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Alternative Safety Methods for Controlling Powdery Mildew in Squash under Field Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. Author MMHR performed the analytical tasks, statistical analysis and wrote the first draft of manuscript. Authors AAE and EKAK designed the study, did the microbiological works and wrote the first draft of manuscript. Author MAA wrote the manuscript and managed the literature search. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Almond (*Prunus dulcis*), camphor (*Cinnamomum camphora*) and clove (*Syzgium aromaticum*) crude oils, silicate, Gram-positive bacterium *Streptomyces griseus* and a fungus *Gliocladium virens* culture filtrates in addition to Topas-100 EC (10% penconazole) were used to study of their effects on powdery mildew caused by the fungus *Erysiphe cichoracearum*. Camphor oil (5 ml/L), clove + camphor + almond (2 and 5 ml/L), *G. virens* filtrate and Topas-100 (240 ppm) completely inhibited the conidial germination after 24, 48 and 72 h. Topas-100 treatment was the most effective treatment in reducing powdery mildew disease severity of squash with an average of 10.34%, followed by clove oil (5 and 10 ml/L), camphor oil (5 and 10 ml/L) and the mixture of clove +

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camphor + almond (5 and 10 ml/L) with averages of (13.70 and 11.43%), (16.31 and 15.84%) and (20.65 and 18.34%), respectively. Concerning the disease incidence, it showed a harmony trend with the disease severity. Squash shoot length, leaf area/plant and the number of leaves/plant increased due to the treatments compared to the control treatment (infected only). The filtrates of *S. griseus* and *G. virens* had moderate effectiveness on the previous parameters. Also, it is clear that the plant fresh and dry weights, number of fruits/plant and weight of fruits/plant increased significantly compared with control treatment of squash plants. The treatments of Topas-100, clove + camphor + almond (10 ml/L and 5 ml/L), *G. virens* and *S. griseus* filtrates had the highest chlorophyll a content with averages of 4.02, 3.95, 3.79, 3.58 and 3.47 mg/g fresh weight, respectively. Also the same arrangement cleared in case of chlorophyll b with averages of 3.34, 3.00, 2.98, 2.83 and 2.75 mg/g, respectively. All treatments used increased the activity of defence-related enzymes (polyphenol oxidase and peroxidase). The activities of pectin methyl esterase, Cx-cellulase and polygalacturonase enzymes were in parallel with the disease severity where the activity of any enzyme from them increased with the increasing of disease severity.

Keywords: Erysiphe cichoracearum; powdery mildew disease; squash; antagonistic microorganisms; essential oils; silicate; enzymes related to defence.

1. INTRODUCTION

Cucurbits play an important role in human nutrition. Cucurbit crops represent a main portion of all vegetables and are grown in different regions in Egypt. Squash is a promising export crop which can be readily produced at a low cost during the winter period of Egypt [1]. Powdery mildew, caused primarily by the fungus *Erysiphe cichoracearum*, may attack all vine crops or cucurbits. The vegetable crops most generally affected are cucumber, gourds, muskmelon, pumpkin, and squash [2]. Powdery mildew occurs on leaves, stems and fruits. Chief epidemics minimize crop yields by causing diminished fruit set, inadequate ripening, fruit cracking and deformation in addition to reducing post-harvest storage time [1].

However, the environmental pollution caused by immoderate use and misuse of agrochemicals, and fear-mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Nowadays, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to artificial chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological controls [3]. The mode of action of the recognized compounds in the tested culture filtrates against the powdery mildew may be attributing to the inhibition of the melanin pigment creation. It was demonstrated, that melanin pigment interrelated with the fungal pathogenicity and could provide the fungi with confident special recovery functions, such as anti-radiation and anti-oxidation [4].

The application of biological control using antagonistic microorganisms has proved to be successful for controlling a range of plant diseases [5]. *Streptomyces griseus* is useful bacteria that produce many antibiotics such as streptomycin, secondary metabolites and volatile compounds [6]. Also, phenol, 2-methyl-5-(1-methylethyl) (Carvacrol) was the major constituent [7]. The confirmed effects of volatile compounds of *S. griseus*, (inhibition of spore germination and mycelium growth) have a potential for the effective control of *Aspergillus flavus, Fusarium oxysporum, A. niger* and *Bacillus cinerea*. Chitinase and lytic enzymes of *S. griseus* can control some fungal plant diseases e.g. *F. oxysporum*, *Alternaria alternata*, *Rhizoctonia solani* and *F. solani* [8]. [9] mentioned that *Streptomycetes* have been found in beneficial associations with plants where they have enhanced plant growth and protected against pests.

The genus *Gliocladium* consists of several species of fungi that are antagonistic to plant pathogens. *G. virens* produces antibiotic metabolites such as gliotoxin which have antibacterial, antifungal, antiviral and antitumor activities [10]. [11] found that *G. virens* appreciably inhibited the radial growth of *A. alternata, Chaetomium* spp., *Penicillium citrinum, A. niger, A. flavus, Rhizopus nigricans* and *F. oxysporum*.

The antimicrobial activity associated with essential oils is attributed to phenolic and terpenoid compounds such as carvacrol, eugenol, thymol and p-cymene which show high antimicrobial activity when tested in pure form [12]. The effect of the essential oils has been shown to be dose dependent resulting in either inhibition of pathogen growth or killing [13]. Furthermore, studies conducted showed that clove oil able to inhibit the mycelial growth of some pathogenic fungi [14].

Silicon (Si) deposition in plant cell walls raised the hypothesis of a possible physical barrier to pathogen penetration [15]. Several studies confirmed the effective action of Si in controlling powdery mildew (*Sphaerotheca fuliginea*) in cucurbits [16]. Cucumber plants inoculated with S. *fuliginea* and grown in nutrient solution supplemented with sodium silicate showed a reduction in spore germination and the number and area of colonies per leaf [17]. The effect of Si on the control of plant diseases, its mode of action and function in several pathosystems are not yet fully understood. There is the hypothesis of a possible physical barrier formation, which is based on the form of Si accumulation in plants, mostly in the cell wall. There may also be increased activity of enzymes involved in plant defense such as peroxidase, polyphenol oxidase, phenyl alanine ammonia lyase and lipoxygenase [18], which, in this case, is considered as a chemical barrier. This work aimed to throw the light on the abiotic and biotic agents as fungicides alternatives in controlling squash powdery mildew by induction the resistance in treated plants.

2. MATERIALS AND METHODS

2.1 Microorganisms

Gliocladium virens GL-21 and *Streptomyces griseus* NBRC 13350 were obtained from the Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt.

2.2 Essential Oils

Almond, camphor and clove oils were obtained from Elcaptain for extracting natural oils herbs (Cairo, Egypt). Almond, camphor and clove oils were prepared at two concentrations (2 and 5 ml/L) for testing *in vitro* and (5 and10 ml/L) for testing *in vivo* and applied at the concentration of 5% using 0.01% of Tween 80 as emulsifier.

2.3 Silicate Solution

Silicate (magnesium silicate, MgO_3Si) obtained from El Gomhoria Company for chemicals, Cairo, Egypt. Silicate was applied at the concentrations of (1 and 2 ppm/L) *in vitro* and (2 and 4 ppm/L) *in vivo.*

2.4 Fungicide

Topas-100(R): (Syngenta, Egypt), 10.0% penconazole (1- [2-(2-4 dichlorophenyl) pentyl] – 1H–1, 2, 4 triazole) was prepared as recommended dose (0.25 ml/L) equivalent to 250 ppm.

2.5 Preparation of Culture Filtrates of Bio-control Agents

Fifty ml of potato dextrose broth (PDB) was taken in 250 Erlenmeyer flask and sterilized. A 4 mm dia. disk of 10 days old culture of *G. virens* was aseptically transferred to cooled broth and incubated at 28±2°C for 10 days. On the 10^{th} day, culture filtrate of this bio-control agent was harvested by filtering through Whatman filter paper No. 42 and repeatedly centrifuged at 9000 rpm to obtain a cell free culture filtrate [19]. The supernatant served as a solution of 100% concentration [20].

Fresh culture of *S. griseus* was inoculated in starch casein broth and incubated at 28°C for 7 days in water bath with shaking. Growth of the organism in the flask was confirmed by turbidity in the broth. The broth culture was centrifuged at 5000 rpm for 20 min and the supernatant was filtered through No. 1 Whatman filter paper. The culture filtrate was used for the determination of antimicrobial activity against the test organisms.

2.6 Laboratory Experiment

The efficacy of treatments was assessed based on the conidial germination of *Erysiphe cichoracearum*. Infected leaves of squash plants were collected from Etay El-Baroud Agric. Res. Station field and conidia spores were harvested from infected leaves using a small paintbrush and water containing 0.01% tween 80. The conidial spores' suspension was filtered through fine nylon membrane 8 µm (Millipore) to remove hyphal aggregates, and then washed twice with sterilized water. Conidial suspension of *E. cichoracearum* had 3×10^4 conidia ml^{-1} using a haemocytometer as spore counter. Susceptible squash cultivar (Eskandarani hybrid) was used to

prepare leaf disks. The leaves (45 days) below the apex that free from disease injury were surface sterilized using 0.1 sodium hypochlorite, rinsed three times in sterile water and air dried, and then leaf disks (15 mm in diameter) were cut and divided into fourteen groups. First, second and third groups were dipped in 2 ml/L concentrations of oils (clove, camphor and almond), fourth, fifth and sixth groups dipped in 5 ml/L for the same oils, seventh and eighth groups dipped in mixtures of three oils (clove + camphor + almond) 2 and 5 ml/L, ninth and tenth groups dipped in 1 and 2 mM/L of silicone solution, eleventh group dipped in *G. virens* culture filtrate, twelfth group dipped in *S. griseus* culture filtrate, threaten group was dipped in fungicide Topas-100 at 240 ppm and fourteenth group was dipped in sterile water and served as a control. All groups were dipped for 5 seconds and allowed to air dry for 2 h, after that two ml of conidial suspensions were sprayed on the disks. Four sprayed disks were distributed in each Petri dish (90 mm in diameter) containing moist filter paper. All treatments had four replicates. The disks, then were incubated at 20°C in darkness. The conidia from incubated leaves were picked on sticky side of cellophane tape to observe the conidial germination. Cellophane tapes were placed on glass slides having a drop of cotton blue in lacto phenol and were examined under microscope [21]. Twenty random microscopic fields were scored for each of the three replications. Percentages of conidial germinations and conidial germination inhibition were calculated according to [20].

The percentage of conidial germination and inhibition % were calculated after 24, 48 and 72 h by using the formula: PG= (A/B) × 100,

Where, PG=percentage of germination, A=number of germinated conidia and B=Total number of conidia examined and conidia inhibition calculates following equation: Inhibited conidia %= (C-T)/C x100, *where*, C=grown conidia of control, T=grown conidia of treatment.

2.7 Field Experiments

Experiments were carried out at Etay El-Baroud Agric. Res. Station during two successive growing seasons; 2014 and 2015. Squash (Eskandarani hybrid) seeds were sown during May and plot was consisted of four rows. Each row was 4 m long and 80 cm wide. Seeds were sown 40 cm apart and three seeds were sown in each hill. After emergence, 15 days old, plants were thinned to one plant per hill. In both seasons the normal agricultural practices of growing squash plant, including amounts of fertilizers and irrigation were followed. The experimental design used was a randomized complete block design. Plants were left to natural infection with powdery mildew then, the plants were sprayed (20 days after sowing) separately with aqueous solutions of oils (clove, almond and camphor) and a mixture of three oils (clove + almond + camphor) at 5 and 10 ml/L. Two groups of pots were sprayed with solutions of silicon (K_2SiO_3) at 1 mM and 2 mM. The thread group was sprayed with culture filtrate of *G. virens* and *S. griseus*. Fungicide Topas-100 was sprayed at 240 ppm and the last group was sprayed with tap water and served as control treatment. All pots were sprayed three times (20, 30 and 40 days after sowing) three replicates were used for each treatment.

2.8 Disease Assessment

Plants were examined periodically and disease measures were determined using the devised scale (0-5) adopted by [22] where:

0= no symptoms appear, **1=** 0.1 to 3% of leaf area covered by the infection, **2=** more than 3 to 10% of leaf area covered by the infection, **3=** more than 10 to 25% of leaf area covered by the infection, **4=** more than 25 to 50% of leaf area covered by the infection **5** = more than 75% of the plant growth covered by the infection and the plants turned to be stunted.

The grown plants were periodically examined for disease symptoms to estimate the severity of the disease and the final averages were recorded using the following formula:

Disease severity (%) = Σ (nxv) / 5 N X 100
Where, n= number of infected leaves in each category, v = numerical values of each category, N = total number of the infected leaves.

Disease incidence (%) = No. of infected plants / Total no. of the plants assessed x 100

2.9 Growth Parameters

Five plants were selected randomly from each replicate unit to measure the vegetative growth characteristics:

a. Plant height (cm) was measured starting from the ground level to the apical meristem of the stem.

b. Number of leaves/plant.
c. Total leaf area/plant (cm^2)
d. Leaves and stems dry weights/plant (g) were gained by drying at 70°C in a forced-air oven till the constant weight.

Total fruits yield: Squash fruits were harvest after 3 day intervals, upon reaching 12-15 cm length for both seasons. The fruits were picked from each single plant separately and the following data were recorded:

a. Number of fruits/plant; the average number of harvested fruits through the entire harvesting period.
b. Yield/plant; the average weight of fruits during the whole harvesting period.
c. Average of fruit weight; calculated by dividing the weight of all fruits/plant by a number of fruits/plant.

2.10 Biochemical Changes Associated with Infection

2.10.1 Pigments estimation

Samples of leaves were cut into small pieces, and 0.25 g fresh piece as one sample was weighed. Pigments were extracted by grinding tissues in acetone, and then filtered to remove the debris. The volume of acetone is restored to 25 ml. The total chloroplast pigments were determined by measuring the optical density at 663 & 645 nm against the blank and calculated using the formula devised by [23]:

Chlorophyll a (mg/g tissue) = 12.7 x (A_{663}) - 2.69 x (A_{645})
Chlorophyll b (mg/g tissue) = 22.9 x (A_{645}) - 4.68 x (A_{645})

2.11 Determination of Enzyme Activities

The sample of one g was homogenized in 2 ml of 0.1 M sodium phosphate buffer (SPB) pH 6.5 at 4°C. These triturated tissues were removed through four layers of cheese cloth and the filtrate was centrifuged at 20,000 rpm at 4°C for 15 min. The supernatant served as an enzyme extract for enzyme assay of polyphenoloxidase and peroxidase using the UV-Vis spectrophotometer (Shimadzu Corporation-MultiSPec-1501).

2.11.1 Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity was carried out according to [24]. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (SPB), 0.2 ml of enzyme extract and 0.2 ml of 0.1 M catechol. Boiled enzyme or distilled water was used as a blank. The polyphenol oxidase activity was expressed as change in absorbance at 495 nm against blank per min/g fresh tissue.

2.11.2 Peroxidase activity

Peroxidase activity was assayed colorimetrically according to the method described by [25] through measuring the oxidation of pyrogallol in the presence of H_2O_2. The reaction mixture consisted of 1.5 ml of SPB, 20 µl of enzyme extract, 1.0 ml of 0.5 M pyrogallol and 480 µl of 1% H_2O_2 solution (v/v). The increase in optical density at 430 nm against blank was continuously recorded every minute. Peroxidase enzyme activity was expressed as change in absorbance per min/g fresh tissue.

2.11.3 Polygalacturonase activity

The polygalacturonase activity was assayed for using the reducing sugar method [26]. The reaction mixture containing 0.8 ml of 1% polygalacturonic acid in 0.2 M acetate buffer solution (pH 4.5) and 0.2 ml of enzyme extract, the reaction tube was incubated at 35°C for 30 min and 0.5 ml of DNS (sodium hydroxide, potassium sodium tartrate) reagent was added. Absorbance was measured at 540 nm. One unit of enzyme activity was defined as the amount of enzyme that released 1 µmol of galacturonic acid per min under standard assay conditions.

2.11.4 C_x-cellulase activity

The method described by [27] was employed as follows; one ml of carboxymethyl cellulose (1%) was added to 1 ml of 0.1 M acetate buffer (pH 6.0) then the enzyme reaction was initiated by adding 1 ml of enzyme extract. The mixture was incubated at 28°C for 30 min; acetate buffer was used as blank. Three ml of Nelson solution was added to the reaction mixture and the mixture was shaken and incubated at 50°C for 15 min. After cooling, 3 ml of the arsenomolybdate reagent was added, carefully mixed and diluted to l0 ml with distilled water. After that the mixture was centrifuged to remove any turbidity. The amount of reducing sugars produced was estimated by determining the absorbance at wavelength 700 nm. A standard curve was plotted using aqueous solutions of D-glucose with concentrations from 10-100 mg/ml.

2.11.5 Pectin methyl esterase (PME) activity

The PME activity was determined according to the method given in [28]. The reaction mixture was formed by mixing 2.3 mL of 0.3% pectin solution in 0.1 M NaCl, 0.5 mL of 0.01% of bromothymol blue in 3 mM sodium phosphate buffer (pH 7.5), and 0.1 mL of enzyme extract. The increase in absorbance at 620 nm was monitored with a constant temperature working at 30°C. PME activity was determined as ΔAbs min^{-1} per g fresh tissue.

2.12 Statistical Analysis

The experimental results were expressed as means of three replicates using SPSS version 17.0 statistical software (IBM Corporation). Comparison of means for *in vitro* antibacterial evaluation was carried out using One-way analysis of variance (ANOVA) and Duncan's multiple range tests. *P* value ≤0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Evaluation of Treatments on Spore Germination

Data presented in Table (**1**) indicate that all treatments (clove, camphor, almond and the mixture of them, silicate, *S. griseous* , *S. virnes*) at the tested concentrations in addition to Topas-100 at the recommended dose significantly decreased the conidial germination of *E. cichoracearum* as compared to control after 24, 48 and 72 h. In case of camphor oil (5 ml/L), clove + camphor + almond (2 and 5 ml/L), *G. virens* filtrate and Topas-100 (240 ppm/L), they inhibited completely conidial germination after the three periods mentioned before. Camphor (2 ml/L) and almond oil (2 and 5 ml/L) inhibited conidial germination until 48 h only. The percentage of conidial germination increased with increasing incubation period up to 72 h. *Syzygium aromaticum* L. (Merrill and Perry) oil exhibited strong inhibitory effects with complete inhibition of mycelia growth in *Botrytis cinerea* [29]. [14] reported that inhibition of mycelium growth of *F. oxysporum, F. redolens* and *F. commune* started at the lowest application rates (0.1µL/mL) of clove essential oil and this inhibition successively increased with the increasing of oil concentration. The main chemical components of clove oils are eugenol, acetyl eugenol, iso-eugenol and β-caryophyllene [30]. These phenolic compounds are responsible for the antibacterial and antifungal properties of essential oil [31]. The activity may be due to their hydrophobicity, responsible for their partition into the lipid bi-layer of the cell membrane, leading to permeability modification and a consequent leakage of cell contents. As typical lipophiles, essential oils can travel through the cell wall and cytoplasmic membrane, disrupt the structure of the different layers of polysaccharides, fatty acids and phospholipids, and permeabilize those [32]. Hegazi and El-Kot [33] reported that ginger, cinnamon and clove oils decreased disease incidence and severity of powdery mildew on *Zinnia elegans* L. Camphor oil was found to be greatly antifungal and most effective against *A. niger* and *A. flavus*. The inhibitory effect of the essential oils was proportional to its concentration [34]. This is in accordance with [35]. Geng et al. [36] documented that 1 mg/mL of bitter almond essential oil (BAEO) could variously inhibit the mycelium linear growth rate of all the tested pathogenic fungi with the inhibition rates of 44.8%~100%. Also, they observed that after spraying BAEO it directly formed an oil membrane layer on the leaf blade surface of cucumber and wheat seedlings. So they speculated that the oil membrane layer could effectively prevent the pathogen spores from penetrating into the tissue, thereby reducing the incidence of infection. Abd-Alla et al. [37] mentioned that sweet almond oil at 2.0% caused a complete reduction of *P. digitatum* linear growth *in vitro*, while bitter almond oil resulting moderate effect to reducing the fungus growth if compared with other treatments and control treatment.

The presence of a large number of alkaloids, phenols, terpenes derivatives compounds and other antimicrobial compounds make the essential oils more précised in their mode action against the plenty variety of pathogenic microorganisms [38]. *G. virens* have significantly inhibited the radial growth of almost all the pathogens tested [11]. Such types of inhibitory effects have also been reported by [39] among others, and are a likely indicator that the antagonistic fungi produce some metabolite(s) that inhibit pathogen growth. Fungal spore germination and mycelium growth of *A. niger*, *A. flavus*, *F. oxysporum* and *B. cinerea* cultures were significantly suppressed in the presence of the volatiles produced by *S. griseus*. The most abundant compounds in volatile of *S. griseus* were Phenol,2-methyl-5-1methylethyl (Carvacrol) [40]. The cytotoxic ability of carvacrol on peroxidant activity can make it an effective antiseptic and antimicrobial agent [41].

Table 1. Effect of some alternative pesticides on the conidial germination of *Erysiphe cichoracearum in vitro*

Treatments	Conc.	Germination of conidia (%)			Inhibition of conidial germination (%)		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Clove	2 ml/L	1.67^{c}±0.54	9.33^{d}±0.54	15.67^{c}±3.03	54.50^{b}±7.14	84.01^{b}±0.32	75.77^{b}±2.82
	5 ml/L	1.00^{d}±0.47	6.67^{d}±2.88	12.00^{cd}±1.25	72.75^{b}±12.37	88.58^{b}±4.72	81.44^{b}±4.79
Camphor	2 ml/L	0.00^{f}±0.00	0.00^{f}±0.00	10.00^{d}±8.16	100.00^{a}±0.00	100.00^{a}±0.00	84.54^{ab}±12.25
	5 ml/L	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00	100.00^{a}±0.00	100.00^{a}±0.00	100.00^{a}±0.00
Almond	2 ml/L	0.00^{f}±0.00	0.00^{f}±0.00	8.67^{de}±1.09	100.00^{a}±0.00	100.00^{a}±0.00	86.59^{ab}±2.20
	5 ml/L	0.00^{f}±0.00	0.00^{f}±0.00	1.67^{f}±0.98	100.00^{a}±0.00	100.00^{a}±0.00	97.42^{a}±1.76
Clove+	2 ml/L	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00	100.00^{a}±0.00	100.00^{a}±0.00	100.00^{a}±0.00
Camphor+ Almond	5 ml/L	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00	100.00^{a}±0.00	100.00^{a}±0.00	100.00^{a}±0.00
S. griseus	100%*	0.33^{e}±0.27	0.67^{e}±0.54	3.33^{ef}±2.72	91.67^{a}±7.14	98.85^{a}±0.95	94.85^{a}±4.40
G. virens	100%	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00	100.00^{a}±0.00	100.00^{a}±0.00	100.00^{a}±0.00
Silicate	1 mM/L	3.67^{a}±0.27	30.67^{b}±1.78	35.00^{b}±2.49	0.00^{d}±0.00	47.42^{c}±2.89	45.88^{c}±8.43
	2 mM/L	2.67^{b}±0.27	24.67^{c}±3.81	30.33^{b}±4.23	27.25^{c}±10.00	57.71^{c}±7.06	53.10^{c}±8.02
Topas-100	240 ppm/L	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00	100.00^{a}±0.00	100.00^{a}±0.00	100.00^{a}±0.00
Infected only		3.67^{a}±0.72	58.33^{a}±3.60	64.67^{a}±2.60	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00

Means followed by the same letter(s) within a column are not significantly different (P≤0.05) according to Duncan's multiple range test **Culture filtrate*

Volatile substances derived from *Streptomyces* sp. and other species of actinomycetes prevent mycelium growth and inhibit spore germination of different fungi [42]. Cyclohexanol, decanol; 2-ethyl-1-hexanol, nonanol, benzothiazole and dimethyl trisulfide are important compounds that inhibit spore germination and mycelium growth of *Sclerotinia sclerotiorum* [43]. Chitinase and lytic enzymes of *S. griseus* can control some fungal plant diseases like *F. oxysporum*, *Alternaria alternata*, *Rhizoctonia solani* and *F. solani* [8]. [7] reported that fungal spore germination and mycelium growth of both *P. chrysogenum* and *B. cinerea* cultures were appreciably suppressed in the presence of the volatile compounds produced by *S. griseus*.

[44] reported that hyphal growth of rice blast fungus was very slow on agar plates containing soluble silicon (liquid potassium silicate) and that hyphal shapes became thick like the knots. Results obtained by [45] recommended that soluble silicon induced physiological changes in the cuticle layer of strawberry leaves after absorption by the plant. In addition, soluble silicate reduced germination of strawberry powdery mildew (*Sphaerotheca aphanis* var. *aphanis*) conidia, formation of appressoria, and possibly the penetration of powdery mildew. Wang Y et al. [46] exhibited that Si significantly inhibited mycelial growth and spore germination of *Trichotethium roseum*, increased cell membrane permeability of hyphal. The inhibiting effect was improved with the concentration of Si increasing. The approach of the identified compounds in the tested cultures against the powdery mildew may be due to the inhibition of the melanin pigment formation [4]. Without such melanin, pathogenic fungi would lose pathogenicity.

3.2 Efficacy of Some Alternative Pesticides against Disease Severity and Disease Incidence of Squash Powdery Mildew under Field Conditions

Results in Table (**2**) show that all the tested treatments appreciably reduced the disease severity and disease incidence of powdery mildew of squash relative to control trial. Topas-100 treatment was the most effective treatment in reducing disease severity with an average of 10.34% followed by clove oil (5 and 10 ml/L), camphor oil (5 and 10 ml/L) and the mixture of clove + camphor + almond (5 and 10 ml/L) with averages of (13.70 and 11.43%), (16.31 and 15.84 %) and (20.65 and 18.34%), respectively. The less effective treatment cleared with Si (2 and 4 ppm) with averages of (35.20 and 32.21%) at the first season (2014). Also, the same trend exhibited in the second season (2015). Concerning the disease incidence it showed a harmony trend with disease severity where Topas-100 treatment had the best effect in decreasing disease incidence with an average of 31.97% in the first season followed by clove oil (5 and 10 ml/L), camphor oil (5 and 10 ml/L) and the mixture of clove + camphor + almond (5 and 10 ml/L) with averages of (36.90 and 35.01%), (42.77 and 40.74%) and (46.96 and 46.19%), respectively. Data at the second season were closed to the first season. These results are in agreement with the findings of many researchers who reported that compounds produced by antagonistic microbes have been shown to have potential antifungal activities. Hegazi and El-Kot [33] reported that ginger, cinnamon and clove oils decreased disease incidence and severity of powdery mildew on *Zinnia elegans,* L. In cucumber, Si reduced the severity of *Podosphaera xanthii* infections by reducing the number of colonies and their size, the conidia germination and the number of haustoria per colony [17] & [47]. Also [1] showed that filtrates of different microbial isolates except for *T. harzianum* were more efficient against powdery mildew in squash than the tested fungicide alone at the suggested levels. The efficacy of the culture filtrates was due to the presence of a mixture of known antifungal compounds. Derbalah AS et al. [48] showed that the fungicide and the tested fungal cultures either alone or combined significantly reduced the disease severity of powdery mildew in okra comparative to control treatment.

Results presented in Table (**3**) reveal that squash shoot length, leaf area/plant and number of leaves/plant increased due to the treatments compared to the control treatment (infected only) in the first season. Topas-100 had the tallest shoot system with an average of 69.70 cm followed by clove oil (10 and 5 ml/L), camphor oil (10 and 5 ml/L) and the mixture of clove + camphor + almond (10 and 5 ml/L) with averages of (69.50 and 68.33 cm), (65.87 and 65.47 cm) and (62.33 and 61.47 cm), respectively. In contrast silicate at the concentrations of 2 and 4 mM/L had the least effect on squash shoot length with averages of 52.77 and 53.70 cm, respectively. In case of leaf area/plant results cleared that Topas-100 had the best effect with

an average of 1824.73 followed by clove oil (10 and 5 ml/L), camphor oil (10 and 5 ml/L) and the mixture of clove + camphor + almond (10 and 5 ml/L) with averages of (1656.93 and 1505.27), (1420.53 and 1388.30) and (1380.77 and 1344.97), but silicate (2 and 4 mM/L) had the less leaf area/plant with averages of 1114.57 and 1113.70, respectively. Also the same trend showed with the number of leaves/plant where Topas-100 had the highest number leaves/plant with an average of 30.11 followed by clove oil (10 and 5 ml/L), camphor (10 and 5 ml/L) and the mixture of clove + camphor + almond (10 and 5 ml/L) with averages of (29.10 and 26.28), (24.46 and 20.85), (22.33 and 21.72) and silicate (4 and 2 mM/L) had the least averages (17.12 and 15.09), respectively. Generally it can be noticed that the filtrates of *S. griseus* and *G. virens* had moderate effectiveness on the previous parameters. The same trend appeared in the second season.

Data in Table (4) show significant increase in the fresh and dry weights of squash plants cv. Eskandarani hybrid compared to control treatment (infected only) in the two seasons 2014 and 2015. Topas-100 treatment had the best plant fresh weight (g) followed by clove oil (10 ml/L), clove oil (5 ml/L), camphor oil (10 ml/L) and camphor oil (5 ml/L) with averages of 276.23, 265.60, 251.90, 233.20 and 230.30 (g), respectively. Also the previous treatments with the same arrangement had the highest plant dry weight (g) with averages of 59.83, 50.17, 47.33, 45.73 and 44.17, respectively. The same trend cleared in case of the second season. From the data presented in Table (5) it is clear that a number of fruits/plant and weight of fruits/plant increased significantly compared with the control treatment (infected only). During the first season Topas-100 had the highest number and weight of fruits/plant followed by clove oil (10 and 5 ml/L) and camphor (10 ml/L) with averages of 9.98, 9.50, 9.21 and 8.54, respectively in case of a number of fruits/plant and 600.70, 562.80, 460.54 and 460.54 (g), respectively in case of weight of fruits/plant. Also data in the second season (2015) are closed to the first one.

Table 2. Effect of some alternative pesticides on the disease severity and incidence of powdery mildew on squash cv. Eskandarani hybrid under field conditions during two successive seasons (2014 & 2015)

Treatments	Conc.	Disease severity (%)		Disease incidence (%)	
		1st year	2nd year	1st year	2nd year
Clove	5 ml/L	13.70^{hi}±0.26	11.20^{fgh}±0.42	36.90^{fg}±0.25	33.47^{efg}±0.27
	10 ml/L	11.43^{ij}±0.28	10.05^{gh}±0.41	35.01^{gh}±0.62	31.14^{fg}±0.31
Camphor	5 ml/L	16.31^{gh}±0.04	14.20^{ef}±0.21	42.77^{de}±0.28	38.50^{de}±0.29
	10 ml/L	15.84^{gh}±0.26	13.35^{ef}±0.31	40.74^{ef}±0.29	36.38^{e}±0.06
Almond	5 ml/L	29.86^{cd}±0.26	26.35^{c}±0.34	49.63^{bc}±0.29	45.08^{bc}±0.29
	10 ml/L	26.92^{d}±0.21	24.25^{c}±0.30	49.18^{bc}±0.02	44.12^{bc}±0.14
Clove+	5 ml/L	20.65^{ef}±0.31	17.27^{d}±0.38	46.96^{bc}±0.52	43.15^{bc}±0.24
Camphor+ Almond	10 ml/L	18.34^{fg}±0.07	16.38^{de}±0.26	46.19^{cd}±0.26	41.36^{cd}±0.33
S. griseus	100%*	21.29^{ef}±0.02	18.28^{d}±0.22	47.99^{bc}±0.18	43.59^{bcd}±0.27
G. virens	100%	22.19^{e}±0.31	19.74^{d}±0.55	47.53^{bc}±0.24	43.48^{bcd}±0.24
Silicate	2 mM/L	35.20^{b}±0.25	49.69^{b}±0.31	51.19^{b}±0.31	47.04^{b}±0.21
	4 mM/L	32.21^{c}±0.36	49.20^{b}±0.30	50.70^{b}±0.30	46.10^{bc}±0.35
Topas-100	240 ppm/L	10.34^{j}±0.07	8.47^{h}±0.26	31.97^{h}±0.34	29.06^{g}±1.01
Infected only		64.46^{a}±1.18	53.70^{a}±2.36	71.92^{a}±0.73	69.22^{a}±0.63

Culture filtrate

Table 3. Effect of some alternative pesticides on shoot length, leaf area and number of leaves of squash plants cv. Eskandarani hybrid in the two seasons 2014 & 2015

Treatments	Conc.	Shoot length (cm)		Leaf area/plant		Number of leaves/plant	
		1st year	2nd year	1st year	2nd year	1st year	2nd year
Clove	5 ml/L	68.33^{a}±0.22	73.23^{a}±0.34	1505.27^{c}±27.75	1471.33^{c}±26.94	26.28^{bc}±0.36	30.87^{b}±0.17
	10 ml/L	69.50^{a}±0.33	74.60^{a}±0.57	1656.93^{b}±17.37	1622.67^{b}±27.34	29.10^{ab}±0.31	34.02^{a}±0.36
	5 ml/L	65.47^{b}±0.28	67.63^{b}±0.18	1388.30^{e}±26.90	1386.97^{d}±27.50	20.85^{ef}±0.52	26.51^{c}±0.47
Camphor	10 ml/L	65.87^{b}±0.26	68.70^{b}±0.29	1420.53^{d}±27.42	1387.53^{d}±23.78	24.46^{cd}±0.33	29.15^{b}±0.31
	5 ml/L	54.67^{f}±0.45	60.13^{f}±0.37	1138.43^{hi}±26.8	1139.33^{g}±16.40	18.09^{fgh}±0.24	21.22^{ef}±0.21
Almond	10 ml/L	58.80^{e}±0.40	62.03^{e}±0.30	1181.77^{h}±26.96	1181.40^{f}±27.89	18.69^{fg}±0.48	22.17^{de}±0.46
Clove+	5 ml/L	61.47^{cd}±0.23	64.22^{d}±0.26	1344.97^{f}±27.14	1343.73^{d}±27.42	21.72^{ef}±0.48	25.12^{c}±0.52
Camphor+ Almond	10ml/L	62.33^{c}±0.34	66.17^{c}±0.31	1380.77^{e}±15.97	1382.97^{d}±16.06	22.33^{de}±0.19	25.98^{c}±0.24
S. *griseus*	100%*	60.60^{d}±0.35	64.23^{d}±0.18	1275.87^{g}±25.96	1275.37e±23.54	19.23e^{fg}±0.32	24.68^{cd}±0.50
G. virens	100%	59.00^{e}±0.47	61.50^{ef}±0.42	1258.97^{g}±23.52	1258.20^{e}±14.11	18.72^{fg}±0.11	22.48^{de}±0.22
	2 mM/L	52.77^{gh}±0.50	57.07^{gh}±0.88	1113.70^{i}±14.03	1112.63^{gh}±14.0	15.09^{hi}±0.33	19.18^{fg}±0.37
Silicate	4 mM/L	53.70^{fg}±0.90	58.17^{g}±1.22	1114.57^{i}±14.38	1113.50^{gh}±28.6	17.12^{gh}±0.32	21.20^{ef}±0.32
Topas-100	240 ppm/L	69.70^{a}±0.40	74.93^{a}±0.22	1824.73^{a}±27.22	1858.77^{a}±26.97	30.11^{a}±0.32	35.33^{a}±0.36
Infected only		51.90^{h}±1.36	56.93^{h}±0.73	1098.70^{i}±14.97	1092.67^{h}±23.23	13.07^{i}±0.17	17.21^{g}±0.14

*Culture filtrate

Table 4. Effect of some alternative pesticides on plant fresh and dry weights of squash plants cv. Eskandarani hybrid in the two seasons 2014 & 2015

Treatments	Conc.	Plant fresh weight (g)		Plant dry weight (g)	
		1st year	2nd year	1st year	2nd year
Clove	5 ml/L	251.90^{b}±0.74	284.07^{ab}±0.54	40.27^{c}±1.22	47.33^{bc}±0.52
	10 ml/L	265.60^{ab}±0.24	284.63^{ab}±0.35	45.43^{b}±2.29	50.17^{b}±0.80
Camphor	5 ml/L	230.30^{c}±0.45	270.03^{ab}±0.71	35.00^{de}±2.64	44.17^{cd}±0.99
	10 ml/L	233.20^{c}±0.60	283.10^{ab}±0.82	38.00^{cd}±1.17	45.73^{bc}±0.55
Almond	5 ml/L	144.13^{fg}±0.67	164.05^{ef}±0.72	27.53^{g}±0.92	30.53^{hi}±1.01
	10 ml/L	149.33^{f}±0.54	166.03^{ef}±0.73	27.87^{g}±0.92	34.50^{fgh}±0.45
Clove+	5 ml/L	172.77^{d}±0.94	190.21^{cd}±0.64	33.07^{ef}±1.22	37.50^{efg}±0.48
Camphor+ Almond	10 ml/L	175.10^{d}±0.59	195.40^{c}±0.38	34.50^{e}±2.26	40.37^{de}±0.99
S. griseus	100%*	162.10^{de}±0.29	182.12^{cde}±0.47	32.73^{ef}±0.97	42.70^{efg}±0.65
G. virens	100%	152.33^{ef}±0.90	172.43^{def}±0.45	30.20^{f}±2.35	34.53^{fgh}±0.33
Silicate	2 mM/L	134.03^{g}±0.67	153.20^{f}±0.29	20.87^{h}±0.70	33.00^{ij}±0.68
	4 mM/L	140.17^{fg}±0.29	160.67^{f}±0.52	25.27^{g}±2.08	32.53^{ghi}±1.01
Topas-100	240 ppm/L	276.23^{a}±0.47	296.37^{a}±0.30	53.40^{a}±1.28	59.83^{a}±0.62
Infected only		110.97^{h}±0.74	128.10^{g}±0.79	18.67^{i}±0.38	23.97^{i}±1.16

culture filtrate

Table 5. Effect of some alternative pesticides on the number of fruits and weight of fruits of squash plants cv. Eskandarani hybrid in the two seasons 2014 & 2015

Treatments	Conc.	Number of fruits/plant		Weight of fruits/plant (g)	
		1st year	2nd year	1st year	2nd year
Clove	5 ml/L	9.21^{a}±0.24	11.03^{ab}±0.26	460.54^{c}±4.71	585.05^{b}±1.21
	10 ml/L	9.50^{a}±0.07	11.34^{a}±0.05	562.80^{b}±13.62	668.50^{a}±11.70
Camphor	5 ml/L	7.94^{bc}±0.17	9.17^{cd}±0.25	414.80^{de}7. ±45	502.50^{c}±2.31
	10 ml/L	8.54^{b}±0.05	10.04^{bc}±0.01	424.54^{d}±1.66	530.11^{c}±2.35
Almond	5 ml/L	5.91^{def}±0.03	7.33^{fg}±0.03	303.54^{g}±0.27	400.20^{ef}±0.57
	10 ml/L	6.17^{def}±0.06	7.88^{fg}±0.01	300.50^{gh}±0.26	406.21^{ef}±0.47
Clove+	5 ml/L	7.30^{cd}±0.03	9.03^{de}±0.01	373.28^{ef}±1.21	465.83^{d}±0.97
Camphor+ Almond	10 ml/L	7.39^{c}±0.03	9.31^{cde}±0.06	393.58^{e}±2.62	480.95^{d}±2.36
S. griseus	100%*	6.73^{cd}±0.13	8.32^{ef}±0.07	361.61^{f}±2.82	464.06^{d}±1.21
G. virens	100%*	6.33^{de}±0.29	8.18^{ef}±0.34	325.24^{g}±0.70	418.83^{e}±1.32
Silicate	2 mM/L	5.17^{fg}±0.03	7.07^{g}±0.01	278.84^{hi} ±0.51	374.88^{fg}±1.70
	4 mM/L	5.67^{efg}±0.23	7.77^{g}±0.23	299.24^{h}±0.26	383.87^{ef}±0.47
Topas-100	240 ppm/L	9.98^{a}±0.01	11.89^{a}±0.06	600.70^{a}±0.25	691.90^{a}±0.27
Infected only		4.81^{g}±0.06	6.54^{g}±0.03	251.99^{i}±3.55	344.19^{g}±1.54

culture filtrate

Field studies indicated that treatment of cumin seeds with cumin, basil and geranium oils significantly increased plant weight, root weight, number of branches and flowers. This enhancement in the growth of plants could be related to the suppression of pathogenic fungi to enable the plant to grow normally or to direct enhancement of the physiological status of the plant making plants more resistant against pathogens [49]. Deore and Sawant [50] reported that *T. viride*, *T. harzianum, T. hamatum, T. longiflorum* and *T. koningii* cultures recorded beneficial effects on guar growth parameters. Saharan and Nehra [51] reported that as quoted for other beneficial interactions, the plant growth-promoting effects related to Streptomyces–plant interactions can be divided into biofertilization, biostimulation, and bioprotection. The production of indole-3-acetic acid by *Streptomyces* spp. has been confirmed in several reports [52] & [37]. Also, *Streptomyces* species isolated from a marine environment exhibited the ability to produce a range of phyto-hormones, including gibberellic acid, and improved the agronomic performance of eggplant (*Solanum melongena*) by influencing its growth parameters; including weight and length of roots [53]. Biotic inducers (propolis extract and filtrates of *B. subtilis* and *T. harzianum*) increased fruit productivity of cucumber (cv. Delta Star) and increased the

activities of defense-related enzymes (peroxidase, polyphenoloxidase and chitinase), but in varying degrees as compared with the control [54]. The integrated treatment of dipping and soil drenching of soluble potassium silicate at 0.4% was the greatest treatment for increasing onion bulb yields under field conditions [55]. In this respect [56] reported that the presence of Si can improve water-use efficiency and photosynthesis rate in plants. Since Si play an essential role in leaves stability and can expose more leaves to light so, it could cause an increase of plant canopy photosynthesis efficiency [57]. Si can cause leaf development and can cause photosynthesis improvement.

Data presented in Table (6) show that all treatments increased leaf chlorophyll a and b contents after each spray compared to the control treatment, but the treatments of Topas-100, clove + camphor + almond (10 and 5 ml/L), *G. virens* and *S.* griseus filtrates had the highest chlorophyll a content with averages of 4.02, 3.95, 3.79, 3.58 and 3.47 mg/g, respectively. Also the same arrangement cleared in case of chlorophyll b with averages of 3.34, 3.00, 2.98, 2.83 and 2.75 mg/g, respectively. Khalifa et al. [55] reported that the application of potassium silicate was found to be effective in increasing the chlorophyll a, b, carotenoids and total chlorophyll concentrations in onion leaves. Potassium silicate 0.4% treated as dipping and soil drench treatment significantly increased the activity of photosynthetic pigments compared to control. Agarie S [58] reported that Si has a significant effect on the photosynthetic rate and prevent the destruction of chlorophyll. Silicon also increased leaf area extends, that cause more light available for photosynthesis implement, whereas, the silicon shortage can cause reduction of chlorophyll amount in the plant leaves. This could be interpreted as the possible effect of Si on the biosynthesis of new chlorophylls and the protection mechanisms of existing chlorophylls against salinity-induced oxidative stress [59].

From the data presented in Figures (1 & 2) it can be noticed that all treatments used increased the activity of enzymes related to defense (polyphenol oxidase (PPO) and peroxidase (PO). Also, it is clear that with increasing the activity of these two enzymes the disease severity of powdery mildew decreased so; Topsin-100 treatment showed the least disease severity and at the same time had the highest PPO and PO activities with averages of 0.216 and 0.094 Abs/min/g fresh tissue, respectively. At the same time clove oil (10 ml/L) had the second grade followed by clove (5 ml/L), camphor (10 ml/L), camphor (5 ml/L) and clove + camphor + almond (10 ml/L) with averages of 0.198, 0.186, 0.179, 0.165 and 0.154 Abs/min/g fresh tissue, respectively for PPO. Also the same arrangement cleared in case of PO but with light differences where clove oil (10 ml/L) had the second grade followed by clove oil (5 ml/L), clove + camphor + almond (10 ml/L), clove + camphor + almond (5 ml/L) and camphor (10 ml/L) with averages of 0.088, 0.085, 0.069, 0.063 and 0.062 Abs/min/g fresh tissue, respectively. The highest activity of enzymes was linked with decreases of infection with the pathogen of powdery mildew disease. These results are in agreement with the results of [33].

Also from data presented in Figures (3, 4 & 5) it is clear that the activities of pectin methyl esterase (PME), Cx-cellulase and polygalacturonase (PG) enzymes were in parallel with the disease severity where the activity of any enzyme from them increased with the increasing of disease severity (DS). Untreated infected plants had the highest PME, Cx-cellulase and PG with averages of 0.48 ΔAbs min^{-1}, 34.38 mg and 6.156 µmol min^{-1}/g fresh tissue, respectively. At the same time Topsin-100 treatment had the least PME, Cx-cellulase and PG values with averages of 0.27 ΔAbs min^{-1}, 25.73 mg and 4.443% µmol min^{-1}/g fresh tissue, respectively followed by clove oil (10 ml/L), clove oil (5 ml/L), camphor oil (10 ml/L) and camphor oil (5 ml/L) and the same trend cleared with PME and Cx-cellulase enzymes.

In this respect [54] reported that and filtrates of *B. subtilis* and *T. harzianum* reduced the disease severity on the treated leaves with powdery mildew caused by *Sphaerotheca fuliginea* in cucumber (cv. Delta Star), and increased the activities of defense-related enzymes (peroxidase, polyphenol oxidase and chitinase) but in varying degrees as compared with the control. Also [33] exhibited that the higher concentrations of all tested essential oils (ginger, cinnamon and clove oils) caused higher activity of peroxidase and polyphenol oxidase than the lower concentrations. Results also showed that spraying zinnia plants with these oils caused higher activity of peroxidase and polyphenol oxidase enzymes. This means that spraying plants with essential oils gave a defense to plants from invasion with the pathogen. Many investigators explained these results since they reported that peroxidase is known to be involved

in the oxidation of polymerization of hydroxyl cinnamyl alcohols to yield lignin and cross-linking iso dityrosine bridges in the cell wall, peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms [60] [61] [62]. Also, [63] and [64] affirmed that the increase in peroxidase activity enhance lignifications in response to infection with pathogens which may restrict fungal penetration. Studies involving powdery mildew in cucumber, Arabidopsis, wheat and strawberry reported an anticipation of Si in the expression of enzymes associated with the plant's defense system and in the production and accumulation of phenolic compounds and phytoalexins at infection sites [16] [65] [66] [67] [68]. It can be assumed that Si helped stimulate accumulation of polymerized phenolics [69] by stimulating the activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase, because Si is known to play a significant role in phenolic metabolism and the biosynthesis of lignin in cell walls [70].

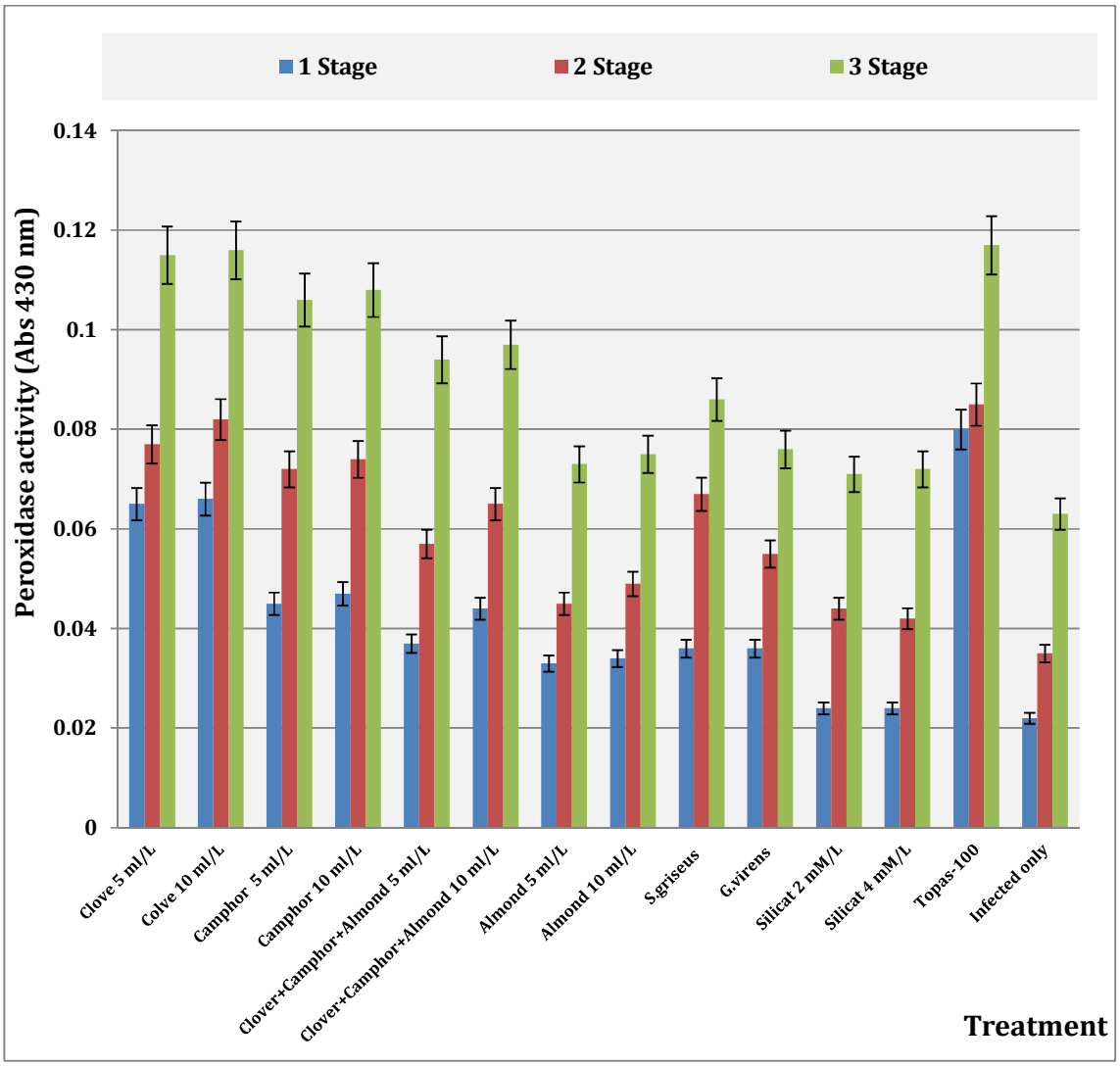

Fig. 1. Effect of some alternative pesticides on peroxidase activity (absorbance @ 430 nm min^{-1}/g fresh weight)

Table 6. Effect of some alternative pesticides on the leaves chlorophyll a and b contents of squash plants cv. Eskandarani hybrid

Treatments	Conc.	Chlorophyll a			Chlorophyll b		
		After 1st spray	After 2nd spray	After 3rd spray	After 1st spray	After 2nd spray	After 3rd spray
Clove	5ml/L	2.97 ^{de}±0.01*	2.18^{d}±0.01	1.19^{e}±0.01	1.99^{f}±0.01	1.82^{e}±0.01	1.42^{c}±0.01
	10ml/L	2.53^{f}±0.28	2.95^{b}±0.03	1.25^{e}±0.02	2.48^{e}±0.03	1.94^{e}±0.02	1.34^{c}±0.05
Camphor	5ml/L	3.25^{cd}±0.04	2.75^{bc}±0.02	1.96^{cd}±0.03	2.65^{de}±0.05	2.55^{cd}±0.02	1.95^{bc}±0.04
	10ml/L	3.45^{c}±0.02	2.81^{b}±0.03	2.10^{c}±0.09	2.77^{d}±0.03	2.69^{c}±0.03	1.94^{bc}±0.02
Clove+ Camphor+ Almond	5ml/L	4.87^{a}±0.04	3.77^{a}±0.05	2.72^{b}±0.05	3.22^{b}±0.01	3.14^{b}±0.01	2.57^{ab}±0.10
	10ml/L	4.88^{a}±0.04	4.02^{a}±0.07	2.96^{ab}±0.10	3.38^{b}±0.06	3.21^{b}±0.05	2.41^{ab}±0.16
S. griseus	100 %**	4.17^{b}±0.01	3.55^{a}±0.14	2.68^{b}±0.09	2.94^{c}±0.05	2.83^{c}±0.02	2.49^{ab}±0.15
G. virens	100 %	4.22^{b}±0.01	3.62^{a}±0.13	2.89^{b}±0.00	3.20^{b}±0.01	3.12^{b}±0.01	2.17^{ab}±0.01
Almond	5ml/L	3.14^{cd}±0.01	2.65^{bc}±0.08	1.22^{e}±0.03	2.54^{de}±0.02	2.32^{d}±0.05	1.56^{c}±0.02
	10ml/L	3.33^{cd}±0.01	2.86^{b}±0.07	1.76^{d}±0.15	2.66^{de}±0.02	2.55^{cd}±0.02	1.60^{c}±0.04
Silicate	2mlM/L	2.75^{ef}±0.02	2.25^{cd}±0.02	1.21^{e}±0.00	1.66^{g}±0.02	1.49^{f}±0.09	0.95^{d}±0.01
	4mlM/L	3.11^{cd}±0.00	2.76^{bc}±0.04	1.82^{d}±70.39	1.76^{fg}±0.02	1.67^{ef}±0.06	1.35^{c}±0.02
Topas-100	240ppm/L	4.98^{a}±0.00	3.89^{a}±0.04	3.18^{a}±0.14	3.70^{a}±0.13	3.61^{a}±0.03	2.72^{a}±0.12
Infected only		2.14^{g}±0.01	1.89^{e}±0.04	1.10^{e}±0.02	0.99^{h}±0.01	1.28^{g}±0.25	0.77^{d}±0.26

mg /g fresh weight
***culture filtrate*

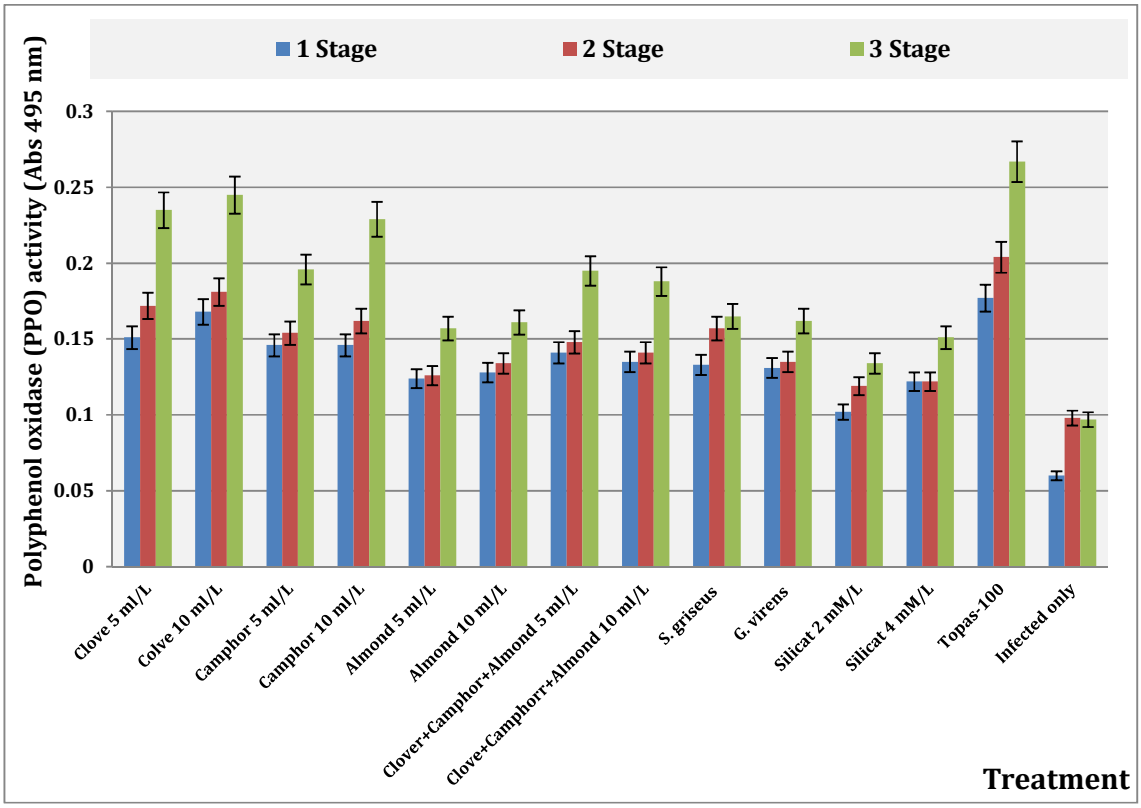

Fig. 2. Effect of some alternative pesticides on polyphenol oxidase activity (absorbance @ 495 nm min^{-1}/g fresh weight)

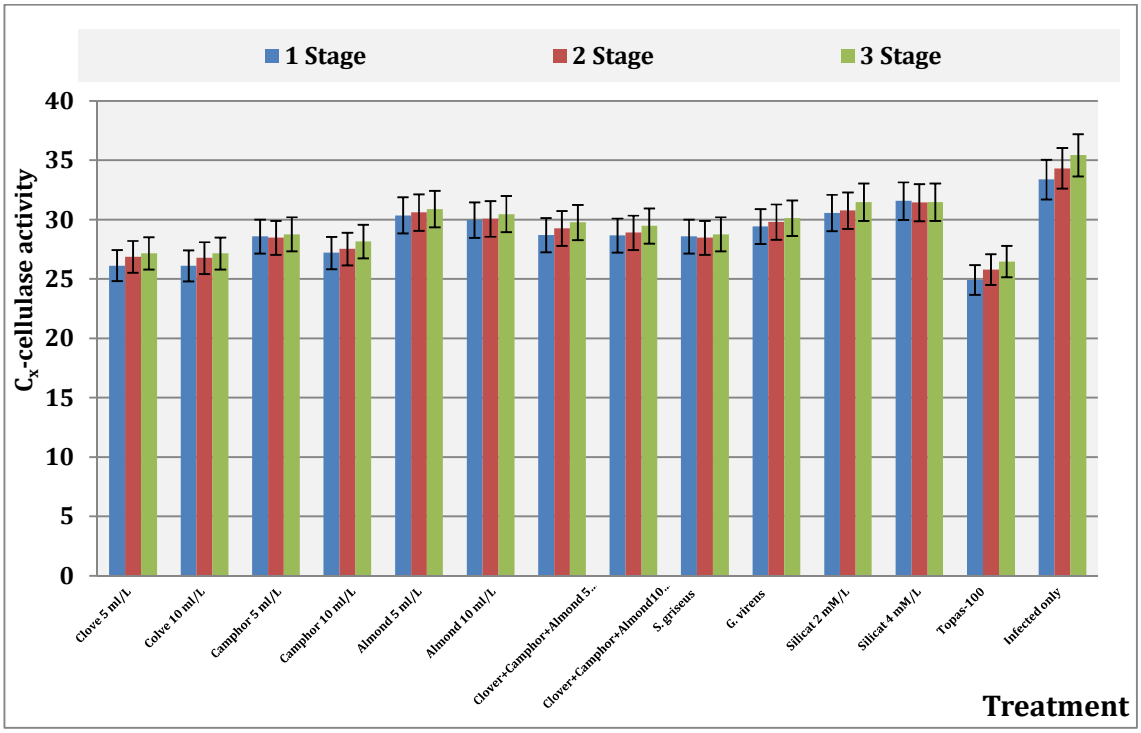

Fig. 3. Effect of some alternative pesticides on Cx-cellulase/g fresh weight

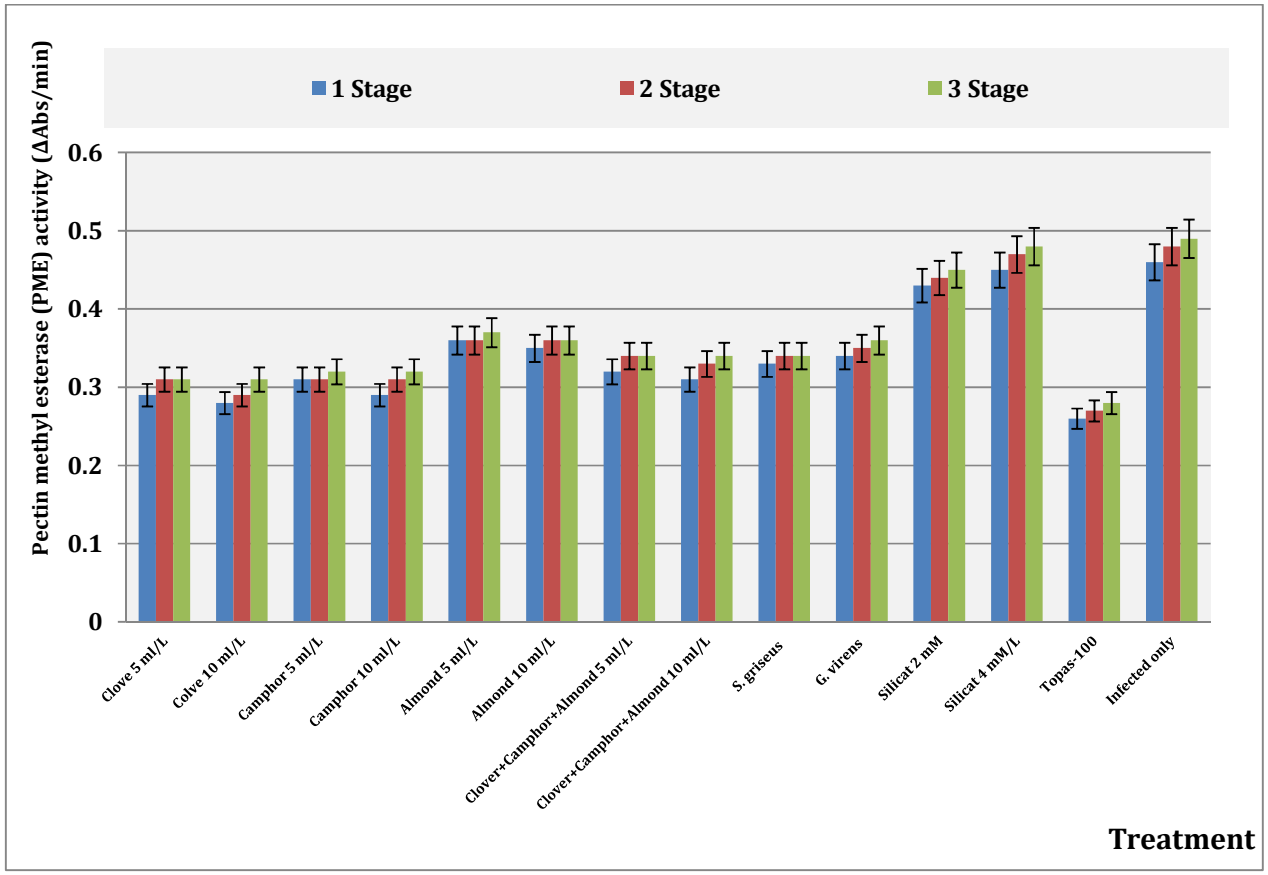

Fig. 4. Effect of some alternative pesticides on pectin methyl esterase/g fresh weight

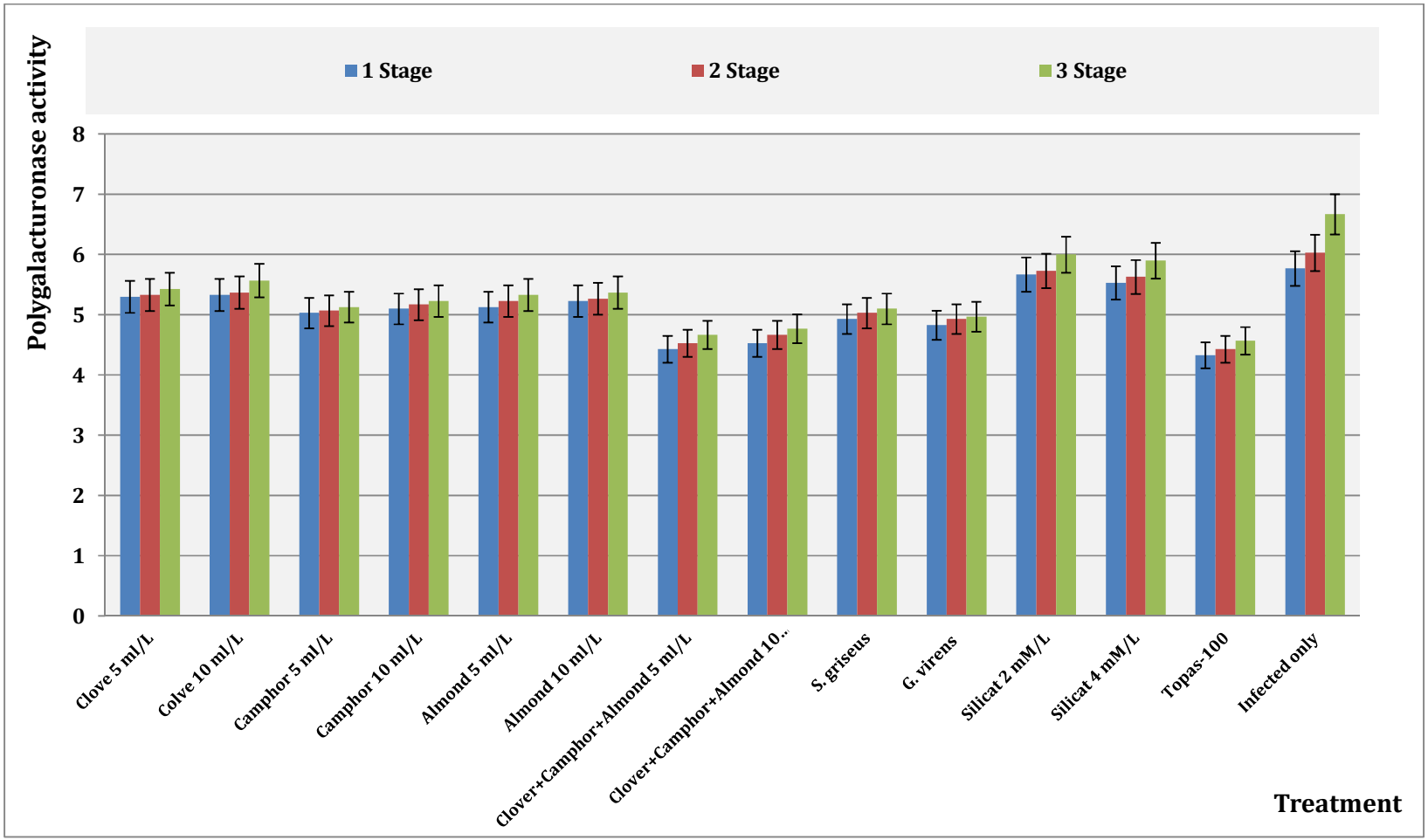

Fig. 5. Effect of some alternative pesticides on polygalacturonase/g fresh weight

4. CONCLUSIONS

The present evaluation clearly indicated that camphor oil was the most effective treatment in reducing powdery mildew disease severity of squash *via* inhibited completely conidial germination after 72 h with an average of 11.43% compared to the positive control treatment with Topas-100. Camphor oil gave the highest chlorophyll a and b contents and increasing the activity of defense-related enzymes.

Nevertheless, it could be concluded that the use of the camphor oil, showed the highest antagonistic activity against *Erysiphe cichoracearum* and powdery mildew disease of squash (cv. Eskandarani hybrid). Therefore, crude oil of camphor could be proposed as an effective, safe and efficient fungicide in biological control of powdery mildew disease of squash under an open field condition in Egypt that is environmentally friendly and safe to farmers and users of the product.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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