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Effect of some essential plant oils , fungicides and inducer resistance chemicals on managing peas downy mildew in Libya

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تأثير بعض الزيوت النباتية الأساسية، والمبيدات الفطرية، والمواد الكيميائية المُحفزة للمقاومة في إدارة مرض البياض الزغبي على البازلاء في ليبيا

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Abstract:
The inhibitory effect of the essential plant oils (EPOs) of cinnamon, citronella, clove, rosemary and thyme, the fungicides Dithan M-45, Kocide 200, Folio-Gold, Privicur-Enargy and Ridomil Gold-MZ and the inducer resistance chemicals (IRCs) , Bion (BTH), chitosan and salicylic acid, on sporangial germination of the fungus like Peronospoa viciae (Berk.) Casp. f. sp. pisi Sydow (formerly P. pisi), the causal of pea downy mildew, was investigated in vitro. The role of the tested materials on management of pea downy mildew was, also, evaluated under greenhouse conditions. The inhibitory effect of the tested EPOs, fungicides and IRCs on sporangial germination of P.viciae f. sp. pisi showed that they caused significant reduction to the germinated sporangia compared with control treatment. In this concern, the tested fungicides were the most efficient ones followed by the EPOs then IRCs. Management of the disease showed the same trend of in vitro assessment when they sprayed on artificially inoculated pea plants with the sporangial inoculum of the pathogen under greenhouse conditions. The reduction in the severity of the infection by pea downy mildew was reflected on the estimated crop parameters, photosynthesis pigments, and percentages of total nitrogen and total crude protein, which considerable increase was recorded due to the treatment with the teste EPOs, fungicides and IRCs.

Keywords: Pea, Downy mildew, Essential plant oils, Fungicides and Inducer resistance chemicals, Photosynthesis pigments, Protein.

الملخص
تمت دراسة التأثير التثبيطي للزيوت النباتية الأساسية *(EPOs)* لكل من: القرفة، والسترونيلا، والقرنفل، وإكليل الجبل (الروزماري)، والزعتر، وللمبيدات الفطرية *(*دياثين*M-45* ، وكوسيد 200، وفوليو-جولد، وبريفيكور-إينيرجي، وريدوميل جولد*MZ*-والمواد الكيميائية المُحفزة للمقاومة*(IRCs)* بيون*BTH* ، والشيتوزان، وحمض الساليسيليك*)*على إنبات الأبواغ للفطر الشبيه بالفطريات *Peronospora viciae (Berk.) Casp. f. sp. pisi Sydow)* المعروف سابقاً بـ *(P. pisi)*، المسبب لمرض البياض الزغبي في البازلاء، وذلك في المختبر *(in vitro)*. كما تم تقييم دور المواد المختبرة في إدارة مرض البياض الزغبي تحت ظروف البيوت المحمية.
أظهرت النتائج أن جميع الزيوت النباتية الأساسية والمبيدات الفطرية والمواد الكيميائية المُحفزة للمقاومة المختبرة تسببت في خفض معنوي في نسبة إنبات أبواغ الفطر مقارنة بمعاملة المقارنة (الكنترول). وفي

هذا الصدد، كانت المبيدات الفطرية المختبرة هي الأكثر كفاءة، تليها الزيوت النباتية الأساسية، ثم المواد الكيميائية المُحفزة للمقاومة. واتبعت نتائج إدارة المرض في البيوت المحمية على نباتات البازلاء الملقحة صناعياً بأبواغ المسبب المرضي نفس اتجاه التقييم المختبري. وقد انعكس الانخفاض في شدة الإصابة بمرض البياض الزغبي إيجابياً على مؤشرات المحصول المقدرة، وأصباغ التمثيل الضوئي، ونسب النيتروجين الكلي والبروتين الخام الكلي، حيث سُجلت زيادة ملحوظة نتيجة المعاملة بالزيوت النباتية الأساسية، والمبيدات الفطرية، والمواد الكيميائية المُحفزة للمقاومة.

الكلمات المفتاحية: البازلاء، البياض الزغبي، الزيوت النباتية الأساسية، المبيدات الفطرية، المواد الكيميائية المُحفزة للمقاومة، أصباغ التمثيل الضوئي، البروتين.

1. Introduction

Pea (*Pisum sativum* L.) belongs to the family Fabaceae (legumes). It is one of the most famous and popular legume crops in Libya for local consumption. The successful management of this pathogens, is essential to reduce its hazard effect on pea plantations. In addition, Libya has a relative advantage of increasing the cultivated area and the produced pod yield.

Pea seeds typically contain 23.1-30.9% protein, 1.5-2.0% fat and tracer number of vitamins, phytic acid, saponins, polyphenols, minerals, and oxalates. The seeds also contain multiple proteins such as globulin, albumin, proline, and glutelin (Kraft and Pfleger 2001). Pea is liable to infection by bacterial, fungal and viral diseases in addition to nematode infection and physiological disorder. However, fungal diseases, especially downy mildew, caused by the fungus like Peronospoa viciae (Berk.) Casp. f. sp. pisi Sydow (formerly P. pisi), severely affected commercial pea production in the protective plantations and the open fields (Harveson et al.,2021). The optimum symptoms of the disease are A grayish white, moldy growth appears on the lower leaf surface, and a yellowish area appears on the opposite side of the leaf. Infected leaves can turn yellow and die if weather is cool and damp. Stems may be distorted and stunted. Brown blotches appear on pods, and mold may grow inside pods. Oospores are formed within diseased tissues, which overwinter in crop debris in the soil which infect seedlings as they emerge in the spring. The oospores can survive in the soil for at least 8 years and attack seedlings as they emerge. The disease then spreads to the surrounding pea plants by air and rain splash. However, the pathogen distributed allover the world and threaten pea plantations. Yield losses can be considerable, particularly in wet seasons, which can be as much as 55% (Olofsson, 1966 and Kraft and Pfleger 2001).Sporangia distributed by wind from neighbouring fields or more remote growing areas are, also important sources of primary inoculum ((Davis and Main, 1986 and Populer, 1981).

The oospores of fungus like P.viciae f.sp. pisi can survive 10-15 years in the soil and also, on pea debris. Infection from these sources can lead to systemic and local leaf infections in volunteer pea seedlings. These seedlings act as a source of infection from which the disease spreads by wind to adjacent plants and fields. The disease can develop quickly when conditions are cold (5-15°C) and high humidity (over 90 per cent RH) for 4-5 days, often when seedlings are in the early vegetative stage. Rain is the major means of spore dispersal and infection. Heavy dew will promote sporulation. Dry, warm weather is un-favorable for the disease. Systemic infection of plants can lead to the disease developing late in the season if conditions are favorable (Olofsson, 1966 and Kraft and Pfleger 2001).

In recent years, the world has suffered from significant pollution caused by numerous pollutants, including agrochemicals, particularly in developing countries. Consequently, the strategy for managing plant pests, especially in vegetables and fruits, increasingly depends on adopting safer alternative methods rather than conventional synthetic pesticides. Scientists are currently focused on developing "green" pesticides that are toxic to target pests while

remaining harmless to the environment and non-target living organisms, or at the very least, producing compounds that rapidly decompose into non-toxic substances.

Modern research highlights the vast potential of phytochemical profiling and biological extracts as sustainable alternatives. Studies on the phytochemical composition and antimicrobial potential of *Hypericum decaisneanum* (Salem & Alhadad, 2026) and the multi-target biological potential of *Catha edulis* ethanolic extracts (Alshawish et al., 2025) demonstrate the richness of plant-derived compounds. Furthermore, biochemical evaluations of *Plantago ovata* leaves (Khalil et al., 2025), *Taraxacum officinale* (dandelion) (Salem et al., 2025), and the lichen *Usnea barbata* (Salem, 2024) have confirmed their efficacy in inhibiting pathogenic growth.

The application of natural substances, such as chitosan and various plant seed oils, has proven effective in controlling *Escherichia coli* and *Staphylococcus aureus* (Kadak & Salem, 2020). Moreover, advancements in analytical chemistry, such as the GC-MS quantification of bioactive isothiocyanates in *Sinapis alba* essential oil, have allowed for the validation of rapid bactericidal kinetics against clinically relevant pathogens (Soof et al., 2025). Alongside these, innovative solutions like antimicrobial polymers offer new mechanisms to combat microbial resistance (Salem & Salem, 2025), which is increasingly critical given the rise of antibiotic-resistant bacteria (Salem, 2025) and their prevalence in clinical and environmental settings (Ben Hsin et al., 2025). Finally, determining the chemical composition and biological activity of plant oils, such as those derived from *Linum usitatissimum* (flaxseed) (Salem & Lakwani, 2024), remains fundamental to substituting harmful chemicals with eco-friendly solutions that ensure environmental safety and human health.

The emergence of downy mildew had a significant impact on the production of pea at multiple scales all over the world including Libya. The infection can destroy the grown pea plants through few days if the Perivale environment condition are suitable for the fungus (Falade ,2021; Shoukry et al.,2021 and Rhouma et al.,2022 and Attia et al.,2025).

Chemical control is highly recommended because downy mildew is an aggressive and destructive disease and satisfactory management without the use of fungicides is unlikely. The role of fungicides in reducing the disease is well known (Mc Grath, 2001) .

Systemic fungicides are needed for control of the underside of leaves. Scientists specializing in the fungicides industry are working tirelessly to find ways to overcome the adaptation of pathogens to existing systemic pesticides. Because these fungicides have targeted activity, additional fungicides must be added to the program when there is a need to manage other diseases such as downy mildew (Keinath et al., 2017 and Rhouma et al.,2022).

Kessmann et al. (1994) reported that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad spectrum disease control and they could be used preventatively to bolster general plant health, resulting in long lasting protection. Iriti and Faoro (2003) reported that Bion was used to induce resistance in bean against rust caused by Uromyces appendiculatus. Histochemical and cytochemical investigations showed that BTH causes hydrogen peroxide (H2O2) accumulation in the treated tissues. H2O2 deposits were localized in situ for the first time in the apoplast of the leaf epidermis. No cell death was detected at BTH concentrations below the phytotoxicity threshold, suggesting that acquired resistance against bean rust is mainly related to the enhanced activity of anionic peroxidases, promoted by H2O2 accumulation, thereby leading to cell wall strengthening. This hypothesis is also supported by the long induction phase required to establish complete resistance.

Bion (BTH) is a systemic acquired resistance elicitor, which reduces many fungal diseases. It has been previously shown that pea rust infection can be reduced by exogenous applications of systemic acquired resistance elicitors such as BTH This protection is known to be related with the induction of the phenol pathway but the particular metabolites involved have not been

determined yet. This suggests fungal growth impairment by both direct toxic effect as well as plant cell wall reinforcement (Barilli et al., 2015).

This work aims to evaluate the efficiency of some EPOs , fungicides and IRCs on sporangial germination in vitro, in addition to evaluate each of them alone under greenhouse condition on management the disease. Also, estimating crop parameters , photosynthesis pigments, and percentages of total nitrogen and total crude protein in the sprayed pea plants with the EPOs , fungicides and IRCs.

2. Materials and Methods

2.1. Effect of some essential plant oils (EPOs), fungicides and inducer resistance chemicals (IRCs), on sporangial germination of *P.viciae* f.sp. *pisi* :

The effect of some EPOs , fungicides and IRCs on the sporangial germination of the fungus like *P.viciae* f.sp. *pisi* was investigated *in vitro*.

2.1.1. Preparation of EPOs:

The essential plant oil of each of cinnamon (*Cinnamomum verum*), citronella (*Cymbopogon nardus*), clove (*Syzygium aromaticum =Eugenia caryophyllata*) , rosemary (*Salvia rosmarinus*) and thyme (*Thymus capitatus)* were stored in dark bottles at 5ºC for further investigation.They diluted to the concentrations of 1, 2 , 3, 4 and 5% using distilled sterile water plus few drops from Tween-20 (to make emulsion).

2.1.2. Preparation of the fungicides tested:

The concentrations of 25, 50, 100, 200 and 300 ppm. of the two contact fungicides Dithan M-45 (mancozeb), Kocide-2000 (copper hydroxide) and the three systemic ones Folio-Gold (Chlorothalonil 50% +Metalaxil-M 3.75%), Privicur-Enargy (Fostyl Aluminium 31% +Promapamocarb hydrochloride 62.5%) and Ridomil Gold-MZ (Mancozib 64% + Metalaxil-M 4%) were prepared depending on their active ingredient .

2.1.3. Preparation of IRCs:

Depending on the molecular weight of the inducer resistance chemicals (IRCs) *i.e.*, Bion (benzothiadiazole; BTH)), chitosan (cellulose with the hydroxyl at position C_2 substituted with an acetamido group) and salicylic acid (monohydroxybenzoic acid) they prepared at 5, 10, 20, 30 and 40 mM.

Pea leaves naturally severely infected by downy mildew were collected from a field located at Azzaytuna district and incubated at 10±1°C until use. The freshly sporangia were collected by sterilized brush from the infected leaves and put in each concentration of EPOs, fungicides and IRCs tested. O.5 m1.of sporangial suspension was placed on each sterilized glass slide, borne on two glass rods in a sterilized Petri-dish containing a piece of wetted cotton by sterilized distilled water to amend high relative humidity. The same was made for a spore suspension put in distilled sterilized water only as control treatment. Each concentration of each of EPOs, fungicides and IRCs was prepared using five Petri-dishes. The dishes were incubated in the dark at 12±1 °C for 24 hour. To kill and fix the germinated sporangia one drop from lacto-phenol cotton blue stain was added at the time of slide examination. The germinated sporangia were counted 24 hours of incubation and the average percentages of germination were calculated for each treatment and recorded. Also, The percentages of germinated sporangia at the beginning of incubation the prepared Petri-dishes were assessed.

2.3. Greenhouse experiment:

Severely infected pea plants were brought from Azzaytuna district in plastic bags provided by a piece of wetted cotton in an ice-box to the laboratory. By a sterilized camel brush the sporangiophores and sporangia were put in a glass beaker (250 ml) containing sterilized distilled water. Sporangial suspension was adjusted to $1X10^3$ sporangium ml^{-1} sterilized water by the aid of haemocytometer just before spraying on the pea plants.

The effect of the tested EPOs *i.e.,* cinnamon, citronella, clove, rosemary and thyme , the fungicides *i.e.,* Dithan M-45, Kocide 2000, Privicur-Enargy, Folio-Gold and Ridomil Gold-MZ and the IRCs *i.e.,* Bion (BTH), chitosan and salicylic acid on the infection of pea downy mildew caused by *P.viciae* f.sp. *pisi* was achieved using artificial inoculation by sporangial suspension of the causal fungus like under greenhouse conditions .

Plastic pots (25 cm in diameter) containing disinfested clay soil by 5% formalin were sown with pea seeds. Five seeds (Little Marvel cv.) were sown in each pot, irrigated and left to grow then thinned into two plants in each pot, ten days after sowing. Five pots were prepared for each treatment. The grown plants (aged three weeks) were sprayed with the tested the EPOs (5%), the fungicides at 250 g, 250 g, 250 , 250 ml. and 200 g, respectively and IRCs (40 mM) five days before the artificial inoculation with sporangial suspension ($1x10^3$ ml^{-1} water) of the pathogen by sterilized plastic sprayer. Also, the plants were resprayed with tested EPOs , fungicides and IRC′s two times by the previous rates 10 and 20 days after the inoculation by the tested pathogen. Two ml of Tween-20 10 ml^{l-} were added to the sprayed materials before spraying them on the plants as adherent and spread material. Plants sprayed with sporangial suspension only without another treatments were left as infected control . Another pea plants sprayed with sterilized water only without another treatments were left as un-infected control. The grown plants were irrigated when it was necessary and fertilized with a local compounded N.P.K. + microelements fertilizer (one g for each pot), three weeks after sowing then 3 times weekly.

The severity of infection by the disease was assessed one week after each spray of the tested treatments and the averages were recorded using a modified 0-4 scale (Falloon *et al.* (1995). Also, some crop parameters were assessed *i.e.,* plant length (cm) .foliage fresh and dry weight (g) , No. of the produced green pod yield and weight of the produced green pod yield $plant^{-1}$.

2.4. Disease assessment:

Pea downy mildew severity was assessed based on the devised scale (0-4) by Falloon *et al.* (1995) using the following formula:

% Disease severity = $(\sum (nxv)/4N) *100$

Where: n= Number of infected leaves in each category,

v= Numerical value of each category and

N= Total number of the examined leaves in the samples 4= Highest numerical value achievable

2.5. Estimation of Photosynthetic Pigments :

The method of Metzner *et al.* (1965) was used to estimate photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) spectro-photometrically. Twenty gram fresh weight of pea leaves, taken from five randomly plants and represent the categories of the disease, were homogenized in 85% aqueous acetone for 10 min. The homogenized tissues were centrifuged (10.000 rpm for 10 minutes), and the supernatant was adjusted to 200 mL with 85% acetone and measured against a blank of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm. Dilutions were used to determine the concentrations of the pigment fractions.

The content of chlorophyll a, chlorophyll b and carotenoids was calculated using the formula of Arnon (1949):

Chlorophyll a (mg ml^{-1}) = $12.7\Delta A663 - 2.69\Delta A645$,

Chlorophyll b (mg ml^{-1}) = $22.9\Delta A645 - 4.68\Delta A663$, and

Carotenoids (mg ml^{-1}) = $4.75\Delta A452.5 - 0.226C$ (a + b),

Where: ΔA is the absorbance at the respective wavelength.

2.6. Estimation of the percentages of total nitrogen and crude protein content in dry pea seeds:

A random sample of dry pea pods was collected from the yield of each treatment of the pot experiment. Total nitrogen percentage (mg g^{-1} pea dry weight) in the seeds was determined according to the method described by Hafez and Mikkelsen (1981). Then the percentage of total protein content in dry pea seeds was calculated by multiplying total nitrogen content by 6.25.

2.7. *Statistical analysis:*

The obtained data were statistically analyzed using the standard procedures for complete randomize block and split designs as reported by Snedecor and Cochran (1989). The averages were compared at 0.05 level using least significant difference (L.S.D.) according to Fisher (1948).

3. Results

3.1. *In vitro* effect of five EPOs on sporangial germination of *P.viciae* f.sp.*pisi*:

Table (1) reveal that the tested EPOs *i.e.*, cinnamon, citronella, clove, rosemary and thyme resulted in significant inhibition to the germinated sporangia of the tested fungus like *P.viciae* f.sp.*pisi* compared with the control treatment. The sporangia of the causal pathogen failed to germinate at the concentration of 5 % of the tested five EPOs as well as at 3% of clove and thyme oils. Clove oil was the best treatment in this concern , being 27.1% sporangial germination, on the average, with 71.4% efficiency followed by thyme oil, being 28.6% sporangial germination, on the average, with 69.8% efficiency, without significant difference. Meanwhile, cinnamon oil was of the lowest efficient one on sporangial germination of the causal pathogen, being 37.3% sporangial germination, on the average with 60.7% efficiency. Citronella and rosemary oils were of intermediate inhibition in this regard, being 36.4 and 36.2 % sporangial germination, on the average, with 61.6 and 61.8 % efficiency, respectively. There was gradual significant increase in the inhibitory effect on the tested EPOs on sporangial germination due to increasing their concentration. Sporangial germination of control treatment recorded 86.8%.

Table 1. Effect of five essential plant oils (EPOs) on sporangial germination of *P . viciae* f.sp. *pisi*, 24 hour after incubation at 12±1 °C.

EPOs	% Sporangial germination at concentration (%)					Mean	% Efficiency
	0.5	1	2	3	5		
Cinnamon	77.0	60.0	37.8	18.6	0.0	37.3	60.7
Citronella	74.2	58.6	34.4	15.0	0.0	36.4	61.6
Clove	63.4	50.4	21.6	0.0	0.0	27.1	71.4
Rosemary	74.0	59.0	33.8	14.0	0.0	36.2	61.8
Thyme	61.6	59.2	22.4	0.0	0.0	28.6	69.8
Control*	86.8	86.8	86.8	86.8	86.8	86.8	----
Mean	70.0	57.4	30.0	9.5	0.0		-----

* Control not included for calculating the means.

The percentages of germinated sporangia at the beginning of incubation was 2.8%.

L.S.D. at 5 % for: EPOs (E) = 2.8, Concentrations (C) = 3.3 and E x C = 4.8.

3.2. *In vitro* effect of five fungicides on sporangial germination of *P.viciae* f.sp.*pisi*:

Data presented in Table (2) indicate that the tested fungicides caused significant reduction to the germinated sporangia compared with control treatment. No germination was occurred at 200 and 300 ppm by Folio-Gold , Previcur-Energy and Ridomil Gold -MZ. In addition, Previcur- Energy was the most efficient one followed by Ridomil Gold-MZ then Folio-Gold, being 15.6, 16.1 and 16.6 % sporangial germination, on the average, without significant differences with 82.0, 81.4 and 80.8% efficiency, respectively. Both Dithan M-45 and Kocide-2000 resulted in lowest figures of efficiency, being 71.3 and 73.0 %, respectively. The inhibitory effect to the sporangial germination was gradually increased significantly by increasing the fungicides concentration. Control treatment recorded 86.6 % sporangial germination.

Table 2. Effect of five fungicides on sporangial germination of *P . viciae* f.sp. *pisi*,24 hour after incubation at 12±1°C.

Fungicides	% Sporangial germination at concentration (ppm)					Mean	% Efficiency
	25	50	100	200	300		
Dithan M-45	51.6	38.4	20.0	10.0	4.4	24.9	71.3
Kocide 2000	50.2	36.0	18.0	9.0	3.6	23.4	73.0
Folio-Gold	42.0	28.6	12.4	0.0	0.0	16.6	80.8
Privicur-Energy	41.8	26.2	10.0	0.0	0.0	15.6	82.0
Ridomil Gold MZ	42.0	27.8	10.6	0.0	0.0	16.1	81.4
Control *	86.6	86.6	86.6	86.6	86.6	86.6	-----
Mean	45.5	31.4	14.2	3.8	1.6	----	-----

* Control not calculated in the mean.

The percentages of germinated sporangia at the beginning of incubation was 2.2%.

L.S.D. at 0.05 for : Fungicides (F)= 2.3, Concentrations (C)=2.3 and F x C = 3.1.

3.3. *In vitro effect of four inducer resistance chemicals on sporangial germination of P. Viciae* f.sp. *pisi:*

Table (3) reveals that the tested IRCs *i.e.*, Bion (BTH), chitosan and salicylic acid caused significant inhibition to the germinated sporangia of the causal fungus like compared with control treatment. The sporangia of the causal pathogen failed to germinate at the concentration of 40 mM of tested IRCs . Meantime, both Bion (BTH) and salicylic acid were more, to somewhat, efficient than chitosan The respective averages of BTH were , being 34.4% sporangial germination, on the average, with 63.7 % efficiency and for salicylic acid were 34.4 % sporangial germination, on the average with 63.7% efficiency. Chitosan resulted in 35.6 % sporangial germination, on the average, with 62.4 % efficiency. The inhibitory effect of the tested IRCs to the sporangial germination was gradually increased significantly by increasing their concentration. Control treatment recorded 86.8% sporangial germination.

Table 3. Effect of three inducer resistance chemicals (IRCs) on sporangial germination of *P .viciae* f.sp. *pisi*, 24 hour after incubation at 12±1 °C.

IRCs	% Sporangial germination at concentration (mM)					Mean	% Efficiency
	5	10	20	30	40		
Bion (BTH)	72.8	51.6	33.4	13.8	0.0	34.3	63.7
Chitosan	74.6	55.6	35.6	12.4	0.0	35.6	62.4
Salicylic acid	71.8	52.0	35.2	13.0	0.0	34.4	63.7
Control *	86.8	86.8	86.8	86.8	86.8	86.8	----
Mean	73.1	53.1	34.7	13.1	0.0		-----

* Control not calculated in the means.

The percentages of germinated sporangia at the beginning of incubation 2.6%.

L.S.D. at 0.05 for: Inducer resistance chemicals (I) = n.s, Concentrations (C) = 3.9 and I x C = 4.4.

3.4. Greenhouse experiment :

2.4.1. Effect of spraying pea plants (Little Marvel cv.) with some EPOs, fungicides and IRC′s on the severity of downy mildew:

Results shown in Table (4) show that significant reduction to the severity of downy mildew was occurred due to spraing the tested EPOs, fungicides and IRC′s on pea plants artificially inoculated with the causl fungus like *P.viciae* f.p. *pisi* compared with inoculated control. In general, the fungicides tested were the most efficient one in reducing the severity of the disease followed by IRCs then EPOs , being 4.6 , 10.5 and 11.7 % disease severity and 86.2, 69.5 and 66.0 %, efficiency. Moreover, the thyme EPO, Previcur- Egergy fungicide and Bion (BTH) IRC were the most efficient treatments in their groups , being 10.3, 3.4 and 10.1 % disease severity and 70.1, 90.1 and 70.6 % efficiency , on the average, respectively. Disease severity for inoculated control recorded 34.4%, on the average. No apparent symptoms were observed on un-inoculated pea plant by the pathogen.

Table 4. Effect of spraying pea plants (Little Marvel cv.) with some EPOs, fungicides and IRCs on the severity of downy mildew under greenhouse conditions.

Treatments	% Downy mildew severity 7 days after spraying			Mean	% Efficacy
	First spray	Second spray	Third spray		
EPOs					
Cinnamon	6.4	15.8	17.7	13.3	61.3
Citronella	6.0	14.1	17.1	12.4	64.4
Clove	5.0	12.8	14.9	10.9	68.3
Rosemary	6.2	13.3	15.2	11.6	68.3
Thyme	4.8	11.5	14.6	10.3	70.1
Mean	5.7	13.5	15.8	11.7	66.0
L.S.D. at 0.05 for : Treatments (T)= 2.7, Downy mildew severity (D) =1.3 and T x D = 3.0.					--------
Fungicides:					
Dithan M-45	3.2	8.5	8.8	6.8	80.2
Kocide 200	2.7	8.0	8.2	6.2	82.0
Previcur-Energy	2.0	4.0	4.2	3.4	90.1
Folio Gold	2.1	4.5	4.6		89.2
Ridomil Gold-MZ	2.1	4.2	4.4	3.7	89.5
				3.6	
Mean	2.4	5.8	6.0	4.7	86.2
L.S.D.at 0.05 for: Treatments (T)= 2.5, Downy mildew severity					----------

(D)=1.0 and T x D = 2.8.					
IRCs :					
Bion (BTH)	4.6	12.4	13.4	10.1	70.6
Chitosan	5.5	12.9	15.0	11.1	67.7
Salicylic acid	5.0	11.8	13.6	10.1	70.6
Mean	5.0	12.4	14.0	10.5	69.5
Control (Infected)*	12.6	40.0	60.6	34.4	-----
Control(Un-infected)*	0.0	0.0	0.0	0.0	--------
L.S.D. at 0.05 for : Treatments (T)= 2.9, Downy mildew severity (D)=1.4 and T x D = 3.2.					-------

* Both controls not included in calculating the mean.

4.3.2.Effect of spraying pea plants (Little Marvel cv.) with some EPOs, fungicides and IRCs on some crop parameters of inoculated plants with *P.viciae* f.sp. *pisi* under greenhouse conditions:

The obtained results (Table 5) exerted significant increase in the assessed crop parameters *i.e*., plant height (cm), foliage fresh and dry weight in addition to number of the produced green pods and weight of the produced green pods plant^{-1}. Due to, the fungicides maintained their high effectiveness in reducing disease incidence, which this resulting in a noticeable increase in the crop parameters of pea plants followed by IRCs then EPOs. The respective averages of plant height (cm), foliage fresh weigh (g) plant^{-1}, foliage dry weight (g) plant^{-1}, No . of green pods plant^{-1} and weight of green pod yield plant^{-1}, for the fungicide tested were 43.0 cm, 289.0 g, 36.9.5 g , 19.1 pod and 51.2 g ; for IRCs were 38.8 cm, 245.7 g, 30.0 g, 14.5 pod and 56.6 g and for EPOs were 36.3 cm, 225.9 g, 26.4 g, 13.7 pod and 44.9 3, respectively. The respective averages for the infected control were 22.6 cv, 203.8 g , 18.9 g, 8.4 pod and 21.0 g, respectively. Un-infected pea plant recorded the highest figures of crop parameters, being 47.0 cm, 348.6 g, 49.8 g, 25.8 pod and 74.0 g, respectively.

Table 5. Effect of spraying with the tested EPOs, fungicides and IRCs on pea plants (Master Pea cv.) artificially inoculated by the causal downy mildew, on the averages of weight of the foliage growth, the foliage dry weigh and weight of pods (g) plant^{-1} in addition to the percentages of total nitrogen and total crude protein (mg g^{-1} dry weight) of dry pea seeds.

Treatments	Average of plant height (cn)	Average weight of the foliage growth (g) plant^{-1}	Average of the foliage dry weight (g) plant^{-1}	Average of the produced green pods (g) plant^{-1}	Average weight of the produced green pods (g) plant^{-1}
EPOs					
Cinnamon	35.4	215.0	24.4	12.8	44.8
Citronella	36.8	220.5	26.0	13.2	55.0
Clove	38.3	236.6	27.6	14.6	55.6
Rosemary	36.5	218.8	25.7	13.0	45.0
Thyme	39.4	238.5	28.1	15.0	56.0
Mean	36.3	225.9	26.4	13.7	44.3

Fungicides:					
Dithan M-45	40.6	260.6	35.0	16.8	63.2
Kocide 200	41.8	264.8	34.8	16.0	63.7
Previcur-Energy	44.9	305.4	38.5	21.8	64.3
Folio-Gold	43.5	302.0	37.3	20.0	64.0
Ridomil Gold-Mz	44.4	312.2	38.9	21.0	66.0
Mean	43.0	289.0	36.9	19.1	64.2
IRCs :					
Bion (BTH)	36.6	246.1	29.5	14.6	56.6
Chitosan	38.8	238.6	28.4	14.0	56.0
Salicylic acid	40.9	252.5	31.8	15.0	57.3
Mean	38.8	245.7	30.0	14.5	56.6
Control (Infected)	22.6	203.8	18.9	8.4	21.0
Control (Un-infected)	47.0	348.6	49.8	25.8	74.0
L.S.D. at 0.05 .	2.9	4.9	1.8	3.1	3.1

4.4. Estimation of Photosynthesis Pigments, total Nitrogen and Total Crude Protein percentages :

Data presented in Table (6) reveal that spraying of the tested materials on pea plants artificially inoculated with the fungus like *P.viciae* f.sp. *pisi* resulted in significant increment in photosynthesis pigments (Chlorophyll –a, Chlorophyll -b and Carotenoids; mg g^{-1} fresh leaves weight) as well as the percentages of total nitrogen and total crude protein (mg g^{-1} seed dry weight) in comparison with inoculated control. As expected, the fungicides tested gave the highest values of photosynthesis pigments and total nitrogen and total crude protein percentages followed by IRCs then EPOs. The respective values for the fungicides tested were 1.49, 1.45 and 0.86 photosynthesis pigments mg g^{-1} fresh leaves weight , 4.16 and 26.03 total nitrogen and total crude protein (mg g^{-1} seed dry weight). The values of IRCs recorded the second rank, being 1.46, 1.36 and 0.84 photosynthesis pigments mg g^{-1} fresh leaves weight , 4.10 and 25.03 total nitrogen and total crude protein (mg g^{-1} seed dry weight), respectively. Meanwhile, the values of EPOs ranked the third order, being 1.43, 1.22 and 0.81 photosynthesis pigments mg g^{-1} fresh leaves weight , 4.06 and 25.37 total nitrogen and total crude protein (mg g^{-1} seed dry weight), respectively.

The infected control had a reduction in photosynthetic pigments, being 1.25, 1.09 and 0.77 (mg g^{-1} fresh leaves weight), respectively. The percentages of total nitrogen and total crude protein of inoculated pea plants recorded, also significant reduction in the percentages of total nitrogen and total crude protein percentages, being 3.15 and 19.69 %, respectively. The giant figures of photosynthetic pigments and total nitrogen and total crude protein (mg g^{-1} seed dry weight) were recorded by the control of the un-infected plants, being 1.59, 1.52 and 0.88 (mg g^{-1} fresh leaves weight) for photosynthetic pigments and 4.25 and 26.56 for total nitrogen and total crude protein percentages, respectively.

Table 6. Effect of spraying artificially inoculated pea plants by the causal pathogen of downy mildew on photosynthesis pigments (chlorophyll a, chlorophyll b and carotenoids) and the percentages of total nitrogen and total crude protein.

Treatments	Photosynthesis pigments (mg g^{-1} fresh leaves weight)			%,Total nitrogen (mg^{-1}g dry weight)	%. Total crude protein (mg g^{-1} dry weight)
	Chlorophyll -a	Chlorophyll -b	Carotenoids		
EPOs					
Cinnamon	1.41	1.20	0.81	4.01	25.06
Citronella	1.41	1.21	0.81	4.05	25.31

Clove	1.45	1.23	0.81	4.08	25.55
Rosemary	1.41	1.21	0.81	4.05	25.31
Thyme	1.47	1.24	0.82	4.10	25.63
Mean	1.43	1.22	0.81	4.06	25.37
Fungicides:					
Dithan M-45					
Kocide 200	1.48	1.45	0.86	4.13	25.81
Previcur-	1.48	1.45	0.86	4.15	25.94
Energy	1.50	1.46	0.86	4.18	26.13
Folio=Gold	1.49	1.45	0.86	4.16	26.00
Ridomil Gold-	1.50	1.46	0.86	4.20	26.25
MZ					
Mean	1.49	1.45	0.86	4.16	26.03
IRCs :					
Bion (BTH)	1.45	1.34	0.83	4.10	25.63
Chitosan	1.45	1.36	0.84	4.10	25.63
Salicylic acid	1.47	1.37	0.84	4.10	25.63
Mean	1.46	1.36	0.84	4.10	25.63
Control (Infected)	1.25	1.09	0.77	3.15	19.69
Control (Un-infected)	1.59	1.52	0.88	4.25	26.56
L.S.D. at 0.05	0.14	0.16	n.s.	0.71	1.45

4. Discussion

Pea (*Pisum sativum* L.) is a globally significant vegetable and pulse crop valued for its high nutritional richness, soil-enriching capabilities, and adaptability across diverse agro-climatic zones. However, its productivity is severely constrained by plant diseases , especially downy mildew, which the pervasive fungal disease primarily caused by the fungus like *Peronospora viciae* (Berk.) Casp. f. sp. *pisi* Sydow (formerly *P. pisi*).

Downy mildew of pea is widely distributed all over the world including Libya. This fungus like causes local infections on leaves, systemic infection of seedlings and pod infections. Mycelia and Oospores of the causal fungus like can be found in seed coats. However, transmission of the infection from the seeds to seedling has not been reported. Oospores in the soil are the most important primary inoculum. The sporangia can be transmitted over long distances (Dixon, 1981).

In general, results of *in vitro* studies revealed that the tested EPOs cinnamon, citronella, clove, rosemary and thyme; the fungicides *i.e.,* Dithan M-45, Kocide 2000, Folio –Gold, Previcur-Energy and Ridomil Gold-MZ and the IRCs *i.e.,* Bion (BTH), chitosan and salicylic acid resulted in significant inhibition to the germinated sporangia of the causal fungus like compared with the control (Infected). The inhibitory effect was increased significantly gradually by increasing the concentration of these materials. In addition, the fungicides were the most efficient ones in this regard followed by EPOs then IRCs. In this regard, the tested EPOs resulted in significant inhibition to the germinated sporangia of the tested fungus like *P.viciae* f.sp.*pisi* compared with the control (Infected) . The sporangia of the causal pathogen failed to germinate concentration at 5 % of the tested EPOs as well as at 3% of clove and thyme oils. Clove oil was the pest treatment in this concern followed by thyme oil. Meanwhile, cinnamon oil was the of lowest efficient one on sporangial germination of the causal pathogen . Meantime, citronella and rosemary oils were of intermediate inhibition in this cocern.

Essential oils (EOs), complex volatile compounds synthesized by plants, represent a vital class of natural products that are increasingly significant in scientific research due to their diverse biological properties and broad-spectrum medicinal applications. This study provides a comprehensive overview of EOs, commencing with a historical perspective and detailing their applications. It systematically catalogs their primary botanical sources, with specific examples of

the most common and important plant families,including *Lamiaceae* (e.g.,sage,oregano,thyme), *Verbenaceae* (vervain), *Magnoliaceae* (magnolia), *Rutaceae* (lemon), *Myrtaceae* (eucalyptus)and *Lauraceae* (cinnamon). A key focus is their antifungal activity, including the bioactive constituents involved and their mechanisms of action, with particular emphasis on their defense against pathogenic fungi (Elshafie *et al.*,2026). Overall, they added that, the antifungal activity of plant EOs and their bioactive constituents results from a coordinated, multi-target mode of action. Key mechanisms include disruption of fungal cell membrane integrity and permeability, leading to leakage of ions and vital cellular components; impairment of mitochondrial function and energy metabolism; induction of oxidative stress through reactive oxygen species (ROS) accumulation; and inhibition of essential enzymatic processes involved in cell wall synthesis, virulence, spore germination, and hyphal development. These effects often act simultaneously and are frequently enhanced by synergistic interactions among individual constituents or between blended EOs. This combined biochemical action disrupts fungal cell balance, slows pathogen growth, and lowers the risk of resistance, thereby supporting the broad-spectrum effectiveness of EOs against plant-decaying and postharvest pathogens.

Lagrouh *et al.* (2017) mentioned that essential plant oils can affect pathogenic fungi through six mechanisms of action: inhibition of electron transport in mitochondria, inhibition of cell division, interference with nucleic acids synthesis and/or inhibition of protein synthesis, and inhibition of efflux pumps. The host plant activates defense processes involving the production of enzymatic and nonenzymatic antioxidants, including the production of soluble sugars, phenols, flavonoids, and hormones (Tarkowski *et al.,* 2019). The antifungal efficacy of EPO also depends on the presence of different active constituents such as monoterpenes, sesquiterpenes, phenols, aldehyde, and ketones, which are interacted to show synergistic, additive, and complementary effects. Terpenoids, alcohols, and phenolic terpenes in an oxygenated form precisely increase the antifungal activity of EOs (Bassolé and Juliani, 2012).

In recent years, the world suffers from great pollution from many pollutants including agrochemicals, especially in the developing countries. So, the strategy of management of plant pests, especially of vegetables and fruits depends on using alternative safe methods for pests management rather than pesticides. Therefore, scientists are trying to produce pesticides that are toxic to pests and do not have a harmful effect on the environment and living organisms, or at least produce pesticides that quickly decompose into non-toxic substances for living organisms.

Chemical management of plant diseases is in most cases efficient in reducing the hazard of most plant disease, but it greatly causes environmental pollution and increase in the accumulated toxic substances in human food chain, especially in case of the fresh fruits. On the other hand, using other trials of disease management, viz. biologicalcontrol, plant extracts and essential oils ,inducer resistance elicitor, sanitary methods and agricultural practices, each alone, are not enough to give adequate results (Rhouma *et al.*, 2022). Therefore, we can use the fungicides early in the growing season to lowering the severity of the infection by the disease , then after spraying another substances such as essential plant oils (EPOs), bioagents, inducer resistance chemicals (IRCs) ….. ect., in order to left a period for the chemical transformation of the sprayed fungicides into non-toxic substances.

Abada and Abdel-Malek (2011) obtained sufficient management for pea downy mildew by using Fungicides and IRCs.

Greenhouse experiment revealed that there were significant reductions to the severity of downy downy mildew with significant increase to the crop parameters , photosynthesis pigments and total nitrogen and total crude protein percentages in dry pea seeds due to spraying of the tested EPOs, fungicides and IRCs compared with the control (Infected) . Meantime, the tested fungicides were the most efficient ones in this regard followed by IRCs then EPOs.

It has been found that IRCs were reported as alternative and/ or safe trial for management of many diseases, especially those of vegetable crops (Abada and Attia, 2017 and Attia *et al.*,2025). Doubrava *et al.* (1988) reported that induction of acquired resistance is persistent and generally is pathogen nonspecific Moreover, Larcke (1981) found that unlike chimicals of phytoalexins accumulations, which are elicited at the site of application, may be responsible for localized protection and induces systemic acquired resistance that sensitizes the plant response rapidly after infection. These responses induced phytoalexins accumulation and lignifications and induce enhance activities of chitinase and P-glucanase (Metranx and Boller, 1986). Furthermore, Kessmann *et al.* (1994) found that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad spectrum disease control and they could be used preventatively to bolster general plant health, resulting in long lasting protection. furthermore, Iriti and Faoro (2003) mentioned that Bion (BTH) was used to induce resistance in bean against rust caused by *Uromyces appendiculatus.* Histochemical and cytochemical investigations revealed that BTH causes hydrogen peroxide (H_2O_2) accumulation in the treated tissues. When H_2O_2 deposits localized in situ for the first time in the apoplast of the leaf epidermis. He noticed that no cell death was detected at BTH concentrations below the phytotoxicity threshold, suggesting that acquired resistance against bean rust is mainly related to the enhanced activity of anionic peroxidases, promoted by H_2O_2 accumulation. Thereby, this leading to cell wall strengthening.

Finally, it is well known that fungicides are the most effective method in controlling pests in general, including fungal diseases. However, due to the danger pf these pesticides pose to the environment, in addition to their accumulation in the human and animal body, scientists have begun attempts to reduce their harmful effects. This involves spraying these pesticides during the early growth stages of plants and well before harvest to allow sufficient time for the pesticides to be converted into compounds harmless to humans and animals. Alternatives to these pesticides, such as essential plant oils, resistance-inducing chemicals, and safe antimicrobials, should be used, along with adherence to proper agricultural practices and health measures during harvesting, to obtain agricultural products free from the harmful effects of pesticides.

5. Conclusion

Pea downy mildew can control by essential plant oils (EPOs), fungicides and inducer resistance chemicals (IRCs). On the other hand, EPOs and IRCs are natural substances that have a long history of use for controlling many fungal diseases. They have a promising action of antimicrobial effect. So, they are used to test their activity by many researchers to see their potential for controlling fungal plant diseases. The obtained results showed the high capability of these natural substances used to act as antifungal agents. Their environmentally friendly characteristics make them interested by the researchers those exploring products that have desirable effects on the target organisms with no or less negative impact on the environment.

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