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Management of Bean Rust by some Bioagents and Essential Plant Oils

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Antagonistic bacteria and fungi occurring on bean phylloplane were isolated and evaluated for their activity as bioagents against *Uromyces appendiculatus* (Pers. ex Pers.) Unger., the causativeof bean rust. Isolates of *Bacillus* spp. and *Trichoderma* spp. were selected. Four isolates of *Bacillus* spp. were purified and identified as *Bacillus megaterium, B. pumilus, B. subtilis* and *B. thuringiensis*. Also,four fungal isolates of *Trichoderma* spp. were purified and identified as *Trichoderma album, T. hamatum, T. harzianum* and *T. viride*. The inhibitory effect of the isolated bioagents as well as the essential plant oils of lemongrass, neem and thyme was assessed *in vitro* on the germination of the urediospore of the causal fungus. The inhibitory effect of *Bacillus* spp. ranged between37.7-53.9%, *Trichoderma* spp. between 39.3 - 58.8% and the essential plant oils between 34.9 - 52.2%. Among *Bacillus* spp., *B. thuringiensis* recorded the highest inhibitory effect on urediospore germination of the causal fungus followed by *B. subtilis* then *B. megaterium and P. pumilus.* Among *Trichoderma* spp., *T. viride* gave the highest inhibition followed by *T. harzianum* then *T. hamatum* and *T. album*. Furthermore, among the essential oils, thyme oil resulted in the highest inhibitory effect followed by lemongrass oil then neem oil. Under greenhouse conditions, spraying bean plants withthe tested bioagents and the essential plant oils, five days before or after inoculation with *U. appendiculatus* urediospore significantly reduced the severity of the disease with significant increase in the produced green pods yield compared to the control. *B. thuringiensis, T.viride* and thyme oil were the most efficient treatments in reducing the severity of the infection with the disease and increasing the number of the produced pods and the weight/plant compared with control treatment. Therefore, they were used in another trial in different alternations in comparison with the fungicide Topas for management the disease. In addition, using of these treatments each alone was of low efficiency and produced low green pod yield. Meanwhile, the alternation among any of *B. thuringiensis* and *T. viride* with thyme oil showed the highest

efficiency in reducing the disease and increasing the produced green pod yield nearby the efficiency of the fungicide Topas.In addition, the three oxidative-reductive enzymes, *i.e.*phenylalanine ammonia-lyase (PAL),peroxidase (PO) and polyphenoloxidase (PPO)were greatly increased in the leaves of all treated plants compared with control treatment. Moreover, plants sprayed with thyme recorded the highest activity of the three enzymes followed by those sprayed with *B. thuringiensis* then *T. viride* and the fungicide Topas.

Keywords: Bean, *Bacillus* spp., biological control, essential plant oils, *Trichoderma* spp. and *Uromyces appendiculatus.*

Bean (*Phaseolus vulgaris* L.) is considered one of the most important food legume crops in Egypt for local consumption and exportation. The economic importance of bean cultivation in the world could be explained by its high nutritional value of vitamins, protein, carbohydrates and some other components. In addition, it improves the soil fertility through nitrogen fixation. As much as 60% of bean production in the developing world occurs under conditions of drought and salinity stresses (Franca *et al.*, 2000). In Egypt, the cultivated area with bean is annually increased due to increasing the demand for local consumption and exportation. Bean is liable to attack by many bacterial, fungal, viral, and nematode diseases in addition to physiological disorders (Hagderon and Inglis, 1986; Lindgren *et al.*, 1995; Abd-El-Khair *et al.*, 2010 and Ragab*et al.*, 2015). However, bean rust is considered one of the major destructive diseases affecting the crop yield (Hagderon and Inglis, 1986; Mmbaga and Stavely, 1996; Jochua *et al.*, 2008; Mersha and Hau, 2008; and Liebenberg and Pretorius, 2010), especially in the north and middle parts of the Delta in Egypt and several countries in the world. Rust is the most important disease of dry beans in South Africa where 100% losses have been reported for rust susceptible varieties (Mukunya and Keya, 1978 and Jochua*et al.*, 2008). In describing the life cycle of the rust pathogen, *Uromyces appendiculatus*(Pers. ex Pers.) Unger., the potential of the sexual stage to produce variation was emphasized. However, with the exception of teliospores germination studies on the High Plains of the USA, nothing is known about the role which this stage plays in most bean production areas with rust problems. Managing plant diseases with fungicides sometimes gives good results. However, improper use of fungicides leads mostly to environmental pollution, disasters throughout the world and the phenomena of resistance to the causal pathogens (Brewer and Larkin, 2005). Therefore, to overcome these difficulties, it is urgent to apply alternative safe efficient methods against such disease or at least rationalization their application. Biological control is considered an important approach of agricultural biotechnology in recent years for controlling many fungal plant pathogens (Deshmukh *et al.*, 2010; Zaher *et al.*, 2013; Abada & Eid, 2013; Abada & Ahmed,2014 and Barakat *et al.*, 2014). *Trichoderma*spp. are the most promising and effective bioagents against various

plant pathogenic fungi (Fahim *et al.*, 1989; Zaher *et al.*, 2013 and Barakat *et al.*, 2014). *Trichoderma*spp., as antagonists for controlling wide range of microbes, were well documented and demonstrated for more than seven decades ago but their use under field conditions came much later (Fahim *et al.*, 1989 and Chet *et al.*, 1997) and their mechanism of mycoparasitism is much more complex, that is nutrient competition, hyperparasitism, antibiosis, space and cell wall degrading enzymes (Abd-El-Khair *et al.*, 2010 and Liebenberg and Pretorius, 2010). Several researchers have reported biological control as an effective method for controlling vegetable diseases to reduce the use of fungicides (Dubos &Bulit, 1981; Mahmoud *et al.*, 2012; Zaher *et al.*, 2013; and Barakat *et al.*, 2014). It was also found that there was a large variety of volatile secondary metabolites produced by *Trichoderma* spp. such as ethylene, carbon dioxide, hydrogen cyanide, aldehydes and ketones which play an important role in controlling many plant pathogens (Faheem *et al.*, 2010; Nagendra & Kumar, 2011and Barakat *et al.*, 2014).

This work aimed to evaluate the efficiency of some bacterial and fungal bioagents as well as some essential plant oils on germination of urediospore of *U. appendiculatus* bean rust. The work was expanded to assess the effect of these treatments on the activity of three oxidative-reductive enzymes, *i.e*. phenylalanine ammonia-lyase(PAL), polyphenoloxidase (PPO)and peroxidase (PO).

Materials and Methods

Plant Materials:

Bean seeds Pronco cv. were obtained from the Legume Crops Res. Dept., Agric. Res. Cent., Giza, Egypt.

The Causal Fungus:

Bean leaves bearing the uredial spores of *Uromyces appendiculatus* (Pers. ex Pers.) were frequently collected from Giza governorate, and were used throughout this study.

Isolation, Purification and Identification of the Antagonists:

Microorganisms naturally occurred on the surfaces of bean leaves were isolated from the phylloplane of healthy plants, collected from Giza governorate using the dilution plate technique. Serial dilution plate technique was used to isolate native antagonistic *Trichoderma* spp. on PDA medium and *Bacillus* spp. on nutrient agar medium (Oedjijono and Dragar, 1993). All the fungal cultures of *Trichoderma* spp. were selected, isolated and purified using the single spore method then identified on the basis of their cultural and microscopic morphological characters (Rifai, 1969

andBissett, 1991). Also, the isolated *Bacillus* spp. were purified and identified depending on the descriptions of Parry *et al*. (1983) and Holt and Krieg (1984).

Source of Plant Essential Oils:

Essential plant oils of lemon grass (*Cymbopogon citrates*), neem (*Azadirachta indica*) and thyme oil (*Thymus capitatus*) were obtained from the International Flavors and Plant Oils Inc., Giza, Egypt. These essential oils were stored in dark bottles at 4ºC for further studies.

Evaluation of the Tested Bioagents and the Essential Plant Oils on Germination of the Urediospore of U. appendiculatus:

Flasks (250 ml.) containing nutrient broth medium were inoculated with loops of the culture of any of the tested bacteria and incubated at 28±2 for 48 h. to grow. The bacterial suspension was adjusted to contain $1x10^2$, $1x10^4$ and $1x10^6$cfu/ml. Also, *Trichoderma* spp. were grown on PDA medium for 7 days. 20 ml. of sterile water were added to each Petri-dish and growth (spores and mycelium) was gently crushed by sterilized camel brush and collected in sterile 500ml conical flask. The collected growth was filtered through 3 layers of cheesecloth and the filtrate was adjusted to contain $1x10^2$, $1x10^4$ and $1x10^6$ conidia using a haemocytometer. Each of the essential oils, lemon grass, neem and thyme was diluted to the concentrations of 10, 20 and 40% using distilled sterile water plus few drops of Tween-20 (to make emulsion).Freshly urediospore of the pathogen were added to each concentration of the tested bacterial and fungal bioagents as well as the essential plant oils. One ml. of uredial suspension was placed on two sterilized slides, borne on two glass rods in a sterilized Petri-dish containing a piece of wetted cotton by sterilized distilled water to provide high relative humidity. The same was made for a spore suspension put in distilled sterilized water only as control treatment. Preparations were incubated in the dark at 25±1°C for 48 hour. Five Petri dishes for each treatment were used as replicates. The percentages of uredial germination were counted in a total of 100 uredospore in each Petri-dish. The germinated urediospore were counted at the initial of the experiment and 48hr after treating with the tested treatments and mean of germination percentage was calculated and recorded for each treatment.

Pot experiment:

The antifungal activity of four*Bacillus*spp. (*B. megaterium, B. pumilus, B. subtilis* and *B. thuringicensis*) and four *Trichoderma* spp. (*T. album*, *T. hamatum*, *T. harzianum*, and *T. viride*) and the three plant essential oils (lemon grass, neem and thyme) was evaluated for their efficiency in controlling bean rust caused by *U. appendiculatus* in pots under artificial inoculation conditions. Four bean seeds, Pronco cv. were sown in each plastic pot (25 cm in diameter) containing formalin sterilized silt soil. The emerged seedlings were thinned into two plants in each pot. Five pot replicates of 35 days old plants for each treatment were sprayed with the tested bioagents, *i.e. Bacillus* spp. ($1x10^6$cfu/ml water),*Trichoderma* spp. ($1x10^6$

spore suspension/ml water) and the three plant oils of lemon grass, neem and thyme (at 40%) at five days before or after artificial inoculation with *U. appendiculatus* urediospore suspension ($1x10^3$uredospore/ml. water). The plants received four sprays with one week interval the first spray was carried out five days before or after inoculation with the urediosporeof the causal fungus. Control plants were sprayed with urediospores uspension of the causal fungus only.

Another experiment was carried out to evaluate the role of the alternation between any of *B. thuringiensis* and *T. viride* with thyme as essential plant oil. Four bean seeds (Pronco cv.) were sown in each plastic pot (25 cm in diameter) containing formalin sterilized silt soil. The emerged seedlings were thinned into two plants in each pot. Five pot replicates of 35 days old plants for each treatment were sprayed with each of *B. thuringiensis*, *T. viride* and thyme each alone and also in alternation in comparison to the fungicide Topas(using the previous concentrations) four sprays with one week interval.Two sets were made for plant treatments, *i.e.* spraying the plants five days before inoculation or after five daysof inoculation with *U. appendiculatus* urediospore suspension, the following treatments were proposed in Table (1).

Control plants were sprayed with urediospore suspension of the causal fungus only. Two grams of the fertilizer Crystalon (1 N: 1P: 1K with traces of the microelements) were added to the grown plants in each pot, 30 and 45 days after sowing.The first spray was carried out in all cases five days before or after artificial inoculation with the urediospore suspension of the tested pathogen. All pots were kept in a greenhouse, where plastic containers filled with water were put surrounding the pots and in the same time the floor of the greenhouse was sprayed with water for three successive days to maintain the humidity necessary for infection process. Disease severity was recorded one week after each spray with the tested materials and the average was recorded. Also, the produced green pods were counted and weighed in each harvest and average numbers of the green pods and their weight (g)/plant were recorded.

Disease Assessment:

The artificially infected plants were carefully examined to estimate the severity of the infection by bean rust depending on the devised scale (0-6) by Godoy *et al.* (1997) using the following formula:

$$\% \text{ Disease severity} = \frac{\sum(n \times v)}{6N} \times 100$$

Where:

n = Number of infected leaves in each category.

v = Numerical values of each category.

N= Total number of the infected leaves.

Table 1. Spraying bean plants with two antagonistic bioagents in alternation with the essential plant oil thyme in comparison with Topas

Treatments	First spray	Second spray	Third spray	Fourth spray
	Plant age (days)			
	35	42	49	56
1	*B.thuringicensis*	*B. huringicensis*	*B. thuringicensis*	*B.thuringicensis*
2	*T. viride*	*T. viride*	*T. viride*	*T. viride*
3	Thyme	Thyme	Thyme	Thyme
4	*B.thuringicensis*	*T. viride*	*B. thuringicensis*	*T. viride*
5	*B.thuringicensis*	Thyme	*B. thuringicensis*	Thyme
6	*T. viride*	Thyme	*T. viride*	Thyme
7	Topas	Topas	Topas	Topas

Estimation of Oxidative-Reductive Enzymes:

The activity of peroxidase (PO), polyphenoloxidase (PPO), and phenylalanine ammonia-lyase (PAL) was measured in leaves collected from pathogen free and -inoculated and bioagent-treated bean plants. Samples were taken after one week from the second treatment of the plants by the different treatments for enzymes assay. One gram of bean leaf sample was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) in ice bath for enzyme assays. The homogenates were then centrifuged at 10,000 g for 10 min. Supernatants were used to analyze the defense-related enzymes, PO, PPO, and PAL activities.

Estimation of PPO Activity:

The activity of polyphenoloxidase (PPO) was determined according to the method proposed by Mayer *et al.* (1965). The reaction mixture contained 200 µl enzyme extract and 1.5 ml of 0.01 M catechol. Activity was expressed as changes in absorbance at 495 $nm \cdot min^{-1} mg^{-1}$protein.

Estimation of PO activity:

To estimate peroxidase activity (PO), 50 µl of enzyme extract were added to 2.85 ml of 0.1M phosphate buffer (pH 7.0) and mixed with 0.05 ml of 20 mMguaiacol reagent (Fu and Huang, 2001). The reaction was started by the addition of 0.02 ml of 40mM hydrogen peroxide to the mixture. Rate of increase in absorbance at 470nm was measured over 1 min. One unit of enzyme activity was defined by the change in absorbance of 0.01 for 1 g fresh weight per minute.

Estimation of PAL activity:

The activity of phenylalanine ammonia lyase (PAL) was determined according to the method of Burrell and Rees (1974). The reaction mixture contained 0.03 ml phenylalanine and 0.2 ml enzyme extract in a total 2.5 ml of sodium borate buffer (pH 8.8). This reaction mixture was kept in a water bath at 37°C for 1h, and 0.5ml of 1M (trichloroacetic acid) TCA was added. The amount of trans-cinnamic acid formed from L-phenylalanine was measured spectrophotometrically at 290nm. Enzyme activity was expressed as microgram of trans-cinnamic acid $h^{-1}mg^{-1}$protein.

Statistical analysis:

Data were statistically analyzed using the standard procedures for complete randomized block and split designs as mentioned by Snedecor and Cochran (1967). The averages were compared at 5% level using the least significant differences (L.S.D) according to Fisher (1948).

Results

Inhibitory effect of the tested bioagents and the essential plant oils on urediospore germination in vitro:

The inhibitory effects of the antagonistic bioagents and the essential plant oils on the germination of *U. appendiculatus* urediospore *in vitro* are shown in Tables (2, 3 and 4). All the tested bioagents and plant oils decreased the germination ofurediosporeof the fungus compared with the control. Urediospores germination was gradually decreased by increasing the concentration of the tested bioagents and the essential plant oils. Data in Table (2) indicate that the antagonistic effect of *Bacillus spp.* against the causal fungus was in the range of 50.85-63.15%uredial germination on the average. In addition, *B. thuringiensis* was the mostefficient in this regard followed by *B. subtilis* then *B. megaterium* and *B. pumilus*, being 50.85, 60.25, 61.9 and 63.15%uredial germination on the average, respectively. Meanwhile, control treatment recorded 90.4% uredial germination. The antagonistic effect of *Trichoderma* spp. against the causal fungus was in the range of 52.25-67.15% uredial germination (Table, 3). *T. viride* gave the highest inhibitory effect followed by *T. harzianum* then *T. hamatum* and *T. album*, being 52.25, 95.40, 65.95and 67.15%on the average respectively, while control treatment recorded 91.2% uredial germination. Data presented in Table (4) show that the three tested essential plant oils, *i.e.* lemon grass, neem and thyme resulted significant inhibitory effect on the germinated urediospore of the causal fungus. Thyme caused the highest inhibitory

effect followed by neem and the lemon grass, being 48.9, 57.55 and 61.85%on the average, respectively. Control treatment recorded 90.8% urediospore germination.

Table 2. Effect of different antagonistic bacteria on the urediospore germination, 48h after incubation at 25±2°C

Bacterial Bioagents	% Spore germination at 1x10* (cfu) concentration				Mean
	0	2	4	6	
B.megaterium	90.4	81.8	51.4	24.0	61.9
B. pumilus	90.4	83.4	52.2	26.6	63.15
B. subtilis	90.4	80.0	50.6	20.0	60.25
B.thuringiensis	90.4	74.2	38.8	0.0	50.85
Mean	90.4	79.85	48.25	17.65	---

* Initial germination percentage was 1.8 %.
L.S.D. at 5 % for:Bacterial bioagents (B) = 2.3, Concentrations(C) =3.0 and B x C= 2.9.

Table 3. Effect of different antagonistic fungi on the germination of urediospore, 48h after incubation at 25±2°C

Fungal Bioagents	% Spore germination at 1x10* (cfu) concentration				Mean
	0	2	4	6	
T. album	91.2	85.4	59.8	32.2	67.15
T. hamatum	91.2	84.6	57.0	31.0	65.95
T.harzianum	91.2	80.8	48.4	17.2	95.40
T. viride	91.2	76.0	41.8	0.0	52.25
Mean	91.2	81.7	51.75	20.1	---

* Initial germination percentage was 1.2 %.
L.S.D. at 5 % for:Fungal bioagents (F) = 2.5, Concentrations(C) =3.3 and F x C= 3.2.

Table 4. Effect of different essential plant oils on the germination of urediospore, 48h after incubation at 25± 2°C.

Essential plant oils	% Spore germination at conc. (%)				Mean
	0	10	20	40	
Lemon grass	90.8	80.6	51.0	25.0	61.85
Neem	90.8	76.8	45.4	17.2	57.55
Thyme	90.8	70.0	34.8	0.0	48.9
Mean	90.8	75.80	43.73	14.07	---

* Initial germination percentage was 1.2 %.
L.S.D. at 5 % for:Essential plant oils (E) = 2.6, Concentrations(C) =3.1 and E x C= 2.8.

The effect of the tested antagonists and the essential plant oils on disease severity and the number and weight of pods under greenhouse conditions:

Spraying bean plants with any of the tested antagonists and the essential plant oils, five days before inoculation with *U. appendiculatus*urediospore, significantly reduced rust severity under greenhouse conditions (Table 5). In addition, the essential plant oils followed by *Trichoderma* spp. then *Bacillus* spp. were in descending order. Moreover, spraying these materials was more efficient in reducing the disease and increasing the produced green pods when the plants were sprayed 5days before inoculation with the urediospore of the causal fungus than those sprayedwith the tested materials 5days after artificial inoculation with the urediospore suspension.

The severity of the disease on plants sprayed with *Bacillus* spp. 5days before inoculation with the causal fungus urediospores was in the range of 8.8-11.0%, while, spraying with *Bacillus* spp. 5 days after inoculation with the causal fungus urediospores was in the range of 13.0-16.1%. Meanwhile, when plants were sprayed with*Trichoderma* spp. 5 days before inoculation with the causal fungus urediospores was in the range of 7.8-12.1%, while those treated 5days after inoculation with the causal fungus urediospores, rust severity was in the range of 12.4-16.8%. Moreover, spraying the essential plant oils 5days before inoculation with the urediospores exhibited disease severity in the range of 6.4-8.4%, while in those treated 5 days after inoculation with the causal fungus urediospores showed 11.2-13.5% rust severity. Control treatment recorded 40.8% disease severity.

The reduction in the severity of the disease was significantly reflected on the produced green pods.Spraying bean plants with two antagonistic bioagents in alternation with the essential plant oil thyme in comparison with Topas 5days before or after inoculation with the pathogen resulted significant reduction in the severity of bean rust,with significant increase in the produced green pods yield (Table 6). Also, spraying these materials was more efficient in reducing the disease and increasing the produced green pods yield when sprayed 5days before inoculation with the causal fungus urediospores than that sprayed with the tested materials 5days after inoculation. Moreover, the alternation between the two bioagents and any of the two bioagents with thyme was more efficient in reducing the disease and increasing the yield of green pods than spraying each alone.Furthermore, the alternation between any of the two bioagents was more efficient in this regard than the alternation between the two bioagents. The fungicide Topas was the superior treatment in this regard. The analogous values were 3.1% for disease severity, 43.0 pod/plant and 236 g green pod yield/plant, while untreated plant recorded 41.6%, 20.4 pod and 120.7

g.on the average, respectively.

Table 5. Effect of spraying bean plants with different antagonistic bioagents and essential plant oils 5 days before or after inoculation with the pathogen on the severity of bean rust (Pronco cv.) as well as the produced green pods yield,greenhouse experiment

Treatments	% Disease severity		Mean	Average No. of green pods/plant		Mean	Average weight of green pod yield (g)/plant		Mean
	Before	After		Before	After		Before	After	
B. megaterium	10.4	15.2	12.8	25.6	23.6	24.6	197.0	185.0	191.0
B. pumilus	11.0	16.1	13.6	25.0	20.0	22.5	194.8	173.2	184.0
B. subtilis	9.2	14.0	11.6	26.1	21.2	24.2	198.1	181.0	189.6
B.thuringiensis	8.8	13.0	10.9	28.0	22.6	25.3	212.5	189.0	200.8
T. album	12.1	16.8	14.5	25.0	21.8	23.4	194.3	185.5	189.9
T. hamatum	11.4	16.6	14.0	27.8	23.6	25.7	209.0	187.0	198.0
T. harzianum	8.3	13.6	11.0	28.8	22.0	25.4	213.4	184.0	198.7
T. viride	7.8	12.4	10.1	30.0	22.6	26.3	218.7	188.7	203.7
Lemon grass	8.4	13.5	11.0	29.0	22.2	25.6	216.0	186.8	201.4
Neem	7.8	12.3	10.1	30.0	22.0	26.0	219.1	186.2	202.7
Thyme	6.4	11.2	8.8	32.8	27.2	30.0	232.3	205.0	218.7
Control	40.8	40.8	40.8	19.4	19.4	19.4	117.5	117.5	117.5
Mean	11.9	16.3	---	27.3	23.2	---	201.9	180.7	---

Before=5day before inoculation with the urediospores.
After=5day after inoculation with the urediospores.

L.S.D. at 5 %for:			
Treatments (T) =	2.6	1.6	3.7
Periods (P) =	1.9	2.2	3.2
and T x P =	3.1	2.8	4.6

Changes in the activity of oxidative-reductive enzymes:

Data presented in Table (7) show the changes in the activity of oxidative-reductive enzymes*i.e.* phenylalanine ammonia lyase (PAL), peroxidase (PO) and polyphenoloxidase (PPO) due to spraying bean plants with *B. thuringienses* and *T. viride* as well as the essential plant oil thyme and the fungicide Topas compared with the untreated control. Data revealed that, in general, activity of the three enzymes,

i.e. PAL, PO and PPO were greatly increased in the leaves of all sprayed treatments compared with control treatment. In addition, plants sprayed with thyme recorded the highest activity of the three enzymes followed by those sprayed with *B. thuringiensis* then *T. viride*, being 1.522, 1.404 and 1.370, respectively. Meanwhile, untreated control leaves recorded the lowest activity followed by those sprayed with Topas, being 0.844 and 1.021, respectively.

Table 6. Effect of spraying bean plants with two antagonistic bioagents in alternation with the essenial plant oil thyme in comparison with Topas 5 days before or after inoculation by the pathogen on the severity of bean rust (Pronco cv.) as well as on the produced green pods yield, greenhouse experiment

	Treatments	%, Disease severity		Mean	Average No. of green pods/plant		Mean	Average weight of green pod yield (g)/plant		Mean
		Before	After		Before	After		Before	After	
1	*B. thuringiensis*(BT)	8.6	14.0	11.3	31.8	24.6	28.2	189.0	171.0	180.0
2	*T. viride*(TV)	7.6	12.8	10.2	33.4	26.0	29.7	198.8	174.2	186.5
3	Thyme (T)	6.0	12.2	9.1	35.0	27.8	31.4	208.1	178.0	193.1
4	BT ,TV, BT ,TV	5.8	11.4	8.6	36.0	29.6	32.8	214.5	182.0	198.3
5	BT , T, BT , T	5.2	7.4	7.3	37.6	31.6	34.6	221.3	189.0	205.2
6	TV, T, TV, T	4.8	6.2	5.5	38.8	33.6	36.2	228.0	195.8	211.9
7	Topas	2.7	3.4	3.1	44.8	42.6	43.7	240.4	232.0	236.2
	Control	41.6	41.6	41.6	20.4	20.4	20.4	120.7	120.7	120.7
	Mean	10.3	13.6	---	34.7	29.5	---	202.6	180.3	---

Before=5day before inoculation with the causal pathogen.
After=5day after inoculation with the causal pathogen.

L.S.D. at 5 % for:

Treatments (T) =	2.3	1.8	4.0
Period (P) =	2.1	2.4	3.7
and T x P =	3.3	2.8	4.3

Table 7. Effect of spraying bean plants with two bioagents and the essential oil thyme in comparison with the fungicide Topas on the activity of oxidative reductive enzymes

Treatments	Activity of enzymes*			Total
	Phenylalanine ammonia lyase (PAL)	Peroxidase (PO)	Polyphenol oxidase (PPO)	
B.thuringiensis	0.417	0.415	0.552	1.404
T. viride	0.414	0.426	0.529	1.370
Thyme	0.492	0.413	0.575	1.522
Topas	0.345	0.301	0.375	1.021
Control	0.228	0.252	0.354	0.84
Total	2.346	1.807	2.255	---

*Expressed as absorption after 30 sec. at appropriate wave length and the values of activity of PAL, PO and PPO at zero time were 0.220, 0.244 and 0.350, respectively.

D i s c uss i o n

Nowadays, most of the countries all over the world suffer from great environmental pollution due to using agrochemicals that cause health hazard. Therefore, production of healthy and safe food free from toxic substances is the desire of the consumer especially that consume freshly like bean. Therefore, to avoid the use of hazard chemicals against diseases, certain protective or curative procedures could be conducted using different non-chemical methods to control such diseases. In this regard, bioagents and essential plant oils were evaluated for management bean rust.

However, in most cases, using such untraditional management methods did not give adequate results when used alone. In this respect, the use of these methods is preferable to use as a mixture or in alternation. All the tested bioagents and plant oils decreased the germination of urediospore of the pathogen compared with the control.Spore germination was gradually decreased by increasing the concentration of the tested bioagents and essential plant oils. The antagonistic effect of *Bacillus* spp. against the causal fungus was in the range of 37.7-53.9% urediospore germination. In addition, *B. thuringicensis* was the most efficient in this regard followed by *B. subtilis* then *B. megaterium* and *B. pumilus*. The antagonistic effect of *Trichoderma* spp. against the causal fungus was in the range of 39.3-58.8 % urediospore germination.In addition, *T. viride* gave the highest inhibitory effect followed by *T. harzianum* then *T. hamatum* and *T. album*. The three tested essential plant oils, *i.e.* lemon grass, neem and thyme resulted in significant inhibitory effect on urediospore germination. In this respect, thyme caused the highest inhibitory

effect followed by neem and the lemon grass. The efficiency of the essential plant oils, *Trichoderma* spp. and *Bacillus* spp. in reducing the severity of the disease and increasing the produced green pods yield was in descending order. Moreover, spraying these materials was more efficient in reducing the disease and increasing the produced green pods when sprayed on the plants 5days before inoculation with the causal fungus than those sprayed with the tested materials 5days after inoculation with the causal fungus. The reduction in the severity of the disease was significantly reflected on the produced green pods. Spraying bean plants with the bioagents *B. thuringiensis* and *T. viride* and the essential plant oil thyme, each alone or in alternations, resulted in significant reduction of disease severity with significant increase in the produced green pods yield.

Furthermore, spraying plants with any of these compounds alone was of low effect compared with spraying them alternatively. However, the fungicide Topas was the superior in this regard followed by using any of the two bioagents and thyme. Several species of the genus *Trichoderma* act as antagonists of other fungi. A number of strains from the *Trichoderma* species, *T. harzianum* are used as biological control agent for the control of soil borne as well as foliar plant pathogens (Schimboch *et al.,* 1994). Six *T.harzianum* strains, five of them isolated from commercial preparations, were evaluated for their capability to control the bean rust fungus *Uromyces appendiculatus* (Pers. ex Pers.) Unger.

Some evidence for an antibiotic interaction between *T. harzianum* and *U. appendiculatus* are discussed.The role of *Trichoderma* spp. in inhibiting the infection by bean rust may bedue to the using of the secreted nutrient materials by the leaves (stimulate the uredospore to recognize the host) by *Trichoderma* spp. and this makes the urediospore of the causal fungus fail to germinate or recognize their host(s). Also, Schirmbock*et al.* (1994) found that there is parallel formation and synergism of hydrolytic enzymes as well as peptaibol antibiotics and molecular mechanisms involved in the antagonistic action of *T. harzianum* against the phytopathogenic fungi. Chemical control is highly recommended because rust is an aggressive and destructive disease and satisfactory control without the use of fungicides is unlikely. The role of fungicides in reducing the disease is well known (Fontem&Bouda, 1998 and Mc Grath, 2001). But due to the great hazard on the human health due to the residue of agrochemicals in the consumed food, fungicides become unlikely to use. Therefore, great efforts by agro-scientists are spent to search about alternative safely methods to management plant pests. In this respect, this work aimed to evaluate spraying bean plants with some bacterial and fungal bioagents and essential plant oils, each alone or in alternation in comparison with the fungicide Topas, on management of bean rust. Essential oils are also considered a

promising alternatives with many having antifungal properties. Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Arras *et al.*, 1993). In this regard, lemongrass (*Cymbopogon citratus* L.) oil was reported to be antifungal against several plant pathogens. Also, thymol is an essential oil component from thyme (*Thymus capitates* L.) has been used in plant disease control of several plants (Plaza *et al.*, 2004 and Klaric*et al.*, 2007). Liebenberg and Pretorius (2010) evaluated the efficacy of selected plant extracts; neem (*Azadirachta indica*) derivatives (neem oil, neem cake powder and neem leaf powder) and the commercial fungicide Kocide DF, against bean rust. Results revealed significant high inhibitory effect on rust severity, incidence and urediospore germinationby neem oil. It is suggested that the partial control of rust obtained by application of essential oils, possibly due to the presence of toxic compounds in large quantities, provides a protective effect (Montes-Bemont and Carvajal, 1998). This evidence does not exclude the possibility that other compounds present in oils in smaller amounts may be contributing indirectly to disease control by inducing plant defense response. The bioactivity of the essential oil may be due to the presence of some highly fungitoxic components in the oil. In fact, basil essential oil has mono-terpenes alcohol as the major components (Adjou-Euloge *et al.*, 2012). Terpenes are hydrocarbons produced from combination of several isoprene units (C_5H_8) and have a hydrocarbon back bone which can be rearranged into cyclic structures by cyclases, thus forming monocyclic or dicyclic structures (Caballero *et al.*,2003). It has been found that, in general, the three enzymes PAL, PO and PPO were greatly increased in the leaves of all treated plants by *B. thuringiensis*, *T. viride*, thyme and the fungicide Topas compared with control treatment. In addition, plants sprayed with thyme recorded the highest activity of the three enzymes followed by those treated with *B. thuringiensis* and *T. viride*. It has been found that the reduction in disease severity was attributed to the increased levels of PAL, PO and PPO enzymes. Farkas and Kiraly (1967) and Morkunas and Gemerek (2007) reported that peroxidase enzyme oxidizes the phenolics to more fungal toxic compounds such as quinines, which inhibit both spore germination and fungal growth. Also, peroxidase was found to be participating in the synthesis of lignin. Moreover, Farkas and Kiraly (1967) and Melo *et al.* (2006) showed that the participation of an endogenous supply of phenolic compound in the plant disease resistance is dependent upon active phenol oxidase system. Protection of plants from plant pathogens by induction of systemic resistance is a new approach and is of much less harmful to the environment and plant products as compared to deadly agrochemicals applied to control plant diseases. Yan *et al.*, (2003) reported that induced systemic resistance was observed in tomato against late blight, caused by *Phytophthora infestans* with *B. pumilus* strain SE34n that was incorporated into the potting medium. Also, *Bacillus megaterium* strain 4 was found to be effective to

control Fusarium wilt of tomato (Terhardt, 1998).*B. thuringiensis* was used to induce systemic resistance in mung bean plants against root colonizing phytopathogenic fungi (Sheikh *et al*., 2006), *B. thuringiensis* strains capable of inducing systemic response to Fusarium wilt of tomato (Akram *et al.*, 2013). Pathogenicity-related proteins are usually quantified to assess the activation of defense system of plants. Plants treated with the two bioagents, the essential oil thyme and the fungicide Topas compared with control treatment showed, also, an increase in PAL, PO and PPO activity. It is well known that PPO is a copper containing enzyme, which is responsible for oxidization of phenolics to highly toxic quinines. This enzyme is also involved in terminal oxidation of diseased plant tissue, and this role of this enzyme is attributed in disease resistance (Kosuge, 1996). On the other hand, PAL is the main enzyme in phenyl propanoid pathway and flavonoid pathway (Scott *et al.*, 1995) and peroxidase enzyme is related with more than one function in plants.

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مكافحة مرض صدأ الفاصوليا باستخدام بعض الكائنات المضادة والزيوت النباتية الطيارة

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تم عزل وتقييم الفطريات والبكتريا الموجودة طبيعيا على أوراق الفاصولياككائنات مضادة للفطر *Uromyce sappendiculatus* (Pers. ex Pers.) Unger المسبب لمرض صدأ الفاصوليا، وتم اختيار عزلات من كل من *Bacillus* spp و *Trichoderma* spp. تم دراسة التأثير المثبط لعزلات الكائنات المضادة وكذلك الزيوت الطيارة لكل من حشيشة الليمون والنيم والزعتر على انبات الجراثم اليوريدية لفطر صدأ الفاصوليا.تم اختبار التأثير المثبط لعزلات بكتريا *Bacillus* spp على إنبات الجراثيم اليوريدية لفطر صدأ الفاصوليا فى المعمل حيث تراوحت بين 37.7-53.9 % وعزلات الفطر *Trichoderma* spp تراوحت بين 39.3 - 58.8 % والزيوت الطيارة تراوحت بين 34.9 – 52.2%.من بين البكتريا، سجلت البكتريا *B. thuringiensis*أعلى معدل لتثبيط إنبات الجراثيم اليوريدية لفطر صدأ الفاصوليا تلاها *B. subtilis* ثم *B. megaterium* و *P. pumilus* ومن بين الفطريات أعطى فطر *T. viride* أعلى معدل تثبيط تلاه *T. harzianum* ثم *T. hamatum*و *T. album*.،ومن بين الزيوت أعطى زيت الزعتر أعلى معدل تثبيط تلاه زيت حشيشة الليمون ثم زيت النيم.تحت ظروف الصوبة، تم معاملة نباتات الفاصوليا بالكائنات المضادة والزيوت النباتية الطيارة قبل خمس أيام أو بعد خمس أيام من عدوى النباتات بالجراثيم اليوريدية لفطر صدأ الفاصوليا وأوضحت النتائج وجود انخفاض ملحوظ فى شدة الإصابة مع زيادة معنوية فى محصول القرون الخضراء بالمقارنة مع معاملة المقارنة.أعطى كل من *B. thuringiensis* و *T. viride* وزيت الزعتر أعلى معدل فى خفض شدة الإصابة بالمرض وزيادة عدد القرون الخضراء ووزنها بالمقارنة مع معاملة المقارنة، لذلك تم استخدامها بالتعاقب مع بعضها البعض بالمقارنة بالمبيد توباس لمقاومة المرض. أدى رش هذه المعاملات منفردة أو متعاقبة مع بعضها البعض إلى حدوث انخفاض كبير وكان أفضل المعاملات رش كل من *B. thuringiensis*ثم زيت الزعتر وكذلك *T. viride* ثم زيت الزعتر حيث سجلت كفاءة تقترب من كفاءة المبيد توباس. سجلت انزيمات الأكسدة الإختزال الثلاثة PAL و PO و PPOزيادة واضحة فى الأوراق المعاملة بالمقارنة مع معاملة المقارنة، سجلت النباتات المرشوشة بزيت الزعتر أعلى نشاط فى الإنزيمات الثلاثة تلاه المعاملة ببكتريا *B. thuringiensis*ثم الفطر *T. viride* ثم المبيد توباس.