



***In vitro* and *in vivo* effect of eco-friendly chemicals on alternaria blight disease (*Alternaria brassicae*) and yield attributes in Indian mustard (*Brassica juncea*)**

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Abstract: The present investigation was done to evaluate the effect of different concentrations of five eco-friendly chemicals *in vitro* and *in vivo*, on the management of alternaria blight and yield attributes in Indian mustard (*Brassica juncea* cv. Varuna). Out of five eco-friendly chemicals, K₂SO₄ 1000 ppm (64.28%) followed by ZnSO₄ 1000 ppm (63.88%) showed maximum inhibition of mycelial growth in comparison to check. 0.5% concentration of KCl (57.06%) followed by CaSO₄ (59.50%) and K₂SO₄ (62.20%) showed significantly maximum effect on spore germination in comparison to check (74.60%). Spore intensity significantly increased by all the treatments except CaSO₄ at 0.5% (40.18%) followed by K₂SO₄ at 0.5% (29.86%) and ZnSO₄ 0.75% (5.11% reduction) in comparison to check. The significantly minimum disease index on leaf over check was found by foliar spray of CaSO₄ at 0.5% (23.58%) followed by CaSO₄ at 1.5% (24.00%) and Na₂B₄O₇·10H₂O at 1.5% (24.08%). Na₂B₄O₇·10H₂O at 0.75% showed significantly lowest disease index (23.91%) on pod followed by K₂SO₄ at 1.5% (25.75%) and KCl at 1.5% (26.00%) in comparison to check. CaSO₄ at 1.0% showed maximum number of primary branches (7.00), number of secondary branches (13.00) and total yield/ha (1917.30 kg/ha) in comparison to check. The results obtained from the present study suggested that K₂SO₄ showed maximum *in vitro* effect on *Alternaria brassicae* and CaSO₄ and Na₂B₄O₇·10H₂O are providing maximum reduction of disease and increase in seed yield/ha that leads to efficient alternaria blight disease management strategies in field condition. These eco-friendly chemicals can protect the crops from alternaria blight diseases and increase the production and productivity of the Indian mustard crop.

Keywords: Alternaria blight, *Alternaria brassicae* (Berk.) Sacc, Eco-friendly chemicals, Mustard

INTRODUCTION

Rapeseed and mustard (*Brassica spp.*) are contributing approximately 25 percent of the India's total oilseed production. Among the oilseed crops, India produced 8.2 mt of rapeseed-mustard from 6.7 mha of land during the 2010-11 seasons (GoI, 2011). Among the diseases, alternaria blight disease caused by *Alternaria brassicae* (Berk.) Sacc has been reported from all the continents of the world and is considered an important constraint in production and productivity of oilseed Brassicas in India, causing up to 47% yield losses (Kolte *et al.*, 1987; Meena *et al.*, 2012). Average yield losses have been reported in the range of 10-70 per cent (Ram and Chouhan, 1998; Shrestha *et al.*, 2005), depending upon prevailing weather and disease situation (Meena *et al.*, 2004; Chattopadhyay *et al.*, 2005). In addition to the direct losses in yield, the disease adversely affects the seed quality by reducing seed size, seed discolouration and reduction in oil content (Kaushik *et al.*, 1984;

Prasad and Lallu, 2006). Alternaria blight disease can be managed by the use of different fungicides viz. Dithane M 45, Rovral 50, Ridomil MZ etc. (Verma and Saharan, 1994; Khan *et al.*, 2007; Sultana *et al.*, 2009) that exerted tremendous pressure on environment and human health.

An increasing consciousness about environmental pollution due to pesticides, human health and development of fungicide-resistant strain in plant pathogens has challenged the plant pathologists to search for eco-friendly non-toxic fungicides for substituting the recommended fungicides in recent years (Meena *et al.*, 2013). Mineral nutrition has long been recognized as an important component of disease management practices (Agrios, 2005). A number of trace elements that are not recognized as essential to plants strongly influence the host-pathogen relationships. Macronutrients and micronutrients can be identified with specific biochemical pathways and their effects on disease offer avenues for elucidating mechanisms of resistance in higher plants. In contrast

to the largely structural, conformational, and osmotic roles of the macronutrients, the micronutrients act as catalysts, cofactors, and inhibitors (Graham, 1983). The eco-friendly approach to overcome biotic stress helped to optimize the economic yields and enable farmers to grow healthy Indian mustard crops (Meena *et al.*, 2011; Meena *et al.*, 2013; Kumar *et al.*, 2014). The present study has been performed to obtain suitable information regarding disease management and precise estimates for yields due to different treatments in Indian mustard. This paper reports *in vitro* and *in vivo* effect of eco-friendly chemicals on management of alternaria blight and yield attributes in Indian mustard.

MATERIALS AND METHODS

Pathogenic fungi: *Alternaria brassicae* infected leaves exhibiting concentric rings, a typical symptoms of alternaria blight, was collected from the field-grown plants of highly susceptible *B. juncea* cv. Varuna from Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (India). The culture of *A. brassicae* was isolated on potato sucrose agar (PSA) and purified by single spore isolation. The pure culture was incubated at $22 \pm 1^\circ\text{C}$, with 12 hrs light and 12 hrs dark. Pathogenicity of the fungal culture was done by infecting 25 days old plants of highly susceptible *B. juncea*.

***In vitro* evaluation of eco-friendly chemicals against *A. brassicae*:** The inhibitory effects of four concentrations i.e. 100, 500 and 500ppm of five eco-friendly chemicals viz. CaSO_4 , KCl, K_2SO_4 , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ and one fungicide, mancozeb as check, were studied. Fifteen-milliliter potato sucrose agar (PSA) medium was poured in each petri plate. Medium without any chemical solution served as control. A 6-mm disc of mycelial growth of *A. brassicae* was placed in the centre of each petri plate and three replications of each concentration were kept. The plates were incubated in an incubator (maintained at $20 \pm 2^\circ\text{C}$ with 12 hrs fluorescent light). The colony diameter measured after 7 and 14 days after inoculation (DAI). Per cent inhibition over control was assessed by following Nene and Thapliyal (1993).

$$R = \{(C - T) / C\} \times 100$$

Where,

R= Per cent inhibition

C = Radial growth of pathogen colony in control

T = Radial growth of pathogen colony in treatments

The effect of five chemicals, each at the concentration of 0.5% on spore germination of *A. brassicae* were investigated on plain water agar plates at 20°C by following the agar plate method (Gattani, 1954). Water agar (1%) was prepared and sterilized. For this purpose, double strength solution of different chemicals of desired strength i.e. 0.5% prepared separately in distilled water. The 10^4 spores ml^{-1} spore suspension was prepared using two-week-old culture in the Blender. One milliliter of each of the spore suspension and chemicals were

mixed and poured on the above mentioned water Agar plates and incubated at 20°C . Germination of spores was recorded after 8 hrs with the help of a compound microscope. Both germinated and un-germinated spores in the several microscopic fields were counted under the low power. In this way total 100 spores were observed for recording effect on spore germination by different chemicals.

Intensity of sporulation of *A. brassicae* on chemically-treated leaves was studied. Infected leaves from each treatment were separated by 6-mm cork borer and surface sterilized with 0.1% mercuric chloride and further washed in sterilized distilled water, dried with a sterilized blotting paper and kept for incubation in moist chamber for 72 hrs at room temperature at about $20 \pm 2^\circ\text{C}$ with continuous light. Sporulation was determined by suspending the sporulating spots in glass vials containing 1 ml of distilled water and lacto phenol (9:1 v/v). The glass vials were then shaken vigorously and spots were scrapped with the help of needle. The spores were counted with the help of haemocytometer.

***In vivo* evaluation of eco-friendly chemicals against**

***A. brassicae*:** To study the effect of three different concentrations of five chemicals viz., CaSO_4 , KCl, K_2SO_4 , ZnSO_4 , and $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, a field experiments were conducted at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (India) which is situated at 29°N latitude and 79.73°E longitude and 243.80 meter above sea level. Two sprays of each chemical of the respective concentrations were given during the entire growth period of plants with an atomizer. First spray of respective chemicals was given on all the leaves of 25 days old plants and the second spray was given after onset of disease. This experiment was conducted in a randomized block design (RBD) with three replications. The plot size for each treatment was $3 \times 2 \text{ m}^2$ where 5 rows of 3 m length were maintained by keeping row-to-row distance 40 cm and plant-to-plant distance 10 cm. Recommended dose of fertilizers of NPK ($\text{N}_{100} : \text{P}_{40} : \text{K}_{40}$) kg ha^{-1} were given in the form of urea, single superphosphate and muriate of potash, respectively.

Data's recorded: Average disease index on leaf due to alternaria blight was taken at 10 days interval at 60, 70, 80 and 90 days after sowing (DAS) and average disease index on pods were recorded at 80 DAS. For recording observations, twenty five leaves and twenty five uniform pods were randomly selected from each replication and rated as per 0-5 rating scale as 0= no symptom, 1= 1-10%, 2= 11-25%, 3= 26-50%, 4= 51-75% and 5= >75% (Verma and Saharan, 1994; Conn *et al.*, 1990) and average disease index was calculated by the following McKinney (1923).

The plant growth and yield components viz. primary branches, secondary branches, number of siliqua/plant, Number of seeds/plant, Seed yield/hectare and oil content (%) were recorded. Five randomly selected plants per plot were taken for observation. The seed samples were analysed for oil content (%) with the help of nuclear magnetic

resonance spectroscope (New Port Analyser) in an oven dried seeds at 60°C.

Statistical analysis: All data were statistically analyzed using an analysis of variance (ANOVA) to determine the least significant difference ($P < 0.05$). The data collected as percentages were processed by an angular transformation.

RESULTS AND DISCUSSION

In vitro evaluation of eco-friendly chemicals against

A. brassicae: The data given in table 1 indicated that all the treatments had inhibitory effect on mycelial growth of *A. brassicae*. All the treatments were significantly different over check at both stages of observation i.e. 7 and 14 DAI. After 7 DAI, mancozeb 1000 ppm showed maximum inhibitory effect (83.33 %) over check. Among the eco-friendly chemicals, zinc sulphate 1000 ppm and borax 100 ppm concentration gave significantly maximum and minimum inhibition of mycelial growth over check i.e. 77.83 percent and 30.36 percent, respectively. The significantly maximum colony diameter was found in Borax 100 ppm (5.40 cm) followed by CaSO₄ 100 ppm (5.30 cm) and CaSO₄ 500 ppm (4.76cm) at 14 DAI (Table 1). The significantly maximum inhibition (%) was recorded in mancozeb 1000 and 500 ppm (81.48 and 74.74 %) followed by K₂SO₄ 1000 ppm (64.28 %) and ZnSO₄ 1000 ppm (63.88%).

All five eco-friendly chemicals were observed for their toxic effect on spore germination of *A. brassicae* (Table 2). KCl at 0.5% showed the significantly lowest spore germination i.e. 57.06 % followed by CaSO₄ at 0.5% (59.50 %) and K₂SO₄ at 0.5% (62.20 %). All chemicals except borax were found significant over check. The minimum per cent reduction of spore germination over check was recorded in borax at 0.5% (3.48 %) and ZnSO₄ at 0.5% (12.78 %).

All the treatments showed increase of sporulation intensity except CaSO₄ at 0.5%, K₂SO₄ at 0.5% and ZnSO₄ at 0.75% (Table 3). The minimum sporulation was recorded in CaSO₄ at 0.5% in which about 40.18 % reduction was observed as compared to check. The maximum sporulation was observed in treatment K₂SO₄ at 1.0% (93.12×10^3 spores/ml) followed by Na₂B₄O₇.10H₂O at 0.5% (44.50×10^3 spores/ml). None of the chemicals was found significantly affecting the sporulation intensity in comparison to check except K₂SO₄ at 1.0% and Na₂B₄O₇.10H₂O at 0.5%.

Kaur (2000) observed that ZnSO₄ was found to be inhibitory to *Albugo candida*, *Peronospora parasitica* and *A. brassicae*. Furuya *et al.* (1999) also found no germination of *Fusarium oxysporum* f sp. *Phaseoli* in soil with higher aluminium contents. It may be chance that heavy ions of metal and non-metal inactivate enzymes required for germination. Vidhyasekaran (1988) reported

Table 1. Effect of different concentrations of some eco-friendly chemicals and mancozeb on the growth of *A. brassicae* on potato sucrose agar (PSA) medium.

Treatment	Concentration (ppm)	Colony diameter (cm)			
		7 DAI	Inhibition (%)	14 DAI	Inhibition (%)
CaSO ₄	100	3.53	41.16	5.30	29.89
	500	3.26	45.66	4.76	37.03
	1000	2.96	50.66	4.43	41.40
KCl	100	2.73	54.50	3.53	53.30
	500	2.53	57.83	3.23	57.27
	1000	2.23	62.83	3.10	58.99
K ₂ SO ₄	100	3.20	46.66	3.90	48.41
	500	2.56	57.33	3.43	54.62
	1000	2.20	63.33	2.70	64.28
ZnSO ₄	100	3.10	48.33	4.56	39.68
	500	2.13	64.50	3.80	49.73
	1000	1.33	77.83	2.73	63.88
Na ₂ B ₄ O ₇ .10H ₂ O	100	4.16	30.66	5.40	28.57
	500	3.40	43.33	4.40	41.79
	1000	3.20	46.66	3.93	48.01
Mancozeb	100	1.86	69.00	4.26	43.65
	500	1.20	80.00	1.93	74.47
	1000	1.00	83.33	1.40	81.48
Check	—	6.00	—	7.56	—
C.D. at 5%		0.11		0.38	

CD- Critical difference; DAI = Days after inoculation.

Table 2. Effect of some eco-friendly chemicals on spore germination of *A. brassicae*.

Treatment	Concentration (%)	Spore germination after 8 hrs (%)	Reduction over check (%)
CaSO ₄	0.5	59.50	20.24
KCl	0.5	57.06	23.51
K ₂ SO ₄	0.5	62.20	16.62
ZnSO ₄	0.5	65.06	12.78
Na ₂ B ₄ O ₇ .10H ₂ O	0.5	72.00	3.48
Check	–	74.60	–
C.D. at 5%		3.56	

CD- Critical difference

Table 3. Effect of some eco-friendly chemicals on intensity of sporulation of *A. brassicae* on naturally inoculated leaves of mustard cv. Varuna.

Treatment	Concentration (%)	Spore intensity ($\times 10^3$ /ml)	Per cent increase (+) or decrease (–) over check
CaSO ₄	0.5	7.25	–40.18
	1.0	14.50	+19.63
	1.5	33.25	+174.33
KCl	0.5	29.25	+141.33
	1.0	17.25	+42.32
	1.5	24.50	+102.14
K ₂ SO ₄	0.5	8.50	–29.86
	1.0	93.12	+668.31
	1.5	37.25	+207.34
ZnSO ₄	0.25	21.00	+73.26
	0.50	36.00	+197.02
	0.75	11.50	–5.11
Na ₂ B ₄ O ₇ .10H ₂ O	0.25	20.00	+65.01
	0.50	44.50	+267.16
	0.75	12.75	+5.19
Check	–	12.12	–
C.D. at 5%		26.53	

CD- Critical difference

that calcium polypectates are resistant to hydrolysis by pectolytic enzymes produced by pathogens. The results from such studies revealed that the differences in spore germination and sporulation intensity may be due to activation of various inducing principles, which might have resulted in various PR proteins, phenolics, phytoalexins and other toxic or stimulatory metabolites (Marschner, 1986; Rouxel *et al.*, 1995).

***In vivo* evaluation of eco-friendly chemicals against *A. brassicae*:** The percent disease index on leaves was observed at 60, 70, 80 and 90 DAS (Table 4). The leaf disease index was found to be highly significant among the treatments, observation intervals and their interactions. The significantly maximum reduction of leaf disease index was recorded in CaSO₄ at 0.5% (36.96 per cent) followed by CaSO₄ at 1.5% (35.84 %) and Na₂B₄O₇.10H₂O at 0.75% (35.63 %) in comparison to check. The leaf disease index was observed maximum in all the treatments of ZnSO₄ i.e. at 0.25% (35.00%),

0.5% (32.91%) and 0.75% (31.91%) respectively. Among the borax, the maximum leaf disease index was found at 0.5% (28.50 %) followed by 0.25% (27.41 %) and 0.75% (24.08 %), respectively.

The percent disease index on pods was observed at 80, 90, 100 and 110 DAS (Table 5). The significantly maximum reduction of disease index on pod was found in Na₂B₄O₇.10H₂O at 0.75% (37.62 %) followed by K₂SO₄ at 1.5% (32.82%) and KCl at 1.5% (32.16 %) over check. The disease index was to be highly significant among the treatments, observation intervals and their interaction. Among CaSO₄ treatments, the minimum disease index on pod was observed at 1.0% (27.91%) followed by 0.5% (31.91%) and 1.5% (32.08%), respectively.

The significantly maximum and minimum numbers of primary branches were observed in CaSO₄ at 1.0% (7.00) and CaSO₄ at 1.5% (5.26), respectively. There was significant effect on number of primary branches due

Table 4. Effect of some eco-friendly chemicals on disease severity of alternaria blight on leaf at different stages of growth of mustard cv. Varuna.

Treatment	Concentration (%)	Disease index (%)				Mean	Reduction over check (%)
		60 DAS	70 DAS	80 DAS	90 DAS		
CaSO ₄	0.5	2.00 (8.13)	10.33 (18.72)	35.66 (36.53)	46.33 (42.89)	23.58 (26.59)	36.96
	1.0	4.00 (11.47)	12.66 (20.73)	47.33 (43.47)	56.66 (48.83)	30.16 (31.13)	19.37
	1.5	4.33 (11.99)	11.33 (19.65)	33.33 (35.22)	47.00 (43.27)	24.00 (27.54)	35.84
KCl	0.5	3.33 (10.49)	10.66 (18.98)	43.66 (41.35)	60.00 (50.77)	29.41 (30.40)	21.38
	1.0	3.66 (11.01)	13.00 (21.12)	39.33 (38.83)	62.66 (52.34)	29.66 (30.83)	20.71
	1.5	3.00 (9.88)	13.33 (21.26)	43.33 (41.16)	62.00 (51.95)	30.41 (31.06)	18.71
K ₂ SO ₄	0.5	4.33 (11.99)	10.33 (18.73)	38.33 (38.24)	63.66 (52.63)	29.16 (30.47)	22.05
	1.0	3.66 (10.95)	10.66 (19.03)	39.00 (38.64)	63.00 (52.53)	29.08 (30.29)	22.26
	1.5	2.66 (9.35)	12.66 (20.83)	45.00 (42.12)	65.66 (54.13)	31.50 (31.61)	15.79
ZnSO ₄	0.25	4.33 (11.99)	13.66 (21.67)	54.33 (47.48)	67.66 (55.34)	35.00 (34.12)	6.44
	0.50	2.33 (8.74)	12.00 (20.22)	52.33 (46.33)	65.00 (53.73)	32.91 (32.26)	12.02
	0.75	3.33 (10.49)	14.00 (21.12)	48.66 (44.23)	62.66 (52.33)	31.91 (32.04)	14.70
Na ₂ B ₄ O ₇ .10H ₂ O	0.25	2.33 (11.01)	10.00 (18.42)	44.33 (41.74)	53.00 (46.72)	27.41 (28.90)	26.73
	0.50	3.66 (11.01)	8.66 (17.07)	39.00 (38.61)	62.66 (52.33)	28.50 (29.76)	23.81
	0.75	4.00 (11.47)	12.66 (20.80)	32.00 (34.44)	47.66 (43.66)	24.08 (27.59)	35.63
Check	–	6.33 (14.56)	17.00 (24.30)	55.33 (48.06)	71.00 (57.42)	37.41 (36.09)	–
Mean		3.58 (10.77)	12.00 (20.17)	43.18 (41.04)	59.79 (50.70)		
C.D. at 5%							
Treatment						1.69	
Interval						0.84	
Interaction						3.38	

CD- Critical difference; Figure in parentheses indicates angular transformed values.

to spray of non-conventional chemicals (Table 6). None of the non-conventional chemicals were found significantly affecting the number of secondary branches and number of seeds/plant. There was significant effect on number of siliqua/plant by spraying of nonconventional chemicals (Table 6). The maximum number of siliqua/plant was found in treatment KCl at 1.0% (748.39) followed by K₂SO₄ at 1.5% (744.24) and CaSO₄ at 0.5% (712.51). The total seed yield/ha increased in all

the treatments over check except K₂SO₄ at 0.5% (1339.06 kg/ha). Among the treatments, maximum seed yield/ha was recorded in case of CaSO₄ at 1.0% (1917.30 kg/ha) followed by Na₂B₄O₇.10H₂O at 0.75% (1642.26 kg/ha) and CaSO₄ at 1.5% (1596.36 kg/ha). There was no significant effect of any nonconventional chemicals on oil content. However, the maximum oil content was observed in case of Na₂B₄O₇.10H₂O at 0.75% (39.75 per cent) in comparison to check (37.20

Table 5. Effect of some eco-friendly chemicals on disease severity of *Alternaria* blight on pod at different stages of growth of mustard cv. Varuna.

Treatment	Concentration (%)	Disease index (%)				Mean	Reduction over check (%)
		80 DAS	90 DAS	100 DAS	110 DAS		
CaSO ₄	0.5	7.00 (15.31)	21.00 (27.25)	45.66 (42.48)	54.00 (47.29)	31.91 (33.08)	16.74
	1.0	4.66 (12.35)	17.33 (24.59)	39.66 (39.03)	50.00 (44.99)	27.91 (30.24)	27.18
	1.5	5.33 (13.34)	22.33 (28.19)	43.66 (41.35)	57.00 (49.02)	32.08 (32.98)	16.30
KCl	0.5	4.00 (11.47)	17.66 (24.84)	39.66 (39.02)	51.66 (45.95)	28.25 (30.32)	26.29
	1.0	7.33 (15.67)	21.66 (27.73)	43.00 (40.97)	56.33 (48.64)	32.08 (33.25)	16.30
	1.5	5.66 (13.75)	19.66 (26.30)	31.00 (33.81)	47.66 (43.66)	26.00 (29.38)	32.16
K ₂ SO ₄	0.5	9.00 (17.38)	25.66 (30.38)	32.66 (34.85)	46.33 (42.89)	28.41 (31.38)	25.88
	1.0	7.66 (16.04)	22.66 (28.42)	39.66 (39.03)	57.00 (49.02)	31.75 (33.13)	17.16
	1.5	7.00 (15.31)	18.66 (25.57)	30.66 (33.62)	46.66 (43.08)	25.75 (29.40)	32.82
ZnSO ₄	0.25	7.33 (15.65)	19.00 (25.83)	44.66 (41.93)	58.00 (49.60)	32.25 (33.25)	15.86
	0.50	7.00 (15.24)	16.66 (24.06)	35.00 (36.26)	55.33 (48.06)	28.50 (30.90)	25.64
	0.75	5.66 (13.68)	21.66 (27.73)	30.00 (33.18)	54.66 (47.67)	28.00 (30.57)	26.95
Na ₂ B ₄ O ₇ .10H ₂ O	0.25	9.00 (17.44)	17.66 (24.88)	29.66 (32.99)	49.33 (44.61)	26.71 (29.97)	30.31
	0.50	7.66 (15.98)	22.00 (27.96)	34.33 (35.84)	52.33 (46.33)	29.08 (31.53)	24.13
	0.75	5.33 (13.29)	15.33 (22.89)	30.00 (33.19)	45.00 (42.12)	23.91 (27.87)	37.62
Check	–	10.66 (19.03)	28.00 (31.91)	48.00 (43.85)	66.66 (54.74)	38.33 (37.38)	–
Mean		6.89 (15.06)	20.43 (26.78)	37.33 (37.59)	53.00 (46.73)		
C.D. at 5%							
Treatment						1.99	
Interval						0.99	
Interaction						3.99	

CD- Critical difference; Figure in parentheses indicates angular transformed values.

per cent) (Table 6).

Graham (1983) reported that macro and micronutrients play an important role in protection by affecting plant susceptibility to pathogens. They may also affect the predisposition of plants to viral diseases, which have been reported to increase or decrease the resistance of asparagus bean to tobacco necrosis virus (Pennazio and Roggero, 1988). However, the induction of resistance to foliar pathogens by a foliar spray of different concentrations

of micronutrient solutions, has not been extensively investigated. The most important feature of these elements in this regard, is their variable valency, which allows them to be involved in oxidation changes and they, therefore, serve to function as co-factor of metallo-protein enzymes such as peroxidase and b-1,3 glucanase (Reuveni *et al.*, 1997).

The present investigation supported the findings of Meena *et al.* (2011) that showed that calcium sulphate,

Table 6. Effect of some eco-friendly chemicals on growth components and yield of mustard cv. Varuna.

Treatment	Concentration (%)	Plant growth and yield components					
		No. of primary branches	No. of secondary branches	No. of siliqua/plant	No. of seeds/plant	Total seed yield/ha (kg)	Oil content (%)
CaSO ₄	0.5	5.73	9.86	712.51	14.06	1560.40	39.25
	1.0	7.00	13.00	628.53	13.40	1917.30	38.97
	1.5	5.26	9.93	451.95	11.26	1596.36	39.02
KCl	0.5	5.46	11.46	442.22	14.66	1491.23	39.21
	1.0	5.73	9.73	748.39	14.06	1510.60	39.24
	1.5	5.40	10.20	466.10	13.86	1505.06	38.97
K ₂ SO ₄	0.5	6.00	9.40	352.49	13.93	1339.06	39.84
	1.0	6.40	11.53	710.99	13.80	1538.26	39.38
	1.5	5.40	9.99	744.24	13.80	1449.73	38.90
ZnSO ₄	0.25	6.00	9.40	454.06	13.13	1397.16	39.59
	0.50	5.86	11.60	402.49	12.66	1330.76	38.81
	0.75	6.26	11.20	658.00	13.00	1510.60	38.72
Na ₂ B ₄ O ₇ .10H ₂ O	0.25	6.06	11.60	568.11	13.80	1427.60	39.28
	0.50	5.73	11.20	488.02	12.93	1388.86	38.85
	0.75	5.86	10.20	317.99	13.60	1642.26	39.75
Check	–	6.40	7.86	592.37	13.33	1355.66	37.20
C.D. at 5%		0.54	NS	97.64	NS	213.10	NS

CD- Critical difference; NS-Not significant

borax and zinc sulphate were found effective against alternaria blight of Indian mustard. Role of calcium in improving tolerance in plants to diseases have been indicated earlier (Reddy, 2001; Agrios, 2005). The foliar application of CaSO₄ at 0.5% concentration induced resistance significantly against alternaria blight of mustard (Kumar *et al.*, 2014). Tewari (1991) examined the black spot lesions on rapeseed leaves by scanning electron microscopy in conjugation with energy-dispersive X-ray microanalysis and revealed that the sequestration of calcium by *A. brassicae*. Therefore, there are possibilities of enhancing resistance to *A. brassicae* in rapeseed by soil or foliar spray of calcium compounds. In the present investigation, CaSO₄ showed remarkable effect on leaf infection but not as much on pod infection. The possible reason for this could be the effect of particular nutrient at different stages of host-pathogen interaction would be likely to be different.

Sharma and Kolte (1994) concluded that under natural conditions, based on the number and size of spot, per cent leaf and pod infection, average disease index on leaf and pod, K-fertilized plants reduced severity of Alternaria blight over N, P and NP fertilized toria plant. Singh (1996) concluded that under field conditions

application of KMnO₄ and KAl(SO₄)₂ showed reduction in disease index of Alternaria blight of rapeseed. Vishwanath (1987) reported that foliar spray of boric acid (0.53%) gave 20-64 per cent disease control of Alternaria blight.

Soil applied sulphur was found to increase resistance against a variety of fungal pathogens on different crops (Klikocka *et al.*, 2005). Agrawal (1999) reported that ZnSO₄ sprayed plants showed maximum grain yield and 1000-grain weight in maize as compared to check. Foliar sprays of Zinc Sulphate at 10⁻³ mmol to chickpea (*Cicer arietinum*) and subsequently challenged against *Sclerotinia sclerotiorum*, causing stem rot in chickpea gave the best result and only 13.6% mortality was recorded after 28 days as compared to 100% in the control (Sarma *et al.*, 2007).

In the present study the treatment CaSO₄ at 1.0% significantly increased the number of primary branches, number of secondary branches, seed yield/plant and total yield/ha (Table 6). Calcium has critical roles in cell division, cell development, carbohydrate movement, neutralization of cell acids, cell wall deposition and formation of pectate salts in the middle lamella (Huber and Arny, 1985).

Conclusion

For plant disease management, increasing public concern about environment and health is proving to be major hindrance in the use of chemical fungicides. Implications of harmful chemicals strongly necessitates the search the new eco-friendly methods for plant disease management. This study showed that potassium sulphate showed maximum *in vitro* effect on *Alternaria brassicae* and calcium sulphate and borax are providing maximum reduction of disease and increase in seed yield/ha in field condition. Therefore, the eco-friendly technologies reported here could help the farmers of India for management of alternaria blight disease and could increase the production and productivity of the Indian mustard crop.

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