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EFFICIENCY OF SOME ABIOTIC AND BIOTIC AGENTS ON *VICIA FABA* L. RUST AND CHOCOLATE SPOT DISEASES

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ABSTRACT

A variety of soil- and air-borne pathogens can attack faba bean plants, leading to rust and chocolate spot diseases that seriously affect plant yield. This study aims to evaluate the efficacy of certain essential oils as abiotic agents and biotic agents as safe alternatives to fungicides for both diseases. Double foliar applications of these essential oils and biotic agents significantly reduced the development of faba bean rust and chocolate spot incidence. The highest reduction in faba bean rust (92.7%) was achieved after spraying with jojoba oil, followed by 88.9% and 85.3% for grape and bitter orange oils, respectively. Furthermore, biotic inducers such as *Bacillus subtilis*, *Trichoderma harzianum*, and *Saccharomyces cerevisiae* reduced chocolate spot incidence by 100.0%, followed by a 72.3% reduction with bitter orange, thyme, black seed, and jojoba oils. The second foliar treatment with *B. subtilis*, *T. harzianum*, black seed oil, jojoba oil, and chitosan increased faba bean protein contents more than the first treatment. Individual foliar sprays with chitosan and grape oils enhanced catalase (CAT) specific activity, with the highest levels achieved after the first and second foliar applications, respectively. *S. cerevisiae* and lemongrass oil induced the highest peroxidase (POD) specific activity after both spray applications. Black seed oil induced the highest chitinase (CHIA) specific activity after the first foliar application, while lemongrass oil achieved the highest level after the second spray. The second foliar application of moringa oil, black seed oil, jojoba oil, chitosan, *B. subtilis*, and *S. cerevisiae* stimulated the formation of new protein bands in treated faba bean plants, which were not found after the first application. Therefore, the abiotic and biotic agents used in this study could be considered good and safe alternatives for resistance against these foliar diseases, reducing the harmful effects of synthetic fungicides.

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INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most substantial grain legume crops, which is a fundamental source of protein for people in different parts of the world (Teshome and Tagegn, 2013). A wide variety of soil- and air-borne pathogens attacks bean plants such as

Uromyces viciae-fabae causing bean rust; one of the most serious diseases. The pathogens of rust survive in soil and straw attacking all aboveground plant parts (Emeran et al., 2005). This disease is widely prevalent in the Middle East, Europe, North Africa and China leading to moderate to huge losses in yield (Sillero et al., 2010; El-Mougy and Abdel-Kader, 2018a). Faba bean rust resistance depends mainly on the causative host resistance which does not extend for long periods due to the high genetic diversity of the pathogen and its ability to develop new strains. Various control methods have been studied to control *U. viciae-fabae* like biological, chemical and cross-protection practices as well as using resistant cultivars (Shahbaz et al., 2023). Chemical resistance to these pathogens achieved perfect outcomes, but at the same time triggers ecological contaminations and elevates pathogens resistance (Hawthorne et al., 2004). Recently, fears from the use of pesticides on human health and the environment have led to the search for environmentally friendly alternatives (El-Mougy and Abdel-Kader, 2018a).

Botrytis fabae Sard*.* and *B. cinerea* Pers. pathogens are the causatives of chocolate spot disease which damages leaves reducing photosynthetic activity and yield of faba bean (Torres et al., 2004; Alnefaie et al. 2023). The incidence of this disease prefers moist warm conditions extending from 4 to 5 days and then quickly propagates through the crop later in the season. It usually develops during flowering and after the canopy has closed leading to abortment of pods, plant damage and massive yield loss (Hawthorne et al., 2004; Baka and El-Zahed, 2023). In Egypt, this disease leads to severe deteriorations of faba bean plants which decrease the production by more than 50% particularly in northern and midst sections of the Delta region (El-Banoby et al., 2013; El-Kholy, 2014; El-Mougy and Abdel-Kader, 2018b).

Utilization of biological control factors as alternative fungicides for disease management has become of great importance. The essential oils of aromatic plants have considerable economic importance due to their antimicrobial activities especially in the field of food industry and pharmaceuticals. There is increasing numbers of studies related to their chemical composition and biological characteristics in addition to the environmental, taxonomic and agricultural factors affecting their quantity and quality (El-Mougy and Abdel-Kader, 2017; Daigham et al., 2023).

Pathogenesis related plant proteins (PRPs) perform significant functions in resistance of different plant diseases. They are wide spread in plants in trace amounts that are greatly increased after pathogens attack (Souza et al., 2017). Defensive enzymes that are related to resistance inducement in plants are part of these proteins like peroxidase (POD), chitinase (CHIA) and catalase (CAT) (Prasannath et al., 2014). POD has been involved in a number of defense concerned operations, like lignifications, hypersensitive responses, cross-linking of phenolics with glycoproteins and aging (Jouili et al., 2011; Begovic et al., 2017). When plants are subjected to pathogen infection, CHIA is greatly expressed and plays significant parts in plant defenses due to its capability of attacking fungal pathogens cell walls (Sharma et al., 2011; Vaghela et al., 2022). CAT catalyzes the H_2O_2 conversion into H_2O and O_2 and plays a considerable role for plant defenses, senescence and aging by protecting them from oxidative damage (Sarker and Oba, 2018; Palma et al., 2020).

This study aims at evaluating the effect of foliar spray of some abiotic agents (essential oils and chitosan) and biotic inducers (*Bacillus subtilis*, *Trichoderma harzianum* and *Saccharomyces cerevisiae*) in controlling faba bean rust and chocolate spot diseases and studying their effects on the protein contents and specific activities of the plant defence enzymes; catalase, chitinase and peroxidase.

MATERIALS AND METHODS

Materials

The most common faba bean cultivar seeds (Giza 3) were obtained from the Vegetable Crops Research Department, Agricultural Research Center, Giza, Egypt. The oils of lemon grass, thyme, moringa, grape, black seed, jojoba and bitter orange and chitosan were acquired from Al-Gamhoria Co. Ltd. for chemicals and medicinal instruments, Cairo, Egypt. Phenyl methyl sulphonyl fluoride (PMSF), bovine serum albumin (BSA), guaiacol, chitin, dinitrosalicylic acid (DNS), N-acetyl glucosamine (NAGA) and Tween 20 were acquired from Sigma Chemical Co. All other chemicals used in the study were of research grade. The candidate antagonistic agents *B. subtilis*, *T. harzianum*, and *S. cerevisiae* were obtained from the culture collection unit, Plant Pathology Department. NRC, Egypt.

Experimental design

The experiment was carried out in a complete randomized design during the autumn growing season 2021/2022 at open greenhouse, NRC, Egypt to estimate the effectiveness of some abiotic and biotic agents as foliar sprays in controlling the faba bean rust and chocolate spot diseases. The required seed germination took place in 4–12 days, and the optimum temperature for germination was 20°C. To calculate disease incidence, a total of sixty seeds were sown for each treatment and the control. The seeds were planted in cleaned and sterile plastic pots with a diameter of 40 cm, each filled with 5 kg of sterile loamy clay soil (6 seeds per pot). Ten replicates were prepared for each treatment, and all pots were placed under open cultivation conditions, following traditional farming practices. For the abiotic treatments, including lemon grass, grape, moringa, bitter orange, thyme, black seed, jojoba oils, and chitosan, solutions were prepared with a concentration of 2% (20 g/L) in water. A few drops of the emulsifier Tween 20 were added to create an emulsion. As for the biotic inducers, *B. subtilis* was sub-cultured in nutrient agar broth medium for 24 hours at 28ºC, and the liquid medium was adjusted to a concentration of 10^8 cfu/ml. *T. harzianum* and *S. cerevisiae* were cultured on Potato dextrose agar (PDA) and Yeast Nitrogen Base (YNB) broth media, respectively, and incubated for seven days at 28ºC. Conidial suspensions were prepared at a concentration of 10^8 spores/ml using a haemocytometer slide.

For comparison, the fungicide Mancozeb WP 75% (3 g/L) and an untreated control (sprayed with sterilized distilled water) were applied. Foliar spray applications were carried out twice, with a 15-day interval starting from the flowering period. Disease incidence percentages for rust and chocolate spot were recorded 15 days after the second spray application. Disease incidence was calculated using the formula (DI %) = (Number of infected plants / Total number of examined plants) × 100.

Faba bean fresh weighed (FWt) leaf samples were collected twice, each time 15 days after a foliar application, for protein and enzyme determination.

Extraction of total proteins

Extraction of proteins was achieved by grinding 1 g FWt faba bean leaves utilizing a mortar including liquid N_2, then mashing the resulted powder for 40 sec in 2 ml 50 M K-phosphate buffer pH 6.5 comprising 1 mM PMSF and centrifugation at 10000 x*g* (30 min, 4°C). The supernatant was kept as crude extract at -20°C (Megahed et al., 2019).

Protein determination

Estimation of proteins was carried out by the method of Bradford (1976) utilizing spectrophotometer (Shimadzu UV-2401).

Enzymes assays

CAT assay reaction consisted of 3 ml [50 mM K-phosphate buffer pH 7.0 + 0.02 M H_2O_2 + the enzyme sample] and the decrease in absorbance was recorded for 3 min at 240 nm. One unit (U) of CAT activity was the enzyme amount needed to convert 1 µmol H_2O_2 (extinction coefficient: 43.6/M/Cm) to H_2O and O_2 per min (Aebi, 1984).

POD activity reaction consisted of 1 ml of 80 mM guaiacol dissolved in K-phosphate buffer pH 6.5 + 100 µl 3% H_2O_2 + 100 µl enzyme extract and monitors the absorbance for 5 min at 470 nm. One POD U is the amount of enzyme yielding 0.001 absorbance increase per min at 25ºC (Johri et al., 2005).

For CHIA assay, colloidal chitin was prepared as reported by Rodriguez-Kabana et al. (1983). CHIA assay mixture [l ml 1% colloidal chitin dissolved in 50 mM Na-acetate buffer pH 6.6 + 1 ml sample] was incubated at 37°C for 1 hr then terminated by 1 ml DNS [0.04 M DNS, 0.02 M phenol, 0.25 M NaOH, 4 mM Na_2SO_3 and 0.7 M $KNaC_4H_4O_6 \cdot 4H_2O$]. For color developing, this mixture was incubated for 10 min at 100°C, centrifuged at 7500 x*g* for 10 min and the absorbance of supernatant was monitored at 540 nm. For unit determination, NAGA standard curve was extracted. One CHIA U is the amount of enzyme which produces 1 µmol NAGA per min (Sadfi et al., 2001).

Electrophoresis on SDS-PAGE

Analysis of proteins was carried out using 12% SDS polyacrylamide gels (Weber and Osborn, 1969; Laemmli, 1970), stained with Coomassie brilliant blue R-250 and analyzed using Syngene™ Ingenius 3 Gel Documentation System software.

Statistical analysis

General Linear Model option of the Analysis System SAS (SAS, 1996) was used to perform the analysis of variance. The statistical analyses were performed according to Bailey, (1997). The standard deviation was calculated from the relation (S.D. = $\sqrt{\sum (M-X)^2 / n-1}$) where Σ = the sum, M = arithmetic mean, X = the individual values, n = the number of individual values.

RESULTS

Disease incidence

Per cent of faba bean rust disease incidence was ranged from 3.3 to 30.0% compared with 35.0% for the fungicide treatment and 45.0% for untreated control (Table 1). The highest protective inducer against rust pathogen invasion was jojoba oil (3.3% incidence) followed by 5.0 and 6.6% for grape and bitter orange oils respectively, while the least protective treatment was 30.0% for the yeast *S. cerevisiae* (Figure 1a). On the other hand, per cent of faba bean chocolate spot disease incidence was ranged from 0.0 to 13.3% compared with 20.0% for the fungicide treatment and 30.0% untreated control. The highest protective inducers against chocolate spot pathogens invasion were the three biotic inducers *B. subtilis*, *T. harzianum* and *S. cerevisiae* (0.0% incidence) followed by 8.3% incidence for bitter orange, thyme, black seed and jojoba oils, while the least protective treatment was 13.3% for chitosan (Figure 1b).

Table 1: The influence of some essential oils and biotic agents on the incidences of faba bean chocolate spot and leaf rust diseases.

Treatment (2% w:v)	Rust		Chocolate spot	
	% Incidence	% Reduction	% Incidence	% Reduction
Lemon grass oil	10.0 e	77.8	10.0 b	66.7
Thyme oil	8.3 f	81.6	8.3 c	72.3
Black seed oil	11.6 e	74.2	8.3 c	72.3
Grape oil	5.0 f	88.9	10.0 b	66.7
Jojoba oil	3.3 g	92.7	8.3 c	72.3
Bitter orange oil	6.6 f	85.3	8.3 c	72.3
Moringa oil	16.6 d	63.1	10.0 b	66.7
Chitosan	8.3f	81.6	13.3 b	55.7
Bacillus subtilis	20.0 c	55.6	0.0 d	100.0
Trichoderma harzianum	16.6 d	63.1	0.0 d	100.0
Saccharomyces cerevisiae	30.0 b	33.3	0.0 d	100.0
Mancozeb WP 75% (3 g/L)	35.0 b	22.2	20.0 a	33.3
Untreated control	45.0 a	0.0	30.0 a	0.0

Mean values within columns followed by the same letter are not significantly different at P≤0.05. The percent reductions in the incidences were calculated relative to untreated controls as describe by Mukhtar et al. (2017).

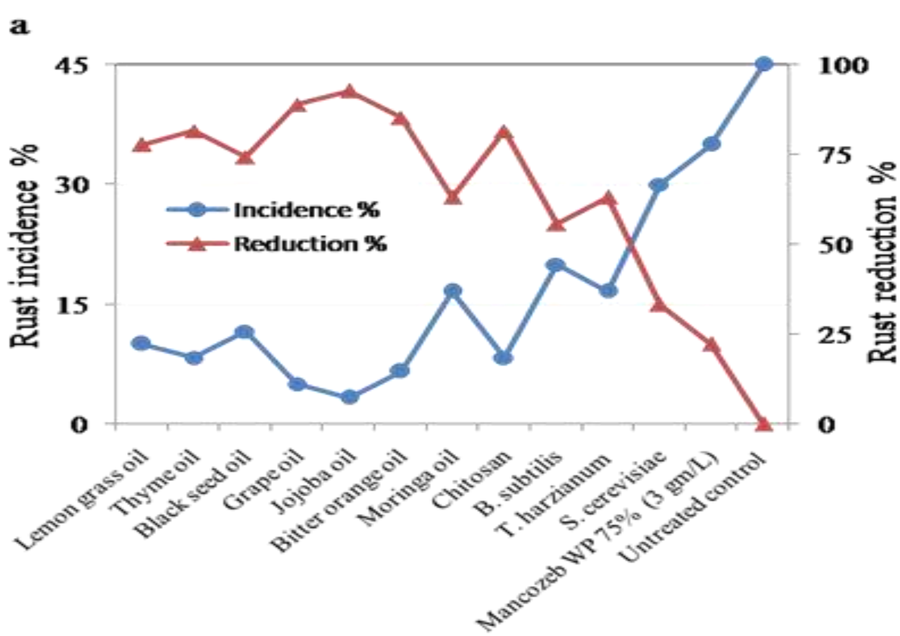


Figure 1a. Effect of foliar spraying of some abiotic and biotic agents on incidence and reduction of faba bean rust disease infection.

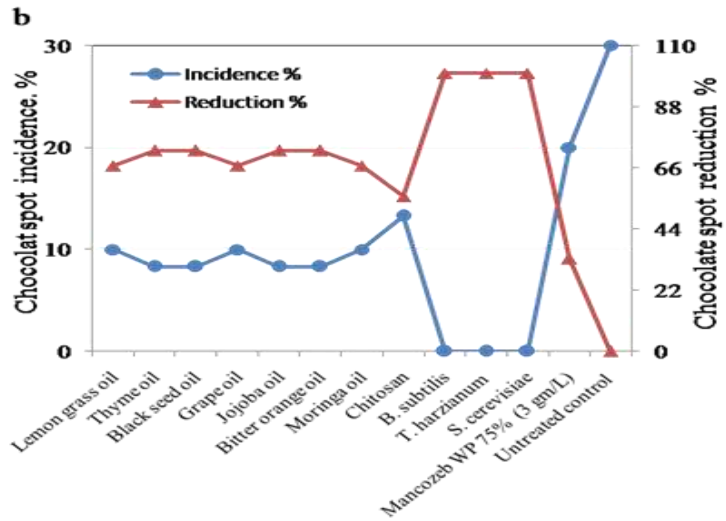


Figure 1b. Effect of foliar spraying of some abiotic and biotic agents on incidence and reduction of faba bean chocolate spot disease infection.

Biochemical parameters

The protein contents of faba bean plants were assessed after treatment with both abiotic and biotic inducers. The application of lemon grass, thyme, grape, bitter orange, moringa oils, and *S. cerevisiae* yeast resulted in a decrease in protein content in treated plants after the second foliar application. In contrast, an increase was observed with treatments using black seed oil, jojoba oil, chitosan, *B. subtilis*, and *T. harzianum*. *S. cerevisiae* induced the highest protein content (0.42 mg/g FWt) after the first foliar application, while black seed oil induced the lowest protein content (0.13 mg/g FWt). After the second foliar application, *B. subtilis* induced the highest protein content (0.54 mg/g FWt), while moringa oil induced the lowest protein content (0.14 mg/g FWt) when compared to the untreated control (Figure 2a).

Distinct CAT, POD, and CHIA specific activities were observed after both foliar application periods. Chitosan exhibited the highest CAT activity (1874.4 U/g FWt), while the lowest specific activity was observed with *S. cerevisiae* (98.1 U/g FWt) after the first foliar spray. Following the second foliar application, grape oil induced the highest CAT activity (1470.8 U/g FWt), while *S. cerevisiae* once again resulted in the lowest activity (305.6 U/g FWt) (Figure 2b).

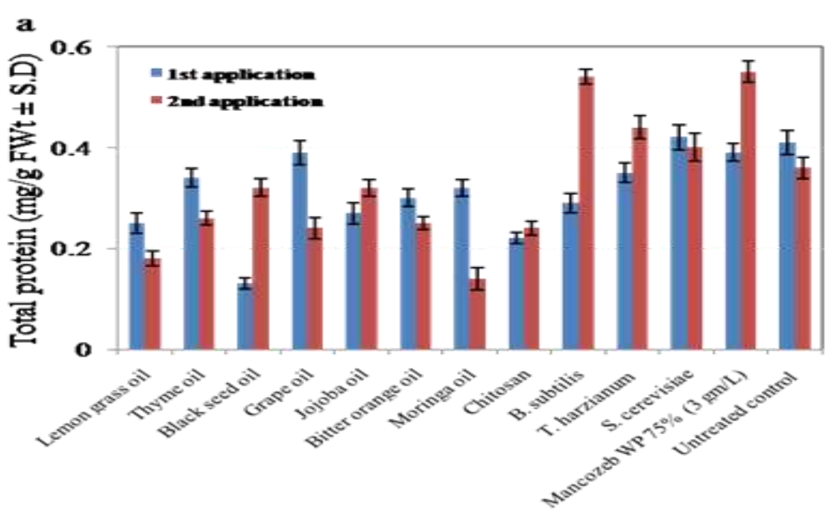


Figure 2a. Effect of foliar spraying of some abiotic and biotic agents on protein contents (mg/g FWt ± S.D) of faba bean plants against rust and chocolate spot infections.

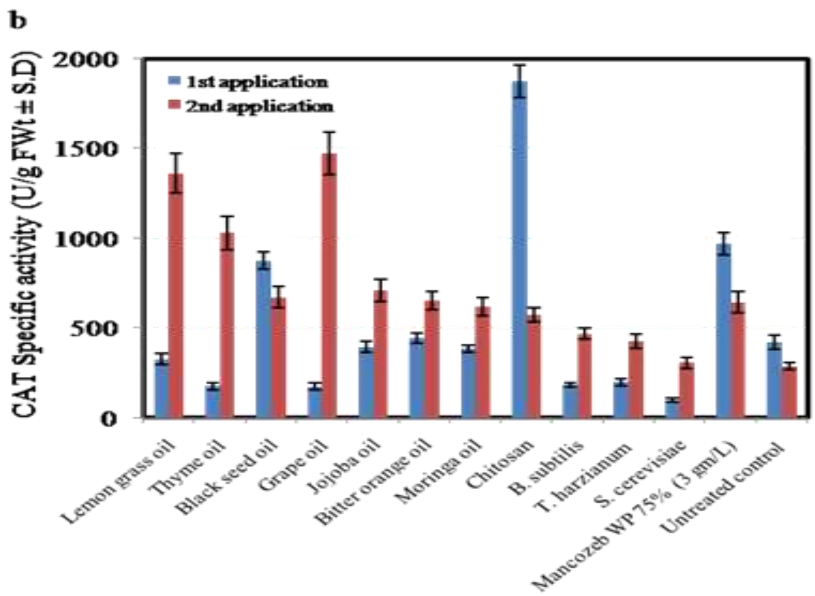


Figure 2b. Effect of foliar spraying of some abiotic and biotic agents on CAT specific activity (U/g FWt ± S.D) of faba bean plants against rust and chocolate spot infections.

S. cerevisiae induced the highest POD activity (383.8 U/g FWt), while the lowest activity was induced by grape oil (114.9 U/g FWt) after the first foliar spray. Following the second foliar application, lemon grass oil led to the highest POD activity (333.3 U/g FWt), while the lowest activity was recorded for *B. subtilis* and *S. cerevisiae* (65.0 U/g FWt) (Figure 3a).

Black seed oil induced the highest CHIA activity (82.2 U/g FWt), while the lowest activity was induced by jojoba oil (28.2 U/g FWt) after the first foliar application. Following the second foliar spray, lemon grass oil recorded the highest CHIA activity (63.6 U/g FWt), while the lowest activity was induced by *B. subtilis* (25.5 U/g FWt) (Figure 3b) when compared to the untreated control.

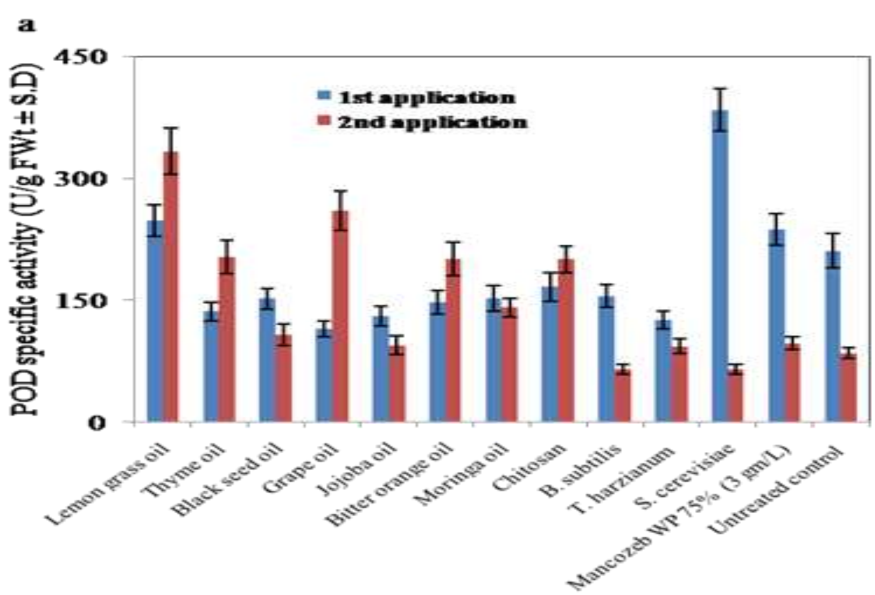


Figure 3a. Effect of foliar spraying of some abiotic and biotic agents on POD specific activity (U/g FWt ± S.D) of faba bean plants against rust and chocolate spot infections.

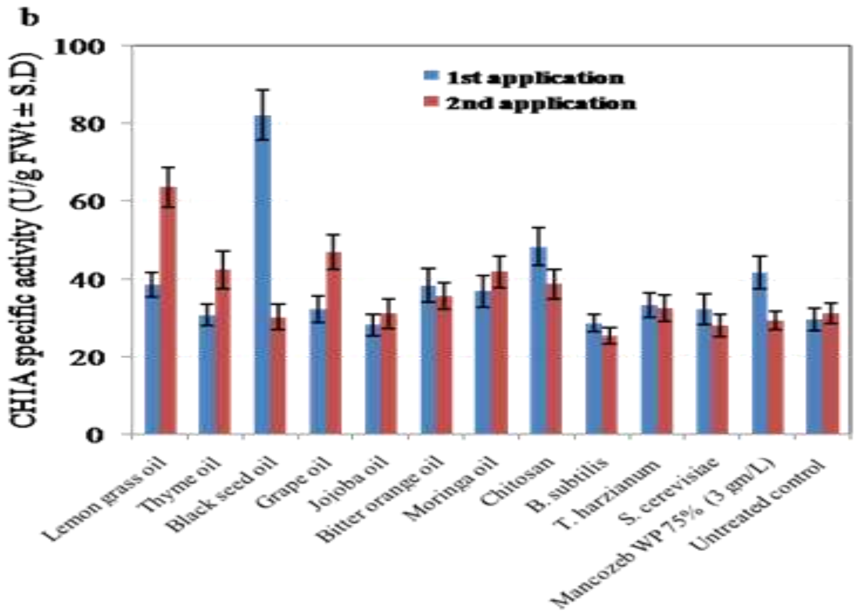
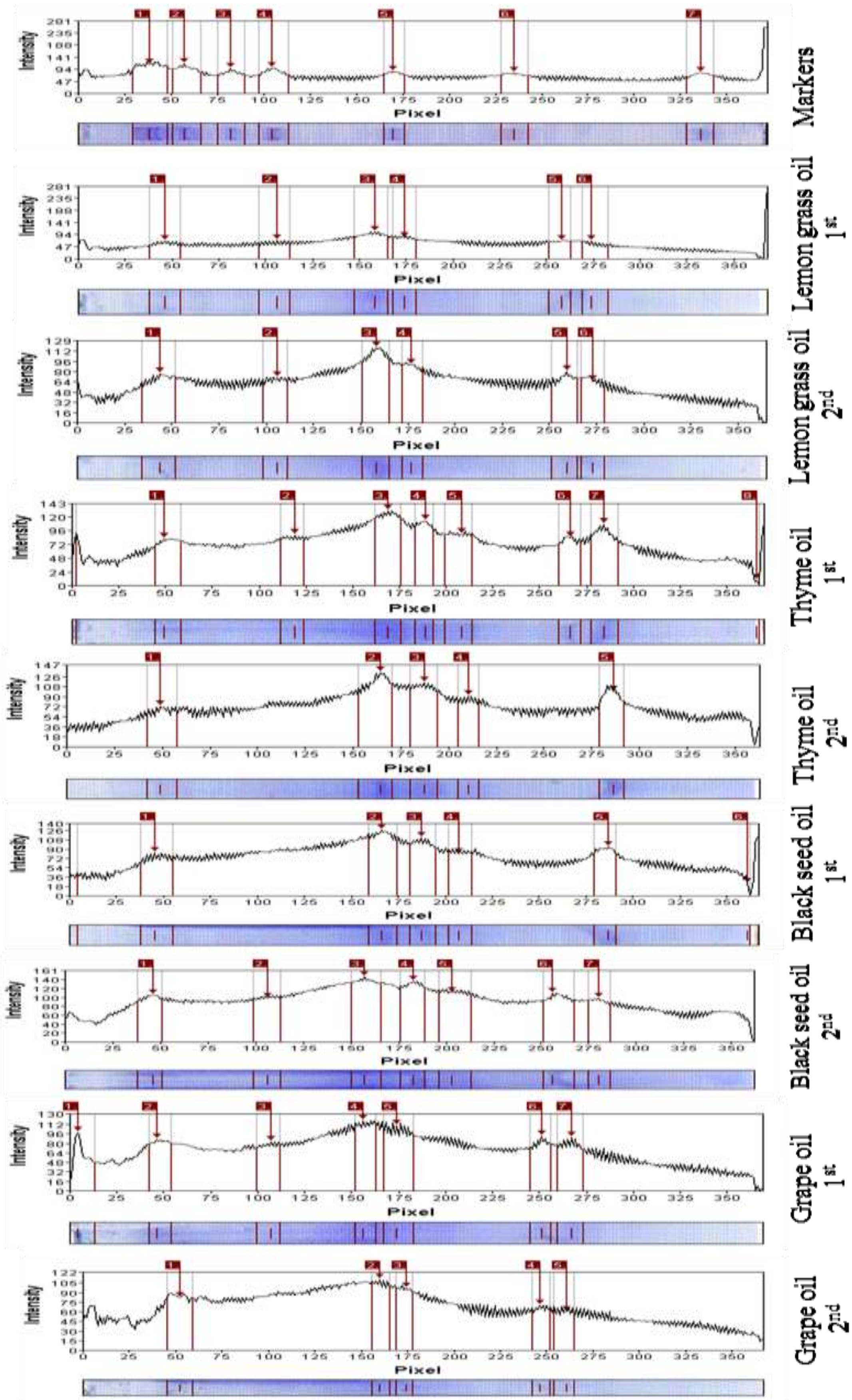


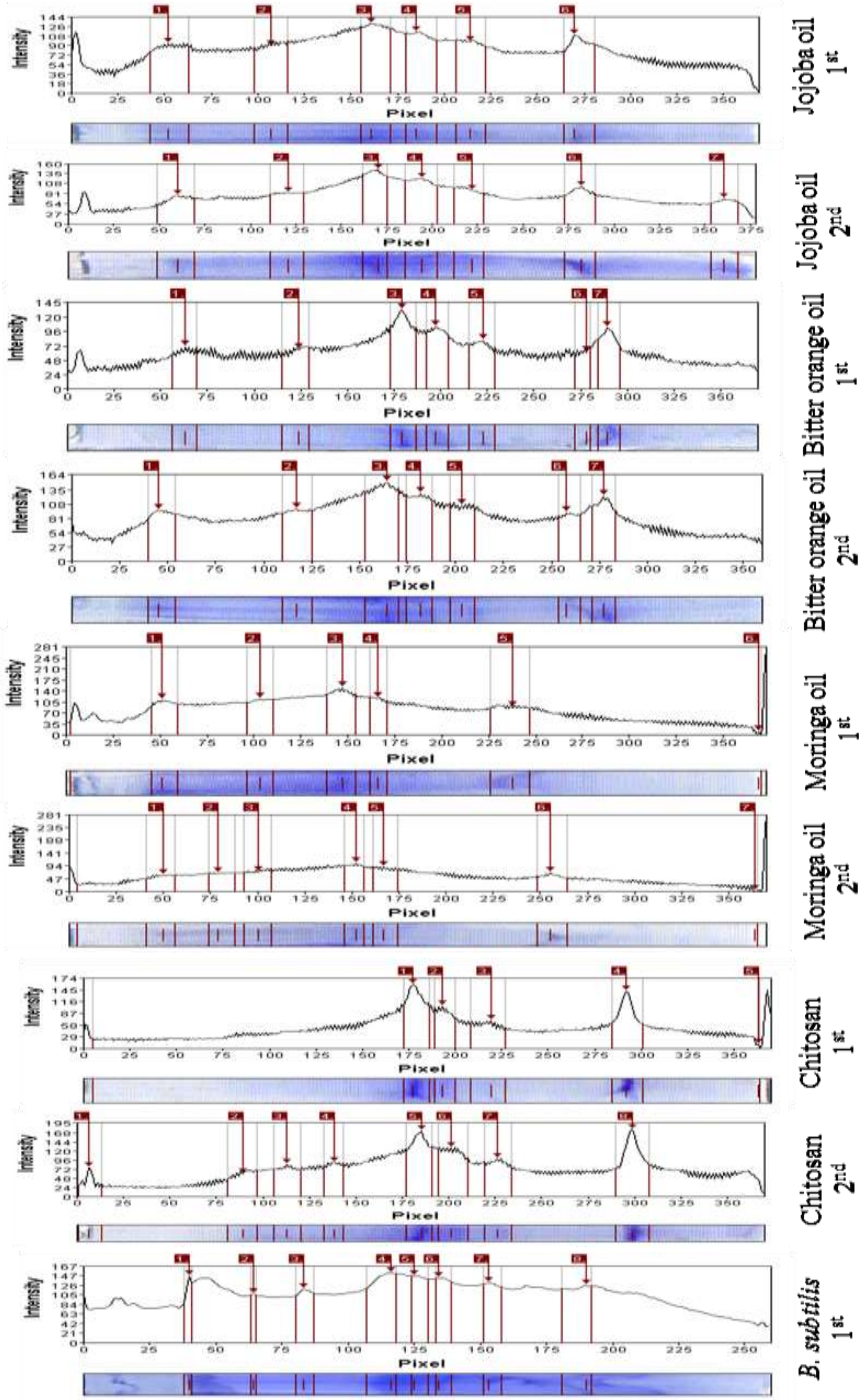
Figure 3b. Effect of foliar spraying of some abiotic and biotic agents on CHIA specific activity (U/g FWt ± S.D) of faba bean plants against rust and chocolate spot infections.

A fixed amount of protein (75 µg) from crude extracts of all treated faba bean plants was used for comparing protein patterns on a 12% SDS-PAGE gel. The gel analysis data revealed that treatments with lemon grass and bitter orange oils resulted in the same number of protein bands with identical molecular weights after both applications. After the second foliar application of moringa oil, black seed oil, jojoba oil, chitosan, *B. subtilis*, and *S. cerevisiae*, new protein bands were observed that were not present after the first application. On the other hand, the application of grape, thyme, jojoba oils, and *T. harzianum* increased the formation of new protein bands after the first sprays, but these bands disappeared after the second ones. Individual applications of bitter orange oil, black seed oil, and chitosan increased the intensity of proteins with the same molecular weight after the second foliar application compared to the first (Figure 4).

DISCUSSION

The production of faba bean crop was greatly limited due to leaf diseases of which are leaf rust and chocolate spot caused by the fungi *Uromyces viciae-fabae, Botrytis fabae* and *B. cinerea* (Ijaz et al., 2018; Lee et al., 2020; Villegas-Fernández et al., 2023). Plant products with antimicrobial properties have notably obtained attention as possible compounds in order to prevent bacterial and fungal growth (Lanciotti et al., 2004). Plant products as essential oils are characterized as having a wide range of volatile components that could be used as alternative anti-bacterial and anti-fungal treatments. In this concern, the effectiveness use of essential oils as antifungal inhibitors and antimicrobial compounds was reported (Arora and Kaur, 1999; Deresa and Diriba, 2023). In the current study, the double foliar applications of all the used abiotic and biotic treatments strongly reduced the development of faba bean rust and chocolate spot incidence. The highest faba bean rust reduction was 92.7% achieved after application of jojoba oil followed by 88.9 and 85.3% for grape and bitter orange oils respectively. Furthermore, the biotic inducers *B. subtilis*, *T. harzianum* and *S. cerevisiae* reduced the chocolate spot incidence 100.0% followed by 72.3% for bitter orange, thyme, black seed and jojoba oils. The essential oils were reported to contain antifungal compounds, specific components and fungitoxic factors against various microorganisms (Voda et al., 2003; Kishore et al., 2007). Thyme, peppermint, lemongrass, mint and clove oils greatly inhibited the growth of *Fusarium oxysporum* mycelia (Ragab et al., 2012; Kouache et al.. 2023). Also, lemongrass oil revealed antifungal action against *B. cinerea*, *Colletotrichum coccodes*, *Rhizopus stolonifer*, *Cladosporium herbarium* and *Aspergillus niger in vitro* (Tzortzakis and Economakis, 2007).


Markers
Lemon grass oil 1st
Lemon grass oil 2nd
Thyme oil 1st
Thyme oil 2nd
Black seed oil 1st
Black seed oil 2nd
Grape oil 1st
Grape oil 2nd
Intensity
Pixel


Intensity
Pixel
Jojoba oil 1st
Jojoba oil 2nd
Bitter orange oil 1st
Bitter orange oil 2nd
Moringa oil 1st
Moringa oil 2nd
Chitosan 1st
Chitosan 2nd
B. subtilis 1st

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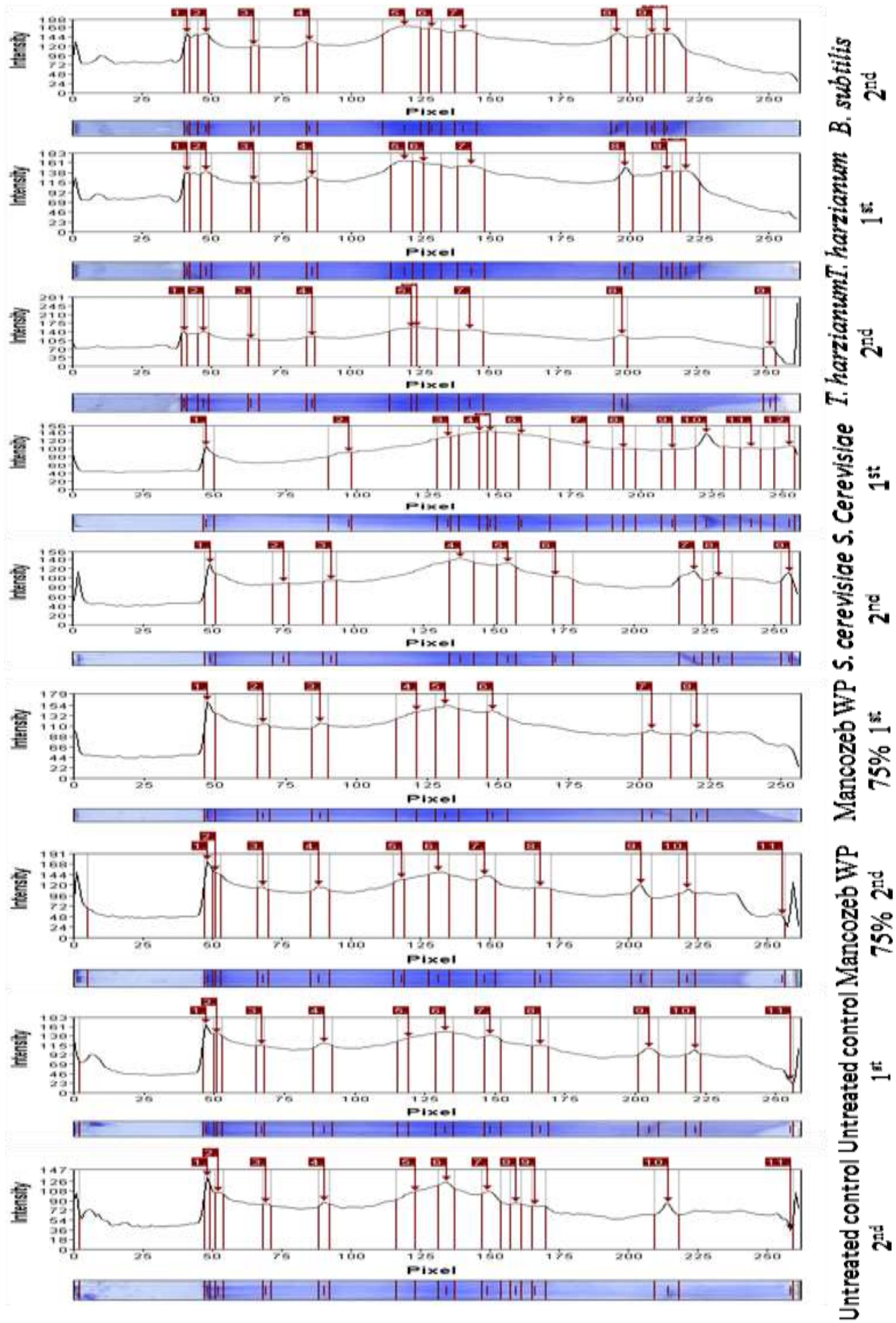

Figure 4. SDS-PAGE electrophoretic gram analysis using Syngene™ Ingenius 3 Gel Documentation System software after both foliar spraying of some abiotic and biotic agents on protein pattern of faba bean plants against rust and chocolate spot infections.

Furthermore, oils of geranium, lemon, mint and rosa prohibited the mycelial growth of *F. oxysporum* f. sp. *phaseoli* and *Rhizoctonia solani* (El-Mougy et al., 2007). A concentration of 2% lemongrass, black seed, thyme and grape essential oils completely inhibited the fungal growth of *R. solani*, *Sclerotium rolfsii* and *F. solani* (El-Mougy et al., 2017). Moreover, using of various fungal and bacterial strains motivated the growth of plants and being utilized as prospect antagonist to challenge many fungal and bacterial invasions (Bendahmane et al., 2012). *Bacillus* spp. was stated to contain various compounds implicated in biocontrol of plant pathogens (Miljaković et al., 2020).

The effects of leaf pathogens can be minimized using biological manipulation with *Trichoderma* (Williamson et al., 2007) and *Saccharomyces* (Iurkiv et al., 2020). Also, *T. harzianum* and some antioxidant agents reduced chocolate spot disease incidence compared to the fungicide Diathane M45 during two successive growing seasons under field conditions (El-Mougy and Abdel-Kader, 2018b). Recently, soil treatment with plant growth-promoting bacteria (PGPB) or mycorrhizae followed by foliar spray of wheat plants with either *T. harzianum* or *Pseudomonas fluorescens* showed announced reduction in severity of foliar diseases like powdery mildew, septoria leaf blotch and stem rust (Mukhtar, 2018; Iabal and Mukhtar, 2020; Azeem et al., 2021; Mukhtar et al., 2021; Abdel-Kader et al., 2023). In addition, our results showed that chitosan foliar spray could reduce chocolate spot and rust diseases incidence down to 13.3 and 8.3%, respectively. In this regards, it was reported that chitosan is well-known as biocontrol agent against plant pathogens. Chitosan has potential in agriculture with regard to controlling plant diseases. It could inhibit fungal growth and development. Chitosan was reported to be active against viruses, bacteria and other pests and exhibits a variety of antimicrobial activities and seems to be faster on fungi and algae than on bacteria (El hadrami et al., 2010). Furthermore, the effect of chitosan as an environmental friendly plant disease control indicated that chitosan has a positive influence on cucumber plant fruit and leaf disease (Sofian et al., 2022).

Plants express several proteins and various enzymes in response to pathogen infection due to their significant functions for plant defense processes. The protein functions include repairing and supporting cell walls, modifying characteristics of the extracellular matrix and synthesizing varied antimicrobial agents (Souza et al., 2017). Plant antioxidant enzymes like CAT and POD perform a significant role in plant defenses and create complex mechanisms for alleviating, neutralizing and scavenging the reactive oxygen species (ROS) (Matamoros et al., 2010; Racchi, 2013; Wu et al., 2014). The plant CHIA is one of the pathogenesis-related proteins which are greatly expressed during infections for performing critical parts versus fungal pathogens (Vaghela et al., 2022). In present study, the 2nd foliar treatment of *B. subtilis*, *T. harzianum*, black seed oil, jojoba oil and chitosan increased faba bean protein content rather than the 1st application compared to other treatments (lemon grass, thyme, grape, bitter orange, moringa oils and *S. cerevisiae*). These treatments act as biosensors for the endogenous salicylic acid synthesis pathway that increased up to 180-fold level. This pathway expresses the mRNA which aids the synthesis of PRPs against infection leading to increasing the plant total protein content (El-Dougdoug et al., 2013). The foliar spray individually with chitosan and grape oils enhanced the highest CAT specific activity 1874.4 and 1470.8 U/g FWt after 1st and 2nd time respectively. *S. cerevisiae* and lemon grass oil induced the highest POD specific activity (383.8 and 333.3 U/g FWt) in both spray times. Black seed oil induced the highest CHIA specific activity (82.2 U/g FWt), after the 1st foliar application while lemon grass oil enhanced the highest level (63.6 U/g FWt), after the 2nd foliar application compared with untreated control. The 2nd foliar application of moringa oil, black seed oil, jojoba oil, chitosan, *B. subtilis* and *S. cerevisiae* stimulated the formation of new protein bands in treated faba bean plants through the fractionation on 12% SDS-PAGE that were not found after the 1st one, while the 2nd application by grape, thyme and jojoba oils and *T. harzianum* caused lysis of some proteins that formed after 1st time. The formation of new protein bands on the gel, and their subsequent disappearance, can be explained on the basis that, under stress and infection conditions, the plant adapted its growth through the formation of either structural or protective proteins necessary for protection. On disappearance of these conditions, the plant proteases break down these proteins after it is no longer needed (Masoud et al., 2023).

CONCLUSION

The foliar spray containing jojoba, grape, and bitter orange oils greatly reduced the incidence of faba bean rust. On the other hand, the biotic inducers *B. subtilis*, *T. harzianum*, and *S. cerevisiae*, in addition to bitter orange, thyme, black seed, and jojoba oils, strongly reduced the occurrence of chocolate spot disease. These treatments induced the formation of new proteins and/or increased the specific activities of defense enzymes. Consequently, such treatments improve the ability of *V. faba* to resist rust and chocolate spot diseases. Therefore, it is recommended to apply these treatments repeatedly during the faba bean growing season to enhance the plant's disease resistance and protect it until the end of the cultivation season. Thus, they can be considered as effective and environmentally friendly alternatives for sustainable disease resistance, reducing the harmful effects of fungicides.

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AUTHORS' CONTRIBUTIONS
All authors contributed to the experimental design, hands on work, discussions, and commented on the manuscript.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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