Egypt. J. Phytopathol., Vol. **41**, No. 2, pp. 35-44 (2013)

Effect of Growth Regulators, Selenium and Potassium Fertilizers on Downy Mildew and Purple Blotch Diseases and Yield of Onion

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Foliar application of indol acetic acid (IAA) and/or indol butyric acid (IBA) to onion cv. Giza 20 under field conditions, significantly reduced the incidence of either downy mildew or purple blotch diseases than the control. Selenium and potassium hydrogen carbonate ($KHCO_3$), also affected the incidence of both diseases. However, spraying potassium hydrogen phosphate (K_2HPO_4) showed high efficacy in reducing downy mildew and purple blotch diseases. Yield of bulbs was increased by 93.5 and 53.3% than the control in response to the application of IAA (400 ppm) at 2010 and 2011 growing seasons, respectively. In the same respect, selenium (50 ppm) resulted 85.5 and 45.3% yield increase than control. Phenolic compounds were increased as compared to the control in response to the different tested treatments. The highest increment was noticed when IBA was applied. This was also recorded in total sugars, while $KHCO_3$ gave the least increase of the total sugars than the control. Galben-Mancozeb was more effective than other treatments in reducing both diseases.

Keywords: Downy mildew, growth regulators, onion, purple blotch, selenium and fertilizers.

Onion (*Allium cepa* L.) is one of the most important vegetable crops all over the world. Early harvest of the Egyptian onions has competitive markets, in both Europe and Arab countries. Downy mildew and purple blotch are destructive diseases attacking onion leaves and causes heavily yield losses. To avoid the harmful effects of fungicides, different methods were applied to control such diseases. Faoro *et al.* (2008) stated that induction of systemic, long lasting and broad-spectrum resistance with natural or systemic compounds represented one of the most approaches to disease control. This phenomenon also known as systemic acquired resistance (SAR) which offered some important advantages without modifications of the plant genome. It is also relatively inexpensive and lakes environmental and toxicological risks and effective against a great variety of plant pathogens. Jamar *et al.* (2007) reported that bicarbonate salts are considered as effective alternative control against *Venturia inaequalis*. Terry *et al.* (2000) and Xue *et al.* (2001) mentioned that although selenium is not required to higher plants; it may exert beneficial role in plants under stress. It also protects plants against fungal infection and from herbivory as reported by Hansen *et al.* (2003). Borsani *et al.* (2001) indicated that selenium plays an important role in the defence response of many plants toward the pathogen attacks. Antioxidant activity against several pathogens was also reported by Galal and Abdu (1996) and Abd El-Megid *et al*. (2004). However, Saleh *et al*. (2009) reported that antioxidants increased the activity of catalase enzyme. Agrios (2005) suggested that in host defence mechanism, phenylalanine amonia lyase (PAL) is a key enzyme involved in the production of basic molecules used for biosynthesis of most of the phenolics, including phytoalexins and lignin.

Deliopoulos *et al*. (2010) indicated that 34 inorganic salts especially, bicarbonates, phosphate, silicates and chlorides; were able to reduce the severity of 49 fungal diseases attacking foliage, stem, fruits and tuber roots of 35 plant species. Walters and Bingham (2007) and Gottstein and Kuc (1989) reported that dibasic and tribasic phosphate salts induced systemic resistance in plants.

This research was designed to study the role of growth regulators, *i.e*. IAA and IBA, antioxidant selenium, potassium hydrogen phosphate (K_2HPO_4) and potassium hydrogen carbonate ($KHCO_3$) that individually applied on onion plants in the field to control either downy mildew or purple blotch diseases, in comparison with the fungicide Galben-Mancozeb.

Materials and Methods

The following experiments were carried out at Meet-Khalaf Experimental Station, Shebin El-Kom, Egypt, during 2009/10 and 2010/11 growing seasons. Nearly similar seedlings of onion cultivar Giza 20 were transplanted in plots 3x3.5m (1/400 feddan) at the first of December of both years. Three plots served as replicates for each treatment. All the agricultural practices, *i.e.* irrigation and fertilization, were accomplished according to Ministry of Agriculture and Land Reclamation recommendations. Onion plants were left for natural inoculation conditions with the different pathogens.

Treatments:

Just before transplanting; onion seedlings were individually dipped for 15 minutes in the different treatments. However, beginning of January 15 the onion plants were sprayed five times, at 15 days intervals, with each of the following:

a) Indol acetic acid (IAA) 200 or 400 ppm.
b) Indol butyric acid (IBA) 200 or 400 ppm.
c) Selenium (antioxidant) 25 or 50 ppm.
d) Potassium hydrogen phosphate (K_2HPO_4) 50 or 100 mM.
e) Potassium hydrogen carbonate ($KHCO_3$) 10 or 20 g/l.
f) Galben-Mancozeb (fungicide) 2.5 or 5.0 g/l.

The untreated control plants received the same amount of sterilized distilled water. Tween 80 (0.5%) was used as surfactant agent in all treatments.

Disease assessment:

Disease index of 0-11 grads suggested by Towsend and Heuberger (1943) was followed. However, disease severity (DS) percentage was calculated according to the following formula:

$$DS (\%) = \frac{\Sigma (n \times v)}{II\ N} \times 100$$

Whereas: n = Number of plants in each category.
v = Numerical value of symptoms of each category.
N = Total number of the inspected plants.
II = Maximum of numerical values of symptoms category.

Yield production:

Random hundred bulbs of the middle rows of each plot were weighed to evaluate the effect of each treatment on the average of yield.

Determination of phenolic compounds:

Free, conjugated and total phenolic compounds were colourimetricaly determined using phosphotungestic phosphomolybdic acid (Folin and Cocaltese) reagents according to Snell and Snell (1953). A standard curve of P. hydroxyl benzoic acid was used to calculate the amount of phenolic compounds in different tested samples. The obtained results were expressed as mg P. hydroxyl benzoic acid equivalent per gram fresh weight.

Determination of sugars:

Reducing, non-reducing and total sugars were determined in a liquid of ethanol-water fractions using Michel DuBois *et al*. (1956) method. The amount of sugar was expressed as mg glucose per gram fresh weight, by means of a standard curve.

Statistical analysis:

Obtained data were statistically analyzed according to the standard procedures mentioned by Snedecor and Cochran (1967). The averages were compared at 5% level using the least significant differences (LSD) according to Fisher (1948).

Results

The effect of growth regulators fertilizers and an antioxidant on two onion diseases were studied under field and natural inoculation conditions. The obtained results can be reviewed as following:

Downy mildew:

All tested compounds sprayed on the cv. Giza 20 onion plants significantly under field and natural inoculation conditions, all tested compounds which were sprayed on cv. Giza 20 onion plants, significantly decreased downy mildew disease incidence than the control; both at 2010 and 2011 growing seasons (Table 1). Growth regulators, *i.e.* IAA followed by IBA, effectively reduced disease severity than selenium, K_2HPO_4 and/or $KHCO_3$. In general, increasing the concentration of any tested compound was more effective in reducing the disease in comparison with the untreated control plants. While the disease severity recorded 22.2 and 11.5% when IAA was applied at 200 and 400 ppm in 2010 compared to 28.3 and 18.5% in 2011, respectively. When Galben-Mancozeb was applied at the concentration of 5.0 g/l, disease severity reached 9.6 and 10.5%, in 2010 and 2011 growing seasons, respectively.

Table 1. Effect of foliar application with individual growth regulators, antioxidant and fertilizers on downy mildew disease of onion (cv. Giza 20) during 2009/10 and 2010/11

Treatment	Concentration	Season / Disease severity			
		2009/10		2010/11	
		Disease severity (%)	Efficacy (%)	Disease severity (%)	Efficacy (%)
IAA	200 ppm	22.2	66.5	28.3	55.6
	400 ppm	11.5	83.3	18.5	71.4
IBA	200 ppm	23.6	65.2	39.2	38.1
	400 ppm	19.5	70.5	15.2	76.2
Selenium	25 ppm	26.6	60.6	27.5	53.8
	50 ppm	22.3	66.7	23.0	61.3
K_2HPO_4	50 mM	28.3	57.7	29.0	54.0
	100 mM	22.6	66.7	25.7	60.3
$KHCO_3$	10 g/l	36.6	45.5	22.3	64.6
	20 g/l	20.7	69.7	12.6	80.0
Control (1) Galben-M	2.5 g/l	11.5	83.3	11.4	82.1
	5.0 g/l	9.6	86.3	10.5	83.3
Control (2) (treatment free)		66.9		63.7	
L.S.D. at 5 %		5.4		0.5	

Purple blotch:

Results present in Table (2) indicated that the tested compounds reduced purple blotch disease incidence. Indol acetic acid also showed better effects in reducing the disease severity of infection at 2010 and 2011 growing seasons. However, potassium hydrogen phosphate (K_2HPO_4) was effective in disease reduction. Meantime, individual application of any of the tested compounds significantly reduced the disease incidence than control. Also, it was noticed that increasing the concentration of the tested compounds was more effective in reducing the severity of infection of *Alternaria porri*. Significant differences could be noticed in all cases except K_2HPO_4 which gave insignificant reduction of purple blotch disease incidence (Table 2).

Yield production:

Yield production was also significantly responded by the different treatments (Table 3). Application of IAA at 400 ppm concentration increased yield production by 93.45 and 53.33% than control (2), in 2010 and 2011 growing seasons, respectively. These were followed by selenium (50 ppm) which increased the yield by 85.84 and 45.33, respectively, in the same growing seasons. Generally, increasing the concentration of the tested compounds results more yield increment.

Table 2. Effect of foliar application with individual growth regulators, antioxidant and fertilizers on purple blotch disease of onion (cv. Giza 20) during 2009/10 and 2010/11

Treatment	Concentration	Season / Disease severity			
		2009/10		2010/11	
		Disease severity (%)	Efficacy (%)	Disease severity (%)	Efficacy (%)
IAA	200 ppm	9.6	41.8	11.0	42.1
	400 ppm	6.3	62.6	9.6	52.6
IBA	200 ppm	12.7	25.0	13.9	29.1
	400 ppm	6.8	62.5	12.0	36.8
Selenium	25 ppm	11.3	29.0	12.0	36.8
	50 ppm	6.3	61.8	9.7	52.7
K_2HPO_4	50 mM	10.0	39.4	11.3	42.3
	100 mM	9.6	41.8	9.3	51.1
$KHCO_3$	10 g/l	11.6	29.7	15.3	21.9
	20 g/l	10.3	37.6	13.3	31.6
Control (1) Galben-M	2.5 g/l	10.8	34.5	10.6	47.4
	5.0 g/l	8.8	46.7	7.6	63.2
Control (2) (treatment free)		16.5		19.5	
L.S.D. at 5%		0.5		5.5	

Table 3. Effect of foliar application with individual growth regulators, antioxidant and fertilizers on plot yield of onion cv. Giza 20 in 2009/10 and 2010/11

Treatment	Concentration	Season / Yield per plot (kg)			
		2009/10		2010/11	
		100 bulb weight (kg)	Increase* (%)	100 bulb weight (kg)	Increase* (%)
IAA	200 ppm	5.33	71.93	5.03	34.13
	400 ppm	6.00	93.45	5.75	53.33
IBA	200 ppm	5.13	65.48	5.60	49.33
	400 ppm	5.23	68.70	5.96	58.93
Selenium	25 ppm	5.30	70.69	5.20	38.60
	50 ppm	5.75	85.48	5.45	45.33
K_2HPO_4	50 mM	3.95	27.40	4.50	20.00
	100 mM	4.03	30.00	4.95	32.00
$KHCO_3$	10 g/l	4.60	48.38	4.00	6.66
	20 g/l	4.80	54.83	4.50	20.00
Control (1) Galben-M	2.5 g/l	4.96	60.00	4.70	25.33
	5.0 g/l	5.75	85.48	6.20	65.33
Control (2) (treatment free)		3.10		3.75	
L.S.D. at 5 %		0.46		3.47	

* Related to the treatment free.

Phenolic compounds:

Individual application of growth regulators, antioxidant and fertilizers increased phenolic compounds in onion plants than the control (Table 4). The most increases (32 and 18%) of free phenols, comparing to control (2), were observed in case of applying IBA and IAA (400 ppm), respectively. Meanwhile, the lowest increases (2 and 5%) of free phenols, comparing to control (2), were recorded when $KHCO_3$ and K_2HPO_4 were sprayed, respectively. IBA followed by K_2HPO_4 caused the highest increase in conjugated phenols, while the opposite results were recorded when Galben-M and/or $KHCO_3$ were used. Total phenolic compounds were also increased in response to the different treatments. The highest increment (62% than control 2) was recorded when IBA was applied, while only 6% increment was observed when $KHCO_3$ was applied.

Table 4. Phenolic compounds mg/g dry matter of onion plants as affected by growth regulators, antioxidant and fertilizers at season 2011

Treatment	Concentration	Phenol contents (mg/g) dry weight		
		Free phenols	Conjugated phenols	Total phenols
IAA	400 ppm	7.1	1.5	8.6
IBA	400 ppm	7.9	2.8	10.7
Selenium	50 ppm	6.6	2.3	8.9
K_2HPO_4	100 mM	6.3	2.4	8.7
$KHCO_3$	20 g/l	6.1	0.9	7.0
Control (1) Galben-M	5.0 g/l	6.4	0.7	7.1
Control (2) (treatment free)		6.0	0.6	6.6

Sugars content:

Results in Table (5) indicate that reducing, non-reducing and total sugars were increased, than control, in response to the different treatments. The increase of reducing sugars ranged from 13% (IBA and selenium) to 33% ($KHCO_3$ and Galben-M). However, non-reducing sugars were highly increased where it was 193%, over the control (2), in case of IBA and 53% in the plants treated with $KHCO_3$. The increase of total sugars was highly responded to the application of IBA which gave 185% over the non-treated control (2) plants. While application of $KHCO_3$ caused the least increase (51%) of total sugars than control (2).

Table 5. Sugars content mg/g dry matter of onion plants as affected by growth regulators, antioxidant and fertilizers at season 2011

Treatment	Concentration	Sugar contents (mg/g) every weight		
		Reducing sugars	Non-reducing sugars	Total sugars
IAA	400 ppm	0.18	2.67	2.85
IBA	400 ppm	0.17	4.27	4.61
Selenium	50 ppm	0.17	3.19	3.36
K_2HPO_4	100 mM	0.19	2.99	3.18
$KHCO_3$	20 g/l	0.20	2.23	2.43
Control (1) Galben-M	5.0 g/l	0.20	2.74	2.94
Control (2) (treatment free)		0.15	1.46	1.61

Discussion

Three treatments, *i.e*. growth regulators, antioxidant and/or potassium fertilizers, were applied at different concentration to determine their effect on downy mildew and purple blotch diseases as well as yield production, phenol and sugar contents of onion (cv. Giza 20).

Results of the present study showed significant reduction in disease incidence was recorded in all tested treatments. The individual application of growth regulators, antioxidant and/or potassium fertilizers enhanced the plant resistance against *Peronospora destructor*; which can be used as an effective disease control treatment (Faoro *et al*., 2008). Although, selenium is not required to higher plants (Terry *et al*., 2000); it may exert a beneficial role in plants under stress (Xue *et al*., 2001). Selenium also protected plants against fungal infection as reported by Hansen *et al*. (2003). However, Borsani *et al*. (2001) indicated that selenium plays an important role in the defense mechanism of many plants to pathogen attack.

Also, the antioxidant toxicity toward several pathogens was reported by Galal and Abdu (1996) and Abd El-Megid *et al*. (2004). Saleh *et al*. (2009) reported that antioxidants increased the activity of catalase. On the other hand, bicarbonate salts are considered the most effective alternative control method against the fungal pathogens (Jamar *et al*., 2007). Suppressing effects of such compounds could be due to one or more of the following mechanisms: (a) direct effect on the pathogen multiplication, development and survival, (b) direct effect on plant metabolism which consequently affect the pathogen food supply and, (c) influence of plant defence responses, which affect the pathogen establishment and spread inside the host. Spraying fertilizers have fast absorption by the plant, high mobility within the tissues and they are safe and low cost nutrient sources as reported by (Horst *et al*., 1992 and Anonymous, 2005).

Application of K_2HPO_4 reduced the incidence of both diseases which could be attributed to its action as fertilizer in side, and increasing cell wall thickness on the other side (Ammar, 2003).

The bulb yield was also significantly increased, and that could be due to diseases management, improving plant growth and optimum metabolism actions; as was indicated also by many researchers (Agrios, 2005; Ammar, 2003; Jamar *et al*., 2007 and Faoro *et al*., 2008 and Youssef and Galal, 2012). However, growth regulators, selenium and used fertilizers resulted such yield increase.

Total phenolic compounds were increased in response to the different treatments. Agrios (2005) suggested that in host defense mechanism, phenylalanine ammonia lyase (PAL) is a key enzyme involved in the production of basic molecules used for biosynthesis of the phenolics, including lignin and phytoalexins. Increasing phenolic compound in plant tissues are good indicator to the host defence against the pathogens (Ammar, 2003).

Increasing sugar contents, in comparison to control (2), could be attributed to the effect of different treatments on plant metabolism and/or nutrition effects of bicarbonate compounds (Ammar, 2003; Jamar *et al*., 2007 and Saleh *et al*., 2009).

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(Received 18/07/2013;
in revised form 12/09/2013)

تأثير منظمات النمو والسلينيوم والتسميد البوتاسي على مرضي البياض الزغبي واللطعة الأرجوانية في البصل

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أدى رش نباتات البصل صنف جيزة-٢٠ تحت ظروف الحقل والعدوى الطبيعية - بأي من إندول حمض الخليك ، إندول حمض البيوتيريك إلى النقص المعنوي لكل من شدة الإصابة بمرضى البياض الزغبي واللطعة الأرجوانية مقارنة بالنباتات غير المعاملة أو تلك المعاملة بالسلينيوم أو كربونات البوتاسيوم. في حين لوحظ أن الرش بفوسفات البوتاسيوم الأحادية قد أدى إلى النقص المعنوي في الإصابة بمرض اللطعة الأرجوانية وقد ازداد محصول البصل بمقدار ٩٣,٥ و ٥٣,٣ % عن محصول النباتات غير المعاملة , عند استخدام اندول حمض الخليك (٤٠٠ جزء في المليون) في العامين ٢٠٠٩/٢٠١٠ و ٢٠١٠/٢٠١١ على التوالى. كما سجلت زيادة المحصول مقارنة بالنباتات غير المعاملة في عامي الدراسة على التوالى. وسجلت زيادة معنوية للمركبات الفينولية والسكريات الكلية كاستجابة لمختلف المعاملات الحقلية ، وكانت أعلى زيادة في الفينولات والسكريات الكلية قد تحققت عند استخدام إندول حمض البيوتيريك في حين أدى استخدام كربونات البوتاسيوم الأحادية إلى أقل زيادة في السكريات الكلية عن النباتات غير المعاملة. وقد أدى استخدام المبيد الفطرى جالبين - نحاس الى اختزال كلا المرضين بصورة أفضل من بقية المعاملات.