Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2019; 8(2): 2246-2250
Received: 14-01-2019
Accepted: 18-02-2019

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# Potential role of chemical elicitors in induced systemic resistance for the effective management of *Alternaria* blight in mustard

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**Abstract**
*Alternaria* blight caused by *Alternaria brassica* (Berk.) Sacc. has been reported to be most wide spread and destructive fungal disease of mustard throughout the world. It has a drastic effect on various yield components of mustard which is reflected in reduction of yield up to the tune of fifty percent. An indiscriminate use of fungicides not only pollutes the environment but has an adverse residual effect on soil health and fertility. Keeping this in mind, a study was conducted for the evaluation of various chemical elicitors for induced systemic resistance against *Alternaria* blight of mustard which were supposed to elicit various proteins, secondary metabolites and enzymes involved in providing resistance against the disease. Among the studied chemical elicitors, Salicylic acid was found to be the best chemical elicitor followed by Chitosan, b-aminobutyric acid (BABA) and 2, 6-dichloro-isonicotinic acid (INA) which was reflected by a decrease in PDI of *Alternaria* blight and correspondingly increase in yield w.r.t. various yield attributing factors. Combined application of Salicylic acid and Chitosan was found to be least effective in controlling the disease which might be due to antagonistic effects of the two studied chemical elicitors. These chemicals in turn were not found to have an adverse effect on plant and soil health. The present study not only validated the role of the studied chemical elicitors in combating the disease but also provided a scope of using these potential chemicals which would trigger production of chemicals for resistance against *Alternaria* blight without an adverse effect on plant and soil, thereby forming an important part of integrated disease management.

**Keywords:** *Alternaria* blight, disease severity, elicitor, induced systemic resistance (ISR), *Alternaria brassica*

## Introduction
India is a major oilseed producer and is the fourth largest oil economy in the world after U.S., China and Brazil accounting for 6.7 % of world production. Mustard together with rapeseed forms an important proportion of the oilseeds across the globe both in terms of cultivated area and yield (Mamgain *et al*., 2014, 2017) [6, 7]. Inspite of India occupying a premier position in the world oilseed production, its productivity is very low. There are various constraints in the production of rapeseed-mustard which hamper the enhanced production of the aforesaid crops. And among these constraints, diseases are the most important limiting factors which decrease the productivity of these crops to a much larger extent than by any other constraint. *Alternaria* blight caused by *Alternaria brassica* (Berk.) Sacc is found to be the most severe disease of rapeseed-mustard with no proven source of transferable resistance in any of the hosts (Meena *et al*., 2010) [8]. Various chemicals have been used indiscriminately to serve the purpose. They however have been found to cause deterioration in the prevalence of *Alternaria* blight but keeping in view the persistent residual effects of these harmful chemicals in plants as well as in the soil, the extensive use of these chemicals is not thoroughly recommended. Various chemical elicitors have been used to induce resistance in plants for *Alternaria* blight. This approach in turn is non-toxic, non-residual and effective method in controlling *Alternaria* blight prevalence and forms an important component of integrated disease management. Although research work is being carried out in this aspect throughout the world but still studies on *Alternaria* blight management in the undulating red and lateritic zone of West Bengal is scanty. The present investigation was carried out to evaluate various potential elicitor chemicals in controlling *Alternaria* blight both in terms of reduction in disease severity as well as in terms of various yield components.

## Materials and Methods
Evaluation of various chemical elicitors against *Alternaria* blight were done at Agricultural Farm, Palli Siksha Bhavana Agricultural Research farm, Sriniketan, Birbhum, West Bengal

using a susceptible variety B-9 for two consecutive years i.e. 2011-12 and 2012-13. The field experiments were carried out in randomized block design (RBD) with four replications for each treatment along with control. Various elicitor compounds viz. Salicylic Acid (SA), Chitosan, combination of salicylic acid and Chitosan, 2, 6-dichloro-isonicotinic acid (INA), b-aminobutyric acid (BABA) were evaluated for their efficiency in controlling the disease. Treatment without elicitor chemicals served as control (i.e. water sprayed plots). The standard concentration of different elicitor chemicals i.e. Salicylic acid (2 ppm), Chitosan (0.2%), combination of salicylic acid and Chitosan (2 ppm followed by 0.2%), 2, 6-dichloro-isonicotinic acid (20 ppm) and b-aminobutyric acid (40 mM) were taken for the induction of systemic resistance. The first foliar application was done at 15 days after sowing with subsequent sprays at a regular time interval of 15 days. The percent disease incidence was recorded at 45 DAS, 60 DAS and 75 DAS (days after sowing). The average percent disease incidence was recorded and was compared with control so as to evaluate the induction of resistance by the application of various elicitor compounds and reduction of percent disease incidence with respect to control.

**Results and Discussion**

The effect of the elicitor compounds on reduction of *Alternaria* blight with respect to the control trial (water spray) as well as on various yield attributing factors is presented and tabulated as follows:

**Table 1:** Effect of different elicitor compounds on PDI and yield (2011-12)

| Treatment No. | Treatments | PDI | % Reduction in PDI over control | Yield (kg/ha) | % Increase in yield over control |
|---|---|---|---|---|---|
| T1 | Salicylic acid (SA) @ 2 ppm | 21.10 (27.33) | 61.10 | 1368.4 | 38.96 |
| T2 | Chitosan @ 0.2 % | 26.50 (30.98) | 51.15 | 1312.2 | 33.25 |
| T3 | SA and Chitosan @ 2ppm + 0.2% | 48.64 (44.22) | 10.34 | 1015.4 | 3.11 |
| T4 | 2,6-dichloro-isonicotinic acid (INA)@ 2 ppm | 32.75 (34.91) | 39.63 | 1210.5 | 22.93 |
| T5 | b-aminobutyric acid (BABA) @ 40 mM | 28.85 (32.49) | 46.82 | 1295.1 | 31.52 |
| T6 | Control | 54.25 (47.44) | 0.00 | 984.7 | 0.00 |
| | SEM(±) | 0.36 | - | 1.19 | - |
| | CD at 5% | 1.10 | - | 3.59 | - |

Note: Figures in parenthesis are angular transformed values

**Table 2:** Effect of different elicitor compounds on PDI and yield (2012-13)

| Treatment No. | Treatments | PDI | % reduction in PDI over control | Yield (kg/ha) | % increase in yield over control |
|---|---|---|---|---|---|
| T1 | Salicylic acid (SA) @ 2 ppm | 22.15 (28.07) | 59.9 | 1375.5 | 39.19 |
| T2 | Chitosan @ 0.2 % | 27.15 (31.40) | 50.85 | 1315.4 | 33.11 |
| T3 | SA and Chitosan @ 2ppm + 0.2% | 49.75 (44.86) | 9.95 | 1025.6 | 3.78 |
| T4 | 2,6-dichloro-isonicotinic acid (INA)@ ppm | 33.45 (35.33) | 39.45 | 1217.5 | 23.20 |
| T5 | b-aminobutyric acid (BABA) @ 40 mM | 29.15 (32.67) | 47.23 | 1301.7 | 31.72 |
| T6 | Control | 55.25 (48.01) | 0.00 | 988.2 | 0.00 |
| | SEM (±) | 0.25 | - | 1.15 | - |
| | CD at 5% | 0.75 | - | 3.46 | - |

Note: Figures in parenthesis are angular transformed values

**Table 3:** Effect of different elicitor compounds on PDI of *Alternaria* blight in mustard (pooled analysis)

| Treatment No. | Treatments | PDI (2011-12) | PDI (2012-13) | PDI(Pooled) |
|---|---|---|---|---|
| T1 | Salicylic acid (SA)* | 21.10 (27.33) | 22.15 (28.07) | $21.61^{a}$ (27.70) |
| T2 | Chitosan | 26.50 (30.98) | 27.15 (31.40) | $26.82^{b}$ (31.19) |
| T3 | SA and Chitosan | 48.64 (44.22) | 49.75 (44.86) | $49.19^{e}$ (44.54) |
| T4 | 2, 6-dichloro- isonicotinic acid (INA) | 32.75 (34.91) | 33.45 (35.33) | $33.10^{d}$ (35.12) |
| T5 | b-aminobutyric acid (BABA) | 28.85 (32.49) | 29.15 (32.67) | $29.00^{c}$ (32.58) |
| T6 | Control | 54.25 (47.44) | 55.25 (48.01) | $54.75^{f}$ (47.73) |
| | SEM (±) | 0.36 | 0.25 | 0.22 |
| | CD at 5% | 1.10 | 0.75 | 0.64 |

Note: Figures in parenthesis are angular transformed values;*Salicylic acid is recommended as best treatment to be used as elicitor compound against *Alternaria* blight for mustard.

Pooled analysis of the data spanning over two years i.e. 2011-12 and 2012-13 (Table 3) indicated that nearly all elicitors proved effective in reduction of PDI. However only a slight reduction in disease severity and increase in yield were observed with combined application of salicylic acid and chitosan. From the pooled data, T1 (Salicylic acid) was found to be the best treatment for reducing the disease (PDI 21.61%) followed by T2 (26.82%), T5 (29.00%), T4 (33.10%) and T3 (49.19%) with respect to T6 i.e. control (54.75%). All the treatments were found to control A*lternaria* blight significantly as shown in Table 3.

The effects of elicitor compounds on yield were also studied and have been presented in Table 4. Salicylic acid was found to give maximum yield (1371.9 kg/ha) followed by chitosan (1313.8 kg/ha), BABA (1298.4 kg/ha), INA (1214 kg/ha), salicylic acid +chitosan (1020.5 kg/ha) and finally control (986.4 kg/ha). Thus maximum reduction in PDI of *Alternaria* blight caused by salicylic acid reflects its effect in maximizing the yield of mustard. The effect of all chemical elicitors on increase in yield was statistically significant (Table 4.10). Chitosan proved to be the second best choice next to salicylic acid as yield enhancing and disease reducing chemical elicitor.

**Table 4:** Effect of different elicitor compounds on yield of mustard (pooled analysis)

| Treatment No. | Treatments | Yield Kg/ha (2011-12) | Yield Kg/ha (2012-13) | Yield Kg/ha (Pooled) |
|---|---|---|---|---|
| T1 | Salicylic acid (SA) | 1368.4 | 1375.5 | 1371.9[a] |
| T2 | Chitosan | 1312.2 | 1315.4 | 1313.8[b] |
| T3 | SA and Chitosan | 1015.4 | 1025.6 | 1020.5[e] |
| T4 | 2, 6-dichloro-isonicotinic acid (INA) | 1210.5 | 1217.5 | 1214.0[d] |
| T5 | b-aminobutyric acid (BABA) | 1295.1 | 1301.7 | 1298.4[c] |
| T6 | Control | 984.7 | 988.2 | 986.4[f] |
| | SEM (±) | 1.19 | 1.15 | 0.83 |
| | CD at 5% | 3.59 | 3.46 | 2.39 |

Note: *Salicylic acid is recommended as best treatment to be used as elicitor compound for obtaining maximum yield in case of mustard

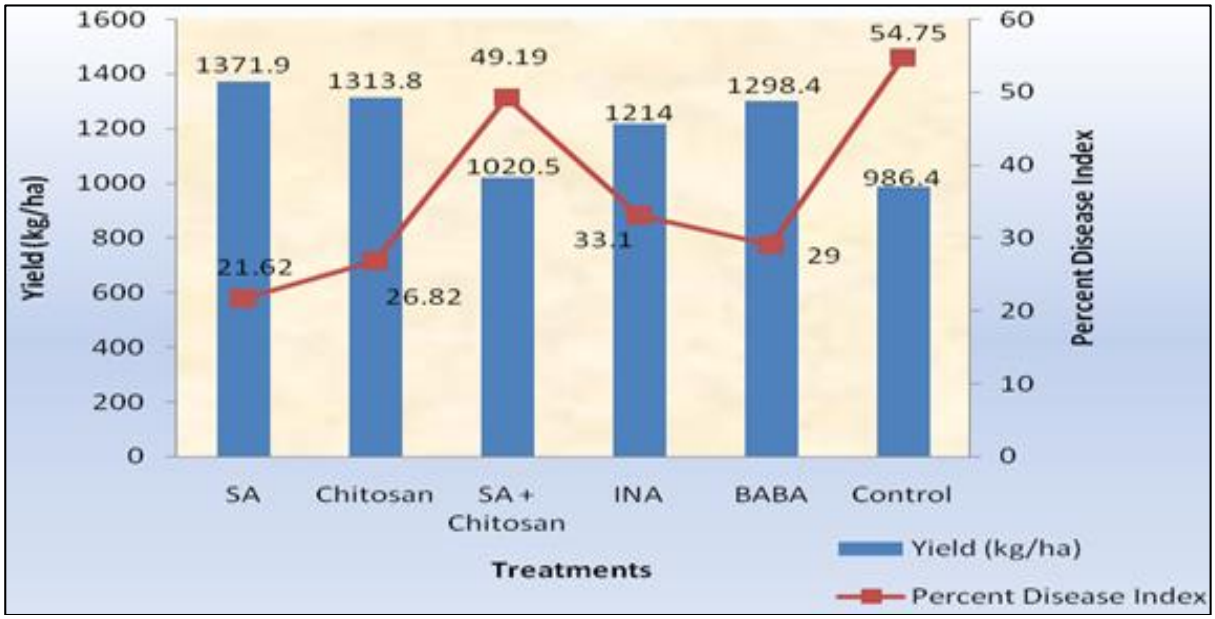


**Fig 1:** Effect of different elicitor compounds on PDI of *Alternaria* blight and yield of mustard (pooled data)

Apart from the effect of studied elicitor compounds on PDI and yield, their effect on various yield components was also studied. Different yield components studied were test weight (Table 5), 100 siliquae weight (Table 6) and number of siliquae per plant (Table 7) which were supposed to be linearly correlated with an increase or decrease in yield of mustard. From Table 5, it was clearly evident that salicylic acid had a pronounced effect on the test weight of mustard seeds (7.74). The seeds treated with salicylic acid were more conspicuous and bold as compared to the seeds in control treatment (4.97) and thus showed a significant impact due to application of salicylic acid. BABA (6.17) showed statistically at par results with Chitosan (6.53) and INA (5.85). However combined application of S.A. and chitosan (5.18) in case of mustard showed statistically at par results to that of control (4.97) making it least preferable treatment in terms of obtaining maximized yield with salicylic acid as the most preferred treatment.

**Table 5:** Effect of different elicitor compounds on test weight of mustard

| Treatment No. | Treatments | Test Weight (g) | | |
|---|---|---|---|---|
| | | 2011-12 | 2012-13 | Pooled |
| T1 | Salicylic acid (SA) | 7.64 | 7.84 | 7.74[a] |
| T2 | Chitosan | 6.48 | 6.58 | 6.53[b] |
| T3 | SA and Chitosan | 5.10 | 5.25 | 5.18[d] |
| T4 | 2, 6-dichloro-isonicotinic acid (INA) | 5.75 | 5.95 | 5.85[c] |
| T5 | b-aminobutyric acid (BABA) | 5.98 | 6.35 | 6.17[bc] |
| T6 | Control | 4.98 | 4.96 | 4.97[d] |
| | SEM (±) | 0.26 | 0.26 | 0.18 |
| | CD at 5% | 0.79 | 0.79 | 0.53 |

Note: Treatments with similar alphabetical letters are statistically at par at p=0.05

The effect of studied chemical elicitors on 100 siliquae weight (Table 6) on pooled basis clearly showed the pronounced effect of Salicylic acid (15.80) on 100 siliquae weight, one of the yield attributing factors of mustard. Chitosan (14.96) proved to be the next best choice after salicylic acid with respect to control treatment (12.18) followed by BABA (13.45). However combined application of S.A. and chitosan (12.35) proved statistically similar with INA (12.87) and control (12.18).

Further studies on effect of chemical elicitors on number of siliquae per plant on pooled basis (Table 7) revealed the superiority of salicylic acid showing maximum number of siliquae per plant (66.6). Chitosan (61.8) and BABA (60.8) showed statistically at par results followed by INA (57.8). However, combined application of salicylic acid and chitosan (54.9) was found to have minimum effect in comparison to control (52.05). Hence, all the treatments were found to have pronounced effect on number of siliquae per plant with

salicylic acid proving to be the best treatment among them. The cumulative effect of studied chemical elicitors on yield and different yield determining components of mustard for each year separately has been depicted in Fig. 2.

**Table 6:** Effect of different elicitor compounds on 100 siliquae weight of mustard

| Treatment No. | Treatments | 100 siliquae Weight (g) | | |
|---|---|---|---|---|
| | | 2011-12 | 2012-13 | Pooled |
| T1 | Salicylic acid (SA) | 15.75 | 15.85 | 15.80[a] |
| T2 | Chitosan | 14.94 | 14.98 | 14.96[b] |
| T3 | SA and Chitosan | 12.25 | 12.45 | 12.35[de] |
| T4 | 2, 6-dichloro-isonicotinic acid (INA) | 12.78 | 12.95 | 12.87[d] |
| T5 | b-aminobutyric acid (BABA) | 13.35 | 13.55 | 13.45[c] |
| T6 | Control | 12.15 | 12.20 | 12.18[e] |
| | SEM (±) | 0.31 | 0.19 | 0.18 |
| | CD at 5% | 0.94 | 0.57 | 0.53 |

Note: Treatments with similar alphabetical letters are statistically at par at p=0.05

**Table 7:** Effect of different elicitor compounds on number of siliquae per plant of mustard

| Treatment No. | Treatments | Number of siliquae per plant | | |
|---|---|---|---|---|
| | | 2011-12 | 2012-13 | Pooled |
| T1 | Salicylic acid (SA) | 65.4 | 67.8 | 66.6[a] |
| T2 | Chitosan | 60.9 | 62.7 | 61.8[b] |
| T3 | SA and Chitosan | 54.5 | 55.4 | 54.9[d] |
| T4 | 2,6-dichloro-isonicotinic acid (INA) | 56.8 | 58.8 | 57.8[c] |
| T5 | b-aminobutyric acid (BABA) | 59.8 | 61.9 | 60.8[b] |
| T6 | Control | 51.7 | 52.4 | 52.05[e] |
| | SEM (±) | 1.23 | 0.63 | 0.69 |
| | CD at 5% | 3.70 | 1.91 | 2.00 |

Note: Treatments with similar alphabetical letters are statistically at par at p=0.05

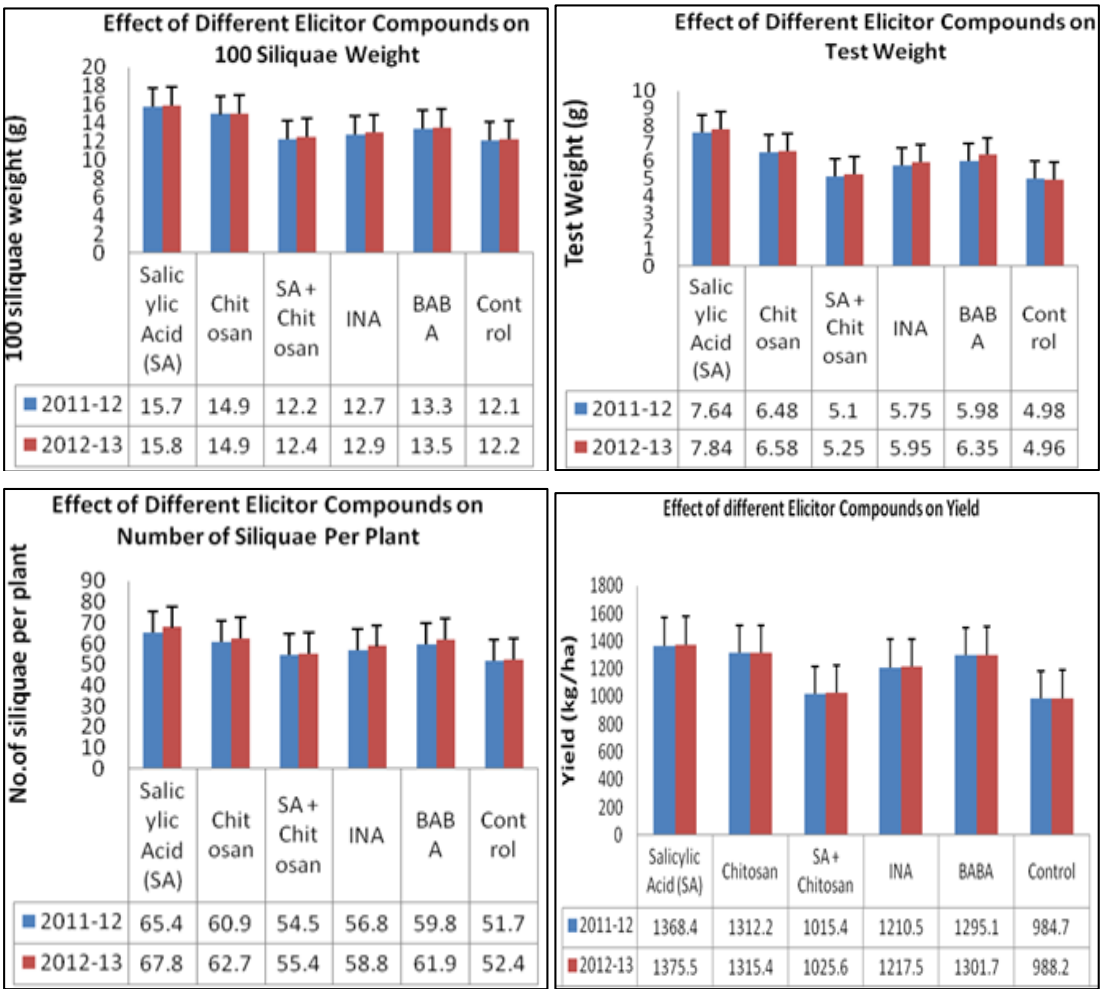


**Fig 2:** Cumulative representation depicting effect of different chemical elicitors on yield and its components of mustard

From the experimental studies, the role of chemical elicitors in reduction of *Alternaria* blight with a corresponding increase in yield in quantitative as well as qualitative characters was validated. Salicylic acid was found to be best treatment to be used as elicitor compound against *Alternaria* blight of mustard. These elicitors are believed to elicit or trigger the production of PR proteins which are

found to be associated with disease resistance (Inbar *et al*., 1998) [5] which is called Induced systemic resistance (ISR). These PR proteins are believed to exert hydrolytic actions (glucanase, chitinase) which suggest a lytic effect on pathogen cell walls built up of glucans or chitins (Gozzo, 2003; Van Loon *et al*., 1997) [3, 9]. Apart from induction of PR proteins, the studied chemical elicitors are also involved in production of are proteinase inhibitors, polyphenol oxidases and phytoalexins and lysozymes (Duffey and Stout, 1996; Holopainen *et al*., 2009) [2, 4]. Induction of peroxidases is yet another defensive system upon elicitor treatment in many plants. Peroxidases are involved in production and polymerization of phenolics, lignification and hypersensitive responses, limiting the possibility of disease spread (Bowles, 1990) [1]. Due to the action of these factors, the oilseed brassicas acquire systemic resistance against *Alternaria* blight which was also validated by the experimental studies.

**Acknowledgements**
Authors are thankful to Pulse and Oilseed Research Station (P.O.R.S.), Behrampore, West Bengal for providing healthy seeds of Binoy (B-9) genotype which is most widely used by the farmers for raising mustard crop.

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