

## COMPARATIVE STUDY OF BION AND SALICYLIC ACID APPLIED THROUGH FOLIAR AND SEEDLING ROOT DIPPING IN TOMATO AGAINST *ALTERNARIA SOLANI*

ASLAM, M.<sup>1\*</sup> – HABIB, A.<sup>1</sup> – SAHI, S. T.<sup>1</sup> – KHAN, R. R.<sup>2</sup>

<sup>1</sup>Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

<sup>2</sup>Department of Entomology, University of Agriculture, Faisalabad, Pakistan

\*Corresponding author

e-mail: [mustansar\\_aslam@live.com](mailto:mustansar_aslam@live.com)

(Received 31<sup>st</sup> Aug 2018; accepted 5<sup>th</sup> Nov 2018)

**Abstract.** *Alternaria solani* is a destructive pathogen to the tomato crop causing heavy losses. The present work was designed during 2015-16 to evaluate the efficacy of Bion and salicylic acid as early blight disease suppressors as well as their use as plant activators under greenhouse conditions. The current study was carried out at field area of Plant Pathology University of Agriculture Faisalabad. Foliar and seedling root dipping application of Bion and salicylic acid (125 ppm and 2 mM) not only reduced the disease severity but also enhanced the plant growth. Maximum disease severity was observed in infected control while minimum disease severity was observed in case of Bion with foliar application in both years. A remarkable increase was observed in Chlorophyll a, b, root fresh/dry weight, photosynthetic rate, shoot fresh/dry weight and growth attributes when *A. solani* inoculated tomato plants were treated with foliar sprays of Bion. A significant healing to cell membrane was observed to reduce the electrolyte leakage in case of Bion and SA application through foliar spray in inoculated plants. Total soluble carbohydrates and total soluble protein contents are increased when Bion was applied through foliar application as compared to seedling root dipping. Increasing trend was observed in 2<sup>nd</sup> year of study. It is concluded that foliar application of Bion could be more effective in managing the *Alternaria solani* as well as in enhancing the growth and yield of tomato.

**Keywords:** *Bion*, physiology, yield, photosynthetic rate, seedling root dipping

### Introduction

Tomato (*Lycopersicon esculentum* L.) is a member of family Solanaceae and ranked as 2<sup>nd</sup> most important vegetable crop around the globe (FAO, 2013). Different pathogens like fungus, nematode, bacteria and virus cause different diseases in the tomato crop. Presently, more than 200 tomato diseases are known worldwide among them *Alternaria solani* causing early blight of tomato is the most destructive of field crops (Chaerani and Voorrips, 2006; Abada et al., 2008) that causes yield and quality reduction of the tomato crop. *A. solani* causes the infections on leaves, petiole fruits, twigs and stem that lead to the defoliation, premature fruit drop and drying of twigs which finally decrease the yield. High humidity and high temperature favor the disease and at fruiting stage fruits are more susceptible to the blight infections (Momel and Pemezny, 2006). Chemicals are used either as protection or as a curative agent for controlling early blight of tomato. The used chemicals found to be severely toxic and cause an augmented hazard for the atmosphere in the circumstance of improper usage or handling (Oostendorp et al., 2001). Systemic acquired resistance in plants has been studied in many different pathosystem for nearly a century (Oka et al., 2000). An unconventional to classic chemical plant protection technique is to induce systemic acquired resistance effects. People globally are conscious about environmental hazards

due to use of costly and toxic chemicals. The increasing public awareness about these problems has stimulated research on the use of biological control agents and development of commercial bio products. To save the nature and the environment, organic amendments and plant extracts are needed to be explored (Hafiz, 2009). SAR can be triggered by some chemicals, including salicylic acid (SA) and its synthetic analogues, such as acibenzolar- S-methyl (ASM), a derivative of the benzo 1,2,3 thiadiazole-7-carbothioic acid-S-methyl ester (BTH). Acibenzolar-S-methyl (ASM, Actigard® or Bion®, Syngenta Crop Protection, Inc., Greensboro, NC) is a plant activator inducing systemic acquired resistance (SAR) to confer protection against a broad spectrum of plant pathogens (Meller Harel et al., 2014; Takeshita et al., 2013). In itself, ASM has no antimicrobial effect, but has been reported to protect several plant species against broad spectrum of pathogens including viral, bacterial and fungal diseases (Tripathi and Pappu, 2015). In tomato and cucumber lower concentration of salicylic acid showed a considerable increase in yield attributes (Larque-Saavedra and Martin-Mex, 2007). According to Shakirova et al. (2003) salicylic acid has a positive effect on growth, physiology and yield because of its influence on other plant hormones. Bion significantly increased the yield in case of moderately resistant tomato cultivars (Pradhanang et al., 2005). Bin does not show any negative effect on yield when tomato plants grow under optimal conditions from transplanting to harvest (Romero et al., 2001). The present study was designed with the aim to evaluate the effect of Bion and salicylic acid against *Alternaria solani* in tomato. In addition their mode of application and their role as plant elicitor to improve physiology, biochemical and yield attributes of tomato plant were also checked.

## Materials and methods

### *Role of Bion and salicylic acid treatments on disease severity (%) caused by Alternaria solani*

This experiment was carried out by selecting a test cultivar (Prescot) due to its high potential for yield, taken from Ayub Agricultural Research Institute Faisalabad. Tomato plants of test cultivar were sown in the greenhouse of Plant Pathology university of Agriculture Faisalabad. Conditions maintained at  $25 \pm 2$  °C during the day time and  $20 \pm 2$  °C at night. The tomato seeds were sown in trays containing commercial peat and vermiculite (1:1) and seedlings were transplanted after 8 weeks into 30 cm diameter pots filled with the mixture of sandy loam soil mixed with compost (1:1). Watering was done twice a week with tap water up to the pot holding capacity. After 30 days of transplanting, *A. solani* suspension was applied (100 ml/plant) at the rate of ( $5 \times 10^6$  conidia/ml) on tomato plants using an atomizer. Bion and salicylic acid (SA) was dissolved in distilled water to give 125 ppm and 2 mM, respectively, and applied to whole plants at the rate of 50 ml for each plant two days prior to inoculation. For seedling root dipping, before transplanting roots of seedlings were dipped in Bion and SA solutions (above mentioned doses) for one hour and transferred to pots. After treatment application, plants were kept in a greenhouse as mentioned earlier. For controls, healthy and diseased control was maintained, which were inoculated or uninoculated but treated with water. This study was carried out under Complete Randomized Design (CRD) and repeated twice under greenhouse conditions in 2015 and 2016. Each pot planted with three seedlings. Three replicates were used, and each

replicate consisted of four pots. Four treatment groups were made (control, control+ *A. solani*, Bion+ *A. solani* and SA+*A. solani*). The disease severity was recorded according to the rating scale of 0 to 9 (Mayee and Datar, 1986).

### ***Effect of Bion and salicylic acid treatments on physiology, biochemical and yield attributes of tomato plants***

This part of experiment was planned to check the effect of Bion and salicylic acid (SA) on physiology, growth, biochemical and yield attributes of tomato plants. Tomato plants were sown in pots in greenhouse conditions. There were six treatment groups as follows: (1) plant without any treatment (healthy control) (2) plant inoculated with *A. solani* (20 ml/plant containing  $5 \times 10^6$  conidia/ml) (3) plants treated with Bion (4) plants treated with SA (5) plants inoculated with *A. solani* and treated with Bion (6) plant inoculated with *A. solani* and treated with SA. After treatment plants were kept in the greenhouse. Plant height, number of flowers/ plant, photosynthetic rate (PR) and number of fruits/plant were calculated over 50 days of transplanting while the yield was measured at the end of the experiment. This experiment was conducted in three way factorial lay out under the CRD.

### ***Biochemical and physiological attributes***

The method used for the quantitative determination of chlorophyll was that of (Vernon and Selly, 1966). The optical density of the plant extract was measured using spectrophotometer (Jenway, 6100 UK microprocessor controlled visible range) of two wave lengths (649 and 665 nm). These are maximum absorption ranges of chlorophyll (a) and (b). The concentrations of chlorophyll (a), (b) and total chlorophyll in plant tissue were measured by using the equations mentioned by Vernon and Selly (1966; Eqs. 1 and 2).

$$\text{Chlorophyll a (mg L}^{-1}\text{)} = 12.7A_{663} - 2.69 A_{645} \quad (\text{Eq.1})$$

$$\text{Chlorophyll b (mg L}^{-1}\text{)} = 20.11A_{649} - 5.18 A_{665} \quad (\text{Eq.2})$$

By adding chlorophyll a and chlorophyll b total chlorophyll was calculated.

The electrolyte leakage percentage was calculated according to the protocol given by Shi et al. (2006). Photosynthetic rate of the top leaf of every plant was recorded with the help of IRGA (Infrared Gas Analyzer) (model, LCA-4; Analytical Development Company, Hoddesdon, England). During data recording, leaf chamber molar gas flow rate  $248 \mu\text{mol s}^{-1}$ , ambient  $\text{CO}_2$  conc. (Cref) was  $352 \mu\text{mol mol}^{-1}$ , ambient pressure (P)  $98.01 \text{ kPa}$ , molar flow of air/leaf area  $221.06 \text{ mol m}^{-2} \text{ s}^{-1}$ , PAR was maximum up to  $1050 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and leaf chamber volume gas flow rate (v)  $380 \text{ mL/min}$ . Total carbohydrates (mg/g) in dry leaves were calorimetrically calculated by following the method given by (Dubois et al., 1956). Protein contents in dry leaves were measured by following the method described by Bradford (1976).

### ***Growth and yield attributes***

55 days after transplanting, plants from each treatment were harvested and data on plant growth variables, including plant height, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight and number of flowers/plant and yield was

determined using standard protocols. Plant height was measured with the help of meter rod. Roots were separated from soil and rinsed with tap water to remove free soil then gently blotted to remove free moisture. To determine the dry weight plant material was dried at 70°C for 48 h in a dry oven. The number of flowers in lower, middle and upper clusters of five randomly selected tomato plants was counted and the mean values were computed and used for further analysis. The number of fruits in lower, middle and upper clusters of five randomly selected tomato plants was counted and the mean values were computed and used for further analysis. The yield/plant was taken at the end of the experiment.

### Statistical analysis

The data analysis was done in three way factorial arrangement under CRD through computer software statstix 8.1 using Fisher's analysis of variance technique and means of treatment were compared by least significance difference (LSD) test at 5% probability level (Steel et al., 1997).

## Results

### Techniques to enhance the resistance against the disease

Bion and salicylic acid applied through foliar and seedling root dipping application methods significantly ( $p \leq 0.05$ ) reduced the disease severity in tomato plants (Table 1).

**Table 1.** Influence of resistance inducers applied through various methods on disease severity (%)

Factors	Disease severity (%)
<b>Method (M)</b>	
Foliar application	30.69 B
Seedling root dipping	35.24 A
<b>Year (Y)</b>	
2015	34.62 A
2016	31.31 B
<b>Treatments (T)</b>	
Healthy control	32.96 B
Infected control	64.50 A
BION	10.96 D
Salicylic acid	23.44 C
<b>LSD (M)</b>	2.23
<b>LSD (Y)</b>	1.11
<b>LSD (T)</b>	1.58
<b>LSD (M × T)</b>	NS
<b>LSD (M×Y)</b>	NS
<b>LSD (T×Y)</b>	NS
<b>LSD (M×T×Y)</b>	NS

Any two means sharing same letters are not significant at  $p \leq 0.05$ . \*\* = Highly Significant at  $p \leq 0.01$ ; \* significant at  $p \leq 0.05$ ; NS = non significant

Maximum decrease in disease severity was noticed in the case of Bion applied through foliar application as compared to infected and healthy control and salicylic acid (SA) treated plants. Increasing trend was observed in 2<sup>nd</sup> year of study (Table 1). Regarding interactive effect all the interactions showed non-significant behavior for the disease severity.

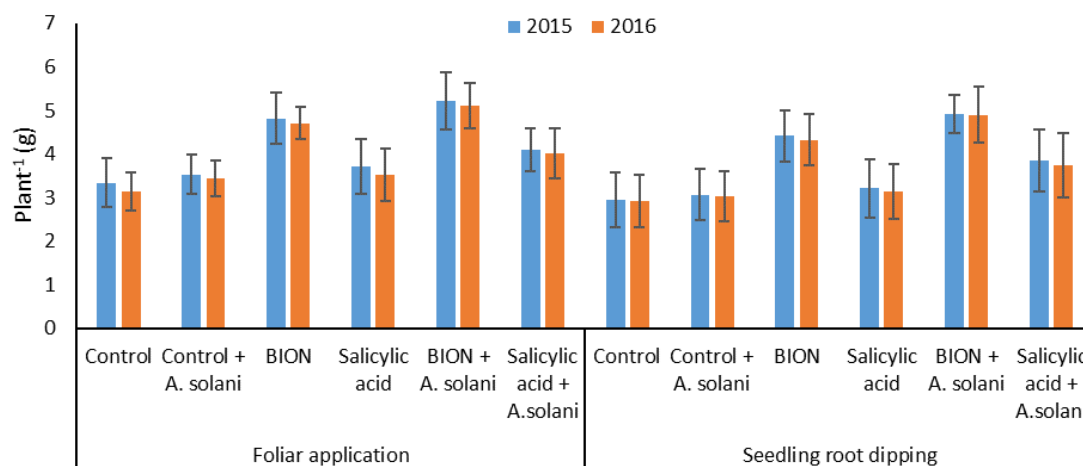
### Growth attributes

All resistance inducers significantly ( $p \leq 0.05$ ) affected the growth attributes of tomato during both years of study (Table 2). Statistically significant increase was observed in all growth attributes in inoculated plants as compared to un-inoculated plants. Maximum fresh and dry biomass of roots and shoots was observed in Bion treated inoculated plants with foliar application followed by seedling root dipping method during both years of study as compared to control. Regarding different treatments following increasing order was observed Bion + *A. solani* > BION > SA + *A. solani* > SA > Control + *A. solani* > Control in all the studied attributes. Positive response of all the growth attributes was observed during 2<sup>nd</sup> year of study. Regarding interactive effect of treatments  $\times$  method  $\times$  year all the factors showed non-significant results except root fresh weight (Fig. 1). However, M $\times$ T showed significant differences ( $p \leq 0.05$ ) for all growth attributes except shoot fresh weight. Regarding the M $\times$ Y both root fresh weight and shoot dry weight showed significant variation. All the growth attributes showed non-significant variation for the interactive effect of T $\times$ Y (Table 2).

**Table 2.** Influence of resistance inducers applied through various methods on growth attributes of tomato plant

Factors	Root fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight
	Plant <sup>-1</sup> (g)			
<b>Method (M)</b>				
Foliar application	4.05 A	0.60 A	25.70 A	4.50 A
Seedling root dipping	3.70 B	0.43 B	22.96 B	4.16 B
<b>Year (Y)</b>				
2015	3.92 A	0.55 A	25.50 A	4.38 A
2016	3.82 B	0.49 B	23.15 B	4.28 B
<b>Treatments (T)</b>				
Control	3.08 F	0.30 F	19.26 F	2.97 F
Control + <i>A. solani</i>	3.26 E	0.40 E	21.43 E	4.00 E
BION	4.56 B	0.64 B	27.42 B	4.90 B
Salicylic acid	3.39 D	0.51 D	23.21 D	4.38 D
BION + <i>A. solani</i>	5.03 A	0.68 A	29.40 A	5.11 A
Salicylic acid + <i>A. solani</i>	3.92 C	0.58 C	25.25 C	4.62 C
<b>LSD (M)</b>	0.21	0.12	2.15	0.02
<b>LSD (Y)</b>	0.07	0.03	1.23	0.02
<b>LSD (T)</b>	0.12	0.02	1.50	0.03
<b>LSD M <math>\times</math> T</b>	**	**	NS	**
<b>LSD M<math>\times</math>Y</b>	**	NS	NS	**
<b>LSD T<math>\times</math>Y</b>	NS	NS	NS	NS
<b>LSD M<math>\times</math>T<math>\times</math>Y</b>	**	NS	NS	NS

Any two means sharing same letters are not significant at  $p \leq 0.05$ . \*\* = highly significant at  $p \leq 0.01$ ; \* significant at  $p \leq 0.05$ ; NS = non significant



**Figure 1.** Interactive effect of year  $\times$  chemicals  $\times$  application methods on root fresh weight as influenced by the different resistance inducers

### Physiological attributes

Bion and salicylic acid significantly ( $p \leq 0.05$ ) affected the physiological attributes of tomato during both years of study (Table 3).

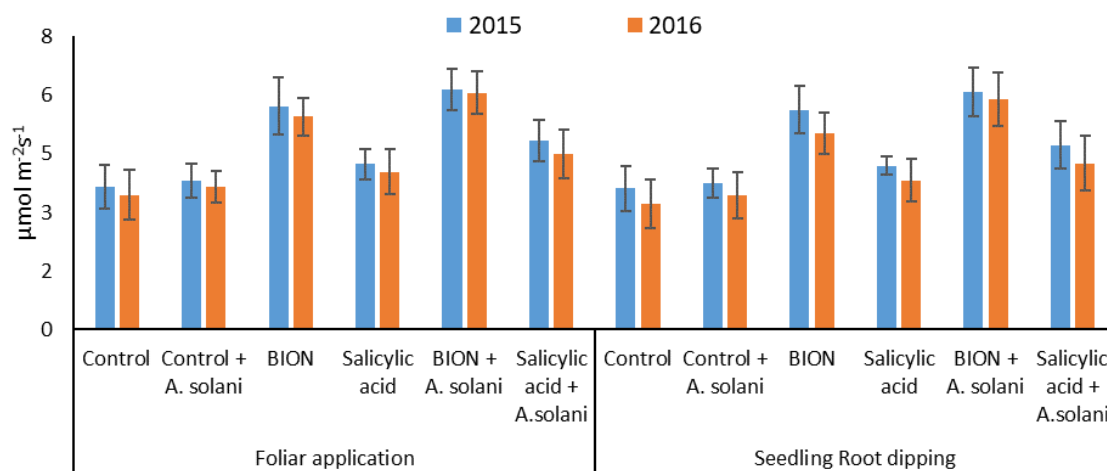
**Table 3.** Influence of resistance inducers applied through various methods on physiological attributes of tomato

Factors	Chlorophyll a (mg L <sup>-1</sup> )	Chlorophyll b (mg L <sup>-1</sup> )	Total chlorophyll	Electrolyte leakage (%)	Photosynthetic rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
<b>Method (M)</b>					
Foliar application	0.57 A	0.18 A	0.75 A	6.58 B	4.62 A
Seedling root dipping	0.51 B	0.15 B	0.66 B	6.69 A	4.47 B
<b>Year (Y)</b>					
2015	0.56 A	0.17	0.73 A	6.84 A	4.69 A
2016	0.52 B	0.16	0.68 B	6.43 B	4.40 B
<b>Treatments (T)</b>					
Control	0.34 E	0.10 F	0.45 F	7.36 B	3.48 F
Control + <i>A. solani</i>	0.46 D	0.13 E	0.59 E	7.88 A	3.65 E
BION	0.66 B	0.20 B	0.87 B	6.83 C	5.45 B
Salicylic acid	0.48 D	0.15 D	0.63 D	6.65 D	4.07 D
BION + <i>A. solani</i>	0.72 A	0.22 A	0.95 A	5.95 E	6.04 A
Salicylic acid + <i>A. solani</i>	0.56 C	0.17 C	0.73 C	5.16 F	4.57 C
<b>LSD (M)</b>	0.03	0.02	0.06	0.05	0.11
<b>LSD (Y)</b>	0.02	NS	0.04	0.23	0.21
<b>LSD (T)</b>	0.04	0.02	0.05	0.11	0.13
<b>LSD M <math>\times</math> T</b>	NS	NS	NS	**	**
<b>LSD M <math>\times</math> Y</b>	NS	NS	NS	**	**
<b>LSD T <math>\times</math> Y</b>	NS	NS	NS	NS	**
<b>LSD M <math>\times</math> T <math>\times</math> Y</b>	NS	NS	NS	NS	*

Any two means within a column sharing same letters are not significant at  $p \leq 0.05$ . \* = significant at  $p \leq 0.05$ ; \*\* highly significant at  $p \leq 0.05$ ; NS = non significant

Statistically significant increase was observed in photosynthetic rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in inoculated plants as compared to un-inoculated plants. The maximum photosynthetic rate (PR) was observed in Bion treated-inoculated plants during both years of study. While minimum PR was noticed in infected control in both years. Regarding different treatments following increasing order was observed Bion + *A. solani* > BION > SA + *A. solani* > SA > Control + *A. solani* > Control in all the studied attributes. Positive response of all the growth attributes was observed during 2<sup>nd</sup> year of study. Maximum chlorophyll *a*, *b* and total chlorophyll were observed in Bion treated inoculated plant with foliar application followed by seedling root dipping method during both years of study (Table 3). Decreasing trend for the physiological attributes was observed during 2<sup>nd</sup> year of study except chlorophyll *b* which showed non-significant behavior in case of years (Table 3). In case of electrolyte leakage (EL) opposite behavior during both years of study. Maximum EL was noticed in infected control in both years. Regarding different treatments following decreasing order was observed for different treatments like Control < Bion + *A. solani* < BION < SA + *A. solani* < SA < Control + *A. solani*. Increasing trend in EL was observed during 2<sup>nd</sup> year of study (Table 3).

Regarding interactive effect of treatments  $\times$  method  $\times$  year all the factors showed non-significant results except photosynthetic rate (Fig. 2). However, M $\times$ T showed significant differences ( $p \leq 0.05$ ) for EL and PR. Regarding the M $\times$ Y both PR and EL showed significant variation. All the physiological attributes showed non-significant variation for the interactive effect of T $\times$ Y except PR (Table 2).



**Figure 2.** Interactive effect of application methods  $\times$  chemicals  $\times$  years on photosynthetic rate as influenced by the different resistance inducers

### Biochemical attributes

Significant increase was observed in total soluble carbohydrate (TSC), total soluble protein (TSP) of tomato after the application of Bion and SA during both years of study (Table 4). Maximum TSC and TSP were observed in Bion treated inoculated plant with foliar application followed by seedling root dipping method during both years of study (Table 4). Regarding interactive effect of treatments  $\times$  method  $\times$  year and M $\times$ T all the factors showed non-significant results (Table 4). Regarding the M $\times$ Y only TSP showed

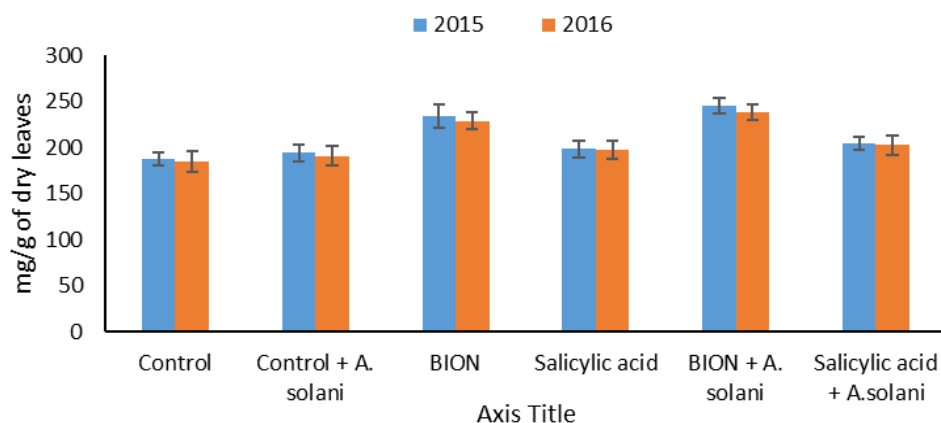


significant variation. While interactive effect of T×Y both TSC and TSP showed significant variation (Figs. 3 and 4).

**Table 4.** Influence of resistance inducers applied through various methods on TSC, TSP of tomato

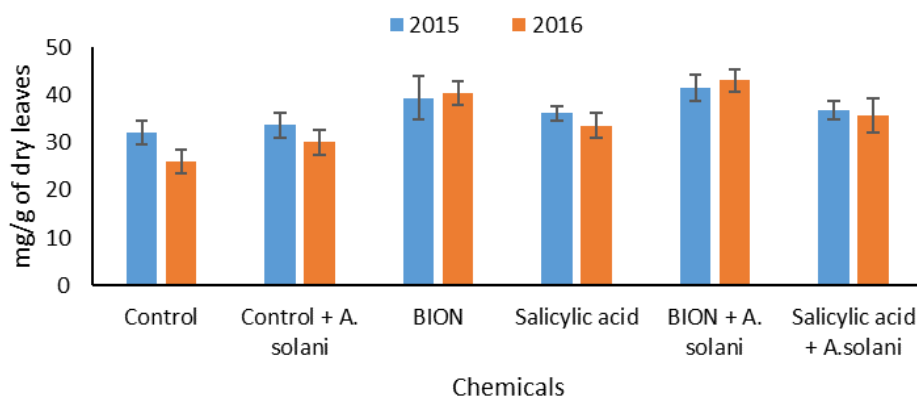
Factors	Total soluble carbohydrate (mg/g of dry leaves)	Total soluble protein (mg/g of dry leaves)
<b>Method (M)</b>		
Foliar application	212.50 A	37.820 A
Seedling root dipping	206.31 B	33.806 B
<b>Year (Y)</b>		
2015	211.26 A	36.599 A
2016	207.54 B	35.027 B
<b>Treatments (T)</b>		
Control	186.60 F	29.124 F
Control + <i>A. solani</i>	192.51 E	31.863 E
BION	233.90 B	39.907 B
Salicylic acid	197.87 D	34.877 D
BION + <i>A. solani</i>	241.81 A	42.287 A
Salicylic acid + <i>A. solani</i>	203.72 C	36.820 C
<b>LSD (M)</b>	2.33	2.98
<b>LSD (Y)</b>	2.22	1.11
<b>LSD (T)</b>	4.11	1.93
<b>LSD (M × T)</b>	NS	NS
<b>LSD (M×Y)</b>	NS	**
<b>LSD (T×Y)</b>	**	**
<b>LSD (M×T×Y)</b>	NS	NS

Any two means within a column sharing same letters are not significant at  $p \leq 0.05$ . \* = significant at  $p \leq 0.05$ ; \*\* highly significant at  $p \leq 0.05$ ; NS = non significant



**Figure 3.** Interactive effect of chemicals × years on total soluble carbohydrates as influenced by the different resistance inducers

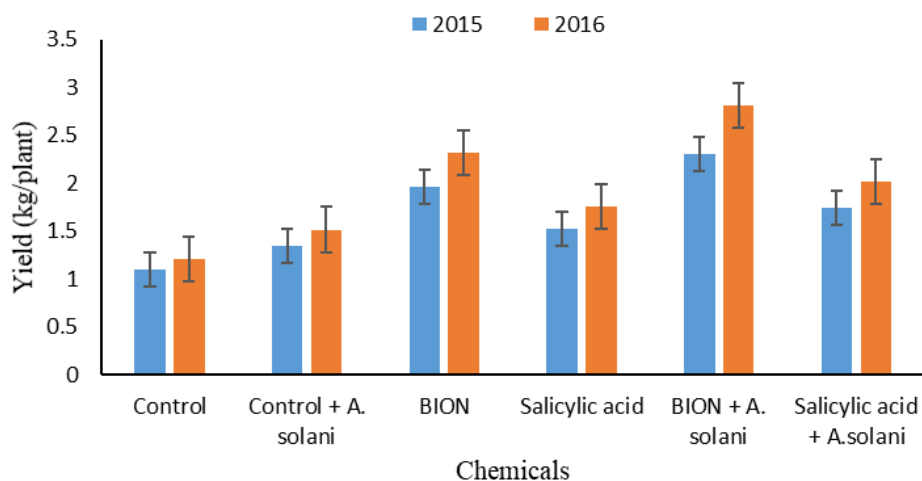




**Figure 4.** Interactive effect of chemicals  $\times$  years on total soluble proteins as influenced by the different resistance inducers

### Yield attributes

Bion and salicylic acid significantly ( $p \leq 0.05$ ) influenced the yield attributes (plant height, number of flowers/plant, number of fruits and yield/plant) of tomato during both years of study except plant height and number of fruits, they showed non-significant response regarding years (Table 5). Significant increase was observed in yield attributes in Bion treated inoculated plants through as compared to un-inoculated plants. Maximum plant height, number of flowers/plant, fruit weight and yield/plant were witnessed in Bion treated inoculated plant with foliar application followed by seedling root dipping method as compared to SA and control. For the interactive effect of chemical  $\times$  method not a single interaction was found significant regarding yield attributes. Regarding interactive effect of treatments  $\times$  method  $\times$  year all the factors showed non-significant results (Table 5). However, M $\times$ T also showed non-significant differences ( $p \leq 0.05$ ) for the factors. Regarding the M $\times$ Y all yield attributes showed significant variation except number of flowers per plant. Yield per plant showed significant variation for the interactive effect of T $\times$ Y (Fig. 5).



**Figure 5.** Interactive effect of chemicals  $\times$  years on yield/plant as influenced by the different resistance inducers

**Table 5.** Influence of resistance inducers applied through various methods on yield attributes of tomato

Factors	Plant height (cm)	Number of fruits/plant	Number of flowers/plant	Yield/plant (kg)
<b>Method (M)</b>				
Foliar application	94.30 A	78.44 A	10.44 A	1.90 A
Seedling root dipping	89.16 B	73.63 B	8.836 B	1.68 B
<b>Year (Y)</b>				
2015	92.22	76.89 A	9.53	1.93 A
2016	91.24	75.19 B	9.75	1.65 B
<b>Treatments (T)</b>				
Control	68.50 F	61.96 F	6.75	1.15 F
Control + <i>A. solani</i>	77.31 E	66.07 E	8.39	1.42 E
BION	106.57 B	85.67 B	10.87	2.13 B
Salicylic acid	87.81 D	69.98 D	9.78	1.63 D
BION + <i>A. solani</i>	115.69 A	92.03 A	12.21	2.55 A
Salicylic acid + <i>A. solani</i>	94.51 C	80.52 C	9.82	1.87 C
<b>LSD (M)</b>	1.17	3.78	0.77	0.16
<b>LSD (Y)</b>	NS	1.10	NS	0.20
<b>LSD (T)</b>	2.04	1.92	NS	0.09
<b>LSD M × T</b>	NS	NS	NS	NS
<b>LSD M×Y</b>	**	**	NS	**
<b>LSD T×Y</b>	NS	NS	NS	**
<b>LSD M×T×Y</b>	NS	NS	NS	NS

Any two means within a column sharing same letters are not significant at  $p \leq 0.05$ . \* = significant at  $p \leq 0.05$ ; \*\* highly significant at  $p \leq 0.05$ ; NS = non significant

## Discussion

In the current study, foliar application of Bion and salicylic acid (SA) minimized the severity of early blight disease symptoms of tomato, during 2015-16, as compared to seedling root dipping method and increasing trend was observed in 2016. These results might be due to protection through Bion that gives quick expression of defense related genes (Bokshi et al., 2003; Buzi et al., 2004). Bion activates SAR mechanism and produces a large number of antimicrobial toxins (Govindappa et al., 2010; Nianlai et al., 2010). Foliar application was found better than seedling root dipping method, this result might be due to SAR signal transduction was difficult to trigger resistance in distant parts of the plants. These results coincide with Scarponi et al. (2000) who found Bion effective in triggering resistance in tomato plants against different pathogens: *Pseudomonas syringae* pv. tomato, bacterial canker (Soylu et al., 2003) and Cucumber mosaic virus (Anfoka, 2000). Results further supported by the work of Achuo et al. (2004) in which they found Bion flooding or foliar application induced resistance in tomato against *B. cinerea* has been found effective.

The result of the present study for both years indicated that Bion caused a significant increase in growth attributes under disease conditions as compared to SA and infected control. These results are might be due to SA which is a plant activator and initiates flowering in plants, increases life span of flower, control ions uptake by roots and

stomatal conductivity (Bhupinder et al., 2003). Bion is a functional analogue of SA and it works just like salicylic acid (Faize and Faize, 2018). Significant increase in all growth parameters and yield (Plant height, number of flowers/plant, roots and shoots fresh and dry weights and fruit yield) was observed in tomato plants treated with Bion followed by salicylic acid and diseased control. The results are in harmony with the findings of Szepesi (2005) in which he described that toxin production by the pathogen might be a reason of the decreased fresh weight of infected tomato shoots, which affected the potassium uptake and stomatal utilities that lead to uncontrolled transpiration and rapid water loss and ultimately plant wilting occurred. However, reduction in shoot dry weight may be due to higher respiration rate and destruction of cell membrane (Orcutt and Nilsen, 2000).

However, application of Bion remarkably increased total chlorophyll contents in inoculated leaves as compared to untreated inoculated control. Results of the current study are consistent with work of Solntsev et al. (2005) who stated that the resistance inducers directly affect the pigment formation and boost the photosynthetic mechanism that results in vigorous growth of the wheat plant. Our findings are in agreement with Nafie and Mazen (2008) they described an increase in chlorophyll contents in soybean leaves triggered by Bion against stem rot disease. *Alternaria solani* infection is responsible of decrease in chlorophyll contents and carotenoids in tomato leaves it might be due to discharge of toxins that leading to oxidative burst resulting in programmed cell death (Howlett, 2006).

In the present study, Bion has increased the photosynthetic rate in inoculated plant as compared to untreated inoculated plants (disease control). These results are might be due to increased action of Rubisco and PEP carboxylase under stress (Popova et al., 2003) and that of CA. The resistance inducers boost up the chlorophyll contents in plants so it might be another reason of high photosynthetic rate.

Our findings showed that Bion and SA application considerably decreased the EL % as compared to diseased control. Electrolyte leakage percent (ELP) depicts the amount of injury to the cell membrane. ELP in diseased plants increased markedly up to 2 times as compared to healthy ones. This phenomenon explains that fungal infection badly ruptures the membrane and reduces the membrane stability, it might be due to the correlation between components of the cell and the amount that oozed out. These results are verified by Hamada and Hashem (2003) they found salicylic acid and Thiamin effective against wheat root rot. These findings verify the efficacy of Bion and SA in improving the cell membrane damage through lessening the fungal infection and also these chemicals induce resistance in tomato plant against fungal pathogens. It may be due to Bion application reduced ELP by making membrane stability better, as revealed from our study data also supported by Agarwal et al. (2005) they conducted a study and found abscisic acid and SA effective against oxidative stress in wheat crop.

Application of resistance inducers increased yield per plant and fruit per plant during both years. Results of present study are consistent with Firoz and Hossain (2000) who conducted an experiment in which they sprayed Bion before a week of ear initiation in rice and observed increase in yield. Hossain et al. (2011) also observed higher grain yield of rice by applying Bion as a foliar spray of rice. Similarly, Pradhanang et al. (2005) documented during bacterial wilt field studies, Bion application enhanced the resistance and also significantly increased the yield of tomato genotypes which were moderately resistant to the bacterial wilt pathogen. Bion application enhanced the yield in optimally grown tomato plants that were free of bacterial spot (Romero et al., 2001).

The maximum number of flower branch/plant and number of fruits/plant was obtained with Bion through foliar application. The maximum TSC and TSP were recorded with Bion with foliar application followed by SA and infected control. Montasser et al. (2012) Carbohydrates in leaves were significantly decreased in TYLCV isolates infected of tested plant cultivars as compared to healthy ones; this may be due to the defense effect of the plant against the virus infection where the strategy altered from defensive to survival as described by Khalil et al. (2014). These results are in harmony with the findings of Radwan et al. (2007), Gupta et al. (2010). Chandra et al. (2007) described that SA and Bion application increased TSS and TSP of cowpea plants.

## Conclusion

Our results indicated that Bion could be eco-friendly and cost effective strategy against *Alternaria solani* in integrated disease management programs. Foliar application of Bion provided better efficiency than seedling root dipping to manage *A. solani*, but still further research is needed under field condition for the confirmation of method success in tomato crop.

## REFERENCES

- [1] Abada, K. A., Mostafa, S. H., Mervat, R. (2008): Effect of some chemical salts on suppressing the infection by early blight disease of tomato. – Egyptian Journal of Applied Science 23: 47-58.
- [2] Achuo, E. A., Audenaert, K. M., Eziane, H., Höfte, M. (2004): The salicylic acid dependent defense pathway is effective against different pathogens in tomato and tobacco. – Plant Pathology 53(1): 65-72.
- [3] Agarwal, S., Sairam, R. K., Srivastava, G. C., Meena, R. C. (2005): Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. – Biologia Plantarum 49: 541-550.
- [4] Anfoka, G. H. (2000): Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum* Mill cv. Vollendung) to Cucumber mosaic virus. – Crop Protection 19(6): 401-405.
- [5] Bhupinder, S., Usha, K. (2003): Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. – Plant Growth Regulation 39(2): 137-141.
- [6] Bokshi, A. I., Morris, S. C., Deverall, B. J. (2003): Effects of Benzothiadiazole and acetylsalicylic acid on  $\beta$ -1-3-glucanase activity and disease resistance in potato. – Plant Pathology. 52(1): 22-7.
- [7] Bradford, M. M. (1976): A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. – Analytical Biochemistry 72: 248-254.
- [8] Buzi, A., Chilosi, G., Magro, P. (2004): Induction of resistance in melon seedlings against soil borne fungal pathogens by gaseous treatments with methyl jasmonate and ethylene. – Journal of Phytopathology 152(8-9): 491-497.
- [9] Chaerani, R., Voorrips, R. E. (2006): Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. – Journal of General Plant Pathology 72(6): 335-347.
- [10] Chandra, A., Anand, A., Dubey, A. (2007): Effect of salicylic acid on morphological and biochemical attributes in cowpea. – Journal of Environmental Biology 28(2): 193-196.

- [11] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956): Colorimetric method for determination of sugar and related substances. – *Analytical Chemistry* 28(3): 350-356.
- [12] Faize, L., Faize, M. (2018): Functional analogues of salicylic acid and their use in crop protection. – *Agronomy* 8(1): 5.
- [13] FAO (2013): Food and Agriculture Organization, United Nations. – <http://faostat.fao.org>.
- [14] Firoz, M. J., Hossain, I. (2000): Induction of resistance to rice against some major diseases with increasing grain yield. – *Bangladesh Journal of Seed Science and Technology* 4(1-2): 37-40.
- [15] Govindappa, M., Lokesh, S., Rai, V. R., Nail, V. R., Raju, S. G. (2010): Induction of systemic resistance and management of safflower *Macrophomina phaseolina* root rot disease by bio control agents. – *Archives of Phytopathology and Plant Protection* 43(1-3): 26-40.
- [16] Gupta, U. P., Srivastava, M., Gupta, U. (2010): Influence of soybean mosaic virus infection on carbohydrate content in nodule of soybean (*Glycine max* L. Merr.). – *International Journal of Virology* 6: 240-245.
- [17] Hafiz, T. B. (2009): Integrated Approach for the Management of Purple Blotch of Onion caused by *Alternaria porri*. – PhD thesis Department of Plant Pathology Sher-e-Bangla Agriculture University Dhaka.
- [18] Hamada, A. M., Hashem, M. (2003): Thiamin and salicylic acid as biological alternatives for control wheat root-rot. – *Egyptian Journal of Agricultural Research* 1: 369-385.
- [19] Hossain, I., Dey, P., Hossain, M. Z. (2011): Efficacy of Bion, Amistar and Tilt in controlling brown spot and narrow brown spot of rice cv. BR11 (Mukta). – *Journal of the Bangladesh Agricultural University* 9(2): 201-204.
- [20] Howlett, B. J. (2006): Secondary metabolite toxins and nutrition of plant pathogenic fungi. – *Current Opinion in Plant Biology* 9: 371-375.
- [21] Khalil, R. R., Bassiouny, F. M., El-DougDoug, K. A., Abo-Elmaty, S., Yousef, M. S. (2014): A dramatic physiological and anatomical changes of tomato plants infecting with tomato yellow leaf curl geminivirus. – *International Journal of Agricultural Sustainability* 10: 1213-1229.
- [22] Larque-Saavedra, A., Martin-Mex, R. (2007): Effect of Salicylic Acid on the Bio-Productivity of Plants. – In: Hayat, S., Ahmad, A. (eds.) *Salicylic Acid. A Plant Hormone*. Springer Publishers, Dordrecht.
- [23] Mayee, C. D., Datar, V. V. (1986): *Phytopathometry Technical Bulletin-1*. – Marathwad Agriculture University, Parabhani, India.
- [24] Meller Harel, Y., Haile Mehari, Z., Rav-David, D., Elad, Y. (2014): Systemic resistance to gray mold induced in tomato by benzothiadiazole and *Trichoderma harzianum* T39. – *Phytopathology* 104: 150-157.
- [25] Momol, T., Pernezny, K. (2006): Florida plant disease management guide: tomato. University of Florida IFAS – <http://edis.ifas.ufl.edu/pdf/PDF/PDF05990.pdf>.
- [26] Montasser, M. S., Al-own, F. D., Haneif, A. M., Afzal, M. (2012): Effect of Tomato yellow leaf curl bigeminivirus (TYLCV) infection on tomato cell ultrastructure and physiology. – *Canadian Journal of Plant Pathology* 34(1): 114-125.
- [27] Nafie, E., Mazen, M. M. (2008): Chemical induced resistance against brown stem rot in soybean: The effect of benzothiadiazole. – *Journal of Applied Sciences Research* 4: 2046-2064.
- [28] Nianlai, C., Qiao, H., Ping, C., XiaoYing, N., Rui, W. (2010): Effects of BTH, SA and SiO<sub>2</sub> treatment on disease resistance and leaf HRGP and lignin contents of melon seedlings. – *Scientia Agricultura Sinica* 43(3): 535-541.
- [29] Oka, Y., Koltai, H., Bar-Eyal, M., Mor, M., Sharon, E., Chet, I., Spiegel, Y. (2000): New strategies for the control of plant parasitic nematodes. – *Pest Management Science* 56(11): 983-988.

- [30] Oostendorp, M., Kunz, W., Dietrich, B., Staub, T. (2001): Induced disease resistance in plants by chemicals. – *European Journal of Plant Pathology* 107: 19-28.
- [31] Orcutt, D. M., Nilsen, E. T. (2000): Influence of Plant Phytopathogens on Host Physiology. – In: Orcutt, D. M, Nilsen, E. T. (eds.) *The Physiology of Plants under Stress: Soil and Biotic Factors*. John Wiley and Sons, Inc., USA, pp. 239-236.
- [32] Popova, L., Ananiewa, E., Hristova, V., Christov, K., Georgieva, K., Alexieva, V., Stoinova, Z. H. (2003): Salicylic acid and methyl jasmonate induced protection on photosynthesis to paraquat oxidative stress. – *Bulgarian Journal of Plant Physiology (Special Issue)*: 133-152.
- [33] Pradhanang, P. M., Ji, P., Momol, M. T., Olson, S. M., Mayfield, J. L., Jones, J. B. (2005): Application of acibenzolar-S-methyl enhances host resistance in tomato against *Ralstonia solanacearum*. – *Plant Disease* 89(9): 989-993.
- [34] Radwan, D. E. M., Fayez, K. A., Mahmoud, S. Y., Hamad, A., Lu, G. (2007): Physiological and metabolic changes of Cucurbita pepo leaves in response to zucchini yellow mosaic virus (ZYMV) infection and salicylic acid treatments. – *Plant Physiology and Biochemistry* 45(6-7): 480-489.
- [35] Romero, A. M., Kousik, C. S., Ritchie, D. F. (2001): Resistance to bacterial spot in bell pepper induced by acibenzolar-S-methyl. – *Plant Disease* 85(2): 189-194.
- [36] Scarponi, L., Buonaurio, R., Bertona, A. (2000): Persistence of acibenzolar-S-methyl and SAR induction in tomato and pepper plants. – *Proceedings of the 5th Congress of the European Foundation for Plant Pathology. Biodiversity in Plant Pathology*. 18-22 September, Taormina (Italy).
- [37] Shakirova, F. M., Sakhabutdinova, A. R., Bezrukova, M. V., Fathudinova, R. A., Fathutdinova, D. R. (2003): Changes in hormonal status of wheat seedlings induced by Salicylic acid and salinity. – *Plant Science* 164: 317-322.
- [38] Shi, Q., Bao, Z., Zhu, Z., Ying, Q., Qian, Q. (2006): Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. – *Plant Growth Regulation* 48(2): 127-135.
- [39] Solntsev, M. K., Franstev, V. V., Karavaev, V. A., Yurina, T. P., Yurina, E. V. (2005): Thermo luminescence of wheat leaves with the plant activator Bion. – *Modern fungicides and antifungal compounds IV: 14th International Reinhardsbrunn Symposium; Friedrichroda, Thuringia, Germany, April, pp. 25-29*.
- [40] Soyulu, S., Baysal, O., Soyulu, E. M. (2003): Induction of disease resistance by the plant activator, acibenzolar-S-methyl (ASM), against bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) in tomato seedlings. – *Plant Science* 165: 1069-1075.
- [41] Steel, R. G. D., Torrie, J. H., Dickey, D. A. (1997): *Principles and Procedures of Statistics. A Biometrical Approach*. – McGraw Hill Co., New York, pp. 178-182.
- [42] Szepesi, Á. (2005): Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt-and osmotic stress. – *Acta Biologica Szegediensis* 49(1-2): 123-125.
- [43] Takeshita, M., Okuda, M., Okuda, S., Hyodo, A., Hamano, K., Furuya, N., Tsuchiya, K. (2013): Induction of antiviral responses by acibenzolar-S-methyl against cucurbit chlorotic yellows virus in melon. – *Phytopathology* 103: 960-965.
- [44] Tripathi, D., Pappu, H. R. (2015): Evaluation of acibenzolar-S-methyl-induced resistance against iris yellow spot tospovirus. – *European Journal of Plant Pathology* 142(4): 855-864.
- [45] Vernon, L. P., Selly, G. R. (1966): *The Chlorophylls*. – Academic Press, New York and London.