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Comparative study on the effect of chemicals on Alternaria blight in Indian mustard – A multi-location study in India

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Abstract

High severity of Alternaria blight disease is a major constraint in production of rapeseed-mustard in India. The aim of this study was to investigate the suppressive potential of chemicals *viz*., zinc sulphate, borax, sulphur, potash and calcium sulphate, aqueous extracts *viz*., *Eucalyptus globosus* (50 g l^{-1}) leaf extract and garlic (*Allium sativum*) bulb (20 g l^{-1}) extract, cow urine and bio-agents *Trichoderma harzianum, Pseudomonas fluorescence* in comparison with the recommended chemical fungicide (mancozeb), against foliar disease Alternaria blight of Indian mustard [*Brassica juncea* (L.) Czern. and Coss] under five different geographical locations of India. Mancozeb recorded the lowest mean severity (leaf: 33.1%; pod: 26.3%) of Alternaria blight with efficacy of garlic bulb extract alone (leaf = 34.4%; pod = 27.3%) or in combination with cow urine (leaf = 34.2%; pod = 28.6%) being statistically at par with the recommended chemical fungicide. Chemicals also proved effective in reducing Alternaria blight severity on leaves and pods of Indian mustard (leaf = 36.3-37.9%; pod = 27.5-30.1%). The effective treatments besides providing significant reduction in disease severity also enabled increase in dry seed yield of the crop (mancozeb = 2052 kg ha^{-1}; garlic = 2006 kg ha^{-1}; control = 1561 kg ha^{-1}).

Key words

Alternaria brassicae, Brassica juncea, Chemicals, Plant extracts

Introduction

In India, Rapeseed-mustard is an important group of edible oilseed crops and contributed around 26.1% of the total oilseed production. Out of 57856 thousand tonnes of rapeseed-mustard seed produced over 30308 thousand ha in the world, India produced 5833 thousand tonnes from 5750 thousand ha (FAO, 2010). Indian mustard [*Brassica juncea* (L.) Czern. and Coss.] contribute about 85% of the total rapeseed-mustard produced in India (Kumar and Chauhan, 2005).

Alternaria blight disease caused by *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schw.) Wiltshire is one among the important diseases of Indian mustard, which has been reported from all the continents of the world, causing 10-70% yield losses depending on the crop species, being high in *B. rapa* with 35-40%

in Indian mustard (Chattopadhyay, 2008), depending on the prevailing environmental factors particularly at critical stages. There is no proven source of resistance against the disease reported till date in any of the host (Kumar and Chauhan, 2005; Chattopadhyay, 2008).

In recent years, an increasing consciousness about environmental pollution due to pesticides and development of fungicide-resistant strain in plant pathogens has challenged the plant pathologists to search for non-toxic fungicides for substituting the recommended chemicals. Fungicides are reported to be able to control the disease (Chattopadhyay and Bagchi, 1994). Alternaria blight disease severity varies with the micro-climatic conditions at the particular location. Application of fungicides at critical stages of 45 and 75 days after sowing for development of the disease has been reported to minimise losses due to the disease and increase benefit for the users (Meena *et al.*, 2004).

Mineral nutrition has long been recognised as an important component of disease management practices (Agrios, 2005). Integration of several management practices *viz.*, planting of cabbage in 01 November at 40 x 40 cm spacing in the field treated with S-Zn-Mg-Mo-B at 30-5-1-1-1 kg ha^{-1} in addition to the recommended rate of NPK and sprayed with chemical fungicide Iprodione (2 g l^{-1}) recorded the best Alternaria blight reduction (93.2%) and increased seed yield by 207.8% over the normal management practices (Hossain and Mian, 2005). The decrease in Alternaria blight severity due to K application at 40 kg ha^{-1} gave consistently increased (68%) seed yield over control (Sharma and Kolte, 1991; Godika *et al.*, 2001).

Sulphur deficiency of oilseed rape negatively affects disease resistance caused by the reduction of sulphur dependent phytoanticipins (Dubuis *et al.*, 2005). Soil applied sulphur was found to increase resistance against a variety of fungal pathogens on different crops (Klikocka *et al.*, 2005). Its negligible toxicity to animals and low toxicity to plants have made sulphur attractive as a chemical control agent; it is also a common component of integrated pest management programmes because of its low toxicity to beneficial insects (Emmett, 2003). The element's possible role in defence and the form, location and levels *in planta* are considered. Sulphur is one of many S-containing defence-related compounds and it is ironic that sulphur deficiency has recently become a widespread nutrient disorder in crops (Singh, 2001), largely due to restrictions on fossil fuel burning. The problem is being addressed by application of sulphur, borax and zinc. However, limited efforts have been made to use plant extracts, bio-agents and non-toxic chemicals for effective management of Alternaria blight of mustard.

Potential of chemical fungicides, promising plant extracts, bio-agents for substituting the recommended chemical fungicides applied as soil application at the time of sowing and foliar spray at critical stages of 45, 75 days after sowing against the Alternaria blight disease of Indian mustard were tested in the multi-location study.

Materials and Methods

Field experiments were conducted in two successive post-monsoon (*rabi*) crop seasons (October - March) of 2004-05 and 2005-06 at five different geographical locations in India *viz.*, Bharatpur (27°12′ N, 77°27′ E, Rajasthan), Ludhiana (30°90′ N, 75°80′ E, Punjab), Hisar (29°15′ N, 75°70′ E, Haryana), Dholi (25°59′ N, 85°75′ E, Bihar) and Pantnagar (29°N, 79°3′ E, Uttarakhand) under the ICAR National Network Project on management of Alternaria blight in field and horticultural crops. Rapeseed-mustard covers all *Brassica* spp. grown in the country including *B. napus* in the Himachal Pradesh state, foothills of Uttarakhand, Jammu & Kashmir, some parts of Punjab state, *B. rapa* var yellow sarson in West Bengal and some parts of Bihar states with around 85% area dominated by *B. juncea* all over the country. Rapeseed-mustard is predominantly cultivated in Rajasthan (50%), Uttar Pradesh (12.3%), Haryana (11.2%), Madhya Pradesh (9.8%), Gujarat (6.5%) and West Bengal (5.1%) states of the country, which all together contribute 95% to the total national production of the crop. The experimental sites represented hot spots for Alternaria blight disease in different dominant Indian mustard growing areas mainly as a field crop under non-limiting soil moisture conditions in semi-arid and sub-humid agro-climatic zones of India.

Twelve treatments including a control plot with only water spray were considered. Experiment was laid out at all the five locations in plots of 5 x 3 m at 30 x 15 cm spacing in randomized block design (RBD) with three replications using popular cultivar Varuna of Indian mustard as the test variety. Experimental plots at all the locations received recommended (NRCRM, 1999) dose of nitrogen (80 kg ha^{-1}) and phosphorus (40 kg ha^{-1}).

Uniform spray solution of required concentration for chemicals at all the locations *viz.*, zinc sulphate @ 2 g l^{-1}, borax @ 5 g l^{-1} and calcium sulphate @ 5 g l^{-1} were prepared from their salts in tap water. Application of potash @ 40 kg and sulphur @ 20 kg ha^{-1} was carried out as basal dose at the time of sowing. Fungal bio-agent *Trichoderma harzianum* (isolate = PBA 1; source = Biocontrol laboratory, Deptt. Plant Pathology, GBPUAT, Pantnagar 263145, India) was grown in autoclaved potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks after inoculating with its actively growing agar plug and incubated for 10 days at 25 $\pm$ 1°C. The fungal growth with the spores was harvested from the broth and churned in warring blender with required amount of sterile distilled water to get spore suspension containing 1 x 10^6 spores ml^{-1}. Bacterial bioagent *Pseudomonas fluorescence* (isolate = PBA 2; source = Biocontrol laboratory, Deptt. Plant Pathology, GBPUAT, Pantnagar 263145, India) was cultured on 100 ml King's broth medium in 250 ml Erlenmeyer flasks. These were incubated at 28 $\pm$ 1°C on shaker at 120 rpm for 48 hr. Spray of formulation having concentration of 1x 10^8 c.f.u. ml^{-1} was prepared. *Eucalyptus globosus* leaf extract @ 50 g l^{-1} and garlic (*Allium sativum*) bulb extract @ 20 g l^{-1} alone and the latter in combination with cow urine @ 5%, cow urine alone @ 5% were used. The plant extracts were prepared after washing the respective plant parts with tap water and rinsing them with sterile distilled water. These were processed with sterile water @ 1 ml g^{-1} of tissue (1:1) in a warring blender and filtered through a double layered cheese cloth. This formed the standard aqueous extract solution. Commonly recommended fungicide mancozeb @ 0.25% a.i. was used as spray for comparison with other treatments tested under study. The treatments were applied as foliar spray at known critical stages for Alternaria blight disease development *i.e.* 45 and 75 days after sowing.

In all the experimental plots, randomly selected ten plants were tagged for observations. Percentage disease severity of Alternaria blight was recorded uniformly at all the locations and experimental plots on 10 randomly selected plants on leaves and pods using standard pictorial rating scale of Conn *et al*. (1990). Dry seed yield was recorded at harvest plot wise in each treatment and replication at all the locations. The data were statistically analysed using analysis of variance to determine the least significant difference ($p<0.05$).

Results and Discussion

All the treatments were found effective in reducing the Alternaria blight severity on the leaves and pods over control (Table 1, 2). Highest reduction in Alternaria leaf blight severity was caused by mancozeb (64.3-19.6%) with similar efficacy of garlic bulb extract alone (61.6-11.9%) or in combination with cow urine (60.1-12.8%), being statistically at par ($p<0.05$) at all locations [except at Pantnagar]. *Trichoderma harzianum* (56.7-8.3%) and *Pseudomonas fluorescence* (45.1-7.7%) followed in order of efficacy over control (Table 1). On pods, lowest Alternaria blight severity was observed in mancozeb (80.1-21.1%) and garlic (68.9-14.1%) bulb extract (at par: $p<0.05$) followed by garlic bulb extract + cow urine (75.6-11.1%) as compared to control (Table 2). Extract of *Eucalyptus* reduced disease severity significantly at Pantnagar (leaves = 24.6%; pods = 43.6%) compared to other centres. The trend of reduction in disease severity matched with an increase in yield (Table 3). Performance of garlic bulb extract foliar spray in increasing seed yield (2006 kg ha^{-1}) was significantly at par ($p<0.05$) with mancozeb (2052 kg ha^{-1}) and significantly superior over the other treatments over locations during both the years. Among the chemicals calcium sulphate gave highest average yield over five locations (1846 kg ha^{-1}) followed by zinc sulphate (1745 kg ha^{-1}).

Garlic aqueous bulb extract has been reported to be effective against Alternaria blight of Indian mustard even earlier (Meena *et al*., 2004). Success of the bioagent *Trichoderma* has been reported earlier against Alternaria blight of oil crop linseed (Mercer *et al*., 1993) as also in Indian mustard (Meena *et al*.,

Table - 1: Effect of different chemicals, plant extracts and bioagents on Alternaria leaf blight severity

Treatment	% Alternaria leaf blight severity*												
	Bharatpur		Ludhiana		Hisar		Dholi		Pantnagar		Mean		Pooled
	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	mean
Mancozeb	14.5	11.1	52.6	32.4	22.2	20.1	33.8	20.3	44.9	57.6	33.6	28.3	30.9
	(22.4)	(19.3)	(63.0)	(34.7)	(27.9)	(26.4)	(31.0)	(26.8)	(50.2)	(49.4)	(34.9)	(31.4)	(33.1)
Cow urine	16.6	13.8	56.8	45.8	43.3	36.7	35.5	18.7	50.0	65.7	40.4	36.1	38.3
	(24.1)	(21.7)	(70.0)	(42.6)	(41.0)	(37.2)	(33.7)	(25.6)	(58.5)	(54.1)	(39.1)	(36.3)	(37.7)
Garlic + Cow urine	16.2	15.6	55.9	45.0	22.3	14.6	29.9	16.0	48.8	64.7	34.6	31.2	32.9
	(23.7)	(23.2)	(68.6)	(43.1)	(27.9)	(22.4)	(25.0)	(23.5)	(56.7)	(53.6)	(35.5)	(33.0)	(34.3)
Garlic	15.5	12.2	56.1	36.6	27.3	20.7	29.3	20.3	47.6	63.6	35.2	30.7	32.9
	(23.2)	(20.4)	(69.0)	(37.2)	(31.3)	(27.0)	(24.0)	(26.8)	(54.7)	(52.9)	(35.9)	(32.9)	(34.4)
P. fluorescens	22.3	19.9	54.9	34.2	32.33	24.1	34.6	13.3	51.0	67.1	39.0	31.7	35.4
	(28.2)	(26.5)	(67.0)	(35.8)	(34.6)	(29.4)	(32.3)	(21.3)	(60.2)	(54.9)	(38.4)	(33.6)	(36.0)
E. globosus	21.7	16.5	55.7	39.9	38.3	29.3	32.8	22.3	46.5	61.3	39.0	33.9	36.4
	(27.8)	(23.9)	(68.2)	(39.1)	(38.2)	(32.8)	(29.3)	(28.2)	(52.9)	(51.5)	(38.4)	(35.1)	(36.8)
T. harzianum	17.6	11.8	53.5	33.5	35.0	27.3	28.9	15.0	50.0	68.3	37.0	31.2	34.1
	(24.8)	(20.1)	(64.6)	(35.4)	(36.3)	(31.0)	(23.3)	(22.8)	(58.7)	(55.7)	(37.1)	(33.1)	(35.1)
Calcium Sulphate	28.6	17.1	58.2	37.6	25.0	16.7	30.4	24.7	48.1	70.5	38.1	33.3	35.7
	(32.3)	(24.4)	(72.2)	(37.8)	(30.0)	(24.0)	(25.7)	(29.8)	(55.6)	(57.1)	(37.9)	(34.6)	(36.3)
Borax	26.5	15.5	58.8	43.8	33.3	25.1	33.2	21.7	50.6	73.9	40.5	36.0	38.2
	(31.0)	(23.2)	(73.3)	(41.4)	(35.3)	(30.1)	(30.0)	(27.0)	(59.5)	(59.3)	(39.4)	(36.3)	(37.9)
Potash + Sulphur	23.5	17.3	54.9	38.6	28.7	20.7	34.6	25.3	49.8	73.1	38.3	35.0	36.6
	(29.0)	(24.5)	(67.0)	(38.4)	(31.9)	(27.0)	(32.3)	(30.2)	(58.2)	(58.7)	(38.0)	(35.8)	(36.9)
Zinc Sulphate	21.4	18.3	56.9	35.5	36.7	28.6	35.7	19.3	49.8	73.9	40.1	35.1	37.6
	(27.5)	(25.3)	(70.3)	(36.6)	(37.3)	(32.3)	(34.0)	(26.1)	(58.3)	(59.2)	(39.1)	(35.9)	(37.5)
Control	40.6	20.9	68.3	47.6	44.3	36.8	38.4	27.7	56.1	81.3	49.5	42.9	46.2
	(39.6)	(27.2)	(78.4)	(43.6)	(41.6)	(37.3)	(38.7)	(31.6)	(67.4)	(64.4)	(44.8)	(40.9)	(42.8)
L.S.D. ($p<0.05$)	2.1	5.5	0.5	0.8	7.1	6.6	2.6	2.9	0.9	1.8	3.3	3.2	2.2

* Figures in parenthesis are angular transformed values and others are actual percent disease severity; mean of three replications

Table - 2: Effect of different chemicals, plant extracts and bioagents on Alternaria pod blight severity

Treatment	% Alternaria pod blight severity*											
	Bharatpur	Ludhiana		Hisar		Dholi		Pantnagar		Mean		Pooled
	2004-05	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	mean
Mancozeb	7.2	50.3	30.1	5.3	3.7	18.1	20.0	44.9	19.1	25.2	18.2	21.7
	(15.6)	(59.2)	(33.2)	(13.3)	(10.9)	(9.7)	(26.5)	(50.2)	(25.9)	(28.3)	(24.4)	(26.3)
Cow urine	7.6	52.6	44.2	22.2	18.1	23.2	10.0	50.0	23.5	31.1	23.9	27.5
	(16.0)	(63.2)	(41.6)	(27.9)	(25.2)	(15.7)	(18.4)	(58.5)	(29.0)	(32.9)	(28.7)	(30.8)
Garlic + Cow urine	6.1	54.7	44.0	16.7	4.3	14.5	16.7	48.8	22.2	28.2	21.8	24.9
	(14.1)	(66.7)	(41.5)	(24.1)	(12.0)	(6.3)	(24.0)	(56.7)	(28.1)	(30.6)	(26.7)	(28.6)
Garlic	5.0	53.3	35.2	8.3	6.7	13.6	19.3	47.6	21.0	25.6	20.5	23.0
	(12.9)	(64.4)	(36.4)	(16.8)	(14.9)	(5.7)	(26.1)	(54.7)	(27.3)	(28.4)	(26.3)	(27.3)
P. fluorescens	7.4	54.3	33.7	12.7	9.8	19.9	8.3	51.0	29.9	29.1	20.4	24.7
	(15.6)	(66.0)	(35.4)	(20.8)	(18.2)	(11.7)	(16.7)	(60.2)	(33.1)	(31.2)	(26.1)	(28.7)
E. globosus	8.6	54.3	38.7	18.7	12.8	16.4	15.7	52.9	19.5	30.2	21.7	25.9
	(17.0)	(66.0)	(38.5)	(25.6)	(21.0)	(8.0)	(23.2)	(46.5)	(26.2)	(32.1)	(27.3)	(29.7)
T. harzianum	5.9	52.8	32.1	16.7	13.7	13.3	10.0	50.0	29.7	27.7	21.4	24.6
	(13.9)	(63.5)	(39.5)	(24.1)	(21.6)	(5.3)	(18.4)	(58.7)	(33.0)	(30.2)	(27.0)	(28.6)
Calcium sulphate	6.7	55.8	36.5	6.7	4.3	13. 6	16.0	48.1	27.0	25.1	20.9	23.6
	(15.0)	(68.4)	(37.2)	(14.9)	(12.0)	(5.7)	(23.5)	(55.6)	(31.3)	(28.8)	(26.2)	(27.5)
Borax	7.5	50.9	42.0	13.3	10.5	18.8	12.3	50.6	28.8	28.2	23.4	25.8
	(15.8)	(60.3)	(40.8)	(21.4)	(18.9)	(10.7)	(20.5)	(59.5)	(32.4)	(30.8)	(28.2)	(29.5)
Potash + sulphur	6.2	55.7	37.5	8.7	5.8	15.6	15.7	49.8	29.0	27.2	22.0	21.6
	(14.3)	(68.3)	(37.7)	(17.1)	(13.9)	(7.3)	(23.3)	(58.2)	(32.6)	(29.6)	(27.1)	(28.4)
Zinc Sulphate	5.1	53.3	34.5	16.7	13.3	24.8	15.0	49.8	30.4	29.9	23.3	26.6
	(12.9)	(64.3)	(35.9)	(24.1)	(21.4)	(17.7)	(22.7)	(58.3)	(33.5)	(31.8)	(28.5)	(30.1)
Control	15.7	59.9	46.8	26.7	19.3	27.7	27.7	56.1	36.1	37.2	32.5	34.8
	(23.3)	(75.0)	(43.2)	(31.2)	(26.1)	(21.7)	(31.7)	(67.4)	(37.0)	(37.1)	(34.5)	(35.8)
L.S.D. ($p<0.05$)	3.7	0.7	0.3	4.9	4.1	2.9	2.7	0.9	1.5	4.4	4.4	2.6

* Figures in parenthesis are angular transformed values and others are actual percent disease severity; mean of three replications

2004). The above results underline the importance of mineral nutrition as a component of disease management practices (Agrios, 2005; Hossain and Mian, 2005). Sulphur deficiency of oilseed rape negatively affects disease resistance caused by the reduction of sulphur dependent phytoanticipins (Dubuis *et al.*, 2005). Soil applied sulphur was found to increase resistance against a variety of fungal pathogens on different crops (Klikocka *et al.*, 2005). Its negligible toxicity to animals and low toxicity to plants have made sulphur attractive as a chemical control agent; it is also a common component of integrated pest management programmes because of its low toxicity to beneficial insects (Emmett, 2003). The element's possible role in defence and the form, location and levels *in planta* are considered. Sulphur is one of many S-containing defence-related compounds and it is ironic that sulphur deficiency has recently become a widespread nutrient disorder in crops (Singh, 2001). In Indian context, more than 50% (10 m ha) of the agricultural soils is zinc-deficient (Singh *et al.*, 2005). Role of calcium in improving tolerance in plants to diseases have been indicated earlier (Reddy, 2001; Agrios, 2005). The problem is being addressed by application of sulphur, borax and zinc. However, this seems to be the possible first report of use of such chemicals *viz.*, sulphur, borax, zinc, *etc.* for effective management of Alternaria blight of mustard.

Disease severity on leaves and pods was higher at most of the locations in 2004-05, except at Pantnagar for blight severity on leaves. Yield in different locations varied over the two seasons and also seemed to vary inversely with disease severity. It seemed that disease severity on pods had a great impact in reducing seed yield in mustard (Chattopadhyay, 2008). This was more evident in 2005-06 at Bharatpur, where yields were high corresponding with no blight severity on the pods. However, a direct relation could not be seen between disease severity and seed yield, which is possibly because yield is a complex polygenic character involving genotype x environment interaction (Thurling, 1991).

Mustard straw is being widely used in the brick kiln as a source of fuel, which gives additional return for the crop produce and encourages farmers for mustard crop cultivation. Farmers traditionally use stem of mustard plant as household fuel for cooking purpose.

Our results from the multi-site two-year study comprehensively proved that chemicals *viz.*, calcium sulphate, borax and sulphur, which significantly reduced the disease severity, could be used to supplement the garlic bulb extract foliar spray, as effective substitutes for mancozeb for better control of disease in Indian mustard crop.

Table - 3: Effect of different chemicals, plant extracts and bioagents on dry seed yield of Indian mustard

Treatment	Yield (kg ha^{-1})*												
	Bharatpur		Ludhiana		Hisar		Dholi		Pantnagar		Mean		Pooled
	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	mean
Mancozeb	1889	4183	1287	2796	1605	1705	1178	1200	1936	2738	1579	2524	2052
Cow urine	1195	3911	730	2657	1520	1635	1000	1333	1480	2264	1185	2360	1773
Garlic + Cow urine	1534	3778	737	2729	1595	1715	1356	1488	1675	2412	1379	2424	1902
Garlic	2050	4233	1130	2603	1585	1698	1156	1200	1744	2664	1533	2479	2006
P. fluorescens	1156	3932	1212	2603	1560	1670	822	1266	1478	2338	1245	2361	1803
E. globosus	1578	3856	1060	2544	1530	1645	1178	911	1916	2701	1452	2331	1892
T. harzianum	1645	4278	1257	2739	1550	1660	1556	1377	1503	2206	1502	2452	1977
Calcium sulphate	1489	3711	1110	2657	1610	1720	1356	1000	1501	2309	1413	2079	1846
Borax	1539	3606	775	2603	1570	1685	844	1244	1418	1894	1229	2206	1718
Potash + Sulphur	1250	3750	1080	2618	1580	1680	800	866	1427	2005	1227	2183	1706
Zinc sulphate	1445	3694	1175	2704	1545	1655	722	911	1434	2168	1264	2226	1745
Control	1117	3500	725	2563	1510	1650	700	822	1215	1813	1053	2069	1561
L.S.D. ($p<0.05$)	274	458	83	NS	49	49	469	30	32	114	211	287	169

*Figures are average of three replications; NS = not significant

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