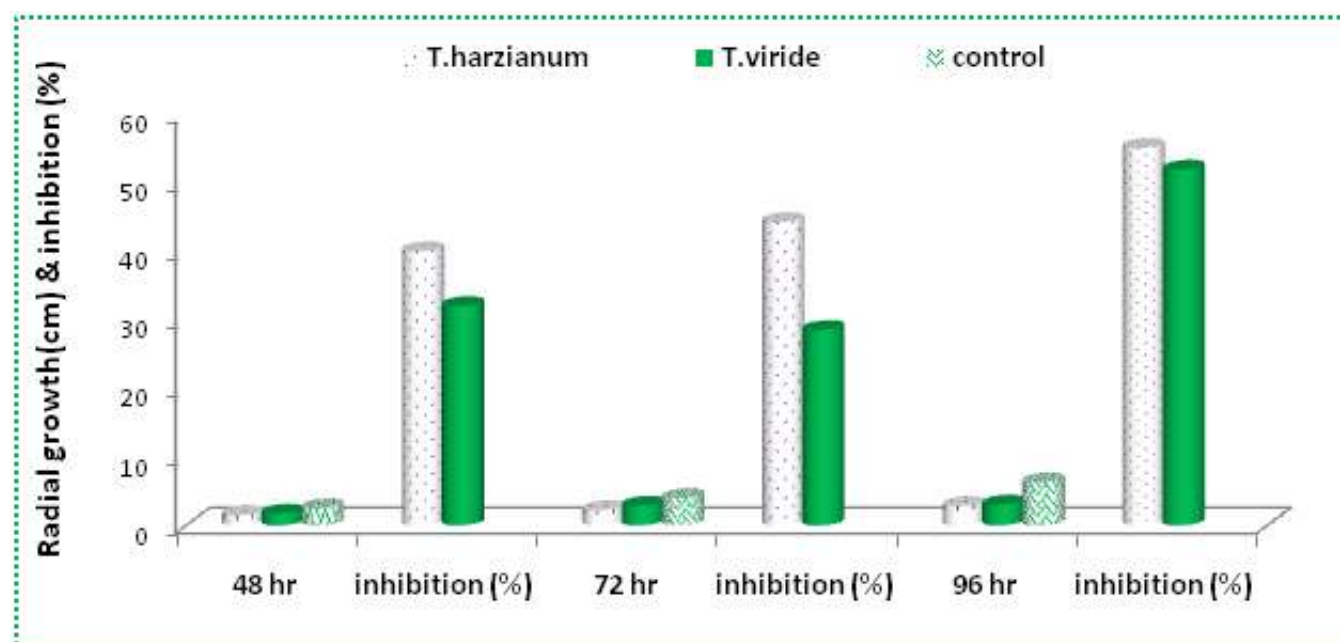
**Table -2: Effect of bio-control agents on radial growth of *Alternaria brassicae* in dual culture**

Treatment	Radial growth (cm)		
	48 hr	72 hr	96 hr
<i>T.harzianum</i>	1.50 (40.00)	2.23 (44.25)	2.83 (55.07)
<i>T.viride</i>	1.70 (32.00)	2.86 (28.50)	3.03 (51.90)
Control	2.50	4.00	6.30
SEM	<b>0.527</b>	<b>0.123</b>	<b>0.103</b>
CD at 5%	<b>1.826</b>	<b>0.426</b>	<b>0.358</b>

**Figure- 2:** Evaluation of bio-control agent *in vitro* on radial growth of *Alternaria brassicae* on PDA (Dual culture plate method).



**Fig. 2(a) Effect of bio-control agents at different time period on growth of *Alternaria brassicae* on PDA**



## CONCLUSION

Survey data illustrated that *Alternaria* blight was prevalent in all the areas where this crop was grown in which oilseed research farm Kanpur gave maximum disease severity and lowest severity was seen in Bagha, Kanpur.

This study confirms in-vitro biological activity of *T. harzianum* and *T. viride* towards

*A.brassicae*. It has shown strong reducing effect on the development of *A.brassicae* with various mechanisms of antagonistic influence. This bio-control agent caused abnormalities in the pathogens's morphology. The strong reducing effect of *T. viride* towards *A.brassicae* can be applied in biological control of this pathogen.

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