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Effect of some Plant Extracts and Antioxidants on Onion White Rot

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White rot of onion is still one of the most destructive diseases in Egypt causing significant losses in bulb yield. The causal organism produces abundant amount of sclerotia in the soil and therefore, it has been established in many parts of Egypt particularly in upper and Middle Egypt. Different methods of disease management were applied; however, the disease is still the main problem of onion in Egypt. Plant extracts, *i.e.* neem and datura, as well as salicylic acid and sodium benzoate antioxidants were tested to control the disease under either greenhouse or field conditions. Obtained results were compared to a fungicidal treatment (Folicur) at different concentrations using the onion (cv. Giza 20). A field experiment was carried out for two seasons (2011 and 2012). Application of plant extracts of neem and datura reduced the rot incidence accompanied by high amount of free phenols and reducing sugars which were higher in the treated plants. Moreover, application of antioxidants reduced the rot incidence.

Keywords: Antioxidants, datura, Folicur, neem, onion, plant extracts and white rot disease.

White rot of onion is still one of the most dangerous diseases in Egypt causing considerable losses in the bulb yield (Melero-Vara *et al.*, 2000 and Abdel-Momen *et al.*, 2000). The causal organism (*Sclerotium cepivorum*) is a soil borne pathogen produces abundant sclerotia in the soil which attack the plant root of the susceptible genotypes (Georgey, 1977; Abd El-Megid, 1994 and Khaled *et al*., 1997). Infection occurs in all plant growth stages particularly in the seedling stage causing severally reduction in the number of the grown plants which consequently reduces the number of bulbs (Melero-Vara *et al*., 2000). Different biological methods such as the use of neem and datura extracts gave significant effect on white rot control (Ghorbanian *et al.*, 2008; Saleh *et al.*, 2009 and Farag *et al.*, 2010). Also, the effect of antioxidants was also studied (Kishore *et al*., 2002; Elad, 1992; Abd El-Megid *et al.*, 2004 and Ahmed, 2013). Moreover, Seed treatment with different plant extracts, *i.e.* neem leaf, garlic clove, marjoram leaf and onion bulbs, significantly reduced damping-off percentage caused by *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *S. rolfsii* on three vegetables over untreated control (Islam and Faruq, 2012).

Therefore, this study was designed to evaluate the effect of some natural plant extracts and some antioxidants on onion white rot incidence in comparison with the application of fungicide.

Materials and Methods

The causal organism:

Samples white rot of onion were collected from Minufiya Governorate, Egypt. The infected plant samples were surface sterilized using 1% sodium hypochlorite for three minutes, rinsed several times in sterilized distilled water, dried between sterilized filter papers and subjected on PDA medium in Petri dishes. The abundant fungal growth was purified using hyphal tip technique. Identification was conducted at the Department of Mycology, Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.

Two medical plant water extracts, *i.e.* neem (*Azadirachta indica*) at the rates of 50 and 75% and datura (*Datura stramonium*) at the rate of 40 and 60%, were tested. Hundred gram of each plant sample were frozen for 24h and blended in 100 ml water to achieve the extract of 100% concentration (Farag *et al.*, 2010). Sodium benzoate and Salicylic acid at the rate 5 mM and 10mM were also evaluated compared to the fungicide Folicur at the rate of 25 ml/l.

Greenhouse experiments:

Formalin sterilized pots (25-cm-diam.) were filled with formalin sterilized clay soil which was infested with *Sclerotium cepivorum* grown on Barley medium at the rate of 3% of the soil weight. Seven days later, seedlings of Giza 20 onion cultivar were transplanted. Fifteen seedlings (60-day-old) were separately treated with any single treatment and transplanted in five pots (replicates). Control treatment included five infested potted soil which planted with 50 untreated onion seedlings. Onion seedlings were dipped for 30 min in the tested preparations of plant extracts. Percentage of infection with *S. cepivorum* was estimated 90 day after transplanting. Obtained data were statistically analysed according to Snedecor and Cochran (1972).

Field experiments:

Under field and natural infection conditions, the onion cv. Giza 20 was cultivated during 2010/2011 and 2011/2012 at Meet-Khalaf (Minufiya), Shebin El-Kom, Egypt. Three plots of 3 x 3.5 m (1/400 feddan) served as replicates for each treatment. The same treatments which were previously applied in the greenhouse experiments were adopted in the field with the same rates and methods of application on onion cv. Giza 20. The experiment was carried out in a complete plot randomize design. Percentages of infection and mean weight of apparently healthy bulbs were recorded after harvest. Data were statistically analysed according to Snedecor and Cochran (1972).

Biochemical assay:

Samples of 90-day-old onion plants were individually assayed as follows:

1- Determination of phenolic compounds:

Total, free and conjugated phenols were colourimetricaly determined using phosphotungstic 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.

Obtained results were expressed as mg P. hydroxyl benzoic equivalent gram fresh weight.

2. Determination of sugars compounds:

Total, reducing and non-reducing sugars were determined in aliquos of the ethanol-water fractions using the method described by Dubois *et al.* (1956) and the amount of sugars were expressed as mg glucose a standard curve.

Results

Greenhouse experiments:

In the greenhouse, all plant extracts and antioxidants as well as Folicur reduced the incidence of white rot disease compared to untreated control. Results in Table (1) show that, at the highest concentration, Folicur, neem and datura were more effective in reducing the incidence of white rot, when their efficacy reached 59.4, 70.3 and 57.7%, respectively, followed by sodium benzoate (40.5%) and Salicylic acid (32.4%).

Table 1. Effect of plant extracts, antioxidants and fungicide applications on white rot of onion (cv. Giza 20) in the artificially infested soil with *Sclerotium cepivorum*

Treatment	Concentration	No. of plants	Infection (%)	Efficacy
Neem	50%	4.2	8.4	43.2
	75%	2.2	4.4	70.3
Datura	40%	4.6	9.2	37.8
	60%	3.2	6.4	57.7
Salicylic acid	5 mM/l	6.0	12.0	18.9
	10 mM/l	5.0	10.0	32.4
Sodium benzoate	5 mM/l	5.6	11.2	24.3
	10 mM/l	4.4	8.8	40.5
Folicur	25 ml/l	2.0	4.0	59.4
Control	Untreated	7.4	14.8	----
L.S.D. at 5%		4.22	8.43	----

Effect of plant extracts and antioxidants on the white rot infection and yield in field:

Data in Table (2) indicate that all tested plant extracts significantly reduced the percentage of white rot disease of onion cv. Giza 20. The efficacy of neem and datura extracts recorded the highest percentages, being 78.8 and 71.7%, respectively, at the highest concentration. In the second season, the efficacy of neem was 72.3% compared to datura extracts (72.04%). These were considered the most effective treatments in reducing the white rot disease (Table 2). It could be observed that onion yield percentage increased in the first year more than that recorded in the second one. The yield of 100 bulbs ranged from 6.63 to 6.50 kg in the first season (2011), meanwhile it reached 6.55 to 6.88 kg in the second one (2012).

Data in Table (2) indicated that the two tested antioxidants significantly reduced the infection percentage. Salicylic acid and sodium benzoate were the most effective treatments, when their efficacy reached 37.9, 43.4 and 28.6, 36.6 at the first season (2011) and 38.5, 55.3 and 41.6, 48.4 at the second season (2012), respectively.

Effect of infection on bulb yield:

It could be observed in Table (2) that the percentage of yield increase of onion in the second year was relatively higher than that was recorded in the first year. The yield increase by increasing antioxidants concentration was also found. The increase ranged from (5.50 and 5.90) to (5.80, 5.62) kg/100 bulbs. Folicur was the superior treatment in suppressing the white rot disease where the percentage of the infected plants was 4.9% compared to 32.2% in the control treatment. Regarding, the yield of onion bulbs, most treatments gave higher yield than control. The highest 100 bulb weight (6.96 kg) was from transplants treated with Folicur while the lowest was from the treatment free (4.63 kg).

Table 2. Effect of plant extracts, antioxidants and fungicide applications on white rot incidence and yield of onion (cv. Giza 20) under field conditions during 2010/11 and 2011/12 growing seasons

Treatment	Conc.	2010/2011			2011/2012		
		Infection (%)	Efficacy	100 bulb weight (kg)	Infection (%)	Efficacy	Estimated 100 bulb weight (kg)
Neem	50%	7.0	77.5	5.88	10.5	67.4	5.80
	75%	6.6	78.8	6.63	8.8	72.3	6.55
Datura	40%	12.0	61.4	5.88	13.6	58.1	5.80
	60%	8.8	71.7	6.50	9.00	72.0	6.88
Salicylic acid	5 mM/l	18.8	37.9	5.25	19.8	38.5	5.50
	10 mM/l	15.5	43.4	5.50	14.4	55.3	5.90
Sodium benzoate	5 mM/l	22.2	28.6	5.25	18.8	41.6	5.52
	10 mM/l	20.0	36.6	5.80	16.6	48.4	5.62
Folicur	25 ml/l	5.0	83.9	6.25	4.9	84.8	6.96
Control	untreated	31.1	-----	4.80	32.2	-----	4.63
L.S.D. at 5%		0.166	-----	0.103	0.44	----	0.49

Biochemical analyses:

1. Phenolic compounds:

Samples of onion plants (different treatments of 2012) were chemically analysed to determine total, free, and conjugated phenols. Results shown in Table (3) clear that total phenols were increased, in comparison with the control treatment, in response to all tested plant extracts and/or antioxidants. In case of neem, datura and salicylic acid, total phenols recorded 56.8, 52.7 and 51.2 mg/g fresh weight, respectively. It was noticed that most of the determined phenols were free, when recorded 31.2, 31.1 and 28.6 mg/g fresh weight, meanwhile the conjugated once recorded 25.6, 21.5 and 22.6 mg/g fresh weight, respectively, for the abovementioned treatments. In the case of Folicur treatment, total phenols reached 34.4 mg/g fresh weight.

2- Sugars compounds:

Data in Table (4) show that sugar contents recorded the lowest concentration in case of datura treatment. Meanwhile, neem and sodium benzoate treatments recorded the highest amounts of reducing (0.189 and 0.189 mg/g fresh) and total sugars (3.18 and 2.85 mg/g fresh), respectively. Folicur treatment recorded the highest amount (4.136 mg) of the non-reducing sugars compared to the control.

Table 3. Effect of plant extracts, antioxidants and fungicide on total phenols (mg/g fresh weight) in naturally infected onion plants (cv. Giza 20) with *Sclerotium cepivorum* under field conditions during 2012 growing season

Treatment	Concentration	Free phenol	Conjugated phenol	Total phenol
Neem	75 %	31.2	25.6	56.8
Datura	60%	31.1	21.5	52.7
Salicylic acid	10 mM	28.6	22.6	51.2
Sodium benzoate	10 mM	18.6	9.9	28.5
Folicur	25 ml/l	20.1	14.3	34.4
Control	----	15.9	6.1	22.0

Table 4. Effect of plant extracts, antioxidants and fungicides on total sugars in naturally infected onion plants (cv. Giza. 20) with *Sclerotium cepivorum* under field conditions during 2012 growing season

Treatment	Concentration	Reducing sugar	Non-reducing	Total sugar
Neem	75 %	0.189	2.991	3.18
Datura	60%	0.125	2.535	2.66
Salicylic acid	10 mM	0.185	3.386	3.57
Sodium benzoate	10 mM	0.189	2.661	2.85
Folicur	25 ml/l	0.164	4.136	4.3
Control	------	0.159	1.571	1.73

D i s c u s s i o n

White rot of onion is one of the most distractive diseases in Egypt. It caused significant losses in bulb yield in Middle and Upper Egypt (Georgey, 1977; Kurtz, 1983; Anonymous, 1985 and Khaled *et al.,* 1997). Many trials were given to reduce the incidence of the disease. Plant quarantine is applied in the area in which the disease was recorded at levels by preventing the movement of the infected transplants to/ or from Middle Egypt (Crowe *et al.*, 1980 and Anonymous, 1985).

Biological control was adapted by Oliveira *et al.* (1984) and Entwistle (1990) using *Trichoderma* spp., which had a significant effect on disease control at the experimental level. In this concern, certain plant extracts, *i.e.* datura and neem, were evaluated as a biocontrol agent. In this research, critical tests were carried out under greenhouse and field conditions to determine the inhibitory effect of neem and datura extracts at different concentrations against onion white rot disease. Obtained results showed significant reduction in the disease incidence in response to apply such extracts. Also, when the amount of phenols (free and conjugate) were determined; it was found that the amount of free phenols was also increased in response to the treatment which support the use of such extracts phenols were found to be involved in the defence mechanism in many plants against disease infection The obtained data are in harmony with those obtained by Ahmed (2013). Abdel-Momen *et al.* (2000) tested the effect of saponin on white rot disease of onion. He found that it was very effective compared to the effect of the fungicide Folicur.

Meena *et al.* (2000) and Ammar (2003) mentioned that increasing phenols in the diseased plants is a physiological reaction for controlling the disease. Moreover, antioxidant compounds such as sodium benzoate, ammonium tartaret and salicylic acid were also tested under greenhouse and field conditions using different concentrations. Obtained results showed also significant decrease in disease incidence. Also, the amount of free phones was also increased the treated plants. Yield of the infected and treated survival plants was also determined. Such results may be due to the positive effects of these treatments both on plant growth improvements and reducing population density of the causal organism units in the soil as reported by many researchers (Galal and Abdou, 1996; Abdel-Momen *et al.*, 2000; Ammar, 2003; Abd El-Megid *et al.*, 2004; Farag *et al.*, 2010 and Islam and Faruq, 2012). It is of logic that improving onion plant growth and minimizing *S. cepivorum* population in the surrounding soil could be the main factors for increasing bulb yield as recorded in the present investigation.

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أدت المعاملة ببعض المستخلصات النباتية ومضادات الأكسدة لشتلات البصل صنف جيزة ٢٠ **قبل الزراعة فى تربة معدة** صناعياً **بالفطر** (سكليروشيوم سيبيفورم) الى تقليل الاصابة بمرض العفن الأبيض وكانت أفضل النتائج عند المعاملة بمستخلص النيم ثم الداتورا (٢٢% و ٣٢%) **إصابة والتى كانت** (٤٤% و ٥٠%) **إصابة عند المعاملة ببنزولت** الصوديوم وحمض السالسيلك على **التوالي**.

وقد أدت المعاملة الحقلية (ميت خلف - محافظة المنوفية) **وتحت ظروف العدوي** الطبيعية عامي ٢٠١١/٢٠١٠ و ٢٠١٢/٢٠١١ بهذه المعاملات الى النقص المعنوى لحدوث المرض والزيادة المعنوية للمحصول . **وكان مستخلص** النيم هو الأفضل فى اختزال المرض وزيادة المحصول حيث حقق نسبة إصابة (٦.٦%) **تلاه مستخلص الداتورا** (٨.٨%) **إصابة** .

لوحظ زيادة الفينولات الحرة فى النباتات المعاملة مقارنة بغير المعاملة وكانت أفضل التقديرات هى المعاملة بمستخلص النيم والداتورا ثم حمض السالسيلك وبصفة عامة كانت الفينولات المرتبطة أقل من الحرة. وكذلك زيادة السكريات الكلية عن السكريات المختزلة وغير المختزلة وبصفة عامة السكريات المختزلة أقل من السكريات الكلية وغير المختزلة .