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CONTROLLING CABBAGE FUSARIUM WILT (YELLOWS) USING TOPSIN M AND SOME COMMERCIAL BIO-FERTILIZER PRODUCTS

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ABSTRACT: Cabbage is one of the most widely cultivated vegetables in Egypt; Fusarium wilt causes significant crop loss in quantity and quality of most vegetable crops. The most frequency and isolated fungus from cabbage roots growing at Faquose District, Sharkia Governorate, Egypt was *Fusarium oxysporum,* followed by *Verticillium albo-atrum*. As for pathogenic activities of *Verticillium albo-atrum* and *Fusarium oxysporum* isolates on cabbage plants (cv. Balady), *Fusarium oxysporum* isolate No.5 gave the highest wilt disease severity. Host range of the tested *Fusarium oxysporum* isolate 5 revealed that cabbage (cv. Balady) was the only infected host. Field experiments were conducted to study the effect of Topsin M70%, Microbin, Rhizobactrin, and Weed-Max (blue-green algae extracts in powder phase) and Oligo-X algae (blue-green algae extracts in liquid phase) on vegetative growth (head diameter and stem height), total yield and wilt disease severity on cabbage (cv. Balady), during the two winter growing seasons of 2015 and 2016 in Sharkia Governorate at Faquose District. Results showed that both head diameter and total yield were increased by Topsin M70% and Microbin application followed by Weed-Max as well as Oligo-X, but the lowest one was Rhizobactrin compared with control. Also, wilt disease severity was decreased without significant effect on stem length. Regarding the effects of applied treatments on biochemical changes in cabbage plants (cv. Balady) under field conditions, all tested treatments reduced the activities of polygalacturonase (PG) and cellulase (CX) enzymes as well as, increased total phenols and total chlorophyll compared with control. In this respect, Topsin M70% and Microbin followed byWeed-Max as well as Oligo-X were the most effective treatments in reducing the activities of PG, CX and increased total phenol contents compared with Rhizobactrin treatment and control.

Key words: Cabbage, Fusarium wilt, chemical control, bio-fertilizers, algae

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important and popular cruciferous vegetables during cold seasons in temperate climates. Cabbage belongs to Brassicaceae family, cultivated for their leaves which are rich in nutrients such as niacin, vitamin C, minerals, fiber and medium quantities of calcium element. Cabbage and other cruciferous have been recognized as important sources of phytochemicals in the diet (**Anil *et al.,* 2018)**. The cultivated area of cabbage in Egypt in 2017 reached about 38,892 faddan, which produced 485,739 ton, with an average production of 12,489 ton/fad. (**Anonymous**, **2017**).

Previous studies reported that wilt diseases are the most widespread and destructive soil-borne diseases, which attack a large number of vegetable species throughout the world. The symptoms expressed when the pathogens infect the roots of susceptible plants and plug the water conducting tissues. Due to vascular wilt diseases 2-90% yield loss have been recorded in wide range of crops (**El-Mougy *et al.,* 2011**).

Soil-borne pathogens, including, *Fusarium oxysporum* f. sp. *conglutinans,* the causal agent of cabbage Fusarium wilt, is a worldwide threat to cabbage production, resulting in severe economic losses and represent major source of biotic stress in the rhizosphere and roots of

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plants **(Booth, 1971)**. Most root pathogens are necrotrophic, that is kill host tissue with toxins, peptide elicitors, or enzymes that trigger host cell lysis and death, thereby providing conditions favorable to pathogen growth. Management of soil-borne diseases are continual challenge to growers, because these organisms live in or near of the rhizosphere and can frequently survive a long period in soil through the formation of resistant survival structures. Also, the structural, physical, and biological complexity of the soil micro-ecosystem in which pathogens interact with plant roots inherently limit the options available for disease control **(Katan, 2017; Milica *et al.,* 2017).**

Currently, chemical control with fungicides is the most effective method for controlling the wilt fungi which can live free in the soil for many years. Application of chemical fungicides has many serious side effects and causes imbalances in the ecosystem if not properly managed. Moreover, the extensive use of fungicides led to develop fungi resistant to fungicides in many areas around the world **(Brent and Holloman, 1998; Alam *et al.,* 2010)**.

Bio-fertilizers play an important role in improving fertility and structure of the soil, plant tolerance to abiotic and biotic factors, secretion of plant growth regulators which help in plant growth, yield production and improve thier quality, protect the plant against attack by pathogens and also it eco-friendly as well as cost effective compared to synthetic fertilizer inputs **(Itelima *et al.,* 2018)**.

The objective of this paper aimed to assess wilt occurrence and to evaluate the effect of certain bio-fertilizers compounds as single treatments on controlling the disease as well as their effects on growth parameters of cabbage. Also the compatible of bio-fertilizer with integrated pest management programs to reduce reliance on fungicides was studied.

MATERIALS AND METHODS

Sampling

Cabbage plants showing typical symptoms of Fusarium wilt disease were collected from three fields located in Faquose District, Sharkia Governorate, Egypt during the winter growing season showing the rotten roots system, and the discoloration of the vascular system .When the stem was cut across at soil level, a brown stain is seen in the vascular system; often this browning is more obvious on one side.

Isolation, Purification and identification of the isolated fungi

Lower stem portions (3 cm long) of each of the collected wilted plants which exhibited different degrees of vascular discoloration were used for isolation of cabbage wilt fungus. Plant samples were thoroughly washed under running tap water, cut into small pieces (0.5 cm), and surface sterilized with dipping in 0.1% sodium hypochlorite for 2 minutes, then washed three times with sterile distilled water. The surface disinfected pieces were dried under laminar flow hood then transferred individually to Petri plates containing potato dextrose agar (PDA) medium and incubated at 25°C for 7 days. The developing fungal colonies were purified by the single spore technique **(Pegg and Brady, 2002; Leslie and Summerell, 2006)**. Identification of choosing *Fusarium* isolates was confirmed at the level of species. In Mycology Research and Disease Survey Dept., Plant Pathology Research Institute, ARC, Giza, Egypt. Formae speciales were identified according to their ability to induce wilt symptoms on different plants of Brassicaceae and other families. The frequency of the isolated fungi was calculated according to the following formula:

$$\text{Fungal frequency percentage} = \frac{\text{No. of isolates of each fungus}}{\text{Total No. of all isolates}} \times 100$$

Pathogenicity Test

Based on frequency number and frequency percentage of the isolated fungi, *Fusarium oxysporum* (nine) and *Verticillium albo-atrum* (three) isolates, were selected to represent the different three fields located in Faquose District, at Sharkia Governorate to be tested for their pathogenic abilities under pot experiment at the Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza.

In this respect, Koch's postulates were carried out successfully to confirm the virulence of the tested *Fusarium oxysporum* and *Verticillium albo-atrum* isolates on healthy transplants of cabbage cv. Balady (Six week old) planted in plastic pots (25 cm Ø), each containing 3 Kg sandy clay soil 1:2 *V/V*. Transplanting and inoculation were carried out as following:

Preparation of *Fusarium oxysporum* and *Verticillium albo-atrum* inocula

Each of the chosen *Fusarium oxysporum* isolates were multiplied on sand corn medium according to **Leslie and Summerell (2006).** Sand and corn grains were mixed at the rate of 1:3 (25.0 g clean washed sand; 75.0 g corn grain)، moistened to 50 percent moisture content, filled in 500 ml glass bottles and then autoclaved at 120ºC for two hours. Fungal discs (5 mm) of pure cultures of each of the tested *Fusarium* isolates were used to inoculate the prepared sand corn grain bottles prior to incubation at room temperature (23± 2°C) for 14 days. These *Fusarium* inocula were used in pathogenicity test trial. For purpose of *Verticillium albo-atrum* artificial inoculation, conidial suspension was adjusted to $6x10^6$ conidia/ml according to **Abdel Ghafour (1989).**

Cabbage transplanting and pathogen inoculation

Cultures of *Fusarium* isolates incubated for 14-days on sand corn medium were used in inoculation prosses as follows : aseptic plastic pots (25 cm) were filled with 3 Kg/pot of previously sterilized sandy clay soil (1:2 *W/W*) with 5% formalin solution, left for 7 days then inoculated with the prepared inocula of *F. oxysporum* at the rate of 3% (*W/W*). Cabbage seedlings were inoculated with *V. albo-atrum* by dipping the roots for 24 hr in the conidial suspension. Pots were irrigated regularly for one week before transplanting with healthy transplants of cabbage cv. Balady (**Whitehead, 1957)**. Six weeks old transplants of cabbage cv. Balady were planted into the previously inoculated pots with the tested isolates at the rate of one transplant/pot. Seedlings immersed for 24 hr in sterile distilled water were transplanted in pots filled with sterilized soil only served as control treatment. The inoculated and un-inoculated pots were irrigated two times weekly. Five replicated pots were used for each treatment. The treatments were arranged in a completely randomized block design. Plants were observed for cabbage wilt symptoms after 3 months from transplanting. Wilt disease severity assessment was calculated according to **Song *et al.* (2004)**.

Disease Assessments of Fusarium Wilt

Three months post transplanting inoculated plants in pathogenicity test, experiment under greenhouse conditions and at the harvesting time in field, cabbage plants were investigated for cabbage wilt disease severity (DS) and some plant growth characters (in field experiment). The wilt disease severity (DS) was also determined according to the following visual scale and description as suggested by **Grattidge and O'Brien (1982).** Plants were uprooted and the lower stem and tap root were longitudinally dissected for examination of internal tissues discoloration. The wilt disease severity (%) (WDS%) was determined and calculated using the following formula of **Song *et al*. (2004):**

$$\text{Wilt disease everity (\%)} = \left(\frac{\sum (nXv)}{5N} \right) \times 100$$

Where:

n = number of infected plants, v= Numerical values of symptoms category, N= total number of plants, 5= maximum numerical value of symptoms catagories.

Where:

0 :No discoloration, 1: 1 to 10% discoloration, 2: 11 to 30% discoloration, 3: 31 to 50% discoloration, 4: 51 to 75% discoloration and 5: 76% to 100 discoloration.

The highest virulent isolate of the tested *Fusarium oxysporum* was isolate 5 which was used in further studies.

Host Range of *Fusarium oxysporum* Isolate 5

This trial was done under greenhouse conditions where, many plant species belong to three different families were evaluated against the highest virulent isolate of *Fusarium oxysporum*. The three different families were :

1- Solanaceae; tomato (cv. Super Strain B) and pepper (cv. California)

2- Cucurbitaceae; squash (cv. Eskandrane) and cucumber (cv. Beta Alfa)

3- Cruciferaceae; broccoli (cv. Heriklation), cauliflower (cv. White Magic) and cabbage (cv. Balady).

The seeds of broccoli (cv. Heriklation) and cauliflower (cv. White Magic) were obtained from Sakata Seeds Company, while the rest of all seeds were obtained from Horticulture Research Institute, ARC, Egypt. In this respect, *Fusarium oxysporum* inocula which were used for inoculation and the transplants which represent different families were prepared as mentioned before. The reaction of the tested plants was indicated by (+) for the infected host plants and (-) for the non-infected ones.

Control of Cabbage Wilt under Field Conditions

The source of controlling materials

Commercial algae extracts, Weed-Max (blue-green algae extracts in powder phase) and Oligo-X algae (blue-green algae extracts in liquid phase) were obtained from Arabian Group for Agricultural Service, 114 King Fysal Street, Giza, Egypt. Commercial bio-fertilizer, Microbin, Rhizobactrin and the Fungicide Topsin M 70% were obtained from Agricultural Research Center (ARC), Giza, Egypt (Table 1).

Application of controlling materials

All previously mentioned controlling materials at the above mentioned dose (Table 1) were used as a root dipping treatment for the transplants of cabbage for 30 minutes before transplanting. Also applied as a soil drench (200 ml/plant) after 4 and 8 weeks from transplanting.

Field Experiment

Field experiments were carried out during two successive winter growing seasons 2015 and 2016 in previously known naturally infested field with *Fusarium oxysporum* f. sp. *conglutinans* in Faquose District at Sharkia Governorate. Randomized complete block design with four replicates was used .The plot was 7 x 6 m^2 (42 m^2= 1/100 fad.). Each plot included 4 ridges and 10 transplants. Six weeks old healthy transplants of cabbage cv. Balady were transplanted on 27th of September, 2015 and 2016 on one side of the ridge at 50 cm spacing. The recommended agricultural practices for cabbage crop were applied. The disease severity was calculated as mentioned before. At the harvest time in 2015 and 2016 growing seasons. The agronomic measurements included head diameter (cm)/ plant, stem height (cm) and total yield (ton/ faddan) were calculated.

Biochemical Changes in Cabbage Plants under Field Conditions

Three months post transplanting of cabbage plants, samples representing the whole plant were taken from each treatment for determining total phenols in addition to cellulase (CX), polygalacturonase (PG) as degrading enzymes and Total chlorophyll (SPAD unit).

Determination of phenolic contents

For total phenols determination, whole fresh plant samples were extracted separately according to **Aneja (2001)**. Total phenols were calculated for each treatment as milligrams of catechol/g fresh weight of the whole plant. The developed color was measured at 520 nm using Spectrophotometer against a reagent blank.

Cellulase (CX) and polygalacturonase (PG) enzymes determination

For determining the degrading enzymes, the crude enzyme extract of the whole plant for each treatment was prepared as recommended by **Aneja (2001)**. Viscosity of the reaction mixture was estimated before incubation (zero time), and after 30 minutes incubation. Loss in viscosity was calculated according to this formula:

Loss in viscosity (%) =T0 – T1/T0 – Tw x 100

Where:

T0 = the time of flow in seconds of the treated mixture at zero time, T1 = the time of flow at a given time interval and Tw = the time of flow at distilled water.

Concerning PG activity determination, 1.2% pectin substrate was added to phosphate buffer solution at pH 5.6 then 2.5 ml of the crude enzyme sample were added to 5 ml buffer and incubated at 30°C. Viscosity of the reaction mixture was estimated before incubation (zero time), and after 30 minutes incubation as mentioned before in CX activity determination.

Table 1. Name, composition and rate of controlling materials of treatments

Name	Composition	Recommended dose in field
Fungicide	Topsin M70% (Thiophanate-methyl)	1 g/L
Biofertilizer	Microbin (*Pseudomonas* spp*, Azotobacter* spp*, Bacillus* spp and *Rhizobium)*	10 ml/L
	Rhizobactrin (*Azotobacter* spp*, Rhizobium* and *Azospirillum* spp)	10 ml/L
Alage	Weed-Max (blue-green algae extracts in powder phase)	2 g/L
	Oligo-X (blue-green algae extracts in liquid phase)	2 ml/L

Determination of total chlorophyll

Total chlorophyll content was measured in fresh leaves determined by using Minolta chlorophyll meter SPAD – 501 as SPAD units (**Yadava, 1986**).

Statistical Analysis

All obtained data were analyzed using analysis of variance (ANOVA) among treatments. Means were compared by the least significant differences (LSD) at p ≤0.05 as described by **Song and Keane (2006)**.

RESULTS AND DISCUSSION

Frequency of Isolated and Identified Wilt Pathogenic Fungi from Cabbage Plants

Results in Table 2 show that 12 fungal isolates were isolated from wilted plants of cabbge collected from three fields located in Faquose District, at Sharkia Governorate. These isolated fungi were identified as *Fusarium oxysporum* nine isolates (3 isolates from each field) and *Verticillium albo- atrum* three isolates (one isolate from each field). Most of the unidentified isolates were non- spore- producing forms.

Fusarium oxysporum was the most dominant among those fungi where it recorded the highest frequency, (65.82%) followed by *Verticillium albo-atrum* (20.71%) Similar results were obtained by **Schnathorst (1981), Okungbowa and Shittu (2012) and Khafagi *et al.* (2018)**.

Pathogenicity Test

The nine pathogenic isolates of *Fusarium oxysporum* was tested for their pathogenic activities on cabbage plants cv. Balady (Tabel 3). In this respect, the *Fusarium oxysporum* isolate 5 gave the highest disease severity (38.3%) followed by isolate 8 (23.6%) and isolate 7 (19.8%). On the other hand, isolate 9 recorded the lowest one (11.1%). Whereas, the three *Verticillium albo-atrum* isolates recorded the lowest disease severity (9.8, 9.3 and 8.6%, respectively).

Vascular wilts are the most important diseases of vegetables and they are caused by pathogenic fungi belonging to *Fusarium* and *Verticillium* genera. These pathogens are a challenge to control because they often survive in soil for long periods and affect the crops throughout the year from across the plant families and the main mode of infection in infected soil. The disease symptoms caused by each pathogenic fungus are often creating confusions **(Agrios, 2005)**.

When healthy plants are grown in infested soil, germ tube of the spore or the mycelium penetrates the root tips directly or enters the root system through wounds or at the point of formation of lateral roots and colonize the cortex of the root, and when it reaches the xylem vessels it enters through the piths. Then the mycelium remains exclusively in the vessels and travels through them, mostly towards the stem and crown of the plant. While, spores or conidia germinate and penetrate the vessels wall and enters into the vessels. Due to this infection the host plant fails to uptake water and nutrition from soil, resulting process is called “vessel clogging”. The leaves of infected plant transpire water more than the water transport by root and stem. Finally, stomata in the leaves will be closed and wilted, and soon followed the death of whole plant **(Koike *et al.*, 2003; McGovern, 2015; Katan, 2017**).

Table 2. The frequency percentage of fungi isolated from cabbage samples collected from Faquose District of Sharkia Governorate

Isolated fungus	Frequency (%)
Fusarium oxysporum (9 isolates)	65. 82
Verticillium albo-atrum (3 isolates)	20.71
Unidentified fungi	13.22

Table 3. Pathogenicity tests of the nine isolates of *Fusarium oxysporum* and 3 isolates of *Verticillium albo-atrum* under greenhouse conditions

Isolate	Code	Wilt disease severity (%)
Fusarium oxysporum	1	13.6
	2	12.3
	3	17.3
	4	16.1
	5	38.3
	6	14.8
	7	19.8
	8	23.6
	9	11.1
Verticillium albo-atrum	1	9.8
	2	9.3
	3	8.6
Control	-	00.0
LSD at 5%		**0.898**

Host range

Results in Table 4 indicate that no symptoms were observed on the Cruciferaceae (Broccoli and Cauliflower), Solanaceae and Cucurbitaceae hosts. Meanwhile, only a typical symptom of Fusarium wilt was detected on Cabbage plant, cv. Balady. Similar results were obtained by **Song *et al.* (1996)**.

Depending on the results of the present work, it is clear that the main causal of wilting symptoms observed on cabbage plants is *Fusarium oxysporum* f. sp. *conglutinans*. Hence, control experiments were carried out using the fungicide Topsin M70% and some bio-fertilizers (Weed-Max, Oligo -X, Microbin and Rhizobactrin) to control the disease.

Control of Cabbage Wilt under Field Conditions

To minimize the crop yield losses due to vascular wilt diseases, approaches like use of bio-control agents through using some commercial bio-fertilizers, are to be included in the disease management practices.

Results in Table 5 indicate that, all treatments significantly reduced the percentages of disease severity. The most effective treatments were Topsin M70% and Microbin followed by Weed-Max as well as Oligo -X. The lowest effect was recorded for Rhizobactrin during the two growing seasons. Similar results were obtained by several workers **(Abdel-latif *et al.*, 2001; Wszelaki and Matthew, 2003; Radovich *et al.*, 2004; El-Sharkawy, 2015)**.

Table 4. Host range of *Fusarium oxysporum* isolate 5 under greenhouse conditions

Host	Cultivar	Reaction
Tomato	Super Strain B	-
Pepper	California	-
Squash	Eskandrane	-
Cucumber	Beta Alfa	-
Broccoli	Heriklation	-
Cauliflower	White Magic	-
Cabbage	Balady	+

Table 5. Effects of different commercial bio-fertilizers and Topsin M 70% on wilt disease severity in 2015 and 2016 growing seasons under field conditions

Treatment	Wilt disease severity (%)	
	2015	2016
Topsin M70%	16.0	13.3
Microbin	24.0	22.7
Rhizobactrin	33.7	32.7
Weed-Max	27.3	26.7
Oligo-X	30.7	29.7
Control	41.3	45.3
LSD at 5%	7.1	7.3

Concerning the effect of the commercial bio-fertilizer products and synthetic compound (Topsin M70%), treatments of dipping transplants and soil applications on total yield and head diameter, results presented in Table 6 indicate that, there were significant differences among the treatments and the control. It is obvious that the highest values of total yield and head diameter were obtained in the first and the second growing season due to treatment by Topsin M70% (11.63 ton/fad. and 26.11 cm in 2nd season), respectively. However the lowest one was Rhizobactrin (6.55 ton/fad. and 21.23 cm in 2nd season), respectively. **El-Mougy and Abdel-Kader (2013)** evaluated the efficiency of two commercial cyanobacteria compounds, Weed-Max and Oligo- Mix, against *Alternaria solani, Sclerotium rolfsii*, *Fusarium oxysporum, Rhizoctonia solani* and *Fusarium solani* and found that they reduced the infection and improved crop yield. However, the detected increment over the treatments was not great enough to reach the level of significance for stem- length. The negative effects of treatments on stem- height might be attributed to the physiological factors special to the cultivar or soil (**Wien and Wurr, 1997**).

The superior effect of Topsin M70% may be due to that fungicides work in a variety of ways. Only limited information suggests that fungicides directly act as activators of resistance, but most of them damage fungal cell membranes or interfere with energy production within fungal cells by interfering with critical cellular processes .So fungicides inhibit fungal growth because it usually includes specific

Table 6. Effect of different commercial bio-fertilizers and Topsin M 70% on the vegetative growth and total yield in 2015 and 2016 growing seasons under field conditions

Treatment	Head diameter (cm)		Stem height (cm)		Total yield (ton/fad.)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Topsin M70%	25.13	26.11	14.67	5.451	10.65	11.63
Microbin	24.89	25.30	13.55	13.97	9.56	10.11
Rhizobactrin	21.15	21.23	2.001	12.30	6.87	6.55
Weed-Max	23.11	24.15	13.15	13.67	9.38	10.85
Oligo-X	22.00	22.97	12.42	12.50	8.59	9.24
Control	19.88	19.47	14.70	14.82	5.60	5.35
LSD at 5%	2.3	2.5	NS	NS	2.7	2.9

NS = Not significant

molecular targets to which the drug binds, such as an enzyme or receptor. Receptor sites have specific affinities for drugs based on the chemical structure of the drug **(Amini and Sidovich, 2010)**. Moreover, **Abdel-Kader *et al.* (2014)** mentioned that Topsin-M70 had the superior effect on wilt incidence (%) of lupine plants comparing with the control treatment under field conditions. Also, **Parsa *et al.* (2013)** confirmed that Topsin M at 0.1% concentration was significantly effective in reducing the disease incidence of Fusarium wilt on vegetable crops especially the eggplant.

The application of bio- fertilizers Microbin (*Pseudomonas* spp.*, Azotobacter* spp.*, Bacillus* spp.*, Rhizobium* spp.), Rhizobactrin *(Azotobacter* spp.*, Rhizobium* spp.*, Azospirillum* spp.) and algae (blue green algae) Weed-Max (powder) and Oligo-X (liquid), when applied as seed or soil treatment, contributes in nutrient cycling and increases crop productivity. Generally, 60 to 90% of the total applied fertilizer is misplaced and the residual 10-40% is taken up by plants. Therefore,bio-fertilizers can be considered as main factor of integrated nutrient management systems for supporting agricultural productivity and a healthy environment by improving fertility and structure of the soil and minimize the sole use of chemical fertilizers **(Adesemoye and Kloepper, 2009)**.

Inoculation with bio-fertilizers, *i.e. Azotobacter* and *Rhizobium* and Vesicular Arbuscular Mycorrhiza gave the highest increase in straw and grain yield of wheat plants with rock phosphate as phosphate fertilizer (**Ritika and Uptal, 2014)**.

Rhizobium spp., *Azospirillum* spp. and blue-green algae; are working by fixing atmospheric nitrogen and converting them to organic (plant usable) forms in the soil and root nodules of legumes, thereby making them available to plants. Nitrogen fixing bio-fertilizers are crop specific bio-fertlilizers (**Choudhury and Kennedy, 2004)**.

Bacillus spp., *Cyanobacteria* (*Azotobacter*) and *Pseudomona*s spp. work by solubilizing the insoluble forms of phosphate in the soil, so that plants can use them. Phosphorus in the soil occurs mostly as insoluble phosphate which cannot be absorbed by plants (**Gupta, 2004)**.

Values of growth, yield and quality parameters of certain plants were significantly increased with bio-fertilizers containing bacterial nitrogen fixers, phosphate and potassium solubilizing bacteria and microbial strains of some bacteria (**Khosro and Yousef, 2012)**.

Results in Table 7 show that the tested treatments, *i.e.* Topsin M70%, Microbin, Rhizobactrin, Weed-Max and Oligo-X reduced the activities of polygalacturonase (PG) and cellulase (CX) enzymes and increased total phenols as well as total chlorophyll (SPAD unit). The obtained results indicated that Topsin

Table 7. Effects of different commercial bio-fertilizers and Topsin M 70% on total chlorophyll (SPAD unit), total phenols (mg/g) and enzyme activity {Cellulase (CX) and Polygalacturonase (PG)} (%) on cabbage in 2015 and 2016 growing seasons under field conditions

Treatment	CX (%)		PG (%)		Total phenols (mg/g)		Total chlorophyll (SPAD unit)	
	2015	2016	2015	2016	2015	2016	2015	2016
Topsin M70%	47.5	41.5	21.4	20.7	5.72	5.817	46.3	46.7
Microbin	43.2	35.4	23.7	23	4.995	5.372	45.8	45
Rhizobactrin	30	25.1	32.2	32.3	4.12	4.139	37.2	38.7
Weed-Max	35.6	42	24.3	23.9	4.85	5.389	43.9	43.9
Oligo-X	27.1	35	25.2	26.6	4.735	4.779	41.1	41.6
Control	62.1	62.2	77.2	76	3.228	3.515	31.3	32.8
LSD at 5%	1.58	1.67	1.45	1.48	0.59	0.41	1.00	0.93

M70% had the best effect in reducing the activities of PG, CX and increasing total phenol contents as well as total chlorophyll (SPAD unit) followed by Microbin and Oligo-X comparing with the Rhizobactrin and control treatments.

These results could be discussed considering the relationship between the successful plant infection with pathogens and cell wall degrading enzymes. Development of Fusarium wilt disease symptoms in effective treatments activated polygalacturonase (PG) and cellulase (CX) enzymes more than of control treatment **(Retig and Lisker, 1975)**.

Effects of fungicides on production of cell wall degrading enzymes were investigated by **Dwivedi and Singh (2015)** who studied the efficacy of two fungicides, *i.e.* Roco (thiophanate methyl 70% WP) as a systemic fungicide and chlorothalonil as a non-systemic fungicide on two pathogens *Fusarium solani* and *Fusarium oxysporum* f.sp. *lycopersici*, on producing of cellulytic and pectinolytic enzyme" *in vitro* and *in vivo*. The production of cellulytic and pectinolytic enzymes was reduced by the two tested fungicides.

It is well known that bio-fertilizer plays an important role in controlling plant diseases, increasing yield and improving the growth and quality of treated plants due to its ability for inducing plant resistance and activating plants to produce more phenolic compounds that inhibiting the growth of pathogen. This inhibition could be attributed to antibiosis, hyperparasitism, production of phenolic compounds inside plants or production of polygalacturinase, cellulase, enzymes which degrade the cell wall and leading to lyses of mycelium of the pathogen **(Weyens *et al.*, 2009)**. Where, phenol content is known to play a key role in plant resistance and acts as a defense mechanism against the invasion of plant pathogens. The obtained results are in agreement with those obtained by **El-Metwally *et al.* (2010)** who reported that Bio-Arc and Bio-Zeid reduced the severity of chocolate spot disease on faba bean, also total chlorophyll reached maximum values with using Bio-Arc and Bio-Zeid. An increase in chlorophyll content has been thought to be due to an increase in the number of chloroplasts in stressed leaves.

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مقاومة الذبول الفيوزاريومي (الإصفرار) في الكرنب باستعمال مبيد التوبسين وبعض مركبات الأسمدة الحيوية التجارية

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الكرنب أحد أهم الخضروات التي تزرع على نطاق واسع في مصر، تتسبب أمراض الذبول في خسارة كبيرة في المحصول من حيث الكمية والنوعية، ووفقًا لدراسة تكرارية الفطريات المعزولة من جذور الكرنب في محافظة الشرقية في ثلاث مناطق بمركز فاقوس، وجد أن الاكثر تكرارا هو فطر *Fusarium oxysporum* ، يليه *Verticillium albo-atrum* وهو الأقل تكرارا، تم عمل اختبار القدرة المرضية لثلاث عزلات من فطر الفرتيسليوم وتسع عزلات من فطر فيوزاريوم أكسيسبوروم الأكثرتكرارا علي شتلات الكرنب صنف بلدي فكانت عزلة فيوزاريوم أكسيسبوروم رقم ٥ هى الأكثر قدرة مرضية، كما أظهرت دراسة المدى العوائلى لعزلة فيوزاريوم أكسيسبوروم رقم ٥ أنها كانت ممرضة فقط لنباتات الكرنب صنف بلدي، تحت ظروف الحقل خلال الموسمين الشتويين 2015 و2016 تم أستخدام التركيز الموصي به للمواد المختبرة لدراسة تأثير المبيد توبسين والمركبين التجاريين الميكروبين والريزوباكتيرين كأسمدة حيوية ومركبين ويد- ماكس والأوليجو– أكس كأسمدة حيوية في صورة مستلخصات طحالب وهذه المواد معروفة بأنها محسنات للتربة الزراعية لمكافحة مرض الذبول (الأصفرار) في الكرنب صنف بلدي، أدت معاملة الكرنب بمركبي الميكروبين والريزو باكتيريين وكذلك مركبي الطحالب ويد- ماكس والاوليجو – أكس والمبيد توبسين سواء بغمر جذور الشتلات قبل الزراعة أو بالإضافة الأرضية لهذه المواد الي الأثر الإيجابى والفعال فى خفض شدة الإصابة بمرض الذبول (الإصفرار) في الكرنب مع وجود اختلافات معنوية فيما بينهم خلال موسمى النموبينما كانت معاملة النباتات بمبيد التوبسين أكثر فاعلية حيث خفضت شدة الإصابة بالذبول مقارنة بالكنترول؛ كما أدت المعاملات إلى حدوث زيادة معنوية فى بعض مواصفات المحصول كطول الساق وقطر الرأس وكذلك المحصول الكلي، أدت المعاملات إلي زيادة تركيز محتوى أوراق الكرنب من الكلورفيل مقارنة بأوراق نباتات الكنترول وخفضت كل المعاملات من نشاطات انزيمى البولى جالاكتورينيز والسليوليز و زادت من كمية الفينولات الكلية مقارنة بالكنترول وفي هذا الصدد كان أفضل المعاملات مبيد توبسين ومركب الميكروبين يليهما مركبي الطحالب ويد- ماكس والأوليجو- أكس بينما كان أقلهم المركب ريزو باكتيريين، وبصفة عامة أثبتت الدراسة إمكانية أستخدام بعض المواد التجارية الأمنة مثل الميكروبين والريزوباكتيرين وايضا مركبي الطحالب ويد- ماكس والأوليجو- أكس كأسمدة حيوية بديلاً عن مبيد توبسين لمكافحة مرض الذبول (الاصفرار) في الكرنب.

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