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Role of Antibiosis in Control of Cabbage Black Rot Caused by *Xanthomonas campestris* pv. *campestris*

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Two different antagonists, *Bacillus subtilis*, *Pseudomonas fluorescens* and Tango fungicide, were contemplated against cabbage black rot caused by *Xanthomonas campestris* pv. *campestris* (Xcc) *in vitro* and *in vivo*. *P. fluorescens* was the most efficient bioagent in repressing of the pathogenic bacterium took after *Bacillus subtilis*. The fungicide Tango was the most potent one in hindering the development of the causal bacterium *in vitro* more than other bioagents. Field experiments during 2014 and 2015 seasons have shown that spraying cabbage plants with the tested bioagents and the fungicide four times resulted in a significant decrease in severity caused by natural infection by the pathogen with a significant increase in yield component. The fungicide was potentially functional than the other bioagents. Tango was the most effective treatment for decreasing the severity of the disease and increasing the produced vegetative components. Meanwhile, the bioagent *B. subtilis* was the lowest in potential and other treatments recorded intermediate figures. Vitamin-C and total phenols content as well as the activity of peroxidase, polyphenoloxidase and chitinase in cabbage leaves infected by *X. c.* pv. *campestris* were greatly low levelled in infected leaves of compared with uninfected or the tested bioagents and fungicide resulted in a considerable increase in these contents compared with the infected leaves.

Keywords: Bioagents, cabbage, chitinase polyphenoloxidase, peroxidase, phenols, and *Xanthomonas campestris* pv. *campestris.*

Cabbage (*Brassica oleracea* L.) is one of the important vegetable crops cultivated word wide (Gopalakrishnan and Rashmi, 2013). In Egypt, its commercial cultivation is governed by various factors among which diseases play an important role. Cabbage is an excellent source of Vitamin-C (44%) and other mineral nutrition's, containing more than 20% of the daily value for each of these nutrients per serving (Terefa, 2017). The crop is attacked by a variety of pathogens both in the nursery and the field. Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (Alvarez, 2000), is one of common and the most dangerous diseases of cabbage.

The bacteria cells are gram-negative, short rods with rounded ends, bacterial (Xcc) colonies were round with entire margins, slightly raised, shiny, butyrous in consistency and light yellow in color on nutrient dextrose agar medium (Pammel, 1895). Bhide (1948) found that on NDA media the bacterium formed round colonies

with entire margins. On agar or gelatin, the bacterium formed smooth, moist, shining, round and flat to curved colonies with entire margins. The growth of bacteria in nutrient or broth media is moderately and showed clouding and yellowish ring without pellicle. The optimum temperature for growth of the organism was from 25 to 30°C (Babadoost, 1999).

Cabbage is susceptible to black rot (Williams, 1980; Mguni, 1996) caused by the bacterium (Xcc) which is considered the most serious disease of cabbage worldwide (Williams, 1980; Alvarez, 2000). Typical symptoms in the field are V-shaped, chlorotic to necrotic lesions starting from leaf margins and blackening of the vascular tissue (Assis *et al.*, 1997; Seebold e*t al.,* 2008). The losses due to the disease may exceed 50% in warm, wet climates. In some cases, the crop may cause a total loss (Maji and Nath, 2015). Relative high humidity 80-100% and temperatures 25-35°C are favorable for disease progress (Griesbach *et al.*, 2003).

The controlling of black rot is difficult in tropical regions, giving bigger attention to the development of other control measures such as biocontrol with antagonistic microorganisms. In recent studies, promising control of black rot in brassicas was achieved when antagonistic bacillus endophytes were used as seed dressing and applied as a root-dip at transplanting (Jalali and Parashar, 1995; Mguni 1996; Pichard and Thouvenot, 1999; Wulff, 2000; Wulff *et al*., 2002a) or as foliar sprays (Assis *et al.,* 1996).

Copper containing fungicides can mostly manage the disease (Krauthausen *et al.* 2011), though, copper resistance to black rot was first identified in 1972 in a Japanese cabbage (Williams *et al.*, 1972). Among several peptide antibiotics, *Bacillus* spp. produce lipopeptides, which are amphiphilic compounds with surfactant activity (Zuber *et al.*, 1993). A surfactant is briefly defined as a material that can greatly reduce the surface tension of water at very low concentration. In the present study, the production of lipopeptides and the role of these compounds in bacillus isolated from plants, that antagonize *X. c*.pv. *campestries* were investigated. *Bacillus* species produce a large number of biological compounds effective against bacteria. Mishra and Arora (2012) reported that *Pseudomonas fluorescens* produced 2, 4-diacetylphloroglucinol and managed black rot in cabbage. Wulff *et al.* (2002b) demonstrated that *Bacillus subtilis* inhibited three strains of Xcc on four Brassica crops (cabbage, cauliflower, rape and broccoli) in different types of soil. The bioagents Bacillus and Pseudomonas spp. played an avital role in plant defense mechanisms against plant pathogens, which increase the activity of some enzymes such as peroxidase, polyphenoloxidase and chitinase. (Li *et al*., 2009 and 2015).

The aim of this study was the isolation and diagnosis of different forms of Bacillus from plants and determination of their efficiency as a bio-control agent against plant pathogens as *X. c.* pv. *campestris*. Supernatants and suspensions containing *Bacillus* spp. were studied for monitoring of antagonistic effect.

Materials and Methods

Isolation, purification and identification of the associated bacteria:

The infected leaves collected from Qalubiya, Behera and Sharkiya governorates (Table 1) were washed with tap water and then air dried. Lesions were surface swabbed with ethyl alcohol (70%), then sodium hypochlorite solution 1.25% for 20 s followed by two successive wash in sterile water. The lesion was macerated in few ml of sterilized water. The homogenate was streaked onto nutrient medium. Three colonies from each lesion were selected randomly. The associated bacterial colonies were purified and identified using the descriptions of Parry *et al.* (1983) and Holt and Krieg (1984). Moreover, the identification of *X. campestris* pv. *campestris* was completed by testing for Xanthomonad determinative features (Table 2) including using of standard bacteriological methods (Schaad *et al*., 2001). The identity of the pathogen was confirmed by testing the sample for pathogenicity on cabbage cv. Sabeany (*Brassica oleracea*), as previously described by O'Garro and Tudor (1994).

Pathogenicity test of X. c. pv. *campestris isolates:*

Cabbage seedlings (cv. Sabeany) of one month old, apparently free from any infection by any disease (grown in Foam trays in a plastic house) were transplanted in pots (25 cm. in diameter) filled with sterile soil. Two transplants were planted in each pot. The two tested strains (Qalubiya 4 & Qalubiya 6) of *X. c*. pv. *campestris* were taken from a 24 h YDC culture then scraped with a sterile pin and punctured. The main veins of 2 newest leaves, as well as near leaf edge by a sterile pin laden with the pathogen (Bila *et al.*, 2013). The infected seedlings were kept under moist condition for two days with polyethylene bags and then left under natural condition. Other cabbage seedlings were free from infection as a control (Maji and Nath, 2015). Symptoms produced by the tested strains were recorded 14 days after inoculation. Also, disease severity was assessed based on a scale ranging from 1 to 9 (1: symptomless, 2: slight symptoms including local necrosis, 3: about one third, 5: about half, 7: about two thirds of the plant covered by typical symptoms, 9: plant dead) as described by Erika *et al*. (2003)

Disease management:

1-In vitro experiment:

The antibiosis of two bioagents (occasionally isolated from cabbage leaves) and one fungicide were determined against *X. c.* pv. *campestris in vitro*. The bacterial suspension of the tested bioagents was adjusted to 10^8 cfu ml^{-1} (Optical Density 660 = 0.06), after growth for 48 h at 28±2°C (Maji and Nath, 2015). The fungicide used was Tango 23% (copper sulphate 8%+ sulfur 28%, 250ml / 100 L water). The concentration was adjusted nutrient broth medium and the concentration of fungicide was prepared at a recommended dose (at a rate of 2.5 ml/L) depending on their active ingredient. Distilled sterile water was used as a control. The pathogenic bacterium was cultured in nutrient broth at 28±2°C for 2 days on a shaker at 150 rpm. In each petri dish (9 cm) 1 ml of suspension from bacterium was added to 15 ml of molten nutrient agar before solidification. Filter paper discs (Whatman No.1,7 mm in diameter) were saturated with the different concentrations of tested bioagents or fungicide. The plates were incubated at 28±2°C for 48 h. Four plates for each

concentration were used and measured diameter of inhibition zone which formed around the disc were estimated. Each treatment was replicated 5 times and the results were averaged (Thornberry, 1960).

2. Field experiments:

Field experiments were carried out at the experimental farm of Fac. Agric. Benha Univ., during 2014 and 2015 growing seasons, where black rot caused by *X. campestris* pv. *campestris* is annually recognized as severe infection. The land was prepared for planting cabbage (*Brassica oleracea* L. cv. Sabeany) as plots of 10.5 m^2 (3.5 x3 m) of five rows, in Complete Randomized Block Design with three plots as replicates. Cabbage seedlings (one month old), apparently free from any infection, grown in Foam trays in a plastic house, were transplanted in the presence of irrigation water on one side of the row. The plants received agricultural practices as a recommended by the Min. of Agric. and kept subject to natural infection. The bioagents, *B. subtilis* and *P. fluorescens* ($1x10^8$ cfu/ml. water) and the fungicide Tango at (250 ml/100L water) were sprayed on the grown plants four times started from mid of each season, by appearance of symptoms of the natural infection with 2 weeks' interval. The sticker material (0.05% Tween 80) was added to the sprayed suspensions plants to adhere components to the treated plants. Disease severity was assessed based on a scale ranging from 1 to 9 (Erika *et al*., 2003).

3- Vegetative measurements:

Following vegetative parameters determinations, heads weight was recorded and total yield was calculated. Heads were weighed (head part was separated from the stem and weighed) , rosette dimensions (mean of two measured diameters, one in a row line, another vertically) and a number of the edible leaves of each plant was considered (Mguni, 1996).

Physiological changes associated with infection:

1-Determination of vitamin C:

Vitamin C or ascorbic acid method based on measurement of the extent to which a 2, 6-dichlorophenol-indophenol dye solution is decolorized by the presence of ascorbic acid was followed (Anonymous, 1975).

Procedure**:**

Two dry test tubes, pipette the requisite volume of sample or standard ascorbic acid solution and make up to 5 ml with 2 % HPO_3. Add 10 ml of the dye solution (0.02 %) with a rapid delivery pipette, shake and take the reading within 15-20 sec. Measure the red color at 518 nm against a blank consisting of 5 ml 2% HPO_3 and 10 ml of water.

2. Estimation of total phenolic compounds:

One gram of cabbage leaves was extracted with 10 ml of 80% methanol at 70°C for 15min. The reaction mixture contained 1 ml of methanolic extracts, 5ml of distilled sterilized water, and 250 µl of Folin-Ciocalteau reagent (1N), and was kept at 25°C. The absorbance of the developed blue color was measured using a spectrophotometer at 725nm. Gallic acid was used as the standard. The number of

phenolic compounds was expressed as mg Gallic acid/g plant material (Zieslin and BenZaken, 1993).

3- Determination of peroxidase:

Peroxidase activity was determined according to the method described by (Allam and Hollis, 1972). The cuvette contained 0.5 ml. 0.1M potassium phosphate buffer at pH 7.0 + 0.3 ml of enzyme extract + 0.3 ml 0.05M pyrogallol + 0.1 ml 1.0% H_2O_2 and distilled water to bring cuvette contents to 3.0 ml. The reaction mixture was incubated at 25°C for 15 minutes, then the reaction was inactivated by adding 0.5 ml. of 5.0% (v/v) H_2SO_4 (Kar and Mishra, 1976). Peroxidase activity was expressed as the increase in absorbance at 430nm/gram fresh weigh/15 minutes.

4- Determination of polyphenoloxidase:

Polyphenoloxidase activity was determined according to a modification of Ishaaya (1971), in a reaction mixture consisting of 0.5 ml phosphate buffer (0.1 M, pH 7), 200 µl enzyme solution and 200 µl catechol solution (2%). Prior to the initiation of the reaction, the substrate and other ingredients of the reaction mixture were separately incubated at the optimum temperature of the reaction (25ºc). The enzyme reaction was initiated by adding catechol solution. then after exactly 1 min, the optical density was determined. Zero adjustment was against sample blank. The phenol oxidase activity was determined as O.D. units$\times 10^3$ at an absorbency of 405 nm.

5- Determination of chitinase Activity:

a. Substrate preparation:

Colloidal chitin was prepared according to Bade and Stinson, (1981) as follows: 4.0 gm of purified chitin powder (Sigma) were suspended in 100 ml water at 4°C and stirred in cold. Concentrated H_2SO_4 (30 ml) at 4°C was added dropwise to the suspension. The cold viscous chitin solution was filtered through glass wool into 1800 ml ice-cold 50% ethanol with rapid stirring. The precipitated colloidal chitin was washed with distilled water to pH 5. It was buffered with phosphate buffer (pH 6.5, 0.2 M) before use as a substrate.

b. Enzyme assay:

The reaction mixture according to Ishaaya and Casida (1974), with some modifications consisted of: 1 ml phosphate buffer (0.2 M, pH 6.5), 200 ml 0.5 % colloidal chitin and 200 ml enzyme solution. After 1.5-hour incubation at 37 ºc, enzyme activity was terminated by boiling test tube. Undigested chitin was sediment by centrifugation for 15 min at 8.000 r.p.m. The supernatant was taken for determination of N-acetylglucoseamine that produced as a result of chitin digestion by Chitinase.

Statistical analysis:

Data were statistically analyzed using the (F) test and the value of LSD (at 5 %) according to Gomez and Gomez (1984).

Results

Isolation and identification of the associated bacteria:

Six bacterial isolates were isolated from the cabbage leaf samples collected from three different governorates Table (1). The isolated bacteria were purified and identified as *Bacillus subtilis* (1-5), *Pseudomonas fluorescens* (2-3) and *Xanthomonas campestris* pv. *campestris* (4-6), respectively.

Table 1. Bacterial isolates from cabbage leaves collected from three governorates, during growing season

Governorate	Isolate code No.
Beheira	1
	2
Sharkiya	3
Qalubiya	4
	5
	6

Confirmation the identification and pathogenic test of X. c. pv. *campestris:*

The two strains (4-6) of the presumptive pathogen were yellow Gram negative of short rods producing Xanthomonadins, Table 2 shows that the tested isolates (Qalubiya 4 & Qalubiya 6) were positive for KOH 3 %, Starch hydrolysis, Catalase activity, Production of H_2S, Growth in 5% NaCl, utilization of Mannose and utilization of Succinic. On the other hand, these isolates gave a different reaction in utilizing the different carbon sources. Pathogenicity test of the two isolates (Qalubiya 4 & Qalubiya 6) of *X. c.* pv. *campestris* was carried out on cabbage seedlings (cv. Sabeany) and hypersensitivity test on tobacco seedlings. Results in Table 3 indicate that *X.c.* pv. *campestris* isolate No.6 of Qalubiya governorate resulted in the highest virulence of the infection to the leaves of the inoculated cabbage plants than isolate No.4. No infection was observed on the un-inoculated cabbage plants.

Table 2. Identification of the isolated bacteria from cabbage

Type of test	Type of reaction					
	Beheira1	**Beheira2**	**Sharkiya 3**	**Qalubiya 4**	**Qalubiya5**	**Qalubiya 6**
Gram reaction	+	-	-	-	+	-
Size	short	short	short	short	short	short
KOH 3 %	+	+	+	+	+	+
Production of pigments	-	+	+	+yellow	-	+yellow
Starch hydrolysis	+	-	-	+	+	+
Gelatin Liquefaction	+	-	-	-	+	-
Catalase activity	+	+	+	+	+	+
Pectate degradation	+	+	+	-	+	-
Fats hydrolysis	-	-	-	-	-	-
Methyl red test (M.R.)	-	-	-	-	-	-
Production of H_2S	-	-	-	+	-	+
Production of indole	-	-	-	-	-	-
Urease production	+	-	-	-	+	-
Nitrate reduction	+	-	-	-	+	-
Growth in 5% NaCl	-	+	+	+	-	+
Growth at 50°C	-	-	-	-	-	-
Levan test	-	+	+	-	-	-
Oxidase reaction	+	-	-	-	+	-
Potato rot	-	-	-	-	-	-
Arginine dihydrolase	+	-	-	-	+	-
Utilization from:						
Lactose	d	+	+	d	d	d
Dextrose	+	+	+	d	+	d
Sorbitol	+	+	+	-	+	-
Mannose	+	+	+	+	+	+
Succinic	-	+	+	+	-	+
Bacterial species	***B. subtilis***	***P. fluorescens***	***P. fluorescens***	***X. campestris***	***B. subtilis***	***X. campestris***

d = different reaction

Table 3. Pathogenicity test of the two isolates of *X. campestries* on cabbage seedlings (cv. Sabeany)

The tested isolates	Location	Type of test	
		Pathogenicity test	hypersensitivity Tobacco
X. campestries No.4	Qalubiya	++	+
X. campestries No.6	Qalubiya	++	+
* No infection was observed on the un inoculated cabbage seedlings.			
- = Avirulent	+ = Virulent	++ = high Virulent	

Inhibition effect of antagonists and fungicide on X. c. pv. *campestries in vitro:*

Results in Table 4 reveal that the two tested bioagents *i.e.*, *B. subtilis* and *P. fluorescens* and one fungicide *i.e.*, Tango caused great inhibition to the causal bacterium *X .c.* pv. *campestris*. *P. fluorescens* was the most efficient followed by *B. subtilis* which was the lowest in this regard, being 2.5 and 1.7 mm, respectively. The fungicide Tango, on the other hand, was the most effective in inhibiting the causal pathogen, being 3.6 mm at the recommended dose (at a rate of 2.5 ml/L), compared with the effect of used bioagents.

Table 4. Inhibition effect of antagonists and fungicide on *X.c.*pv. *campestries*, two days after incubation at 28± 2º *in vitro*

Treatments	Inhibition zone (mm)
Tango	3.6
P. fluorescens	2.5
Bacillus subtilis	1.7
Control	0.0
LSD at 5%	0.1264

Effect of some antagonists and fungicide on cabbage black rot in vivo:

Data in Table 5 reveal that all tested bioagents and Tango fungicide had a significant decreasing effect to black rot incidence and disease severity caused by *X.c.* pv. *campestries* during the two growing seasons 2014 under field conditions. In this respect, Tango fungicide treatment followed by *P. fluorescens* scored high significant decrease in disease incidence percentage (0.0 and 17.1%, respectively) and disease severity percentage (0.0 and 7.9%, respectively). Meanwhile, the bioagent *B. subtilis* was the lowest efficient treatment, being 29.6%. In general, the tested fungicide was more efficient in this regard than the bioagents (Efficacy%), where, Tango fungicide treatment followed by *P. fluorescens* scored the highest treatment efficacy in comparison with control.

Table 5. Effect of some antagonists and fungicide on cabbage black rot assessments *in vivo* under field conditions during 2014 and 2015 growing seasons

Treatment	Disease incidence percentage				Disease severity percentage			
	2014	2015	Mean	Efficacy %	2014	2015	Mean	Efficacy %
Tango	0.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0
P. fluorescens	16.6	17.6	17.1	79.34	7.7	8.1	7.9	90.30
Bacillus subtilis	29.0	30.2	29.6	64.25	19.2	20.0	19.6	75.95
Control	82.4	83.3	82.8	0.0	80.4	82.7	81.5	0.0
L.S.D at 5%	0.1548	0.1787	0.1488		0.2605	0.3996	0.2977	

Efficacy % calculated based on the mean of disease incidence and disease severity percentage in two seasons for comparison all tested treatments with a control.

Effect of some antagonists and fungicide on cabbage parameters under field conditions during 2014 and 2015 growing seasons:

As regard to the effect of applied treatments on cabbage vegetative parameters, data in Table 6 reveal that most of treatments significantly increased cabbage parameters as head weights, rosette diameter and edible leaves number during 2014 and 2015 growing seasons.

Table 6. Effect of some antagonists and fungicide on cabbage parameters under field conditions during 2014 and 2015 growing seasons

Treatment	Head weight (kg)			Rosette diameter (cm)			Edible leaves (No.)		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
Tango	5.45	5.34	5.39	106.3	102.6	104.4	20.0	19.0	19.5
P. fluorescens	4.54	4.28	4.41	85.0	85.3	85.1	17.0	16.0	16.5
B. subtilis	2.62	2.43	2.52	63.8	64.1	63.9	15.0	14.0	14.5
Control	1.40	1.45	1.42	74.0	74.3	74.1	13.0	12.0	12.5
L.S.D at 5%	0.0019	0.0019	0.0017	1.03	0.2189	0.6633	1.938	1.003	1.375

Results in Table 6 indicate that spraying cabbage plants with the tested bioagents, *i.e.*, *P. fluorescence*, *B. subtilis* and Tango fungicide four times resulted in a significant decrease in severity of the natural infection caused by *X. c.* pv. *campestries*, with a significant increase in the produced yield parameters compared to the control. In general, the tested fungicide was more efficient in this regard than the bioagents. In addition, Tango was the most efficient treatment for increasing heads weight (5.39 kg), rosette diameter (104.4 cm) and edible leaves number (19.5 leaf). Meanwhile, the bioagent *B. subtilis* was the lowest efficient treatment, head weight (2.52 kg), rosette diameter (63.9 cm) and edible leaves number (14.5 leaf),

respectively. The other treatments recorded intermediate figures. The plants of the control recorded 1.42 kg,74.1 cm and 12.5 leaf on the average respectively.

Effect of some antagonists and fungicide on biochemical alterations in cabbage:

Table 7 reveals that total phenol contents as well as the activity of peroxidase, polyphenoloxidase and chitinase in cabbage leaves infected by *X.c.* pv. *campestries* were higher compared with the uninfected ones. In this respect, the fungicide Tango resulted in the highest increase in the content of the total phenols (682.33 µg GAE/g fresh weight) compared with the other treatments except the control. The antagonistic *B. subtilis* caused recognized increase in the activity of the enzymes of peroxidase (229.67 µg fw/min/g), polyphenoloxidase (125.67 µg fw/min/g) and the enzymes chitinase (1637.00 µg NAGA x 10^3/min/g fresh weight) compared with the other treatments.

Table 7: Effect of some antagonists and fungicide on cabbage biochemical changes

Treatments	Vitamin C	Total Phenols	Peroxidase	Polyphenoloxidase	Chitinase
	µg A.A./g FW	Mg GA/DW	O.D 430 FW /min/g	O.D 405 FW /min/g	µg NAGA X 10^3/g FW
Tango	172.33	682.33	194.67	108.33	1495.33
P. fluorescens	120.67	421.67	121.00	116.00	1610.00
Bacillus subtilis	130.00	354.33	229.67	125.67	1637.00
Control*	219.33	1408.00	561.00	234.33	1641.00
L.S.D at 5%	1.68	1.038	1.86	1.922	1.91

*Uninfected plants by the causal bacterium.

The amount of vitamin-c in the leaves due to using Tango, *P. fluorescens* and *Bacillus subtilis* treatments reached 172.33,120.67 and 130.0 µg A.A./g fresh weight, respectively compared with the uninfected leaves. Uninfected plants by the causal bacterium (control) recorded high figures for total phenols, vitamin-c and the three enzymes.

Discussion

Symptoms of cabbage black rot caused by *Xanthomon campestris* pv. *campestris* appear in the field as V-shaped, chlorotic to necrotic lesions starting from leaf margins and blackening of the vascular tissue (Seebold *et al.,* 2008). Isolation trials from cabbage leaves showing typical symptoms of bacterial black rot collected from Qalubiya, Behera and Sharkiya governorates yielded 6 bacterial isolates. Isolated bacteria were purified and identified as *B. subtilis*, *P. fluorescens* and *Xanthomonas campestris* pv. *campestris.* Identification of two isolated strains bacterial have proven identity with *X.c*.pv. *campestris*. The two strains were identified and confirmed by morphological, biochemical and physiological tests as designated by (Schaad *et al*., 2001). The causal bacterium was isolated by the authors from cabbage plants showing bacterial black rot and conform with those of Wulff *et al.*(2002a); Griesbach *et al.* (2003); Bila *et al.* (2013); Maji and Nath (2015). Results

of pathogenicity test showed that *X.c*.pv. *campestris* isolate of Qalubiya 6 origin gave high virulent infection to the inoculated cabbage plants. Meanwhile, isolate of Qalubiya 4 origin resulted in the low virulence of the infection. No symptoms appeared on control plants. Re-isolations made from artificially infected plants yielded the bacterium originally inoculated. Field experiments through growing seasons 2014 and 2015 showed that spraying cabbage plants with the tested bioagents *i.e., B. subtilis, P. fluoresence* and fungicide Tango four times resulted in significant decrease in severity of the natural infection by bacterial black rot with a significant increase in the produced yield components compared with the control. In general, the tried fungicide was more efficient in this regard than the bioagents. Meanwhile, the bioagent *B. subtilis* was the lowest effective treatment and the others noted middle figures. While, Mguni (1996) and Assis *et al.* (1998), noted that using biocontrol under greenhouse and growth chamber conditions, they shown a reduction of black rot incidence up to 90.3% in cabbages treated with *Bacillus* spp., including the strain BB and in a 90-day field experiment conducted in Zimbabwe. Massomo *et al.* (2004) evaluated the efficacy of bacillus strains from Tanzania as a biocontrol against the plant pathogenic bacteria Xcc in cabbage and the effect of the method of an application under field conditions. They indicated that the disease incidence and disease severity of black rot in susceptible cultivar heads were significantly decreased. Umesha and Roohi (2017) showed that *P. fluorescens* was very efficient in reducing the disease as compared to INA. Thus, *P. fluorescens* could be used as a biocontrol agent, in the integrated disease management program to control black rot of cabbage. Abdel-Kader *et al.* (2013) evaluated the use of *P. fluorescens* on the foliar disease incidence of some vegetables under plastic house conditions in the greenhouse as a best biocontrol agent. Mishra and Arora (2012) confirmed that the rhizospheric strains of Pseudomonas managing black rot of cabbage and observed that Pseudomonas strain (TO7) isolated from infected brassica plant with *X. c.* pv. *campestris* was significantly inhibiting growth of (Xcc) *in vitro* and *in vivo.*

Stall *et al.* (1986) found that copper bactericides control the sensitive strains but the copper-tolerant strains were difficult to control with copper only. Although, the tolerant one are more successfully controlled by the mixture of a copper bactericide and mancozeb or maneb. Fixed copper is often in combination maneb and mancozeb have been the principle chemicals for managing bacterial spot caused by *X.c.pv. vesicatoria* on pepper (Mirik *et al.*, 2007). Similar results were obtained by Marco and Stall, 1983; Mc Carter, 1992 and Ju-Hee *et al.,* 2015. It has been found that the gotten data showed that vitamin-c and total phenols content as well as the activity of peroxidase, polyphenoloxidase and chitinase in cabbage leaves infected by *X.c*.pv. *campestris* and treated with the tested bioagents and fungicide were greatly higher than those of the control. Ascorbic acid or vitamin C, is the most common sulfite alternative. It acts as a reducing agent, preventing quinone accumulation by reducing it back to the original phenolic form before they are able to polymerize and form pigments (Queiroz *et al.*, 2008).

Regarding the activity of enzymes, data exposed that spraying of cabbage plants with the tried bioagents and fungicide resulted in considerable increase in the

activity of peroxidase, polyphenoloxidase and chitinase. It is known that these enzymes can play an important role in plant defense mechanisms against the infection by plant pathogens. Data displayed that the enzymatic activity in treated cabbage plants was increased than those in the control. Many plant enzymes are involved in defense mechanisms against plant pathogens. while other oxidation phenols contribute in the formation of defense barriers for reconstruction the cell structure (Avdiushko *et al.*, 1993). In addition, experimentally supported the idea that peroxidase and chitinase play a defense role against invading pathogens (Yang *et al.*, 2008 and Fan *et al.*, 2016). In addition, phenololic compounds in plants are among the most influential and widely distributed by-products. Such compounds have regulated disease resistance in many crop plants. There is a lot of research that establishes that a high-level phenolic material is positively proportional to the degree of resistance of plants against the diseases of plants. Phenolic compounds are ubiquitous secondary metabolites in plants, which are important in many aspects of plant life, especially during their interaction with the environment (Lattanzio, 2013). Some phenolics play an important role in plant defense responses to pathogen attacks.

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تقييم بعض العوامل الحيوية في مكافحة مرض العفن الأسود في الكرنب الذي تسببه بكتيريا زانسومونس كامبيستريز

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تم تقييم تأثير كلا من بكتيريا باسيلس سبتيلس وبكتيريا زيدومونس فلورسنس ومبيد التانجو في مكافحة مرض العفن الأسود في الكرنب الذي تسببه بكتيريا زانسومونس كامبيستريز في المعمل والحقل. وتعد بكتيريا زيدومونس فلورسنس هي الأكثر فاعلية في مقاومة البكتيريا المسببة للمرض في حين ان مبيد التانجو هو الأكثر فاعلية في منع تطور وانتشار البكتيريا الممرضة للكرنب في الحقل ويلي ذلك الكائنات المضادة. ففي التجارب الحقلية التي أقيمت في الموسم الزراعي خلال عامي ٢٠١٤/٢٠١٥ م أوضحت النتائج ان رش نباتات الكرنب بالكائنات المضادة المختبرة والمبيد المستخدم أربع مرات ادت الي نتائج معنوية في اختزال المرض المتسبب عن العدوي الطبيعية بالبكتيريا الممرضة للكرنب بالإضافة إل زيادة ملحوظة في القياسات المختلفة للمحصول مقارنة بالنباتات الغير معاملة. وكان المبيد أكثر فاعلية عن الكائنات المضادة حيث يعتبر مبيد التانجو هو الأكثر فاعلية في تقليل الإصابة بالمرض وزيادة القياسات الخضرية للمحصول في حين ان الكائنات المضادة مثل باسيلس سبتيلس هي الأقل فاعلية لمقاومة المرض ولكن بعض المعاملات الأخرى تعتبر ذات فاعلية متوسطة. وجد ان محتوي أوراق الكرنب المصابة ببكتيريا زانسومونس كامبيستريز من فيتامين سي والفينولات الكلية ونشاط انزيمات البيروكسيديز والبولي فينول اوكسيديز والشيتينيز كانت اقل بكثير من الأوراق غير المصابة. كما أدت العوامل البيولوجية المختبرة والمبيد الفطري إلى زيادة كبيرة في هذه المحتويات مقارنة بالأوراق المصابة.

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