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Promising Integrated Management Strategy of Garlic Rust (*Puccinia porri* G. Wint.) with Improved Productivity Using Benzothiadiazole (BTH), Essential Oils and Fungicides

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ABSTRACT

The essential oils (EOs) of lemongrass, clove, neem, and thyme, as well as the fungicides Dithane M-45®, Kocide-2000®, Sumi-8®, and Topas®, resulted in a significant reduction to the *in vitro* uredial germination percentage of *Puccinia porri*, the causal pathogen of garlic rust. The tested fungicides, in general, had a higher germination inhibitory effect than the essential oils as it ranged between 14.2-34.9% for the fungicides but ranged between 17.1-19.8% for the essential oils. Sumi-8® fungicide was the most efficient one in this concern followed by Topas® then clove oil. Meanwhile, Dithane M-45® was the lowest efficient one followed by Kocide-2000® then neem oil. Under greenhouse conditions, an integrated management plan consisted of soaking garlic seed cloves in 50 mM of Bion® (Benzothiziazole; BTH), a synthetic analogue of salicylic acid as plant resistance inducer, for two hours just before planting then spraying the grown plants with each of the tested essential oils, fungicides individually or their binary combinations, three days post challenge inoculation with the urediospores of *Puccinia porri* was applied. All treatments significantly reduced the severity of rust disease with a significant increase in the produced bulb yield compared with the control. Garlic plants grown from cloves soaked in BTH were highly protected against the pathogen and had improved productivity estimated as bulb weight (g) plant^{-1} compared to control plants soaked in distilled water, either sprayed with tested EOs and fungicides or not. The combinations between the sprayed fungicides and EOs were of higher efficacy in lowering the severity of the disease and increasing the bulb weight (g) plant^{-1} compared to spraying any of them individually. The estimated alkaloids, flavonoids, and phenolic compounds in the bulbs of garlic plants sprayed with the tested EOs and fungicides recorded considerable increments compared with both control treatments.

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the Alliaceae family and is the second most important *Allium* species, next to onion, which grows in all temperate to subtropical and mountainous tropical areas as an important spice and medicinal plant (Rabinowitch, 2002). Garlic is one of the most widely cultivated bulb crops in Egypt. Egyptian garlic is exported to Arab, African, Eastern and Western European countries. In 2017, the cultivated area with

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garlic in Egypt reached 29242 feddans, which increases annually due to the increased demand for local consumption and exportation. In addition to its economic value, garlic has a social impact because it is grown by small farmers.

Among the constraints of garlic production, biotic factors are the major problems affecting yield and productivity, as well as the quality of this high-value crop. Garlic plant is liable to be attacked by many bacterial, fungal, viral, and nematode diseases in addition to physiological disorders (Schwartz and Mohan, 1995; Agrios, 2005; Mengesha, *et al.*, 2015; Khalil and Abdel-Rahman, 2018). However, fungal diseases, especially rust caused by *Puccinia porri* G. Wint. (Formerly *Puccinia allii*) considered one of the major destructive diseases, especially in the northern Egyptian governorates, where high humidity prevails for severe infection, which causes a great reduction in the bulb yield (Koike *et al.,* 2001; Endalew *et al.,* 2020; Kassaw *et al.,* 2021).

P. porri is an air-borne, obligate parasitic fungus that occurs on most aerial-grown parts of the plant but is most often observed on the leaves. It infects garlic during the bulb formation stage. Initial symptoms include small white to yellow flecks and spots. The spots enlarge and become oval- or diamond-shaped and take on an orange cast as uredia. The raised, orangey uredia pustules cover the foliage quickly when conditions are favorable to the repeated infection. In severe cases, the leaves become yellow, shrivel, and die. Later in the season, the uredia turn to black pustules due to the formation of telia, making the infected plants look rusty. These telia may or may not break through the epidermis. Finally, the foliage becomes unable to convert energy in its usual efficient style, bulbs are small but still edible (Koike *et al.,* 2001).

The fungus may overwinter on volunteer garlic and onions, or on wild hosts. The rust is autoecious, and same-season reinfection by urediospores is common. The disease spreads quickly through touching leaves, wind, and rain. Infection rates are highest at cool temperatures (10°-15°C) and 100% relative humidity, caused by excessive irrigation, fog and rain. Together, wet foliage and mild temperatures create the perfect environment for urediospores to multiply during the growing season, causing severe losses, especially in spring season. rain, fog, or irrigation are excessive (Anikster *et al.*, 2004).

Chemical control can be achieved by preventive treatments with mancozeb, propiconazole, tebuconazole, or azoxystrobin if sprayed at 10-day intervals (Rochecouste, 1984; Blum and Gabardo, 1993; Koike *et al.*, 2001).

BTH; Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester, trade name Bion®, is a salicylic acid (SA) synthetic analogue that, when applied to plant tissue, functions for the inactivation of catalases and ascorbate peroxidase, the two major H_2O_2 scavenger enzymes, increasing the H_2O_2 pool in treated tissues (Wendehenne *et al.*, 1998). Consequently, it enhances acid peroxidase activity involved in cell wall strengthening (Stadnik and Buchenauer, 2000).

Essential oils (EOs) are plant-derived compounds, which originally are complex volatile compounds synthesized by aromatic plants in order to protect themselves against diseases and are considered safe for humans and animals (Isman, 2000). Regarding plant disease management, EOs and their derivatives are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use. Hence, EOs became a powerful alternative to conventional fungicides. (Arras *et al*., 1993; Sokovic *et al.,* 2009; Oliveira *et al*., 2019).

To provide garlic crops with preventive as well as a curative effect against infection with *P. porri*, and to reduce the residual effect of fungicides in consumed garlic bulbs, this investigation was carried out aiming to evaluate the efficiency of integrated application of BTH, certain essential oils (EOs), i.e., lemongrass, clove, neem, and thyme and certain systemic fungicides on the fungus *P. porri*, the causal organism of garlic rust *in vitro* and *in* *vivo*. The research also pertained to the effect of these treatments on the leaf content of alkaloids, flavonoids, and total phenols content in the treated plants.

MATERIALS AND METHODS

1-Plant Materials:

Garlic bulbs (Cv. Sids-40) were obtained from Horticultural Research Institute, Vegetables Department, El-Dokki., Giza, Egypt.

2-Fungal Pathogen:

For this study, garlic leaves bearing the uredial sori of a virulent isolate of *Puucinia porri* were frequently collected from Toukh county, Kalubia governorate.

3-Source of Plant Essential Oils*:*

Essential oils of lemongrass (*Cymbopogon nardus*), clove [*Syzygium aromaticum* (=*Eugenia caryophyllata*)], and thyme (*Thymus vulgaris)* were obtained from the International Flavors and Plant Oils Inc., Giza, Egypt. The essential oils were stored in dark bottles at 5±1ºC for further investigation.

4-Evaluation of the Tested Essential Oils (EOs) and Fungicides on Germination of the Urediospores of *P. porri*:

The effect of four essential oils (EOs) *i.e.,* lemongrass, clove, neem, and thyme, and four fungicides *i.e.,* Kocide-2000® (copper hydroxide), Dithane M-45® (mancozeb), Sumi-8® (diniconazole) and Topas® (penconazole) on the germination of urediospores of *P. porri* was carried out *in vitro*. The tested EOs were diluted to the concentrations of 0.5, 1.0, 1.5, and 2.0% using distilled sterile water plus a few drops from Tween-20 (to make emulsion).

The concentrations of 50, 100, 250, and 500 ppm of the tested fungicides were prepared depending on their active ingredient**.**

Freshly collected *P. porri* urediospores were added to each concentration of both the essential oils and fungicides. Urediospore suspension (one m1) was placed on two sterilized slides, borne on two glass rods in a sterilized Petri dish amended with a wetted cotton piece by sterile distilled water to provide suitable relative humidity. Also, for the control treatment, the same occurred for a spore suspension kept in distilled sterile water only. Prepared dishes were laid out in an incubator at 22±2 °C for 24 hours. Five Petri dishes were prepared as replications. The percentages of uredial germination were calculated as a count of germinated spores in a total of 100 urediospore in each Petri dish by an optical microscope (10X magnification) and examining 100 spores per replicate. The mean value was calculated and recorded for each treatment.

5-Pot Experiment:

Antifungal activity of the four tested essential oils (EOs) of lemongrass, clove, neem, and thyme, and the four commercial fungicides *i.e.,* Kocide-2000®, Dithane M-45®, Sumi-8®, and Topas® were evaluated for their efficacy on the management of garlic rust caused by *P. porri* in a greenhouse experiment under artificial inoculation conditions. Similar-sized garlic cloves (Cv. Sids-40) were soaked in 50 mM Bion® (BTH) and water for two hours just before planting. Then three soaked cloves were planted in each plastic pot (25 cm in diameter) containing autoclaved Nile silt soil, at the beginning of December 2021. Four replication pots were used for each treatment. The plants were left to grow under greenhouse conditions and inoculated with the uredial spore suspension (10^3 spore ml^{-1} water) using a hand atomizer. Inoculated plants with the causal fungus were sprayed with the tested EOs i.e., lemongrass, clove, neem, and thyme (at 15%) and fungicides i.e., Kocide-2000®, Dithane M-45®, Sumi-8® and Topas® at 200g, 250 g, 35 ml, and 35 ml, each alone and in binary combinations, three days after artificial inoculation with the causal fungus. The same spray treatments were repeated three times at two weeks intervals. Plants of control treatment soaked in BTH, or water were sprayed with urediospores suspension of the causal fungus only without any treatments.

The severity of the infection by garlic rust was assessed one week after each spray with the tested materials and the average was recorded using the scale (0-5) devised by Koike *et al.* (2001). The produced garlic bulbs were harvested and left for 2 weeks in a shaded place to air-dry, and the neck of each bulb was cut (2 cm long above the bulb) with a cutter then weighed and the average bulb weight (g) plant^{-1} was recorded.

6-Disease Assessment:

Garlic rust severity was recorded by using a rust severity scoring scale of 0–5; where, 0 = 0% (non-infected leaves), 1 = 1 to 10% infected leaves, 2 = 11 to 25% infected leaves, 3 = 26 to 50% infected leaves, 4 = 51 to 75% infected leaves, and 5 = 76 to 100% infected leaves with the surface covered by rust lesions as suggested by Koike *et al.* (2001). The disease severity was scored for treated as well as untreated plants. The percentage of severity index (PSI) was computed using the following formula follows for data analysis:

$$\% \text{ Severity index} = \frac{\sum(n \times v)}{5N} \times 100$$

Where:

n = Number of infected leaves in each category.

v = Numerical values of each category.

N = Total number of infected leaves.

7-Estimation of the Alkaloids, Flavonoids, and Phenolic Compounds in Garlic Leaves of Plants Sprayed with the Tested EOs and Fungicides:

Five fresh bulbs were randomly picked from each treatment with the tested EOs and fungicides as well as negative (un-inoculated with the causal fungus) and positive (inoculated with the causal fungus) controls and washed with distilled water, air dried for two weeks and ground into a fine powder using sterile pestle and mortar under laboratory condition. Fifty grams of the powder were separately mixed with 500ml of distilled water and ethanol in a sterile conical flask and let stand for 3 days with intermittent shaking. The mixture was filtered using filter paper and concentrated in a water bath at 70°C for 3 hours. Each extract was kept in a sterile container and refrigerated at 4±1°C for the following assessments.

7.1-Quantitative Analysis:

Alkaloids and flavonoids were determined by a method described by Adeniyi *et al*. (2009). Meanwhile, the Folin-Ciocalteu procedure was used to determine phenol content (Zieslin and Ben-Zaken, 1993).

8-Statistical Analysis:

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

RESULTS

1-*in vitro* Inhibitory Effect of The Tested Essential Oils (EOs) And Fungicides on The Uredial Germination of *P. porri*:

The inhibitory effect of the tested EOs and fungicides on the germination of *P. porri* urediospores *in vitro* is presented in Figures (1 and 2).

Data shown in Figure (1) reveal that significant reduction to the germinated urediospores ranged between 20.4-35.7% occurred by the tested EOs *i.e.,* lemongrass, clove, neem and thyme. In addition, clove oil was the most efficient one in this respect followed by thyme then lemongrass and neem, being 20.4, 28.6, 33.8 and 35.7 % uredial germination of efficiency 78.8, 70.3, 64.9 and 62.9%, respectively.

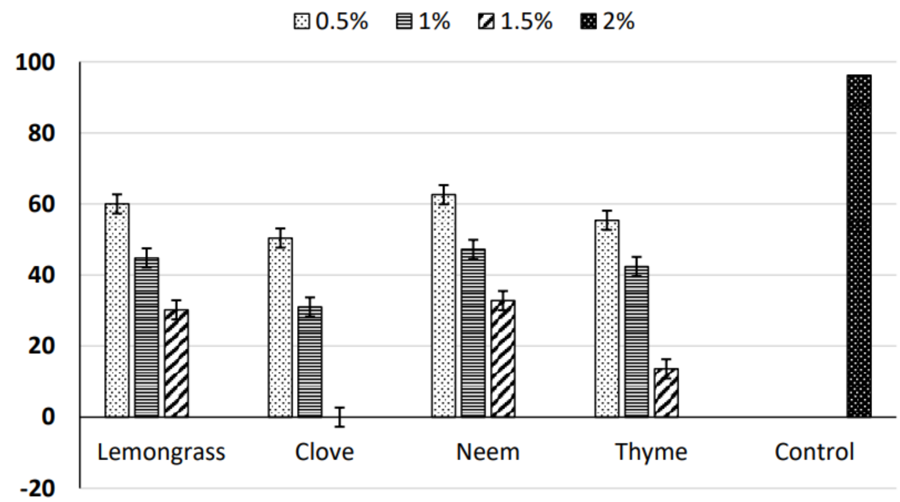

Fig.1: Effect of different essential oils on the urediospores germination (%) of *Puccinia porri in vitro*, 24h. after incubation at 22±2ºC.

Results (Fig. 2) indicate that significant reduction to the germinated urediospores of the causal fungus ranged from 16.0- 35.5% occurred by the tested fungicides *i.e.*, Kocide-2000®, Dithane M-45®, Sumi-8®, and Topas®. In addition, Sumi-8® fungicide was the most efficient fungicide followed by Topas® then Kocide-2000® and Dithane M-45®, being 16.0, 17.4, 33.1, and 35.5% uredial germination with an efficiency of 85.1, 84.1, 66.1 and 64.3%, respectively. Control treatment recorded 96.2% uredial germination.

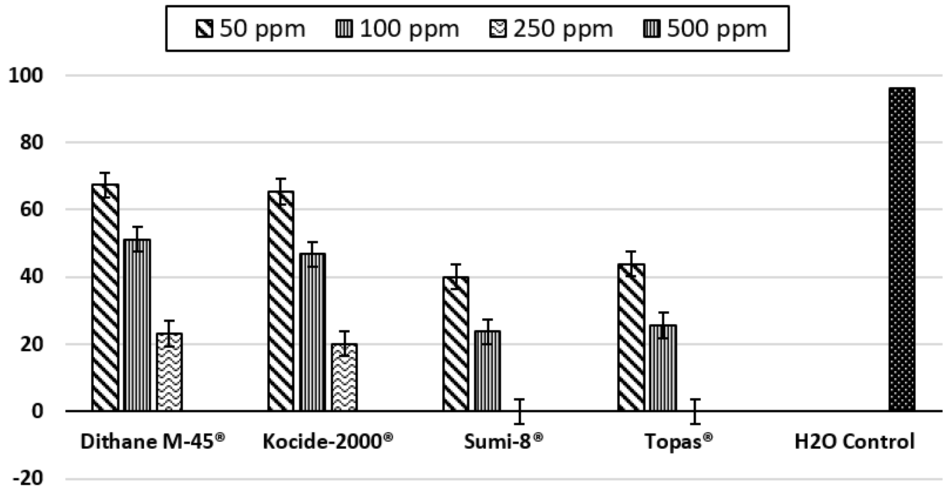

Fig.2: Effect of different fungicides on the urediospores germination (%) of *Puccinia porri in vitro*, 24h. after incubation at 22±1ºC.

2. Greenhouse Experiment:

The effect of spraying the tested fungicides, *i.e.*, Dithane M-45®, Kocide-2000®, Sumi-8® and Topas®, and the EOs *i.e.*, lemongrass, clove, neem, and thyme, each alone or in combinations on the severity of the infection by garlic rust of plant grown from cloves soaked in BTH or water as well as the weight of the produced bulb plant^{-1}, under greenhouse conditions, is presented in Table (1).

Spraying garlic plants, grown under greenhouse conditions, with any of the tested fungicides and EOs, each alone or in combinations three days after inoculation with the causal fungus (*P. porri*) resulted in a significant reduction of the disease severity with a significant increase in the produced bulb yield plant^{-1} (Table 1). However, the individual fungicide treatments were generally more efficient than each individual EO individually in reducing plant mortality and increasing the produced bulb yield. The sprayed plants with the tested fungicides *i.e.*, Dithane M-45®, Kocide-2000®, Sumi-8®, and Topas® recorded 8.7, 9.1, 5.5 and 5.8% disease severity, on average and 93.9, 92.6, 113.7 and 108.4 g bulb weight plant^{-1}, on average compared with the plants sprayed with the tested EOs *i.e.*, lemongrass, clove, neem, and thyme, which recorded 10.7, 10.3, 10.0 and 10.5% disease severity, on average and 89.9, 91.6, 89.5 and 91.2 g bulb weight plant^{-1}, on average, respectively. Moreover, spraying the plants with the combination of EOs and fungicides 3 days after inoculation with the causal fungus was more efficient in reducing the disease and increasing the produced bulb yield than those sprayed with any of them alone. In all cases, plants grown from soaked cloves in BTH were the lowest figures of rust severity (29.4%, on average) and had the highest bulb weight (g) plant^{-1} (71.4 g plant^{-1}, on average) compared with plants grown from cloves soaked in water, 44.6% and 58.5 g, on average, respectively. In addition, no apparent symptoms were observed on garlic plants grown from cloves soaked in BTH and sprayed by the combination of any of the systemic Sumi-8® or Topas® fungicides and the tested EOs.

The highest increase in bulb weight (g) plant^{-1} was more obvious when the tested Sumi-8® and Topas® fungicides were sprayed in combinations with any of the tested EOs.

Table 1. Effect of spraying garlic plants (Cv. Sids-40) grown from cloves soaked in BTH with combinations of four fungicides and four EOs after challenge inoculation with *P. porri* on the severity of rust and plant yield under greenhouse conditions.

Treatments	Plants grown from cloves soaked in:					
	BTH			Water		
	Rust Severity (%)	Rust control efficacy (%)	Average weight of bulbs (g) plant^{-1}	Rust Severity (%)	Rust control efficacy (%)	Average weight of bulbs (g) plant^{-1}
Kocide-2000® (KO)	**7.1**	74.1	**94.0**	**10.3**	76.9	**93.8**
Dithane M-45® (DI)	**7.6**	86.4	**93.0**	**10.6**	76.2	**92.2**
Sumi-8® (SU)	**4.0**	84.4	**114.6**	**7.0**	84.3	**112.7**
Topas® (TO)	**4.6**	84.4	**109.5**	**7.1**	84.1	**107.3**
Lemongrass (LG)	**9.3**	68.4	**90.3**	**12.0**	73.1	**89.4**
Clove oil (CL)	**8.9**	69.7	**92.3**	**11.0**	75.3	**90.8**
Neem (NE)	**9.4**	68.0	**90.0**	**11.8**	73.5	**89.0**
Thyme (TH)	**9.2**	68.7	**91.8**	**11.8**	73.5	90.3
KO + LG	**5 .2**	82.3	**110.9**	**9.1**	79.6	**110.0**
KO + CL	**4.4**	85.0	115.0	**7.5**	83.2	113.0
KO + NE	**5.1**	82.7	110.0	**9.3**	79.1	109.0
KO + TH	**5.5**	81.3	112.6	**9.0**	79.8	111.5
DI + LG	**5.5**	81.3	108.0	**9.4**	78.9	107.0
DI + CL	**5.0**	83.0	114.4	**8.5**	80.9	113.0
DI + NE	**5.3**	82.0	110.0	**9.4**	78.9	108.5
DI + TH	**5.8**	80.3	112.8	**9.8**	78.0	111.0
SU + LG	**0.0**	100.0	139.0	**3.2**	92.8	136.0
SU + CL	**0.0**	100.0	144.0	**2.6**	94.2	139.8
SU + NE	**0.0**	100.0	140.0	**3.8**	91.5	137.4
SU + TH	**0.0**	100.0	141.8	**3.6**	91.9	137.1
TO + LG	**0.0**	100.0	138.0	**3.3**	92.6	136.2
TO + CL	**0.0**	100.0	142.1	**2.8**	93.7	139.0
TO + NE	**0.0**	100.0	138.8	**2.8**	93.7	137.8
TO + TH	**0.0**	100.0	140.0	**2.7**	93.9	137.8
Control	**29.4**		**71.4**	**44.6**		**58.5**
Mean	**9.8**		**151.8**	**10.6**		**150.7**

3-Estimation of the Alkaloids, Flavonoids, And Phenol Compounds in Garlic Cloves of Plants Sprayed with The Tested EOs and Fungicides:

Spraying garlic plants with any of the tested EOs *i.e.*, lemongrass, clove, neem, and thyme and fungicides *i.e.*, Dithane M-45®, Kocide-2000®, Sumi-8®, and Topas® 3 days after inoculation with the causal fungus resulted in a considerable increment in the alkaloids, flavonoids and phenol compounds in garlic cloves compared with both control plants (Figs. 3, 4 and 5). In addition, the highest increment in these compounds was achieved by the EOs, being 7.4, 7.5, 7.4 and 7.5% for alkaloids; 2.23, 2.25, 2.23 and 2.34% for flavonoids and 0.81, 0.83, 0.80 and 0.82% for total phenols compared with the tested fungicides, which contained 7.1, 7.2, 7.3 and 7.3% for alkaloids; 2.17, 2.18, 2.2 and 2.2% for flavonoids and 0.75, 0.77, 079 and 0.79% for total phenol, respectively.

A low increase in these compounds was recorded for the inoculated control plants with the pathogen than the un-inoculated ones.

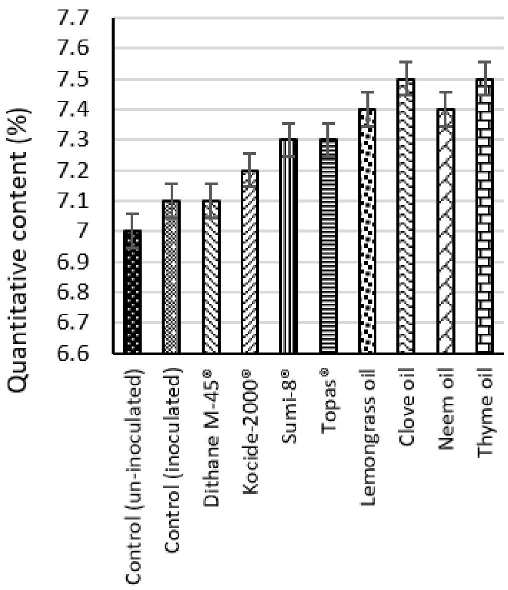

Fig. 3: Effect of tested EOs and fungicides on the quantitative alkaloids content of garlic cloves extract**.**

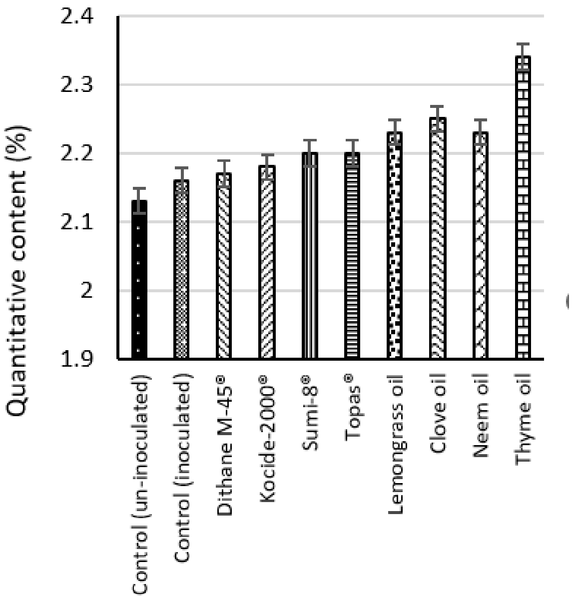

Fig.4: Effect of tested Eos and fungicides on the quantitative flavonoides content of garlic cloves extract.

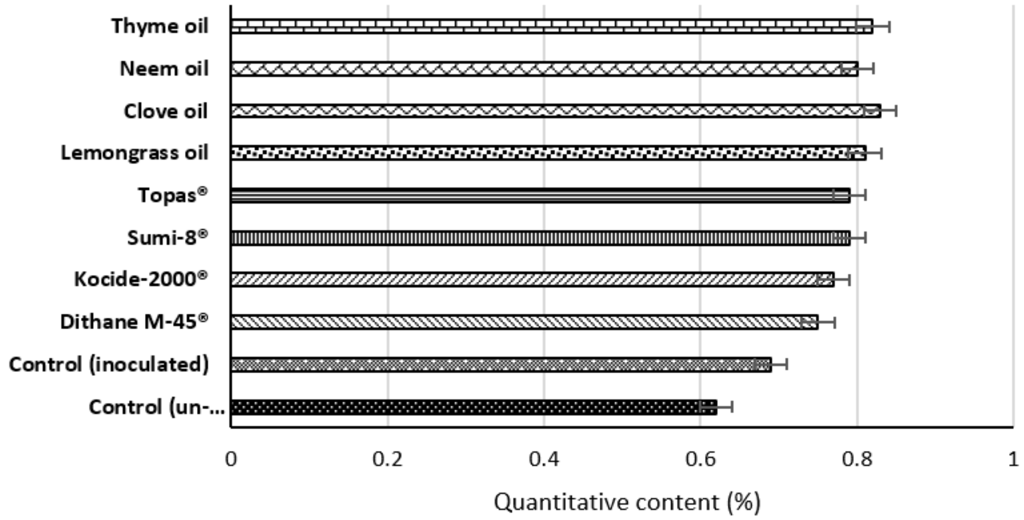

Fig. 5: Effect of tested EOs and fungicides on the quantitative total phenols content of garlic cloves extract.

DISCUSSION

Garlic (*Allium sativum* L.) production is highly affected by biotic and abiotic factors. Among the infectious diseases, fungal diseases are the major problems affecting yield and productivity, as well as the quality of this high-value crop. The rust disease caused by *Puccinia porri* G. Wint. is one of the most destructive diseases. The disease does not attack the garlic bulb directly, but it damages the leaves which, indirectly, negatively impacts the size and quality of bulbs of the infected plants at harvest thereby reducing its marketability (Koike *et al.*, 2001).

Chemical control using fungicides is the most common method for white rot management. Several synthetic fungicides, i.e., mancozeb, propiconazole, tebuconazole and azoxystrobin have been reported to be effective against this pathogen (Rochecouste, 1984; Blum and Gabardo, 1993; Koike *et al.*, 2001). In most cases, managing plant diseases with fungicides turn out essential due to its high effectiveness in combating diseases, considering a sufficient pre-harvest interval to reduce its residual effect on plants, especially vegetables and fruits.

The anti-fungal effect of essential oils (EOs) 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, or mitochondrial structure disorganization. Furthermore, EOs 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; Dewitte *et al.*,2019).

In this study, *in vitro* experiments revealed that a significant reduction in the *P. porri* urediospores germination percentage ranged from 14.2-33.9% occurred by the tested EOs and 16-35.5% occurred by the fungicides tested. Meantime, clove oil and Sumi-8® fungicide were the most efficient ones in this regard.

It has been found that spraying garlic plants, grown under greenhouse conditions, with any of the tested fungicides and EOs, each alone or in combinations three days after inoculation with the causal fungus (*P. porri*) resulted in a significant reduction in the severity of the disease with a significant increase to the produced bulb weight plant-^{1}. Meantime, the fungicides were more efficient than EOs in reducing plant mortality and increasing the produced bulb yield. Moreover, spraying the plants with the combination of EOs and fungicides 3 days after challenge inoculation with the causal fungus was more efficient in reducing the disease and increasing the produced bulb yield than those sprayed with any of them alone. Notably, there is a positive correlation between the urediospore germination reduction rate and the concentration increase of all examined EOs and fungicides. At the highest concentrations, most of them positively inhibited the uredial germination of the pathogen (Figures 1 and 2). McGrath *et al.* (2019) sprayed essential oils, as safe materials to combat the disease, after spraying plants with fungicides to obtain a crop free from the effect of the pesticides or reduce them to a safe limit.

In the current study, the individual fungicide treatments were generally more efficient than each individual EO individually in reducing plant mortality and increasing the produced bulb yield. To date, fungicides, particularly systemic, are the most efficient method to manage plant diseases. Sumi-8® is a systemic fungicide with 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 many crops (Fontem and Bouda,1998; McGrath, *et al.,* 2019). The efficacy of mancozeb, hexaconazole, chlorothalonil, azoxystrobin, propiconazole and difenoconazole against garlic rust (*P. porri*) has been reported by different investigators (Worku *et al.,* 2016; Kassaw *et al.,* 2017; Kassaw *et al.,* 2021; Negash *et al.,* 2018.; Kifelew *et al.,* 2019).

In this concern, the fungicidal effect of EOs against several plant pathogens is widely reported (Behtoei *et al.,* 2012; Amini *et al.,* 2016; Varo *et al.,* 2017). To name just a few, lemongrass (*Cymbopogon citratus* L.) oil was reported to have antifungal activity against several plant pathogens. Also, thymol is an essential oil component from thyme (*Thymus capitates* L.) and has been used for the management of plant diseases (Plaza *et al.,* 2004; Klaric *et al.,* 2007). Clove oil has demonstrated toxicity to various plant-pathogenic fungi and bacteria and showed suppression to various plant diseases (Beg and Ahmad, 2002; Bowers and Locke, 2004; Kishore *et al*., 2007; El-Habbak and Refaat, 2019). *in vivo* evaluation of the efficacy of neem (*Azadirachta indica*) derivatives (neem oil, neem cake powder and neem leaf powder) against bean rust (*Uromyces appendiculatus*) proved a significantly high inhibitory effect on rust severity, incidence and urediospores germination (Liebenberg and Pretorius, 2010).

The variability in the antifungal activities of EOs may be attributed to their physical, molecular, and chemical characteristics and the sensitivity of the pathogens to the quantitative differences in the constituents of each oil (Stevic *et al.,* 2014). The antifungal components of EOs have shown that the oils consist of 20 to 60 components at various concentrations, where 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).

In the entire study, all plants grown from soaked cloves in BTH had the lowest rates of rust severity and the highest bulb weight plant^{-1} compared to plants grown from cloves soaked in water. Furthermore, no apparent symptoms were observed on garlic plants grown from cloves soaked in BTH and then sprayed by the combination of any of the systemic Sumi-8® or Topas® fungicides and the tested EOs. BTH was proven to induce resistance against plant diseases with three main advantages: it has a broad spectrum and a low environmental impact. Furthermore, the risk probability regarding the selection of pathogen-resistant strains to BTH is null.

Considerable increments in the alkaloids, flavonoids and phenolic compounds were recorded in garlic cloves of plants sprayed with the tested EOs and fungicides compared to both control plants. EOs provided the highest increments in those compounds as compared to the tested fungicides. Previous investigations reported that in reaction to pathogen attacks in plant cells, some plant hydrolyzing enzymes (glycosidases) act to readily convert inactive bound forms of certain phenolic compounds into biologically active antimicrobial compounds. In such circumstances, free phenolic compounds are likely to be considerably more toxic to the invading organism than the bound forms. In addition, the participation of an endogenous phenol compound in plant disease resistance is dependent upon the active phenol oxidase system (Lattanzio *et al.*, 2006; Melo *et al*., 2007; Farkas and Kiraaly, 2008; Attia *et al.,* 2022).

It is noteworthy that low increases in the alkaloids, flavonoids and phenolic compounds occurred in the control plants challenged with the pathogen than the un-inoculated ones. This might be explained by the fact that even if pre-formed phenolic compounds are present in healthy plants at levels that are anticipated to be antimicrobial, their levels may increase further in return for pathogen challenge. Ali and Ibrahim (2009) found that the qualitative phytochemical screening of *Allium sativum* aqueous and ethanol extracts indicated the presence of alkaloids, terpenoids, flavonoids, steroids, phenols, anthraquinones, saponins, tannins and glycosides. It is well known that phenolic compounds content is the compounds whose quantity is raised when a plant comes under invasion by a pathogen (Waterman and Mole, 1995).

CONCLUSIONS

In recent years, essential oils have been renowned as a crucial component of agricultural biotechnology for managing a lot of plant pathogens, and as a sustainable and ecologically acceptable complete or partial alternative to conventional disease management methods. Based on the findings of the present investigation, BTH as a resistance inducer could replace or work simultaneously with conventional fungicides for the management of rust disease in garlic crops. The tested fungicides and essential oils (EOs) resulted in a considerable reduction in the germinated urediospores of *P. porri,* the causal pathogen of garlic rust *in vitro.* In addition, these materials caused a significant reduction in infection under greenhouse conditions when used alone or in combinations. However, combinations were more efficient, especially when each of Sumi-8® or Topas® fungicides (systemic) was combined with any of the tested EOs. Moreover, tested fungicides and EOs caused an increment in the quantitative values of the alkaloids, flavonoids and phenol compounds in garlic cloves.

Concerning perfect disease management with an increased focus on safety, using an integrated management plan consists of pre-planting soaking clove seeds in 50 mM Bion® (BTH) then spraying the grown plants with clove or thyme (at 15%) *combined with* Sumi-8® (35ml), for four times with two weeks intervals is considered the preferred treatment for controlling garlic rust under greenhouse conditions. Field experiments are needed to evaluate this management strategy to control garlic rust under uncontrolled environmental conditions.

Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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