ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 6, December 2013

Fungicides Alternatives as Plant Resistance Inducers against Foliar Diseases Incidence of Some Vegetables Grown under Plastic Houses conditions

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Abstract - Evaluating the efficacy of fungicides alternatives treatments as plant resistance inducers against foliar diseases incidence was carried out as spray treatments on growing cucumber, tomato and pepper plants under commercial plastic houses at Dokki and Haram locations, Giza governorates. The evaluated foliar spray treatments were applied four times with fifteen days intervals starting at thirty days after transplanting. The obtained results revealed that plant spray with treatments, [Calcium chloride (20mM) + S. cerevisiae $10x10^{10}$cfu/mL (10ml/L) + Chitosan (0.05mM)] and [Potassium bicarbonate (20mM) + Thyme oil (5ml/L)] resulted in the highest reduction in foliar diseases, powdery and downy mildews as well as early and late blight diseases incidence and severity of cucumber, pepper and tomato plants and increased produced yield as well. Moreover, the obtained results revealed that the plants applied with resistance inducers treatments gave the best increase in the total protein contents as well as the activity of Peroxidase, Polyphenol oxidase, Phenylalanine ammonia-lyase, chitinase and ß-1,3-Glucanase enzymes which escalating defense response for diseases resistance in sprayed cucumber, pepper and tomato plants. On the light of the present study it could be suggested that the usage of combined application of the bio-agent S. cerevisiae and/or resistance plant inducers might be used as easily applied, safely and cost effective control methods against such foliar plant diseases. Successful development of such compounds as antifungal would not only provide a potent tool for control of vegetables foliar diseases, but also could promise success in multipurpose bio-rational alternatives to conventional fungicides for the management of other plant diseases.

***Index Terms*—Cucumber, downy mildew, early blight, late blight, pepper, plant resistance inducers, plastic houses, powdery mildew, tomato.**

I. INTRODUCTION

Growing vegetables under protected cultivation system is an important practice in Egypt [1]. Powdery and Downy mildews are the most serious foliar diseases attacked cucumber and pepper plants grown in plastic houses. Powdery mildew disease is one of the most serious plant diseases, causing large yield losses in a number of crops [2]. Early and Late blights caused by *Alternaria solani* and *Phytophthora infestans* are the most important diseases attacking potato plants [3,4,5,6]. Control of these diseases depends mainly on fungicidal treatments. In order to avoid the environmental pollution fungicide alternatives are needed [7,8]. A successful disease-control program could involve just a single practice, but the long term reduction of disease losses generally requires the application of several control measures. The best way to ensure success of a disease- management program is to use integrated disease-control measures [9]. Some chemicals were reported as resistance inducers against plant diseases. Several new agrochemicals are in developments that have activity on the pathogens that cause downy mildew diseases. Economical control depends on establishing an overall disease management system for the entire farm. Keeping careful records of the crops planted, the problems encountered, and the pesticides alternatives used are important. Generally, IPM is regarded as the use of environmentally safe practices to reduce the disease incidence and development or use of multiple control tactics integrated into a single pest control strategy [10]. For example, different natural products, *i.e.*, biocontrol agents, plant extracts and natural compounds were used as an IPM program to control powdery mildew of greenhouse crops [9,11]. Since economic thresholds have not been established for most plant pathogens, an IPM takes a somewhat different approach in plant disease control. Salts have been previously studied as foliar applied control agents for powdery mildews on various horticultural crops. In this regard, it was found that potassium bicarbonate applications were effective in reducing the severity of powdery mildew on *E. japonica* and pumpkin [12,13]. In pot experiment, under artificial infestation with pathogenic fungus, application of sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity [14]. They added that Calcium chloride proved higher efficacy for reducing both disease incidence and severity than that of sodium bicarbonate when applied either alone or combined with *Saccharomyces cerevisiae*. Moreover, Chiosan, in recent years, the importance of chito-saccharides as plant growth promoting and disease control agents has been emphasized [15,16]. Chitosan has been shown to induce defense responses in different plants [17,18]. Also, Chitosan oligomers was found to induce defense responses in grapevine leaves and a stimulation of chitinase and *ß*-1,3-glucanase activities [16]. These findings encouraged us to evaluate the potential use and the efficacy of foliar sprays of single or integrates of natural compounds as biological control (*S. cerevisiae*), mineral salt ($CaCl_2$, K_2HPO4), resistance chemical inducers (Chitosan) and essential oil (Thyme) on grown vegetables to provide acceptable control level of powdery and downy mildews under greenhouse conditions. Therefore, the main objective of the present study was foliar diseases control with eco-friendly

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 6, December 2013

environment by investigating the efficacy of foliar spray with some plant resistance inducers treatments under commercial plastic houses conditions. Thus, the present study was carried out to manage foliar diseases using fungicides alternatives and to assess the induction of different defense enzymes in treated vegetable plants in response to the fungicides alternatives application

II. MATERIALS AND METHODS

This experiment was carried out on growing vegetable under protected cultivation system in commercial plastic houses of Ministry of Agriculture and Soil Reclamation, A.R.E. at Dokki and Haram locations. The cultivated vegetables were Cucumber (at Dokki plastic house location); Tomato and Pepper (at Haram plastic house location). The cultivated vegetables received traditional agriculture practices, *i.e.* irrigation, fertilizers, etc. Different plant resistance inducers were sprayed 3 times with 15 days intervals after transplanting [19]. The tested fungicide alternatives as plant resistance inducers were as follows:

- Calcium chloride (20mM) + *S. cerevisiae* ($10x10^{10}$cfu/mL) + Chitosan (0.05mM)
- Potassium bicarbonate (20mM) +Thyme oil
- Control (fungicide treatment)

(A) Plastic houses experiment:

Assessment of foliar diseases incidence and severity

The experimental plastic house consists of 5 rows, each (0.9 x 60m, width x long) divided into 3 parts 20m long each, and every part considered as one replicate. Five replicates were used for each particular treatment in complete randomized design. The growing vegetables were sprayed with proposed treatments 3 times with 15 days intervals after transplanting [19]. At all locations, the growing vegetables in the experimental plastic houses received only the recommended pesticides against harmful insects, *i.e.* aphids, trips, white fly, etc. as needed. Meanwhile, only the check control received traditional programs for controlling foliar diseases which recommended by the follow up committee of Protected Cultivation Administration Office, Ministry of Agriculture and Soil Reclamation. Monitoring and scouting of foliar diseases incidence, *i.e.* powdery and downy mildews of cucumber and pepper and early and late blights of tomato were recorded till the end of growing season. Percentages of disease incidence and severity were recorded at 120 days of transplanted date. Accumulated obtained yield was also recorded.

Disease assessment:

- **Disease incidence:**

Percentage of each foliar disease incidence was recorded as the number of diseased plants relative to the number of growing plants for each treatment, then the average of disease incidence was calculated.

- **Disease severity:**

Percentage of each foliar disease severity was recorded as following equation:

$$D.S.\% = \frac{\Sigma (n \times c)}{N} X100$$

Whereas: D.S. = Disease severity %
n = Number of infected leaves per category
c = Category number
N= Total examined leaves

Disease severity scale from 0 to 4 according to [20] was followed, whereas: 0 = No leaf lesions; 1 = 25% or less; 2 = 26-50 %; 3 = 51-75 %; and 4 = 76-100% infected area of plant leaf.

At the end of growing season the accumulated yield was calculated for each particular treatment in both experimental and control treatment.

(B) Laboratory tests:

Assess the induction of different defense enzymes:

Determination the activity of different enzymes responsible for diseases resistance in sprayed vegetable plants with plant resistance inducers (fungicides alternatives) grown under natural protected plastic houses was carried out. Determination of protein content, and the activity of peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, chitinase and β-1,3-Glucanase enzymes was determined in sprayed and control plants as follows:

- **Extraction of total proteins:**

Cucumber, tomato and pepper leaves were collected 10 days after inducers application and proteins were extracted according to [21]. One gram fresh weight was ground in a mortar with pestle containing liquid nitrogen. The resulting powder was macerated for 30 sec in 3 ml extraction buffer [50 mM sodium phosphate buffer, pH 6.5. One mM phenylmethylsulfonyl fluoride (PMSF)], then centrifuged at 20,000 g for 25 min at 4 °C. The supernatant was divided in different parts and kept in ice at -20 °C for the following determination.

- **Determination of protein content:**

The protein content was determined according to [22] with the coomassie brilliant blue G-250 as protein assay reagent. 500 µl of protein assay reagent added to 500 µl of distilled water containing the protein sample.

After mixing, the absorbance was recorded at 595 nm within one hr against a blank control in 1 cm light path cuvette using Shimadzu UV-2401 PC UV-Vis recording spectrophotometer (Molecular Biology Lab., NRC). A standard curve was constructed by using Bovine serum albumin (BSA) as standard protein (Fig.1).

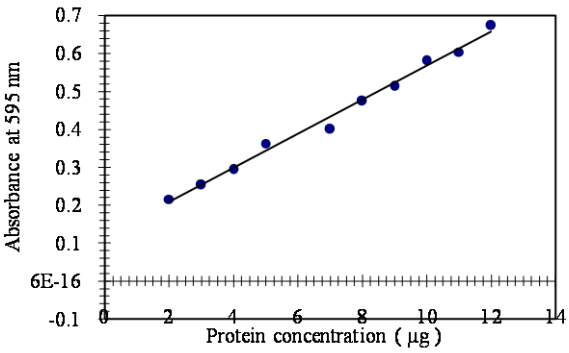

Fig. (1) Standard curve of the protein concentration using bovine serum albumin as a standard protein

- **Kinetics Determination of enzymes:**

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
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The activity of all enzymes was determined spectrophotometric as unit/1 gm fresh weight by measuring the change in absorbance at different weave lengths refers to enzymes using Shimadzu UV-2401 PC UV-Vis recording spectrophotometer (Molecular Biology Lab., NRC).

Spectrophotometric determination of peroxidase activity

Peroxidase activity is routinely assayed by measuring the change in absorbance at 470 nm due to guaiacol oxidation in the presence of hydrogen peroxide and the enzyme was assayed every 30 sec intervals using Shimadzu UV-2401 PC UV-Vis recording spectrophotometer in a 1 ml light path cuvettes. The reaction miture (unless otherwise stated) contained in a volume of 3 ml : 8 µmoles hydrogen peroxide, 60 µmoles guaiacol, 50 µmoles sodium acetate buffer, pH 5.6 and peroxidase at concentrations which gave a linear response over a period of 3 min. The reaction was initiated by introducing the enzyme and mixing, all assays were carried out at 25 °C. A unite of peroxidase activity was defined as that amount of enzyme which cause one O.D. change per minute [23].

Spectrophotometric determination of polyphenol oxidase activity

Polyphenol oxidase activity was determined by measuring the initial rate of quinine formation, as indicated by an increase in absorbance at 420 nm, [24]. One unite of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min. The sample cuvette contained 2.95 ml of 20 mM Catechol solution in 0.1 M phosphate buffer, pH 6.0 and 0.05 ml of the enzyme solution. The blank sample contained only 3 ml of substrate solution.

Spectrophotometric determination of phenylalanine ammonia-lyase activity

Phenylalanine ammonia-lyase activity was determined according to [25]. Two hundred µL of sample crude extract was transferred to a 3 ml light path cuvettes containing 2.5 ml of 0.03 M L-Phenylalanine dissolved in 0.05 M sodium borate buffer, pH, 8.8. The reaction mixture was then incubated in controlled water bath at 37 °C for 1 hr, after cooling the unit of phenylalanine ammonia-lyase activity was calculated as one O.D. change per minute at 290 nm.

Spectrophotometric determination of chitinase activity

The chitinase activity was determined by colorimetric method of [26] by using colloidal chitin as a substrate and dinitrosalicylic acid as reagent to measure reducing sugars. The colloidal chitin substrate was prepared from chitin powder as the method described by [27], by milting 25 gm of chitin and then suspended in 250 ml of 85 % phosphoric acid and kept at 4 °C for 24 hr. The mixture was blended in 2 L of distilled water and then centrifuged at 2500 g for 20 min. This washing step repeated twice and the colloidal chitin suspension in the final wash was adjusted to pH, 7.0 and collected by centrifugation and stored at 4 °C. Chitinase activity was assayed in test tube containing the reaction mixture consists of 1 ml of 1 % colloidal chitin in 50 mM sodium acetate buffer pH, 6.6 and 1 ml sample. The mixture was incubated for 1 hr in controlled water bath at 37 °C and centrifuged 2500 g for 20 min. the concentration as mM of N-acetyl-glucose amine (NAGA) in the supernatant was determined using absorbance at 540 nm and the unit of chitinase activity was calculated by the expressed N-acetyl-glucose amine equivalent released/gram fresh weight tissue/60 minutes.

Spectrophotometric determination of β-1,3-Glucanase activity

The enzyme solution (100 µL) was mixed with 200 µL of 0.2 % (w/v) laminarin (Sigma, USA) dissolved in 0.1 M sodium phosphate buffer, pH 6.0 and incubated at 30 °C for 30 min. The reaction was terminated by adding dinitrosalicylic acid solution and boiling the reaction mixture for 5 min. The absorbance at 540 nm was measured and the unit was defined as the amount of the enzyme that released reducing sugar equivalent to 1 µg glucose per min under the above conditions [28].

Statistical analysis

All carried experiments in plastic house experiments were set up in Completely Randomized Design (CRD). The data collected were analyzed by MSTAT-C program [29]. The means differences were compared by Least Significant Difference test (LSD) at 5% level of significance. Moreover, analysis of variance (ANOVA) test was used to analyze some other obtained data. General Linear Model option of the Analysis System SAS [30] was used to perform the analysis of variance. Duncan's Multiple Range Test was used for means separation [31].

The statistical analysis procedures were kindly carried out by Statistical Consulting Office, National Research centre, Egypt.

III. RESULTS AND DISCUSSION

(A) Plastic houses experiment:

Assessment of foliar diseases incidence and severity

Powdery and Downy mildews as well as early and late blights are the most serious foliar diseases attacked cucumber, pepper and tomato plants grown in plastic houses. Traditionally, the controls of the foliar diseases have been done with the use of resistant cultivars, seeds free of pathogen and fungicides. The last one, at a short time, has it's advantages, but for a long period of time, can cause problems due the residues accumulation and environmental pollution [10]. Thus, with the objective to find new technologies, ecologically or environmentally safer, for the control of plant diseases, mainly in organic growth, alternative methods for the control of phytopathogens are been development. This kind of alternative methods are been investigated by our 'Biological and Alternative Control of Plant Diseases' research group [32, 33]. Under natural conditions, the growing cucumber, pepper and tomato in plastic houses treated with fungicides alternatives spray comparing with traditional fungicides for the purpose of controlling foliar diseases. The growing plants received two treatments, [calcium chloride + *S. cerevisiae* + chitosan] and [potassium bicarbonate + thyme oil] as fungicides alternatives. The obtained results in Table (1) showed the Downy, Powdery mildews and early, late blights incidence of grown cucumber, pepper and tomato in plastic houses at Dokki and Haram locations. Presented data revealed that all applied treatments have positive effect on foliar diseases incidence comparing with control. Announced highly significant effect of applied treatments resulted in reduction

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
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in diseases incidence. Percentage of both Downy and Powdery mildews incidence at 120 days of growth were 12.2, 11.4% ; 10.2, 11.4% and 18.4, 14.2% ; 12.4, 9.6% for cucumber and pepper plants at applied treatments, [Calcium chloride + *S. cerevisiae* + Chitosan] and [Potassium bicarbonate + Thyme oil] comparing with 58.8,57.8% and 39.6,38.6% in control treatment, in respective order. The illustrated data in Fig (2) showed that the highest reduction in disease incidence calculated as 79.2, 82.3% and 53.5, 67.8% for Downy and Powdery mildews of cucumber and pepper plants and 85.7, 90.5% of tomato plants at the applied treatment, [Calcium chloride + *S. cerevisiae* + Chitosan]. Meanwhile, treatment [Potassium bicarbonate + Thyme oil] caused reduction in Downy and Powdery mildews of cucumber and pepper calculated as 80.6, 80.2% and 64.1, 75.1% as well as 76.7, 72.3% of tomato plants at growth periods of 120 day, respectively. Furthermore, the applied treatments showed significant suppressive effect on the Downy, Powdery mildews of cucumber, pepper and early, late blights of tomato comparing with control (Table 2). Presented data revealed an drastic suppressive effect recorded as 0.4, 0.4% ; 1.2, 0.8% as well as 0.2, 0.4% disease severity (DS) when the treatment [Calcium chloride + *S. cerevisiae* +Chitosan] was applied on cucumber and pepper and tomato plants against downy, Powdery mildews and early, late blights diseases development. As for applied treatment, [Potassium bicarbonate + Thyme oil] the recorded disease severity (DS) of downy, Powdery mildews and early, late blights diseases development were 0.4, 0.5% ; 0.0, 0.6, 0.5% and 0.4, 0.4%, respectively. Meanwhile, at control treatment the disease severity (DS) of downy, Powdery mildews and early, late blights on cucumber and pepper and tomato plants were recorded as 2.4, 2.2% ; 2.8, 2.1% and 1.6, 2.3%, respectively.

Table (1) Percentage of foliar diseases incidence in response to application of different formula against foliar diseases of cucumber, Pepper and tomato grown in plastic houses under protected cultivation system

Treatment	Cucumber		Pepper		Tomato	
	Downy mildew	Powdery mildew	Downy mildew	Powdery mildew	Early blight	Late Blight
Calcium chloride + *S. cerevisiae* + Chitosan	12.2 c	10.2 c	18.4 b	12.4 b	3.3 c	7.4 b
Potassium bicarbonate +Thyme oil	11.4 c	11.4 c	14.2 c	9.6 c	5.4 b	7.8 b
Control	58.8 a	57.8 a	39.6 a	38.6 a	23.2 a	28.2 a

Mean values within each column followed by the same letter are not significantly different ($P \leq 0.05$).

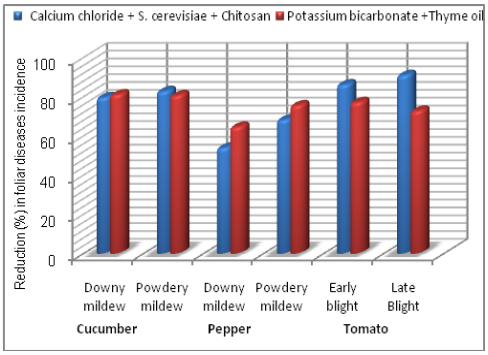

Fig. (2) Reduction (%) in foliar diseases incidence in response to application of different formula against foliar diseases of cucumber, Pepper and tomato grown in plastic houses under protected cultivation system

Table (2) Percentage of foliar diseases severity in response to application of different formula against foliar diseases of cucumber, Pepper and tomato grown in plastic houses under protected cultivation system

Treatment	Cucumber		Pepper		Tomato	
	Downy mildew	Powdery mildew	Downy mildew	Powdery mildew	Early blight	Late Blight
Calcium chloride + *S. cerevisiae* + Chitosan	0.4 d	0.4 b	1.2 b	0.8 c	0.2 c	0.4 c
Potassium bicarbonate +Thyme oil	0.4 d	0.5 b	0.6 c	0.5 c	0.4 c	0.4 c
Control	2.4 a	2.2 a	2.8 a	2.1 a	1.6 a	2.3 a

Mean values within each column followed by the same letter are not significantly different ($P \leq 0.05$).

On the other hand, disease severity of downy and Powdery mildews of cucumber and pepper plants dramatically reduced in response to chemical plant resistance inducers as foliar application. Illustrated data presented in Fig. (3) showed high suppress in downy and powdery mildew diseases severity recorded at 120 day of plant growth as 83.3, 81.8% and 57.1, 61.9% for both diseases at the applied treatment, [Calcium chloride + *S. cerevisiae* + Chitosan], [Chitosan + Thyme oil] as well as 83.3, 77.2% and 78.5, 78.1% for both diseases at the applied treatment, [Potassium bicarbonate +Thyme oil], for cucumber and pepper plants in respective order. Also, data in Fig (3) show that reduction in early and late blights diseases severity of tomato recorded as 87.5, 82.6% and 75.0, 82.6% at applied treatments, [Calcium chloride + *S. cerevisiae* + Chitosan], [Chitosan + Thyme oil] and [Potassium bicarbonate +Thyme oil], respectively. Application of different formula of fungicides alternatives as plant resistance inducers as foliar spray resulted in reduction of foliar diseases incidence and severity which reflected positively in plant stand and its healthy growth as well as its yield. The obtained yield of cucumber and pepper plants in response to foliar application with different formula in plastic houses under protected cultivation system (Dokki and Haram locations) was presented in Table (3). Presented data revealed that the highest recorded accumulated yield 1.417 and 1,018 Ton/ plastic house was obtained from plants

ISSN: 2277-3754
ISO 9001:2008 Certified
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sprayed with [Calcium chloride + *S. cerevisiae* + Chitosan] followed by 1.378 and 0.968 Ton/ plastic house for plants sprayed with the treatment, [Potassium bicarbonate +Thyme oil], respectively. Meanwhile, the yield of control plants was recorded as 1,063 and 0.786 Ton/ plastic house. As for Tomato plants grown in plastic house at Haram location, the accumulated yield (Table 2) was recorded as 1,713 and 1,622 Ton/ plastic house for the applied treatments, [Calcium chloride + *S. cerevisiae* + Chitosan], [Potassium bicarbonate +Thyme oil], respectively. Meanwhile, the yield of control plants was recorded as 1,234 Ton/ plastic house.

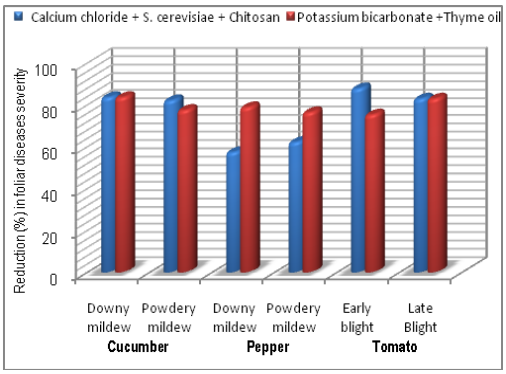

Fig. (3) Reduction (%) in foliar diseases severity in response to application of different formula against foliar diseases of cucumber, Pepper and tomato grown in plastic houses under protected cultivation system

Illustrated data by Fig. (4) Represent the increase (%) in the yield of cucumber, pepper and Tomato in response to foliar application with different formula of chemical plant resistance inducers in plastic houses under protected cultivation system at Dokki and Haram locations. At Dokki location, the accumulated cucumber yield increased over control plants by 33.3 and 29.5% at applied treatments, [Calcium chloride + *S. cerevisiae* + Chitosan], [Potassium bicarbonate +Thyme oil], respectively. Moreover, at Haram location the applied treatments at the same previous order caused an increase in accumulated obtained yield over control of pepper and tomato plants calculated as 29.5, 23.0% and 44.6, 36.9%, respectively.

Table (3) Obtained yield of cucumber, pepper and tomato in response to foliar application of different formula in plastic houses under protected cultivation system

Treatment	Cucumber		Pepper		Tomato	
	Average Yield Kg/row	Yield Ton/plastic house	Average Yield Kg/row	Yield Ton/plastic house	Average Yield Kg/row	Yield Ton/plastic house
Calcium chloride +*S. cerevisiae* +Chitosan	283.4 c	1,417	203.6 c	1,018	342.6 c	1,713
Potassium bicarbonate +Thyme oil	275.5 b	1,378	193.5 b	0.968	324.4 b	1,622
Control	212.6 a	1,063	157.2 a	0.786	236.8 a	1,234

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).

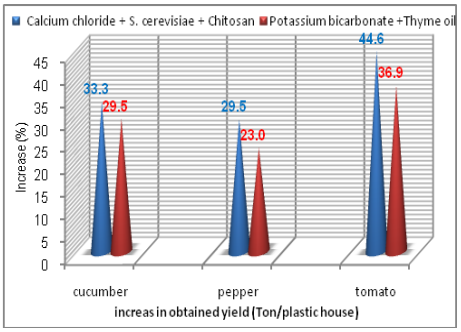

Fig. (4) Yield increase (%) of cucumber, pepper and tomato in response to foliar application of different formula in plastic houses under protected cultivation system

A successful disease-control program could involve generally requires the application of several control measures [9]. Generally, IPM is regarded as the use of environmentally safe practices to reduce the disease incidence and development or use of multiple control tactics integrated into a single pest control strategy [10]. The use of different approach in plant disease control, i.e., bio-control agents, plant extracts and natural compounds were used as an IPM program to control powdery mildew of greenhouse crops [9,11]. Among other control measures the use of compound that induce a systemic plant resistance which were successfully used against several plant diseases incidence affected either plant root or shoot systems. There are several fungicides alternatives commercially used for induction of plant resistance against viruses, bacteria, and fungal infections. In this concern, salts have been previously studied as foliar applied control agents for powdery mildews on various horticultural crops, *e.g.* cucumber, grape, nectarine, mango, and rose can be reduced through foliar applications of phosphate and potassium salts [34,35]. Also, reducing the severity of powdery mildew on *E. japonica* and pumpkin is achieved by application with potassium bicarbonate [12,13]. Sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity of tomato plants in pot experiment under artificial infestation with pathogenic fungus [14]. They added that Calcium chloride proved higher efficacy for reducing both disease incidence and severity than that of sodium bicarbonate when applied either alone or combined with *Saccharomyces cerevisiae*. Induction of local and systemic resistance to powdery mildew in cucumber using phosphate foliar applications [35, 36]. Moreover, using calcium administered as plant nutrient has been reported to be important for resistance to bacterial wilt [37]. The use of plant resistance inducers in combination with bio-agents was subjected to evaluation in many reports. In this regards, an interesting alternative to fungicide application for plant disease control involves the use of some organic and inorganic salts with antimicrobial properties generally used in food processing and preservation. Selected organic and inorganic salts are active antimicrobial agents and have been widely used in the food industry. Many of these salts are effective against a range of micro-organisms; most of them have low mammalian toxicity and therefore have potential for postharvest disease control. Salt treatments can inhibit plant pathogens or suppress mycotoxin production [38,39].

ISSN: 2277-3754
ISO 9001:2008 Certified
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Also, [40] found that from field experiments, that spraying cantaloupe plants three times with fungicides in alternation with another three sprays with any of calcium chloride or salicylic acid resulted in significant reduction in the disease severity with significant increase in the fruit yield when compared with unsprayed (check) plants. Similar reports conducted the efficacy of chemical inducers application individual or combined with bio-agents against plant pathogens were cited in literature. The use of sodium bicarbonate alone to control postharvest decays of fruit has its limitations [41], but it can be combined with other alternative treatments to synthetic fungicides, resulting in the control that is superior to individual treatments alone. For example, sodium bicarbonate was successfully used in combination with bacterial and yeasts bio-control agents to enhance control of postharvest decays on citrus, pome, and stone fruits [42,43]. These reports are clearly demonstrated in the present study and show that the application of *S. cerevisiae* enhanced the control of foliar vegetables diseases when combined with either calcium chloride spray. Many researchers have shown that calcium plays an important role in the inhibition of postharvest decay of fruits [44,45], and in enhancing the efficacy of postharvest bio-control agents [46,47]. In the USA, found that foliar-applied Ca was found to enhance both disease control and dry bean yield [48]. Also, [49] suggested that Ca may be a nutritional supplement that increases plant resistance to white mold. Another record, [50] stated that incidence and severity of white mold on dry bean were significantly reduced with application of calcium chloride and calcium silicate. As for Chiosan, in recent years, the importance of chitosaccharides as plant growth promoting and disease control agents has been emphasized [15,16]. CHN (β-1-4 linked *N*-gluco-samine) has been shown to induce defense responses in different plants [17,18]. Chitosan oligomers was found to induce defense responses in grapevine leaves, as evidenced by an accumulation of stilbene phytoalexins, *trans*- and *cis*-resveratrol, ε-viniferins, and piceids, and a stimulation of chitinase and *β*-1,3- glucanase activities [15]. They added that the combination of Chitosan and CuSO4 increased phytoalexin production. This elicitor capacity of Chitosan and/or $CuSO_4$ appeared to be associated with an induced protection of grapevine leaves against gray mold and downy mildew diseases. Also, Chitosan enhanced the accumulation of pathogenesis related-proteins such as ss-1,3-glucanase, chninase and PR14 in treated and upper untreated tomato leaves [51]. Their studies with chitosan against tomato late blight suggested thatchitosan displays dual effects: (a) direct interference in developmental stages of *P. infestans* and (b) by lesion formation, leading to disease resistance mechanisms. Moreover, several workers suggested two different mechanisms of chitosan molecule and target microorganism interaction: the first is the adsorption of chitosans to cell walls leading to the cell wall covering, membrane disruption and cell leakage; the second is the penetration of chitosans into living cells leading to the inhibition of various enzymes and interference with the synthesis of mRNA and proteins [52,53]. Essential oils as natural alternatives that are user friendly and demonstrate low toxicity to humans are desirable to be tested either alone or in combination in the present work. Thyme oil applied in combination showed effective reduction in foliar diseases incidence. In this regards, several investigators reported the antifungal effect of essential oils. Thyme and Egyptian geranium oils are considered antimycotic natural compounds may be useful for inhibition of mold fungi on wood in service or during storage of building materials [54]. Moreover, [55] had the first report on the use of Thymol for controlling a plant disease under field conditions, which indicated that this compound pro-vided effective control of bacterial wilt on susceptible tomato cultivars. Also, Thymol has been reported to have fungicidal activities and fumigation with thymol has been used for control of postharvest fungal diseases [56, 57]. Modes of action of the antibacterial property of thymol appeared to include disruption of bacterial cell membrane integrity by altering protein reactions [58, 59]. The use of fungicides alternatives such as induces resistance compounds and bio-agent microbes to control plant disease and enhance crop production are desirable for the following reasons: 1) chemical pesticides are being severely restricted; 2) the public is demanding reduced pesticide use; and 3) pesticides alternatives are effectively and more safely used. Therefore, in the present study, analysis of plants response towards fungicide alternatives was carried out using induction of several key marker enzymes associated with plant defense mechanism. Some defenses are constitutive, such as various pre-formed antimicrobial compounds, whereas others activated by elicitors recognition. Recognition factors by host plant starts one or more signal transduction pathways that activate several of plant's defenses, thus inhibiting ability of pathogen to colonize plant [60,61]. Response of treated cucumber, pepper and tomato plants was evaluated in terms of induction of defense-related marker enzyme activity, namely, peroxidase (POX), polyphenol oxidase (PPO), ß-1,3 glucanase and chitinase. This evaluation is stated in the following second part of the present study.

(A) Laboratory tests:

Assess the induction of different defense enzymes:

Results of determination the protein content and enzymes activities responsible for diseases resistance in sprayed vegetable plants with plant resistance inducers (fungicides alternatives) grown under plastic houses are presented in Figs. (5 and 6).

Protein content:

Protein content was determined in the tested cucumber, pepper and tomato plants treated with different chemical inducers. Chemical inducers produced an increasing in protein content in all treated plants, Fig. (5), related to BSA as standard protein. The cucumber plants had a highly content of protein in all treatment compared with other treated plants under the study (pepper and tomato). The treatment, [Potassium bicarbonate +Thyme oil] induced highly protein content in cucumber and pepper plants (1465.7 and 1645.7 µg /g FW), while the highly content in tomato referred to Treatment of [Calcium chloride + *S. cerevisiae* + Chitosan] which induced 790.0 µg/g FW.

Kinetics Determination of enzymes:

ISSN: 2277-3754
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The spectrophotometric activities of all enzymes including peroxidase, Polyphenol oxidase, Phenylalanine ammonia-lyase, Chitinase and *β*-1,3-Glucanase activities were increased with all chemical inducers in the induced cucumber, pepper and tomato plants, Fig. (6).

Peroxidase activity:

Peroxidase activity was increased in all tested plants (Fig. 6). The treated cucumber, pepper and tomato plants with the treatment [Potassium bicarbonate +Thyme oil] had the highly level of peroxidase activities (141.0; 142.4 and 129.8 unit/g FW). On the other hand, the lower increase in peroxidase activities were induced by applied treatment, [Calcium chloride + *S. cerevisiae* + Chitosan]. Meanwhile, peroxidase activities were recorded as 65.5, 73.7 and 18.8 in cucumber, pepper and tomato plants in control treatment, respectively.

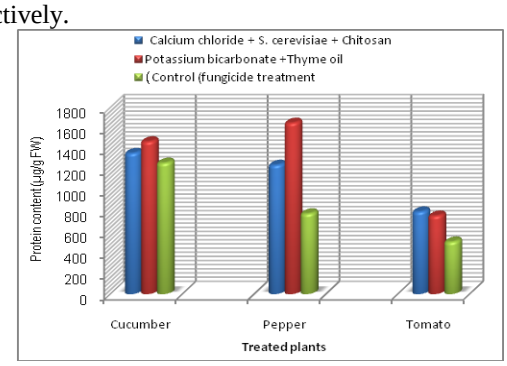

Fig. (5) Effect of chemical inducers on protein content of cucumber, pepper and tomato plants

Polyphenol oxidase activity:

All different inducers produced an increasing in polyphenol oxidase activity in all treated plants under study with different levels (Fig. 6). All solanaceae plants (pepper and tomato) have highly level of polyphenol oxidase activity compared with cucurbitaceae plant (cucumber) and the tomato plants were recorded one fold of enzyme activity comparing with pepper plant. The highest level of polyphenol oxidase activity was determined in treatment, [Potassium bicarbonate +Thyme oil] which recorded as (333.3; 2557.7; 3933.3 unit/g FW) in cucumber, pepper and tomato plants, respectively. Meanwhile, the lower records of polyphenol oxidase activity in the same plants were (333.3; 2557.7; 3933.3 unit/g FW) in respective order at treatment, [Calcium chloride + *S. cerevisiae* + Chitosan]. At for fungicides treated plants the recorded activity of polyphenol oxidase was 44.4, 488.8 and 320.0 unit/g FW for cucumber, pepper and tomato, respectively.

Phenylalanine ammonia-lyase activity:

Phenylalanine ammonia-lyase activities were increased in cucumber, pepper and tomato plants treated with individual chemical inducers, Fig. (6). All applied inducers increased enzyme level in sprayed plants. The treated pepper and tomato plants have a more enzyme level more than cucumber plants, the more effective chemical inducers in treated plants were [Potassium bicarbonate +Thyme oil] followed [Calcium chloride + *S. cerevisiae* + Chitosan] treatments. Phenylalanine ammonia-lyase activities were recorded as 634.2, 249.1 and 605.3 unit/g FW and 542.6, 199.5 and 589.7 unit/g FW, respectively for both applied treatments. The less Phenylalanine ammonia-lyase activities was recorded at fungicide treatment [control] were as 240.8, 53.7 and 292.0 unit/g FW of cucumber, pepper and tomato, respectively.

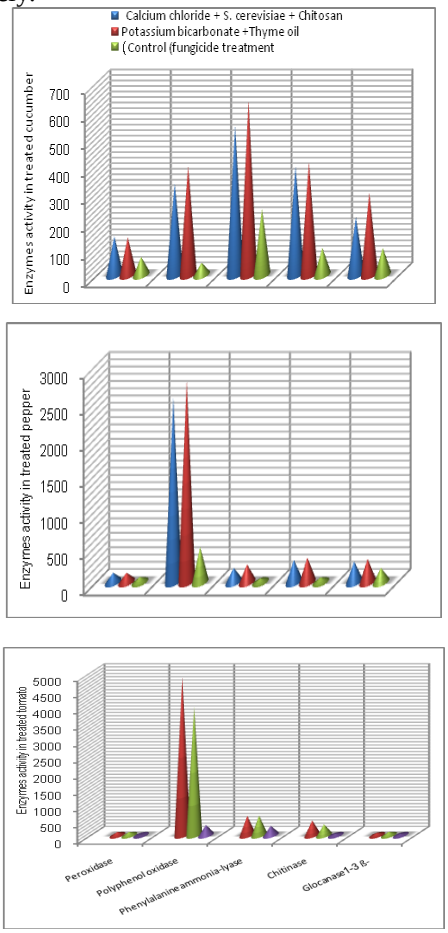

Fig. (6) Effect of chemical inducers (fungicides alternatives) on enzymes activity in treated cucumber, pepper and tomato plants.

Chitinase activity:

The presented data in Fig. (6) revealed that the different chemical inducers induced different levels of chitinase activities in all tested plants. The highest level produced 409.6, 342.4 and 453.6 unit/g FW by applied treatment, [Potassium bicarbonate +Thyme oil] in cucumber, pepper and tomato followed treatment of [Calcium chloride + *S. cerevisiae* + Chitosan] which recorded 395.2, 311.2 and 338.4 unit/g FW for the same plants in respective order. Moreover, the lowest chitinase activities were 99.2, 68.8 and 56.8 unit/g FW for cucumber, pepper and tomato, respectively at fungicide treatment [control].

Determination of *β* -1,3- Glucanase activity:

The data presented in Fig (6), showed that, the different and individual chemical inducers induced different levels of *β*-1,3-Glucanase activities in the tested sprayed plants. Cucumber and pepper have more highly enzyme level than tomato plants which has low level. The highest level produced by treatment [Potassium bicarbonate +Thyme oil] which recorded as 298.3, 322.1 and 106.2 unit/g FW (Fig. 6) in cucumber, pepper and tomato, respectively. Meanwhile,

ISSN: 2277-3754
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the lower levels were induced by treatment [Chitosan + Thyme oil] as 209.6, 289.0 and 73.1 as well as 99.1, 202.6 and 48.9 unit/g FW in fungicide treatment [control] in respective order for cucumber, pepper and tomato plants. Induced resistance is defined as an enhancement of the plants defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. Pre-treatment of plants with avirulent pathogens (biotic inducers) or chemical compounds (abiotic inducers) can enhance resistance to subsequent attack not only at the site of treatment, but also in tissues distant from the initial infection sites. Typically, this inducible resistance system known as systemic acquired resistance (SAR) is effective against diverse pathogens including viruses, bacteria and fungi [62]. Defense related genes encode a variety of proteins including enzymes controlling secondary metabolism, pathogenesis related proteins (PR) and regulatory proteins that control the expression of other defense related genes [63]. The defense gene products include polyphenol oxidase (PPO), peroxidase (POD) that catalyzes the formation of lignin, and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis. Other defense enzymes include pathogenesis-related proteins (PRs) such as ß -1, 3-glucanases and chitinases, which degrade the fungal cell wall and cause lysis of fungal cell. Chitin and glucan oligomers released during degradation of fungal cell wall act as an elicitor that elicit various defense mechanisms in plants [64]. Induction of defense proteins makes the plant resistant to pathogen invasion [65], and has been correlated with defense against pathogen invasion in cucumber [66] and tomato [67]. Polyphenol oxidizes (PPO) catalyze the oxygen-dependent oxidation of *o*dihydroxyphenols to *o*-quinones, which are more toxic to pathogens than the former. Direct toxicity of quinones against pathogens has also been proposed [68]. The induction of resistance in plants involves the activation of defense latent mechanisms [69] in response to the treatment with elicitor agents, protecting against subsequent infection by pathogens. Among the non-conventional elicitors can be included the extracts of medicinal plants and essential oils [70], as well as the alternative control of plant diseases [71,72]. Moreover, [69] reported that the activities of peroxidase, polyphenoloxidase and ß-1,3 glucanase, and the content of total proteins and chlorophylls were altered in plants treated with *P. sanguineus* extract. They added that, changes in the activities of peroxidase have being frequently correlated to the answer of resistance or susceptibility in different pathosystems. The peroxidase is responsible for the remove of atoms of hydrogen of the hydroxyl cinnamic alcohols groups, whose radical polymerize to form the lignin. This polymer, together with cellulose and other polysaccharides occurring in the cell wall of the superior plants, works as a physical barrier to the pathogen penetration. In the present work, the protein content was significantly altered both in the leaf treated with the *P. sanguineus* extracts as in the non-treated leaf, however, there was a faster response on protein synthesis in the 4th leaf. This result could be related to the age of the leaves in the moment of treatments application and pathogen inoculation, since the 4th leaf has probably more physiological activity than 3rd one, optimizing the protein synthesis and plant resistance response [73]. Protein synthesis could be related with the increase of the demand for substrates, necessary to the production of plant defense mechanisms induced by *P. sanguineus* treatment. Among the proteins, there are the pathogenesis related proteins (PR-proteins) which are induced in plant tissues due to inoculation with pathogens/microorganisms, systemically or local, as well as with treatments with chemical agents [74]. The activation of protein synthesis leads to a phase of plant resistance [73]. Thereafter, [75] verified reduction in protein content of bean plants when treated with *Bacillus cereus*, contrary to the treatment with fungicides alternatives, demonstrating specificity in the physiological response of this host to the elicitor treatment. Peroxidases are a well-known class of PR proteins and induced in host plant tissues by pathogen infection. They belong to PR-protein [65] and are expressed to limit cellular spreading of infection through establishment of structural barriers or generation of highly toxic environments by massively producing ROS and RNS [76]. Polyphenol oxidase, are involved in the oxidation toxic PCs into quinones (antimicrobial compounds) and lignifications of plant cells during the microbial invasion. These enzymes are also may participate in the inducible defense reaction and hypersensitivity in inducting resistance of plants to fungi, viruses and bacteria. Also, polyphenol oxidase, increased in resistant potato varieties but not in susceptible varieties which suggests a resistance mechanism to the enzyme [77]. POX and PPO through the oxidation of phenolic compound to quinines causing increase in antimicrobial activity during high microbial invasion. In the resistance induction, the increment of ß-1,3-glucanase is related with the plant defense. This enzyme hydrolyzes ß -1,3-glucan, which, together chitin, is the main component of fungal cell wall [78]. In another pathosystem [79] was observed increase in specific activity of ß -1,3-glucanase in common bean treated with *P. sanguineus* extract and challenge with anthracnose caused by *Colletotricum lindemuthianum*.These enzymes act up on the fungal cell wall resulting in degradation and loss of inner contents of cells [80]. In present study, ß -1, 3-glucanase has been highly induced by application of fungicides alternatives treatments. Therefore, these results indicate the possibility of involvement of ß -1, 3-glucanase in defense of chickpea against wilt. A direct role for ß-1, 3-glucanase in defense of plants against pathogens has been proposed because the substrates for these enzymes are major components of cell walls of many fungi [94]. Furthermore, ß -1,3 glucan and chitin, polymer of N-acetylglucosamine (NAG) are major cell wall components of many fungi. Since -1,3 glucanase and chitinases have been shown to be capable of attacking cell wall of fungal pathogens, these enzymes have been proposed as direct defense enzymes of plants [81]. We observed an increase in chitinase and ß -1,3 glucanase activity indicating plants ready mechanism to ward off pathogen by directly degrading the pathogen cell wall and in turn protecting the plant. Hence the high level accumulation of PR-Protein, Po, PPO, PAL, Chitinase and ß -1, 3-glucanase in plant leaves might have collectively contributed to the induced resistance in sprayed plants

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against *foliar diseases*. Results of infectivity tests (Tables 1 and 2), shows that disease infection was lower in treated vegetable plants with fungicides alternatives compared to control (fungicides treatment).

IV. CONCLUSION

In present work can be concluded that the fungicides alternatives reduce both foliar diseases incidence and severity of cucumber, pepper and tomato by increasing the activity of defense enzymes peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase, ß-1,3-glucanase and chitinases , local and systemically. Additionally, physiological changes in the content of protein were verified, probably due the apparatus energy synthesis required for plant defense mechanism involved in the reduction of theses disease. In this way, the use of fungicides alternatives for the control of such plant diseases in organic growth shows promising.

V. ACKNOLEDGEMENT

This work was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No. 1059.

REFERENCES

[1] Anonymous, 2009. 'Yearbook of statistics of Ministry of Agriculture.' Agricultural Economical and Statistical Department, Arab Republic of Egypt: Cairo, (2009), [In Arabic].

[2] Kiss, L. 2003. A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manage Sci., 59: 475-483.

[3] Cook, L.R. and Deahi, K.L. 1998. Potato-blight global changes and new problems. Pesticides, 9: 22-28.

[4] Khurana, S.M.P. 1998. Potato diseases & crop protection: Changing scenario. J. Indian Potato Association, 25: 1-7.

[5] Abd-El-Kareem, F., Abd-Alla, M.A. and El-Mohamedy, R.S.R. 2001. Induced resistance in potato plants for controlling late blight disease under field conditions. Egypt. J. Phytopathol., 29(2): 29-41.

[6] Abd-El-Kareem, F., Abd-Alla, M.A. and El-Mohamedy, R.S.R. 2002. Induced resistance in potato plants for controlling Early blight disease under field conditions. Egypt. J. App. Sci., 17(2): 51-66.

[7] Rauf, B.A. 2000. Seed-borne disease problem of legume crops in Pakistan. Pakistan J. Sce. And Indust. Res., 43: 249-254.

[8] El-Mougy, N.S., Abd-El-Karem, F., El-Gamal, N. G. and Fotouh, Y.O. 2004. Application of fungicides alternatives for controlling cowpea root rot diseases under greenhouse and field conditions. Egypt. J. Phytopathol., 32(1-2): 23-35.

[9] Dik, A., Wubben, J., Elad, Y., Kohi, J. and Shtienberg, D. 2002. Combination of control methods against powdery mildew diseases in glasshouse-grown vegetables and ornamentals. IOBC WPRS bulletin, 25: 5-8.

[10] Zinkernagel, V., Tischner, H., Hausladen, H., Habermeyer, H., Taborsky, V., Polak, J., Lebeda, A. and Kudela, V. 2002. Practical application of integrated disease management. Plant Prot. Sci., 38: 212-222.

[11] Napier, D. and Oosthuyse, S.A. 2000. Use of monopotassium phosphate in the integrated control of powdery mildew. Rivista di Frutticoltura e di Ortofloricoltura, 62(6): 57-58.

[12] Ziv, O. and Zitter, T.A. 1992. Effects of bicarbonates and film forming polymers on cucurbit foliar diseases. Plant Dis., 76: 513-517.

[13] Ziv, O. and Hagiladi, A. 1993. Controlling of powdery mildew in Euonymus with polymer coatings and bicarbonate solutions. Hort. Science, 28: 124-126.

[14] El-Mougy, N.S. and Abdel-Kader, M.M. 2009. Salts application for suppressing potato early blight. Journal of Plant Protection Research, 49(4): 353-361.

[15] Trotel-Aziz, P., Couderchet, M., Vernet, G. and Aziz, A. 2006. Chitosan stimulates defense reactions in grapevine leaves and inhibits development of Botrytis cinerea. Eur J Plant Pathol., 114: 405-413.

[16] Aziz, A., Trotel-Aziz, P., Dhuicq, L., Jeandet, P., Couderchet, M. and Vernet, G. 2006. Chitosan Oligomers and Copper Sulfate Induce Grapevine Defense Reactions and Resistance to Gray Mold and Downy Mildew. Phytopathology, 96: 1189-1194.

[17] Chang, M.M., Hadwiger, L.A. and Horovitz, D. 1992. Molecular characterization of a pea beta-1,3-glucanase induced by Fusarium solani and chitosan challenge. Plant Mol. Biol., 20: 609-618.

[18] Hadwiger, L.A., Ogawa, T. and Kuyama, H. 1994. Chitosan polymer sizes effective in inducing phytoalexin accumulation and fungal suppression are verified with synthesized oligomers. Mol Plant Microbe Interact, 7: 531-533.

[19] Aleandri, M.P., Vincenzo Tagliavento, R.R., Magro, P. and Chilosi, G. 2010. Effect of chemical resistance inducers on the control of Monosporascus root rot and vine decline of melon. Phytopathol. Mediterr., 49: 18–26.

[20] Cohen, Y., Gisi, U. and Mosinger, E. 1991. Systemic resistance of potato plants against Phytophthora infestans induced by unsaturated fatty acids. Physiol. Mol. Plant Pathol., 38: 255-263.

[21] Lanna, A.C., Oliveira, M.G.A., Barros, E.G. and Moreira, M.A. 1996. Kinatic parameters of leaf lipoxygenase pool from normal soybean genotypes and from a line devoid of seed lipoxygenase. Rev.Bras.Fisiol.Vegetal., 8: 87-92.

[22] Bradford, M.M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

[23] Johri, S., Jamwal, U., Rasool, S., Kumar, A., Verma, V. and Qazi, G.N. 2005. Purification and characterization of peroxidases from Withania somnifera (AGB 002) and their ability to oxidize IAA. Plant Science, 169: 1014-1021.

[24] Coseteng, M.Y. and Lee, C.Y. 1978. Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. Journal of Food Science, 52: 985.

[25] Guleria, S. and Kumar, A. 2006. Qualitative profiling of phenols and extracellular proteins induced in mustard (Brassica juncea) in response to benzothiodiazole treatment. J. Cell Mol., Biol., 5: 51-56.

[26] Boller, T. and Mauch, F. 1988. Colourimetric assay for chitinase. Methods in Enzymology, 161: 430-435.

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 6, December 2013

[27] Ried, J.D. and Ogrd-ziac, D.M. 1981. Chitinase over producing mutant of Servatia marcescens. Appl. and Environ. Microbiol., 41: 664-669.

[28] Sun H., Yang, J., Lin, C., Huang, X., Xing, R. and Zhang, K.Q. 2006. Purification and properties of a β-1,3-glucanase from Chaetomium sp. that is involved in mycoparasitism. Biotechnology Letters, 28: 131-135.

[29] MSTAT-C. 1988. MSTAT-C, a microcomputer program for the design, arrangement and analysis of agronomic research. Michigan State University, East Lansing.

[30] SAS 1988. Statistical Analysis System. User's Guide: Statistics (PC-Dos 6.04). SAS Institute Inc., Cary, NC, USA.

[31] Winer, B. J. 1971. Statistical Principles in Experimental Design. 2nd ed. MiGraw-Hil Kogakusha, LTD, 596 pp.

[32] Abdel-Kader, M.M., El-Mougy, N.S. and Lashin, S.M. 2012. Efficacy of Different Plant Resistance Inducers against Downy and Powdery mildew Diseases of Pepper under Plastic Houses Conditions. Journal of Applied Sciences Research, 8 (7): 3415-342.

[33] El-Mougy, N.S., Abdel-Kader, M.M., and Aly, M.D.E. 2012. Application of plant Resistance Inducers for Controlling Early and Late Blights of Tomato under plastic houses conditions. Journal of Applied Sciences Research, 8 (7): 3406-3414.

[34] Belanger, R.R., Dik, A.J., Menzies, J.G. 1997. Powdery mildew: Recent advances towards integrated control. In: Boland GS, Kuykendall LD (eds) Plant-microbe interactions and biological control. Marcel Dekker, New York, USA.

[35] Reuveni, M., Agapov, V. and Reuveni, M. 1996. Controlling powdery mildew caused by Sphaerotheca fuliginea in cucumber by foliar sprays of phosphate and potassium salts. Crop Prot., 15: 49-53.

[36] Mucharromah, E. and Kuc, J. 1991. Oxalates and phosphates induce systemic resistance against disease caused by fungi, bacteria and viruses in cucumber. Crop Prot., 10: 265-270.

[37] Yamazaki, H. and Hoshima, T. 1995. Calcium nutrition affects resistance of tomato seedlings to bacterial wilt. Hort. Science, 30: 91-93.

[38] Roinestad, K.S., Montville, T.J. and Rosen, J.D. 1993. Inhibition of trichothecene biosynthesis in Fusarium tricinctum by sodium bicarbonate. J. Agric. Food Chem., 41: 2344-2346.

[39] Singh, S.N. and Chand, L. 1993. Inhibition of aflatoxin production by garlic extract and sodium bicarbonate. Crop Res., 6: 149-154.

[40] Ashour, A.M.A. 2009. Effect of Application of some Systemic Fungicides and Resistance Inducing Chemicals on Management of Cantaloupe Powdery Mildew Disease. Egypt. J. Phytopathol., 37: 1-8.

[41] Palou, L., Smilanick, J.L., Usall, J. and Vinas, I. 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate and sodium bicarbonate. Plant Dis., 85: 371-376.

[42] Smilanick, J.L., Margosan, D.A., Mlikota, F., Usall, J. and Michael, I.F. 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis., 83: 139-145.

[43] Janisiewicz, W.J. and Peterson, D.L. 2005. Experimental Bin drenching system for testing biocontrol agents to control postharvest decay of apples. Plant Dis., 89: 487-490.

[44] Conway, W.S. and Sams, C.E. 1985. Influence of fruit maturity on the effect of postharvest calcium treatment on decay of Golden Delicious apples. Plant Dis., 69: 42-44.

[45] Conway, W.S., Sams, C.E., McGuire, R.G. and Kelman, A. 1992. Calcium treatment of apples and potatoes to reduce postharvest decay. Plant Dis., 76: 329-333.

[46] Conway, W.S., Abbott, J.A. and Bruton, B.D. 1991. Postharvest calcium treatment of apple fruit to provide broad-spectrum protection against postharvest pathogens. Plant Dis., 75: 620-622.

[47] Wisniewski, M., Droby, S., Chalutz, E. and Eilam, Y. 1995. Effects of Ca2+ and Mg2+ on Botryris cinerea and Penicillium expansum in vitro and on the biocontrol activity of Candida oleophila. Plant Pathology, 44: 1016-1024.

[48] Venette, J.R. 1998. Calcium micronutrient may reduce white mold of dry beans. Proceedings of the Sclerotinia Workshop, Minnesota ND, In-Service Extension Worksho, Fargo.

[49] Gulya, T.J. and Miller, J.F. 2007. Advances in the development of sunflower germplasm with resistance to both Sclerotinia stalk rot and head rot, and the possible role of calcium in Sclerotinia resistance. Proceedings of the 2007 National Sclerotinia Initiative Annual Meeting. Minneapolis.

[50] Paula Júnior, T.J., Vieira, R.F., Teixeira, H. and Carneiro, J.S. 2009. Foliar application of calcium chloride and calcium silicate decreases white mold intensity on dry beans. Tropical Plant Pathology, 34: 171-174.

[51] Atia, M.M.M., Buchenauer, H., Aly, A.Z. and Abou-Ziad, M.I. 2005. Antifungal activity of chitosan against Phytophthora infestans and activation of defense mechanisms in tomato to late blight. Biological agriculture & horticulture, 23: 175-197.

[52] Chircov, S.N. 2002. The antiviral activity of chitosan (review). Applied Biochemistry and Microbiology, 38: 1-8.

[53] Zheng, L. and Zhu, J. 2003. Study on antimicrobial activity of chitosan with different molecular weights. Carbohydrate Polymer, 54: 527-530.

[54] Yang, V.W. and Clausen, C.A. 2007. Antifungal effect of essential oils on southern yellow pine. International Biodeterioration & Biodegradation, 59: 302-306.

[55] Momol, M.T., Olson, S.M., Pradhanang, P.M. and Jones, J.B. 2005. Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. Plant Dis., 89: 497-500.

[56] Liu, W.T., Chu, C.L. and Zhou, T. 2002. Thymol and acetic acid vapors reduce postharvest brown rot of apricots and plums. Hort. Science, 37: 151-156.

[57] Paster, N., Menasherov, M., Ravid, U. and Juven, B. 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J. Food Prot., 58: 81-85.

[58] Juven, B.J., Kanner, J., Schved, F. and Weisslowicz, H. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. Appl. Bacteriol., 76: 626-631.

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 6, December 2013

[59] Walsh, S.E., Maillard, J.Y., Russell, A.D., Catrenich, C.E. and Charbonneau, D.L. 2003. Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. J. Appl. Microbiol., 94: 240-247.

[60] Blondelle S. E. and K. Lohner, "Combinatorial libraries: a tool to design anti-microbial and antifungal peptide analogues having lytic specificities for structure-activity relationship studies," Biopolymers, vol. 55, no. 1, pp. 74–87, 2000.

[61] Bent A. F., "Plant mitogen-activated protein kinase cascades: negative regulatory roles turn out positive," Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 3, pp. 784–786, 2001.

[62] Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996) Systemic acquired resistance. Plant Cell. 8, 1809-1819.

[63] Dixon RA, Harrison MJ, Lamb CJ (1994) Early events in the activation of plant defense responses. Annu Rev Phytopathol. 32: 479-501.

[64] Frindlender M, Inbar J, Chet I (1993) Biological control of soil borne plant pathogens by a ß-1, 3-glucanase producing Pseudomonas cepacia. Soil Biol Biochem. 25: 1211–1221.

[65] Van Loon, L. C. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. European Journal of Plant Pathology, 103 (9): 753–765.

[66] Rasmussen, J.B. 1991.) Systemic induction of salicylic acid accumulation in cucumber after inoculation with Pseudomonas syringae. Plant Physiol., 97: 1342-1348.

[67] Bashan, Y., Okon, Y. and Henis, Y. 1985. Peroxidase, polyphenol oxidase and phenols in relation to resistance against pseudomonas syringae pv tomato in Tomato. Can. J. Bot., 65: 366-372.

[68] Mayer, A.M. and Harel, E. 1979. Polyphenol oxidases in plants. Phytochem., 18: 193-215.

[69] Stangarlin, J.R., Kuhn, O.J., Toledo, M.V., Portz, R.L., Schwan-Estrada, K.R.F. and Pascholati, S.F. 2011. Plant Defense against Pathogens. Scientia Agraria Paranaensis, 10(1): 18-46.

[70] Schwan-Estrada, K.R.F. and Stangarlin, J.R. 2005. Extracts and Essential Oils of Medicinal Plants in the Resistance Induction against Plant Pathogens. In: Cavalcanti LS, Di Piero RM, Cia P, Pscholati SF, Resende MLV, Romeiro RS (ed.) Resistance induction in plants against pathogens and insects. Piracicaba: FEALQ, pp.125-138.

[71] Assi, L. 2005. Control of Colletotrichum lindemuthianum (Sacc. Et Magn.) Scrib, in common bean (Phaseolus vulgaris L.) by Pycnoporus sanguineus (L. ex. Fr.) extract. M. Sc. thesis. Western Parana State University.

[72] Peiter-Beninca, C., Franzener, G., Assi, L., Iurkiv, L., Eckstein, B., Costa, V.C., Nogueira, M.A., Stangarlin, J.R. and Schwan-Estrada, K.R.F. 2008. Phytoalexin Induction and Peroxidase Activity in Sorghum and Soybean Treated with Basidiocarp Extracts of Pycnoporus sanguineus. Arquivos do Instituto Biológico, 75: 285-292.

[73] Larcher, W. 2000. Plant Ecophysiology. São Carlos: Ri Ma.

[74] Guzzo, S.D. 2003. Pathogen Related Proteins. Revisão Anual de Patologias de Plantas, 11: 283-332.

[75] Kuhn, O.J. 2007. Resistance Induced in bean Plants (Phaseolus vulgaris) by Acibenzolar-Smethyl and Bacillus cereus: Physiological and Biochemical Aspects, Growth and Production Parameters. PhD thesis. ESALQ.

[76] Passardi, F., Cosio, C., Penel, C. and Dunand, C. 2005. Peroxidases have more functions than a Swiss army knife, Plant Cell Reports, 24 (5): 255–265.

[77] Tomiyama, K. 1963. Physiology and biochemistry of disease resistance of plants. Annu. Rev. Phytopathol., 1:295-324.

[78] Cornelissen, B.J.C. and Melchers, L.S. 1993. Strategies for Control of Fungal Diseases with Transgenic Plants. Plant Physiology, 101: 709-712.

[79] Baldo, M. 2008. Histological and Biochemical Aspects of the Resistance Induction in Bean Plants and Antifungal Activity by Pycnoporus sanguineus. Master thesis. Western Paraná State University.

[80] Benhamou, N., Lafontaine, P.J. and Nicole, M.1994. Seed treatment with chitosan induces systemic resistance to Fusarium crown and root rot in tomato plants. Phytopathology, 84: 1432-1444.

[81] Abeles, F. B., Bosshart, P., Forrence, L. E. and Habiz, W. 1970. Preparation and purification of glucanase and chitinase from bean leaves, Plant Physiology, 47(1): 129–134.

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