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Suppression of Powdery Mildew Disease (*Erysiphe betae*) on Sugar Beet by using Some Natural Substances and Essential Oils

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THE OBTAINED results from evaluation of the response of 25 sugar beet cultivars (100 days old) to the disease, explaining that, the disease severity of powdery mildew was high in the first tested season, while, it was low in the second season. The information of climatic conditions showed that, high differences in maximum and minimum temperature centigrade and relative humidity (RH) % between two tested seasons during the months, which were the disease development within them in (December, January, February and March),), therefore, the maximum and minimum temperature centigrade were high and the relative humidity % was low and suitable for disease distribution during first season comparing with the second season. Overall, the obtained results indicated that, FD17B4010, MK4199 and Alanya-KWS cultivars, were recorded the least disease severity % (High resistant) against *Erysiphe betae* infection and can be recommended in recent cultivation. In the contrast, Hammond, Dipendra-KWS, Frappina-KWS, SV-2173 and Carma cultivars, were the most susceptible cultivars (recorded the highest disease severity %) which can be recommended eliminated and not be planted under Egyptian climatic conditions. The highest efficacy treatments were the spraying with Frankincense at 2500 ppm and essential oils of Pelargonium *graveolens* at 10ml/L. While. Chitosan nanoparticles at 500ppm and essential oil of *Cymbopogon nardus* at 10ml/L followed these treatments in their efficacy, and all tested treatments were similar/ or near to Tobas fungicide application. These tested treatments are less expensive, eco-friendly and may replace recommended tested fungicides totally or partially. Therefore, spraying of these treatments significantly reduced the powdery mildew disease severity, increased the size of sugar beet roots as well as increased the yield per feddan. Moreover, the efficacy role(s) of these tested compounds may be resulted from the enzymatic antioxidant activation mechanism which reacted by increasing enzymatic activities of PPO and POX and / or consequently the alternative of PH values of some biosynthesis. Results obtained from scanning electron microscope (SEM) of the sprayed samples with all tested treatments indicated reducing in mycelium density and formation of the pathogen structures and their unable to produce either of conidia and/or conidiophores of tested fungus (*E. betae*) and finally, plasmolysis of pathogen cells and their structures on the leaves of plants. Indeed, it is very suitable for applications where the tested treatments in this study to minimize or prevent the chemical fungicides that were contaminated the environment and harmful to public health.

Keywords: *Erysiphe betae*, Scanning electron microscope, *Pelargonium graveolens*, *Cymbopogon nardus*, Chitosan nanoparticles and Frankincense.

1. Introduction

Sugar beet (*Beta vulgaris* L.) is the most important sugar crop worldwide and it can be grown in different climatic conditions. In Egypt, sugar beet is the second crop for production of sugar after sugar cane (Eweis *et al*., 2006). The *E. betae* pathogen on sugar beet foliage causes damages in yield of roots which negatively affected on the total crop production. The disease spread and severity is largely dependent on climatic conditions during the planting year. Climatic conditions are playing important role on the spread and growth of this disease, as the distribution of this disease is enhanced in humidity

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ranges from 40 to60% accompanied by a moderate temperature 22 to 32 °C (Draycott, 2006 and Kontradowitz and Verreet, 2010). The temperature ranged from 15°-30°C, and under conditions at low relative humidity is very suitable for infection and colony, (El-Fahar and Abou El-Magd 2008; Gado 2013; Neher and Gallian 2013) they also added that, under high disease severity by *E. beata*, production of root yield is decreased down to 22% as well as sucrose percent in the roots reduced to 13%, as well as deteriorating yield quantity and quality.

Using some safe alternative methods to control some diseases has become quite important. Therefore, the effect of chitosan nanoparticles, Frankincense (boswellic acids) and some essential oils on reduce severity of some plant diseases and their mechanisms in disease restricted remain important (Hamidpour *et al*., 2013, Mark et al., 2019, María et al., 2022; Elsharkawy *et al*., 2022). El-Kady and El-Mansoub (2020) revealed that some growth regulators which tested decreased severity (%) of disease and enhanced growth, yield of sugar beet crop compared to untreated (control). In recent years, chitosan and its derivatives have attracted much attention as antimicrobial agents against fungi, bacteria, and viruses and as elicitors of plant defense mechanisms (Chirkov 2002 and Rabea *et al*., 2003). found that, chitosan decreased development of powdery mildew disease on sugar beet from 43.5% to 77% Efficacy of chitosan enhanced with increased level of its concentration, Takai *et al.,* (2002). Raafat *et al*, (2008) found that chitosan action started from teichoic acids and potential extraction of membrane lipids (predominantly lipoteichoic acid), leading finally to bacterial death. Ali and Joshi (2011) and Avelelas *et al.,* (2019), Malerba and Cerana (2016) demonstrated that, changing in size and surface charge of chitosan nanoparticles resulted in significant variation in their antimicrobial activity. On the other hand, Hamidpour *et al.,* (2013) found that, Frankincense (boswellic acids) have antimicrobial activities against many microorganisms such as gram positive and gram-negative bacterial strains as well as fungi. Ismail *et al*. (2014) reported that, extracts of frankincense showed high antimicrobial activity as compared with control. Liu *et al.* (2016) found that, Frankincense oil treatment led to mycelium growth inhibition and spore germination of many pathogens. Mohamed *et al.* (2021) showed that, total bacteria, total fungi and E. coli were linearly reduced in case of supplemented Frankincense compared with control. El-Nagar *et al,* (2022) cleared that, frankincense at 3 g/L level showed good effect on restricting of chocolate spot disease. Vita *et al.* (2020) showed that, the frankincense has great activity against all reference strains of tested bacteria and fungi. Khalid *et al.* (2018) found that, all tested essential oils treatments decreased the severity of powdery mildew disease, and significantly increased also total phenols, total soluble solids and sucrose percentages. Elsharkawy *et al.,* (2022) suggested that chitosan nanoparticles might be an eco-friendly strategy to enhance growth and restrict leaf rust disease.

In recent years, the use of medicinal plants due to their ability to be natural molecules could be devoid of side effects and also can be used as the lowest cost. Among the many types of these substances which include volatile substances or essential oils (Balahbib *et al.* 2020). Therefore, Rozwalka *et al*. (2010) found that conidia of some fungi were damaged after being treated with essential oil and led to decreasing of conidia germinate. Moreover, Elouadi *et al*. (2022) found that, all essential oils which were tested reduced the incidence of powdery mildew disease on Zinnia elegans. Fatouh *et al*. (2011) found that, the increase in sugar beet yield was the highest with spraying by Citronella essential oil at 5.0 ml/l., which increase the yield of sugar beet more than 11.5 %. This treatment increased also the percent of total soluble solids roots by 6.7% while, TSS was slightly increased with Citronella at 2.5 ml/l (Desoky *et al*. 2020; El-Saadony *et al.* 2021). Pereira *et al*. (2012) found that, essential oil of citronella activates the plants defense system and making controls brown eye spot in coffee plant, In the same item, Wen-Ru et al. (2013) demonstrated that, treatment with citronella oil destroyed and damaged the cell wall of *A. niger* hyphae. Chen *et al*. (2014) showed that, essential plant oil citronella inhibited growth of *A. alternata in vitro* and *in vivo*, scanning electron microscopy examination revealed abnormal mycelial morphology. Hussein *et al.* (2015) illustrated that, the extracted essential oils from Cymbopogon genus and their chemical components are known to have antifungal effect against a wide range of importance plant pathogenic fungi species. Moreover, Ebrahim and Helmy (2016) found that, the application of essential oils resulted in high content of phenols, sugar, protein, and antioxidant enzymes (Da Cruz *et al*., 2015; Dela Cueva and Balendres 2018) added that, the essential oil from *C. winterianus* plants has an inhibitory effect on growth of *F. solani* and *C. acutatum* pathogens, because it has direct toxic action to conidia of tested fungi. Ghazi *et al.,* (2018) they reported that, charcoal rot disease severity was decreased as a result of essential oil treatments compared with the control treatment. Elouadi *et al.* (2022) and Mangalagiri *et al*. (2021) summarized that, *Pelargonium graveolens* essential oil are very suitable antifungal against *Botrytis cinerea*, and other tested fungi. Therefore, essential oils are characterized by their low toxicity, biodegradability, and they do not persist in the environment and can be recommended as alternatives to synthetic fungicides to protect some plants from phytopathogenic fungi (Hafez *et al*, 2021; Abdelrasheed *et al.* 2021) and some other biotic challenges such as viral pathogens (Abdelkhalek *et al.*, .2020) bacterial (Nehela, and Killiny 2020) and fungal (Nehela *et al.* 2021; Atallah *et al.*, 2022) phytopathogens. The important changes in reaction of many plants to diseases are via induction of enzymatic defense machinery (Nehela *et al*. 2021; Abdelrhim *et al.*, 2021; El-Nagar *et al*., 2022).

Enzymatic defense machinery resulted from increased some enzyme activity such as POX, PPO and other enzymes. The effect of studied substances on the activity of some enzyme's oxidation, and its action which related by enhancing plants to resist pathogens was evaluated (Hafez *et al,* 2021 and Sehsah *et al*., 2022).

The scanning electron microscope test has been obtained by many studies, including Jackowiak *et al*., (2005) and Sehsah *et al.*, (2022), to test the effect of application of some substances on the growth of pathogens and their structures. Scanning electron microscope (SEM) test of leaves samples treated with considerable plant extracts showed that, the fungal structures were reduced and couldn't be able to produce both of conidia and conidiophores structures. El-Nogoumy *et al.*, (2022) showed that, scanning electron microscope test of leaves, which treated can be summarized in the reducing of fungal hyphae, conidiophores, conidia, and plasmolysis were found in fungal cells and spores on tested leaves. In this study, we are looking for find efficient and eco-friendly alternatives that may replace fungicides for controlling the tested disease. In addition to understanding the mechanism(s) of tested treatments on pathogen inhibition and its structures on leaves treated with tested essential oils and natural substances, which will be examined by scanning electron microscopy as compared with untreated ones.

2- Materials and Methods

1-Evaluation of some sugar beet cultivars against powdery mildew disease under field conditions

Twenty-five sugar beet cultivars (namely Smart Djrba KWS, Smart Jell KWS, SV2173,9k887, Indira-KWS, Gregoria-KWS, Allanya- KWS, B 8141, Carma, Posfidon, Melooia, Fantazia, LP 17b4011, FD17B4010, FD18B4018, MK 4199, MK 4200, Hammond, Vangeus, SHRB21802, Pintea, Zeppeun, SI21801, Dipendra-KWS and Frappina-KWS) were utilized in this investigation for infection with powdery mildew disease. The disease severity percentage was documented as recorded by **McGrath *et al.*, (1996)** after 100 days from sowing.

2-Evaluation of some tested treatments against powdery mildew disease in field.

The experiment was performed at Sakha Research Farm, Sakha Agricultural Research Station during 2020/ 2021 and 2021/ 2022 successive seasons. The susceptible variety 'Pleno' was used in these experiments, which designed in a complete block randomized design with 3 replicates, each treatment contained six replicates

3-Essential oils extraction and oil constituents

Fresh herbs of geranium, *Pelargonium graveolens*, Family Geraniaceae and citronella grass *Cymbopogon nardus* Poaceae Family were obtained from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Center (ARC), Egyptian Ministry of Agriculture and Land reclamation. These plants were selected on the basis of previous knowledge of their antifungal potentials (**Chen *et al*., 2014; Dela Cueva and Balendres, 2018; Džamić *et al*., 2014; and Moutaouafiq *et al*., 2019)**. Citronella grass and geranium essential oils were extracted by hydro distillation using a Clevenger type apparatus for 3 hours. The extracted oils were dried in anhydrous sodium sulfate then stored at 4 ^{0}C in tightly sealed dark-glass bottles. Chromatographic analysis (GC) was determined for geranium and citronella grass essential oils at Medicinal and Aromatic Plants Research Department Lab., Horticulture Research Institute, (ARC) Giza, Egypt which were investigated by DsChrom 6200 Gas Chromatograph prepared with a flame ionization detector for separation of volatile oil constituents. The analysis circumstances were as follows: The chromatograph apparatus was connected with capillary column BPX-5, 5% phenyl (equiv.)

polysillphenylene-siloxane 30 m x 0.25mmID x 0.25 µm film. Temperature program ramp increases at a rate of 10 Cº / min from 70 to 200 Cº. Flow rates for gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. The temperature of the detector and injector were 300 Cº and 250 Cº, respectively. The acquired chromatogram and report of GC analysis were investigated to calculate the percentage of essential oils constituents.

Both extracted essential oils were used at 10ml/L. Frankincense (boswellic acids) was used at 2500 ppm and Chitosan nanoparticles at 500ppm (were obtained from Elgamhoria co.), Fungicide Topas 100, EC, from Syngenta Co. was used at recommended dose, 1 mL/ L and control treatment. Each treatment contained two rows 4 M. length and 60 cm distance. Each row is planted with 20 hills at 20 cm between them. All agricultural practices were applied as recommended. Treatments were sprayed three times, with ten-days in between, the control treatment was sprayed with only water. The first spray was performed after 90 days from sowing date when disease symptoms started to be detected. Disease severity was recorded after 20 days from the third spray and rated on the 0-4 scale of **McGrath *et al.*, (1996)**, where 0: no powdery mildew symptoms present; 1: 1-25%; 2:26-50%; 3: 51-75%; 4:76-100% of leaf area covered by powdery mildew and average disease rating at each treatment was estimated. Efficiency percentages were calculated according to the following formula: Efficiency percentage = Control- Treatment /Control ×100. At harvest, TSS % and sucrose % were evaluated in fresh roots using a sacarometer and refrectometer according to **AOAC (2000)** and **McGinnis (1982),** respectively. Purity % was also estimated according this formula: Purity % = Sucrose % / TSS % x 100. Yield /kg for 5 root /plot were also estimated.

3- Scanning Electron Microscopy (SEM) Test

The reactions of used tested application on conidia, conidiophore and spore formations, as well as the development of *E. betae* on leaves of sugar beet, were estimated using scanning electron microscopy (SEM) test as formerly described by Manzali *et al.* (1993). Interaction sites (spots) were done and discs of 1 cm^2 were taken for SEM test using Jeol model JSM-5500lv (Tokyo, Japan) in the Electron Microscope Unit, Nanotechnology Institute, Kafrelsheikh University. Samples, illustrating the interaction region, were appointed with osmium oxide, and then dehydrated, using a serial dilution of ethyl alcohol and then finally acetone. Processed samples were then dried, using a critical point drier, coated with gold using a sputter coater, then the samples were investigated.

4-Determination of polyphenol oxidase and peroxidase enzymes activity

Extraction of enzyme and assay: 24 h after application, leaf samples were taken from each treatment, either healthy and/or infected, were taken for assay activity of peroxidase and polyphenol oxidase enzyme. Leaves tissue was grounded in a mortar in 0.1 M sodium phosphate buffer at pH 7.1 to enzyme extracts. 4 layers of cheese cloth were used to strain the retrieved tissues. The filtrates were centrifuged at 3000 rpm and at 20 min. The considered crude enzyme extract were collected from clear supernatants. The oxidation of pyrogallol into pyrogalline in available hydrogen peroxide was done to test peroxidase activity, as stated by **Allam *et al.*, (1972).** Absorbance at 425 nm changes were calculated every one min for up to four min to estimate the activity of peroxidase. Also, the polyphenol oxidase (PPO) activity was spectrophotometrically estimated at 495 nm after five minutes using the same spectrophotometer recommended by **Maxwell and Batman (1967)**. .

5-The used method to calculate the disease severity %:

Powdery mildew rate from 0 to 4 as recorded by McGrath *et al*., (1996), was used to estimate the severity of disease, where R0 = no powdery mildew colony, R1 = 1 - 25%, R2 = 26-50%, R3 = 51-75%, and R4 = 76-100% colonies/leaf, which covered by powdery mildew and disease rating at each treatment was estimated. The severity of disease was estimated according to the following equation:

severity of disease (%) = {Σ (rating no.) x (no. leaves were found in rating category) x(100)} / (Total no. of leaves)x (highest rating value).

The treatment efficiency percentage in for the reduction severity of powdery mildew was calculated according to the last equation.

6- Statistical analyses

Data were statistically analysed by WASP1 which was developed by Ashok Kumar Jangam and Pranjali Thali at ICAR Research Complex for Goa, India program, the means and analysis of variance were further estimated using the least significant difference test (LSD) at 5%, according Snedecor and Cochran (1980).

Results and Discussion

Gas chromatographic analysis of *Pelargonium graveolens* essential oil revealed the presence of 24 components of which 10 components of them account for (87.9%) of the essential oil which were identified by the retention times obtained from pure reference compounds. The major components identified were citronellol (28.42%), geraniol (21.59%), linalool (9.84%), citronellyl formate (7.22%), eugenol (6.63%) and geranyl formate (5.63) these results were in accordance with (Džamić *et al*., 2014 and Moutaouafiq *et al*., 2019). Additionally, gas chromatographic analysis of *C. nardus* (L.) revealed the presence of 14 components of which 9 components of them accounting for (93.28%) of the essential oil were identified and the citronella essential oil was mainly composed of citronellal (27.64%), geraniol (24.51%), myrcene (20.77%), linalool (4.96%) and citronellol (4.70%) which was in accordance with formerly published studies **(Chen *et al.*, 2014)**.

Data obtained in Figure 1 clearly displayed that, the disease severity % recorded high values during season 2020, compared with season 2021. On the other hand, and within the two tested seasons, the lowest disease severity was recorded with FD17B4010, MK4199 and Alanya-KWS cultivars, while, the highest disease severity, were recoded with Carma, Dipendra-KWS, Frappina-KWS, SV-2173 and Hammond cultivars, and in the same direction, the moderate disease severity during two tested seasons, were recorded with B8141, MK 4200 and pintea cultivars. On the other hand, the rest of the tested sugar beet cultivars obtained high variances in disease severity % between two tested seasons. The differences in reaction of tested sugar beet cultivars in disease severity % by tested disease during two tested seasons, directed to study effect of different in climatic conditions during the tested seasons on disease severity % of powdery mildew disease. Data obtained in Table 2 cleared that, high differences in maximum and minimum temperature centigrade between two tested seasons during the months, which were the disease development within them (December, January, February and March), therefore, the maximum temperature centigrade ranged from 22.88 to 21.08 and from 19.85 to 17.72 centigrade, while the minimum temperature centigrade ranged from 12.08 to 10.56 and from 10.87 to 7.06 centigrade during 2020/2021 and 2021/2022 growing seasons respectively. The obtained results also indicated that, the lowest maximum and minimum temperature centigrade (19.85 and 7.06 respectively) during the months, which the disease development within them in season 2020/2021 were quite important for increasing disease severity % by *E. betae* fungus causal agent of powdery mildew disease of sugar beet. On the other hand, the low relative humidity % during first season (63.83 %) was considerable and suitable for distribution and enhancement of infection by tested disease, while, the reverse was true in the case of second season (Table 1).

Table 1. Maximum, minimum temperatures centigrade, and relative humidity% during disease development during two successive seasons.

Years	Months	Maximum temperature, C°	Minimum temperature, C°	Relative humidity (%)
2020/ 2021	December 2020	22.88	12.08	69.44
	January 2021	21.68	10.98	70.25
	February 2021	21.08	10.56	57.28
	March 2021	22.78	10.73	58.38
	The mean	22.105	11.87	63.83
2021/2022	December 2021	19.85	10.87	74.50
	January 2022	17.72	7.06	70.81
	February 2022	18.88	8.62	76.38
	March 2022	19.63	7.16	69.44
	The mean	19.02	8.427	70.032

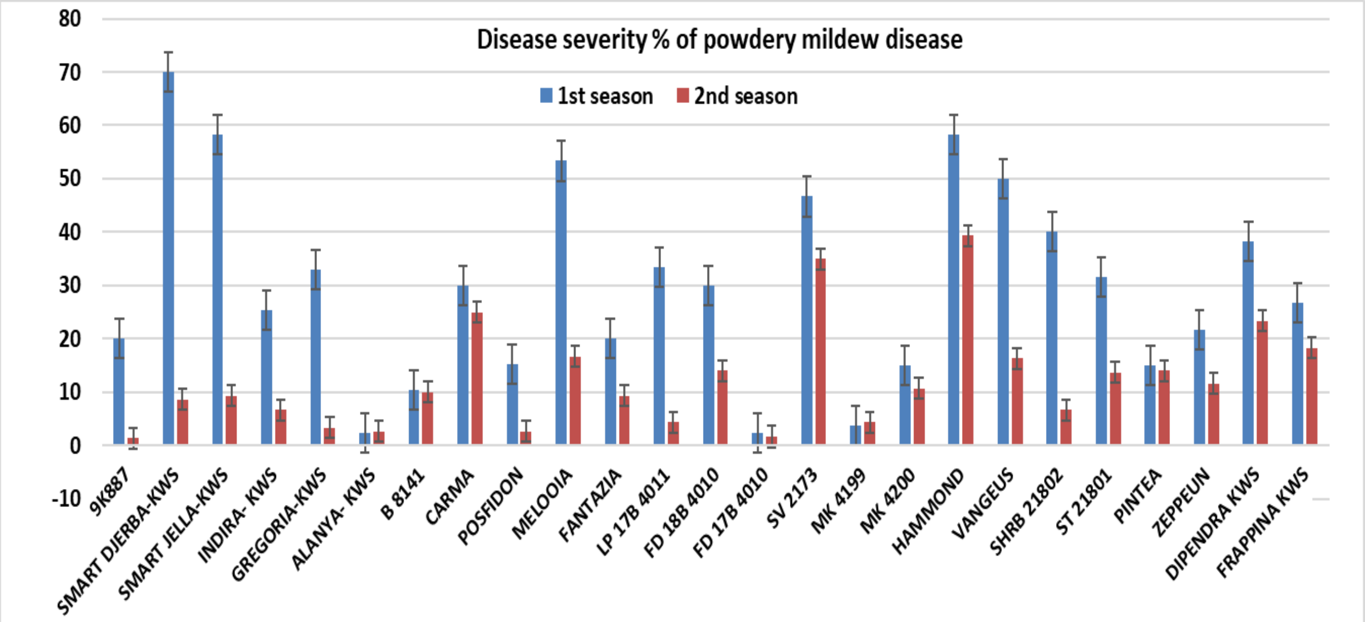

Fig. 1. Disease severity% of powdery mildew disease on 25 sugar beet cultivars in two tested seasons.

Initially, the susceptibility of 25 sugar beet cultivars were tested under the natural infection of the pathogen. These cultivars reported wide differences in their reaction to the tested disease. These results are in the same direction with many previous research which was carried out on the selected disease, which were found a wide difference in the reaction of sugar beet cultivars to tested disease that was due to the genetic variation between those cultivars. Therefore, **El-Nogoumy *et al.*, (2022)** demonstrated that, the cultivars, which were tested against sugar beet powdery mildew disease were classified as follows: 1- 10 cultivars were least susceptible, 2- 10 cultivars detected as susceptible to the disease, and 3- contained six cultivars that had higher disease severity and were recorded as highly susceptible to tested seasons. The differences in reaction of tested sugar beet cultivars in disease severity % of tested disease during seasons 2020/2021 and 2021/2022, directed to study the effect of different cultivars in climatic conditions during the two cultivated seasons on disease severity % of tested disease. The obtained results indicated that, the low maximum and minimum temperature centigrade during the months which the disease development within them in season 2020/2021 were quite important for increasing disease severity % by *E. betae* causal agent of powdery mildew disease of sugar beet. Overall, low relative humidity % during first season was considerable and suitable for distribution and enhancement of infection by tested disease, while, the reverse was found in case of second season (Table 1). These results are in agreement with findings obtained by **Kontradowitz and Verreet, 2010**, they found that, disease severity is dependent on climatic condition during planting season. Climatic conditions are playing a vital importance role on the spread of this disease, as the distribution of this disease is enhanced in rate of humidity from 40 to 60% with moderate temperature ranging from 22 to 32 °C**. Draycott, 2006** added that, the temperature ranges from 15°-30°C, and low relative humidity is very suitable for infection and colony. Therefore, the control of disease is achieved by considerable applications of increasing plant spacing, planting tolerant sugar beet cultivars, and avoiding increasing the dose of nitrogen and irrigation **(Gado 2013).**

Efficacy of spraying by tested treatments against powdery mildew disease in sugar beet in the field conditions

The effectiveness of application by foliar used substances including the essential plant oils *Pelargonium graveolens* at 10ml/L and *Cymbopogon nardus* at 10 ml/L and natural substances of Frankincense at 2500 ppm and Chitosan nanoparticles at 500ppm, fungicide Topas 100, EC 1 mL/L, or sprayed with water (control) against tested disease in sugar beet was estimated in field conditions. In general, all treatments, which tested were significantly reduced the severity percent of powdery mildew disease as compared with control during both tested seasons (Table 2 &3). The most efficient treatments in controlling powdery mildew disease were the foliar application of Frankincense at 2500 ppm and essential oil of *Pelargonium graveolens* at 10ml/L in both tested seasons (Table 2&3), followed by plants sprayed with essential oil of *Cymbopogon nardus* at 10 ml/L and Chitosan nanoparticles at 500 ppm and all of them were slightly lower than Topas 100 fungicide treatment. Results showed that foliar spraying by Frankincense at 2500 ppm and essential oil of *Pelargonium graveolens* at 10ml/L significantly decreased the percent severity of powdery mildew disease on sugar beet, it recorded 10.33 and 11.66 % respectively, compared with 29.33 % in control treatment, under 1st spray during season 2020/2021 followed by plants sprayed with essential oil of *Cymbopogon nardus* at 10ml/L and Chitosan nanoparticles at 500ppm, which recorded 17.00 and 14.66 % respectively (Table2). The same direction was observed in the case of 2nd spray and 3rd spray in both tested seasons (Table 2 and 3). Results summarized that foliar spraying by Frankincense at 2500 ppm and *Pelargonium graveolens* at 10ml/L significantly decreased the percent of disease severity of tested disease on sugar beet, compared with control under 1st spray during season 2020/ 2021, followed by plants sprayed with essential oil of *Cymbopogon nardus* at 10ml/L and Chitosan nanoparticles at 500ppm. Previous researchers were in the same direction as follows**:** **Ali and Joshi (2011) and Avelelas *et al.*, (2019)** demonstrated that, changing in size and surface charge of chitosan nanoparticles led to significant differences in their antimicrobial activity. The chitosan products were characterized by their antioxidant, metal chelation, and antifungal properties. **Malerba and Cerana (2016)** added that, chitosan application enhances the immunity of plants to be more tolerant to many of soil and foliar pathogens as well as induces root nodulation.

Table 2. Disease severity% of disease and efficacy % of tested substances against *Erysiphe betae* on field conditions during 2020/2021 season.

Treatments	**1st spray**		**2nd spray**		**3rd spray**	
	Disease severity (%)	Efficiency (%)	Disease severity (%)	Efficiency (%)	Disease severity (%)	Efficiency (%)
Pelargonium graveolens at 10ml/L	11.66d	60.24c	14.33d	70.55c	15.66d	77.41c
Cymbopogon nardus at 10ml/L	17.00b	42.03e	18.66b	61.65e	21.66b	68.75e
Frankincense at 2500 ppm	10.33e	64.78b	11.66e	76.03b	13.33e	80.77b
Chitosan nanoparticles at 500ppm	14.66c	50.02d	15.33c	68.49d	18.66c	73.08d
Fungicide Topas 100, EC, at 1 mL/ L	6.66f	77.29a	8.00f	83.55a	9.66f	86.06a
control	29.33a	0.00f	48.66a	0.00f	69.33a	0.00f
`LSD	0.455	0.224	0.455	0.527	0.310	0.642

Means followed by a common letter (s) are not significantly different at the 5% level by DMRT

On the other hand, **Hamidpour *et al.*, (2013)** found that, Frankincense (boswellic acids) have antimicrobial activities against many microorganisms such as gram-positive and gram-negative bacterial strains and fungi. **Ismail *et al*. (2014) and Elsayed *et al*., (2023)** found that, extracts of frankincense showed high antimicrobial activity as compared with untreated plants and it can be used in restriction of many bacterial diseases caused by many bacterial strains. **Mohamed *et al.*, (2021)** showed that, total bacteria, total fungi and *E. coli* were linearly reduced in case of supplemented Frankincense compared with control. **El-Nagar *et al.*, (2022)** showed that, frankincense at 3g/L resulted high effect on restricted severity % of chocolate spot disease, and also increasing germination percentage as well as increased yield per plot, weight of 100 seeds, protein, total chlorophyll, and significantly increased polyphenol oxidase and peroxidase enzymes activity. **Vita *et al.*, (2020)** showed that, the frankincense has great activity against all reference strains of tested bacteria and fungi. **Khalid *et al*., (2018)** studied the efficacy of foliar application of chitosan, ascorbic acid and salicylic acid to induce systemic resistance against powdery mildew disease, and found that, all tested applications decreased the disease severity. Moreover**, Ebrahim and Helmy (2016)** noted that, the application of essential plant oils from neem and soybean as resistance inducers in sugar beet plant against powdery mildew disease and prevented the disease incidence. while, **Da Cruz *et al*., 2015,** and **Dela Cueva & Balendres, 2018** added that, the essential oil from *C. winterianus* plants inhibited on the growth of *F. solani* and *C. acutatum* pathogens, because it has direct toxic action against the conidia of tested fungi.

Table 3. Disease severity% and efficacy % of tested treatments against *E. betae* on field conditions during 2021/2022 season.

treatments	**1st spray** **Disease severity** (%)	**Efficiency** (%)	**2nd spray** **Disease severity** (%)	**Efficiency** (%)	**3rd spray** **Disease severity** (%)	**Efficiency** (%)
Pelargonium graveolens at 10ml/L	8.66cd	53.59c	11.33c	61.80c	13.00c	70.22c
Cymbopogon nardus	10.66b	42.87e	12.33b	58.42d	15.33b	64.88e
Frankincense at 2500 ppm	7.33d	60.71b	9.33c	68.54b	11.00d	74.80b
Chitosan nanoparticles at 500ppm	10.00bc	46.40d	12.66b	57.31e	14.66b	66.42d
Fungicide Topas 100, EC, at 1 mL/ L	3.66e	80.38a	4.66e	84.28a	6.33e	85.50a
Control	18.66a	0.00f	29.66a	0.00f	43.66a	0.00f
LSD	1.356	0.556	0.984	0.485	1.358	0.618

Means followed by a common letter (s) are not significantly different at the 5% level by DMRT

While, **Ghazi *et al.* (2018)** indicated that, charcoal rot severity of disease was decreased as a result of essential oils substances compared with the control treatment. The application of tested essential plant oils not only reduced the fungal infection but also increased plant figure and growth parameters. **Elouadi *et al*., (2022) and Mangalagiri *et al*., (2021)** summarized that, *Pelargonium graveolens* essential oil is very suitable antifungal treatment against *Botrytis cinerea*, and other tested fungi. Therefore, essential oils are characterized by their low toxicity, biodegradability, and protect plants from phytopathogenic fungi.

Effect of treatments on percent of (TSS) and sucrose in sugar beet roots

Percent of total soluble solids and sucrose content were estimated in treated fresh root of sugar beet. The role of tested applications on the economic value and quality of sugar beet roots, (tables 4 & 5). Percent of total soluble solid was significantly enhanced in all applied treatments which tested comparing by control, with the superiority Frankincense at 2500 ppm and essential plant oil *Pelargonium graveolens* at 10ml/L during the 2020/2021 and 2021/2022 seasons compared with control treatment (Table 4 & 5). Frankincense at 2500 ppm and essential oil of *Pelargonium graveolens* at 10ml/L showed the highest sucrose percentage, was as recorded 19.89 and 18.92% in the first season and 19.93 and 18.66 in the second season, respectively. While, TSS% values ranged from 22.56 to 23.87 % compared with 21.45 % in control treatment during the first season of 2020/2021, and ranged from 22.89 to 25.65 % compared with 21.75 % in control during the second season of 2021/2022. On the other hand, the obtained results in Table 4 showed that, the highest yield per five roots were obtained under all tested treatments (ranged from 4.470 to 5.430 kg**)** compared with control treatment (4.334 kg), with the superiority of Frankincense at 2500 ppm and essential oil of *Pelargonium graveolens* at 10ml/L treatments. The same trend was obtained during the second season of 2021/2022 (Table 5)

Table 4. TSS (%), sucrose (%), purity (%) and yield/5 roots, under tested treatments during 2020/2021 season.

Treatments	**TSS (%)**	**Sucrose (%)**	**Purity (%)**	**Yield/5 roots kg.**
Pelargonium graveolens at 10ml/L	23.87b	18.92b	79.26a	4.850ab
Cymbopogon nardus at 10ml/L	22.56d	17.65c	78.23bc	4.787ab
Frankincense at 2500 ppm	25.23a	19.89a	78.83ab	5.430a
Chitosan nanoparticles at 500ppm	23.51bc	18.11bc	77.03d	4.470b
Fungicide Topas 100, EC, at 1 mL/ L	23.18c	17.99c	77.61cd	4.535b
Control	21.45e	16.69d	77.80c	4.334b
LSD 0.05	0.502	0.917	0.756	0.656

Means followed by a common letter (s) are not significantly different at the 5% level by DMRT.

The obtained results illustrated that, total soluble solid percentage was enhanced in all treatments which tested compared with control, with the superiority of Frankincense at 2500 ppm and essential oil of *Pelargonium graveolens* at 10ml/L in each tested season. These results agree with findings of Fatouh et al., (2011) who found that, the increase in sugar beet yield was highest with spraying with Citronella essential oil at 5.0 ml/l. which enhanced the sugar beet yield. In the same trend, Ebrahim, and Helmy **(2016**) noted that, the application of essential oils from neem and soybean and salicylic acid (SA) as inducers for resistance in sugar beet plant resulted in high content of sugars, phenols, protein, and antioxidant enzymes. Khalid et al., (2018) added that foliar treatment with chitosan, and/or salicylic acid significantly increased each of total phenols, total soluble solids and sucrose %. El-Nagar et al., (2022) also found that spraying with frankincense at 3 g/L, resulted in highest yield per plot (kg) production, weight of 100 seeds, protein, and total chlorophyll El-Khateeb and Ketta (2019). El-Nogoumy et al., (2022) illustrated that, treatments of sugar beet by some essential oils and plant extracts against powdery mildew, enhanced of sucrose and total soluble solids %, as well as enhanced also the activity of peroxidase and polyphenol oxidase enzymes.

Table 5. TSS%, sucrose %, purity % and yield/5 roots, under tested treatments during 2021/2022 season.

Treatments	**TSS (%)**	**Sucrose (%)**	**Purity (%)**	**Yield/5 roots (kg)**
Pelargonium graveolens at 10 ml/L	24.13b	18.66b	77.36d	5.250a
Cymbopogon nardus at 10 ml/L	22.89c	18.23b	79.64a	4.750b
Frankincense at 2500 ppm	25. 65a	19.93a	77.69cd	5.320a
Chitosan nanoparticles at 500ppm	24.33b	18.99b	78.05bc	4.830b
Fungicide Topas 100, EC, at 1 mL/ L	23.12c	18.34b	79.32a	4.880b
Control	21.75d	17.02c	78.25b	4.540c
LSD 0.05	0.603	0.852	0.446	0.269

Means followed by a common letter (s) are not significantly different at the 5% level by DMRT

Use of scanning electron microscopy (SEM) test to understand the interaction among the tested treatments and *E. betae* fungus on leaves of sugar beet.

Our results which obtained from SEM examination indicated that the foliar treatment by essential oils of *Pelargonium graveolens* at 10ml/L and *Cymbopogon citratus* at 10ml/L and natural substances of Frankincense at 2500 ppm and Chitosan nanoparticles at 500ppm significantly reduced the fungal mycelium density, conidia and conidiophores, especially on leaves treated with essential oil of *Pelargonium graveolens* at 10ml/L and natural substance Frankincense at 2500ppm, followed by plants treated with essential oil of *Cymbopogon citratus* and Chitosan nanoparticles at 500ppm. Similarly, the all tested treatments decreased the amount of conidia which formed by the fungus and exhibited the ability of the powdery mildew pathogen to production conidia and conidiophores. Moreover, the tested treatments also caused plasmolysis and formation of the conidia and conidiophores of the pathogen (Figures 2 and 3). Additionally, to understand about the kind of mechanisms by tested treatments under this study which effected on fungal morphology, many morphological characteristics of tested fungus were also observed on powdery mildew spots infection on treated leaves of plants as compared with control ones by using scanning electron microscopy test. The examination included each of growth, conidia, conidiophores, mycelium and conidia formation of the fungus. The obtained results exhibited that, the treated leaves contained conidia appeared to be abnormal, and other construction appeared to be damaged. The revers was completely true in case of control treatment which appeared the morphological characteristics of tested fungus in normal and very standard forms. Previous research suggests that**,** the citronella essential oil damaged the hyphae cell wall of *A. niger*. Subsequently, large cytoplasm loss led to the hyphae was being collapsed, squashed, and destroyed of organelles. Similarly, citronella essential oil could lead to the rupture of the hard cell wall and kill the conidia. Therefore, the citronella essential oil provides promising safe and environmentally friendly fungicide in the future **(Wen-Ru *et al*., 2013)**. In the same direction**, Chen *et al.* (2014)** reported that, citronella oil inhibited growth of *A. alternata in vitro* and *in vivo*. Therefore, it could be considered as important and suitable promising natural substance for controlling the black rot disease in cherry tomato, they also added that, scanning electron microscopy showed considerably abnormal and damaged mycelia morphology of the fungus. **Sehsah *et al*., (2022**) added that, scanning electron microscope observation of sugar beet leaves treated with some plant extracts against powdery mildew disease displayed that, the fungal structures were abnormal and impossible to produce both conidiophores and conidia. Some plant extracts restrict the growth of fungi and their ability to sporulation because contact with it. Our results were also in agreement with findings the findings of **El-Nogoumy *et al*., (2022) and Hamden *et al*., (2023)** they found that, scanning electron microscope examination of treated leaves by some plant extracts against powdery mildew of sugar beet exhibited the decreasing formation of fungal hyphae, conidia and conidiophores. Plasmolysis to fungal cells and spores were also occurrence on the surface of infected leaves. It can be believed that the ability of the development of resistant strains of tested treatments is very low because available much more of its modes of action.

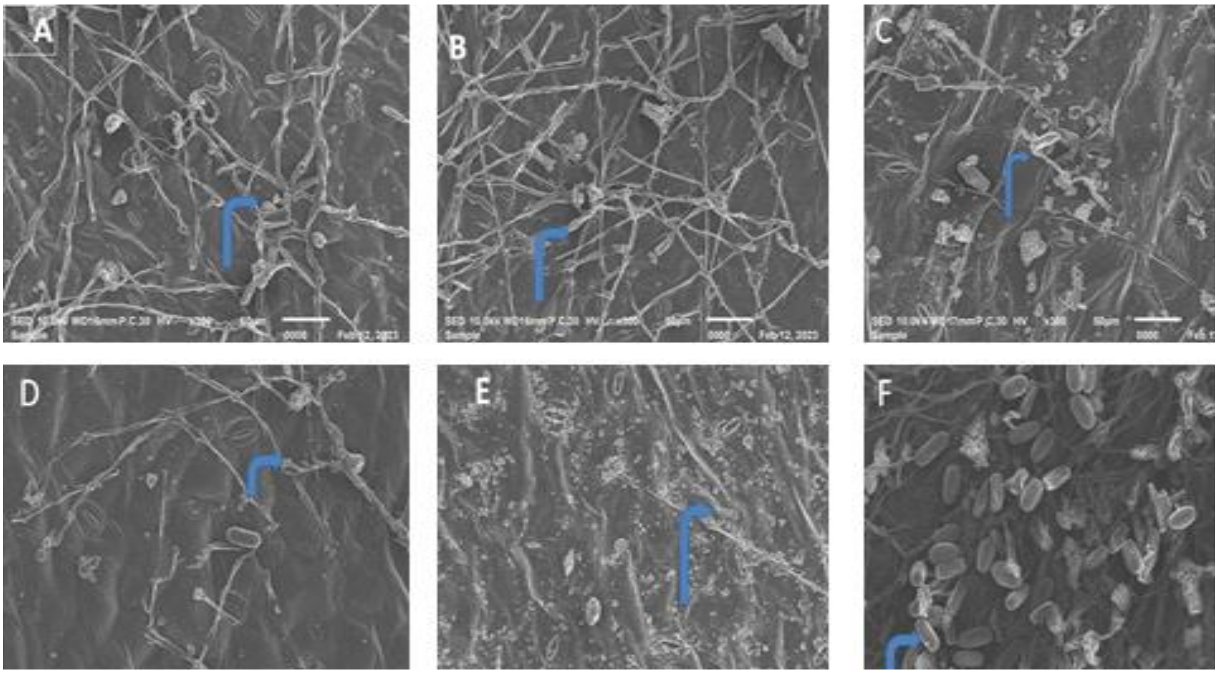

Fig. 2. Effect of the tested treatments on the mycelium growth, conidia and conidiophore formation of powdery mildew disease causal agent (*E. betae*) on sugar beet with scanning electron microscopy (SEM) at X 300. (A) Frankincense (boswellic acid) at 2500 ppm, (B) *Pelargonium graveolens* at 10ml/L, (C) Chitosan nanoparticles at 500ppm, (D) *Cymbopogon nardus* (Citronella) at 10ml/L, (E) Fungicide Topas 100 at 1ml/L and (F) control treatment.

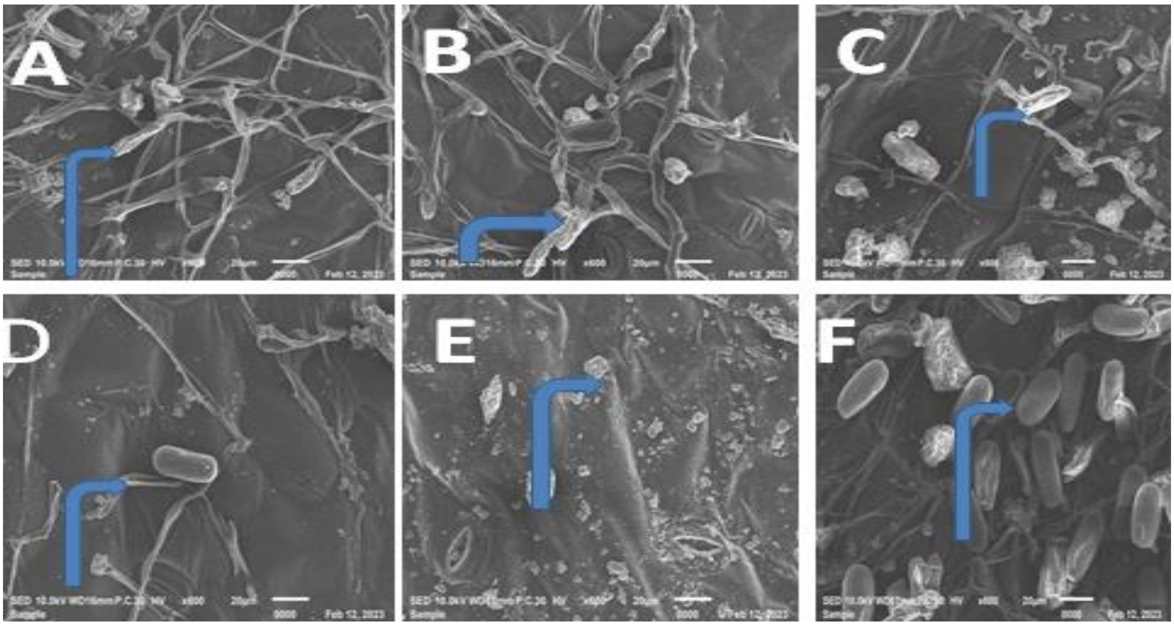

Fig. 3. Effect of tested treatments on the mycelium growth, conidia and conidiophore formation of powdery mildew causal agent (*E. betae*) on sugar beet with scanning electron microscopy (SEM) at X 600. (A) Frankincense (boswellic acid) at 2500 ppm, (B) *Pelargonium graveolens* at 10ml/L, (C) Chitosan nanoparticles at 500ppm, (D) *Cymbopogon nardus* (Citronella) at 10ml/L, (E) Fungicide Topas 100 at 1ml/L and (F) control treatment.

Effect of treatments on (POX) and (PPO) activity on infected sugar beet plants

Interestingly, foliar spraying by Frankincense at 2500 ppm and essential oil of *Pelargonium graveolens* at 10ml/L resulted in the highest POX and PPO (Fig. 4) activities followed by essential oil of *Cymbopogon citratus* and Chitosan nanoparticles, at 6 days post second spray. Therefore, foliar spraying of tested treatments significantly enhanced the activity of tested enzymes (Fig. 4). In general, the positively affected the enzymatic activity of POX and PPO at all tested treatments on infected treated leaves of sugar beet, especially after 6 days post second spray. This increase in activity of tested defence enzymes helps to obtain more understanding for the effectiveness of tested treatments to decrease the aggressiveness of the fungus under study, moreover the ability of enhancement some of the good qualities, such as the content of total soluble solids and sucrose percentage of sugar beet. These results are in the same direction with reported by **Elouadi *et al.* (2022)** who tested certain essential oils against powdery mildew disease on *Zinnia elegans*, they found that, the peroxidase and polyphenol oxidase activities were enhanced as a result of treatment by testing essential oils on plants.

El-Dengawy *et al*., (2016), Hafez *et al*. (2021) and **Sehsah *et al*. (2022)** added that, activates some enzymes such as polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), peroxidase (POX), and other enzymes resulted in defence machinery mainly relying on treated plants. Finally, it could be summarized that, to more understand the positive and better results of treatments which were used to prevent powdery mildew disease on sugar beet, the efficacy of the treatments which studied the enhancement of some defence enzymes, such as POX and PPO, was evaluated, and showed its action was related to stimulating plants to become resistant to the pathogens.

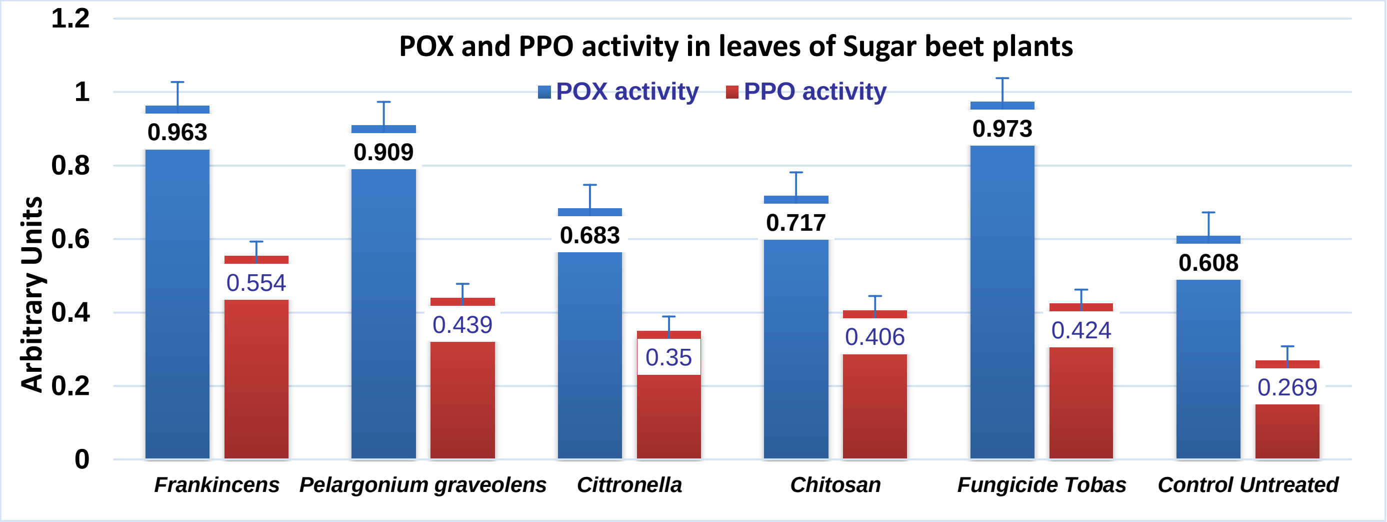

Fig. 4. Effect of tested treatments on peroxidase (POX), polyphenol oxidase (PPO) activity 6 days post second spray.

Conclusions

Our results showed that, environmental conditions play an important role in the distribution of powdery mildew disease in sugar beet. Foliar application of essential oils of *Cymbopogon nardus* (Citronella grass) at 10ml/L and *Pelargonium graveolens* at 10ml/L, and natural substances Frankincense (boswellic acids) at 2500 ppm and chitosan nanoparticles at 500 ppm significantly restricted infection of sugar beet by the tested disease caused by *E. betae*. These applications are eco-friendly, less costly, and may replace partially commercial fungicides. All the tested treatments enhanced the weight of roots and their quality by increasing the percent of TSS and sucrose. The effectiveness of these compounds may be due to increasing activation of enzymatic machinery, which is expressed by higher activities of POX and PPO enzymes and/or the favourable of SA biosynthesis. Scanning electron microscope (SEM) examination showed decreasing in mycelium density and formation of the fungal structures and their incapacity to produce either conidiophores or conidia of *E.betae* fungus. Therefore, further studies are required to explain the chemical analysis of natural substances and their molecular mechanisms of essential oils as well as natural substances on the *E. betae* fungus interactions.

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استخدم بعض المواد الطبيعية والزيوت العطرية لمكافحة مرض البياض الدقيقي ببنجر السكر

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تم تقييم خمسه وعشرون صنفا من البنجر للإصابة بمرض البياض الدقيقي في موسمي ٢٠٢١/٢٠٢٠ و٢٠٢٢/٢٠٢١ تحت الظروف الحقلية بعد ١٠٠ يوم من الزراعة وبينت النتائج المتحصل عليها زيادة كبيرة في الشده المرضية في الموسم الزراعي الأول بينما كانت منخفضه في الموسم الثاني. وبالعودة الي بيانات الارصاد الجوية تلاحظ وجود اختلاف كبير في درجات الحرارة الصغرى والكبرى وقيم الرطوبة النسبية في موسمي الزراعة خلال شهور تكشف وظهور المرض (ديسمبر ويناير وفبراير ومارس), فقد تلاحظ ارتفاع في درجات الحرارة الصغرى والكبرى وإنخفاض في الرطوبة النسبية (ظروف مناسبه لتكشف وظهور المرض) في الموسم الأول بينما كان العكس صحيح خلال الموسم الثاني. كذلك بينت النتائج المتحصل عليها ان الاصناف ام كي ٤١٩٩ و الصنف اف دي ١٧ بي ٤٠١٠ والصنف الانيا كي دبيو اس قد سجلت اقل شده مرضيه بالبياض الدقيقي (أصناف مقاومه للمرض) وبزلك يمكن اعتمادها للزراعة في المواسم المقبلة. وعل الجانب الاخر فان الاصناف كارما وديبيندراكي دبيو اس وفرابينا كي دبيو اس واس في ٢١٧٣ وهاموند قد سجلت اعلي شده مرضيه (حساسة للإصابة بالمرض) وبذلك يوصي باستبعادها وعدم زراعتها تحت الظروف المصرية. وعند اختبار المواد المستخدمة في هذه الدراسة وهي الزيوت العطرية لنباتات العتر و وحشيشة السيترونيلا بمعدل ١٠سم^{٣}/لترماء وكذلك المواد الطبيعية فرانكينسينس عند ٢٥٠٠ جزء في المليون والشيتوزان في صورة النانو عند تركيز ٥٠٠ جزء في المليون والمبيد الفطري توباس بمعدل ١سم^{٣}/لتر ماء (التركيز الموصي به), عند اختبار المواد السابقة لمكافحة مرض البياض الدقيقي في البنجر تحت الظروف الحقلية فقد بينت النتائج المتحصل عليها ان جميع المواد السابقة كانت فعاله بدرجات متفاوتة في انخفاض معنوي في الشده المرضية بالبياض الدقيقي مقارنة بالكنترول خلال موسمي الزارعة تحت الدراسة. وكانت المعاملة بالمادة الطبيعية فرانكنسينس والزيت العطري لنبات العتر أكثرهم فاعليه في مكافحة المرض بينما كان الشيتوزان والزيت العطري حشيشة السيترونيلا في المرتبة الثانية في مكافحة المرض. وكل المعاملات السابقة كانت تقترب من او تتساوي مع المبيد الفطري توباس في الفاعلية في مكافحة المرض. لذلك فان المعاملات السابقة تكتسب أهميتها في انها مواد أقل تكلفه وصديقه للبيئه ويمكن أن تحل جزئيا أو كليا محل المبيدات الفطرية المستخدمة في مكافحة الفطر المسبب للمرض. وبالإضافة الي ان هذه المواد قللت كثيرا من الشده المرضية بالفطر فإنها ايضا أدت إلي زيادة في وزن الجذور وزيادة نسبة السكر والمواد الصلبة الذائبة وبالتالي زيادة محصل الفدان من البنجر سواء في الكم او الجودة. ومن جهة اخري فان المعاملة بهذه المواد ادت الي زيادة في نشاط انزيمات الأكسدة المرتبطة بالمقاومة للأمراض وهي انزيمات البيروكسيديز والبولي فينول اكسيديز في الاوراق المعاملة بها. لذلك فان ميكانيكية تأثير هذه المواد في مكافحة المرض اما أن تكون عن طريق زيادة نشاط انزيمات الأكسدة واما أن تكون عن طريق تنشيط إنزيم الفينيل الانين امونيا لياز والذي يؤدي الي تنشيط وتكوين حامض السالسيلك . كذلك بينت نتائج المسح بالميكروسكوب الالكتروني على الاوراق المصابة المعاملة أن المعاملات السابقة أدت الي إنخفاض في كثافة الفطر وتشوهه في التراكيب الفطرية وعدم مقدرتها على انتاج الحوامل الجرثومية وجراثيم الفطر وكذلك بلزمة خلايا الفطر وفسادها على الاوراق المعاملة. وفي النهاية فأننا نوصي بإنتاج هذه المواد المختبرة في هذا البحث تجاريا وإستخدامها لمكافحة مرض البياض الدقيقي في بنجر السكر والابتعاد أو التقليل من إستخدام المبيدات الفطرية التي تؤثر على صحة الإنسان وتلويث البيئة.

الكلمات المفتاحية: إيريسيف بيتا، فرانكنسينس، العتر، حشيشة السترونيلا، الشيتوزان والمسح بالميكروسكوب الإلكتروني.