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Effect of some fungicides alternatives on reducing disease severity of Stemphylium Blight in onion

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ABSTRACT

Onion production suffered from decreasing in productivity due to some fungal diseases during growing season. One of the most destructive diseases exposed onion is Stemphylium leaf blight (SLB) caused by the fungus *Stemphylium vesicarium* (Wallr.) Simmons, (teleomorph: Pleospora allii. (Rabenh.) Ces. and de Not). It had wide host range and wide range of variability in disease symptoms expression depending on cultivars, environmental conditions etc., now a days the high cost of using fungicides and fungal strains that are resistant to the effect of fungicides, which were formed as a result of the excessive use of pesticides and the environmental pollution associated with the use of pesticides, Among the alternatives of fungicides are the inducers of plant systemic acquired resistance (SAR) and mineral salts. In this work, the effect of Salicylic acid (SA), Potassium phosphate dibasic (PD) as inducers for (SAR), Sodium bicarbonate (SBC), Potassium carbonate (PC), Sodium carbonate (SC) salts was studied on reducing the incidence and severity of natural infection of SLB with improving vegetative growth of onion. The results showed that (SA) followed by (PD) gave significant differences in reducing both of natural disease incidence and disease severity for Stemphylium leaf blight, and increasing vegetative growth of onion in comparison with control, also both SA and PD increased activity of peroxidase and catalase enzymes in treated plants. All used salts (SBC, PC, and SC) significantly reduced both disease incidence and disease severity, increasing vegetative growth of onion plants and increase enzymes activity in comparison with control. So it can concluded that using the above treatments will be economic and ecofriendly alternatives in Stemphylium blight disease management.

Keywords: sodium bicarbonate, potassium carbonate, peroxide, catalase, biotic stress.

1. Introduction

Onion (*Allium cepa* L.) is one of the most valuable *Allium* crops, with a total world production of approximately 125 million tonnes in 2019 (FAOSTAT, 2020). Onion is one of the main important and oldest vegetable crops grown in Egypt. The Egyptian onion is famous all over the world for its superior quality and early appearance in European markets. Onion although primarily is grown for food, it is also used as traditional medicine (Hussein, *et al.,* 2007). Onion bulbs are rich source of minerals like phosphorus, calcium and carbohydrates besides being rich in proteins and vitamin C. Onion contains chemical compounds with potential anti-inflammatory, anti-cholesterol and anti-cancer properties (Slimestad *et al.,* 2007). During the last years the international cultivated area with onion profoundly has been decreased because of the serious damage of the crop yield due to infection by several diseases that attack onion during the growing season (Schwartz and Mohan, 1996). Stemphylium leaf blight caused by *Stemphylium vesicarium* (Wallr.) Simmons, (teleomorph: *Pleospora allii*. (Rabenh.) Ces. and de Not) is one of the most destructive fungal diseases in Allium species. The host range of the pathogen varies among different crops such as garlic (Suheri and Price, 2000), chilli pepper, (Vitale *et al.,* 2017), leek, (Suheri and Price 2000), asparagus (Foster, 2018), pear (Llorente and Montesinos, 2006 and Köhl *et al.,* 2008). Hassan *et al.* (2020) reported that Stemphylium blight caused by *Stemphylium vesicarium* is the most destructive disease of onion crop and poses a grave threat to the very existence of its cultivation in Kashmir. Wright *et al.,* (2019) indicated that the outbreak of onion leaf blight happened in New Zealand was caused by *Stemphylium vesicarium* and it may be that warm,

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and wet summer possibly contributed to this outbreak. Hassan, *et al.* (2007) isolated *Stemphylium vesicarium* for first time in Egypt. Abo-Elyousr *et al.* (2017) reported that in Egypt, Stemphylium blight is one of the most important disease attacked onion plant and sometimes causes 100% loss especially in seed production. Gedefaw *et al.* (2019) reported for a first time *Stemphylium vesicarium* as causal agent for *Stemphylium* leaf blight in Ethiopia. The disease incidence was up to 95.2% and severity up to 4.67 where in some fields it has been causing early plant senescence and reduced bulb size. The disease is characterized by appearance of small yellow to orange streaks which soon develop into elongated, spindle shaped to ovate elongated diffused spots surrounded by pinkish margins, oval lesions that turned brown as they expanded eventually coalescing and killing affected leaves. The disease can cause severe damage, especially to the onion seed crop and losses of about 80-85% on the crop by affecting leaves and seed stalk leaves affected by *Stemphylium* blight (Tomaz and Lima, 1988; Shishkoff and Lorbeer 1989). Under natural epiphytotics, *S. vesicarium* has been found to express a wide range of variability in disease symptoms expression depending upon the onion cultivars, environmental conditions etc. as observed by various workers (Hosen *et al.,* 2009; Arzanlou *et al.,* 2012; Nisha, 2013). According to Thind (2001) disease initiated as small, yellow to pale orange flecks which develop in the middle of leaf and soon became elongated, spindle shaped to ovate spots surrounded by characteristic margins. Later these spores turn grey at centre with the development of conidiophores and conidia and giving the leaves a brightened appearance. Sharma and Sharma (1999) reported that infection caused by Stemphylium vesicarium occurred on the leaves and inflorescence stalks of onion in the form of small, light yellow to brown water-soaked streaks in the middle of leaves which soon became spindle shaped to ovate-elongate.

Salicylic acid (SA) has been recently included in the category of plant hormones for proper plant growth and tolerance to both biotic and abiotic stresses, the accumulation of SA within the plant is an important defensive signal in plants that is essential for inducing systemic acquired resistance (SAR) (Carr *et al.,* 2010). Thus, SA is an endogenous growth regulator of a phenolic nature, which is involved in the regulation of several physiological processes in crop plants such as stomata closure, ion uptake, inhibition of ethylene biosynthesis and transpiration, seed germination, yield, glycolysis, and flowering, (Amin *et al.,* 2007 and Khan *et al.,* 2007). Aldesuquy *et al.* (2015) reported that the applied of salicylic acid induced additional increase in activity of defense enzymes (i.e., peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase) in infected faba bean plants with *Botrytis fabae*, and the most effective treatment in enhancement faba bean resistance was salicylic acid. Onion plants treated by Salicylic Acid (SA) and inoculated with *Stemphylium vesicarium* showed significantly higher peroxidase, poly phenoloxidase, SA content and phenolic content than inoculated water-treated plants (Abo-Elyousr *et al.,* 2009). Spraying salicylic acid caused significant increase in most growth characters, photosynthetic pigments content/ leaves, yield and its quality, total soluble sugars, total free amino acids, total phenols and total indoles. Amin *et al.,* (2007) and Bhasker *et al.,* (2020), revealed that spraying onion plants with Salicylic acid (SA), performed superior in terms of growth, development, yield and Stemphylium blight disease incidence and intensity. SA partially involved in post-harvest management of onion. Monopotassium phosphate (KH_2PO_4) and dipotassium phosphate (K_2HPO_4) are salt compounds that are used as a food additive. KH_2PO_4 and K_2HPO_4 have been approved as generally recognized as safe (GRAS) for use in human food by the U.S. Food and Drug Administration. Potassium phosphates are used as fertilizer and the principal source of phosphorus for plants in agriculture, but in some cases, when used as a foliar spray, disease resistance of plants is improved. In this regards, many investigations reported the use of potassium salts as a chemical agent for induction of plant resistance (Stromberg, and Brishammar, 1991; Yurina *et al.,* 1993). Hussein *et al.* (2007) reported that all resistance inducers i.e. Bion, K_2HPO_4 and salicylic acid) treatments resulted in significant reduction of disease severity. Kamal *et al.* (2008) concluded that application of simple non-toxic chemical solutions as di-potassium phosphate and Bion can control Stemphylium leaf blight of onion. EL-Ashmony (2021) revealed that potassium bicarbonate ($KHCO_3$) had significant inhibitory effects on growth parameters of Sclerotium rolfsii or in sunflower root/collar. Inhibitory effects were increased by enhancing $KHCO_3$ concentration. Carbonic acid salts, such as sodium carbonate (Na_2CO_3, soda ash) and sodium bicarbonate ($NaHCO_3$, baking soda), are common food additives allowed with no restrictions for many applications by European and North American regulations (Lindsay, 1985). Sodium bicarbonate is classified as generally recognized as safe by the United States Food and Drug Administration and also proposed exempt from residue tolerances on all agricultural commodities by

the United States Environmental Protection Agency. Both sodium carbonate and sodium bicarbonate were listed as approved ingredients on products labeled "organic" as proposed by the United States Department of Agriculture. The antimicrobial activity of these compounds has been described in vitro (Corral *et al.,* 1988), first described for sodium bicarbonate to control postharvest green and blue molds of citrus was by Barger in 1928 (Barger, 1928). Both salts can be useful tools to manage postharvest decay because they are inexpensive, readily available, and can be used with a minimal risk of injury to the fruit (Smilanick *et al.,* 1999). The most effective chemicals used to control lettuce foliar diseases were sodium carbonate, ammonium sulfate, calcium chloride and potassium dibasic phosphate that decreased leaf spot severity. Sodium carbonate had a higher inhibiting activity against foliar diseases of lettuces. Muhanna and Elwan (2020). Chi and Anh (2019) found that sodium bicarbonate, and apple cider vinegar (ACV) completely inhibited the growth of *Colletotrichum musae*, causal agent of postharvest anthracnose of Banana fruits *in vitro* but they failed to control the anthracnose disease development on treated fruits compared to untreated fruits (control) *in vivo*.

The aim of this study evaluation of Salicylic acid (SA), Potassium phosphate dibasic (PD) as inducers for (SAR), Sodium bicarbonate (SBC), Potassium carbonate (PC), Sodium carbonate (SC) salts was studied on reducing the incidence and severity of natural infection of Stemphylium leaf blight with improving vegetative growth of onion.

2. Materials and Methods

2.1. Effect of SAR inducers and salts on Stemphylium leaf blight disease under field conditions

Onion is a winter vegetable crop. The planting dates differ according to the cultivation area. This research was conducted in the Beni-Suef governorate. The planting was carried out with seedlings of the Giza white variety, and the planting date was in the middle of October for two growing seasons 2020/2021 and 2021/2022, with all the recommended agricultural practices. The seedlings were planted around the drip irrigation line 10cm. between the seedlings. The experiment was conducted to study the effect of some fungicides alternatives to reduce the incidence and severity of natural infection of *Stemphylium* blight. The experiment was designed with five spray treatments (1- Salicylic acid (SA) $C_7H_6O_3$; 2- Potassium phosphate dibasic (PD) K_2HPO_4; 3- Sodium bicarbonate (SBC) $NaHCO_3$; 4- Potassium carbonate (PC) K_2CO_3 ; 5- Sodium carbonate (SC) Na_2CO_3). Spraying was carried out with a concentration of 0.5g/liter of water, for all materials and each treatment was applied in 3 replicates, in addition to the control treatment in which no spraying materials were used. All materials used in the spray were purchased from Al-Gomhoria Company for medicines and medical supplies. The treatments were sprayed 4 times, the first treatment was after 30 days of transferring the seedlings to the field then every 15 days, and the infection rate was estimated as soon as the symptoms of infection appeared, the ratio of infection was estimated by calculating the number of infected plants in relation to the total number of plants grown in each treatment, and the severity of the infection is also estimated based on the scale by Mishra and Singh (2019) as follow:

0 No disease symptom
1 A few spots towards tip covering 10 percent leaf area
2 Several purplish brown patches covering up to 20 percent of leaf area
3 Several patches with paler outer zone covering up to 40 percent leaf area
4 Leaf streaks covering up to 75 percent leaf area or breaking of the leaves from center
5 Complete drying of the leaves or breaking of leaves from center

The plants were uprooted three days after the last spray. To find out the effect of spraying with treatments on the rate of plant growth and the percentage of some components inside the plant that are affected by infection or related to the plant's resistance to infection. Measurements of vegetative growth (Plant height, leaf length, bulb length, plant weight, leaf weight, bulb weight, number of leaves/plant), the percentage of peroxidase and catalase enzymes activity in leaves were measured.

2.2. Enzyme assays

2.2.1. Peroxidase (PO)

Assay (based on the oxidation of pyrogallol to purpurogallin in the presence of H2O2) was determined according to the method described by Allam and Hollis (1972). The reaction mixture contained 0.5 mL of 0.1 M potassium phosphate buffer solution at pH 7.0;0.3 mL enzyme extract; 0.3 mL 0.05M pyrogallol and 0.1 ml 1.0% H2O2. The mixture was completed with distilled water up to 3 ml. Enzyme extract was replaced by distilled water in the control blank cuvette. The absorbance of 1 mL was recorded, and peroxidase activity was expressed as the change in absorbance at 425nm /15 minute/gram fresh weight.

2.2.2. Catalase (CAT)

Activity is measured spectrophotometrically using the method with ammonium molybdate (Goth 1991). The reaction mixture consisted of 65 mM H2O2, phosphate buffer, and enzyme extract was incubated at 37 ◦C for 1 min. By adding the 32.4 Mm, ammonium molybdate, the reaction was stopped and the complex of molybdate and H2O2 was measured against blank at 405 nm. One unit of CAT is the amount of enzyme that decomposes 1 μM of H2O2 per minute; CAT activity is expressed as U mg−1of protein.

2.3. Data analyses

The experimental data was statistically analyzed by costat version 2.

3. Results and Discussion

3.1. Effect of SAR inducers and salts on diseases incidence and severity of Onion

Stemphylium blight caused by *S. vesicarium* is one of the most destructive fungal diseases in allium species due to its host range among different crops and the wide range of variability in disease symptoms expression depending upon cultivars, and environmental conditions. To evaluate the effect of foliar spraying with treatments on disease incidence and severity.

Data in table (1) showed significant differences between treatments in reducing both diseases incidence (DI) and diseases severity (DS) in compared with control, the natural infection in S2 (58 DI and 3.7 DS) was higher than S1 (49 DI and 3 DS), while results of the treatments were better in second season than the first in reducing both DI and DS. Salicylic acid (SA) was the superior treatment in reducing DI (23, 21) and DS (1 and 1.3) in both seasons respectively, followed with Potassium Phosphate Dibasic (PD; DI; 27, 25) and DS (1.3 and 1.7) for S1 and S2. (PC) and (SC) respectively gave moderate effect in decreasing both DI and DS in the two seasons, while (SBE) resulted the lowest effect in reducing DI and DS with significant differences with control DI (42.7,39), DS (2,2.3) in both growing seasons.

Table 1: Effect of spraying with (SA), (PD), (SBE), (PC), and (SC), on (DI) Percentage and (DS)

Treatment	DI %		DS	
	S1	S2	S1	S2
Salicylic acid	23	21	1	1.3
Potassium phosphate dibasic	27	25	1.3	1.7
Potassium carbonate	30.3	28	1.7	2
Sodium bicarbonate	42.7	39	2.3	2.7
Sodium carbonate	32	31	2	2.3
control	49	58	3	3.7
LSD 5%	2.02	1.8	0.64	0.98

3.2. Effect of SAR inducers and salts on vegetative growth of Onion

The results in Table 2 indicate that the treatments had a significant effect on the vegetative growth rates of plants, and the differences were significant between the treatments and the untreated control.

Salicylic acid was the most effective in increasing all growth rates than the control, followed by the treatment with Potassium phosphate dibasic, then potassium carbonate and sodium carbonate, also the treatment with sodium bicarbonate was the lowest effective treatment on growth rates relative to the treatment. It was noticed that no difference between the treatment with Potassium phosphate and potassium carbonate in the plants height (plant height 77.8 cm, leaf height 66.8 cm, and bulb height 10.67 cm) for PD while the results recoded (plant height 76 cm, leaf height 66.3 cm and bulb height 10 cm) also the obtained results showed no significant differences between the treatment with Potassium carbonate and Sodium bicarbonate in bulb height (10 cm and 9.33 cm) also between Sodium bicarbonate and Sodium carbonate in leaf weight (61.67 cm and 62 cm).

Table 2: Effect of spraying with SA, PD, SBE, PC and SC, on vegetative growth of onion plants as average of combined data over two seasons.

Vegetative growth Treatment	Total weight (g)	Leaf weight (g)	Bulb weight (g)	Leaf no.	Total plant height (cm)	Leaf height (cm)	Bulb height (cm)
Salicylic acid	117	77	40	11.7	78.5	67	11.3
Potassium phosphate dibasic	112	75	36	10.7	77.8	66.8	10.7
Potassium carbonate	96	67	28.8	9.3	76	66.3	10
Sodium bicarbonate	86. 3	61.7	22.7	7.3	68.5	59.2	9.3
Sodium carbonate	93	62	31	8.3	74	65.7	8.3
Control	74	51.7	23	5.7	62	56	6
LSD 5%	1.8	0.9	2.9	0.9	1.6	1.7	1

Field experiments were conducted to study the effect of some alternatives to fungicides where the obtained results showed noticeable superiority of these alternatives in reducing the severity and incidence of natural infection of Stemphylium blight and significant increasing in vegetative growth for untreated plants, which gives an advantage in use over fungicides. Where the results of using Salicylic acid showed significantly differed than control and the other treatments in reducing the incidence and severity of infection, as well as an increase in vegetative growth, these effects may be due to it is recently included in the category of plant growth hormones, as an important signal molecule belonging to an extra ordinary diverse group of plant phenolic compound synthesized under different biotic stresses, on other hand (SA) is distributed in wide range of plant species and act as a defensive signal that is essential for elicitor triggered immunity and the establishment of Systematic Acquired Resistance (SAR) by inducing particular enzymes involved in biosynthetic reactions (Vlot *et al.,* 2009; Bideshki and Arvin, 2010; Carr *et al.,* 2010), while Koo *et al.* (2020) found that, salicylic acid (SA) indeed is a key plant hormone regulating plant immunity. In addition, SA can regulate many different responses, such as tolerance to abiotic stress, plant growth and development, and soil microbiome. The data in combatable with Hussein *et al.,* (2007) where they reported that, salicylic acid treatments resulted in significant reduction of Stemphylium blight severity. Also Bhasker *et al.* (2020) revealed that, spraying onion plants with Salicylic acid performed superior in terms of growth, development, yield and Stemphylium blight disease incidence and intensity, in the same line, Aldesuquy *et al.* (2015) reported that salicylic acid was the most effective treatment in enhancement faba bean resistance to *Botrytis fabae.* As explained by Mohamed, and Amer, (2014) that, soaking seeds in salicylic acid significantly decreased both damping-off and root rot of squash and cantaloupe.

Phosphates are used as fertilizer and it is the principal source of phosphorus for plants in agriculture, but in some cases, when used as a foliar spray, disease resistance of plants was improved. In this study Potassium phosphate dibasic (K_2HPO_4) gave good results in decreasing both DI and DS of Stemphylium blight and increasing all vegetative growth in comparing with other treatments and control, this results in agree with Muhanna and Elwan, (2020) where they reported that, Potassium dibasic phosphate inhibited mycelial growth of *Alternaria alternate*, *Helminthosporium sp*., *Stemphylium botryosum*, and *Curvularia lunata* isolated from lettuce to less extent. Also potassium dibasic phosphate decreased severity of lettuces leaf spot. Arslan (2015) was evaluated antifungal activity of monopotassium phosphate (KH_2PO_4) and dipotassium phosphate (K_2HPO_4) as possible

alternatives to synthetic fungicides for control phytopathogenic fungi. The percentage of mycelial growth inhibition ranged from 0 to 100% for K_2HPO_4, The obtained data suggested that KH_2PO_4 and K_2HPO_4 should be used as a protective fungicide, and could become natural alternatives to synthetic fungicides for control of phytopathogenic fungi. In the same trend, using Carbonate and bicarbonate salts in this study significantly decreasing both DI and DS of natural infection with Stemphylium blight in comparison with control and increasing vegetative growth than control plants. These results harmonious with (Zaman *et al.,* 2019) who suggested that, potassium, sodium and calcium salts have significant effects on yield and quality of many vegetables and fruits parallel with its effects as an alternative of fungicides. Also Gomaa *et al.,* (2021) who found that, calcium carbonate, potassium and sodium bicarbonate salts increased the vegetative growth characters on onion plants. Abd-El-Kareem (2007) indicated that, the most effective treatments were potassium or sodium bicarbonate which reduced the early blight incidence in potato plants under green house and field conditions on other hand Chitinase activity was increased in potato plants treated with both salts.

3.3. Effect of tested SAR inducers and salts on peroxidase and catalase

The catalase and peroxidase levels in onion plants after treatment with Salicylic acid (SA), Potassium phosphate dibasic (PD) as systemic acquired resistance inducers, Sodium bicarbonate (SBC), Potassium carbonate (PC), Sodium carbonate (SC) were determined. As seen in Figure 1, catalase and peroxidase concentrations were affected by all tested salts and inducers significantly. Peroxidase concentrations were recorded 1.31 with salicylic acid compared with control (7.64), while catalase enzyme was recorded 0.84 in compared with control (8.58). the obtained data were agreed with Mahmoud *et al.,* 2006 who reported that, Salicylic acid recorded highest content of phenol compounds and highest increase of oxidative enzymes (peroxidase and polyphenoloxidase) activity in roots of peanut plants. Catalase plays an important role in plant resistance, and protecting cells from the toxic effects of H_2O_2 (Lebeda *et al.,* 2001), it was demonstrated that peroxidase and catalase play an important role as an active signaling molecule leading to different cellular responses (Kovalchuk, 2010) depending on the site of its production and also on the interaction between this ROS and specific hormones i.e., abscisic acid, auxins, ethylene, salicylic acid, nitric oxide, (Ślesak *et al.,* 2007).

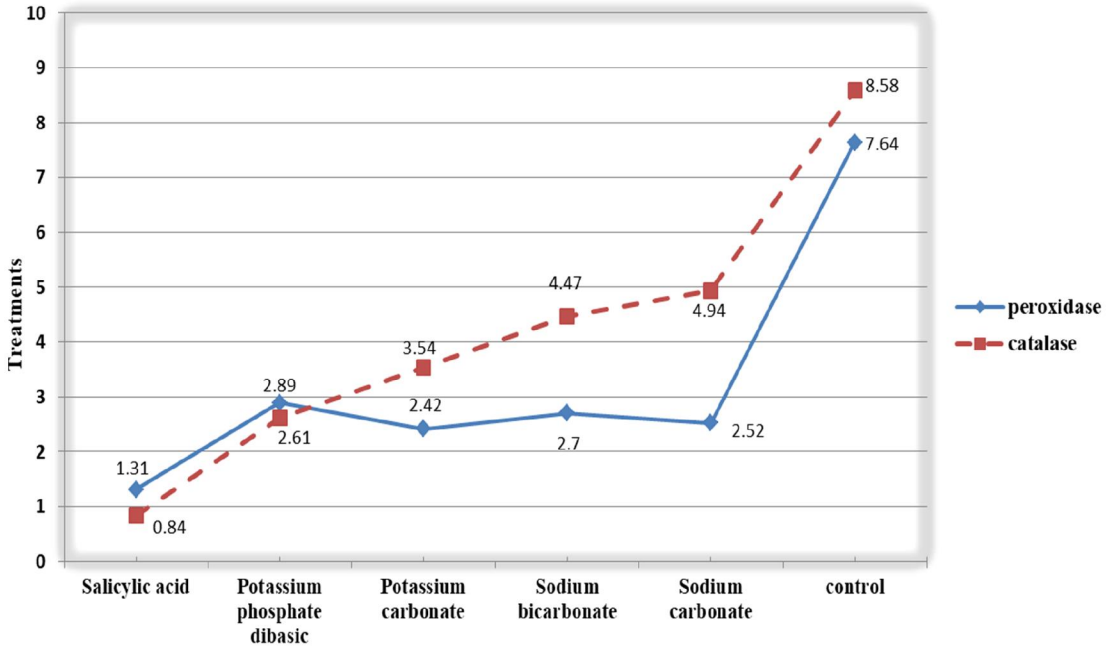

Fig. 1: Effect of spraying with (SA), (PD), (SBE), (PC), and (SC), on Activity of peroxidase and catalase enzyme

Conclusion

On the basis of the above findings of the present investigation the effects of Salicylic acid (SA), Potassium phosphate dibasic (PD) as systemic acquired resistance inducers, Sodium bicarbonate (SBC), Potassium carbonate (PC), Sodium carbonate (SC) salts on reducing the incidence and severity of natural infection of Stemphylium leaf blight with improving vegetative growth of onion were found positive and significant in both growing seasons. The maximum decreasing of disease severity and incidence was recorded with was recorded in treatment with Salicylic acid, where the vegetative growth rates of plants were also the maximum the same treatment when recorded 117g, 77g and 40g in Total weight, Leaf weight and Bulb weight respectively, while the results recorded 78.5 cm, 67 cm and 11.33 cm in total plant height , leaf height and bulb height respectively, which were statistically similar with other treatments in comparison with control. The obtained data from this study concluded that using the above treatments will be economic and ecofriendly alternatives in Stemphylium blight disease management.

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