BIOLOGICAL CONTROL OF FUSARIUM WILT DISEASE IN SWEET BASIL PLANT

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ABSTRACT

Ocimum basilicum L. (Sweet basil) infected with Fusarium oxysporum was studied from three Governorates (Assuit, Beni-Suif and Fayoum); the highest infection of the basil presented in Beni-Suif Governorate. There are four treatment for control of basil wilt disease, fungicide (Topsin M (1.5 and 3 mg/L), bioagent (Bioark (2.5 and 5 mg/L), silicon (potassium silicate 2 & 4 mg/L) and nanoparticles (Lemon grass 8 ml/L & Citronella 8 ml/L). Topsin M (3 mg/L) had the highest significant in increasing of Ocimum length infected with Fusarium in two seasons (2nd season (39.75cm than 1st season 39.00 cm), where the nano Citronella 8 ml/L gave the lowest efficiency on the plant length in two seasons (34.23&34.82cm) for 1st and 2nd seasons. Bioagent (Bioark 5mg/L) considered the most significant treatment in increasing the branches fresh weight (40.53&41.32 mg) for 1st and 2nd seasons, while the highest effect of bioagent for branches dry weight (12.65 &13.20 gm) for 1st and 2nd seasons. Root length, root fresh weight and root dry weight; bioagent (Bioark 5 mg/L) gave the highest effect on root length (12.43&12.98 cm) root fresh weight (15.48 &16.01mg) and root dry weight (6.68 &6.94 mg) for first and second season respectively. Finally, essential oil content in Ocimum tissues infected with Fusarium was highest using Bioark 5 mg/L treatment into two seasons (0.07mg) for first and second seasons.

Keywords: Ocimum basilicum, Fusarium oxysporum, Topsin M, Bioark, potassium silicate, Lemon grass, Citronella

INTRODUCTION

Ocimum basilicum, popular known as Basil or Sweet Basil, is a common herb that belongs to Lamiaceae family. Studies have shown many pharmacological effects in several diseases, with potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties (Sakr and Al-Amoudi, 2012). Studies have reported that rosmarinic acid (RA) is the most biologically active compound present in Basil related to this activities (Javanmardi *et al*., 2002; Lee and Scagel, 2009; Shiga *et al*., 2009).

Ocimum species is cultivated for medicinal purposes, and for religious belief. It has long been documented as a diverse and rich source of essential oils. At the same time, it is used in cooking for its flavor and fragrance, so the fresh or dried leaves add to many foods, such as rice, pasta, and salads (Mabey *et al*., 1988).

Fusarium wilt is a production constraint in basil and its occurrence is reported from different parts of the world. The disease is caused by Fusarium oxysporum. It is one among the 120 host-species, individual strains (formae species) of the wilt pathogen, Fusarium oxysporum (Fox). During 2000's, a new-wilting disease has impaired 20 – 40 % of yields of basil (Ocimum basilicum L.) in Taiwan. Wilt and crown rot of sweet basil (Ocimum basilicum), caused by Fusarium oxysporum f. sp. basilici, represents a major problem on this crop. Infected plants with Fusarium showed stunting root/stem rot, defoliation, dieback, vascular discoloration and wilting (Al-Hatmi *et al*., 2014).

To effectively manage basil downy mildew while reducing the potential for fungicide resistance development, it is important for conventional basil

growers to (i) initiate a regular fungicide maintenance program prior to the arrival of the pathogen, (ii) limit the number of applications of high-risk fungicides applied during the production season, and (iii) rotate fungicides with different modes-of-action as indicated by their Fungicide Resistance Action Committee (FRAC group) (Gilardi *et al*., 2012; Homa *et al*., 2014).

Biocontrol is the most promising strategy due to presenting advantages related to safety, longevity, environmental conservation and low cost-effectiveness with a high return (Wang *et al*., 2013). Applying bioagent after secondary solid fermentation from organic material and biocontrol agent has been reported to be a timely method for controlling many soil borne diseases (Zhao *et al*., 2014; Wang *et al*., 2013).

The combination of white rot infection with silicon nutrition in the form of PS (potassium silicate) via the nutrient solution can improve the impact of disease infection on membrane leakage and lipid peroxidation. The addition of PS can mediated a reduction in lipid peroxidation that is attributed to its regulation of antioxidant activity in plants (Lamb and Dixon, 1997).

The aim of this investigation is studying several tools to control wilt disease of sweet basil by Fusarium in Egypt based on different ways (fungicide (Topsin), bioagent (Bioark), silicon (potassium silicate) and nanoparticles (Lemon grass and Citronella)) and the efficiency of these control on plant growth parameters

MATERIALS AND METHODS

The sweet basil infection with Fusarium wilt disease were studied in three governorates (Assuit, Beni-Suif and Fayoum) in two seasons (2014/2015 and 2015/2016) and the percentage of infection were recorded.

Isolation and identification of the associated fungi to infected plant and their frequencies (%): Basil showing wilt symptoms seedlings was collected from the studied governorates in addition to the healthy seedlings and sterilized with sodium hypochlorite (1%) for 3 min. and left to dry for 6 hrs. and transferred to PDA plates and incubated at 25±2ºC for 7 days. The growing fungi were purified and identified by means of comparison with the description sheets of Commonwealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications as well as publications of Ellis, 1971; Moubasher, 1977; Nelson *et al*. (1983); Booth, 1985; Burrges *et. al*., 1988; and Singh *et al*., 1991 according to their morphological and cultural characteristics. Percentage of frequency per each fungus was also determined as follows:

$$\% \text{ frequency} = \frac{\text{No. of isolates per each fungus}}{\text{Total no. of the isolated fungi}} \times 100$$

Control Studies: Four control agents were used fungicide (one fungicide (Topsin M) with two concentration (1.5 and 3 mg /L)), bioagent (bioark with two concentrations 2.5 mg/L and 5 mg /L), silicon (potassium silicate with two concentrations 2mg/L and 4 mg /L), finally nano control using lemon grass (8 ml/L) and Citronella(8 ml /L). Different controls were used for Fusarium wilt basil disease. The healthy seedlings and seedlings infected with Fusarium were treated with two concentrations of Topsin M and transplants in field plots (3x4 m) for two seasons. After 60 and 120 days from

transplanting, plant growth parameters (plant height (cm), branches fresh and dry weights, root length, root fresh and dry weights, in addition to essential oil of basil leaves produced from each treatment was extracted with steam distillation and determined according to Guenther (1961).

Infection percentage of wilt disease was determined as follows:

$$\text{Infection percentage} = \frac{Wilted\ plants}{Total\ Plants} X\ 100$$

Statistical analysis: The data obtained were statistically analyzed and computed giving L.S.D. according to Sendecor and Cochran (1989).

RESULTS

Identification of the associated fungi to infected basil and their frequency (%): There are 158 isolates from seven isolated fungi. The mean frequency percentages of the isolated fungi were 100 %. The highest frequency percentage was recorded for Fusarium oxysporum (81 %) with 18 isolates, where the lowest percentage of frequency was reported for Pythium sp. (0.60 %) with one isolate as shown in Table (1).

Table (1): Mean value of number of isolates of associated fungi to infected basil and their frequency (%).

Fungi	No. of isolates	Frequency %
Btryodiplodia theobromae	2	1.30
Fusarium oxysporum	128	81.00
Fusarium semitectum	4	2.50
Fusarium solani	7	4.40
Macrophmina phaseolina	3	2.00
Pythium sp.	1	0.60
Rhizooctonia solani	13	8.20
LSD 0.05	1.4879	1.4879

Percentage of infection of basil in the study Governorates: The infection percentage of downy mildew disease in basil infected with Fusarium in three Governorates under study was shown in Table (2). Highest mean value of percentage of infection of Ocimum basilicum (basil) with Fusarium oxysporum was found in second season (31.8 %). The highest percentage of infection was presented in Beni-Suif Governorate in the two seasons (31.6 % for first season and 38.4 % for the second season)

Table (2): Infection percentage of Ocimum infected with Fusarium through two seasons in three Governorates.

Governorates	Infection %	
	2014/2015 season	2015/2016 season
Assuit	24.40	29.20
Beni-Sueif	31.60	38.40
Fayoum	20.60	27.80
LSD 0.05	3.9309	5.0561

Effect of different treatments on plant height (cm): Table (3) showed that fungicide control using Topsin M (3 mg/L) had the highest significant in plant length increase in two seasons, it recorded 38.58 cm and 39.41 cm length of plant after 60 days and 120 days respectively for the first season compared to the compared to the control infected with fungus (28.20 and 0.00 respectively). For second season, Also Topsin M (3mg/L) gave the highest effect in increasing of plant length with value 39.29 cm and 40.20 cm after 60 and 120 days respectively. The lowest effect in plant length was found by using was found by using nano control Citronella 8 ml/L in two seasons with mean value 34.23 cm for first season and 34.82 cm for second season compared to control infected with

Table (3): Effect of treatments on plant height (cm) of Ocimum (sweet basil) infected with Fusarium through first and second seasons.

Treatment		1st season			2nd season		
		60 days	120 days	Mean	60 days	120 days	Mean
Fungicide	Topsin M 1.5 mg / L	36.66	37.9	37.28	37.32	38.48	37.9
	Topsin M 3 mg / L	38.58	39.41	39.00	39.29	40.20	39.75
Bioagent	Bioark 2.5 mg / L	35.55	37.77	36.66	36.21	38.6	37.41
	Bioark 5 mg / L	36.45	38.23	37.34	37.84	39.58	38.71
Silicon	Potassium silicate 2mg /L	34.85	35.67	35.26	35.65	36.22	35.93
	Potassium silicate 4mg /L	35.33	36.17	35.75	36.15	35.88	36.52
Nano	Lemon grass 8 ml/L	34.43	34.93	34.68	34.95	35.60	35.28
	Citronella 8 ml/L	34.00	34.47	34.23	34.68	34.95	34.82
Control	Control without fungus	44.80	48.41	46.60	24.63	46.81	46.22
	Control infected	28.20	0.00	14.10	29.65	0.00	14.52
Mean		35.89	34.30	35.09	36.68	34.73	35.70
L.S.D. (0.05)	Treatment (T)	0. 52			0.58		
	Day (D)	0. 24			0.26		
	TXD	0.74			0.82		

Effect of different treatments on branches fresh weight (gm): Table (4) showed that bioagent (Bioark 5mg/L) gave the highest effect in increasing of branches fresh weight in two seasons with mean value of 40.53 gm and 41.32 gm for first and second seasons respectively compared to control infected

with fungus 9.37 and 10.42 gm for 1st and 2nd seasons respectively. The lowest significant effect of treatments was reported by using nano control Citronella 8 ml/L in the two seasons with 33.28 and 34.11 gm for 1st and 2nd seasons respectively compared to the control infected with fungus.

Table (4): Effect of treatments on branches fresh weight (gm) of Ocimum (sweet basil) infected with Fusarium through first and second seasons

Treatment		1st season			2nd season		
		60 days	120 days	Mean	60 days	120 days	Mean
Fungicide	Topsin M 1.5 mg / L	36.10	34.46	35.28	36.58	35.09	35.34
	Topsin M 3 mg / L	40.72	39.68	40.20	41.18	40.40	40.79
Bioagent	Bioark 2.5 mg / L	40.38	38.40	39.39	41.22	38.84	40.03
	Bioark 5 mg / L	41.61	39.44	40.53	42.35	40.29	41.32
Silicon	Potassium silicate 2mg /L	35.03	34.48	34.76	35.65	36.21	35.43
	Potassium silicate 4mg /L	37.90	36.68	37.29	38.31	37.46	37.89
Nano	Lemon grass 8 ml/L	34.40	33.68	34.04	35.00	34.35	34.68
	Citronella 8 ml/L	34.20	32.37	33.28	34. 80	33.41	34.11
Control	Control without fungus	45.20	47.58	46.39	47.04	47.35	47.20
	Control infected	18.74	0.00	9.37	20.83	0.00	10.42
Mean		36.43	33.48	34.95	37.20	34.24	36.22
L.S.D. (0.05)	Treatment (T)	1.20			4.21		
	Day (D)	0. 54			1.88		
	TXD	1.70			5.95		

Effect of different treatments on branches dry weight (gm): Table (5) showed that bioagent (Bioark 5 mg/L) had the highest significant effect in increasing of dry weight of branches (gm) in two seasons and the increasing was found after 60 days than 120 days. Bioark (5mg/L) effect in second

season higher than first season with mean value (13.2 gm) and (12.65 gm) respectively. The lowest significant effect of treatment was observed by using nano Citronella 8 ml/L in the two seasons with mean values of 10.1gm and 10.72 gm for 1st and 2nd seasons respectively compared to control infected with fungus 3.57 and 4.16 gm respectively.

Table (5): Effect of treatments on branches dry weight of Ocimum (sweet basil) infected with Fusarium through first and second seasons

Treatment		1st season			2nd season		
		60 days	120 days	Mean	60 days	120 days	Mean
Fungicide	Topsin M 1.5 mg / L	12.38	12.55	11.96	12.69	12.41	12.42
	Topsin M 3 mg / L	12.88	11.83	12.36	13.18	12.43	12.81
Bioagent	Bioark 2.5 mg / L	11.77	11.37	11.57	12.14	11.66	11.90
	Bioark 5 mg / L	13.00	12.30	12.65	13.49	12.90	13.20
Silicon	Potassium silicate 2mg /L	11.86	11.28	11.57	12.10	11.63	11.89
	Potassium silicate 4mg /L	12.24	11.87	12.06	12.67	12.38	12.52
Nano	Lemon grass 8 ml/L	10.79	10.40	10.59	11.02	10.25	10.88
	Citronella 8 ml/L	10.21	9.99	10.1	10.82	10.62	10.72
Control	Control without fungus	14.02	13.14	13.58	14.15	14.53	14.34
	Control infected	7.15	0.00	3.57	8.32	0.00	4.16
Mean		11.63	10.37	11.00	12.06	10.91	11.48
L.S.D. (0.05)	Treatment (T)	0.47			0.69		
	Day (D)	0.21			0.31		
	TXD	0.57			0.98		

Effect of different treatments on root length (cm), fresh weight and dry weight (gm): Table (6), showed that the highest significant increase of treatments on the root length, root dry weight and root fresh weight was appeared by using bioagent treatment (Bioark 5 mg/L) for two seasons. Bioark fertilizer showed higher increase in root parameters in 2nd season than 1st season.

For root length, Bioark (5 mg/L) gave the highest increase 12.43 and12.98 cm for 1st and 2nd respectively compared to the control infected with fungus 0.00 for root fresh and dry weights, Bioark (5gm/L) gave the most significant increase in 2nd seasons 16.01 and 6.94 gm compared with control infected with fungus (0.00).

Nano control using Citronella 8 ml/L gave the lowest significant of treatments on root length, root fresh and dry weights in two seasons compared to the control.

Table (6): Effect of treatments on root (length, fresh weight and dry weight) of Ocimum (sweet basil) infected with Fusarium through first and second seasons.

Treatment		Root length (cm)		Root fresh weight (mg)		Root Dry weight (mg)	
		1st	2nd	1st	2nd	1st	2nd
Fungicide	Topsin M 1.5 mg / L	11.87	12.11	14.31	14.85	6.24	6.45
	Topsin M 3 mg / L	12.31	12.46	15.03	15.47	6.5	6.98
Bioagent	Bioark 2.5 mg / L	12.20	12.55	14.60	15.05	6.00	6.25
	Bioark 5 mg / L	12.43	12.98	15.48	16.01	6.68	6.94
Silicon	Potassium silicate 2mg /L	11.42	11.72	13.29	12.25	5.72	6.00
	Potassium silicate 4mg /L	11.78	12.05	13.69	13.82	5.77	5.96
Nano	Lemon grass 8 ml/L	11.49	11.92	12.80	12.73	5.43	5.55
	Citronella 8 ml/L	10.77	11.11	11.64	11.80	4.81	5.07
Control	Control without fungus	14.49	13.84	17.63	16.00	6.49	7.26
	Control infected	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.05		0.33	0.62	0.53	0.55	0.43	0.52

Effect of different treatments on essential oil content of Ocimum plant: Table (7) showed that the highest significant increase in essential oils content of Ocimum basilicum plant infected with Fusarium oxysporum was reported by using bioagent (Bioark 5 mg/L), while the lowest significant effect was reported by using nano Citronella 8 ml/L in two seasons.

Bioark 5gm/L and Topsin M 3 gm/L gave the highest effect in oil content with mean value 0.07 gm compared to control infected with fungus 0.01 gm.

The lowest effect was reported by using nano Citronella 8 ml/L in two seasons with mean value 0.03 gm compared to the control infected with fungus.

Table (7): Effect of treatments on essential oil content of Ocimum (sweet basil) infected with Fusarium through first and second seasons.

Treatment		1st season			2nd season		
		60 days	120 days	Mean	60 days	120 days	Mean
Fungicide	Topsin M 1.5 mg / L	0.06	0.05	0.6	0.06	0.04	0.06
	Topsin M 3 mg / L	0.08	0.06	0.7	0.08	0.06	0.07
Bioagent	Bioark 2.5 mg / L	0.08	0.05	0.06	0.07	0.05	0.06
	Bioark 5 mg / L	0.09	0.06	0.07	0.08	0.06	0.07
Silicon	Potassium silicate 2mg /L	0.05	0.3	0.04	0.04	0.03	0.04
	Potassium silicate 4mg /L	0.06	0.4	0.05	0.05	0.03	0.04
Nano	Lemon grass 8 ml/L	0.05	0.03	0.04	0.04	0.04	0.04
	Citronella 8 ml/L	0.03	0.02	0.03	0.03	0.03	0.03
Control	Control without fungus	0.1	0.09	0.1	0.1	0.09	0.1
	Control infected	0.02	0.01	0.01	0.02	0.00	0.01
Mean		0.06	0.05	0.06	0.06	0.04	0.05
L.S.D. (0.05)	Treatment (T)	0.055			0.0094		
	Day (D)	0.024			0.0042		
	TXD	0.078			0.0133		

DISCUSSION

Sweet basil (Ocimum basilicum L.) is an economically important herb crop in several Mediterranean countries. Basil is also an important fresh and processed (frozen and pesto sauce) crop. The intensified use of cultivation systems, coupled with increasing restrictions on the use of fungicides, led to severe epidemics. The recent widespread outbreaks of Fusarium wilt (Davis *et al*., 1993).In this investigation the infection of basil with wilt disease was highest in Beni-Suif Governorate.

Topsin-M is fungicide widely available for commercial purpose amended at different concentrations in PDA inhibited growth of the fungus. Topsin M (Methyl thiophenate) is a systematic fungicide used for control of diseases such as powdery mildew and downy mildew of basil and other plants (Singh, 1991). Topsin M (3 mg /L) gave the highest efficiency in plant length increasing infected with Fusarium in 1st and 2nd seasons, this result was in agreement with result of Hilal *et al.* (2008). This may due to the direct effect of Topsin M on the fungal growth, reducing or preventing the fungal pathogenicity and changing the soil microflora profile; the fungicides were recommended to control wilt disease of many hosts (Hilal *et al*., 2003).

Natural safety bioagent causes various promotion effects on plants. It is considered as a natural source of cytokinins which stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and B-vitamin. It also releases CO_2 which reflected in improving net photosynthesis. Bioagents are applied as seed or soil inoculants, they multiply and participate in nutrient cycling and benefit crop productivity (Singh *et al*., 2011). In general, 60% to 90% of the total applied fertilizer is lost and the remaining

10% to 40% is taken up by plants. In this regard, microbial inoculants have paramount significance in integrated nutrient management systems to sustain agricultural productivity and healthy environment (Adesemoye and Kloepper, 2009).

The significant effect of bioagent may be due to the effect of different strain groups and nutrients mobilizing microorganisms which help in availability of metals and their forms in the composted material and increased levels of extracted minerals (El-Kramany *et al*., 2000).Using bioagents produce antifungal antibiotics and growth substances that improved seed germination and plant stand (Shende *et al*., 1977) and plant growth regulators (Reddy *et al*., 1991).In this study bioagent (Bioark 5 mg/L) gave the highest significant increase in branches fresh and dry weights; in addition to increasing in root length and root fresh and dry weights. Also, Bioark gave the highest significant increasing in oil content of Basil infected with Fusarium oxysporum. Bioagents promote growth by increasing the supply or availability of primary nutrients to the host plant (Lakshmi Kumari *et al.*, 1975)

The addition of a specific biological control agent to compost was reported leading to a substrate with a broader-range suppressive effect (Qiu *et al*., 2012; Pugliese *et al*., 2011; Shoda, 2000), and such substrate has been widely used to control soil borne diseases, especially Fusarium wilt disease (Alabouvette *et al*., 2006). In conclusion, bioagent (Bioark 5 mg/L) was the most effective control in increasing the branches fresh and dry weights; root length, root fresh and dry weights; in addition to oil content of infected basil with Fusarium oxysporum.

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المكافحة الحيوية لمرض الذبول لنبات الريحان المتسبب عن فطر الفيوزاريوم

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المستخلص

تهدف هذه الدراسة الى دراسة نبات الريحان المصاب بفطر الفيوزاريوم اوكسيسبورم في ثلاث محافظات (أسيوط– بني سويف– الفيوم)، حيث كانت أعلى نسبة اصابة للنباتات في محافظة بني سويف.ولقد تم استخدام اربع معاملات وذلك للسيطرة والحد من مرض الذبول الناتج للاصابه بهذا الفطر، وكانت على النحو التالي: المبيد الفطرى (توبسين ١,٥ و ٣ مللي جرام /لتر)، المخصب العضوي (بيوأرك ٢,٥ & ٥ مللي جرام / لتر)، السيلكون (سليكات البوتاسيوم ٢ & ٤ مللي جرام/لتر) وزيت النانو (حشيشة الليمون(٨ مللى /لتر) و السترونيلا (٨ مللي / لتر) .

من الدراسة وجد أن المبيد الفطرى (توبسين) كان له اعلى تأثير في زياده طول النبات المصاب بالفطر (٣٩,٧٥ سم للموسم الثاني و ٣٩,٠٠ سم للموسم الاول) بينما ان السترونيلا كان له اقل تأثير علي طول النبات (٣٤,٢٣ سم و ٣٠,٨٢ سم) لكل من الموسمين الأول والثاني بالترتيب. المخصب العضوى (بيوأرك) يعتبر اكثر المعاملات تاثيرا في زياده وزن الافرع الطازج والجاف في كل الموسمين الاول والثاني. بالاضافه لتأثير البيو أرك علي زيادة كل من طول الجذر والوزن الطازج والجاف للجذر في الموسمين. وكان بالغ التأثير للمعاملة بالمخصب العضوي بيوأرك (٥ ملليجرام/لتر) في زيادة محتوى النبات المصاب من الزيوت الاساسية (٠,٠٧ جم) في كل من الموسمين.

الكلمات المفتاحية: الريحان – فطر الفيوزاريوم اوكسيسبورم – بيوآرك – سيليكون – نانو زيت