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Using some systemic resistance inducers to protect onion plants from Purple blotch disease

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ABSTRACT

Purple blotch caused by *Alternaria porri* (Ellis), Ciffen, causes serious problems in onion plants as a major limiting factor in onion cultivation where attacks older leaves and causes destructive damage to bulbs as well as seed crop. Fungicides application is the major way to manage this disease. But the use of fungicides has become a high cost to use, one of the obstacles to export due to the residual effect of fungicides, which causes the rejection of export shipments, in addition to the impact of the pollutant on the environment and humans. Therefore, it has become necessary to search for safe alternatives to fungicides to avoid the negative effects of fungicides. In this research, Plant systemic resistance inducers such as chemical, biological and mineral salts were used. Salicylic acid and Potassium Dibasic Phosphate as chemical inducers, while *Bacillus megaterium, Pseudomonas fluorescens* and *Azotobacter chroococcum* bacteria were used as bio-inducers. Sodium and Potassium bicarbonate mineral salts as well as Omega3 and Vetanool commercial compound to evaluate its effects in reducing the incidence and severity of Purple Blotch natural infection, enhancement vegetative growth, increasing the activity of oxidative enzymes (Peroxidase, Catalase), Potassium and Phosphor. All treatments that used significantly effected in decreasing Purple blotch incidence and severity in compared with fungicides and untreated control.

Keywords: *Bacillus megaterium;* Potassium; Phosphor; *Pseudomonas fluorescens*; *Azotobacter chroococcum*

1. Introduction

Onion has most important properties of antioxidant, anticancer, antimicrobial, anti-diabetic and anti-asthmatic. It is attacked by wide range of diseases worldwide, most of these diseases are caused by fungal diseases, while the others are caused by bacterial, nematode, viral and phytoplasma. Purple blotch is the most destructive one among fungal diseases; it caused by *Alternaria porri* (Ellis) Cif. which causing about 80 to 90 % losses (Yadav, *et al.* 2013; Mamgain, *et al*. 2013; Ashwini and Sathishkumar, 2014). Purple blotch disease of Allium spp. crops caused by *Alternaria porri* has remained a major concern for both farmers and research fraternity as it severely damages the crops and drastically reduces the yield. Priya *et al*. (2016) revealed that, purple blotch caused by *Alternaria porri* (Ellis) Ciffen consider as a major limiting factor in cultivation of onion it causes extensive damage to bulbs as well as seed crop. Abo-Zaid *et al.* (2020) suggested that disease severity was increased in the bulb produced from previous infected crop, the number of flower stalk was reduced, and its disease severity was increased. Alternaria spp. are producing specific and nonspecific toxins, where the host-Selective Toxins (HSTs) are toxic only to host plants. Pathogenicity of Alternaria is due to production of these toxins which mainly secondary metabolites that damage plant tissues by causing leaf necrosis. The symptoms appear after 1–4 days of infection and bulb rot begin, then eventually turn into dark reddish-purple and brownish/black lesions. Many factors such as season, sowing time, humidity and temperature and crop stage have a huge impact on the progression of purple blotch disease Dar *et al.* (2020). Purple blotch symptoms were described by many researchers, initial symptoms appear on older

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leaves and plants which more susceptible to late infection in the summer when fungal spores are blown from infested debris. The fungus is disseminated among fields by splashing water and wind, and overwinters on infested crop debris; the pathogen may also be seed-borne. Lesions are elongate, small, sunken and whitish with a purple center. Concentric light and dark zones later appear over part or all of the purple area. These blotches may enlarge up to four inches long and become covered with spores then leaves wilt and die. Storage symptoms appear as a dark yellow to spongy rot of outer or inner scales of bulbs. This disease can reduce bulb yield up to 20% or more. Angela Madeiras, (2014) reported that, symptoms of purple blotch often begins on older leaves as small, sunken, water soaked lesions with light centers. Lesions enlarge as disease progresses and turn purple to brown, often with yellow rings that create a distinctive bull's-eye pattern. Leaves turn yellow/brown and wilt, and may be girdled. Young leaves become more susceptible with the bulb matures. Bulbs may become infected through neck wounds, and seeds produced from infected bulb showed reduction in germination percentage. Yields may be reduced due to undersized bulbs and diseased bulbs may rot in storage. Bulb rot symptoms begin as soft, water-soaked areas; even bulbs turn dark reddish-purple, and then brown/black. Abo-Zaid *et al.* (2020) reported that *Alternaria porri* (Ellis) causes serious problems in onion plants. It is transmitted with naturally infected seeds and bulbs from the previous crop to infect the new flower stalk of the next crop. Results showed that disease severity was increased in the bulb produced from previous crop, the number of flower stalk was reduced, and its disease severity was increased. Seeds produced from infected bulb showed reduction in germination percentage. He *et al.* (2016) suggested that, plant disease management faces growing challenges due to (i) increasing demands for total, safe and diverse foods to support the booming global population and its improving living standards; (ii) reducing production potential in agriculture due to competition for land in fertile areas and exhaustion of marginal arable lands; (iii) deteriorating ecology of agro-ecosystems and depletion of natural resources; and (iv) increased risk of disease epidemics resulting from agricultural intensification and monocultures. Therefor future plant disease management should aim to study the pesticide alternatives as approaches to avoid the hazards of chemical pesticides either on environment or customer. Salicylic acid a natural molecule plays an important role in regulating a number of physiological processes in plants. Its exogenous application has promoted plant performance under biotic and abiotic stresses (Senaratna *et al.,* 2000). Mahmoud *et al.* (2016) found that, treated onion plants with chemical inducers salicylic acid (SA), Indol Butyric Acid (IBA) at different concentrations showed a significant reduction of downy mildew and purple blotch severity and increased onion bulb yield, and the increase of chemical inducers concentration caused more reduction in disease severity of both downy mildew and purple blotch. Data clearly indicated that phenolic contents (free, conjugated, and total phenols) and activity of the oxidative enzymes (Peroxidase, Polyphenol Oxidase and Catalase) were affected by chemical inducers. Foliar spray of low concentration of salicylic acid promote and influence the growth, development, differentiation of cells, and tissues of plants and enhanced significantly plant's growth parameters, plant height, number of leaves per plant, total chlorophyll content, bulb diameter, average weight of bulb, total bulb yield, marketable bulb yield as compare to higher dose and water spray (Helgi and Rolfe 2005; Nangare *et al.* 2018). Mansha *et al.* (2019) reported that, plants infected with *Alternaria porri* and treated with salicylic acid (SA) had significantly higher PAL, PO, PPO activity, and phenolic contents than control, this proved that application of non-toxic chemical solutions like SA can control purple blotch of onion by the modification of biochemical attributes. Orober *et al.* (2002) reported that, spray application of dipotassium hydrogenphosphate (K_2HPO_4) was effective in inducing a high level of systemic protection against *Colletotrichum lagenarium* causal agent of anthracnose in cucumber plants. Jabnoun-Khiareddine, (2016) reported that, Potassium Sorbate (PS), Potassium Bicarbonate (PB) and Dipotassium Hydrogenphosphate (DPHP) deferred in their antifungal activity against *Fusarium oxysporum f. sp. lycopersici* (FOL), *F. oxysporum f. sp. radicis-lycopersici* (FORL), *F. solani*, *Verticillium dahliae* (VD), *Rhizoctonia solani*, *Colletotrichum coccodes*, Pythium aphanidermatum, Sclerotinia sclerotiorum, *Botrytis cinerea* and *Alternaria solani*, and effects on tomato growth. *P. aphanidermatum, S. Sclerotiorum* and *B. cinerea* being the most sensitive to all salts. PB suppressed Fusarium wilt severity, while DPHP and PS significantly decreased Botrytis, Rhizoctonia, Alternaria and Anthracnose fruit rots. PS significantly increased plant height, root and aerial part fresh weights and had improved root fresh weight. Saleh, (2013) reported that, spraying potassium hydrogen phosphate (K_2HPO_4) showed high efficacy in reducing downy mildew and purple blotch diseases. Beneduzi *et al.* (2012) referred to the effect of PGPR Bacteria which colonize plant roots and promote plant growth, can occur as an antagonism to soil-borne pathogens or by induction the systemic resistance against pathogens throughout the entire plant. PGPR produced many substances related to pathogen control and promote growth of many plants such as siderophores and antibiotics. Rhizobacteria induce resistance through the salicylic acid-dependent systemic acquired resistance SAR pathway, or require Jasmonic Acid and ethylene perception from the plant for induced systemic resistance ISR. Rhizobacteria belonging to the genera Pseudomonas and Bacillus are well known for their antagonistic effects and their ability to trigger ISR. Rhizobacteria might be useful in formulating new inoculants with combinations of different mechanisms of action, leading to a more efficient use for biocontrol strategies to improve cropping systems. Bacteria that reduce the incidence or severity of plant diseases are often referred as biocontrol agents whereas those that exhibit antagonistic activity toward a pathogen are defined as antagonists (Kamilova *et al.* 2005; Neeraja *et al.* 2010; Maksimov *et al.* 2011; Van Loon, 2007). Savitha, *et al*. (2014) reported that, purple blotch of onion was successfully management through the application of bio-agents, botanical and fungicides. Positive and significant effect was found with the seed treatment using *Pseudomonas fluorescens* followed by two sprays of difenconazole. Yousefi *et al.* (2011) reported that, using combination of foliar spray with salicylic acid (SA) and soil drench with *Bacillus subtilis*, prior to fungal infection, exhibited reduction of *Fusarium oxysporum f. sp. radicis cucumerinum* infection and increased plant growth as compared to control and other treatments. Abo-Zaid *et al.* (2020) reported that, all treatments including, *Bacillus sp*., showed stimulation of germination for onion seed compared with plants in the control treatment. Many biological approaches such as plant extracts and bio-control agents were found partially effective for controlling purple blotch (Dar *et al*. 2020; Yadav *et al.* 2013; Vásconez *et al.* 2020; Kildea *et al.* 2008). This study was conducted to investigate the inducing effect of chemical inducers, inorganic salts, and Plant Growth Promoting Rhizobacteria (PGPR) as a bio inducers in compare with pesticides and untreated plants in reducing naturally incidence and severity for purple blotch disease and enhancement growth parameters of onion plants.

2. Material and Methods

This experiment was carried out in the New Salhia area, Sharkia Governorate, during the winter planting seasons 2020/2021 and 2021/22 in first of November using onion seedlings, Giza red variety. The cultivated land has a long history of growing onions and in the last season, the farm faced a great loss in the onion crop as a result of the severe infestation with purple blotch in spite of increasing the quantity and types of fungicides used. Therefore, it was chosen to apply the research treatments to compare the effect of them with the effect of farm treatments (fungicides) and the control not treated with any treatment. Cultivation was done on 120 cm wide terraces and irrigation system was drip irrigation, the distance between the irrigation lines was 30 cm, the distance between the seedlings 10 cm. The number of treatments 10, 9 for research treatment and the tenth was the farm treatment which using deferent kind and doses of fungicides. The research and farm treatments were separated by a number of uncultivated lines to avoid the spread of fungicides on the research treatments. Each treatment 5 replicates and the length of each replicate 3 meters. The transactions were as follows

Salicylic acid (SA) $C_7H_6O_3$ - Potassium Dibasic Phosphate (PDP) K_2HPO_4 - Sodium Bicarbonate (SBC) $NaHCO_3$ - Potassium bicarbonate (PBC) ($KHCO_3$) -– *Azotobacter chroococcum* (Azc) - *Pseudomonas fluorescens* (Pf)*; Bacillus megaterium* (Bm); Vetanool (Ve) Commercial Compound; Omega 3(Om) Commercial Compound; Untreated control (Unc.); Farm Treatment (FT).All chemical used in the spray were purchased from Al-Gomhoria Company for medicines and medical supplies. Spraying was carried out with a concentration of 1g/2 liters of water, for all salts and inducers treatment. Omega 3 purchased from Green line agricultural investment company. Used as 2cm/L. water. Vetanool purchased from Dakahlia for agricultural projects company. Used as 2cm/L. water.

2.1. Bacterial growth

The bacterial isolates were cultured on Nutrient Agar Media (5 g peptone, 3 g beef extract/yeast extract, 15 g agar, 5g sodium chloride / 1L. distilled water). Spraying treatment of the bacteria, it is done using a liquid nutrient media, and the bacterial concentration is 1x108 CFU. g-1, First spray treatment begin 30 days after transplanting the seedling to the field and repeated 3 times 15 days interval diseases incidence and severity recorded two days after each spray and the samples for estimate (vegetative growth, activity of Peroxidase PO, Catalase CAT enzymes, Potassium K and phosphor P concentrations in plant), collected 2 days after the last spray.

2.2. Potassium and Phosphor Measurement

Leaves samples were taken at harvesting from each treatment and the N, P, and K percentages were determined in the dry leaves. Their dry weights were determined following drying in a drying chamber to a constant weight at 75oC for 72 hours. After dryness, the plant samples were milled and stored for analysis as reported. However, 0.5g of the leaves powder was wet-digested with H2SO4–H2O2 mixture according (Lowther, 1980).

Phosphorus Content: Total phosphorus determined by the Vanadomalayaite yellow method as given by Jackson (1973) and the intensity of color developed read in a spectrophotometer at the wavelength of 405nm.

Potassium content: Potassium was determined according to the method described by Jackson (1973) using a Beckman Flame photometer.

2.3. Enzyme Assays

Peroxidase (PO): Assay (based on the oxidation of pyrogallol to purpurogallin in the presence of H_2O_2) 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; 0.3mL 0.05M pyrogallol and 0.1 ml 1.0% H_2O_2. The mixture was completed with distilled water up to 3ml. Enzyme extract was replaced by distilled water in the control blank cuvette. The absorbance of 1mL was recorded, and peroxidase activity was expressed as the change in absorbance at 425 nm /15minute/gram fresh weight.

Catalase (CAT): Activity is measured spectrophotometrically using the method with Ammonium Molybdate (Goth 1991). The reaction mixture consisted of 65 mM H_2O_2, phosphate buffer, and enzyme extractwas 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 H_2O_2 was measured against blank at 405 nm. One unit of CAT is the amount of enzyme that decomposes 1 µM of H_2O_2 per minute; CAT activity is expressed as Umg-1of protein.

2.4. Data analyses

The experimental data was statistically analyzed by COSTAT Version 2.

2.5. Disease assessment

Diseases Incidence (DI) for treatments was recorded as percentage of diseased plants in order to total number of plants cultivated in treatment replicates. Summation of total No. of diseased plants in each replicate/total No. of cultivated plants in treatment replicates×100

Disease Severity (DS): was according to the scale, (0: No disease symptoms; 1: few spots towards tip covering 10 percent leaf area; 2: Several dark purplish brown patch covering up to 20 percent leaf area. 3: Several patches with paler outer zone covering up to 40 percent leaf area; 4: Leaf streaks covering up to 75% leaf area or breaking of the leaves from center; 5: Complete drying of the leaves or breaking of the leaves from center. % Disease Intensity (DI) was calculated by using the following formula (Wheeler, 1969). % Disease Index=Sum of Numerical Ratings/Number of observations x100/5.

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3. Results

3.1. Effect of tested inducers, inorganic salts, Commercial Compounds and biocontrol agents on Purple blotch natural infection

Purple blotch caused by *Alternaria porri* (Ellis) Cif. causes serious problems in onion plants, It can infect all aboveground parts of the plant in addition to the bulb, it can cause about 80 to 90 percent accountable losses (Yadav *et al.* 2013). Data in table (1) revealed that there was significant differences among treatments and farm treatment and control in reducing DI in first season but there was no significant differences between (*B. megaterium,* 23.8; *P. fluorescens,* 22.2), (Omega3, 52.8, Vetanool 52.2, farm treatment 53 and control 55.2). All treatments achieved significant differences between them and between the farm treatment and the control treatment in reducing disease severity of infection. *P. fluorescens*, (22.2, 19.2), *B. megaterium* (23.8, 22.4), Salicylic acid (32.2, 17.4), and Potassium phosphate (34.4, 31) had superior effect in reducing both DI and DS in compare with other treatments, followed by *A. chroococcum* (41.8, 27.8), Sodium bicarbonate (43.2, 25.2) and Potassium bicarbonate (46.60, 24.4). The last effective treatment in reducing both DI and DS in compare with farm treatment and untreated control were Omega3 (52.8, 44.8) and Vetanool (52.2, 50).

Table 1: Effect of inducers (SA, PDP), inorganic salts (SBC, PBC), Commercial Compounds (Om, Ve) and (Azc, Bm, Pf) Bacteria, on DI and DS of Purple blotch natural infection

Treatments	First season		Second season	
	DI	DS	DI	DS
Salicylic acid (SA)	32.2	17.4	34.4	20.6
Sodium bicarbonate (SBC)	43.2	25.2	44	26.2
Potassium phosphate dibasic (PDP)	34.4	31	36.8	31.4
Potassium bicarbonate (PBC)	46.6	24.4	46.8	25.8
Omega 3 (Om)	52.8	44.8	51.6	46.2
Vetanool (Ve)	52.2	50	51.8	48
Azotobacter chroococcum (Azc)	41.8	27.8	36	28.8
Bacillus megaterium (Bm)	23.8	22.4	29	23.4
Pseudomonas fluorescens (Pf)	22.2	19.2	24.2	20.2
Farm treatment (FT)	53	47	53.6	51
Untreated Cont. (Unc.)	55.2	52.4	61.6	72.4
LSD 5%	2.9	1.8	3.4	2.5

In second season, results showed significantly variation in reducing both DI and DS that due to natural infection of Purple blotch. This clearly variation appears in the data recorded by treated onion plants with *P. fluorescens*, (24.2, 20.2), *B. megaterium,* (29, 23.4), Salicylic Acid, (34.4, 20.6), *A. chroococcum*, (36, 28.80) and Potassium phosphate (36.8, 31.4) in compared to the results recorded with farm treatment (53.6, 51) and control plants (61.6, 72.4) for both DI and DS respectively. On other hand spraying onion plants with Sodium and Potassium bicarbonate significantly decreasing both DI and DS for Purple blotch natural infection in compared with farm treatment and untreated control Sodium bicarbonate recorded (44 DI, 26.2 DS) while Potassium bicarbonate recorded (46.8 DI,25.8 DS). Spraying plants with Omega3 and Vetanool only decreasing significantly DI and DS in compared with untreated control, Omega3 recorded (DI 51.6, DS 46.20) while Vetanool recorded (DI 51.8, DS 48) and untreated control recorded (DI 61.6, DS 72.4). So it is clear from these data that *P. fluorescens*, *B. megaterium, A. chroococcum* gave highly significant redaction for both naturally DI and DS of Purple blotch followed by Potassium phosphate dibasic while Sodium and Potassium bicarbonate gave moderate redaction.

3.2. Effect of tested inducers, inorganic salts, Commercial Compounds and biocontrol agents on Onion vegetative growth under natural infection of Purple blotch

Data in table (2) indicated that all treatments could have a significant effect on vegetative growth of onion plants. *B. megaterium* (Bm)*, P. fluorescens* (Pf), *A. chroococcum* (Azc) enhancement significantly all vegetative growth recorded in compared with farm treatment, untreated control, and all other treatments, followed by Omega3, Sodium and Potassium bicarbonate. Salicylic acid, Potassium phosphate dibasic and Vetanool increase significantly vegetative growth of onion plants compared to the results of the untreated control and the results of the farm treatments. *Bacillus megaterium* recorded highly increasing on plant height (88.89cm.) leaf height (79.3cm.) Bulb height (9.68cm.) followed by *Pseudomonas fluorescens* and *Azotobacter chroococcum* Otherwise Pf recorded highly increasing in plant weight (157.6g) leaf weight (107 g) Bulb weight (50.6g)

Table 2: Effect of inducers (SA, PDP), inorganic salts (SBC, PBC), Commercial Compounds (Om, Ve) and (Azc, Bm, Pf) Bacteria, on Onion vegetative growth under natural infection of Purple blotch (average of combined data over two seasons)

Treatments	Plant height (cm)	Leaf height (cm)	Bulb height (cm)	Plant weight (g)	Leaf weight (g)	Bulb weight (g)
Salicylic acid (SA)	77.7	69.4	8.4	121.1	78.5	42
Sodium bicarbonate (SBC)	80.3	72.7	7.6	138.5	95.6	42.6
Potassium phosphate dibasic (PDP)	75.3	68.4	6.7	129.3	77.7	51.62
Potassium bicarbonate (PBC)	80.5	75.4	6.7	152.6	105.7	46.5
Omega 3 (Om)	83.5	75.7	7.5	133.4	85.7	47.6
Vetanool (Ve)	73.8	67.4	6.4	132.3	91.3	41
***Azotobacter chroococcum* (Azc)**	85	76.3	8.8	153.3	110.2	43.3
***Bacillus megaterium* (Bm)**	88. 9	79.3	9.7	155.5	108.3	47.15
***Pseudomonas fluorescens* (Pf)**	86.3	77	9.3	157.6	107	50.6
Farm treatment (FT)	61.1	56. 6	4.6	81.5	56	25.7
Untreated Cont. (Unc.)	55.4	51.3	4.1	35.6	23.5	12.7
LSD 5%	0.6	0.5	0.5	0.5	0.5	0.9

3.3. Effect of tested inducers, inorganic salts, Commercial Compounds and biocontrol agents on PO, CAT enzymes and K, P content

Data in table (3) revealed that SA increasing activity of both PO (1.12) and CAT (0.09) enzymes and onion plant content from K (45ppm) and P (1.189mg/g) followed by Pf (1.22 PO), (0.09 CAT), (38ppm K), (1.123 mg/g P), Bm (1.45 PO), (1.24 CAT), (36ppm K), (0.977 P), Azc (1.45 PO), (1.24 CAT), (36ppm K), (0.886mg/g P), on other hand effect of PDP, SBC and PBC in activation PO, CAT enzymes and increased plant content from K and P were moderate and Om and Ve had the lowest effect in compared with FT and Unc. which recorded (5.75 PO), (6.75 CAT), (32ppm K), (0.768mg/g P) and (7.64 PO), (9.98 CAT), (29ppm K), (0.669mg/g P) respectively.

Table 3: Effect of inducers (SA, PDP), inorganic salts (SBC, PBC), commercial compounds (Om, Ve) and (Azc, Bm, Pf) bacteria on PO, CAT enzymes and K, P content of Onion plants under Purple blotch natural infection

Treatments	Peroxidase PO	Catalase CAT	K. ppm	P. mg/g
Salicylic Acid (SA)	1.1	0.1	45	1.2
Sodium Bicarbonate (SBC)	2.4	1.7	33	0.8
Potassium Phosphate Dibasic (PDP)	2.2	1.5	34	1.1
Potassium Bicarbonate (PBC)	2. 9	1.6	31	0.9
Omega3 (Om)	4.5	2.6	30	0.7
Vetanool (Ve)	4.1	2.9	30	0.7
***Azotobacter chroococcum* (Azc)**	1.8	1.6	32	0.9
***Bacillus megaterium* (Bm)**	1.5	1.2	36	0.9
***Pseudomonas fluorescens* (Pf)**	1.2	0.1	38	1.1
Farm treatment (FT)	5.8	6.8	32	0.8
Untreated Cont. (Unc.)	7.6	9.9	29	0.7

4. Discussion

Onion is one of the most important export crops in Egypt; its productivity and exportation is restricted by several diseases, Purple Blotch disease caused by *Alternaria porri* (Ellis) Ciffen is one of the most destructive disease causing accountable losses rich about 80% to 90% in onion plants. It is consider as a major limiting factor in cultivation of onion. It can infect all aboveground parts of the plant causes extensive damage to bulbs as well as seed crop. Also it has wide host range and wide variability in symptoms, for this Purple Blotch management consider as uneasy processes to deal with, especially in the case of using fungicides, due to its residual which polluting the environment and plants, high costs of their use, the emergence of fungal strains resistant to fungicide effect. Looking for new alternatives to manage purple blotch is a good approach especially when dealing with exporting abroad. Induced disease resistance can be defined as the process of active resistance dependent on the host plants, physical or chemical barriers are activated by biotic or abiotic agents (Meena *et al.* 2001). Induction of systemic resistance sensitizes the plant to respond rapid after infection. These responses include Phytoalexin accumulation, Phenols, Lignifications and activation of many enzymes such as Peroxidase, Polyphenoloxidase,Catalase and Chitinase (Meena *et al.* 2001; Ibrahim *et al.* 2013 and Mahmoud *et al.* 2014).

Salicylic acid (SA) is a phenolic phytohormone could act as a systemic signal that triggers local systemic resistance response, as a key regulator of the signaling network in plants under abiotic and biotic stresses, significantly increased activity of PAL, PO, PPO enzymes, and phenolic contents, supplement SA exogenously stimulated systemic resistance and promoted plant performance under biotic and abiotic stresses (Chen *et al.* 1999; Mansha *et al.* 2019; Saikia *et al*. 2003; Chandra *et al*. 2007; Senaratna *et al*. 2000). Dipotassium hydrogen phosphate KHPO the most studied phosphate salts for their antifungal properties, it is an ideal candidates for fungal disease management as they are fast absorbed by the plant, they have high mobility within tissues and have low cost nutrient source. Furthermore, phosphates exhibit antifungal activity through the induction of systemic acquired resistance; thus, providing long-lasting plant protection and are also effective against many fungal pathogens. Induction of resistance by KHPO was associated with localized cell death in leaves of plants treated with phosphate salt. Cell death is observed, resulting in visible spots with the naked eye similar to spots caused by infection. The appearance of these spots is accompanied by an increase in the hypersensitivity response (HR) that leads to the activation of the pathogen-induced SAR. Phosphate-mediated cell death is preceded by rapid generation of superoxide and hydrogen peroxide. An increase in the levels of free and conjugated salicylic acid was detected as another result of phosphate treatment (Reuveni *et al.* 1996; Orober *et al.* 2002; Deliopoulos *et al.* 2010). The foregoing shows the good results that were obtained during this study as a result of treated onion plants with Salicylic acid $C_7H_6O_3$ and Potassium phosphate dibasic (PDP) K_2HPO_4, which gave a noticeable suppuration in Purple Blotch DI and DS under natural infection for the two seasons, enhancement vegetative growth of onion plants, as well as an increase in the activity of PO, CAT enzymes and the accumulation of potassium and phosphorous inside the plant. These results in agreement with Pradhan *et al.* (2018) revealed that, invariably exogenous application of salicylic acid SA significantly reduced the incidence of purple blotch disease as compared to their control plots, which clearly demonstrated the efficacy of SA towards induced tolerance to disease in onion. Mahmoud *et al.* (2016) reported that, treated onion plants with chemical inducers salicylic acid, bion and indol butyric acid, significantly reducing downy mildew and purple blotch severity and increased onion bulb yield, data clearly indicated that phenolic contents (free, conjugated, and total phenols) and activity of the oxidative enzymes (peroxidase, polyphenol oxidase and catalase) were affected by chemical inducers. Mansha *et al*. (2019) reported that, plants infected with *Alternaria porri* and treated with salicylic acid (SA) had significantly higher PAL, PO, PPO activity, and phenolic contents than control, this proved that application of non-toxic chemical solutions like SA can control purple blotch of onion by the modification of biochemical attributes. Nangare *et al.* (2018). The foliar application of salicylic acid at lower concentration gives significantly maximum plant height, number of leaves per plant, total chlorophyll content, bulb diameter, average weight of bulb, total bulb yield, marketable bulb yield as compare to higher concentration and control (Saikia, 2003). Salicylic acid showed the highest protection of chickpea seedlings against wilting caused by *Fusarium. oxysporum f. sp. ciceri*. Saleh, (2013) reported that, spraying potassium hydrogen phosphate (K_2HPO_4) showed high efficacy in reducing downy mildew and purple blotch diseases. Orober *et al.* (2002) reported that, spray application of dipotassium hydrogenphosphate (K_2HPO_4) was effective in inducing a high level of systemic protection against *Colletotrichum lagenarium* causal agent of anthracnose in cucumber plants.

Bacteria that reduce the incidence or severity of plant diseases are often referred as biocontrol agents whereas those that exhibit antagonistic activity toward a pathogen are defined as antagonists. The activities of antagonistic bacteria in rhizospher can be summarized; (1) synthesis of hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases, that can lysing of pathogenic fungal cells (2) competition for nutrients and suitable colonization of niches at the root surface (3) regulation of plant ethylene levels through the ACC-deaminase enzyme, which can act to modulate the level of ethylene in a plant in response to stress imposed by the infection (4) production of siderophores and antibiotics. On other hand Bacteria that colonize plant roots and promote plant growth, their effects can occur as an antagonism to soil-borne pathogens or by induction the systemic resistance against pathogens throughout the entire plant. Rhizobacteria induce resistance through the salicylic acid-dependent systemic acquired resistance SAR pathway, or require Jasmonic acid and ethylene perception from the plant for induced systemic resistance ISR. Rhizobacteria belonging to the genera *Pseudomonas* and *Bacillus* are well known for their antagonistic effects and their ability to trigger ISR. *Rhizobacteria* might be useful in formulating new inoculants with combinations of different mechanisms of action, leading to a more efficient use for biocontrol strategies to improve cropping systems. Otherwise PGPR promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents. (Beneduzi *et al.* 2012; Kamilova *et al.* 2005; Neeraja *et al.* 2010; Van Loon 2007; Ahemad and Kibret 2014; Gupta, and Kaushal, 2017). Bacteria *Azotobacter chroococcum* (Azc), *Bacillus megaterium* (Bm), and *Pseudomonas fluorescens* (Pf), were used in this study, which gave highly significant redaction for both naturally DI and DS of Purple Blotch, significantly regulate vegetative growth, increase enzymes activity and increase accumulation of K and P. in compared with other treatment, FT and Untreated control. The obtained results in harmonize with Kildea *et al.* (2008). *Bacillus megaterium* isolated from barley leaves and grain consistently retarded *septoria tritici* blotch (STB) caused by *Mycosphaerella graminicola* in field trails. Utkhede *et al*. (1983) reported that, *Bacillus subtilis* applied as seed treatments singly or in combination with broadcast chemicals significantly reduced white rot of onions in unsterile muck soils in a controlled- environment chamber. Savitha *et al*. (2014) reported that, onion purple blotch can be successfully controlled by seed treatment with *Pseudomonas fluorescens* and fungicides spray. Yadav *et al.* (2013) found that *Pseudomonas fluorescens* followed by *Bacillus subtilis* effective against *Alternaria porri a*nd recorded significantly reduced in mean disease intensity and increased the bulb yield over unsprayed control. Vásconez *et al*. (2020) concluded that, *Bacillus megaterium*-based biopreparations, can control diseases symptoms caused by *Alternaria japonica* on leaves and inflorescences of *Brassica oleracea* and showed significant differences between treatments. *B. megaterium can* produce lipopeptide metabolites it case inhibition to phytopathogenic fungi growth. Mergawy *et al.* (2022) Adding biofertiizer *Azotobacter chroococcum* and spraying tomato plants with bioagents reduced the disease incidence and disease severity of Tomato (*Lycopersicon esculentum* Miller) early blight caused by *Alternaria solani*. *T. harzianum* and *B. subtilis* with biofertilizer significantly increased the activities of defense-related enzymes i.e., catalase, polyphenoloxidase, peroxidase and the total content of phenols under greenhouse conditions.

In this study, spraying onion plants with bicarbonate salts (Potassium and Sodium) significantly reduced both DI and DS of Purple Blotch, enhancement vegetative growth increasing activity of PO and CAT enzymes in compared with FT and Untreated control, and these results in agree with. EL-Ashmony (2021) suggested that, potassium bicarbonate (KHCO3) have significant inhibitory effects either on growth parameters of *Sclerotium rolfsii* or in sunflower root/collar. Inhibitory effects were increased by enhancing KHCO3 concentration. Erper *et al.* (2011) concluded that, potassium bicarbonate was an alternative chemical agent for controlling *R. solani* and *S. sclerotiorum.* Also, $KHCO_3$ was found to have negative effects on *Trichoderma* sp. Jabnoun-Khiareddine *et al.* (2016) found that, Potassium Bicarbonate (PB) can inhibited fungal growth and greatest inhibition achieved using the highest concentration, also PB causing varied degree in protected tomato plants against wilts caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), *Verticillium dahliae* (VD) while PB-based treatment significantly lower Rhizoctonia tomato fruit rot. The lowest effect in reducing both Di and DS recorded by Omega3 and Vetanool with significant differences in compered with untreated control but in convergence with the results of the farm treatment especially in reducing DI, on other hand they increase vegetative growth and activity of enzymes and this may be due to their active component.

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