Journal of Oilseed Brassica, 5(1) : 78-82, Jan 2014

Impact of elicitor spray on Alternaria blight severity and yield of *Brassica juncea* and *Brassica napus* species

Meenakshi Thakur[1*], BS Sohal[1] and PS Sandhu[2]

[1]*Department of Biochemistry, College of Basic Sciences and Humanities*
[2]*Department of Plant Breeding and Genetics, College of Agriculture,*
Punjab Agricultural University, Ludhiana 141 004
**Corresponding author: thakurmeenakshi94@gmail.com*
(Received: 14 January 2013; Revised: 20 April 2013; Accepted: 21 September 2013)

Abstract

The effect of two elicitors *viz*., salicylic acid (SA) and benzothiadiazole (BTH) was studied to control Alternaria blight caused by *Alternaria brassicae* in *Brassica juncea* and *B. napus* varieties. The elicitors were applied alone, and in combinations *viz.*, 10 ppm BTH (treatment-1), 50 ppm SA (treatment-2), 3 ppm BTH + 33 ppm SA (treatment-3), and 7 ppm BTH + 17 ppm SA (treatment-4). Fungicide-blitox and water-sprayed control were used as treatment-5 and treatment-6, respectively. Both the treatment combinations of elicitors i.e. treatment-3 and treatment-4, were effective in reducing the disease severity in both *B. juncea* and *B. napus* on all weeks of observation as compared to control and fungicide. In 13 week old *B. juncea* plants, treatment-3 showed 33.9% reduction in disease severity as compared to control. In 11 week old *B. napus* plants, treatment-4 showed 38.3% less disease severity than control. There was 28.4% and 22.9% increase in seed yield of *B. juncea* plants treated with treatment-3 as compared to control and fungicide treated plants, respectively. In *B. napus*, treatment-4 showed 27.1% and 21.5% increase in seed yield as compared to control and fungicide treated plants, respectively.

Key words: *Brassica juncea, B. napus, disease severity index, seed yield*

Introduction

Brassica species are now the largest oilseed crop in the world oilseed production followed by soybean, peanut and sunflower (Anonymous 2009). Out of 58 million tonnes of estimated rapeseed-mustard produced over 30 million hectares (ha) in the world, India produces 6.41 million tonnes from 5.53 million ha with 1159 kg/ha productivity (GOI 2010). India stands third next to Canada and China for the production of rapeseed-mustard in the world.

Brassica species occupy an important position in world agriculture as a source of oil, vegetable, forage, green manure and condiment. *Brassica* seed oil is used mainly for edible purposes, but also used as industrial lubricant and as a base for polymer synthesis. Its defatted meal (cake) is used as organic manure and as a source of protein in animal feeds (Bhowmik 2003). *Brassica juncea* (Indian mustard or raya), a predominant *rabi* oilseed crop, is not only an important source of oil but also widely used as spice for seasoning of food. It is a good source of protein (28-36%), and phenolic antioxidants like sinapic acid and sinapine. *Brassica napus* (rapeseed) is grown for production of animal feed, vegetable oil for human consumption, and biodiesel. Rapeseed meal contains approximately 40% of protein which rates among the nutritionally best plant proteins.

Brassica species suffer from a number of foliar diseases including Alternaria blight [causal organism-*Alternaria brassicae* (Berk.) Sacc.] which is the most devastating disease causing yield losses of upto 35-38% (Kolte and Singh, 1997). There are various options available for the farmers to protect their crop from diseases including cultivation of resistant cultivars, biological control, crop rotation, and use of chemical fungicides. Although the use of fungicides can control the spread of this disease, but these have poor compliances and may constitute health hazards. The pathogen constantly changes its nature and the resistant cultivars may become susceptible with time (Chaudhary *et al.*, 2001). Recently, a new technology for plant disease management is being adopted where host plants' own defence system is activated with the aid of low molecular weight synthetic molecules called elicitors (Cohen *et al.*, 1999). This method is the alternative of fungicides in plant protection as there is more awareness of deleterious effect of fungicides on the natural ecosystem (Vimala and Suriachandraselvan, 2009). Examples of elicitors include salicylic acid (SA) and its functional analogue benzothiadiazole (BTH; also known as acibenzolar-S-methyl [ASM]), which have been shown to elicit systemic acquired resistance in a wide range of plant-pathogen interactions (Karthikeyan *et al.*, 2009; Abdel-Monaim *et al.*, 2011; Farouk and Osman, 2012; Mandavia *et al.*, 2012). The purpose of the present study, therefore, was to assess the efficacy of the elicitors alone, and their combinations on induction of resistance against Alternaria blight in *B. juncea* and *B. napus* varieties, and to compare their efficacy with the widely used fungicide for disease control.

Materials and Methods

The seeds of *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6) were procured from the Oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana, India. A field experiment was conducted in *rabi* (winter) season 2011-12 at the Experimental Farm, PAU, Ludhiana. The seeds were sown in field at row spacing of 30 cm in case of *B. juncea* and 45 cm in case of *B. napus* in plot size of 3 x 4 m^2. After 3 weeks of sowing, thinning of plants was done to ensure plant-plant distance of about 10 cm in both the varieties. The fertilizers and irrigation were applied according to the recommended agronomic practices.

Treatment details

The following treatments were used in RBD with three replications :

Treatment-1 10 ppm benzothiadiazole (BTH)

Treatment-2 50 ppm salicylic acid (SA)

Treatment-3 3 ppm BTH + 33 ppm SA

Treatment-4 7 ppm BTH + 17 ppm SA

Treatment-5 0.25% Blitox (fungicide)

Treatment-6 Control (water-spray)

The elicitors were applied as a foliar spray once every week as first sign of Alternaria blight appeared on *B. juncea* and *B. napus* plants. Disease incidence was recorded every week after spray. Twenty plants from each plot were chosen at random and used to estimate the percentage of the leaf area affected. Disease severity index (DSI) was calculated for each plot by summing the scores of twenty leaves and analyzing using rating scale described by Conn *et al.* (1990). The value was expressed as percentage using the following formula :

$$\text{DSI (\%)} = \frac{\text{Sum of all ratings x 100}}{\text{No. of leaves examined x Maximum rating score}}$$

Scale (0-6) used for rating :

Rating	Symptoms of Alternaria blight on leaves
0	No infection
1	Up to 5% leaf area covered
3	5-10% leaf area covered
5	11-25% leaf area covered
7	26-50% leaf area covered
9	More than 50% leaf area covered

Data were subjected to analysis of variance (ANOVA) using SPSS (version 16). Tukey's test was used to test the significance of difference between the treatment means.

Results and Discussion

Disease severity

Table 1 shows the effect of foliar spray of elicitors

Table 1. Effect of foliar spray of elicitors and fungicide on Alternaria blight severity on *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6)

Plant age	Per cent disease severity index					
(week)	Treatment-1	Treatment-2	Treatment-3	Treatment-4	Treatment-5	Treatment-6
			B. juncea (cv. PBR-91)			
10	26.4 ± 0.9[b]	27.0 ± 0.8[b]	19.0 ± 1.3[a]	22.8 ± 1.5[ab]	27.5 ± 1.7[b]	27.4 ± 4.3[b]
11	27.3 ± 0.8[bc]	27.5 ± 1.8[c]	20.3 ± 1.0[a]	23.3 ± 1.0[ab]	28.7 ± 2.0[c]	30.0 ± 1.8[c]
12	27.5 ± 1.5[c]	28.5 ± 0.6[c]	20.9 ± 1.2[a]	23.9 ± 1.0[b]	29.0 ± 0.5[c]	30.2 ± 0.8[c]
13	28.9 ± 0.5[b]	28.9 ± 0.8[b]	23.0 ± 1.0[a]	28.5 ± 1.1[b]	29.3 ± 0.8[b]	34.8 ± 0.8[c]
14	32.5 ± 1.5[b]	32.8 ± 0.8[b]	27.3 ± 0.8[a]	31.9 ± 0.9[b]	37.7 ± 1.8[c]	41.0 ± 1.7[c]
15	37.4 ± 2.0[b]	42.8 ± 1.3[c]	33.2 ± 1.6[a]	33.0 ± 1.1[a]	44.3 ± 0.6[c]	45.6 ± 0.5[c]
16	42.3 ± 2.5[ab]	46.7 ± 4.2[bc]	38.0 ± 2.0[a]	35.7 ± 2.5[a]	49.0 ± 2.6[bc]	52.0 ± 3.6[c]
			B. napus (cv. GSC-6)			
10	22.5 ± 1.5[a]	23.3 ± 1.8[a]	21.8 ± 1.0[a]	21.8 ± 0.8[a]	29.8 ± 1.5[b]	31.7 ± 0.8[b]
11	26.2 ± 1.3[a]	26.8 ± 2.1[a]	24.3 ± 1.8[a]	22.7 ± 0.8[a]	31.7 ± 1.8[b]	36.8 ± 1.8[c]
12	29.8 ± 1.8[ab]	32.3 ± 1.6[bc]	27.2 ± 2.0[a]	28.4 ± 1.8[ab]	35.0 ± 1.0[cd]	38.0 ± 0.9[d]
13	30.9 ± 0.8[ab]	32.4 ± 1.0[bc]	27.9 ± 1.7[a]	30.0 ± 2.5[ab]	36.2 ± 1.3[cd]	38.5 ± 1.0[d]
14	31.7 ± 1.8[ab]	33.3 ± 1.3[b]	28.7 ± 1.0[a]	31.3 ± 1.5[ab]	40.9 ± 0.8[c]	42.2 ± 0.7[c]
15	33.0 ± 0.5[a]	36.3 ± 1.0[b]	32.8 ± 1.3[a]	32.7 ± 1.0[a]	41.3 ± 1.5[c]	42.8 ± 0.9[c]
16	37.7 ± 2.5[a]	42.0 ± 2.0[a]	37.0 ± 1.0[a]	38.7 ± 1.5[a]	47.7 ± 2.5[b]	50.0 ± 2.0[b]

*Data represent mean ± SD of three replications. Different letters indicate significant difference between treatments at p = 0.05, according to Tukey's test.

and fungicide on Alternaria blight severity on *B. juncea* and *B. napus* plants. In both *B. juncea* and *B. napus*, as compared to the fungicide-treated and control, disease severity indices were considerably lower in elicitor-sprayed plants. In both the species, disease severity increased with the age of plants. The combinations of elicitors *viz.*, treatment-3 (3ppm BTH + 33 ppm SA) and treatment-4 (7ppm BTH + 17ppm SA) were more effective in controlling disease severity compared to other treatments.

B. juncea plants treated with treatment-3 showed the least disease severity indices from 10 to 14 weeks after sowing. Treatment-3 also showed least disease severity in 10, 12, 13, 14 and 16 week old *B. napus* plants. Treatment-4 treated *B. juncea* plants showed least disease severity in 15 and 16 week old plants. Treatment-4 showed minimum disease severity in 10, 11 and 15 week old *B. napus* plants.

In both *B. juncea* and *B. napus*, as expected, water-sprayed control (treatment-6) had the significantly higher disease severity indices, followed by treatment-5 and treatment-2. In *B. juncea*, treatment-3 was more effective in controlling the disease followed by treatment-4 and treatment-1, while in *B. napus*, both treatment-3 and treatment-4 were equally effective followed by treatment-1. Most of the reduction in disease severity (33.9%) was shown by treatment-3 in 13 week old *B. juncea* plants as compared to water-sprayed control plants. The same treatment exhibited most of the reduction in disease severity (34.1%) in 11 week old *B. napus* plants as compared to control plants. Treatment-4 also showed 38.3% decrease in disease severity in 11 week old *B. napus* plants than water-sprayed control plants. Godard *et al.* (1999) observed that BTH (0.45-0.5mg/ml) induced downy mildew (causal organism-*Peronospora parasitica*) resistance in 30-day old plants of cauliflower (*B. oleracea* var *botrytis*). Babu *et al.* (2003) reported about 50% reduction in bacterial leaf blight caused by *Xanthomonas oryzae* in rice plants treated with BTH @ 100µg/ml. The results indicated that bacterial leaf blight resistance can be induced even in genetically susceptible cultivars through BTH application. Abd-El-Kareem *et al*. (2004) also studied the effect of various chemical inducers i.e. ethephon, dichloroisonicotinic acid (INA) and K_2HPO_4 as foliar spray treatments for controlling powdery mildew and Alternaria leaf spot of squash; INA (100-150mg/L) reduced the severity of powdery mildew and Alternaria leaf spot by 94.2% and 56.9%, respectively and increased the yield by 66.7% in plants treated with INA, ethephon and K_2HPO_4. El-Hendawy *et al.* (2010) observed that oxalic acid and ascorbic acid treatments decreased chocolate spot disease severity in faba bean upto 79.4% and 71.3%, respectively under field conditions. On barley, the combination of acibenzolar-S-methyl (ASM) + â-aminobutyric acid (BABA) + *cis*-jasmone (CJ) was demonstrated to provide better control of *Rhynchosporium secalis* than any of the elicitors used singly (Walters *et al*. 2010). Oxley and Walters (2012) examined the effect of combination of ASM + BABA + CJ on oilseed rape (*B. napus*) for the control of light leaf spot caused by *Pyrenopeziza brassicae*. It was observed that elicitor combination was more effective in reducing light leaf spot than fungicide.

Seed yield and 1000-seed weight

The effect of foliar spray of elicitors on seed yield and 1000-seed weight of *B. juncea* and *B. napus* plants is shown in Table 2. Fungicide treated plants and water-treated control plants produced significantly lower yield in both *Brassica* species as compared to elicitor treated plants. In *B juncea*, plants treated with combination of elicitors (treatment-3 and 4) produced maximum yield. In *B. napus*, maximum yield was produced in plots treated with elicitors *viz.*, treatment-1, 2, 3 and 4. There was 28.35% and 22.91% increase in seed yield of *B. juncea* plants treated with treatment-3 as compared to control and fungicide treated plants, respectively. In *B. napus*, treatment-4 showed 27.12% and 21.50% increase in seed yield as compared to control and fungicide treated plants. Also, *B. juncea* and *B. napus* plants treated with elicitors exhibited higher 1000-seed weight compared to fungicide-treated and water-sprayed control plants.

Sharathchandra *et al.* (2004) reported that foliar spray of chitosan to 7-day-old pearl millet seeds increased the plant height, earhead length and seed weight under greenhouse conditions. Asghari-Zakaria *et al*. (2009) investigated the effect of chitosan application on growth and minituber yield of *Solanum tuberosum* and it was observed that 500mg/L chitosan resulted increase in minituber number and yield as compared to the control.

Application of elicitors stimulates plant defence related genes, reduces infection on plants, and thus increases plant yield. On the basis of this research, it can be concluded that elicitors might offer potentially new approach to control Alternaria blight in *Brassica* species.

Acknowledgements

The authors are grateful to Department of Science and Technology for research fellowship (INSPIRE) to the first author for Ph.D.

Table 2. Effect of foliar spray of elicitors on seed yield and 1000-seed weight of *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6).

Treatments	*B. juncea* (cv. PBR-91)		*B. napus* (cv. GSC-6)	
	Yield (g/m^2)	1000-seed weight (g)	Yield (g/m^2)	1000-seed weight (g)
Treatment-1	300.2^{bc}	4.38^{b}	275.8^{b}	2.93^{b}
Treatment-2	301.7^{bc}	4.41^{b}	274.6^{b}	3.11^{b}
Treatment-3	324.9^{c}	4.60^{b}	284.1^{b}	3.14^{b}
Treatment-4	308.7^{c}	4.43^{b}	295.6^{b}	3.16^{b}
Treatment-5	264.4^{ab}	4.12^{ab}	243.3^{a}	2.83^{a}
Treatment-6	253.2^{a}	3.57^{a}	232.6^{a}	2.81^{a}

*Different letters indicate significant difference between treatments at $p = 0.05$, according to Tukey's test.

References

Abd-El-Kareem, F, El-Mougy, NS, El-Gamal, NG and Fotouh, YO. 2004. Induction of resistance in squash plants against powdery mildew and Alternaria leaf spot diseases using chemical inducers as protective or therapeutic treatments. *Egypt. J. Phytopathol.* **32:** 65-76.

Abdel-Monaim, MF, Ismail, ME and Morsy, KM. 2011. Induction of systemic resistance in soybean plants against *Fusarium* wilt disease by seed treatment with benzothiadiazole and humic acid. *Not. Sci. Biol.* **3**: 80-89.

Anonymous 2009. Agricultural data, FAOSTAT. Available at Food and Agriculture Organization of the United Nations. http://faostat.fao.org/

Asghari-Zakaria, R, Maleki-Zanjani, B and Sedghi, E. 2009. Effect of *in vitro* chitosan application on growth and minituber yield of *Solanum tuberosum* L. *Plant Soil Environ.* **55:** 252-256.

Babu, RM, Sajeena, A, Vijayasamundeeswari, A, Sreedhar, A, Vidhyasekeran, P and Reddy, MS. 2003. Induction of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in rice by treatment with acibenzolar-S-methyl. *Ann. Appl. Biol.* **143:** 333-340.

Bhowmik, TP. 2003. Oilseed Brassicas constraints and their management. CBS Publishers and Distributers, New Delhi, pp 1-2.

Chaudhary, MHZ, Sarwar, N and Chaughati, FA. 2001. Biochemical changes in chickpea plant after induction treatment with simple chemical for systemic resistance against ascochyta blight in the field. *J. Chem. Soc. Pakistan* **23:** 182-186.

Cohen, Y, Reuveni, M and Baider, A. 1999. Local and systemic activity of BABA (DL-3-aminobutyric acid) against *Plasmopara viticola* in grapevines. *Eur. J. Plant Pathol.* **105:** 351-361.

Conn, KL, Tiwari, JP and Awasthi, RP. 1990. A disease assessment key for *Alternaria* blackspot in rapeseed and mustard. *Can. Plant Dis. Survey* **70:** 19-22.

El-Hendawy, S, Shaban, W and Sakagami, JI. 2010. Does treating faba bean seeds with chemical inducers simultaneously increase chocolate spot disease resistance and yield under field conditions? *Turk. J. Agric. For.* **34:** 475-485.

Farouk, S and Osman, MA. 2012. Alleviation of oxidative stress induced by spider mite invasion through application of elicitors in bean plants. *Egypt. J. Biol.* **14**: 1-13.

Godard, JF, Ziadi, S, Monot, C, Le Corre, D and Silue, D. (1999) Benzothiadiazole (BTH) induces resistance in cauliflower (*Brassica oleracea* var *botrytis*) to downy mildew of crucifers caused by *Peronospora parasitica*. *Crop Prot.* **18:** 397-405.

GOI (Government of India) 2010. Agriculture Statistics at a Glance 2010. Agriculture Statistics Division, Department of Agriculture and Cooperation, Ministry of Agriculture, GOI, New Delhi.

Karthikeyan, G, Doraisamy, S and Rabindran, R. 2009. Induction of systemic resistance in black gram (*Vigna mungo*) against urdbean leaf crinkle virus by chemicals. *Arch. Phytopathol. Plant Prot.* **42**: 1-15.

Kolte, SJ and Singh, MP. 1997. Management of threatening plant diseases of national importance. Malhotra Publishing House, New Delhi, pp 87-95.

Mandavia, MK, Raval, L, Mandavia, C and Karkar, C. 2012. Effect of brassinolide and salicylic acid on biochemical parameters and yield of groundnut. *Indian J. Agric. Biochem.* **25**: 20-24.

Oxley, SJP and Walters, DR. 2012. Control of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) with resistance elicitors. *Crop Prot.* **40:** 59-62.

Sharathchandra, RG, Raj, SN, Shetty, NP, Amruthesh, KN and Shetty, HS. 2004. A chitosan formulation Elexa induces downy mildew disease resistance and growth promotion in pearl millet. *Crop Prot.* **23:** 881-888.

Vimala, R and Suriachandraselvan, M. 2009. Induced resistance in bhendi against powdery mildew by foliar application of salicylic acid. *J. Biopesticides* **2:** 111-114.

Walters, DR, Paterson, L and Havis, ND. 2010. Control of foliar diseases of spring barley using resistance elicitors. In: Proc Crop Prot. Northern Britain, pp 91-96.