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Compatibility between Chemical Inducers and Amistar Top Fungicide for Controlling Onion Downy Mildew and Purple Blotch Diseases

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Three chemical inducers salicylic acid (SA), bion and indol butyric acid (IBA) at different concentrations were applied under field trials to control onion downy mildew and purple blotch and study their effectiveness on onion bulb yield during two successive seasons (2014/2015 and 2015/2016). Chemical inducers were used as a foliar treatment only as well compiled with Amistar Top SC 32.5% (Azoxstrobin + difenoconazole) as a dipping for onion transplants in permanent field. Recommended fungicide Amistar Top was the best treatment recorded the highest percentage efficacy in reducing downy mildew and purple blotch diseases severity compared to other treatments. Treatment with Chemical inducers showed a significant reduction of downy mildew and purple blotch severity and increased onion bulb yield. In this respect, the highest reduction in disease severity and increase in onion bulb yield were detected with IBA at 200 ppm treatments followed by bion treatment during the two growing seasons. In general, disease severity of downy mildew and purple blotch showed more reduction when onion transplants were dipped in the fungicide before planting. Spraying with IBA at 200 ppm on onion plants treated their transplants by dipping in fungicide tested was the best treatment in reducing downy mildew and purple blotch diseases severity followed by bion treatment at 8 mM. Increase the concentration of the chemical inducers caused more reduction in disease severity of both downy mildew and purple blotch diseases. Data clearly indicated that phenolic contents (free, conjugated, and total phenols) and activity of the oxidative enzymes (peroxidase, polyphenol oxidase and catalase) were affected by the three tested concentrations of any chemical inducer. But, applying fungicide as a dipping treatment with different concentrations of chemical inducers had no significant effect on increasing the phenolic contents or the activity of oxidative enzymes in leaves of onion plants during the two growing seasons, 2014-2015 and 2015-2016.

Keywords: *Allium cepa L,* Amistar Top SC 32.5%, bion, chemical inducers, downy mildew, fungicide, IBA, purple blotch and SA.

Onion (*Allium cepa* L.) is the most widely cultivated *Allium* species in Egypt (Mahmoud *et al.*, 2016). Productivity of onion is affected by many biotic and abiotic stresses. For commercial cultivation of onion, foliar diseases play the key role in reducing the bulb yield and quality of production. Among the various foliar diseases affecting leaves and bulbs, downy mildew caused by *Peronospora destructor* (Berk). Caspary and purple blotch caused by *Alternaria porri* (Ellis) Ciferri are the most devastating and prevalent one (Gupta *et al.,* 2011 and Tripathy *et al.*, 2014). The destructiveness of all these diseases varies widely with locality and season, depending on how often and how long onion foliage is wet by dew (Gupta *et al.*, 2011 and Tripathy *et al.*, 2014).

Downy mildew is one of the major leaf diseases affecting the production of onion bulbs, as well as for onion seeds in the second year which may causes local infection on onion leaves or be systemic and infects the entire plant (Schwartz and Mohan, 2008). Yield reductions of onion bulbs due to downy mildew outbreaks may range from 30% up to 70% when the environment is conducive for the disease (Maude, 1990). Yields are strongly reduced by *P. destructor* during seed production, where 60– 70% losses in India may occur (Sharma *et al.,* 2002). Moreover, seeds may be infected by *P. destructor,* acting as primary inoculum for the next season (Rondomanski, 1971). Purple blotch is one of the most devastating diseases, commonly reported in almost all onion growing areas of the world, which causes heavy loss in onions under field conditions (Mustafijur *et al.,* 2015). The disease is however more severe on seed crop as compared to bulb crop causing sometimes 100% loss of the seed production with average yield loss ranges from 32 to 80% (Tomaz and Lima, 1988 and Conn and Tewari, 1990).

One of the important management strategies for controlling these diseases is by fungicide applications. Multiple applications of fungicides are usually required to keep onion leaves below damaging levels (Mathur and Sharma, 2006 and Gaikwad *et al.*, 2014). People globally are conscious about environmental deterioration due to use of costly and toxic spray chemicals. So, to save the nature and getting balanced environment, reduce of fungicides usage is needed by finding alternative tools or in some cases reduce of the number of fungicides sprays or their rate (Akter *et al.*, 2015).

Induced disease resistance can be defined as the process of active resistance dependent on the host plants, physical or chemical barriers are activated by biotic or abiotic agents (Meena *et al.,* 2001 and Walters *et al.,* 2007). Some compounds, *e.g.* ethylene, nicotinic acid, salicylic acid, butyric acid, K_2HPO_4 and KH_2PO_4 have been shown to induce resistance in plants (Mandal *et al.,* 2009, Hussien, 2011, Ibrahim *et al.,* 2013 and Mahmoud *et al.,* 2014). Induction of systemic resistance sensitizes the plant to respond rapid after infection. These responses include phytoalexin accumulation, phenols, lignifications and activation of many enzymes such as peroxidase, polyphenoloxidase, catalase and chitinase (Meena *et al.,* 2001, Ibrahim *et al.,* 2013 and Mahmoud *et al.,* 2014).

The present study was conducted to investigate the effect of integration between fungicides as dipping treatment and chemical inducers as spraying treatment for reducing onion downy mildew and purple blotch diseases.

Materials and Methods

1. Foliar treatments:

The effectiveness of three chemical inducers, *e.g.* salicylic acid (SA) and bion at 2, 4 and 8 mM and indol butyric acid (IBA) at 50; 100 and 200 ppm on disease severity was studied. Each chemical inducer was applied with or without the fungicides dipping treatment of onion transplants. Chemical inducers were used as a foliar spray after 40 and 70 days from transplanting, where the fungicide Amistar top (Azoxstrobin+difenoconazol) was used as a dipping treatment for the transplants at the rate of 75 ml/100 L. Meanwhile, Amistar top was also applied as foliar spray, 40 days after transplanting, at the rate of 75 ml/100 L. for four times at 15 days intervals.

2. Biochemical changes associated with induced resistance:

Samples of leaves were taken after two days from the second foliar spray and extracted according to Mahmoud *et al.* (2014), then activities of the oxidative enzymes, *i.e.* peroxidase (PO); polyphenoloxidase (PPO) and catalase (CAT) were determined according to Allam and Hollis (1972); Matta and Dimond (1963) and Maxwell and Bateman (1967), respectively and assayed using a spectrophotometer at 425, 495 and 240 nm., respectively. The reaction substrates of the oxidative enzymes were pyrogallol, catechol and H_2O_2 for determining activity of peroxidase; polyphenoloxidase and catalase, respectively. Other samples were extracted in soxhlet units using 75% ethanol for 10-12 hrs then used to determine phenol compounds as described by Snell and Snell, (1953), The Phenol contents were also calculated as milligrams equivalent of catechol /g fresh weight of onion leaf, respectively.

3. Field experiments:

Field experiments were carried out during the two successive growing seasons 2014/2015 and 2015/2016 in naturally infested field with *Peronospora destructor* and *Alternaria porri* the causal of downy mildew and purple blotch, respectively. The experiment was carried out in Gemiza Experimental St., A.R.C., Gharbia governorate. Randomized complete block design with four replicates was used and the plot was 3.0 x 3.5 m^2 (10.5 m^2 = 1/400 feddan). Each plot included 6 rows. Sixty day-old transplants of onion cultivar (Giza 20) were transplanted per each plot at the recommended spacing 10 cm X 10 cm, within each row on the first week of December, at approximately 75 plants/row. The recommended agricultural practices for onion crop were applied.

4. Disease and yield assessment:

a) Disease severity of downy mildew was recorded after three months from transplanting (at first week of March), while purple blotch was recorded after four months from planting (first week of April). One hundred leaves from each plot were chosen as randomized samples to determine disease severity of both studied diseases and was monitored using (0-8) scale and recorded according to the method described by Townsend and Heuberger (1943) as follows:

0 = no infection (leaves are completely healthy), 1= 1-2 spots per onion leaf, 2 =3-5 spots per onion leaf, 3 =6-10 spots per onion leaf, 4= 25% of leaf surface

was attacked, 5= 35 - <50% of leaf surface was attacked, 6= 50% of leaf surface was attacked, 7= 75% of leaf surface was attacked, 8 more than 75% of leaf surface was attacked. Disease severity index of downy mildew and purple blotch was estimated using the following formula:

$$D.S.I. = \frac{\Sigma\,(n\ xv)}{ZN} \times 100$$

Where:

D.S.I. = Disease severity index, n = Number of leaves in each category, v = Numerical value of each category, Z = Numerical value of highest category and N = Total number of leaves in the sample.

b) Onion bulb yield as kg/plot (10.5 m^2) was weighted in all treatments after harvesting.

c) Treatment efficiency (%) in reducing the disease infection was calculated as follows:

% Treatment efficiency = {(Control-treatment)/Control} X 100

% Chemical inducer efficiency to fungicide efficacy (spray treatment) =

{Ch. Inducer efficiency / fungicide efficacy (spray treatment)} X 100

8. Statistical analysis:

The obtained data were statistically analyzed by analysis of variance (ANOVA) using MSTAT-C program version 2.10 (1991). Means were separation by Duncan test at $P < 0.05$ level.

Results

1. Effect of foliar spraying with some chemical inducers, applied with or without the transplants with the fungicide:

1.1. On downy mildew disease:

Data presented in Tables (1&2) show that, there was a significant effect of all treatments at their tested concentrations in reducing of downy mildew disease severity during seasons 2014-2015 and 2015-2016 compared to untreated control treatment. In general dipping transplanting of onion in Amistar Top fungicide before planting gave the highest effect in reducing downy mildew disease.

Data in Table (1) also indicate that, IBA treatments followed by bion were the most effective treatments in reducing disease severity and recorded the highest efficacy compared to SA treatments during the two seasons. In this respect IBA at 200 ppm with dipping fungicide application treated was the best treatment in reducing downy mildew disease severity followed by bion at 8 mM under the same conditions during seasons 2014-2015 and 2015-2016 compared to other treatments.

Data also indicate that there was positive correlation ship between increasing the concentration of treatments and their effect on reducing disease severity during the two successive seasons 2014-2015 and 2015-2016. Data in Tables (1&2) clearly show the ability of some inducers treatments especially with dipping in fungicide treat to be near the fungicide efficiency (spray treatment) in reducing downy mildew disease severity. In this respect, IBA at 200 ppm was the nearest one to fungicide efficiency in reducing disease severity of onion downy mildew (93.8%) during season 2014-2015 while it gave 94.9% during season 2015-2016.

Table 1. Effect of Amistar Top and some chemical inducers on onion downy mildew severity under field conditions after 3 months from planting during season 2014/2015

Treatment			2014-2015		
Dipping	Foliar	Conc.	Disease severity	Efficacy [x]	Fungicide Efficacy [y]
Amistar Top 75 ml/100	SA	2 mM.	25.94 d	56.92	65.1
	SA	4 mM.	21.91 e	63.62	72.8
	SA	8 mM.	18.15 f	69.86	79.9
	Bion	2 mM.	21.12e	64.93	74.3
	Bion	4 mM.	17.58 gf	70.81	81.0
	Bion	8 mM.	14.21 ih	76.40	87.4
	IBA	50 ppm	16.95 gfh	71.85	82.2
	IBA	100 ppm	13.58 i	77.45	88.6
	IBA	200 ppm	10.87 j	81.95	93.8
	Amistar Top	300 ml/fed.	5.22 k	91.33	104.5
	Dipping only		42.91 b	28.74	32.9
None dipping treatment in fungicide	SA	2 mM.	32.22 c	46.50	53.2
	SA	4 mM.	28.21 d	53.16	60.8
	SA	8 mM.	22.87 e	62.02	71.0
	Bion	2 mM.	25.88 d	57.02	65.2
	Bion	4 mM.	21.88 e	63.67	72.8
	Bion	8 mM.	17.33 gf	71.22	81.5
	IBA	50 ppm	22.12 e	63.27	72.4
	IBA	100 ppm	16.22 gifh	73.07	83.6
	IBA	200 ppm	14.87 gih	75.31	86.2
	Amistar Top	300 ml/fed.	7.58 k	87.41 [z]	100.0
	Control		60.22 a	-	-

[x] % Treatment efficiency = ((Control-treatment)/Control) X 100
[y] % Chemical inducer efficiency to fungicide efficacy (spray treatment [z]) =
(Ch. Inducer efficiency / fungicide efficacy (spray treatment [z])) X 100

Table 2. Effect of Amistar Top and some chemical inducers on onion downy mildew severity under field conditions after 3 months from planting during season 2015/2016

Treatment			2015-2016		
Dipping	Foliar	Conc.	Disease severity	Efficacy[x]	Fungicide Efficacy[y]
Amistar Top 75 ml/100	SA	2 mM.	21.53 de	59.29	67.8
	SA	4 mM.	19.32 ef	63.47	72.6
	SA	8 mM.	15.35 hg	70.98	81.2
	Bion	2 mM.	18.09 gf	65.80	75.2
	Bion	4 mM.	15.22 hg	71.22	81.4
	Bion	8 mM.	12.55 hi	76.27	87.2
	IBA	50 ppm	14.69 h	72.23	82.6
	IBA	100 ppm	11.22 ji	78.79	90.1
	IBA	200 ppm	8.99 jk	83.00	94.9
	Amistar Top	300 ml/fed.	4.12 l	92.21	105.4
	Dipping only		33.95 b	35.81	40.9
None dipping treatment in fungicide	SA	2 mM.	26.53 c	49.84	57.0
	SA	4 mM.	22.90 d	56.70	64.8
	SA	8 mM.	18.32 f	65.36	74.7
	Bion	2 mM.	23.77 dc	55.06	63.0
	Bion	4 mM.	20.80 def	60.67	69.4
	Bion	8 mM.	14.23 hi	73.10	83.6
	IBA	50 ppm	19.85 ef	62.47	71.4
	IBA	100 ppm	14.58 h	72.43	82.8
	IBA	200 ppm	12.33 hi	76.69	87.7
	Amistar Top	300 ml/fed.	6.63 lk	87.46[z]	100.0
	Control		52.89 a	-	-

[x] % Treatment efficiency = ((Control-treatment)/Control) X 100

[y] % Chemical inducer efficiency to fungicide efficacy (spray treatment [z]) = (Ch. Inducer efficiency / fungicide efficacy (spray treatment z)) X 100

1.2. On purple blotch disease:

Data illustrated in Tables (3&4) indicate that disease severity of purple blotch was significantly decreased by different treatments of chemicals induce resistance in onion plants and recommended fungicide Amistar Top during two successive seasons compared to untreated control treatment. In general, disease severity of purple blotch was highly affected when onion transplants dipped in fungicide before planting. Data in Tables (3&4) also, show that recommended fungicide Amistar Top was the best treatment recorded the highest percentage efficacy in reducing purple blotch disease severity compared to other treatments.

On the other hand, IBA treatments followed by bion were the most effective treatments in reducing disease severity and recorded the highest efficacy compared to other SA treatments during the two growing seasons 2014-2015 and 2015-2016. In this respect, spraying plants dipped their transplants in the fungicide tested with IBA at 200 ppm was the best treatment in reducing purple blotch disease severity followed by bion at 8 mM.

Table 3. Effect of fungicide Amistar Top and some chemical inducers on onion purple blotch disease severity under field conditions during season 2014/2015

Treatments			2014-2015		
Dipping	Foliar	Conc.	Disease severity	Efficacy[x]	Fungicide Efficacy[y]
Amistar Top 75 ml/100	SA	2 mM.	12.01 fe	70.60	79.0
	SA	4 mM.	9.23 hg	77.41	86.6
	SA	8 mM.	6.45 kji	84.21	94.2
	Bion	2 mM.	9.89 fg	75.79	84.8
	Bion	4 mM.	7.58 hji	81.44	91.1
	Bion	8 mM.	6.22 kji	84.77	94.9
	IBA	50 ppm	7.22 hji	82.33	92.1
	IBA	100 ppm	5.40 kj	86.78	97.1
	IBA	200 ppm	3.12 ml	92.36	103.4
	Amistar Top	300 ml/fed.	1.89 m	95.37	106.7
	Dipping only		36.85 b	9.79	11.0
None dipping treatment in fungicide	SA	2 mM.	18.7 c	54.22	60.7
	SA	4 mM.	15.98 d	60.88	68.1
	SA	8 mM.	13.41 e	67.17	75.2
	Bion	2 mM.	16.07 d	60.66	67.9
	Bion	4 mM.	12.88 e	68.47	76.6
	Bion	8 mM.	10.12 fg	75.23	84.2
	IBA	50 ppm	13.52 e	66.90	74.9
	IBA	100 ppm	10.6f g	74.05	82.9
	IBA	200 ppm	8.42 hgi	79.39	88.9
	Amistar Top	300 ml/fed.	4.35 kl	89.35[z]	100.0
	Control		40.85 a	-	-

[x] % Treatment efficiency =((Control-treatment)/Control) X 100
[y] % Chemical inducer efficiency to fungicide efficacy (spray treatment [z]) =
(Ch. Inducer efficiency / fungicide efficacy (spray treatment [z])) X 100

Data also indicated that, there was a relation between inducer material concentrations and their effect on purple blotch disease severity. Data clearly indicated that, increasing the concentration of inducer material led to increase their effect in reducing the disease severity (Tables 3&4). Data clearly showed the ability of some tested inducer materials to give more efficiency than tested fungicide (spray treatment) in reducing purple blotch disease (Tables 3&4). In this respect, IBA at 200 ppm with dipping application was the best one in the growing season 2014-2015 (103.4%) while, it gave 123.1% in the second season 2015-2016.

Table 4. Effect of fungicide Amistar Top and some chemical inducers on onion purple blotch disease severity under field conditions during season 2015/2016

<table>
<tr><th colspan="3">Treatments</th><th colspan="3">2015-2016</th></tr>
<tr><th>Dipping</th><th>Foliar</th><th>Conc.</th><th>Disease severity</th><th>Efficacy^x</th><th>Fungicide Efficacy^y</th></tr>
<tr><td rowspan="11">Amistar Top 75 ml/100</td><td>SA</td><td>2 mM.</td><td>10.22 ehgf</td><td>67.19</td><td>82.7</td></tr>
<tr><td>SA</td><td>4 mM.</td><td>8.14 ihg</td><td>73.87</td><td>91.0</td></tr>
<tr><td>SA</td><td>8 mM.</td><td>5.98 ij</td><td>80.80</td><td>99.5</td></tr>
<tr><td>Bion</td><td>2 mM.</td><td>8.25 ihg</td><td>73.52</td><td>90.5</td></tr>
<tr><td>Bion</td><td>4 mM.</td><td>6.02 ij</td><td>80.67</td><td>99.3</td></tr>
<tr><td>Bion</td><td>8 mM.</td><td>4.12 kj</td><td>86.77</td><td>106.8</td></tr>
<tr><td>IBA</td><td>50 ppm</td><td>5.55 ij</td><td>82.18</td><td>101.2</td></tr>
<tr><td>IBA</td><td>100 ppm[1]</td><td>2.14 kl</td><td>93.13</td><td>114.7</td></tr>
<tr><td>IBA</td><td>200 ppm</td><td>0.00 l</td><td>100.00</td><td>123.1</td></tr>
<tr><td>Amistar Top</td><td>300 ml/fed.</td><td>0.00 l</td><td>100.00</td><td>123.1</td></tr>
<tr><td colspan="2">Dipping only</td><td>27.25 b</td><td>12.52</td><td>15.4</td></tr>
<tr><td rowspan="11">None dipping treatment in fungicide</td><td>SA</td><td>2 mM.</td><td>16.4 5 c</td><td>47.19</td><td>58.1</td></tr>
<tr><td>SA</td><td>4 mM.</td><td>12.75 ed</td><td>59.07</td><td>72.7</td></tr>
<tr><td>SA</td><td>8 mM.</td><td>9.85 hgf</td><td>68.38</td><td>84.2</td></tr>
<tr><td>Bion</td><td>2 mM.</td><td>14.0 5 d</td><td>54.90</td><td>67.6</td></tr>
<tr><td>Bion</td><td>4 mM.</td><td>10.8 egfd</td><td>65.33</td><td>80.4</td></tr>
<tr><td>Bion</td><td>8 mM.</td><td>8.15 ihg</td><td>73.84</td><td>90.9</td></tr>
<tr><td>IBA</td><td>50 ppm</td><td>12.3 edf</td><td>60.51</td><td>74.5</td></tr>
<tr><td>IBA</td><td>100 ppm</td><td>9.75 hgf</td><td>68.70</td><td>84.6</td></tr>
<tr><td>IBA</td><td>200 ppm</td><td>7.98 ih</td><td>74.38</td><td>91.6</td></tr>
<tr><td>Amistar Top</td><td>300 ml/fed.</td><td>5.85 ij</td><td>81.22^z</td><td>100.0</td></tr>
<tr><td colspan="2">Control</td><td>31.15 a</td><td>-</td><td>-</td></tr>
</table>

^{x} % Treatment efficiency =((Control-treatment)/Control) X 100
^{y} % Chemical inducer efficiency to fungicide efficacy (spray treatment ^{z}) =
(Ch. Inducer efficiency / fungicide efficacy (spray treatment ^{z})) X 100

1.3. On onion bulb yield:

Data presented in Table (5) indicate that all tested treatments caused significant increases in bulb yield during the two successive seasons 2014/2015 and 2015/2016 compared to untreated control.

Applying fungicide in dipping treatment with different concentrations of chemical inducers was significantly effective for increasing onion bulb yield if compared with without fungicide dipping treatment. Also, increasing concentration of chemical inducers gave more and significantly effective for increasing onion bulb yield.

The superior treatments in this regard were obtained by IBA at 200 ppm with dipping fungicide application followed by bion at 8 mM during two growing seasons 2014/2015 and 2015/2016 (Table 5).

Table 5. Effect of fungicide Amistar Top and some chemical inducers on onion bulb yield kg/plot under field conditions during seasons 2014/2015 and 2015/2016

Treatments			2014-2015		2015-2016	
Dipping	Foliar	Conc.	Bulb yield[y]	Increases[z] (%)	Bulb yield[y]	Increases[z] (%)
Amistar Top 75 ml/100	SA	2 mM.	25.5 fge	64.52	28.9 ll	55.38
	SA	4 mM.	31.2 bdec	101.29	35.2 gifh	89.25
	SA	8 mM.	33.3 bdec	114.84	37.5 gdfce	101.61
	Bion	2 mM.	30.2 fbdec	94.84	34.1 gihj	83.33
	Bion	4 mM.	32.4 bdec	109.03	36.5 gifhe	96.24
	Bion	8 mM.	34.8 bdac	124.52	39.2 dce	110.75
	IBA	50 ppm	33.1 bdec	113.55	37.3 gdfhe	100.54
	IBA	100 ppm	36.1 bac	132.90	40.6 dc	118.28
	IBA	200 ppm	39.3 ba	153.55	44.2 b	137.63
	Amistar Top	300 ml/fed	43.4 a	180.00	48.8 a	162.37
	Dipping only		20.3 gh	31.29	30.97 lkj	33.87
None dipping treatment in fungicide	SA	2 mM.	21.4 fgh	38.06	24.3 m	30.65
	SA	4 mM.	27.9 fgdec	80.00	31.5 lkj	69.35
	SA	8 mM.	30.4 bdec	96.13	34.3 gihj	84.41
	Bion	2 mM.	26.8 fgde	72.90	30.3 lk	62.90
	Bion	4 mM.	29.3 fdec	89.03	33.1 ikj	77.96
	Bion	8 mM.	32.1 bdec	107.10	36.2 gifhe	94.62
	IBA	50 ppm	30.1 fbdec	94.19	34.0 ihj	82.80
	IBA	100 ppm	33.5 bdec	116.13	37.8 dfce	103.23
	IBA	200 ppm	36.2 bac	133.55	40.8 c	119.35
	Amistar Top	300 ml/fed	39.0 ba	151.61	43.9 b	136.02
	Control		15.5 h	-	18.6 n	-

[y]Onion bulb yield kg/plot (10.5m2 = 1/400 fed.)
[z]Increases related to the control

2. Effect of foliar spraying with some chemical inducers, applied with or without fungicide treatment on biochemical changes in onion plants:

2.1. On phenolic contents in onion leaves:

Results presented in Tables (6&7) indicate that, phenol contents including the free, conjugated and total phenols were obviously higher in leaves of onion plants which treated with any tested chemical inducers than at untreated control during the two growing seasons 2014/2015 and 2015/2016.

The highest phenolic contents were induced by IBA treatment at 200 ppm and SA treatment at 8 mM. Data clearly indicated that, phenol contents were affected by three tested concentrations of each chemical inducer. But, applying fungicide as dipping treatment with different concentrations of chemical inducers had no significant effect in increasing phenolic contents (free, conjugated and total phenols) in leaves of onion plants during the two growing seasons 2014-2015 and 2015-2016.

Table 6. Effect of fungicide Amstar Top and some chemical inducers on phenol contents (milligrams equivalent of catechol /g) in onion leaves during season 2014/2015

Treatments			2014-2015		
Dipping	Foliar	Conc.	Free	Conjugate	Total
Amistar Top 75 ml/100	SA	2 mM.	11.69 g	5.75 egf	17.44 i
	SA	4 mM.	14.75 fde	5.9 egf	20.65 h
	SA	8 mM.	16.4 bdec	6.66 edc	23.06 dfe
	Bion	2 mM.	14.94 fde	6.33 edf	21.27 gh
	Bion	4 mM.	15.63 fdec	7.37 bac	23.00 dfe
	Bion	8 mM.	16.19 fbdec	7.45 bac	23.64 dfe
	IBA	50 ppm	16.25 fbdec	7.66 ba	23.91 dce
	IBA	100 ppm	17.31 bac	7.82 ba	25.13 bc
	IBA	200 ppm	18.38 a	8.15 a	26.53 a
	Amistar Top	300 ml/fed	11.44 g	5.38 gf	16.82 i
	Dipping only		9.31 hi	2.77 h	12.08 j
None dipping treatment in fungicide	SA	2 mM.	11.25 g	5.43 gf	16.89 i
	SA	4 mM.	14.22 f	5.54 gf	20.01 h
	SA	8 mM.	15.81 fbdec	6.26 edf	22.34 gf
	Bion	2 mM.	14.44 fe	6.00 egf	20.64 h
	Bion	4 mM.	15.08 fde	6.99 bdc	22.31 gf
	Bion	8 mM.	15.60 fdec	7.03 bdc	22.91 fe
	IBA	50 ppm	15.72 fbdec	7.30 bac	23.23 dfe
	IBA	100 ppm	16.73 bdac	7.43 bac	24.40 dc
	IBA	200 ppm	17.75 ba	7.72 ba	25.74 ba
	Amistar Top	300 ml/fed	11.01 hg	5.06 g	16.28 i
	Control		8.89 i	2.48 h	11.60 j

Table 7. Effect of fungicide Amistar Top and some chemical inducers on phenol contents (milligrams equivalent of catechol /g) in onion leaves during season 2015/2016

Treatments			2015-2016		
Dipping	Foliar	Conc.	Free	Conjugate	Total
Amistar Top 75 ml/100	SA	2 mM.	11.44 i	5.68 ghf	17.13 gh
	SA	4 mM.	14.44 gh	5.83e ghf	20.27 ef
	SA	8 mM.	16.06 ed	6.58 edc	22.64 ebdc
	Bion	2 mM.	14.63 ghf	6.25 edf	20.88 edfef
	Bion	4 mM.	15.30 gef	7.28 bac	22.58 ebdc
	Bion	8 mM.	15.85 de	7.36 abc	23.21 ebdac
	IBA	50 ppm	15.91 fbde	7.56 ba	23.47 bdac
	IBA	100 ppm	16.95 bac	7.72 ba	24.68 bac
	IBA	200 ppm	17.99 a	8.05 a	26.04 a
	Amistar Top	300 ml/fed	11.20 i	5.31 h	16.51 h
	Dipping only		9.12 j	2.74 i	11.86 i
None dipping treatment in fungicide	SA	2 mM.	11.01 i	5.36 gh	16.58 h
	SA	4 mM.	13.92 h	5.48 ghf	19.63 gf
	SA	8 mM.	15.48 def	6.18 egdf	21.93 edfc
	Bion	2 mM.	14.13 h	5.92 egf	20.26 ef
	Bion	4 mM.	14.76 ghf	6.90 bdc	21.90 edfc
	Bion	8 mM.	15.27 gef	6.95 bdc	22.48 ebdfc
	IBA	50 ppm	15.39 gef	7.20 bac	22.80 ebdc
	IBA	100 ppm	16.38 dc	7.33 bac	23.96 bac
	IBA	200 ppm	17.37 ba	7.62 ba	25.26 ba
	Amistar Top	300 ml/fed	10.77 i	5.00 h	15.98 h
	Control		8.70 j	2.45 i	11.39 i

2.2. On some oxidative reductive enzymes in onion leaves:

Results in Tables (8&9) show that, catalase (CAT), peroxidase (PO) and polyphenoloxidase (PPO) activities in onion leaves increased as result of using any chemical inducer treatments than the untreated control during two growing seasons 2014/2015 and 2015/2016.

In general, applying fungicide as dipping treatment with different concentrations of chemical inducers had no significant effect for increasing enzymes activities in leaves of onion plants. Data clearly indicated that, enzymes activities were affected by three tested concentrations of each chemical inducer. In this respect, the highest activities in all determine enzymes were induced by IBA at 200 ppm and SA at 8 mM during the two growing seasons 2014-2015 and 2015-2016.

Table 8. Effect of fungicide Amistar Top and some chemical inducers on enzymes activities in onion leaves during seasons 2014/2015

Seasons			2014-2015		
Dipping	Foliar	Conc.	PO	PPO	CAT
Amistar Top 75 ml/100	SA	2 mM.	1.169 f	0.482 f	0.327 j
	SA	4 mM.	1.475 e	0.608 ed	0.413 hi
	SA	8 mM.	1.640 ebdac	0.676 ebdac	0.459 dce
	Bion	2 mM.	1.494 ed	0.616 ed	0.418 ghi
	Bion	4 mM.	1.563 edc	0.644 edc	0.437 ghfe
	Bion	8 mM.	1.619 ebdc	0.667 ebdc	0.453 dfe
	IBA	50 ppm	1.625 ebdc	0.670 ebdac	0.455 dfe
	IBA	100 ppm	1.731 bac	0.714 bac	0.484 bc
	IBA	200 ppm	1.838 a	0.758 a	0.514 a
	Amistar Top	300 ml/fed	1.144 f	0.472 f	0.320 j
	Dipping only		0.931 g	0.384 gh	0.261 k
None dipping treatment in fungicide	SA	2 mM.	1.146 f	0.473 f	0.321 j
	SA	4 mM.	1.446 e	0.596 e	0.405 i
	SA	8 mM.	1.608 ebdc	0.663 ebdc	0.450 dfe
	Bion	2 mM.	1.465 e	0.604 e	0.410 i
	Bion	4 mM.	1.532 edc	0.631 edc	0.428 ghfi
	Bion	8 mM.	1.587 edc	0.654 ebdc	0.444 gfe
	IBA	50 ppm	1.593 edc	0.657 ebdc	0.446 fe
	IBA	100 ppm	1.697 bdac	0.700 bdac	0.475 dc
	IBA	200 ppm	1.802 ba	0.743 ba	0.504 ba
	Amistar Top	300 ml/fed	1.122 f	0.463 f	0.314 j
	Control		0.913 g	0.376 h	0.256 k

Table 9. Effect of fungicide Amistar Top and some chemical inducers on enzymes activities in onion leaves during season 2015/2016

Seasons			2015-2016		
Dipping	Foliar	Conc.	PO	PPO	CAT
Amistar Top 75 ml/100	SA	2 mM.	1.144 e	0.472 e	0.320 e
	SA	4 mM.	1.463 cd	0.603 d	0.409 dc
	SA	8 mM.	1.573 b	0.649 bdac	0.440 bdac
	Bion	2 mM.	1.494 cbd	0.616 dc	0.418 dc
	Bion	4 mM.	1.530 cbd	0.631 bdc	0.428 bdc
	Bion	8 mM.	1.559 cb	0.643 bdac	0.436 bdac
	IBA	50 ppm	1.578 b	0.651 bdac	0.441 bdac
	IBA	100 ppm	1.702 a	0.702 ba	0.476 ba
	IBA	200 ppm	1.741 a	0.718 a	0.487 a
	Amistar Top	300 ml/fed	1.129 e	0.466 e	0.316 e
	Dipping only		0.882 f	0.364 f	0.247 f
None dipping treatment in fungicide	SA	2 mM.	1.122 e	0.463 e	0.314 e
	SA	4 mM.	1.434 d	0.591 d	0.401 d
	SA	8 mM.	1.542 cb	0.636 bdac	0.431 bdac
	Bion	2 mM.	1.465 cd	0.604 d	0.410 dc
	Bion	4 mM.	1.500 cbd	0.619 d	0.420 bdc
	Bion	8 mM.	1.528 cbd	0.630 bdac	0.427 bdc
	IBA	50 ppm	1.547 cb	0.638 bdac	0.432 bdac
	IBA	100 ppm	1.669 a	0.688 bac	0.467 bac
	IBA	200 ppm	1.707 a	0.704 ba	0.477 ba
	Amistar Top	300 ml/fed	1.107 e	0.457 e	0.310 e
	Control		0.865 f	0.357 f	0.242 f

Discussion

Regarding to the effect of treatments by chemical inducers on onion diseases severity of downy mildew and purple blotch, results showed that, there was a significant effect of all treatments tested at different concentrations in reducing studied diseases and consequently increasing the bulb yield during two successive seasons 2014/2015 and 2015/2016 compared to untreated control. This agrees with many investigators who stated that, the inducer resistance has an important role in controlling of plant diseases (Mandal *et al.,* 2009; Hussien 2011; Ibrahim *et al.,* 2013 and Mahmoud *et al.,* 2014). IBA and bion treatments showed appreciable increases in the phenolic contents and increased the activity of catalase (CAT), peroxidase (PO) and polyphenoloxidase (PPO) in onion leaves that led to reduce the severity of onion downy mildew and purple blotch diseases by enhancing activities of the defense related enzymes moreover it increased phenolic contents including the free, conjugated and total phenols. This is in agreement with Khalifa, 2003, Chowdhury (2003), Mahmoud *et al*. (2011) and Ragab *et al.* (2012) who stated that, IBA showed increase in the total phenol, calcium and increase the activity of many enzymes included peroxidase, polyphenoloxidase, catalase and pectolytic enzyme. While, bion has the ability to enhance the activities of the defense related enzymes chitinase, *B*-(1, 3)-gluconase and peroxidase (Siegrist *et al.*, 1997; Abou-Taleb, 2001; Mosa 2002 and Mahmoud *et al.*, 2014). Moreover, in many plants investigated so far, bion treatment is associated with increases in activities of many classes of pathogenesis-related protein (Gorlach *et al.*, 1996; Schweizer *et al.*, 1999 and Abou-Taleb, 2001).

The results of this study indicated that recommended fungicide Amistar Top was the best treatment for reducing disease severity of downy mildew and purple blotch under field conditions during the two growing seasons (2014-2015 and 2015-2016) compared to untreated control treatment. This is in accordance with Survilienė *et al.* (2008) and Mahmoud *et al.* (2013) who stated that Amistar SC 25% and Amistar Top SC 32.5% gave significant suppression in disease severity of onion downy mildew and purple blotch and increased the marketable onion yield. Moreover, some reports suggested that fungicides containing active ingredients such as azoxystrobin, benalaxyl, dimethomorf, metalaxyl and Difenoconazole are the most effective resulted in control of onion downy mildew (Robak and Adamicki, 2007 and Mahmoud *et al.*, 2013).

In the present study, using fungicide Amistar Top as dipping treatment in combinations with different concentrations of chemical inducers used as spraying treatment were significantly effective for reducing diseases severity of downy mildew and purple blotch diseases under naturally conditions in field trials compared with untreated control (without any treatment). This is in agreement with Spencer *et al.* (2003) whom reported that, treated the transplants for many crops *i.e.* brassicas, soybean, peas and sunflower with fungicide led to more control of downy mildew diseases. That might be due to the infection with downy mildew fungi happens by two ways: first way onion transplant infected by the mycelium or oospores which left on plant remains in the field after harvesting (systemic infected) and the second way the plant leaf infected by sporangium spores (Local infection). In this respect, the first ways affected by using application Amistar Top as dipping treatment and give more effect for controlling downy mildew (Spencer *et al.*, 2003).

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التوافق بين المستحثات الكيماوية والمبيد الفطري أميستار توب في مكافحة مرضي البياض الزغبي واللطعة الأرجوانية في البصل

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تم استخدام ثلاث مركبات من المستحثات الكيماوية وهى حمض السليسليك والبيون وحمض الأندول بيوتريك بتركيزات مختلفة وتم تقييمها تحت ظروف الحقل لمقاومة مرضى البياض الزغبى واللطعة الأرجوانية في البصل ومدى تأثيرهم على إنتاجية محصول البصل للموسمين 2015/2014 و 2016/2015. استخدمت المستحثات الكيماوية رشاً على الأوراق كما تم تطبيقها مع نقع الشتلات في المبيد الفطري أميستار توب قبل الزراعة في الحقل و بدون نقعها مرة أخرى. أعطى المبيد الموصى به أميستار توب أعلى خفض في شدة الإصابة بمرضى البياض الزغبي واللطعة الأرجوانية في البصل مقارنة بباقي المعاملات كما أن استخدام المستحثات الكيماوية أعطت تقليلاً معنوياً في شدة الإصابة بمرضى البياض الزغبى و اللطعة الأرجوانية وأدت إلى زيادة محصول البصل، ووجد أن حمض الأندول بيوتريك كان الأعلى تأثير في خفض شدة الإصابة وزيادة المحصول يليه البيون للموسمين 2015/2014 و 2016/2015 على التوالي. بصفة عامة وجد أن نقع الشتلات قبل الزراعة بالمبيد الفطري أدت إلى خفض معنوي في شدة الإصابة بمرضى البياض الزغبي و اللطعة الأرجوانية . وفي هذا الصدد وجد أن نقع الشتلات بالمبيد الفطري مع استخدام حمض الأندول بيوتريك عند تركيز 200 جزء في المليون كانت المعاملة الأعلى تأثيراً في خفض شدة الإصابة بمرضى البياض الزغبى واللطعة الأرجوانية في البصل يليها استخدام البيون عند تركيز 8 ملليمول. كما وجد أنه بزيادة التركيز في المستحثات الكيماوية يزيد الخفض في شدة الإصابة بمرضى البياض الزغبى واللطعة الأرجوانية في البصل. أوضحت النتائج أن كل التركيزات المستخدمة من المستحثات الكيماوية أثرت في كميه المركبات الفينولية (الحرة – المرتبطة – الكلية) وزيادة أنشطة الأنزيمات المؤكسدة (بيروكسيديز- بولي فينول أوكسيديز – الكتااليز) بينما المعاملة بالنقع باستخدام المبيد الفطري الموصى به مع تركيزات المستحثات الكيماوية كانت غير معنوية في زيادة المحتوى من المركبات الفينولية وأنشطة الأنزيمات المؤكسدة في أوراق البصل خلال موسمي الزراعة 2015/2014 و 2015 /2016.