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PHYSIO-PATHOLOGICAL STUDIES ON THE EFFECT OF FERTILIZATION AND PLANT WATER EXTRACTS ON ONION (*Allium cepa* L.) INFECTED WITH DOWNY MILDEW DISEASE

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ABSTRACT: *Additional fertilization with actosol macroelements, actosol microelements and/or compost; significantly reduced the incidence of onion downy mildew disease(ODM) on both tested onion cultivars (Giza 6 and Giza 20). Increasing the fertilizer dose gave more disease reduction and improved growth characteristics, photosynthetic pigments, water relations and yield production. Generally, the best results were obtained when actosol macroelements (10 ml/l) was applied. Application of plant water extracts on onion plants at intervals of 15 days, significantly decreased the infection with the fungus. Both of neem or mint plant extracts at conc. 10 % showed the best results for disease reduction, growth characteristics, photosynthetic pigments, water relations and yield production. The best yield production was obtained by the application of actosol macroelements and /or neem plant extract.*

Key words: *Downy mildew, Peronospora destructor, fertilization, actosol, compost, plant extracts, growth, photosynthetic pigments, water relations and yield production.*

INTRODUCTION

Onion (*Allium cepa* L.) is an important vegetable crop in Egypt as well as in many other countries. It plays a very important role in the Egyptian agricultural economy where it occupies good position among the exportation crops; both to European and Arabic countries. The cultivated total area with onion crop during season 2014/2015 in Egypt was approximately 183,916 feddans produced about 2,691,919 tons of onion bulbs with average (14,637 tons/feddan). However, there are about 12,853 feddans intercropped with laden onion crop, produced about 151,816 tons of bulbs (11,812 tons/feddan). As for onion seed production, about 1,943 feddans were cultivated and produced 5,381 tons with average 277 tons/feddan (According to Economic Affairs Sector – Ministry of Agriculture and Land Reclamation, Egypt, 2014-2015). Due to good qualities and excellent characters of Egyptian onions, continuous studies have been achieved in order to improve and increase its yield of bulbs, which have great exported value and seeds, which are considered the initial source of cultivated onion every year. Some diseases attack onion plants during the different growth stages causing great yield losses. Out of them, downy mildew (ODM) caused by *Peronospora destructor* (Berk.) is considered very important disease. In order to control this disease using ecofriendly methods and /or plant extracts were individually applied. Tico (1952) stated that sources of plant nutrients for onions produced bulbs of high keeping qualities and protected the plants from fungal diseases. However, Geard (1959) got promising control of ODM by the avoidance of excessive nitrogen application. Taek *et al.,* (2001) showed that onion plants supplied with the slow-release fertilizers exhibited better growth and low downy mildew infection compared to the control. Such fertilizers increased soluble solid content, the number and thickness of scaly leaves and the total marketable yield of early and late maturing onions. Aly *et al.,* (2003) indicated that both the composed cow and chicken manures significantly reduced powdery and downy mildew incidence and severity. Heminder and Garampalli (2012) used composts and dry powder biomass to compare their potential to suppress downy mildew disease caused by *Perenosclerospora sorghi*. They found that compost at the rate of 4% or more suppressed the disease. Abd El-Megid *et al.,* (2001) investigated the efficacy of the aqueous cold dry-leaf extracts in controlling downy mildew and purple blotch of onion in Egypt; in comparison with the fungicide Ridomil plus. They found that black-cumin extract significantly decreased DM disease incidence than control and the extract (3 g/l) had approximately equal effect as Ridomil plus. Tiwari and Srivastava (2004) studied the efficacy of some plant extracts i.e., neem (*Azadirachta indica*), eucalyptus (*Eucalyptus globutens*), against the onion pathogens. All extracts exhibited significant antifungal activity. Kofoet and Fischer (2007) demonstrated that plant extracts, salts and micro-organisms can reduce disease severity and incidence of downy mildew in several vegetables. Kamalakannan and Shanmugam (2009) found that leaf extract of neem (*Azadirachta indica*) was effective in controlling maize downy mildew.

On the other hand, studies on the effect of plant extracts on the changes in the physiological and biochemical aspects were found to be fewer. Gado (2013) found that commercial plant extracts *i.e.*, Sincocin and Agrispon caused a great reduction in disease severity and a great increase in root growth and yield component of sugar beet infected with powdery mildew. Also, Dominic *et al.,* (2016) studied the influence of plant extracts selected based on their antifungal activities on shoot dry weight, plant height and leaf area of french beans (*Phaseolus vulgaris* L.) infected with rust (*Uromyces appendiculatus*).

This study aimed to find out environmental friend control methods for such diseases, such as organic fertilization and plant extracts under field conditions with studying the changes induced in morphological and physiological aspects as well as yield of onion plants under these conditions.

MATERIALS AND METHODS

Under field and natural inoculation conditions, these experiments were conducted in new reclaimed land at El-Khatatba, Menoufia governorate, both at 2014 and 2015 growing seasons. A complete randomized block design with three replicates was followed. Each replicate (1x2 m) included 50 onion plants (20 cm apart) of either Giza 6 or Giza 20 cultivars. Nearly equal size seedlings were planted at December, 20 of both years. The recommended N-P-K fertilizers were applied and dropping irrigation system was used. Lanit was used to control onion fly. Before planting, the seedlings were dipped, separately, in each tested material for 15 minutes and sprayed every 15 days with the same solution. However, in compost treatment, the used compost was applied to the soil once just before planting. The following treatments were distinguished in order to control downy mildew disease of onion.

1. Fertilization: Individual treatments of compost and/or actosol were conducted to study their efficacy on ODM disease and related characteristics. Actosol is nontoxic liquid organic fertilizer containing 2.9% humic acid available for all vegetable and field crops. Actosol macroelements contained NPK (10-10-10) while actosol microelements contained Fe, Zn, Cu, Mn etc. + humic acid. Compost used in this study contained ground rice straw + 20% animal residues + 10% pentonite + 0.5% phosphate stone + 0.5% sulfur and 2% urea. Compost was added to the soil, just before plating, at the rate of 5, 10 and 15 kg/plot. While actosol preparations were sprayed on onion plants at the concentrations of 0.5, 5 and 10 cm/l monthly, starting one month after planting.

2. Plant water extracts: Hundred grams of the leaves of mint (*Mentha sprgi*), neem (*Azadirachta indica*) and basil (*Ocimum basilicum*) were seperatly boiled in distilled water for half an hour. then completed for one liter with sterilized distilled water. The concentrations of each extract (2.5, 5 and 10%) were individually used to find out their effect on ODM disease and other physiological aspects. Application of each treatment was carried out every month to Giza 6 and/or Giza 20 onion cultivars at the rate of 5 ml/plant. Control plots received sterilized distilled water with 3% Tween 80 (Mutwally *et al.,* 2010).

Data recorded: The results of severity of infection, growth characteristics, photosynthetic pigments, permeability, transpiration rate, osmotic pressure, water relations and yield production as affected with the different treatments were estimated as following:

1.Severity of infection: Severity of infection was determined four times using the scale of 0-9 according to the area of the leaves covered with the disease symptoms where: (O: healthy plants and 9: up to 100%). The formula of Soliman *et al.,* (1988) were used for this estimation as following:

Severity of infection = (a x b) / (N x K) x100

Where:

a = Number of the diseased plants

b= Infection rate (0-9)

N= Total number of the plants/plot

K= Total infection rates

2. Physiological aspects:

2.1. Growth characters:

Plant height (cm), dry weight of whole plant (dried in an electric oven at 70^{o}C for 72 h) g/plant.

Leaf area (cm2/plant) was estimated using the formula of TEL *et al.,* (1996) leaf area was determined on subsample of four plants per plot: during the first stages of the growth cycle, the green leaf (non-senescent length) (*l*) and the maximum width (*w*) of earth leaf blade were measured and leaf shape as a cone.

LA = л l W/ 2

2.2. Photosynthetic pigments:

Chlorophyll a, b and carotenoids were determined from fresh leaves, using spectrophotometer method reported by Fadeel (1962) as follow: a known fresh weight of leaves (0.5 g) was homogenized in 15 ml of 85% aqueous acetone for 10 minutes. The homogenate material was centrifuged and the supernatant was made up to the volume of 5 ml with 85% acetone then measured at three wave lengths of 662, 644and 440 nm using SPEKOL spectrophotometer (Model: SP/02 DP). Pigment concentrations were calculated according to the following equations:

Chl, a = 9.784 x E 662 – 0.99 x E 644 (mg/l)

Chl, b = 21.426 x E 644 – 4.63 x E 662(mg/l)

Careteniods = 4.695 x E 440 – 0.268 (a+b) (mg/l).

The concentration of pigments was then expressed in mg/g D.Wt.

2.3. Water Relations:

The total water content in leaves were determined using the methods described by Gosev (1960). Relative water content (RWC) and leaf water deficit (LWD): Equal leaf discs (1 cm) were cut from 90 days old plants, weighed to give the fresh weight (FW) floated on water for 6 hours until they reweighed (turgidity weight) and final oven dried at 70 °C for 72 hours to reach a constant weight. Relative water content and leaf water deficit (LWD) were calculated using the following formula according to Kalapos (1994):

RWC % = [(Turgid weight – Fresh weight) / (Turgid weight – Dry weight)] x 100

LWD % = 100 – RWC

Osmotic pressure: Values of total soluble solids of the cell sap were obtained for the pressed sap of the leaf using the Abbe Refrectometer and the osmotic pressure values (bar) were calculated by using special tables according to the method described by Gosev (1960).

Transpiration rate: was determined using the weight method described by Kreeb (1990).

Membrane integrity (Permeability): Membrane integrity was determined according to Yan *et al.,* 1996 as follow: A portion of the excised young fresh leaves was washed then put in a beaker containing double distilled water. The beakers were kept at 30 °C for 3 hr, then conductivity (C_1) of the solution was measured by the conductivity meter (Model: CD-4301). After boiling the samples for 2 min, their conductivity (C_2) was measured again when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated according to the formula: [EC % = (C_1/C_2) x 100].

3. Yield production:

At the end of the experiments (6 months), the harvested onion bulbs of each plot were weighed and yield production was estimated as kg/plot.

Statistical analysis: The data were statistically analyzed and significance among means was assessed by least significant difference (LSD) at 5% probability level using PROC ANOVA of the SAS program v.9 (Anonymous, 2002).

RESULTS

Under field and natural inoculation conditions, both Giza 6 and Giza 20 onion cultivars were subjected to different treatments in order to control downy mildew disease caused by *Peronospora destructor* (Berk.). These experiments were conducted at 2014 and 2015 growing seasons. The tested treatments included fertilization with actosol and compost, plant water extracts, as well as the severity of infection, photosythenthic pigments, water relations and yield production were estimated to find out the treatments effect.

1. Effect of fertilization with Actosol and compost:

1.1. Severity of infection:

Results shown in Table (1) indicate that application of any tested fertilizer significantly decreased the severity of infection with *Peronospora destructor* than control; Actosol macro-elements (10.0 ml/l) gave the best results where they were 4.0, 8.0, 12.0 and 20% at season 2014 and 4.0, 8.0, 12.6 and 18.0% at 2015 for Giza 6 cultivar, after 2, 3, 4 and 5 months from planting, respectively. Severity of infection with downy mildew in control plants recorded 14.0, 22.8, 44.9 and 64.0% at 2014 and 13.3, 20.0, 40.0 and 62.3% at 2015, in the same respect. The decrease in severity infection was about 71.4, 64.9, 73.3 and 68.8% of the owning controls at 2, 3, 4 and 5 months in season 2014, 69.9, 60, 68.5 and 71.1% in season 2015. Nearly similar results were obtained for Giza 20 onion cultivar. Such results were observed by Aly *et al.,* (2003) and Goncalves *et al.,* (2004).

1.2. Growth characteristics:

The effect of fertilization with actosol and/or compost on the growth characters of onion plants was studied. Results present in Table (2) clear that the application of any concentration of the fertilizer compounds improved the growth of onion plants significantly compared to the control plants. Plant height of Giza 6 cv. control plants averaged 51.2 cm. while it reached 80 cm when actosol macro-elements was applied at the concentration of 10 ml/l. Leaf area ranged from 153.29 cm2/plant (Compost 5 kg/plot) to 188.97 cm2/plant (actosol mac. 10 cm/l). On the other hand, Average leaf area of control plants was 94.80 cm2 only. Total fresh weight of an onion plant of control treatment averaged 46.72 gm. However, it recorded 91.95, 90.01 and 86.44 g., respectively when actosol macro. (10 ml/l), actosol micro. (10 ml/l) and compost (15 kg/plot) were applied. In the same

Table (1): Severity of infection with *Peronospora destructor* on Giza 6 and Giza 20 onion cultivars as effected by Actosol and compost treatments at 2014 and 2015 growing seasons.

Treatments	Concentration	Giza 6								Giza 20							
		Season 2014				Season 2015				Season 2014				Season 2015			
		After 2 months	After 3 months	After 4 months	After 5 months	After 2 months	After 3 months	After 4 months	After 5 months	After 2 months	After 3 months	After 4 months	After 5 months	After 2 months	After 3 months	After 4 months	After 5 months
Actosol (macro-elements)	0.5 cm/l	9.33	15.30	24.00	30.00	8.00	14.00	22.40	26.00	8.00	14.00	22.00	28.60	7.33	13.33	20.00	22.00
	5.0 cm/l	7.33	12.00	19.20	25.73	6.00	11.20	19.60	22.00	6.00	10.60	18.00	20.00	6.00	10.00	16.40	19.60
	10.0 cm/l	4.00	8.00	12.00	20.00	4.00	8.00	12.60	18.00	4.00	7.46	12.00	16.00	4.00	7.20	11.20	15.60
Actosol (micro-elements)	0.5 cm/l	10.00	18.00	28.26	34.33	9.33	17.60	26.00	30.00	9.33	16.40	27.87	32.00	10.00	15.60	24.00	29.37
	5.0 cm/l	8.00	14.40	22.00	28.00	7.60	13.33	21.60	24.00	7.33	13.60	22.00	26.00	8.00	12.00	20.60	16.00
	10.0 cm/l	6.00	10.00	18.40	22.00	5.60	11.20	19.33	22.00	6.00	11.20	19.60	20.00	7.33	10.00	16.40	13.60
Compost	5 kg/plot	10.60	20.00	30.00	36.00	10.00	18.00	28.60	33.60	10.00	18.00	30.00	36.33	10.20	17.20	28.00	32.00
	10 kg/plot	8.00	16.33	26.00	30.00	7.60	14.40	24.00	30.00	8.00	14.60	24.00	30.00	8.60	15.60	22.00	26.00
	15 kg/plot	6.20	14.00	23.33	26.00	6.00	12.00	20.00	26.60	7.33	12.00	20.00	24.00	7.33	14.00	19.60	20.00
Control		14.60	22.80	44.93	64.00	13.33	20.00	40.00	62.33	14.00	27.20	47.33	59.20	12.00	22.00	35.60	53.33
LSD at 5%		1.21	2.48	2.55	3.05	1.45	2.17	2.89	3.31	1.45	2.17	3.25	3.47	1.45	2.43	2.55	3.68

Table (2): Effect of Actosol and compost on some growth characteristics of Giza 6 and Giza 20 onion cultivars under disease stress conditions at 2015 growing season.

Treatments	Concentration	Giza 6			Giza 20		
		Plant height (cm)	Leaf area (cm^2/ plant)	Whole plant dry weight (g/plant)	Plant height (cm)	Leaf area (cm^2/ plant)	Whole plant dry weight (g/plant)
Actosol (macro-elements)	0.5 cm/l	64.38	168.83	7.87	70.42	187.94	7.97
	5 cm/l	67.73	180.78	8.04	74.30	202.44	8.01
	10 cm/l	67.95	188.97	8.23	74.93	208.38	8.36
Actosol (micro-elements)	0.5 cm/l	63.87	157.49	7.28	67.25	168.93	7.45
	5 cm/l	64.27	165.10	7.69	67.51	173.54	7.79
	10 cm/l	67.55	180.28	7.91	69.81	186.32	8.08
Compost	5 kg/plot	62.22	153.29	7.25	65.18	160.78	7.34
	10 kg/plot	64.48	161.97	7.57	65.05	167.27	7.69
	15 kg/plot	66.00	173.20	7.38	67.92	181.27	7.46
Control		46.45	94.80	7.54	50.06	110.04	3.77
LSD at 5%		2.20	8.99	0.46	3.14	10.84	0.46

respect, total dry weight averaged 8.23, 7.91 and 7.38 g/plant, while it was 4.54 g in the case of control. Shoot: 10.0 ml/l root ratio was also increased significantly than control, in response to the different fertilizer applications. As for Giza 20 onion cultivar, similar observations were noticed. Such results are in agreement with those obtained by Goncalves *et al.,* (2004).

1.3. Photosynthetic pigments:

Chlorophyll a, chlorophyll b and carotenoids pigments of onion Giza 6 and Giza 20 cvs. were determined in 90 days old leaves of disease stressed onion plants. Results in Table (3) show that the application of any tested fertilizer significantly increased such pigments than control. Total chlorophyll of control plants was 1.58 mg/g dry weight. These were 4.10, 4.15 and 4.03 mg/g dry weight, respectively when actosol macr. (10 ml/l), actosol micr. (10 ml/l) and compost (15 kg/plot) were applied. These treatments achieved increases in total chl. by 159.5, 162.7 and 155.1% over the control, respectively. Carotenoids were 1.16, 1.65 and 1.09 mg/g dwt, respectively with the abovementioned treatments and 0.59 mg/g dwt in the control plants with % increase 96.6, 179.7 and 84.7 over the untreated plants. Ratio of total chlorophylls to carotenoids showed significant increment than control in response to variable fertilizers.

1.4. Water relations and membrane integrity:

Results of water relations and membrane integrity in the leaves of disease stressed onion cultivars Giza 6 and Giza 20 are shown in Table (4). Total water content in both onion cultivar leaves was significantly

Table (3): Effect of Actosol and compost on photosynthetic pigments content in the leaves of Giza 6 and Giza 20 onion cultivars under disease stress conditions at 2015 growing season.

Treatment	Concentration	Giza 6					Giza 20				
		Chl. a mg/g DW	Chl. b mg/g DW	Total Chl. a+b mg/g DW	Carotenoids mg/g DW	Total Chl./ Car. Ratio	Chl. a mg/g DW	Chl. b mg/g DW	Total Chl. a+b mg/g DW	Carotenoids mg/g DW	Total Chl./ Car. ratio
Actosol (macro-elements)	0.5 cm/l	2.21	1.51	3.63	1.62	3.12	2.16	1.59	3.75	1.51	2.51
	5 cm/l	2.35	1.54	3.89	1.16	3.37	2.35	1.68	4.03	1.43	2.87
	10 cm/l	2.48	1.62	4.10	1.16	3.52	3.75	1.79	5.54	2.52	2.23
Actosol (micro-elements)	0.5 cm/l	2.23	1.46	3.69	1.08	3.40	2.11	1.38	3.48	1.26	2.82
	5 cm/l	2.35	1.54	3.89	1.09	3.56	2.22	1.65	3.87	1.32	2.99
	10 cm/l	3.72	2.43	4.15	1.65	3.72	3.61	2.69	6.30	2.08	3.09
Compost	5 kg/plot	2.10	0.52	3.62	1.07	3.38	2.04	1.41	3.45	1.73	2.77
	10 kg/plot	2.22	0.60	3.83	1.08	3.54	2.16	1.49	3.66	1.53	2.42
	15 kg/plot	2.35	0.68	4.03	1.09	3.71	2.28	1.72	4.00	1.50	2.70
Control		0.93	0.65	1.58	0.59	2.68	0.96	0.63	1.60	0.59	2.71
LSD at 5%		0.03	0.13	0.15	0.02	0.08	0.03	0.05	0.18	0.12	0.40

Table (4): Effect of Actosol and compost applications on water relations and membrane integrity in leaves of Giza 6 and Giza 20 onion cultivars under disease stress conditions at 2015 growing season.

Treatments	Concentration	Giza 6						Giza 20					
		Total Water content (%)	Leaf water def. (%)	Relative water content (%)	Osmotic pressure C.S. (bar)	Transpiration mg/cm^2 h	Membrane integrity %	Total Water content (%)	Leaf water def. (%)	Relative water content (%)	Osmotic pressure C.S. (bar)	Transpiration mg/cm^2 h	Membrane integrity %
Actosol (macro-elements)	0.5 cm/l	95.31	31.77	68.23	6.20	15.38	89.99	95.30	31.62	68.38	6.00	18.59	90.38
	5 cm/l	95.72	30.27	69.73	6.50	14.87	88.45	95.54	31.12	68.88	6.40	12.00	88.88
	10 cm/l	95.83	30.31	69.70	6.60	14.45	78.26	95.81	30.87	69.13	7.00	14.34	89.28
Actosol (micro-elements)	0.5 cm/l	95.21	34.64	65.36	6.00	15.55	89.79	95.19	33.77	66.24	6.10	17.34	89.99
	5 cm/l	95.32	33.60	66.40	6.20	15.82	88.45	95.32	33.60	66.40	6.60	16.80	90.38
	10 cm/l	95.41	32.98	67.03	6.50	13.15	88.67	95.39	31.29	68.71	6.90	15.29	88.88
Compost	5 kg/plot	94.88	35.60	64.40	6.00	15.42	89.35	94.88	35.60	64.40	5.90	20.92	89.57
	10 kg/plot	94.97	34.93	65.07	6.20	16.22	87.74	94.80	35.09	64.91	6.00	17.10	89.79
	15 kg/plot	95.08	34.38	65.62	6.30	15.64	86.53	95.08	34.38	65.62	6.50	15.18	90.38
Control		83.08	61.07	38.93	4.50	5.38	72.45	83.42	55.70	44.30	4.80	8.46	72.45
LSD at 5%		0.58	0.06	3.07	0.34	5.81	0.50	1.12	4.74	4.75	0.90	5.44	0.53