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Influence of Humate Substances and Fungicides on the Control of Onion Downy Mildew

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DOwny mildew of onion (*Allium cepa*), caused by *Peronospora destructor*, is one of the most important onion diseases that mainly controlled by fungicides. This investigation aimed to evaluate the effect of foliar application of two forms of humic substances (HS) individually or in combinations with two fungicides against downy mildew, growth, photosynthetic capacity and metabolism changes of onion. The trials were carried out during the growing seasons 2016 and 2017. Final disease severity (FDS%) andarea under disease progress curve (AUDPC) were used to assess the potential of twoforms of (HS), and two fungicides to control downy mildew of onion. All treatments resulted in minimizing the FDS (%) and AUDPC, compared to control treatment, during the two seasons. The most effective treatment was (HS)-leonardite combined with fungicide Ridomil Gold 68% (WP), which exhibited a significant decrease in FDS (%) and AUDPC in relation to other treatments and control. All treatments improved onion growth parameters, *i.e.* bulb diameter, plant height, dry weight/plant, dry matter, average yield compared to untreated control plants. The chemical changes in treated plants, *i.e.* enzymes activities, total carbohydrates, total nitrogen (%), total soluble solids (TSS %) were also assessed. Principal component analysis gave evidence to the importance of all parameters in this study, especially FDS (%), as a reliable indicator for evaluation of these materials against downy mildew disease.

Keywords: *Allium cepa*, downy mildew, fungicides, humate sources and *Peronospora destructor*.

Downy mildew of onion (*Allium cepa*) caused by *Peronospora destructor* (Berk.) Casp is an economically important disease causing heavy losses in yield quantity and quality up to 60-75% (Mirakur *et al.,* 1978). This plant pathogen is distributed worldwide causing both early and severe defoliations and growth reduction of bulbs in field and under storage conditions (Surviliene *et al.,* 2008). Moreover, it has a devastating effect especially when weather conditions are cool and humid with long dew periods (Ryley, 1989). Without chemical application, an economic onion production would not be possible since the fungicides represent a powerful tool for controlling onion downy mildew (Whiteman and Beresford, 1998). Comprehensively, metalaxyl and cymoxanil proved to be the most effective in minimizing the disease severity less than 88% (Iqbal *et al.,* 2009). Furthermore, it was demonstrated that the mixture of metalaxyl and mancozeb was also effective in controlling the disease (Tahir *et al.*, 1990).

Humic substances (HS) composed of three fractions, humic acid (HA), fulvic acid (FA) and humin have been known as a plant growth stimulator through the increasing cell division, optimizing uptake of nutrients and water, as well as stimulating soil beneficial microorganisms (Chen *et al.,* 2004). Humic acids and FAs as well as, other humates supplemented into soil by organic amendment can influence either directly or indirectly on some physiological and biochemical processes occurring in plants and rhizosphere (Shusheng *et al.,* 2008). Along to their use as fertilizers, HAs and FAs are environmentally safe alternatives to synthetic fungicides and have a beneficial effect in controlling plant diseases (Kamel *et al.,* 2014). Efficacy of bio-fertilizers also has been proven to be effective against plant diseases such as; Fusarium wilt (Shusheng *et al.,* 2008). Kamel *et al.* (2014) stated that fulvic acid (FA) is rich in sulphur, which is considered a potential control agent against powdery and downy mildews of cucumber plants.

Nowadays, in Egypt, farmers adopting utility of (HS) in their commercial fields at a large scale, because of its beneficial effects on plant growth. So, there is an ingoing need for a scientific basis to explore its effects on soil and plant.

Therefore, the main objective of this investigation was to evaluate the effect of the foliar application of two forms of (HS) separately or in combinations with two fungicides against downy mildew, growth, photosynthetic capacity and metabolism of onion.

Materials and Methods

Plant materials:

Onion seeds cv. Giza-20 were obtained from the Onion Research Department, Field Crops Research Institute, ARC, Egypt. Field experiments were carried out during the seasons of 2016 and 2017 at Basyoun, Gharbiya Governorate, Egypt.

Humic substances (HS):

Compost manure was obtained from Agricultural Wastes Training Center, Moshtohor, Qalyubiya Governorate, Egypt as well as humate leonardite was obtained from commercially (Delta Bio Tec Company), Egypt. The main physical and chemical properties of the compost manure and humate leonardite were determined according to the standard methods described by Jackson (1973).

Extraction of (HS):

Extraction of (HS) from compost manure and humate leonardite was carried out following the method described by Sanchez *et al*. (2002).

Raising of nursery and transplantation of seedling:

Seeds of onion cv. Giza-20 were sown in nursery beds prepared from well-manure soil. Sixty days after sowing, healthy seedlings were transplanted in the field. Farmyard manure, super phosphate and ammonium sulphate were applied at the time of land preparation at the recommended dose rate, 200 kg and 100 kg/ feddan, respectively. Urea was applied 30-days after transplanting with a rate of 80 kg/feddan. The agricultural practices were applied as recommended by the Ministry of Agriculture and Land Reclamation, Egypt.

Experimental design:

Randomized complete block design (RCBD) with three replicates was followed to carry out these experiments. The experimental unit consisted of five rows with 2-m long. Each row contained 60 bulbstransplants with 70 cm width (2 ×3.5 m). The eight treatments Table 1 and two fungicides in Table 2 were carried out as soon as the first symptom of downy mildew on onion plants was observed. After that, these treatments were repeated 5 times, with 7 days intervals using the mentioned dose for each treatment (Table 1).

Table 1. Humate substances (HS) and fungicides and their doses used in the study

No.	Treatment	Dose
1	Humate leonardite	200 ppm
2	Humate plants	200 ppm
3	Ridomil Gold 68 WP	2.5 g/l
4	Folio Gold 53.75% SC	1 L/fedden(2.5ml/l)
5	Humateleonardite + Ridomil Gold 68 WP (50:50%)	100 ppm+ 1.25 g/l
6	Humate plants + Ridomil Gold 68 WP (50:50%)	100 ppm+ 1.25 g/l
7	Humate leonardite + Folio Gold 53.75% SC	100 ppm+ 1.25 ml/l
8	Humate plants + Folio Gold 53.75% SC	100 ppm+ 1.25 ml /l

Table 2. The fungicides, active ingredients and their doses used in this study

No.	Fungicide	Active ingredients	Recommended dose
1	Ridomil Gold 68 WP	Metalaxyl + Mancozeb	2.5g/l
2	Folio Gold 53.75% SC	Mefenoxam 3.75% + Chlorothalonil50%	2.5ml/l (1 L /feddan)

Disease assessment:

Disease severity (DS %) was recorded four times, with 7 days intervals, during each of the two successive growing seasons following the method adopted by Mohibullah (1992). The scale of the disease ranged from 1 - 9. The area under disease progress curve (AUDPC) for each treatment was calculating using the equation proposed by Pandey *et al*. (1989).

Assessmentof plant growth parameters and yield components:

Plant growth parameters, *i.e.* bulb diameter (cm), plant height (cm), dry weight (g) and dry matter (%) were measured. Bulb diameter (cm) was measured using a caliper at the maximum swollen part of the bulb. Plant height (cm) was also measured from the base of swelling sheath to the top of the longest tubular blades**.** Dry matter (DM %) was calculated using the formula of Nieuwhof *et al.* (1973): DM % = sample dry weight / sample fresh weight X 100

Additionally, yield components, *i.e.* number of total bulbs/plot and weight of total bulbs/plot (kg) were determined, during the two seasons under study.

Determination of biochemical compounds:

Total carbohydrates were determined in the aqueous extract according to Dubois *et al*. (1956). Total chlorophyll content was determined using the SPAD-501 portable leaf chlorophyll meter (Minolta Corp) for greenness measurements in the 5^{th} apical fully expanded leaf (Yadava 1986). Total nitrogen percentage was estimated according to Jackson (1973). Total soluble solids (TSS %) were determined using a Carlzeiss hand Refractometer according to Anonymous (1975).

Enzymatic activities:

Prophylactically treated onion plants were sampled and analyzed for the activity of peroxidase and polyphenoloxidase, as indicators of fungal activities. The activity of peroxidase was measured colorimetrically based on the oxidation of pyrogallol to pyrogalline and using H_2O_2 at 425 nm (Thimmaiah 1999). Polyphenoloxidase activity was determined using colorimetric method of Maxwell and Bateman (1967).

Statistical analysis:

Analysis of variance (ANOVA) of the data was performed using WASP software. The least significant difference (LSD) at 5% level of significant was used to compare treatment means. Descriptive statistics and PCA of plant growth parameters, yield components, biochemical compounds and enzyme activities were performed using statistical product and service solutions (SPSS 22). The used technique of PCA was factor-loading matrix extracted from a Varimax rotation with Kaiser Normalization of main components.

Results

The effect of (HS) and fungicides (individually or in combinations) on the downy mildew of onion:

Final disease severity (FDS%) and area under disease progress curve (AUDPC) were used to assess disease development on treated and untreated onion plants. The treatment of (HS)-leonardite combined with the commercial fungicide (Ridomil Gold 68 WP, 50:50%) was significantly effective in controlling downy mildew compared with the check. This treatment revealed a significant decrease in FDS (%) and AUDPC, in the pooled data of seasons 2016 and 2017 with values; 18.3 and 273.8, respectively (Fig.1), compared to the control treatment, which displayed the highest values of FDS (73.2%) and AUDPC (1200.00), in the pooled data of the two seasons, the remaining treatments significantly reduced FDS (%) and AUDPC compared to the control treatment but with less order.

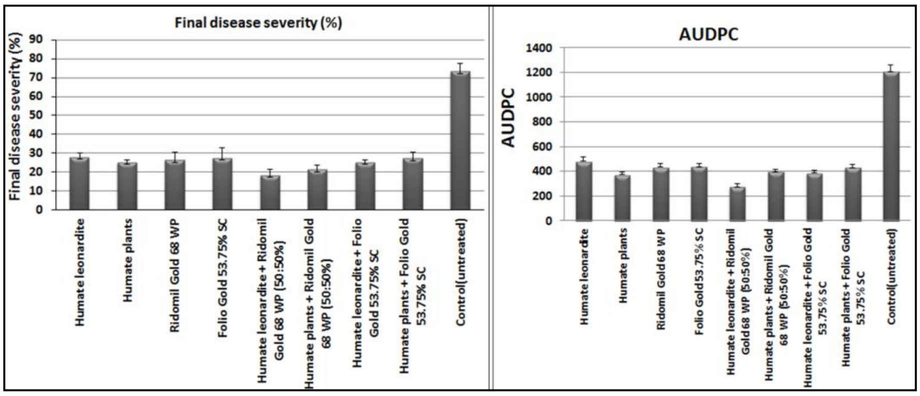

Fig. 1. Effect of two (HS) and two fungicides (individually or in combinations) on downy mildew, expressed as FDS (%) and AUDPC in the pooled data of seasons 2016 and 2017.

The effect of (HS) and fungicides (individually or in combinations) on the growth and yield quantity of onion plants:

The obtained results indicated that foliar application of all the tested (HS) and fungicides, either separately or mixed could potentially improve bulb diameter (cm), plant height (cm), dry weight (g) and dry matter (%) of the treated onion plants in relation to untreated plants (control) (Fig. 2). The application of the two forms of (HS) alone or in combination with the two synthetic fungicides revealed a significant increase in bulb diameter (11.4 to 14.1 cm) relative to control plants (7.05 cm), in pooled data of seasons 2016 and 2017. Likewise, the application of (HS)-plants combined with fungicide Folio Gold and Ridomil Gold significantly increased plant height, being 66.2 and 63.7 cm, respectively in comparison with the control treatment (25.2 cm). The plants treated with (HS)-leonardite mixed with Ridomil Gold revealed also a significant increase in dry weight (4.3g) compared to the control plants (1.2 g) in the pooled data of seasons 2016 and 2017. Regarding the dry matter, it was clear that all treatments increased the dry matter from 5.04 to 6.6 % in relation to the control treatment (4.27 %).

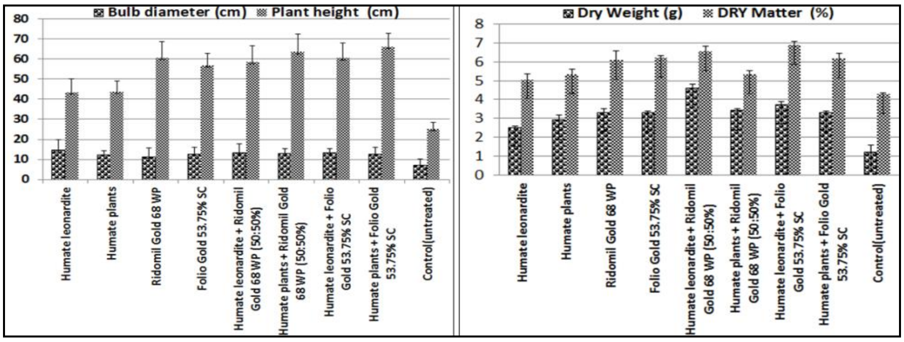

Fig.2. Effect of two (HS) and two fungicides (individually or in combinations) on bulb diameter, plant height, dry weight and dry matterof onion plants under field conditions in the pooled data of seasons 2016 and 2017.

Also, the differences between any of the tested treatments concerning the total number of bulbs and weight bulbs/plot of onion plants throughout the means of 2016 and 2017 were not significant, but there were significant differences between treatments and the control (Fig. 3).

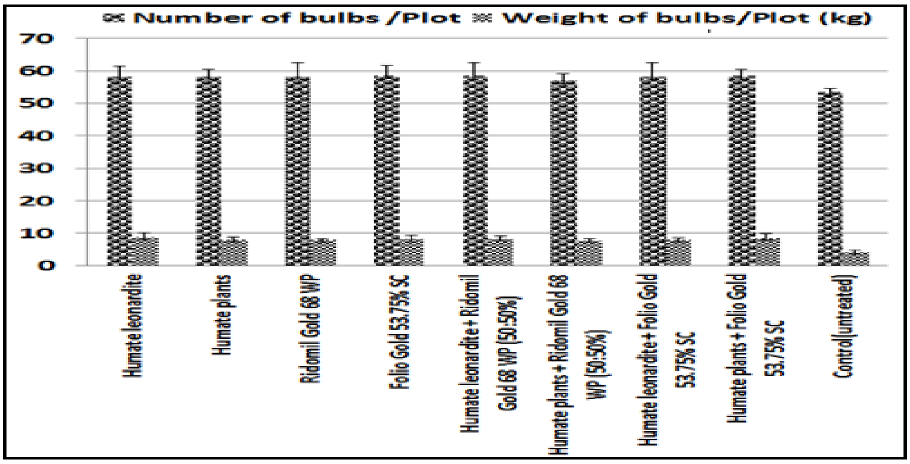

Fig. 3. Effect of two (HS) and two fungicides (individually or incombination) on number of bulbs/plot and weight bulbs/plot of onion plants grown under field conditions in the pooled data of seasons 2016 and 2017.

Effect of (HS) and fungicides (individually or combined) on some biochemical compounds of onion plants:

Data presented in Table 3 explain that application of (HS) individually or with fungicides in the pooled data of seasons 2016 and 2017, significantly increased the biochemical compounds of treated onion plants, *i.e.* total carbohydrates, total nitrogen (%), total soluble solids (TSS %) and total chlorophyll in relation to control. However, based on the statistical analysis there were no significant differences among most of the treatments but still showing significant differences than the control. Almost all treatments increased the total carbohydrates.The plants treated with Ridomil Gold individually or in combination with (HS)-plants exhibited the higher values in total carbohydrates. It was clear that plants treated with (HS)-plants combined with Ridomil Gold displayed a significant increment in total nitrogen and TSS % in themean of seasons. Whereas, the highest value in total chlorophyll was obtained in plants treated with (HS)-plants combined with Ridomil Gold fungicide.

Table 3. Effect of two (HS) and two synthetic fungicides (individually or incombination) on some biochemical compounds of onion plants, grown under field conditions in the pooled data of seasons 2016 and 2017

Treatment	Total carbohydrates (mg/g dry weight)	Total nitrogen (%)	Total soluble solids (TSS) (%)	Total Chlorophyll (SPAD)
Humateleonardite	1.54	12.32	12.64	12.48
Humate plants	1.40	13.45	13.35	13.29
Ridomil Gold 68 WP	1.63	13.21	13.37	13.40
Folio Gold 53.75% SC	1.35	12.93	13.08	13.01
Humateleonardite + Ridomil Gold 68 WP (50:50%)	1.42	12.66	13.06	12.86
Humate plants + Ridomil Gold 68 WP (50:50%)	1.68	13.55	14.16	13.85
Humateleonardite + Folio Gold 53.75% SC	1.37	12.75	13.65	13.20
Humate plants + Folio Gold 53.75% SC	1.47	12.34	13.06	12.70
Control (untreated)	0.15	8.12	8.61	8.36
$L.S.D_{0.05}$	0.21	0.37	0.70	0.78

Effect of (HS) and fungicides (individually or in combination) on the activity of antioxidant enzymes:

Data presented in Table 4 reveal in general that foliar application of (HS) and either fungicide individually or in combination caused an increment in the activity of peroxidase and polyphenoloxidase enzymes in the treated onion plants compared to untreated (control) plants. Onion plants treated with (H)-leonardite, displayed the highest enzymatic activity of peroxidase that reached to 0.323 mg/ml. In contrast, untreated (control) displayed the lowest enzymatic activity of peroxidase, *i.e.* 0.067 in the pooled data of seasons 2016 and 2017. On the other hand, the plants treated with the fungicide Ridomil Gold (0.046) exhibited the highest activity of polyphenoloxidase compared to the control onion plants (0.004).

Table 4. Effect of two (HS) and two synthetic fungicides (individually or in combination) on the enzymatic activity of peroxidase and polyphenoloxidase in onion plants, under field conditions inthe pooled data of seasons 2016 and 2017

Treatment	Enzymatic activity	
	Peroxidase (mg/ml)	Polyphenoloxidase (mg/ml)
Humate leonardite	0.323	0.036
Humate plants	0.053	0.006
Ridomil Gold 68 WP	0.073	0.046
Folio Gold 53.75% SC	0.196	0.016
Humate leonardite + Ridomil Gold 68 WP (50:50%)	0.116	0.013
Humate plants + Ridomil Gold 68 WP (50:50%)	0.143	0.029
Humate leonardite + Folio Gold 53.75% SC	0.143	0.003
Humate plants + Folio Gold 53.75% SC	0.103	0.013
Control(sprayed with water)	0.067	0.013
$L.S.D_{0.05}$	0.033	0.004

Studied characteristics of onion plants against downy mildew using principal component analysis (PCA):

Principal component analysis was performed on disease parameters, plant growth parameters, yield components, biochemical compounds and enzymatic activities in response to onion downy mildew (Figs. 4A and B). Interpretation of the principal components (PCs) was aided by inspection of the factor-loading matrix extracted from a Varimax rotation with Kaiser Normalization of main components to identify factors responsible for the grouping of the dataset. As shown from scree plot graph of eigen values and loadings plot (Figs. 4A and B), the first twocomponents, *i.e.* FDS (%) and AUDPC have eigen values more than one. Therefore, the two components were extracted describing approximately 93.99% in the pooled data of 2016 and 2017 seasons. Accordingly, principal component analysis gave evidence to the importance of the disease parameters, especially FDS (%), as it was considered a good and more reliable indicator for evaluation of these materials under study.

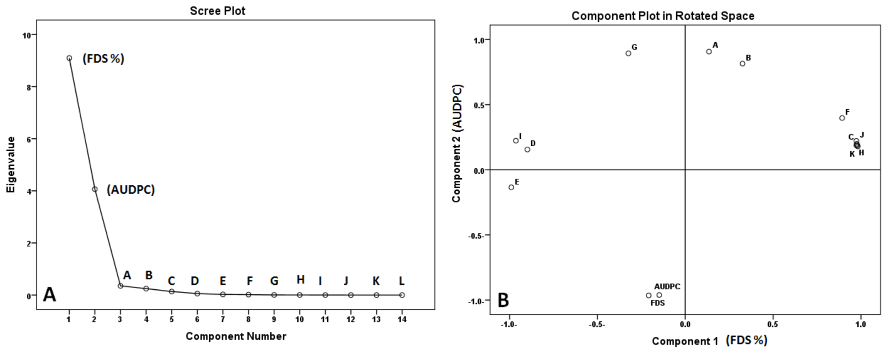

Fig. 4. The scree plot of eigenvalue (A) and loadings plot (B) for the component number of disease parameters, plant growth parameters, yield components, biochemical compounds and enzymatic activities in response to onion downy mildew in the pooled data of 2016 and 2017 growing seasons.

Details: FDS (%), AUDPC, A: Bulb diameter, B: Plant height, C: Dry weight, D: Dry matter, E: Number of total bulbs, F: Weight of total bulbs, G: Total carbohydrates, H: Total chlorophyll, I: Total nitrogen, J:. Total soluble solids (TSS).K: Peroxidase and L: Polyphenoloxidase.

Discussion

Humic acid is a suspension that can be applied successfully in many areas of plant production as stimulant of resistance of many host plants against diseases and pests (Scheuerell and Mahaffee 2004). Our results supported by similar findings obtained in previously conducted studies stated the potential use of (HS) in controlling plant diseases. For example; the study of Hahlbrock and Scheel (1989), revealed that fulvic acid (FA) which is a fractions of humic substances (HS) mostly consists of a mixture of phenolic compounds which play a major role in plant defense mechanism. Likewise, Kamel *et al*. (2014) stated that (FA) is rich in sulphur, which is considered a potential control agent against powdery and downy mildews. Zhang (1997) found that the foliar application of (FA) enhanced the production of some enzymatic and non-enzymatic antioxidants such as α-tocopherol, α-carotene, superoxide dismutase and ascorbic acid in turf grass species.

The *in vitro* study of Moliszewska and Pisarek (1996) displayed that (FA) and (HA) potentially suppressed *Alternaria alternata* and *Fusarium culmorum* in PDA medium. The previous studies also emphasized that (HA) could potentially inhibit mycelial growth and spore germinations of different plant pathogenic fungi, *i.e. Pythium ultimum*, *Fusarium culmorum*, *Alternaria alternata* and *F. oxysporum* (Loffredo *et al*., 2007). They attributed this suppressive effect to the presence of some fungitoxic compounds and functional properties, especially $COOH^-$ group content and elemental composition, but the physiological mechanism has not been well established.

Furthermore, the *in vitro* study of Loffredo *et al*. (2007) displayed that (HS) significantly reduced the radial growth and spore germination of *Fusarium oxysporum* f. sp. *melonis* and *Fusarium oxysporum* f. sp. *lycopersici.* Schiavon *et al*., (2010) showed that the treatment with (HS) enhanced the expression of the phenylalanine (tyrosine) ammonia-lyase (PAL/TAL) that catalyzes the first committed step in the biosynthesis of phenolics, by converting phenylalanine to trans-cinnamic acid and tyrosine to p-coumaric acid. The authors concluded that the stimulatory effects of (HS) on plant secondary metabolism provide an innovative approach to explore plant responses to stress.

Results presented herein revealed that the foliar application of all humic substances (HS) forms combined with the two fungicides significantly improved consistently bulb diameter, plant height, dry weight and dry matter compared to the control plants. These findings are coincided with those of Malik and Azam (1985) who found that the foliar spray with (HA) increased root length and leaf area index of wheat (Figliolia *et al*., 1994). Also, Tan and Nopamornbodi (1979) indicated that (HA) was beneficial to shoot and root growth of corn plants. This hypothesis appears to be confirmed by the findings of O'Donnell (1973) who found that (HA) from leonardite exhibits auxin-like effects. Similarly, Piccolo *et al.* (1992) confirmed our suggestion that humic acid could be used as a growth regulator to regulate hormone level, improve plant growth and enhance stress tolerance. Moreover, Singaroval *et al*. (1993) claimed that the increase in dry matter production with (HA) might be due to its direct action on plant growth auxin activity, contributing to increase in the dry matter.

More recently, numerous experimental data have shown that (HS) and its different fractions may affect plant growth and development, involving specific structural and physiological responses to (HS) applications (Canellas and Olivares 2014). El-Ghamry *et al*. (2009) reported that all morphological characteristics; yield components, macronutrients content as well as chlorophyll content of faba bean were significantly increased by foliar application of (HA). The enhancement of Nitrogen uptake/assimilation and N metabolism in plants treated with (HS) had been well documented. Farnia and Moradi (2015) stated that the application of (HA) increased the TSS % in tomato plants under heat stress.

The most described effect of (HS) is the promotion of plant root system. However, plant metabolisms, diverse and complex enzymatic machinery related with a plethora of cell processes and more recent changes on secondary metabolism by (HS) are being increasingly documented. Muscolo *et al.* (1993) reported that the treatment of *Nicotiana plumbaginifolia* with humic extracts increased slightly the peroxidise activity. In a similar study, Manas *et al*. (2014) found that the foliar application of (HA) increased significantly the polyphenol oxidase of pungent pepper**.** Furthermore, Lotfi *et al*. (2015) found also that the foliar application of (FA) improved the peroxidase activity in rapeseed plants. Nardi *et al*. (2007) showed that (HS) affected the enzyme activities glucokinase, phosphor glucose isomerase, PPi-dependent phosphorfructokinase and pyruvate kinase related to glycolysis and the tricarboxylic acid cycle (TCA). Conversely, these enzymes activities were not investigated in the current study. Summing up the results, the factor-loading matrix, extracted from a Varimax rotation with Kaiser normalization gave clear evidence to the importance of all parameters in this study, especially FDS (%).

As a conclusion, all treatments under study minimized FDS (%) and AUDPC as well as improved onion growth parameters compared to control treatment, during the two growing seasons. The most effective treatment was (HS)-leonardite combined with fungicide Ridomil Gold 68% (WP).

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تأثير المواد الدوبالية (الهيومية) والمبيدات الفطريـة علي مكافحة مرض البياض الزغبي في البصل

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يُعتبر مرض البياض الزغبي في البصل المتسبب عن الفطر *Peronospora destructor* ، أحد أهم أمراض البصل والتي تتم مكافحتة بالمبيدات الفطرية. ويهدف هذا البحث إلى تقييم تأثير شكلين من المواد الدوباليه (الهيومية) علي المجموع الخضري سواء منفصلة اومخلوطة مع مبيدين فطريين ضد مرض البياض الزغبي وتأثيرها كذلك علي النمو والقدرة على التمثيل الضوئي والإنتاج الأيضي في البصل. أجريت التجارب خلال موسمي ٢٠١٦ و ٢٠١٧ وتم أخذمقياسين وبائيينللمرض وهي النسبة المئوية لشدة المرض (FDS%) والمساحة الواقعة تحت منحني الإصابة المرضي (AUDPC) لتقييم شكلين للهيومات (HS) ، مبيدين فطريين بشكل منفصل أو مخلوطة ضد البياض الزغبي في البصل. حققت كل المعاملات تحت الدراسة خفضاً في النسبة المئوية لشدة المرض (FDS%) والمساحة الواقعة تحت منحنى الإصابة المرضي (AUDPC) مقارنة بمعاملة الكنترول خلال موسمي الدراسة. وكان أكثر المعاملات تأثيراً هي هيومات الليونارديت مخلوطة مع مبيد ريدوميل جولد ٦٨ % (WP) والتي أظهرت خفضا معنويا فيالقياسات الوبائية السابق ذكرها مقارنة بباقي المعاملات والكنترول. أيضا كل المعاملات حسنت من مقاييس نمو نباتات البصل مثل قطر البصلة ، ارتفاع النبات ، وزن النبات الجاف ، وزن المادة الجافة ، متوسط المحصول مقارنة بالنباتات الغير معاملة. وكذلك تم قياس التغيرات الكيميائية مثل النشاط الانزيمي ، الكربوهيدرات الكلية ، نسبة النيتروجين الكلي ، نسبة المواد الصلبة الكلية (TSS%) ولوحظت فروق معنوية بين المعاملات والكنترول. وبتحليل مصفوفة الإرتباط بين كل من المقاييس تحت الدراسة فقد أتضح أهمية تلك المقاييس وخاصة النسبة المئوية لشدة المرض النهائية (FDS%).