تأثير رش بعض المبيدات الفطرية والزيوت النباتية الأساسية على مكافحة مرض صدأ الفاصوليا

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المُلخّـــــص :

أدّت كـــلا من المبيدات الفطرية الدايثين م-45 وكوسيد-2000 والسومى -8 والتوباس والزيوت النباتية الأساسية للسترونيلا والقرنفل والزعتر في انخفاضا معنويا للجراثيم اليوريدية النابتة للفطر *Uromyces apendeculatus* المسبب لمرض صـــدأ الفاصوليا .

بالإضافة لذلك ، فقد أحدثت المبيدات الفطرية النسب الأعلى من التثبيط للجراثيم اليوريدية للفطر أكثر من الزيوت الأساسية النباتية المستخدمة ، وقد تراوحت نسب التثبيط للمبيدات الفطرية بين 14.2-34.9 % بفعالية بين 64.3-85.1 % والزيوت الأساسية النباتية بين 17.1-19.8 % بفاعلية بين 79.4-82.0 %. وكان المبيد توباس هو الأكثر فعالية (85.1 %) تلاه المبيد سومى-8 (84.1%) ثم زيت القرنفل (82.0 %) ، بينما كان المبيد دايثين م-45 هو الأقل فاعلية (64.3 %) تلاه المبيد كوسيد-2000 (66.3 %) .

أدّى رش نباتات الفاصوليا من المبيدات الفطرية والزيوت النباتية المختبرة قبل أو بعد العدوى بالجراثيم اليوريدية للفطر المسبب للمرض إلى حدوث انخفاضا معنويا في شدة الإصابة بالمرض مع حدوث زيادة معنوية في محصول القـــرون الخضراء الناتج مقارنة بمعاملة المقارنة ، وكان المبيد توباس وزيت القرنفل هما الأكثر فاعلية في خفض شدة الإصابة بالمرض مع حدوث زيادة عدد القـــرون الخضراء ووزنها عند المقارنة بالمعاملات الأخـــرى.

تم تسجيل زيادة محسوســـــة للمركبات الفينولية الكلية والنسب المئوية للنتروجين والبروتين الموجودين في القرون الخضراء للنباتات التي تم رشها بالمبيدات الفطرية والزيوت النباتية المختبرة مقارنة بمعاملة المقارنة.

Role of some fungicides and essential plant oils on management of snap be*an* rust in Libya

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ABSTRACT

Significant reduction to the germinated urediniospores of *Uromyces apendeculatus*, the causal of bean rust disease, was occurred by the fungicides Dithane M-45, Kocide-2000, Sumi-8 and Topas as well as the essential oils of citronella, clove and thyme *in vitro*. Moreover, the tested fungicides resulted the highest inhibitory effect to the germinated urediniospores of the

causal fungus than the tested essential plant oils. The inhibitory effect of the tested fungicides ranged between 14.2-34.9% and efficiency ranged between 64.3-85.1% , while in case of the essential plant oils ranged between 17.1-19.8 % and efficiency ranged between 79.4-82.0*%* . Topas fungicide was the most efficient one in this regard (85.1%), followed by Sumi-8 (84.1%) then cove oil (82.1%). Meanwhile, DithaneM-45 was the lowest efficient one (64.3%), followed by Kocide-2000 (66.3%).

Greenhouse experiment revealed that spraying of snap bean plants with the tested fungicides and essential plant oils, three days before or after inoculation with *the* urediniospores of the causal fungus led to a significant reduction to severity of the disease with a significant increase to the produced green pods yield compared with the control. The most efficient treatments in reducing the severity of the infection with the disease and increasing the number of the produced pods and their weight plant^{-1} were Topas fungicide and clove oil when compared with the other treatments. Meantime spraying of any of the tested fungicides and the essential plant oils alone led to a low efficiency and produced low green pods yield . Meanwhile, the alternation of spraying the tested fungicides two sprays firstly then spraying the essential oils another two sprays was of high efficacy in lowering the disease and increasing the produced green pods yield nearby the efficiency of both Sumi-8 and Topas fungicides, each alone.

Noticeable increase the total phenolic compounds in the leaves of snap bean and the percentages of nitrogen and protein content in the green bean pods of the sprayed plants by the tested fungicides and essential plant oils was occurred compared with the control treatment.

Key words: Bean, rust, essential plant oils, fungicides, green pod yield, % nitrogen, *%* protein, phenolic compounds .

INTRODUCTION

Snap bean (*Phaseolus vulgaris* L.) is considered one of the most important food legume crops all over the world including Libya. Nutritional value of snap beans can't be denied as these are an excellent source of protein, carbohydrates, water-soluble fibers, vitamins and antioxidants. As much as 60% of bean production in the developing world occurs under conditions of drought and salinity stresses **(Hagderon and Inglis , 1986 ; Schwartz *et al.*, 2010 and Azmeraw and Hussien, 2017).**

In Libya, the cultivated area with snap bean is annually increased due to the increasing of its demand to the local consumption. Snap bean is liable to be attack by many bacterial , fungal viral , nematode diseases in addition to physiological disorder **(Hagderon and Inglis,1986 ; Zyton and Ahemed, 2016 and Azmeraw and Hussien, 2017)**) . However, fungal diseases , especially rust is considered one of the major destructive diseases affecting the crop yield **(Hagderon and Inglis,1986; Jochua *et al.*, 2008 ; Mersha and Hau , 2008 and Liebenberg and Pretorius, 2010)**, in several countries in the world including Libya , where high humidity is prevail .

Bean rust infection can occur on most aerial grown parts of bean plant but is most often observed on the leaves. Symptoms, also, occur on pods and sometimes on branches and stem .The spots firstly appear as small brown dots containing a brown powder, which are the urediniospores of the pathogen. Finally, the spots become larger and spores turn black. If the infection by the disease is more than 79.0 %, the loss will be 37.0% **(Hagderon and Inglis,1986).**

Good results could be achieved when fungicides are used for managing plant diseases, but in most cases the use of pesticides is indispensable due to its high effectiveness in combating diseases, taking into account that it is given a sufficient period after spraying to collect the crop to reduce its residual effect on plants, especially vegetables and fruits. In order to increase the time between spraying pesticides and collecting the crop, it is possible to spray safe materials to combat the disease as is followed in this research by spraying vegetable oils twice after spraying plants with fungicides in order to obtain a crop free from the effect of the used pesticides or reduce them to a safe limit.

Nowadays, essential plant oils, are a powerful alternative to conventional fungicides. for managing plant diseases, where they are safe. The fungicidal effect of these compounds against several plant pathogens is widely reported **(Behtoei *et al.*, 2012; Amini *et al.*, 2016 and Varo *et al.*, 2017)**. However, the mode of action of these compounds is not yet completely unraveled. The anti-fungal effect includes the suppression of spore germination and reduction of hyphal growth. This can be attributed to the fact that the application of essential oils can lead to changes in cell wall composition, plasma membrane disruption, and mitochondrial structure dis-organization. Furthermore, essential oils can interfere with the enzymatic reactions of the mitochondrial

membrane, such as respiratory electron transport, proton transport, and coupled phosphorylation steps (**Rasooli *et al.*, 2006; Kishore *et al.*, 2007 and Dewitte *et al.*,2019)**. In this concern, clove oil has demonstrated toxicity to various microbes, including plant-pathogenic fungi and bacteria **(Beg and Ahmad, 2002; Bowers and Locke, 2004 and Kishore *et al.*, 2007).**

This investigation aimed to evaluate the efficiency of some fungicides and essential plant oils on the fungus *U.appendiculatus* , the causal of snap bean rust *in vitro* and *in vivo.* The research pertained the effect of these treatments on the total phenols content in the leaves in addition to % total nitrogen and % protein in the green snap bean seeds.

MATERIALS AND METHODS

Source of seeds*:*

Bean seeds (Bolesta cv) were purchased from at store at Tripoli-Al-Karimiya, Libya.

Fungal pathogen:

Snap bean leaves bearing the uredial sori of *Uromyces appendiculatus* were frequently used to collect the pathogen.

Evaluation of the tested fungicides and the essential plant oils *on* the germination percentage of the urediniospores *of U. appendiculatus*:

The inhibitory effect of different four fungicides *i.e.*, Kocide-2000 (copper hydroxide), DiathameM-45 (mancozeb), , Sumi-8 (diniconazole) and Topas (penconazole **)** and three essential plant oils *i.e.*, citronella (*Cymbopogon nardus*), clove [*Syzygium aromaticum* (=Eugenia caryophyllata)], and thyme (*Thymus vulgaris*) on the urediniospore germination was carried out *in vitro.*

Tested fungicides were prepared **at t**he concentrations of 50, 100 , 250 and 500 ppm depending on their active ingredient.

Citronella, clove and thyme essential plant oil was diluted to the concentrations of 50, 100, 150 and 250 ppm using sterilized distilled water plus few drops of Tween-20 to make emulsion.

The freshly urediniospores of *U. appendiculatus* were added to each concentration of both fungicides and the essential plant oils. One m1 of uredial suspension was placed on two sterilized slides, borne on two glass rods in a sterilized Petri-dish containing wetted cotton piece by sterilized distilled water to raise the relative humidity. The same procedure was occurred for a spore suspension kept only in distilled sterilized water and

severed as control treatment. All prepared dishes were incubated in an incubator at 25±2 °C for 48 hour. Five replicated Petri-dishes were used for each treatment. The uredial germination percentage was counted in a total of 100 urediniospore in each Petri-dish by an optical microscope (10X magnification) and examining 100 spores per each replicate. The average of each treatment was recorded.

Greenhouse experiment:

Greenhouse experiment was carried out to assess the antifungal activity of the four tested commercial fungicides *i.e.*, Kocide-2000, Dithane M-45 , Sumi-8 and Topas as well as the three plant essential oils of citronella, clove , and thyme for their efficacy on managing snap bean rust caused by *U.appendiculatus* in pots (25 cm in diameter) under artificial inoculation conditions Tween 20 has been added as a spreading adhesive material to the sprayed fungicides and essential plant oils at the rate of 0.5 ml l^{-1} water. Bolesta cv. seeds were sown in plastic pots at the rate of 4seeds pot^{-1} containing formalin sterile silt soil. After two weeks, the emerged seedlings were thinned into two plants in each pot. Five replicates of 35 days old plants for each treatment were sprayed at the rate of 100 ppm with the tested fungicides and the three essential plant oils at three days before or after artificial inoculation with the causal fungus urediniospores suspension (1x10 ^{3} urediniospore ml^{-1} water). The plants were sprayed firstly three days before or after the inoculation with the urediniospores of the causal fungus then three sprays with two weeks interval. The plants of control treatment were sprayed only with urediniospores suspension of the causal fungus.

The severity of the disease was assessed one week after each spray with the tested materials and the average was recorded for each material. The produced green pods were counted, harvested and weighed in each harvest and the average number of green pods and their weight (g) $plant^{-1}$ were recorded.

Disease assessment:

The artificially inoculated plants with the urediniospores of the causal pathogen were carefully examined to assess the severity of the infection by snap bean rust using the devised scale (0-6) by **Godoy *et al.* (1997)** using the following formula:

(nxv)

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

Where:

n = Number of infected leaves in each category.

v = Numerical values of each category.

N = Total number of the infected leaves.

Biochemical studies

Determination of total phenolic compounds:

Five g snap bean leaves representing each treatment were extracted with 50 ml of 80% methanol at 70 °C for 15 mins. The reaction mixture contained 5 ml of methanolic extracts, 25 ml of distilled sterilized water and 250 µL of Folin–Ciocalteu reagent (1 N). This solution was kept at 25±1°C. The absorbance of the developed blue color was measured using a spectrophotometer at 725 nm. Gallic acid was used as the standard. This estimation was carried out 15, 30 and 45 days after each treatment with the fungicides and essential plant oils, the pathogen, and the control. The amount of phenolic compounds was expressed as mg gallic acid g plant^{-1} material **(Zieslin and Ben-Zaken, 1993).**

Determination of nitrogen and protein content in snap bean seeds:

Snap bean pods were randomly taken from the yield of each treatment. The percentage of nitrogen in the green seeds was determined according to the method described by **Hafez and Mikkelsen (1981)**. In addition, protein percentage was calculated by multiplying nitrogen content by 6.25.

Statistical analysis:

Results obtained were statistically analyzed using the standard procedures for split design as mentioned by **Snedecor and Cochran (1989).** The averages were compared at 0.05 level by using least significant differences (L.S.D) according to **Fisher (1948).**

RESULTS

***In vitro* evaluation of the inhibitory effect of the tested fungicides and essential plant oils on the germinated urediospores of *Uromyces appendiculatus* :**

In vitro evaluation of the inhibitory effect of the test fungicides and the essential plant oils on the germination of *U. appendiculatus* urediniospores is shown in Tables (1 and 2) .

Data presented in Table (1) indicate that the usage of the tested fungicides *i.e.*, Kocide-2000, DithaneM-45, Sumi-8 and Topas resulted in significant reduction of the germinated urediniospores ranged from 14.2-33.9 %. In addition, Topas fungicide was the most efficient in this respect followed by Sumi-8 then Dithane M-45. The analogous *values were* 14.2, 15.1, 32.0 and 33.9 % uredial germination with efficiency of 85.1, 84.1, 66.1 and 64.3%, respectively.

Table (2) indicates that the tested essential oils *i.e.*, citronella, clove oil and thyme resulted a significant reduction of the germinated urediniospores of the causal fungus, being 19.8, 17.1 and 18.9 % uredial germination with efficiency of 82.0, 80.1and 79.4 , respectively. Gradual decrease to the germinated urediniospores was occurred by increasing the concentration of the tested fungicides and essential plant oils. The percentage of the geminated urrediniospores in control treatment was 95.0%

Table 1. *In vitro* effect of four fungicides on *the* germinated urediniospores of *U. appendiculatus*, 48 h after incubation at 25±1 °C.

Fungicides	% Uredial germination* at concentration (ppm) 50	100	250	500	Mean	% Efficiency
DithaneM-45	65.0	49.2	21.4	0.0	33.9	64.3
Kocide-2000	62.4	46.6	19.0	0.0	32.0	66.3
Sumi-8	38.4	22.0	0.0	0.0	15.1	84.1
Topas	36.2	20.4	0.0	0.0	14.2	85.1
Control**	95.0	95.0	95.0	95.0	95.0	-----
Mean	50.5	34.6	10.1	0.0	-----	-----

* Initial germination percentage was 1.8 %., **Control not included in calculating the mean. L.S.D. at 5 %: Fungicides (F)= 2.8, Concentration (C)= 3.7, FxC = 4.2.

Table 2. *In vitro* effect of different essential plant oils on the germinated Urediniospores of *U. appendiculatus*, 48 h after incubation at 25± 1°C.

Essential oils	% Uredial germination* at concentration (ppm) 50	100	150	250	Mean	% Efficiency
Citronella	40.8	23.2	14.4	0.0	19.6	79.4
Clove	36.4	20.0	12.0	0.0	17.1	82.0
Thyme	39.2	22.4	14.0	0.0	18.9	80.1
Control**	95.0	95.0	95.0	95.0	95.0	-----
Mean	46.2	21.9	13.5	0.0	-----	-----

* Initial germination percentage was 1.2 %, **Control not included in calculating the mean.

L.S.D. at 5 % for: Essential plant oils (E) = 2.6, Concentration(C)=3.1 and ExC= 2.8.

Greenhouse experiment:

The obtained data of the greenhouse experiment concerning the effect of the tested fungicides and the essential plant oils on the severity of snap bean rust and the number and weight of green pods, under greenhouse conditions are presented in Table (3).

Under greenhouse conditions, spraying snap bean plants with any of the tested fungicides and the essential plant oils , three days before inoculation with *U. appendiculatus* significantly reduced rust severity (Table, 3). In addition, the fungicides were more efficient than the essential plant oils in reducing disease mortality and increasing the produced green pods yield. Moreover, spraying these materials was more efficient in reducing the disease and increasing the produced green pods yield when sprayed 3 days before inoculation with the causal fungus than those sprayed with the tested materials 3 days after inoculated with the causal fungus. The severity of the disease of plants sprayed with the tested fungicides alone *i.e.*, DithaneM-45, Kocide-2000, Sumi-8 and Topas was lowered than the plants sprayed with the tested essential plant oils *i.e.*, citronella, clove and thyme, being 13.1, 14.4, 8.5 and 8.2% for fungicides **,** respectively **and was** 17.4, 15.8 and 15.6% for the essential plant oils, respectively (Table, 3)**.**

When the tested essential plant oils were spraying two sprays after spraying the tested fungicides two sprays great reduction to the severity of the disease in comparison with spraying each of them only four sprays was resulted. The highest increase was recorded when any of the essential plant oils *i.e.*, citronella, clove and thyme were sprayed two sprays after spraying Topas fungicide two sprays, being 6.0, 6.1 and 6.0%, disease severity respectively. Meanwhile, the lowest bi-combination was found when the plants sprayed with DithaneM-45 and citronella (11.2%).

Severity reduction of the disease was reflected on the number and weight of the produced green pods, where remarkable increase to the produced green pod yield was recorded. The variability in the number of the produced green pods was not high, but their weight recorded greet variation. In addition, the

produced green pods of plants sprayed with the tested fungicides alone was, also, lower than the plants sprayed with the tested essential plant oils alone. On the other hand, the bi-combination between Sumi-8 and Topas fungicides and the essential oils resulted in producing the highest effect on increasing the number of green pods (31.5-34.2 pod) and their weight (**170.6** -133.7 g) compared with all the other treatments. Meanwhile, spraying Sumi-8 and Topas fungicides alone was of moderate effect in this regard.

Table 3**.** Effect of spraying snap bean plants with four fungicides and three essential plant oil, each alone and following the fungicides by the essential oils, 3 days before or after *U. appendiculatus* inoculation on the severity of snap bean rust (Bolesta cv.) in addition to the produced green pods yield, greenhouse experiment.

Treatments	% Rust severity of plants treated 3 days before or after the artificial inoculation		Mean	Number of green pods plant^{-1} of plants treated 3 days before or after the artificial inoculation		Mean	Weight of green pods yield (g) plant^{-1} of plants treated 3 days before or after the artificial inoculation		Mean
	Before	After		Before	After		Before	After	
Kocide-2000 (KO)	**12.9**	**13.1**	**13.1**	**25.0**	**24.8**	**24.8**	**24.2**	**144.0**	**142.9**
DithaneM-45 (DI)	**13.2**	**14.2**	**14.2**	**24.6**	**24.4**	**24.5**	**30.2**	**141.8**	**141.4**
Sumi-8 (SU)	**13.8**	**8.5**	**8.5**	**24.4**	**23.8**	**24.1**	**30.6**	**142.6**	**165.9**
Topas (TO)	**14.5**	**8.2**	**8.2**	**23.8**	**23.2**	**23.5**	**23.2**	**140.2**	**168.0**
Citronella (CI)	**8.0**	**17.4**	**17.4**	**30.4**	**30.0**	**30.2**	**24.1**	**166.3**	**139.8**
Clove oil (CL)	**9.0**	**15.8**	**15.8**	**30.0**	**31.0**	**30.5**	**24.3**	**165.4**	**141.2**
Thyme (TH)	**7.8**	**15.6**	**15.6**	**31.0**	**30.2**	**30.6**	**25.5**	**168.5**	**140.2**
CO then CI	**8.7**	**10.1**	**10.1**	**30.2**	**30.0**	**30.1**	**27.2**	**167.5**	**161.3**
	16.8	**10.6**	**10.6**	**23.8**	**23.6**	**23.7**	**28.0**	**140.0**	**159.8**
	18.0	**10.0**	**10.0**	**22.6**	**22.4**	**22.5**	**27.4**	**139.6**	**160.0**
	15.2	**11.2**	**11.2**	**24.6**	**24.4**	**24.5**	**27.5**	**142.3**	**160.5**
	16.4	**10.1**	**10.1**	**23.6**	**23.4**	**23.5**	**27.5**	**140.0**	**159.5**
	14.7	**10.5**	**10.5**	**24.8**	**24.6**	**24.7**	**32.6**	**140.4**	**160.0**
	16.4	**6.8**	**6.8**	**23.6**	**23.4**	**23.5**	**31.5**	**140.0**	**173.7**

Treatments	% Rust severity of plants treated 3 days before or after the artificial inoculation Before After	Mean	Number of green pods plant^{-1} of plants treated 3 days before or after the artificial inoculation Before After	Mean	Weight of green pods yield (g) plant^{-1} of plants treated 3 days before or after the artificial inoculation Before After	Mean
CO the CL	9.6	7.2	25.6	32.7	161.7	172.8
CO then	10.6	6.9	24.4	34.2	160.9	173.4
TH	10.0	6.0	27.4	33.5	160.4	171.5
DI the CI	11.1	6.1	27.0	34.2	159.2	170.6
DI then CL	9.6	6.0	28.6		161.0	171.5
DI the TH	10.4		27.4		160.0	
SU then CI	10.8		27.8		160.0	
SU then CL	11.6		27.0		159.0	
SU the TH	9.8		28.0		160.6	
TO then CI	10.4		27.0		159.4	
TO then	9.9		28.0		161.0	
CO	11.0		27.0		159.0	
TO then	6.4		33.0		174.4	
TH	7.1		32.2		173.0	
	7.0		32.0		173.0	
	7.4		31.0		172.6	
	6.5		33.0		173.8	
	7.2		32.4		173.0	
	5.8		34.8		172.0	
	6.2		33.6		171.0	
	6.0		34.0		171.0	
	6.2		33.0		170.2	
	5.8		34.8		172.0	
	6.2		33.4		171.0	
Control*	36.8 37.6	37.2	16.0 15.6	15.8	66.4 65.6	66.0
Mean	9.8 10.6	-----	29.0 28.1	------	151.8 150.7	-----

* Control not included in the mean

L.S.D. at 5 % for:			
Treatments (T)	=	2.3	2.8 4.0
Period (P)	=	ns	ns ns
TxP	=	3.3	2.8 5.3

Biochemical studies

Estimation of total phenolic compounds:

Spraying of any of the tested fungicides and essential plant oils on snap bean plants 3 days before inoculation with the causal fungus of rust resulted in remarkable increase in the total phenolic compounds compared with the unsprayed control plants (Table, 4). This increase was gradually increased by increasing the time of inoculation with the pathogen *i.e.,* 15 , 30 and 30 days, being 0.45, 0.63 and 0.70 mg g plant^{-1} leaves , on the average, respectively. In addition, the highest increase in the total phenolic compounds was occurred by the essential plant oils, being 0.59, 0.60 and 0.59 mg/ g plant leaves, on the average compared with the tested fungicides, being 0.54, 0.55,0.57 and 0.57 mg g plant^{-1} leaves, on the average, respectively. In the meantime, the low increase was recorded for the un-inoculated control plants, followed by inoculated control, being 0.42 and 0.49 mg g plant^{-1} leaves, on the average, respectively.

Determination of nitrogen and protein content in snap bean seeds:

The percentages of the estimated nitrogen and protein content in snap bean seeds (Table,4) were greatly increased due to spraying the tested fungicides and essential plant oils on snap bean plants 3 days before inoculation with the causal fungus of rust. The corresponding figures for fungicides were 1.43, 1., 1.46 and 1.47 % total nitrogen; 8.9 9.0, 9.1 and 9.2 % protein. The corresponding values for the essential plant oils were 1.47. 1.48 % total nitrogen and 1.47; 9.2, 9.3 and 9.2 % protein, respectively compared with un-inoculated and inoculated controls, being 1.40 and 1.22 % total nitrogen and 8.8 and 7.6 % protein, respectively.

DISCUSSION

The world, in recent years,, suffers from high environmental pollution including agrochemicals that causes health hazard to all alive organisms. Hence, the production

of healthy and safe food staff free from toxic agrochemicals is the desire of the consumer, especially those consume freshly like snap bean. On the other hand, it is well known fungicides, especially systemic ones, until now are the efficient method to management plant diseases **(Fontem and Bouda, 1998 and Mc Grath *et al.*, 2019).**

Table 4. Effect of spraying four fungicides and three essential plant oils on pathogen-treated snap bean plants (Bolesta cv) on the content of phenolic compounds and the percentages of nitrogen and protein content of snap bean pods.

Treatments	Gallic acid in mg g plant^{-1} leaves after (days) of inoculation with the pathogen			Mean	% Total nitrogen	% Protein
	15	30	45			
DithaneM-45	0.42	0.56	0.64	0.54	1.43	8.9
Kocide-2000	0.42	0.58	0.66	0.55	1.44	9.0
Sumi-8	0.44	0.62	0.69	0.57	1.46	9.1
Topas	0.44	0.62	0.69	0.57	1.47	9.2
Citronella oil	0.45	0.62	0.70	0.59	1.47	9.2
Clove oil	0.45	0.63	0.71	0.60	1.48	9.3
Thyme oil	0.45	0.63	0.70	0.59	1.47	9.2
Control (un-inoculated)	0.36	0.44	0.47	0.42	1.40	8.8
Control (inoculated)	0.39	0.50	0.58	0.49	1.22	7.6

In most cases, synthetic fungicides are usually used as effective, dependable and economical control measures to control fungal diseases. However, the indiscriminate use of chemical fungicides has resulted in several problems, such as toxic residues in food. So, the timing of spraying the fungicides, especially system ones, is very important to produce healthier food. Because of, eradicative action, fungicides application also provide a chemical toxic barrier against pathogens and are thus unavoidable means of managing many plant diseases. In this respect, the tested fungicides were sprayed firstly two sprays then the essential plant oils another two sprays with two weeks intervals in all sprays. Therefore, there have been a long period after spraying the fungicides and thus this period is sufficient to convert the fungicides sprayed inside the plant to non-toxic substances, or at least their concentration decreases significantly to reach the safety level. In present investigation, fungicides and essential plant oils were evaluated for management of bean

rust. Thyme oil and its major phenolic constituents thymol and carvacrol are clearly active against most fungal species tested **(Carmo *et al.*, 2008 and Gallucci *et al.*, 2014).**

It has been found that, all the tested fungicides and essential plant oils decreased the germinated urediniospores of *the causal fungus* compared with the control. This decrease was gradually decreased by increasing the concentration of the tested fungicides and essential plant oils.The inhibitory effect of the tested fungicides *i.e.*, DithaneM-45, Kocide-2000, Sumi-8 and Topas against *the causal fungus* was in the range of 14.2-39.9 % (64.3 % efficiency) uredial germination . In addition, Topas fungicide was the most efficient in this regard followed by Sumi-8 then Kocide-2000 and Dithane M-45, being 85.1, 84.1, 66.3 and 64.3 % efficiency , respectively.

The systemic fungicide Topas is of fast uptake and penetration and strong translaminar and acropetal translocation. It controls both primary and secondary infections with long-lasting preventative and curative activity for the control of powdery mildews and rusts of grapes, vegetables, fruit-crops, hops, tobacco and ornamentals.

The three tested essential plant oils ,*i.e.* citronella, clove oil and thyme resulted in a significant inhibitory effect to the germinated urediniospores of the causal fungus . In this respect, clove oil caused the highest inhibitory effect, followed by citronella then thyme.

The variability in antifungal activities of the essential plant oils might be due to the physical, molecular, and chemical characteristics of the EOs and the sensitivity of the pathogens to the quantitative differences in the constituents of the oil (**Stevic *et al.* , 2014**). Studies on the antifungal components of EOs have shown that the oils consist of 20 to 60 components at various concentrations (**Bakkali et al., 2008**). Their most common constituents are terpenes and aromatic and aliphatic compounds, especially alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers (**Bakkali *et al.*, 2008**). For example, major components of *Thymus vulgaris* (thyme EO) were thymol (48.9%) and p-cymene (19.0%) (**Sokovic *et al.*, 2009**).

Although the present study found that spore germination was inhibited by the tested three essential plant oils, the levels of inhibition were different. This could be attributed to differences in the mode of action of the oils, and spore

sensitivity to the oils. Infection and spread of fungal pathogens occur mainly through spores. Therefore, inhibition of sporulated spores is desirable for targeting the pathogen to prevent or slow down intra-plant and interplant disease spread.

The efficiency of the tested fungicides and essential plant oils in reducing disease 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 3 days before inoculation with the causal fungus than those sprayed with the tested materials 3 days after inoculated with the causal pathogen. The reduction in the severity of the disease was significantly reflected on the produced green pods. **Sharma *et al.* (2019)** revealed that under *in vitro* conditions minimum urediniospore germination was recorded using zineb + hexaconazole (0.69%) in comparison with germination in the control (45.26%). Minimum germ tube length (15.48µm) was observed in case of zineb + hexaconazole as when compared with the germ tube length in control (149.4 µm). Under *in vivo* conditions, though all the fungicides could significantly reduce rust severity in comparison with unsprayed control treatment (49.33%), yet sprays of azoxystrobin (0.1%) was significantly most effective in reducing the disease severity to 4.64 % and also resulted in the maximum green pod yield (6.80 kg plot^{-1}), whereas the yield of control plot was 3.45 kg plot^{-1}. Azoxystrobin in addition to the fungicidal activity also exhibited some phytotonic effect which delayed the crop senescence and attributed to enhanced yield of the crop.

Mancozeb, hexaconazole, chlorothalonil, azoxystrobin, propiconazole and difenoconazole efficiency against bean rust (*U. appendiculatus*) have been reported by different workers **(Modesto, 2005; Shukla and Sharma, 2009 and Sharma *et al.*, 2019)**.

Due to the hazard effect of fungicides when used in disease management, essential plant oils are considered of a promising alternative with having many 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 and Oliveira *et al.*, 2019)**. In this regard, lemongrass (*Cymbopogon citratus* L.) oil was reported to be antifungal

activity against several plant pathogens. Also, thymol is an essential oil component from thyme (*Thymus capitates* L.) has been used as plant disease control of several plants **(Plaza *et al.*, 2004 and Klaric *et al.*, 2007)**. Meantime, **Liebenberg *et al.* (2010)** found that *in vivo* evaluation of 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 was conducted. The present data revealed significantly high inhibitory effect on rust severity, incidence and urediniospores germination by citronella 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 **(Oliveira *et al*., 2019)**. 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. Also, **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. Meantime, peroxidase was found to be participate in the synthesis of lignin. Moreover, **Farkas and Kiraly (1967) and Melo *et al.* (2006)** declared that the participation of an endogenous supply of phenolic compound in the plant disease resistance is dependent upon active phenol oxidase system.

Lombardo *et al.* (2016) and Zhang *et al* . (2019) reported that the essential plant oils can be extracted from many plant species and have reportedly shown antimicrobial actions against many fungal that damage the grown plants.The use of essential plant oils for managing plant diseases are increasingly globally and becoming popular among consumers because of their health benefits and environmental-friendly features **(Oliveira *et al.*, 2019)**, where their secondary metabolites are found abundantly in natural plant extracts **(Lombardo *et al.*, 2016).**

The essential plant oils affect microorganisms by altering the integrity and stability of cell membranes, leading to the leakage of amino acids and genetic materials (DNA and RNA), so interrupting the normal growth of pathogenic cells and deforming the cells **(Guo *et al.*, 2017)**. In addition, essential plant oils are specifically known for their mechanisms of action that alter the integrity of cell membrane of the pathogen, thereby leading to

protein leaks, changes in the growth of microorganisms and disfigurations in their shape**)**. In general, the mode of inhibitory action by essential plant oils involves cytoplasmic granulation, rupture in the cellular membrane and inactivation of enzymes **(Campo *et al.*, 2003)**.

Using of essential plant oils in management of many plant diseases was previously successfully applied by many authors (**Klaric *et al.*, 2007; Lombardo *et al.*, 2016; Zyton and Ahmed, 2016; Oliveira *et al.*, 2019 ; Zhang *et al*., 2019 and Ranjbar *et al* ., 2022)** .

The obtained results of spraying any of the tested fungicides and essential plant oils on snap bean plants 3 days before inoculation with the causal fungus of rust recorded remarkable increase in the total phenolic compounds, % total nitrogen and % protein compared with the unsprayed control plants. This increase was gradually increased by increasing the time of inoculation with the pathogen. In addition, the highest increase in the total phenolic compounds was occurred by the essential plant oils compared with the tested fungicides.

Phenolic and polyphenolic compounds are ubiquitous in plants and play an important function in non-host resistance to filamentous fungi. The term "Phytoanticipin" has been proposed to distinguish these preformed antifungal compounds from phytoalexins, which are synthesized from distant precursors in response to pathogen attack. Some antibiotic phenolic compounds are found in plant cells as inactive bound forms but are readily converted into biologically active antibiotics by plant hydrolyzing enzymes (glycosidases) in reaction to pathogen attack. In such circumstances, free phenolic compounds are likely to be considerably more toxic to the invading organism than the bound forms **(Lattanzio *et al.* ,2006)**. Moreover, even if preformed antifungal phenolic compounds are present in healthy plants at levels that are anticipated to be antimicrobial, their levels may increase further in return to challenge by pathogens. It is well known that phenolic compounds content are the compounds whose quantity is raised when a plant comes under invade by a pathogen **(Waterman and Mole, 1995). Lattanzio *et al.* (2006); Melo *et al*. (2006) and Farkas and Kiraly (2008)** mentioned that the participation of an endogenous outfit of phenol compound in the plant disease resistance is dependent upon active phenol oxidase system.

The metabolic changes that occur in host tissues during a necrotrophic plant/fungal interaction have been poorly investigated. Whereas carbon metabolism reprogramming and photosynthesis disturbances have been studied, data on plant amino acids stores during infection are scarce **(Dulmero *et al.*, 2009). In this regard, Reddy *et al.* (2005)** reported that the increase in soluble nitrogen may be associated to increase hydrolysis of proteins. Moreover, **El-Sayed (2017) and Attia *et al.* (2022)** found that there was considerable increase in the percentages of total nitrogen and protein content of faba-bean and pea seeds due to controlling damping-off and root-rot diseases compared with untreated plants.

CONCLUSION

Nowadays, essential plant oils have been recognized as a crucial component of agricultural biotechnology for controlling a lot of plant pathogens, and as a sustainable and ecologically acceptable as alternative and / or combination to conventional disease management methods. According to the obtained results, praying any of the tested essential plant oils two sprays after spraying the tested fungicides two sprays caused great reduction to the germinated urediniospores and severity of rust and considerable increase in phenolic compounds, % total nitrogen and % protein. This method of controlling could be used to effectively cure snap bean to the rust disease.

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