Pathological Studies on Fruit Rots of Cucurbits

By

Safaa El-Said El-wan

B.Sc., in Agric. (Plant Pathology), 1992

M.Sc. in Agric. (Plant Pathology), 1999

Fac. Agric., Zagazig Univ.

THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Plant Pathology

Agricultural Botany Department

Faculty of Agriculture, Moshtohor

Benha University

2012

SUPERVISION COMMITTEE

Pathological Studies on Fruit Rots of Cucurbits

By

Safaa El-Said Elwan

B.Sc., in Agric. (Plant Pathology), 1992

M.Sc. in Agric. (Plant Pathology), 1999

Fac. Agric., Zagazig Univ.

Prof. Dr. Abd El-Moneim I. I. El-Fiki
Professor of Plant Pathology,
Agric. Botany Dept.,
Fac. Agric., Banha Univ.

Prof. Dr. Gehad M. D. El Habbaa
Professor of Plant Pathology,
Agric. Botany Dept.,
Fac. Agric., Banha Univ.

Dr. Fatma Sayed Ahmed
Assistant Researcher,
Plant Pathology Institute,
ARC, Giza, Egypt

Plant Pathology Branch

Agricultural Botany Department

Faculty of Agriculture, Moshtohor

Banha University

APPROVAL SHEET

Pathological Studies on Fruit Rots of Cucurbits

By

Safaa El-Said Elwan

B.Sc., in Agric. (Plant Pathology), 1992

M.Sc. in Agric. (Plant Pathology), 1999

Fac. Agric., Zagazig Univ.

This Thesis for Ph.D. degree in plant pathology has been approved by:

Prof. Dr. Mohamed M. Amaar
Professor of Plant Pathology,
Agric. Botany Dept.,
Fac. Agric., Menofyia Univ.

M. M. Amaar

Prof. Dr. Abd El-Moneim I. I. El-Fiki
Professor of Plant Pathology,
Agric. Botany Dept.,
Fac. Agric., Banha Univ.

Sfiki A M

Prof. Dr. Gehad M. D. El Habbaa
Professor of Plant Pathology,
Agric. Botany Dept.,
Fac. Agric., Banha Univ.

Gehad M. El-Habbaa

Prof. Dr. Fathy Gad Mohamed
Professor of Plant Pathology,
Agric. Botany Dept.,
Fac. Agric., Banha Univ.

Mohamed, F. G.

Date: 6 /2 / 2012

ACKNOWLEDGMENT

Firstly my unlimited thanks to "Allah"

a word of gratitude is not enough towards the great effort and help of the main supervisor ***Prof. Dr. Abd El-Moneim Ibrahim Ismaeil El-Fiki,*** Professor of Plant Pathology, Agric. Bot. Dept., Fac. of Agric., Benha Univ., for his constructive supervision, valuable advices, kind guidance and for his help in putting thesis in its final form. He gave me his time and effort to introduce this thesis in the best form .He was kind enough to share me my problems during this work.

Deepest thanks and appreciation are to ***Prof. Dr. Gehad Mohamed Dessouky El-Habbaa*** Professor of Plant Pathology, Agricultural Botany Dept., Faculty of Agriculture, Benha University, for his great support during preparation this work. He gave me his time and efforts to introduce this work in the best form and it was a pleasure to work under his supervision.

I would like to express my deepest thanks and gratitude to ***Dr. Fatma Sayed Ahmed,*** Assistant Researcher of Plant Pathology, Institute of Plant Pathology, Agricultural Research Center, Giza for suggesting the topic of the current thesis, her valuable advices and kind guidance through this study.

Special thanks also to ***Dr. Azza Nafee Mohamed Ali,*** Assistant Researcher of Plant Pathology, Storage Diseases Department, Plant Pathology Institute, Agric. Research Center, Giza for her valuable help during preparing this work.

Gratitude is extended also to ***all staff members*** of Plant Pathology Branch, Agric. Botany Dept., Fac. Agric., Benha Univ. and all the members of Vegetable Diseases Department, Plant Pathology Institute, Agricultural Research center, Giza, for their kind help.

Name: *Safaa El-Saied El-wan*

Degree: *Doctor of Philosophy*

Title: Pathological Studies on Fruit Rots of Cucurbits

Department: Agricultural Botany, Plant Pathology

Abstract:

Our study was designed to combat rot the fruits of cucurbits. Surveying the natural disease incidence of the most cucumber and squash fruit rots occurrence in many locations of seven governorates (*i.e.* Beheira, Kafr El-Sheikh, Qalubiya, Ismailia, Sharkiya, Dakahliya and Giza) was performed. All laboratories, greenhouse and field experiments were carried out during autumn and spring of 2003 and 2004 seasons.

During survey, 152 and 81 fungal isolates were isolated from naturally infected cucumber and squash rotted fruits. They included *Alternaria* ssp., *B. cinerea, F. solani, Mucor* spp., *Penicillium* spp., *Pythium* spp. and *Sclerotinia sclerotiorum.*

Promise means for fruit rots control were achieved in the present investigation using combination –as integrated control- of some fungicides, organic acids and salts, plant extracts, kombucha tea filtrate, cold, modified atmosphere storage conditions, and UV-treatments.

Positive correlation between pathogenesis related proteins (as enzymes) activities were recorded between inhibition of disease severity and some treatments.

CONTENTS

INTRODUCTION

INTRODUCTION

Cucurbits considered one of the most important and widely distributed vegetable crops in Egypt and all over the world. The cultivated area has been increased to face the increasing demand of cucurbits, which they are mostly tropical or subtropical plants.

Family Cucurbitaceae belongs approximately about 90 Genera, but three are grown in Egypt in open fields or under protected cultivation, *i.e. Cucumis* spp. (cucumber, melon and snake cucumber), *Cucurbita* spp. (squash& pumpkin) and *Citrullus* spp. (watermelon). They are grown throughout the year round in many localities in Egypt, either in open fields and/or protected cultivations. In 2009/2010 seasons(*), the area cultivated with cucurbits was 150327 feddan yielded 1289642 tons (average 8.579 ton/fed.). The area cultivated with cucumber and squas was 67234 feddan (average yield 55.507 ton/fed.) and 83093 feddan (average yield 44.224 ton/fed.), respectively.

These fresh cucurbits are important for the human nutrition and health, which contains flavor and essential nutrients such as vitamins and minerals. They are also a major source of complex carbohydrates, antioxidants and anti-carcinogenic substances (**Arul, 1994**).

---

* Economic Statistic Reports, Agricultural statistics, Ministry of Agriculture and Land Reclamation, Economic Affairs Sector, 2010.

Protected cultivation is considered a modern agricultural system in Egypt during the last few decades. The great need to intensify agricultural production in Egypt and to encourage young graduates to start their own business had resulted in fast distribution of greenhouses. Meantime, this particular trend has been gradually increased in the new reclaimed lands. It is preferred also in certain geographic areas where weather conditions of days and nights are sharply variable and water sources are not adequate. Therefore, the purpose of growing crops under protected cultivations is mainly to protect the plants from the adverse environmental conditions as well as diseases and pests (**Hanan *et al.*, 1978**). Their products are usually very suitable for exportation, and this increases the income of the growers. In addition, local market has recently attracted to this production due to the relative good price offered, particularly for some vegetables such as cucumber, tomato and pepper. Such specific products are seasonally produced, but they are now available during all the year after the realizable increase of protected cultivation.

Several fungal diseases are usually attack cucurbits, *i.e.* cucumber, melon, watermelon, and squash during different growth stages in relation to successive cropping periods resulting great yield losses. These diseases are powdery, downy mildews, grey mould, white mould, Fusarium wilt, stem and fruit rots anthracnose and root rot (**Bedlan, 1986** and **1992; Siviero and Motton, 2000**). Meantime, cases of fruit rot disease on cucurbits

have been frequently occurred during different seasons in both open fields and protected cultivations causing considerable losses in fruit yield. Thus, the present study was conducted to study the followings:

1) Surveying the incidence of fruit rot disease on cucumber and squash at different localities in Egypt.
2) Isolation and identification of fungi associated with the infected cucumber and squash fruits collected from different cultivated areas in Egypt.
3) Studying the pathogenicity of the different isolates on the susceptible cucumber and squash cultivars.
4) Studying the response of different cucumber cultivars and cucurbit to fruit rot diseases.
5) Studying some of the factors affecting the incidence of fruit rot on the tested cucurbits.
6) Studying the enzymatic activity of the tested mould fungi in healthy and infected cucumber and squash fruits *in vitro* and *in vivo.*
7) Determining the efficacy of some bio-agents in controlling the cucurbit fruit rot diseases *in vitro* and *in vivo.*
8) Studying the effect of fungicides on growth of fruit rot pathogens *in vitro.*
9) Studying the effect of induced resistance on fruit rots disease under greenhouse conditions.
10) Studying the chemical analysis of infected and healthy fruits of some cucurbits.

REVIEW OF LITERATURE

REVIEW OF LITERATURES

1. Symptoms and the causal agents of cucurbits fruit rots:

Wasfy (1967) found that fruits of cucumber, squash, pumpkin, snake cucumber and watermelon in Egypt were infected with many fungi *i.e. B. cinerea*, *Choanephora cucurbitarum*, *F. solani*, *Phytophthora derceslera*, *Pythium aphanidermatum*, *Rhizopus stolonifer* and *Sclerotinia sclerotiourum*. Pathogenicity tests under field conditions were confirmed.

Sommer *et al.* (1974) found that grey mould rot (*B. cinerea*) developed during transportation and marketing within a period of 7 days at 5°C. The disease resulted mostly from pre-harvest infection.

Assawah *et al.* (1984) identified the fungi causing pre harvest rot diseases of squash during a field survey at Mosul (Iraq) showing the occurrence of blossom blight (*Choanephora cucurbitarum*), soft rot (*F. solani*) or (*Pythium aphanidermatum*) and grey mould (*B. cinerea*). Post harvest diseases occurred in the market, included rots caused by *F. solani, P. aphanidermatum, B. cinerea, Rhizopus stolonifer* and *Alternaria alternata*.

Zhukovskaya *et al.* (1984) reported that *B. cinerea* was the main pathogen of grey mould disease of cucumbers and tomatoes in the glasshouse. The description depends on disease symptoms and morphological criteria of the pathogen.

Hawthorne (1988) isolated fourteen fungi from rots of stored *Cucurbita maxima* and *C. moschata* cultivars. He identified them as pathogens of the fruits. The major pathogens were *F. solani* and *Didymella bryoniae* while, the minor pathogens included *B. cinerea*.

Reddy and Reddy (1989) isolated *F. equiseti, F. oxysporum, F. semitectum* [*F. pallidoroseum*], *F. solani, F. moniliforme* [*Gibberella fujikuroi*] and *F. scirpi* from soft rots of cucumber, watermelon and melon.

Rath *et al.* (1990) collected >300 rotten samples of 7 cucurbits including cucumber and *Cucurbita maxima*. The results revealed that 171 of the samples were rotted by fungi and 145 samples were rotted by non-fungal agents. The fungi were identified as *Aspergillus flavus, A. niger, Fusarium oxysporum, F. solani, Geotrichum candidum, Phytophthora. sp., Rhizoctonia solani, Rhizopus arrhizus* and *Rhizopus stolonifer*.

Correll *et al.* (1991) observed fruit rot of pumpkin (*Cucurbita maxima*) periodically in Arkansas during 1990. Field observation of pumpkin cv. Halloween had a high incidence of fruit rot losses, which were estimated about 30%. Initial symptoms included soft, sunken areas on the fruit. Lesions often became water-soaked, and mycelium was observed frequently on the fruit surface. Lesions didn't always occur on the part of the pumpkin in contact with the soil and were not associated with clear wounds.

Extensively colonization fruit collapse completely. *Fusarium equiseti* was consistently isolated from symptomatic fruit.

Bedlan (1992) mentioned that the most important diseases of cucumber are powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*), downy mildew (*Pseudoperonospora cubensis*), grey mould (*B. cinerea*), white mould (*Sclerotinia sclerotiorum*), wilt (*Fusarium oxysporum* f. sp. *cucumerinum*), stem and fruit rot (*Didymella bryoniae*), anthracnose (*Colletotrichum lagenarium*), root rot (*Pythium debaryanum*) and storage rot (*Penicilium oxalicum*).

Sonoda *et al.* (1993) found that lesions were observed on 5% of mature fruits of squash (*Cucurbita pepo*) cv. Oragetti at Florida, USA. *Fusarium semitectum* f. sp *pallidoroseum* and *Didymella bryoniae* were frequently isolated and *F. oxysporum* and *Colletotrichum gloeosporiodes* were occasionally isolated from separate lesions and their pathogenicity was confirmed. No lesions were observed on fruit of adjacent *C. pepo* cv. Spaghetti.

Albornett *et al.* (1994) isolated *F. solani* and *Fusarium* sp., from melons in packing houses and exportation enterprises in Venezuela.

Yucel (1994) reported that greenhouse grown cucumber was attacked by *Pseudoperonospora cubensis*, *Sphaerotheca fuliginea*, *Erysiphe cichoracearum*, *F. oxysporum* f.sp. *cucumerinum*, *Phytophthora* spp., *Rhizoctonia solani* and *B. cinerea* during survey of 1989-91 in Turkey.

Zhu and Zhang (1995) studied postharvest disease of marrow squash [*C. pepo var. oleifera*]. The results showed that *F. solani* and *Colletotrichum lagenarium* [*C. orbiculare*] were the cause of 2 main diseases of marrow squash during storage. In Aug. and Sep., severe fruit rot occurred.

Elmer (1996) noticed unusual symptoms on cucurbits exclusively on pumpkins (*Cucurbita maxima*) cv. Howden. The lesions decreased market ability of the pumpkins and were described as a pre-harvest dry, hard rot (type 1) or a post harvest soft, sunken rot (type 2). *Fusarium acuminatum, F. equiseti* were isolated from type 1 lesions, and *F. equiseti* and *F. solani* were isolated from type 2 lesions.

Park *et al.* (1996) examined the incidence of the major diseases of cucurbit plants including grey mould (*B. cinerea*) in 3 important cucurbit crops, *i.e.* melon, watermelon and cucumber cultivating areas in Kyungbuk province, Korea Republic, during 1992 to 1995. The incidence of grey mould on the three crops was related to culture conditions such as density of plant stand and ventilation.

Garcia-Jimenez *et al.* (1997) observed the root, crown and fruit rots of squash (*Cucrubita pepo*) in eastern provinces of Valencia and Astellon (Spain). The isolated fungi were identified as *F. solani* based on colony morphology on potato sucrose agar and fungal morphology on Bilag's medium. Pathogenicity was confirmed by inoculation of squash (cv. Dulce de Horno) seedlings

and mature fruit. Stem isolates were pathogenic on fruit and fruit isolates were pathogenic on stems. On the basis of these results and disease symptoms in the field the fungus was classified as *F. solani* f. sp. *cucurbita* race 1.

Bourbos *et al.* (1997) found that *F. solani* f.sp. *cucurbitae* causes serious damage in greenhouse cucumbers (*Cucumis sativus*).

Kim *et al.* (1998) reported that grey mould (*B. cinerea*) was considered one of the main diseases of cucumber, tomato and strawberry at the major fields in Keyongbuk area (Korea republic) from 1996 to 1997.

Armengol *et al.* (2000) reported on the basis of pathogenicity tests and disease symptoms in the field that fruit rot of squash (*C. moschata*) caused by *F. solani* f. sp., *cucurbitae* race 1.

Maklad (2004) described the symptoms of cucurbits fruit rot as water-soaked area and turned from light brown to grey growth on the fruit surface. *B. cinerea, Alternaria* spp., *Pythium* spp., *Rhizopus nigricans*, *Rhizoctonia solani*, *Penicillium* spp., *Mucor* spp., *Fusarium* spp., *Aspergillus* spp., and *Sclerotinia scleratiorum* were isolated. Pathogenicity test was confirmed.

Boughalleb *et al.* (2005) isolated 291 isolates which were identified as *F. solani*. These isolates were identified as *F. solani* f. sp. *cucurbitae* (Fsc.) and races 1 and 2 characterized on the basis of pathogenicity tests on watermelon seedlings and muskmelon fruits.

Mehl and Epstein (2007) mentioned that *F. solani* f.sp., *cucurbitae* causes a fruit rot of cucurbits and is classified into two races that are actually distinct species: *F. solani* f.sp. *cucurbitae* race 1 and race 2.

Castroagudin *et al.* (2009) reported that *F. solani* f.sp. *cucurbitae* caused disease incidence ranged from 50 to 75% of pumpkin (*Cucurbita pepo*). This is thought to be the first report of *F. solani* f.sp. *cucurbitae* as the causal agent of pumpkin fruit rot in the state.

Yu *et al.* (2009) reported that the disease investigation and identification of cucumber pathogens in the greenhouse were cucumber powdery mildew (*Oidium*. sp.), cucumber downy mildew (*Pseudoperonospora cubensis*), cucumber gray mould (*B. cinerea*),

Jamiolkowska (2011) reported that *Cucurbita pepo* L. var. *giromontina* is infected by a number of pathogens *i.e. Alternaria alternata*, *B. cinerea*, *Fusarium avenaceum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *Rhizoctonia solani* and *Trichoderma hamatum*. Actually, natural products such as plant extracts are more and more frequently used in plant protection from pathogens.

2. Inoculum potential and pathogenicity:

Fantino *et al.* (1989) reported that *F. solani* f. sp. *cucurbitae* race 2 on squashes was established in pathogenicity tests on susceptible squash cultivars.

Choi *et al.* (1990) mentioned that disease severity of grey mould on cucumber caused by *B. cinerea* was increased with the increasing of spore concentration of inoculum. It reached 70% of infected leaf area with 5×10^6 spores/ml. Spore age had no effect on disease severity.

Maklad (2004) pointed out that fruit rot disease severity increased by increasing inoculum density. The lowest concentration (100 spores/ml) was sufficient to cause the disease on cucumber. Poathogenicity test revealed that all tested isolates of *B. cinerea* were pathogenic to the tested variety.

Rampersad (2009) identiffied *F. solani* by morphological observations and pathogenicity tests. Symptoms began as water-soaked lesions on pumpkin (*Cucurbita pepo*) of any age at the point of contract with the soil.The disease progressed to a soft rot with leakage and whole fruit collapase. A dark brown, soft decay also developed at the base of the main vines.

3. Varietal reaction:

Dautel (1978) compared twelve cucumber cultivars with standard cv. Bambina in a three year trial (1975-77) for yield, earliness, quality, ability to regenerate, vigor and susceptibility to

B. cinerea and *Mycosphaerella melonis* under protected cultivation. Cultivars Uniflora D, Pandorex, Sandra-Ampex and Reform were rated the least susceptible ones as well as produced higher and good quality fruits than cv. Bambina.

McCall and Willumsen (1991) evaluated eight glasshouse cucumber cultivars to early yield, total yield, quality, market value and taste in relation to disease susceptibility. They found that cv. Aminex had the highest total yield and market value, despite the highest susceptibility to *B. cinerea* and *Penicillium* spp.

Maklad (2004) concluded that *B. cinerea* was pathogenic to all the tested cucumber, cantaloupe and squash cultivars. The wounded fruits of these different cucurbits showed higher percentages of disease severity compared with unwounded fruits. Different cultivars of the above-mentioned cucurbit hosts showed different levels of disease severity.

4. Factors affecting the severity of cucurbit fruit rot:

a- Induced resistance:

Induced resistance is a phenomenon explained by which the plant can utilize the own defense mechanism to increase the level of resistance without alteration the plant genome. It has been demonstrated in many plant species against a broad spectrum of pathogens, which include many important phytopathogenic fungi as well as bacteria and viruses **(Kuć, 1982)**. However, the systemic acquired resistance (SAR) in plants is briefly reviewed focusing on

the systemic signaling pathway including the role of salicylic acid and the use of exogenously applied agents to induce resistance **(Kerssmann *et al.*, 1994)**. There are two types of induced resistance, *i.e.* local acquired resistance (LAR) which can be obtained on the pretreated part of the plant, and systemic acquired resistance (SAR) which develop in the tissues distant from the site of prior including treatment **(Kerssmann *et al.*, 1994** and **Deverall, 1995).**

Akutsu *et al.* (1987) reported that spraying cucumber leaves of cv. Sagami Hanjiro with conidia of *B. cinerea* in the presence of KH_2PO_4 solution containing glucose arrested the spreading of grey mould lesions by the defense reactions of the epidermal cells *e.g.* deposition of well-developed papillae and hyper sensitive cell death. They suggested that KH_2PO_4 enhances the penetration activity of *B. cinerea* but dose not suppress the resistant reaction in cucumber leaves.

Abd-El-Rehim *et al.* (1987) showed that treating the inoculated squash (*Cucurbita pepo* cv. Askandrani) with calcium chloride gave significant increase in the germination percentage of seeds and reduced the infection with *F. solani*.

Elad (1992) tested eighteen free radical scavengers (antioxidants) for their ability to control grey mould. Most of them significantly reduced the disease in at least one of the test hosts (leaves of tomato, pepper, bean, aubergines, or rose flowers and cucumber fruits). However, the effective concn., varied between 0.1 and 10.0 mM. In this respect, Butylated hydroxytoluene

(BHT), tannic acid, ascorbic acid and dimethyl sulfoxide (DMSO) at a conc. of 1.0 mM controlled grey mould of tomato fruits. All these compounds except BHT controlled the disease on cucumber fruits. Four to 6 compounds reduced linear growth of *B. cinerea* isolates in culture at a conc. of 1.0 mM, and 6 more compounds were effective at 10.0 mM. However just 5 compounds inhibited conidial germination at the high conc., alone. Gluconic acid lactone, thiourea and propyl gallate reduced *Sclerotinia sclerotiorum* on lettuce by 51-76%.

Takuo *et al.* (1993) indicated that enzymes activity plays an important role in plant disease resistance through increasing of plant defense mechanisms which considered that the main tool of cultivar resistance. The production of pecteolytic and celluloytic enzymes was more obvious in the susceptible cultivars than in the resistant ones.

Horst *et al.* (1994) pointed out that potassium bicarbonate ($KHCO_3$) inhibited the fungal growth of *B. cinerea* and affected the morphology of conidia.

Palmer *et al.* (1994 a and b) reported that potassium bicarbonate ($KHCO_3$) and ammonium bicarbonate (NH_4HCO_3) had a good inhibitory effect on growth of *B. cinerea* and affected the germination of conidia.

Chardonnet and Doneche (1995) studied the role of calcium in protecting plant tissues from infection by *B. cinerea* in cucumber fruit. They suggested that calcium treatment of

cucumber fruit prior to infection can increase the cell-wall-bound Ca and therefore decrease pectin digestion by fungal pectinolytic enzymes. The role of calcium in protecting plant tissue from infection by *B. cinerea* consisting of an increase in cell-wall-band Ca occurred in the plant tissue after an injury or infection.

Reuveni *et al.* (1995) used phosphate salts in controlling plant diseases, which provides further evidence that may facilitate applying simple non-toxic chemical solutions. It has low costs, low toxicity, environmental safety and nutrient value, which make them ideal foliar fertilizer, and can be used practically in the field for disease control.

Ooswtendrop *et al.* (1996) noticed that the plant activator Bion induced resistance in cucumber plants against fungal and bacterial diseases as in SAR.

Pieterse *et al.* (1996) stated that systemic acquired resistance was a pathogen induced defense mechanism in plants. They also concluded that the resistant state was dependent on endogenous accumulation of salicylic acid (SA) and was characterized by the activation of genes encoding pathogenesis related proteins.

Raum (1997) mentioned that Bion-g is a new plant activator that can improve the natural resistance of plants to the diseases.

Ruess *et al.* (1997) reported that Bion-g is the first compound of a new generation of crop protection agents, which activate plant defense mechanism called SAR. This plant resistance can be activated by biotic and abiotic agents and results in a systemic

protection of the entire plant against a spectrum of disease caused by fungi and bacteria. Also, Bion g copies this natural biological phenomenon and provides reliable and commercially acceptable protection in several crops against a number of diseases.

Galal *et al.* (2000) reported that benzoic acid, salicylic acid and tannic acid have direct antifungal activity on *Fusarium moniliform, F. oxysporum* and *F. solani* on media.

Nabil (2000) found that spraying squash plants with oxalic acid (OA) and salicylic acid (SA) at 10 mM induced resistance by reducing infective virus particles by 38.3 and 37.5% respectively.

b. Plant extract:

Zedan (1993) reported that the water extract of *Eucolyptus* spp. (leaves or cortex), *Psidium guajava, Morus alba* and *Ficus nitida* (leaves) inhibited the different characters, spore germination and physiological aspects of many fungi.

Abd El-Megeed and Khafagi (1998) used some plant extracts *i.e.* guava, lemon, scented and white mulberry, as seed treatment to control root rot and wilt diseases of watermelon cv. Giza-1. Good results were obtained in either survival plants or high quantity and quality of yielding under greenhouse and field conditions. Also, some biochemical related resistance such as plasma membrane; lipid peroxidation and accumulation of phenolic compounds were increased with enhancing resistance.

Abd El-Moneim (2001) found that the plant extracts *i.e.*, neem, garlic and camphor at 5% were effective in increasing the survival of cucumber plants and decreasing the disease severity either pre or post emergence damping- off. The most effective extract was neem and garlic whereas; camphor was the least effective one.

Nada (2002) found that hot water extracts of blue gum and thyme were the best treatments in increasing total phenol contents and in decreasing values of infection parameters of pepper and squash if compared with check treatment.

Schmitt (2002) found that plant extracts from *Reynoutria sachalinensis* (*Fallopia sachalinensis*) induce local resistance in a variety of crops against different plant pathogens. In cucumber, tomato and grape and in ornamentals, infection with powdery mildew (*Sphaerotheca fuliginea*) or grey mould can be reduced to a large degree by regular application of the inducer. Besides reduced infestation with pathogens, higher yields were recorded and morphological changes, such as darker green of leaves or enforced main shoot and flower production were found.

Abou-Jawdah *et al.* (2004) tested extracts of nine plant species for their efficacy against seven plant pathogenic fungi *i.e.*, *B. cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f.sp. *melonis*, *Rhizoctonia solani* and *Sphaerotheca cucurbitae*. Extracts of the three plants *i.e.*, *Origanum syriacum*, *Micromeria nervosa* and *Plumbago maritima*, showed the highest

levels of *in vitro* activity against spore germination and mycelial growth of the tested fungi. Preventive sprays with extracts of *O. syriacum*, *M. nervosa*, *P. maritima* and *I. viscosa*, applied at concentrations ranging between 4 and 8% to squash and cucumber seedlings, gave efficient protection against grey mold caused by *B. cinerea* and powdery mildew caused by *S. cucurbitae*.

Morsy *et al.* (2009) reported that plant extracts of garlic and onion reduced linear growth of *F. oxysporum*, *F. solani* and *S. rolfsii*, which associated with cucumber damping-off disease in the newly reclaimed lands. Pre-and post-emergence damping-off were decreased by soaking cucumber seeds in the extracts of garlic or onion at 6% concentration for 60 minutes before sowing. Natural infection by cucumber powdery mildew was decreased by spraying garlic or onion extracts at concentration of 9% and increased of length, fresh and dry weight of shoots and roots as well as number of flowers/plant compared with control. Intercropped cucumber with garlic or onion decreased percentage of pre- and post-emergence damping-off as well as increased leaves number and number of flowers/plant.

c. Modified atmosphere

Ishii (1989) found that growing plants under film that excludes ultra-violet radiation below 380-390 nm markedly decreased spore production in some disease organisms. Results are discussed for experiments with *B. cinerea*.

Elad *et al.* (1992a) characterized microclimatic conditions, which affect epidemics of *B. cinerea* on cucumber crops were grown in unheated polyethylene greenhouses. From 14 to 9 days before the appearance of symptoms, plants were predisposed to infection by low (<9°C) or high (>24°C) temperature and by dryness. Infection occurred 7-8 days before symptoms were visible and was promoted by high humidity (>91% RH) and temperature of 9-24°C.

Watada (1997) monitored quality changes, microbial population and respiration rates in freshly sliced fruits and vegetables (spinach, broccoli, carrots, zucchini [marrows], peaches, honeydew melons and strawberries) stored in a low-O2 controlled atmosphere (CA; 0.5-2.0% O2, 5-10% CO2) to simulate modified atmosphere packaging conditions. CA storage reduced the total microbial population after 10 days, but did not reduce the quality of fresh-cut fruits and vegetables, compared to storage in air. CA storage also reduced the respiration rate of fruits and vegetables, but did not generally affect their respiratory quotients. It is concluded that a low-O2 atmosphere in modified atmosphere packages can be beneficial, providing the minimum O2 concentration is maintained.

Krishna *et al.* (1998) used carbon dioxide to deliver fungicides into greenhouses. Three experimental aerosol formulations for the control of powdery mildew in cucumbers and grey mould in tomatoes (caused by *Sphaerotheca fuliginea* and *B. cinerea* respectively), severity of powdery mildew in cucumbers

was reduced by 65%, and incidence of grey mould on tomatoes was reduced by 68% when treated with these formulations.

Galvis and Morais (2001) evaluated firmness and pectin methyl esterase (PME) activity in pears (cv Rocha) after 9 months of storage in controlled atmosphere (CA) followed by various periods of exposure to air at room temperature. The free calcium content was also evaluated in tissues. Fruit firmness decreased with increasing time of air exposure for all four different CA storage conditions tested. After 9 days of air exposure, fruits stored in 2% O_2+1.5% CO_2 were less firm than control fruits (stored in air) and showed higher PME activity. In spite of normal textural changes being observed with increasing time of exposure to air at room temperature, the underlying metabolism might have been affected by CA storage.

Galvis and Morais (2002) reported that after 9 days of air exposure, fruits stored in 2% O_2+1.5% CO_2 were less firm than control fruits (stored in air) and showed higher PME activity.

Park and Jung (2005) found that pretreated kiwifruits before storage with 30% CO_2 for 1, 2 and 3 days reducd the rate of fruit decay caused by *B. cinerea.*

Fernandez and Martinez (2006) reported that low temperature storage or chilling injury (CI) in pickling cucumber fruit (*Cucumis sativus*. L. cv. 'Tropico' and in the cv. 'Perichan 121') affected the onset of the symptoms of disorders and associated rot.

d- Effect of Fungicides

Kim *et al.* (1990) reported that Prochloraz applied as a foliar spray at 1.56, 6.25, 25 and 100 µg a.i./ml reduced cucumber grey rot effectively, with control values of 35.7, 57.1, 85.7 and 92.9%, respectively. Triadimefon and triforine were less effective at all tested concn. Mycelial growth of *B. cinerea* was inhibited by Prochloraz at 2 µg/ml compared with 20 µg/ml for triadimefon and triforine. Prochloraz had no effect on the respiration of *B. cinerea* at <2 µg/ml, but reduced the rate at 20 µg/ml. Prochloraz did not affect mycelial growth during the 1st 3 h of treatment, but inhibited it significantly at 2 µg/ml after 3 h. Prochloraz also affected cell membrane permeability at 2 µg/ml after 10 h incubation, inducing an increase in conductivity of diluted potato dextrose broth inoculated with a spore suspension of the fungus.

Vozenilkova and Zvara (1990) tested fungicides for control of *Sclerotinia sclerotiorum*, *B. cinerea*, *Cladosporium cucumerinum* and *Didymella bryoniae* on greenhouse grown cucumbers. Rionilan 50 WP [vinclozolin], Rovral 50 WP [iprodione] and Topsin M 70 WP [thiophanate-methyl] were effective against *S. sclerotiorum*.

Elad and Zimand (1991) found that applying *Trichoderma harzianum* alone or in combination with Iprodione, diethofencarb, Carbendazim or Vinclozolin was capable of controlling *B. cinerea* under commercial conditions on greenhouse cucumbers and in vineyards. The tank mix of *T. harzianum* with Iprodione was more effective than either of the agents alone.

Yunis *et al.* (1991) conducted greenhouse trials during winter 1987-88. The number of diseased female fruits of cucumber was reduced by diethofencarb + carbendazim (2.5 mg/ dm3 each) by 93% and by tebuconazole (2.5 mg/dm3) (phytotoxic when alone) or tebuconazole (1 mg/dm3) + dichlofluanid (4 mg/dm3) by 54-57%. Vinclozolin (5 mg/dm3) + chlorothalonil (25 mg/dm3) significantly reduced disease incidence on fruits by 40%. During winter 1988-89, tebuconazole + dichlofluanid (1.5 + 6 and 3 + 12 mg/ dm3) and RH7592 (1 mg/dm3) significantly reduced diseased fruits (30-71%).

Elad *et al.* (1992b) surveyed 15 plastic greenhouses with cucumbers, tomatoes and strawberries at 12 sites during Jan.-Mar. 1989, for the presence of fungicide-tolerant strains of *B. cinerea* using a fungicide-amended Botrytis-selective medium. Tolerance of Benzimidazoles and Dicarboximides was frequent at most sites. Tolerance of Carbendazim + Diethofencarb was found only in the 8 sites where a mixture of these fungicides had been used against grey mould. It was found only in cucumber greenhouses that had been sprayed with the fungicide mixture Carbendazim + Diethofencarb against grey mould. Isolates of this phenotype were pathogenic in artificial inoculation of cucumber cotyledons treated with carbendazim, iprodione or carbendazim + diethofencarb.

Xu (1994) stated that Carbendazol [carbendazim] dust directly spread on young stems effectively controlled grey mould of cucumber (*B. cinerea*).

Ono *et al.* (1997) monitored the sensitivity of 365 isolates of *B. cinerea* collected from 31 protected cucumber fields and 28 open vineyards in Yamanashi Prefecture, Japan, during 1994-96 to benzimidazoles, dicarboximides and diethofencarb. On cucumber, frequency of strains resistant to a diethofencarb mixture was as high as 50%, and one third of them were highly resistant to diethofencarb.

Yabuki *et al.* (1997) monitored the fungicide sensitivity of *B. cinerea* using a rapid sensitivity test on protected fruit vegetables in Kanagawa Prefecture, Japan. Although a strain of the fungus, which was resistant to both a diethofencarb-benzimidazole mixture and a diethofencarb-dicarboximide mixture, was frequently detected on tomato and cucumber in 1995, the frequency of the resistant strains was reduced in 1996.

Kim *et al.* (1998) isolated 2397 isolates of *B. cinerea* from infected plants of strawberry, tomato and cucumber from several areas in the Korea Republic during 1994-96. The tolerance of these isolates against some fungicides was examined. The isolation frequency of phenotypes tolerant of carbendazim, procymidone and diethofencarb were 69.9, 43.7, and 31.8%, respectively.

Park and Yu (1998) examined the sensitivity of 60 isolates of *B. cinerea* collected from tomatoes and cucumber to Dicarboximide fungicides, Procymidone and Vinclozolin, *in vitro* during May-June, 1994 in vinyl houses in Sedo-myon, Puyo-gun, Chungnam Province, Korea Republic. Of 60 isolates, 55 were

tolerant *in vitro* tests on PDA containing the fungicides. Diethofencarb + carbendazim were effective in controlling grey mould on cucumber leaves when inoculated with both mycelia and spores of the isolates.

Kalamarakis *et al.* (2000) mentioned that Fluazinam is a new active ingredient for controlling grey mould, belonging to the novel broad spectrum phenylpyridinamine fungicides. They studied the effect of Fluazinam on one wild type and four strains of *B. cinerea*, which had been isolated from vegetable crops in Greece, and were resistant to benzimidazoles and/or dicarboximides and to the mixture of benzimidazoles (carbendazim) + phenylcarbamates (diethofencarb). *In vitro*, Fluazinam was found to be highly active against strains of *B. cinerea*, which were sensitive or resistant to benzimidazoles or exhibited multiple resistance to benzimidazoles, dicarboximides and to the mixture carbendazim + diethofencarb. No cross-resistance was observed between Fluazinam and the market products Benomyl, Iprodione or Carbendazim + Diethofencarb. Preventive applications of Fluazinam *in vivo* completely inhibited infections of cucumber seedlings by all the above-mentioned resistant strains of *B. cinerea*. Benomyl and Iprodione did not effectively control the benzimidazole and dicarboximide-resistant strains. The mixture of carbendazim+diethofencarb insufficiently controlled the strain of *B. cinerea* with moderate resistance to benzimidazoles. The results of this investigation indicate that it should be possible to use

Fluazinam as an alternative in resistance management programmes against grey mould.

Kim *et al.* (2001) collected a total of 2109 isolates of *B. cinerea* from infected plants of strawberry, tomato, and cucumber in Korea Republic from 1994 to 1996. Based on *in vitro* tests for mycelial growth on potato-dextrose agar containing fungicides, the isolates were classified into six phenotypic groups: SSR, SRR, RSS, RRS, RSR, and RRR, representing sensitivity (S) or resistance (R) to carbendazim, procymidone, and diethofencarb. In that order the isolation frequencies of the SSR, SRR, RSS, RRS, RSR, and RRR phenotypes were 28.7, 1.1, 28.8, 39.4, 1.0, and 0.9%, respectively. In the competitiveness tests, carbendazim-sensitive phenotypes (SSR and SRR) were found to be more competitive than the resistant ones (RSS and RSR), whereas, the procymidone-resistant phenotypes (SRR and RRS) appeared to be more competitive than the sensitive ones (SSR, RSS, and RSR). There was no consistent dominance in competitiveness between the diethofencarb-resistant and sensitive phenotypes. The RSR phenotype was the least competitive among the five phenotypes.

Petsikos *et al.* (2003) reported that no cross-resistance was observed between pyrimethanil or cyprodinil and the fungicides Benomyl, Iprodione or Carbendazim+Diethofencarb. *In vitro*, both Anilinopyrimidine fungicides were effective against strains of *B. cinerea* resistant to benzimidazoles and/or dicarboximides and against a wild type strain insensitive to diethofencarb. Preventive applications

of anilinopyrimidines completely protected young cucumber plants and fruits that were inoculated with all strains of *B. cinerea*.

Moyano *et al.* (2004) surveyed forty-seven greenhouses of vegetable crops in south-eastern Spain at the beginning of the epidemic (January 2000) to compare sensitivity of *B. cinerea* populations to pyrimethanil (an anilinopyrimidine fungicide) after 4 years of treatment with an unexposed population from a 1992 collection. No resistance build-up to pyrimethanil was developed in *B. cinerea* populations after exposition of 4 years to the fungicide. Isolates resistant to pyrimethanil in the *in vitro* assay caused visible lesions on cucumber leaf discs treated with the fungicide. No significant differences in fitness (growth or sporulation) between resistant and sensitive isolates were obtained. The 307 isolates collected in January 2000 were tested *in vitro* using discriminatory doses to estimate the frequencies of resistance of *B. cinerea* to benzimidazoles (carbendazim), dicarboximides (procymidone), N-phenylcarbamates (dietho-fencarb), and anilinopyrimidines (pyrimethanil). Of the 307 isolates collected, 90% were resistant to benzimidazoles, 77% to dicarboximides, 23% to N-phenylcarbamates and 12% to anilinopyrimidines (in this case of 165 isolates). Dicarboximide and benzimidazole cross-resistant isolates were found in each of the surveyed greenhouses and accounted for 65.8%. Fourteen percentage of the population were resistant to dicarboximides, benzimidazoles and N-phenylcarbamates, and 3% were also resistant to anilinopyrimidines.

Markoglou *et al.* (2006) reported that pyraclostrobin inhibited strongly Oxygen uptake in whole cells in the wild-type strain and mutants of *B. cinerea* with moderate and high resistance to azoxystrobin, fluoxastrobin, trifloxystrobin and picoxystrobin, but not to famoxadone. They reported also that pyraclostrobin play as inhibitor of mitochondrial electron transport at the cytochrome. Moreover, no effect of pyraclostrobin resistance mutation(s) on fungitoxicity of the hydroxyanilide fenhexamid, the phenylpyrrole fludioxonil, the benzimidazole benomyl, and to the phenylpyridinamine fluazinam, which affect other cellular pathways was observed.

Smolinka and Kowalska (2006) evaluated efficacy of plant extracts of the green parts of potato, tomato, Indian mustard, rape, Lucerne, *Camelina sativa,* and fruits of elder (*Sambucus nigra*) in controlling *B. cinerea, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Fusarium sp.* which infecting the French bean [*Phaseolus vulgaris*]. They found that the most toxic extracts, especially on *Fusrium sp., B. cinerea* and *R. solani,* were obtained from green parts of potato, tomato, and rape.

Shi *et al.* (2007) reported that Chlorphenomizole is a new fungicide active against several fungal pathogens. *In vitro* trials (1000, 800, 500, 100, 50, 20, 10, 8.9, 8ml/L 90% chlorphenomizole, water as control) were conducted to investigate its effect on *Cladosporium cucumurnim* and *Fusarium oxysporum* by spore germination trial, *Sclerotinia sclerotiorum* and *Phytophthora capsici*

by microbiostatic ring, *B. cinerea* by detached leaves and a pot trial conducted on *Sphaerotheca fuliginea*. Results showed that chlorphenomizole at the rate of 500 mg/litre exhibited high activity against more than 80% pathogens. Results of greenhouse trials in cucumber cv. Shandongmici conducted in the period 2004-05 were reported. On cucumber, 20% chlorphenomizole applied at the rate of 40 mg/litre at intervals of 7 days three times showed outstanding levels of efficacy at 80% on *B. cinerea* and 84% on *Cladosporium cucumurnim*.

Nasreen and Ghaffar (2010) studied the effect of fungicides, microbial antagonists and oilcakes in the control of *F. solani* the cause of seed rot, seedling and root infection on bottle gourd, bitter gourd and cucumber *in vitro* and *in vivo*. Complete inhibition of colony growth of *F. solani* was observed where fungicides *viz*., Aliette, Benlate and Carbendazim at 100 ppm were used. Carbendazim completely eradicated seed borne infection of *F. solani* in bitter gourd and gave maximum reduction in cucumber and bottle gourd. Root infection was completely checked by Benlate and Carbendazim in bitter gourd and was best controlled by Aliette, Topsin-M and Carbendazim in bottle gourd and cucumber. *F. solani* infested seeds of bottle gourd, cucumber and bitter gourd reduced seedling mortality and root infection when sown in mustard and neem cake amended soil. Mustard cake was found most effective at all ratios followed by neem and castor cake.

Takagaki *et al.* (2010) reported that Pyribencarb methyl {2-chloro-5-[(E)-1-(6-methyl-2-pyridylmethoxyimino) ethyl] benzyl} carbamate, is a novel fungicide having excellent activity against a wide range of plant pathogenic fungi, especially grey mold diseases caused by *B. cinerea*. Pyribencarb exhibited not only a preventive effect but also a curative effect. When spraying was performed 48 hr after inoculation (after visible symptoms appeared), Pyribencarb also showed strong inhibitory activity against lesion development by cucumber grey mold that was significantly superior to its preventive activity.

e. Enzymatic studies:

1- Pectinolytic enzymes activities:

Kim *et al.* (1997) measured the activities of polygalacturonase, laccase and intra- and extra-cellular β-glucosidase produced by 20 *B. cinerea* isolates in liquid culture media containing cucumber cell walls as a carbon source and their relationships to pathogenicity were analyzed. No significant correlations between enzyme activity and the pathogenicity of *B. cinerea* were found. Immuno-blot analysis of the culture filtrate using antibodies against exo-polygalacturonase revealed that only one band with a molecular weight of 66 kDa was detected among 34 tested isolates. It appears that these enzymes may not be the primary factors in determining the pathogenicity of *B. cinerea*.

Rha *et al.* (2001) found that the pathogenic fungus, *B. cinerea* causing grey mould disease in a variety of plant species, secretes at least four polygalacturonases (PGs), cell wall degrading enzymes. Among them, we prepared polyclonal antibody against purified 66-kDa exo-PG in rabbit. Immunoblot analysis revealed that the antibody recognized two exo-PGs, 66 kDa and 70 kDa in molecular mass, secreted from *B. cinerea* cultured in the medium containing citrus pectin as a carbon source. By immunohistochemical analysis, the expression of exo-PGs was identified in cucumber leaves inoculated with spores of *B. cinerea*. The exo-PGs were observed 9 h after inoculation, and the amount of exo-PGs increased with time in the leaves. The exo-PGs were induced by polygalacturonic acid as well as its monomer, galacturonic acid *in vitro*. The expression of 66-kDa exo-PG (exo-PG I) increased with time of culture, while 70-kDa exo-PG (exo-PG II) was transiently expressed soon after the start of culture. Therefore, exo-PGs might play an important role in pathogenesis at an early stage of infection as well as in tissue maceration of host plant.

El-Habbaa (2003) reported that four isolates of the grey mould fungus *B. cinerea* obtained from infected cucumber, pepper and strawberry fruits and rose petals had the ability to produce cell wall degrading enzymes. Secretion of cellulase, xylanase and PG enzymes was affected, in general, by the isolate source, pH value, carbon source and incubation temperature. *Botrytis* isolates varied in the production of PG enzyme *in vivo* and this was depending on

the infected plant tissues. PG isozyme analysis exhibited different 7 to 13 PGs isozyme bands varied in their intensities. In cultural filtrates, Bc-1 isolate showed 7 & 13 while Bc-2 isolate showed 10 & 9 isozyme bands at pH 6.8 and pH 5, respectively. Thus, degree of intensity and number of PG isozyme bands in the cultural filtrates of the two isolates were variable and affected by pH value. In tissues of carrot, strawberry, cucumber and pepper infected with Bc-1, 10, 9, 13 and 12 PGs isozyme bands were observed, respectively. Ability of *B. cinerea* isolates to attack different hosts and plant organs was discussed in the light of their PG isozymes.

Veronesi *et al.* (2009) showed that the enzymatic activities (cellulose, pectinase, pectin lyase and protease) of isolated *F. solani* f. sp. *cucurbitae* race 1 isolates from crown and foot rot disease of zucchini in Bologna province since 2004 showed high variability among the strains. Cellulase and pectin lyase were correlated with disease incidence or disease severity. Pectinase values were very low and did not vary among the strains, whereas protease levels were high, from 6.44 to 24.28 µg/µL.

Ahmed (2010) found that *B. cinerea* was more active in producing PG enzyme than *S. sclerotiorum*. Also, *B. cinerea* was less active in producing Cx than *S. sclerotiorum* at 15 days of incubation *in vivo*. Moreover, the loss% in viscosity was increased by increasing the reaction time from 15 to 30 min.

2- Oxidative enzymes activities:

Biochemical changes associated with plant resistant have been studied on different healthy and infected hosts. In this respect, oxidative enzymes are a biochemical marker for resistance in several host-pathogen systems.

Turner (1965) showed that secretion of polyphenoloxidase enzyme was considered an indicator of resistance in a number of plants to fungal pathogens. Comparison of polyenoloxidase activity in varieties susceptible or resistant to a particular pathogen frequency showed this activity to be higher in those plants showing resistance.

El-Khadem *et al.* (1985) found that oxidative enzymes and ascorbic acid oxidase in healthy cucumber plants were higher in leaves of the resistant variety than in the susceptible ones. It also was higher in inoculated leaves than in healthy ones.

Reuveni and Kue (1989) explained the association of peroxidase with systemic resistance to *Cladosoporium cucumerinum* infection. They found that the induction of systemic induced resistance on cucumber which is induced by *Colletotricum lagenarium* in cucumber cultivars resistant or susceptible to *C. cucumerinum* is associated with enhanced peroxidase activity.

El-Toony (1992) reported that *Alternaria alternata* infection of some cucurbit plants increased the activity of pectin methyl

esterase (PME), polyglacturonase (PG) and cellulase (Cx) in extracts of the infected tissues comparing with check treatment.

Reuveni *et al.* (1992) found that peroxidase activity in uninfected muskmelon plants was used to predict the resistance and susceptibility of 527 plants as cultivars or breeding lines and crosses of susceptible and resistant plants. Peroxidase activity was increase with time in both susceptible and resistant plants. The ratio of activity in infected or uninfected leaves was increased over the time in the susceptible plants. This ratio however, was lowered or remained unchanged in the resistant.

Yurina *et al.* (1993) exhibited that peroxidase activity was related to resistant and to tolerance against powdery and downy mildew of cucurbits. Resistant varieties of cucurbits had higher activity of the enzyme than the susceptible ones.

Mohamed *et al.* (1995) found that peroxidase activity in healthy cucumber plants was higher in the moderately cultivars, than in the highly susceptible one while polyphenoloxidase and catalase activities were higher in the healthy plants of the highly susceptible cultivar. Infection increased the activity of peroxidase, polynoloxidase and catalase in both cultivars.

Faccoli and Schlyter (2007) found that phenols are important in conifer resistance to fungi associated with bark beetles and as markers for resistance to beetle mass-attacks.

Hassan *et al.* (2007) revaled that, citric and benzoic acids were the most effective ones, since they recorded the lowest

percentages of disease severity of *Botrytis fabae* and/or *B. cinerea* on faba bean plants and the highest levels of peroxidase activities. Moreover, pretreated faba bean plants showed some new isozymes and increment in the density of original isozymes, especially in infected plants.

Shang *et al.* (2007) showed that chitosan decreased disease grey mould of *B. cinerea* index of cucumber seedlings by 39.4%, increased phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) activities, enhanced total polyphenol, flavonoid and lignin content. Chitosan played an important role in inducing disease resistance of cucumber seedlings with the optimum concentration at 200 mg.

f- Effect of initiated amino acids:

Phytoalexins are antimicrobial, low-molecular-weight secondary metabolites that are both synthesized by and accumulated in plant cells as a result of the interaction between the metabolic systems of the host and a fungal parasite, and that require *de novo* expression of the enzymes involved in their biosynthetic pathway **(Müller, 1956** and **Paxton, 1980)**. For example, the major phytoalexin in alfalfa is the pterocarpan medicarpin, synthesized from phenylalanine via the isoflavonoids pathway. The elicitation of medicarpin in alfalfa cell suspension cultures exposed to crude elicitor from cell walls of *Colletotrichum lindemuthianum* is preceded by increases in the activity of all 11 enzymes required for its biosynthesis and their transcriptional activation. The genes encoding

L-phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and chalcone reductase (CHR) were the most rapidly activated, within 10-20 min after the elicitation of the isoflavonoids biosynthetic pathway **(Dixon *et al.*, 1995** and **Salles *et al.*, 2002)**. Phytoalexins are substances not detectable before infection, and are considered to inhibit the further development of most attacking pathogens of the species under consideration.

Jun *et al.* (1988) reported that total RNA prepared from healthy cucumber leaf, stem, fruit and root tissues were hybridized to the ascorbate oxidase c DNA colne under stringent conditions. The results show that 5 reved an abundant in fruit and stem tissues and allow abundance in leaf and root tissues.

Zimmerli *et al.* (2001) found that the non-protein amino acid β-aminobutyric acid (BABA) protects numerous plants against various pathogens. Protection of Arabidopsis plants against virulent pathogens involves the potentiation of pathogen-specific defense responses. BABA-treated Arabidopsis were found to be less sensitive to two different strains of the gray mold fungus *B. cinerea*. Treatments with benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester, a functional analog of salicylic acid (SA), also markedly reduced the level of infection.

Ma *et al.* (2007) sequenced the complete two-component histidine kinase gene (Bos1) from eight dicarboximide-resistant and six-sensitive field isolates of *B. cinerea*. Comparisons in the predicted amino acid sequences of Bos1 showed that each two

isolates had a single point mutation at amino acid position 365 from an isoleucine to serine (I365S) or to an asparagine (I365N). Three isolates were characterized with a change from glutamine to proline at position 369 (Q369P) as well as an asparagine to serine at the position 373 (N373S). These mutations located within the 90-amino-acid repeats of Bos1 have been reported previously.

Wayne and Benny (2010) reported that separation and quantitation of amino acids derivatives is reliable tool to examine the inter play of genetic potential and growing conditions on the levels of physiologic amino acid pools in cucurbits.

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Survey of cucumber and squash fruit diseases:

Diseased samples of naturally infected cucumber (*Cucumis sativus*) and squash (*Cucurbita pepo*) fruits showing identical fruit rot (grey mould and fusarium rot) symptoms were collected from greenhouses during autumn and spring of 2003 and 2004 seasons. Samples were collected from different areas representing seven governorates. *i.e.* Beheira, Kafr El-Shikh, Qalubiya, Ismailia, Sharkiya, Dakahliya and Giza. The infections were estimated as percentages of diseased fruits comparing to total of healthy ones.

2. Isolation, purification and identification of the associated fungi:

The associated fungi with rotted cucumber and squash fruits collected from different locations were isolated and identified. Small pieces of diseased fruits were cut, sterilized in 0.3% sodium hypochlorite solution for one minute, washed several times in sterilized distilled water and dried between sterilized filter papers. The sterilized pieces were directly transferred to Petri dishes containing potato dextrose agar (PDA) medium, then incubated at 22±2°C for 3-7 days. The plates were examined daily and each of the emerged fungi was transferred on fresh PDA plates. Isolated fungi were purified using single spore or hyphal tip techniques. Identification of the isolated fungi was carried out in the Vegetable

Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, according to **Barnett and Hunter (1998).** The number of detections and % frequency of each isolated fungus was calculated.

3. Pathogenicity tests:

The pathogenicity test of the isolated fungi were carried out under the laboratory conditions using healthy mature fruits of cucumber (cv. Sinai I) and squash (cv. Eskandarani). The selected fruits were carefully chosen free from mechanical disorders or diseases as far as possible. Then, they were surface sterilized as before. Each fruit was inoculated with culture disc (5 min in diameter) with each fungus and three replicates with 5 fruits for each replicate were used for each fungus. The inoculated fruits were placed in polyethylene bags and incubated at room temperature (22±2°C). The check was left without fungal inoculation. Percentage of infection and disease severity index of rotted fruits were recorded 7 days after inoculation.

Disease assessment:

Disease readings were determined for fruits according to a modified disease index depending on the average diameter of the affected area of fruits. The following numerical rates were suggested to facilitate visual determined symptoms and to give a satisfactory comparison:

0 = healthy fruit with no grey mould rot.
1 = scattered small grey mould rotted spots.
2 = grey mould including about 25% to 50% fruit area.
3 = more than 50% of the infected fruit area.

Readings were calculated according to **Townsend and Heuberger (1943)** as follows:

$$\text{Disease index\%} = \frac{\sum (n \times r1) + (n \times r2) + (n \times r3)}{N3 \times 100}$$

Where, n is the number of fruits in each numerical rate; r_1, r_2 and r_3 are ratings, and N is the total number of inoculated fruits multiplied by the maximum numerical rate. Also, the percentage of infected fruits was estimated.

4. Effect of inoculum potentials:

Two isolated fungi from cucumber rotted fruits (*B. cinerea* and *F. solani*) collected from cucumber greenhouses at Ismaelia district were used in this study. Each fungal isolate was grown on PDA medium in Petri dishes at 25°C for 7 days. Spore suspensions were prepared by flooding fungal colonies with about 10 ml of 0.02% Tween 20 in sterile distilled water (**Bautista-Baños *et al.*, 2001**), gently agitating with a sterile glass rod and filtering through glass wool. After filtration through double layers of cheesecloth the obtained spore suspensions were adjusted originally to be containing $1.0x10^6$ spores/ml by using haemocytometer slide then several concentrations *i.e.* $7.0x10^3$, $6.0x10^3$, $5.0x10^3$, $4.0x10^3$, $3.0x10^3$, $2.0x10^3$ and $1.0x10^3$ spores/ml were prepared by dilution

in sterilized distilled water. Spore suspension at each given concentration was sprayed on the cucumber or squash fruits using hand atomizer, they were placed polyethelen bags. Cucumber or squash fruits sprayed with sterile distilled water served as control. Three replicates with 5 fruits each were used for each particular treatment. All treatments were incubated at room conditions (22±2°C). Percentages of infection and disease severity were recorded one week after inoculation.

5. Cultivar reactions (responses):

In this study, two isolated fungi (*B. cinerea* and *F. solani*) from cucumber rotted fruits as the main casuals of fruit rot disease were used. Fruits of seven cultivars of cucumber *i.e.* Sinai I, Fysal, Delta star, Samar, Shams, Heikal, New Star and in addition to the squash cultivar "Eskandarani" were used to investigate their responses against infection with the tested pathogenic fungi. Apparently healthy fruits were rinsed several times in sterilized distilled water, dried between sterilized filter papers and surface sterilized by ethyl alcohol 70% for 2 minutes. Each fruit was inoculated by spraying with spore suspension containing $6x10^3$ spores/ml using hand atomizer. Three replicates with 5 fruits in each were used as a treatment. The inoculated fruits were put in full dishs and covered by polyethelen shet and incubated at room temperature (22±2°C). Response of different tested cultivars in term of percentage of infection with the

two tested fungi and disease severity index of rotted fruits were recorded 7 days after inoculation.

6. Factors affecting cucumber and squash fruit rot diseases severity:

6.1. Plant age:

Effect of plant age on fruit rots incited by the two tested pathogenic fungi was studied. Seeds of cucumber (cv. Sinai I) and squash (cv. Eskandarani) were planted in the greenhouses of Kaha Research Station. Healthy looking 3-days old cucumber and squash fruits (uniform in size) were harvested after 45, 60, 75 days from planting and taken to laboratory. The selected fruits were rinsed several times in sterilized distilled water, dried between sterilized filter papers and surface sterilized by ethyl alcohol 70% for 2 minutes. Half of the selected fruits were gently wounded by streaking with a sterilized fine needle on one side of each chosen fruit. The wounded and unwounded fruits were inoculated as above described with a spore suspension containing 6000 spores/ml of each one of the tested fungi. Each treatment was represented by four replicates and three fruits were used for each replicate. The inoculated fruits were kept under glass jars at room temperature (22±4°C) for ten days. Percentages of disease incidence and disease severities of fruit rots were recorded as above described.

6.2. Fruit age:

In this study cucumber (cv. Sinai I) and squash (cv. Eskandarani) fruits with different ages *i.e.* 1, 2, 3, 4 and 5 days were harvested from plants grown under greenhouse conditions at Kaha Research Station. Healthy looking fruits were rinsed several times in sterilized distilled water, dried between sterilized filter papers and surface sterilized by ethyl alcohol 70% for 2 minutes. The collected fruits were inoculated as above described with a spore suspension containing 6000 spores/ml of the two tested pathogenic fungi. Each treatment was represented by four replicates and three fruits were used for each replicate. The inoculated fruits were kept under glass jars at room temperature (22±4°C) for ten days and fruit rot disease severity was recorded as above described.

6.3. Storage temperature:

Cucumber and squash fruits were carefully harvested and healthy ones were selected, washed and surface sterilized as described before. Then, the fruits were inoculated as above described by spraying them with spore suspensions prepared from *in vitro* grown cultures of each one of the tested pathogenic fungi. Inoculated fruits were incubated at different temperatures *i.e* 2, 5, 7, 24°C. Thirty fruits in 3 replicates were used for each particular treatment. The fruit rot disease severity was estimated as previously described after storage for 4 and 8 days.

6.4. Modified atmosphere (using CO_2):

In this study, healthy looking cucumber fruits (cv. Sinai I) were harvested from cucumber plants grown under El-Haram greenhouses at Giza and brought to the laboratory. The chosen fruits were surface sterilized and inoculated by spraying them with a spore suspension (6000 spores/ml) of either of the two tested fungi. The inoculated fruits were stored at 22±4°C for 10, 15 and 21 days under controlled conditions containing 10, 15 or 20% of CO_2 in clothed polyethylene bags. Each treatment has three replicates with five fruits in each replicate. The untreated inoculated fruits served as control. The development of fruit rot disease severity for each treatment was recorded as above described.

6.5. Exposure to UV radiation:

Samples of cucumber fruits cv. Sinai I were collected from location of El-Haram greenhouses at Giza and brought to the laboratory. The healthy looking fruits were surface sterilized and inoculated with spore suspension of either tested pathogenic fungi as above described. The inoculated fruits were exposed to UV radiation at either 280 or 320 nm for 1 or 2 hours for each. The inoculated UV-exposed fruits were placed in tightly sealed polyethylene bags. Inoculated but not UV-exposed fruits served as control. Three replicates (5 fruits/each) for each treatment were used. The fruit rot disease severity was recorded after 21 days as above mentioned.

7. Effect of fungicides:

7.1. Effect of some fungicidal treatments on growth of tested fungi *in vitro*:

In this study, seven different fungicides as clear in **Table, (1a)** were used at the concentrations of 25, 50, 100, 150, 200, 250, 300, 400 and 500 ppm (based on their active ingredients) to investigate their effects on the linear growth of the two tested fruit rot pathogenic fungi.

Table (1a): Trade name, common name (between bracts), chemical composition and industrial companies of different fungicides used.

Trade name	Common name	Chemical composition	Company
Ronilan 50% w.p.	Vinclozolin	3- (3.5-dichlorophenyl)-5- viny P-5-methyl-1.3-oxazolidine- 2,4-dione	Sumitomo
Teldor	Fenhexamide	N-(2.3-Dichloro-4hydroxy phenyl)11MethyyllcycpoHexaMecar box amide	Bayer
Copral 50%WP	Copper oxychloride 50%WP	Di Coppers Chloride Tri, hydroxide $CuCl_2$ 3Cu $(OH)_2$	Kafer Al-Zyat
Redomil M 2587	Methoxya Mitalaxil+ mancozib	N-2,6- Dimethyl N-2-Cetyl-Dit- alani nmethyl- ester	Syngenta
Sumisclex 50% W.P.	Procymidone	N- (3.5-dichlorophenyl)- 2 diemthylcyclopropane- 1,2-dicarboximide	Sumitomo
Rovral	Iprodione	3-(3, 5-dichlorophenyl)-N-(1-methylethyl) 2, 4-dioxo-1-imidazolidine-carboxamide.	Bayer
Tecto 45% FL.	Thiabendazole	2-(4- Thiazolyl)benzimidazole.	Syngenta

The amount of a tested fungicide required to make a known concentration was added to autoclaved warmed (45-50°C) Czapek's agar medium immediately before solidification. The treated medium for each fungicidal treatment was poured into four Petri dishes. Fungicide-free medium was used as control. Petri dishes with or without fungicide were inoculated with mycelial disc (5 mm in diameter) of 5 days old cultures of the two tested fungi. Four replicates were prepared for each treatment, as well as for control. All dishes were incubated at 20±2°C for 7 days then diameter of fungal growth of each particular treatment was measured and recorded.

7.2. Effect of pre-harvest fungicidal treatments on controlling fruit rot-diseases:

Cucumber (cv. Sinai I) and squash (cv. Eskandrani) plants were grown under conditions of El-Haram greenhouses at Giza. The grown plants were sprayed five times at 7 days intervals with a concentration of 250, 500 and 750 ppm that was prepared for a known fungicides tabulated in **Table (1a).** At harvest, any bruised and wounded fruits were discarded. The selected fruits from each fungicidal treatment as well as from the control (unsprayed plants) were washed thoroughly with tap water followed by 5% sodium hypochlorite for 5 minutes, then left to air dried. Sterilized cucumber and squash fruits were artificially wounded and sprayed with spore suspension (6×10^3 spores/ml) of each isolate using hand atomizer. Similarly uninoculated, but immersed in sterile water were served as check, for

each treatment, three replicates of 30 fruits (10 fruits/each) were used. Fruits were allowed to dry and then stored at 20±2°C. The fruits were examined daily until full development of disease among check fruits, and then fruit rot disease severity was measured and recorded.

8. Effect of pre-spraying cucumber and squash plants with some chemicals and salts:

8.1. Effect on fruit rot infections after harvesting:

In this study, Cucumber (cv. Sinai I) and squash (cv. Eskandarani) seeds were sown under greenhouse conditions of Research Station at El-Haram, Giza, ARC. The transplants were sprayed with aqueous solutions of salicylic acid (SA), oxalic acid (OA), sodium sulfate (Na_2SO_4), magnesium sulfate ($MgSO_4$), lithium sulfate ($LiSO_4$) or potassium dihydrogen phosphate (K_2HPO_4), Bion, Ethephon, Catechol and Calcium chloride ($CaCl_2$) **(Table, 1b)** at 7 days after sowing with concentration of 250, 500 and 1000 ppm for each particular chemical inducers.

In control treatment, plants were sprayed with sterilized distilled water instead of chemical inducers. After 45 days from sowing, the cucumber and squash fruits were harvested, surface sterilized by wiping with ethyl alcohol 70% followed by rinsing several times with sterilized distilled water then dried between two sterilized towels. All fruits were inoculated as above mentioned with fungi B. cinerea or F. solani then stored under room temperature (20±2°C). The fruit rot disease severity as affected by tested chemical inducers was determined as described above 10 days after inoculation.

Table (1b): Tested chemical inducers, their formula and molecular weights

Chemical products	Molecular formula	MW (g/mol)
Salicylic acid	$HO.C_6H_4.COOH$	138.12
Oxalic acid	$HOOC.COOH._2H_2O$	126.07
Lithium sulfate monohyderate	$Li_2SO_4.H_2O$	127.95
Potassium dihydrogen phosphate	KH_2PO_4	136.08
Sodium sulfate	Na_2SO_4	142.04
Magnesium sulfate	$MgSO4.7H_2O$	246.45
Calcium chloride	$CaCl_2$ (monohydrate)	128.999
Bion	1,2,3-Benzothiadiazole-7-carbothioicacid, S-methyl ester (135158-54-2)	210.276
Ethephon	2-chloroethylphosphonic acid ($C_2H_6ClO_3P$)	144. 5
Catechol	1,2-dihydroxbenzene ($C_6H_6O_2$)	110.1

8.2 Effect on changes in protein patterns in leaves of cucumber and squash plants

Poly-acrylamide gel electrophoresis (PAGE) was used to determine the quantitative changes that occur in the soluble proteins of leaves of cucumber and squash plants previously sprayed with some tested resistance inducers *i.e.*, Bion, SA, OA, KH_2PO_4, $CaCl_2$ at 7 days after transplants (**Broglie *et al.*, 1986**).

Protein extraction:

Leaves of cucumber and squash treated with some tested resistance inducers 30 days old were taken. Two grams from each

sample were ground in 0.05 M sodium acetate buffer + sea sand with a mortar in liquid nitrogen at 4°C. After that 50 mg of the extract plus 0.7 ml of ES solution (4% SDS, 5% sucrose and 50% mercapto ethanol) were shacked for 10 min at room temperature with gentle stirring. The extract was centrifuged at 18,000 rpm for 30 min and the clear supernatant was heated at 100°C for 2–5 min and then cooled to room temperature. Proteins were precipitated by adding cold (-20°C) acetone (8X volume of the supernatant). Protein content of the supernatant was determined using the method described by **Ekramoddoullah and Davidson (1995)**.

Staining of protein bands:

Silver staining method of protein bands was used as described by **Hochstrasser *et al.* (1988)** as follow:

- 300 ml H_2O+300 ml ethanol, then, shaking for 20 min. 3 times
- 400 ml H_2O+0.08 g Sod. Boronhydrate, then, shaking for 1 min.
- Wash for 20 sec. 3 times,
- 400 ml H_2O+0.8 g Silver nitrate + 300 µL formalin, shaking for 20 min.
- Wash for 20 sec. 2 times.
- 400 ml H_2O+12 g Sod. Carbonate + 400 µL formalin + 8 ml Sod. Bornehydrate, then shaking until protein bands appear.
- Put the gel in fix (storage) solution.

Protein separated by SDS-PAGE was transferred to Immobilon-P membranes as described by **Matsudaira (1987).**

Gel analysis

Protein or DNA gel was scanned for band Rf using gel documentation system (AAB Advanced American Biotechnology 1166 E. Dr. Valencia, Unit-6C, Fullerton CA 92631). The different molecular weights of bands were determined against PCR marker Promega-G317 by un-weighted pair-group method based on arithmetic mean (UPGMA).

8.3. Effect on changes in analysis of the free amino acids in plant leaves

Hydrolysis of Amino Acids:

This work has been done at the Amino Acid Analyzer Lab., at Faculty of Agriculture Research Park, Cairo Univ. Automatic Acid Analyzer AAA 400 (INGOS Ltd) was used. Twelve samples were used in this work. Acid hydrolysis was carried out according to the method of **Block *et al.* (1958)**. The dried grinded sample (100 mg) was hydrolyzed with 6 N HCl (10ml) in a sealed tube at 110°C in an oven for 24 hours. The excess of HCl was then freed from 1 ml hydrolyzed under vacuum with occasionally addition of distilled water, then evaporated for dryness. The HCl free residue was dissolved in exact (2 ml) of diluting buffer (0.2M, pH2.2).

Preparation of diluting solution of buffer 0.2 M Na, pH 2.2:

The used buffer for diluting both samples and standards to the required concentration was prepared according to the following recipe: Citric acid (14.0g/l); Sodium chloride (11.5g/l); Thiodigrycol (5.0ml/l) and Sodium azide (0.1g/l).

8.4. Effect on biochemical components in fruits inoculated with fruit rot pathogens:

In this study, cucumber (cv. Sinai I) and squash (cv. Eskandarani) seeds were sown under greenhouse conditions of Research Station at El-Haram, Giza, ARC. The transplants were sprayed with aqueous solutions of four different resistance inducers chosen randomly *i.e.,* Bion, Calcium chloride ($CaCl_2$), Catechol and Ethephon at 500 ppm as well as they sprayed also with two different fungicides from those previously tested *i.e.,* Copral and Teledor at 500 ppm at 7 days after sowing. In control treatment, plants were sprayed with sterilized distilled water instead of chemical inducers. After 45 days from sowing, the cucumber and squash fruits were harvested, surfaced sterilized by wiping with ethyl alcohol 70% followed by rinsing several times with sterilized distilled water then dried between two sterilized towels. All fruits were inoculated as above mentioned with either *B. cinerea* or *F. solani* and then stored under room temperature (20±2°C).

8.4.1. Changes in phenolic compounds:

A known amounts (5g) of cucumber fruits were cut into small portions, immediately plunged into 95% boiling ethanol for 10 min., in order to kill the tissues then extracted for 10-12 hrs in soxhlet units using 75% ethanol till the percolate was colorless. The combined ethanol extracts were filtered and rotary evaporated to near dryness at 60°C. The dried residues were redissolved in a known volume (5ml) of 50% isopropanol alcohol. The later isopropanol extracts were used

for determining free, total and conjugated phenols using Folin and Ciocalteu's phenol reagent as described by Snell and Snell (1953). Phenolic compounds were calculated as milligrams equivalent of catechol/5g fresh weight of leaves.

8.4.2. Changes in total amino acids:

Total amino acids were determined using the method of analysis described by Muting and Kaiser (1963). The ethanolic extract (0.1ml) was placed into tube containing 1.5ml ethanol/acetone mixture (1:1 v/v) + 1ml phosphate buffer (pH 6.5) and 2.0ml 0.5% ninhydrin solution in n-butanol. The tube was placed into boiling water bath for 10 minutes, then immediately cooled in ice water and the mixture volume was made up to 10 ml. with absolute methanol. The developed color was measured at 580 nm using spectrophotometer (Spectronic 20-D) against a reagent blank. Data were obtained referring to standard pure glycine curve.

9. Effect of pre-spraing cucumber and squash plants with plant extracts and kombucha tea preparations

9.1. Effect on the infection with fruit rot-pathogens:

In this study, different preparations of plant extracts and kumbusha tea were used. The used plant extracts were prepared by blending a known weight (200 g) of a known plant parts (Table, 1c) in an electric blender with 200 ml of sterilized distilled water. The resulted homogenates were squeezed in two layers of cheesecloth then centrifuged at 3000 rpm for 5 minutes. The clear supernatants were considered as 100% crude extract and diluted with sterilized

distilled water to make 10 and 20% concentrations. One of tested material was known as kombucha (fermented tea). Kombucha culture was kindly provided by Dr. Mohamed Hafez, Associate Prof. of Plant Pathology, Faculty Agric., Benha Univ. Kombucha tea is made by combining its culture, with a mixture of black tea, and sugar. The ingredients are allowed to "ferment", usually for 15 days.

Table (1c): Plant and plant parts used in preparing the tested plant extracts and Kombusha tea the present study

English name	scientific name	Arabic name	Used parts
Henna	*Lawsonia inermis* L.	حناء	Leaves
Nigella	*Nigella sativa* L.	حبة البركة	Seed
Garlic	*Allium sativum* L.	ثوم	Cloves
Fermented tea *	*Kombusha tea* *	كومبوشا	Filtrates *
Thyme	*Thymus vulgaris* L.	زعتر	Leaves
Ginger	*Zingiber officinale* L.	زنجبيل	Rhizome
Carnation	*Dianthus caryphlus* L.	قرنفل	Cloves
Marjoram	*Majorana hortensis* L.	بردقوش	Leaves

* Prepared according to (http://www.kombucha.org)

The cucumber (cv. Sinai I) and squash (cv. Eskandrani) plants were grown under greenhouse conditions at El-Haram, Giza. The cucumber and squash plants were sprayed at beginning of flowering stage then at 7 days intervals with 10 or 20% concentration of a known preparation. Control plants were sprayed at the same times with sterilized distilled water. Three days after the last spraying, the healthy looking cucumber and squash fruits were harvested, taken to the laboratory, surfaced sterilized, inoculated as above-mentioned with *B. cineria* or *F. solani* then stored under room temperature (20±2°C). Cucumber and squash fruits which harvested from

unsprayed plants (control) were surface sterilized as usual then inoculated as above described with either pathogens. Three replicates with five fruits of each served as a particular treatment. Fruits of each replicate for a known treatment were placed in polyethylene bags and stored under room temperature (20±2°C) then fruit rot disease severity was determined as described above 10 days after inoculation.

9.2. Effect on the enzymes activities in fruits inoculated with fruit rot pathogens

In this experiment, the activities of both pectolytic (PG and PME) and oxidative (PPO and PRO) enzymes were determined in tissues of the cucumber and squash fruits harvested from plants of the above experiment. All harvested fruits were inoculated with the tested pathogens. Enzyme activities were done after 15 days after inoculation. Fruit samples (50 g) were taken from each particular treatment, blended for 15 minutes in a warning blender containing the same amount of distilled water (50 ml), then filtered through muslin and centrifuged at 3000 rpm for 20 minutes at 6°C. The clear supernatant fluids were used for pectolytic (PG and PME) enzyme assays.

9.2.1. Activity of the pectolytic enzymes:

A. Pectin methyl esterase (PME) activity

Pectin methyl esterase (PME) activity was determined by the titration method described by Kertesz (1951) as follows: Five ml of crude enzyme preparation of each particular treatment plus five ml 1.2% aqueous high meoxyl pectin buffered at pH 5.6 were allowed to

react for 4 hours at 30°C after that titrated back to pH 5.6 with 0.01 N (NaOH) solution. The reaction was continued for 20 hours after which the pH was readjusted to pH 5.6 and the total volume of 0.01 N (NaOH) required over the 24 hours was calculated. A check was carried out in which 5 ml of boiled crude enzyme was added to 5 ml buffer substrate. The difference between the volume "in ml" of 0.01 N (NaOH) required for the crude enzyme and that required for the boiled check, gave an approximate indicator of the relative activities of the PME enzyme in each particular treatment. Efficiency of different treatments to reduce PME activity comparing to the untreated control was calculated. Contamination with microorganisms was prevented by the addition of two drops of chloroform to each reaction mixture tube.

B. Polyglactorinase (PG) activity:

The activity of polyglactorinase (PG) was determined using the viscometric method described by **Matta and Dimond (1963)** by calculating the loss in viscosity of the substrates in Ostwald viscometer at 30°C. The substrate used for measuring PG activities was 1.2% pectin in phosphate buffer solution at pH 5.6. The crude enzyme sample (2.5 ml) was added to 5 ml buffer, and then incubated at 30°C. A check was run in which 2.5 ml of boiled sample was substitute. After 5, 10, 15, 20 and 60 minutes of incubation, viscosity was measured and the percentage of loss in

viscosity was recorded using the following equation proposed by **Bell *et al.* (1955).**

$$\text{Activity \%} = \frac{(T_0 - T_t)}{T_0 - T_w} \times 100$$

Where: T_0 = Time of flow (in seconds) of the reacted mixture at zero time; Tt = Time of flow (in seconds) at a given time intervals of the sample and Tw = Time of flow (in seconds) of the distilled water

9.2.2. Activity of the oxidative enzymes:

Oxidative enzymes activity was determined in inoculated cucumber and squash with the two tested fungi. Enzyme extracts were obtained by grinding the tissues in 0.1 M sodium phosphate buffer at pH 7.1 (2 ml/g fresh weight) in china mortar. Plant tissue (g) was homogenized in a mortar with 0.2 M Tris HCl buffer (PH 7.8) containing 14 MMB. Mercapto ethanol rate of 1/3 w/v.

The extracted tissues were stained through four layers of cheesecloth. The filtrate was centrifuged at 3000 rpm for 15 minutes at 6°C. The supernatant was served in the refrigerator at -20°C till determination of enzymes **(Tuzun *et al.*, 1989).**

A. Polyphenoloxidase (PPO) activity:

For polyphenoloxidase determination, a colorimetric method proposed by **Matta and Dimond (1963)** was used. The reaction mixture consists of 1.0 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0 and 1.0 ml of 10 M Catechol brought to final volume of 6.0 ml with distilled water. The activity of

polephenoloxidase was expressed as the change in absorbance/min/g fresh weight at optical density 495 nm.

B. Peroxidase (PRO) activity:

Peroxidase activity was determined according to Allam and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425 nm. The reaction mixture consists of 0.5 ml of 0.1 M sodium phosphate buffer solution at pH 7.0, 0.5 ml enzyme extract, 0.3 Pyrogullol, 0.1 ml 1.0% H_2O_2 brought to final volume of 3.0 ml with distilled water. Peroxidase activity was expressed as the change in absorbance/min/g fresh weight.

Peroxidase and polyphenoloxidase assays were carried out using (Spectronic 601). The control cuvette contained the same solution except that the substrate solution was replaced by distilled water. Readings were recorded every 30 S for 5 minute in case of peroxidase and polyphenoloxidase.

Statistical analysis:

Statistical analysis was done according to procedure of ANOVA reported by **Snedecor and Cochran (1999).**

RESULTS

EXPERIMENTAL RESULTS

1. Survey of cucumber and squash fruit diseases:

Data illustrated in **Table (2)** and **Fig. (1)** stated that, the fruit rot disease severity (DS) on cucumber (*Cucumis sativus*) and squash (*Cucurbita pepo*) fruits under greenhouses conditions was varied between tested locations. The DS on cucumber and squash fruits, in most cases, was obviously higher in 2003 than 2004 seasons. The cucumber fruit rot recorded 17.2 and 14.66% whereas, squash fruit rot recorded 12.02 and 9.59% in seasons 2003 and 2004, respectively.

Table (2): Surveying of disease severity (DS) percentages of rotted fruits of cucumber and squash during 2003 and 2004 seasons.

Localities	Cucumber			Squash		
	2003	2004	Mean	2003	2004	Mean
Beheira	20.45	17.50	18.98	15.00	13.00	14.00
Kafr El-Sheikh	15.35	16.33	15.84	14.16	12.50	13.33
Qalubiya	19.14	16.18	17.66	8.33	5.00	6.67
Giza	17.50	15.00	16.25	9.16	8.33	8.75
Ismaelia	14.16	10.83	12.50	10.0	4.16	7.08
Sharkiya	15.83	12.80	14.32	14.16	11.66	12.91
Dakahliya	18.00	14.00	16.00	13.3	12.50	12.90
Mean	17.20	14.66	15.93	12.02	9.59	10.80

Concerning locations, the DS on cucumber and squash fruits, respectively recorded 18.98 and 14.0% at Beheira, 16.0 & 12.9% at Dakahliya, 16.25 & 8.75% at Giza, 12.5 & 7.08% at Ismaelia, 15.84 & 13.33% at Kafr El-Sheikh, 17.66 & 6.67 Qalubiya and 14.32 & 12.91% at Sharkiya.

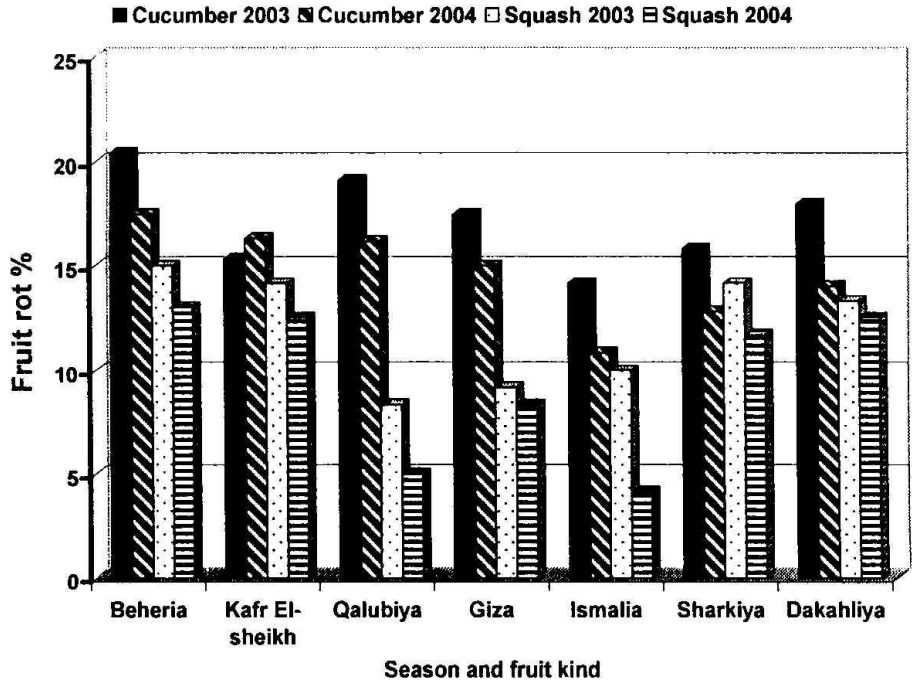


Fig. (1): DS% of rotted fruits of cucumber and squash at different locations during 2003 and 2004 seasons

The DS on cucumber fruits at Beheira recorded the highest percentage (20.45%) followed by Qalubiya (19.14%), Dakahliya (18.0%) and Giza (17.5%) at 2003 season and Beheira (17.5%), Kafr El-Sheikh (16.33%) and Qalubiya (16.18%) at 2004 season, respectively. Also, the highest DS on squash fruits, was recorded at Beheira (15.0%) followed by Kafr El-Sheikh and Sharkiya (14.16%) and Dakahliya (13.3%) at 2003 season and Beheira (13.0%) followed by Kafr El-Sheikh and Dakahliya (12.5%) and Sharkiya (11.66%), respectively at 2004 season.

2. Frequency of different fungi associated with rotted fruits of cucumber and squash:

Data in **Table (3)** and **Fig. (2)** show that, 152 and 81 fungal isolates were found to be associated with the cucumber and squash rotted fruits, respectively. These associated fungal isolates included *Alternaria* spp., *B. cinerea, F. solani, Mucor* spp., *Penicillium* spp., *Pythium* spp. and *Sclerotinia sclerotiorum.*

Table (3): Number of detections (N) and Frequency (F %) of fungi associated with the rotted cucumber and squash fruits collected from different localities.

Associated fungi	Cucumber fruits		Squash fruits	
	N	F %	N	F %
Alternaria spp.	25	16.45	15	18.52
B. cinerea	53	34.87	27	33.33
F. solani	32	21.05	16	19.75
Mucor spp.	3	1.97	2	2.47
Penicillium spp	6	3.95	4	4.94
Pythium spp.	9	5.92	5	6.17
Sclerotinia sclerotiorum	24	15.79	13	16.05
Total	152	100.00	81	100.00

Out of these fungi, *B. cinerea* recorded the highest frequency on rotted cucumber fruits (34.87%) followed by *F. solani* (21.05%), *Alternaria* spp. (16.45%), *S. sclerotiorum* (15.79%), *Pythium* spp. (5.92%), *Penicillium* spp. (3.95%), and *Mucor* spp. (1.97%), respectively. Also, *B. cinerea* recorded the highest frequency on rotted squash fruit (33.33%) followed by *F. solani* (19.75%), *Alternaria* spp. (18.52%), *S. sclerotiorum* (16.05%), *Pythium* spp. (6.17%), *Penicillium* spp. (4.94%), and *Mucor* spp. (2.47%), respectively.

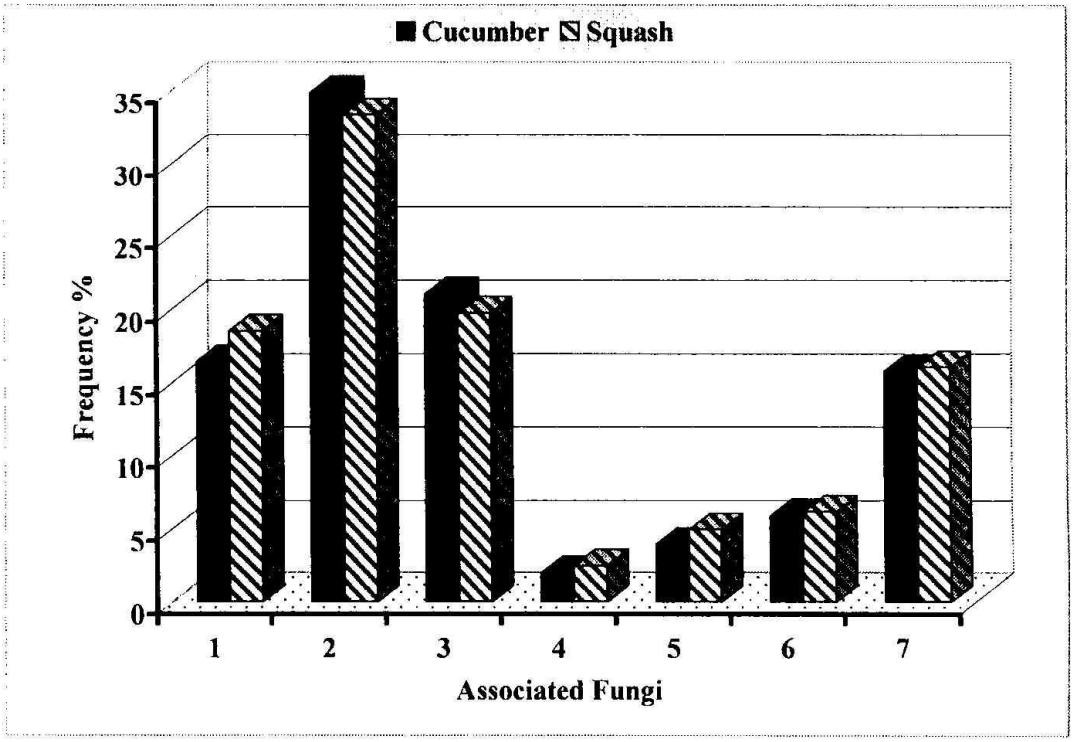


Fig. (2): Frequency of associated fungi with the rotted cucumber and squash fruits. 1) *Alternaria* spp., 2) *B. cinerea,* 3) *F. solani,* 4) *Mucor* spp., 5) *Penicillium* spp., 6) *Pythium* spp. and 7) *S. sclerotiorum*

3. Pathogenicity tests:

The pathogenic abilities of different isolates of *B. cinerea* and *F. solani*, which represent the highest frequency from different localities and causing fruit rot on cucumber and squash fruits were investigated. Obtained results prove that DS on either cucumber or squash fruits was significantly affected by the tested pathogen, the location from which was isolated as well as the interaction between these two factors.

As for cucumber, data in **Table (4)** and **Fig. (3)** show that the Fruit Rot Disease Severity, in general, was significantly higher in case of *B. cinerea* (16.7%) than in case of *F. solani* (13.9%). The

isolated fruit rot pathogens (*B. cinerea* and *F. solani*) from Beheira recorded the highest average of DS (23.4%) followed by those from Ismaelia (23.0%), Kafr El-Sheikh (20.3%), Sharkia (17.1%) whereas the lowest DS was recorded on fruits from Dakahliya location (6.6%). Regarding interaction between pathogens and locations, it is clear that the highest DS was incited by *B. cinerea* isolated from Ismaelia (27.9%) and Beheira (24.7%) and *F. solani* isolated from Beheira (22.1%), Kafr El-Sheikh (19.0%) and Ismaelia (18.1%) without significant differences which only noticed between the later two isolates.

Table (4): Pathogenic ability of *B. cinerea* and *F. solani* from different localities on cucumber fruits after two weeks

Isolation locality	DS% on Cucumber			DS% on Squash		
	B. cinerea	*F. solani*	Mean	*B. cinerea*	*F. solani*	Mean
Beheira	24.7	22.1	23.4	9.5	6.5	8.0
Kafr El-Sheikh	21.6	19.0	20.3	14.0	9.5	11.7
Kalubia	7.5	9.2	8.3	9.3	9.3	9.3
Giza	10.2	6.7	8.4	6.7	6.3	6.5
Ismaelia	27.9	18.1	23.0	27.3	26.7	27.0
Sharkia	19.9	14.3	17.1	28.7	13.7	21.2
Dakahliya	5.1	8.2	6.6	7.7	9.8	8.7
Mean	16.7	13.9		14.7	11.7	8.0
L.S.D at 0.05						
Fungi (F)		0.420			0.307	
Location (L)		0.267			0.195	
F x L		2.949			2.149	

With regard to squash fruits, the same data **(Table, 4)** revealed that *B. cinerea* was more pathogenic than *F. solani* where they recorded DS of 14.7 and 11.7%, respectively. The isolated fruit rot

pathogens from Ismaelia recorded the highest DS (27.0%) followed by those from Sharkia (21.2%), Kafr El-Sheikh (11.7%), whereas the lowest DS was recorded on squash fruits at Kalubia (9.3%), Dakahliya (8.7%), Beheira (8.0%) and Giza (6.5%), respectively.

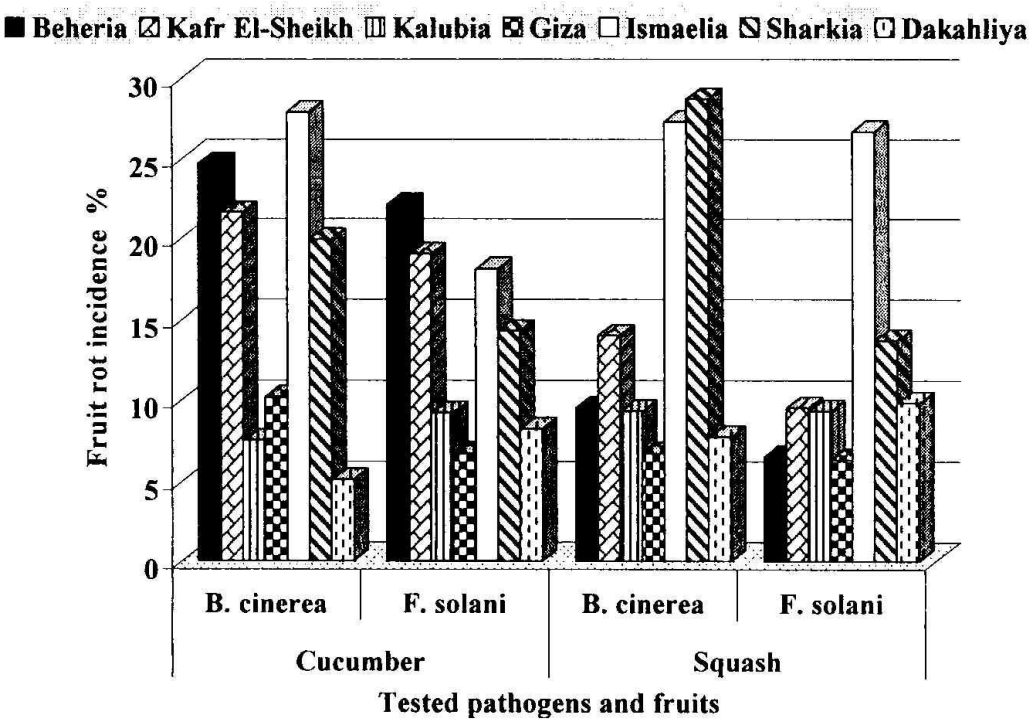


Fig. (3): DS% of fruit rot caused by *B. cinerea* and *F. solani* from different localities on cucumber and squash fruits after two weeks from inoculation.

Regarding pathogen/location interaction, the highest DS was caused by *B. cinerea* isolated from Sharkia (28.7%), Ismaelia (27.3%) and *F. solani* isolated from Ismaelia (26.7%) without significant differences between them. While, the remained interactions between location and pathogen recorded DS ranged between 6.3 and 14.0%.

4. Inoculum potential of the most virulent fungi:

In this study, spore suspension containing 1000-7000 spores/ml were prepared for each tested fruit rot pathogen and used to inoculate cucumber and squash fruits. Data in **Table (5)** and **Fig. (4)** proved that DS on cucumber or squash fruits were significantly varied according to the tested pathogen, inoculum concentration and the interaction between them. *B. cinerea* was more pathogenic than *F. solani* either on the cucumber or on the squash fruits. The DS caused by *B. cinerea* recorded 27.1 and 28.0% while *F. solani* recorded 23.46 and 19.94% on cumber and squash fruits, respectively.

Regardless pathogens, DS on cucumber or squash fruits progressively increased, in most cases, as tested inoculum concentration increased. The DS increased from 17.8 to 52.5% (cucumber fruits) and from 14.05 to 51.0% (squash fruits) as inoculum concentrations increased from 1000 to 7000 spores/ml. The results indicate also that, the highest significant increase of DS was recorded when inoclum concentration increased from 6000 to 7000 spores/ml (*B. cinerea* on cucumber and squash fruits) and *F. solani* (on cucumber fruits) while increasing inoculum concentration of *F. solani* from 5000 to 6000 spores/ml caused the highest significant increase in the DS comparing with any other pairs. Between concentrations of 6000 and 7000 spores/ml, *B. cinerea*, DS increased from 32.5 to 55.0% on cucumber (difference is 22.5%) and on squash from 40.0 to 66.0% (difference is 26.0%)

whereas, DS caused by *F. solani* on cucumber was increased from 30.0 to 50.0% (difference is 20.0%).

In case of *F. solani* on squash fruits, the highest significant difference in DS occurred when inoculum concentration increased from 5000 (25.0%) to 6000 spores/ml (35.0%), the difference in between was 10.0%). These results emphasized that DS was affected significantly by the interaction between pathogens and inoculum concentrations on either cucumber or squash fruits. In most comparisons, the differences between any pairs of these interactions were significantly positive.

Table (5): Effect of different inocula levels of *B. cinerea* and *F. solani* on percentage of rots on cucumber and squash fruits.

Inoculum Con. spore/ml	Cucumber		Squash	
	B. cinerea	*F. solani*	*B. cinerea*	*F. solani*
1000	20.0	15.5	17.6	10.5
2000	25.0	18.7	22.1	15.0
3000	27.5	20.5	25.0	17.5
4000	26.0	25.5	25.0	20.5
5000	30.5	27.5	28.0	25.0
6000	32.5	30.0	40.0	35.0
7000	55.0	50.0	66.0	36.0
Control	0.0	0.0	0.0	0.0
Mean	27.1	23.5	28.0	19.9
L.S.D at 0.05				
Fungi (F)		0.278		0.284
Inoculum (I)		0.177		0.181
F x I		1.945		1.991

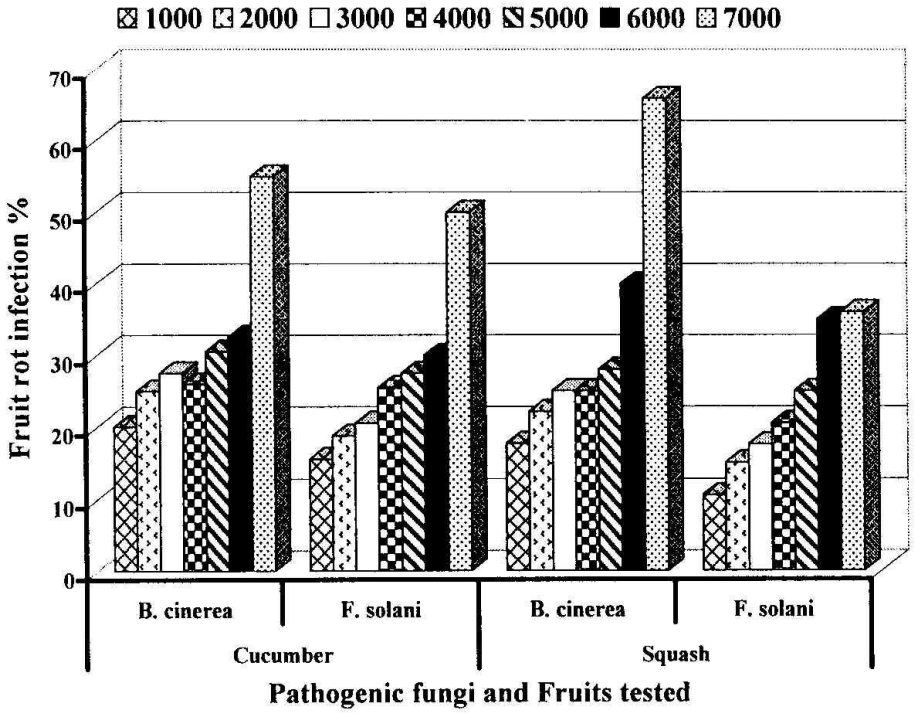


Fig. (4): Fruit rot disease incidence as affected by inoculum levels (1000 to 7000 spores/ml) of *B. cinerea* and *F. solani* on cucumber and squash fruits

5. Response of different cultivates of cucumber and squash against the tested fruit rot causal fungi:

In this study, fruits of seven cultivars of cucumber and one of squash were investigated for their responses against infection with *B. cinerea* and/or *F. solani*. Data in **Table (6)** and **Fig. (5)** proved that *B. cinerea* caused significantly higher average of DS (10.5%) than *F. solani* (7.7%).

Regardless tested pathogens, DS of fruit rot diseases on the fruits of squash and cucumber cultivars were significantly varied. As for cucumber, fruits of Heikal cultivar seemed to be the most resistant, recorded the lowest DS (4.3%) followed by Delta Star cultivar (6.4%), Shams cultivar (8.1%), New Star cultivar (8.3%),

Fysal and Sinai I cultivars (11.5%) and Samar cultivar (13.8%) whereas, DS on squash fruits recorded 8.9%. Also, the DS was significantly affected by the interactions between tested fungi and cucurbitaceous cultivars.

Table (6): Fruit rot disease incidence on different cucumber cultivars and squash due to infection with *B. cinerea* and *F. solani* 7 days after incubation at (22+2°C).

Tested Cultivar	% rotted fruits caused by		Mean
	B. cinerea	*F. solani*	
Sinai I	13.2	9.9	11.5
Fysal	12.0	11.0	11.5
Delta star	6.5	6.3	6.4
Samar	15.5	12.2	13.8
Shams	10.2	6.0	8.1
Heikal	5.2	3.5	4.3
New star	11.5	5.0	8.3
Eskandarani (squash)	10.3	7.5	8.9
Mean	10.5	7.7	9.1
L.S.D .at 0.05			
Fungi (F)		0.23	
Cultivars (C)		0.15	
F x C		1.64	

As above mentioned, the lowest DS was recorded on cucumber fruits of Heikal cultivar either infected with *B. cinerea* (5.2%) or *F. solani* (3.5%) whereas fruits of cucumber of Samar cultivar recorded the highest significant DS *i.e.* 15.5 and 12.2% for the same two pathogens, respectively.

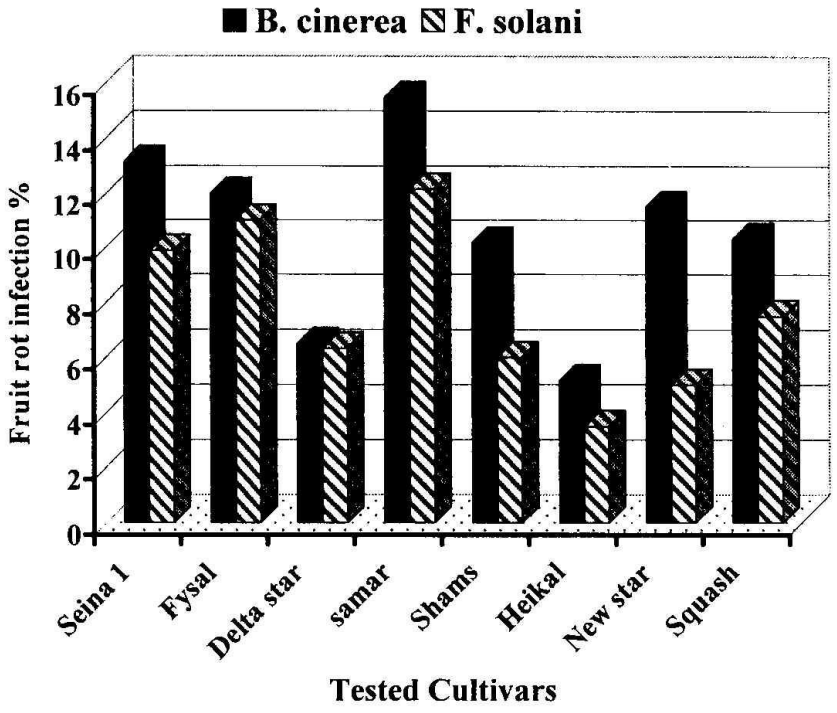


Fig. (5): Responses of different cucumber cultivars and squash against infection with *B. cinerea* and *F. solani*.

6. Factors affecting cucumber and squash fruit rot diseases severity:

6.1. Effect of plant age and fruit wounding:

In this study, marketable size, wounded and unwounded, cucumber and squash fruits harvested from 60, 75 and 90 days old plants were used. Data in **Table (7)** and **Fig. (6)** proved that DS on cucumber and squash fruits was significantly affected by tested fungi, treatments (plant age and fruit wounding). Regardless treatments, DS caused by *B. cinerea* on cucumber fruits (5.56%) was significantly higher than that caused by *F. solani* (4.74%). On the contrary, DS caused by *B. cinerea* or *F. solani* was significantly equal on squash fruits (10.69%).

Table (7): Effect of plant age and fruit wounding on % fruit rot disease severity caused by *B. cinerea* and *F. solani* on the cucumber and squash fruits after incubation for 10 days at 22±4°C.

Treatments		Cucumber fruits		Squash fruits	
Wound	Age	*F. solani*	*B. cinerea*	*F. solani*	*B. cinerea*
Wounded	60d	2.70	4.25	6.56	3.97
	75d	4.31	7.50	16.26	8.12
	90d	9.01	8.49	23.10	9.26
Unwounded	60d	0.83	1.59	3.90	2.40
	75d	2.40	3.53	6.30	6.73
	90d	9.20	8.00	8.00	7.40
Mean		4.74	5.56	10.69	10.69
L.S.D.at 0.05					
Fungi (F)			0.063		0.159
Treatment (T)			0.040		0.101
F x T			0.439		1.112

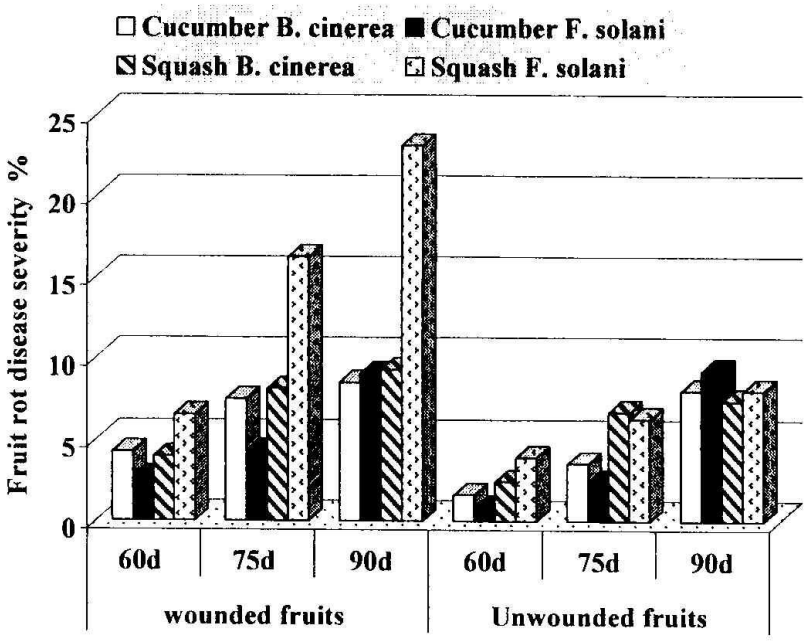


Fig. (6): Effect of plant age and fruit wounding on the fruit rot disease severity caused by *B. cinerea* and *F. solani* on the cucumber and squash fruits 10 days after incubation at 22±4°C.

Regardless pathogens, the Fruit Rot Disease Severity (FRDS), with few exceptions, significantly increased as plant age increased and it was significantly higher on the wounded than the unwounded cucumber or squash fruits. In cucumber fruits, the DS average on fruits harvested from 60, 75 and 90 days old plants recorded 3.48, 5.91 and 8.75% on the wounded fruits while they recorded 1.21, 2.97 and 8.6% on the unwounded fruits, respectively. Thus, the differences between DS averages at 60 and 75 but not at 90 days old cucumber plants were significantly varied. Similar trend of DS was also observed on squash fruits but differences at different plant ages were significantly varied between the wounded and unwounded squash fruits. The DS averages on squash fruits harvested from 60, 75 and 90 days old plants recorded 5.27, 12.19 and 16.18% on the wounded and 3.15, 6.52 and 7.7% on the unwounded fruits, respectively.

6.2. Effect of fruit age:

In this study, cucumber and squash fruits of 1, 2, 3, 4 and 5 days-old were inoculated with either *B. cinerea* or *F. solani* to investigate the effect of fruit age on the FRDS. Data in **Table (8)** and **Fig. (7)** proved that DS on cucumber and squash was significantly affected by tested pathogen, and the fruit age. In general, DS was significantly higher on fruits inoculated with *B. cinerea* than those inoculated with *F. solani*. This was true either on the cucumber or squash fruits. Regardless fruit age, *B. cinerea* and *F. solani*, respectively recorded 8.49 and 4.41% DS on the

cucumber and 7.86 and 4.32% on squash fruits. The DS was gradually and significantly increased as fruit age increased.

Table (8): Effect of fruit age on the cucumber and squash fruit rot disease severity caused by *B. cinerea* and *F. solani.*

Fruit age (days)	Cucumber		Squash	
	B. cinerea	***F. solani***	***B. cinerea***	***F. solani***
1	4.83	2.17	1.33	1.17
2	6.33	2.57	6.00	3.00
3	7.50	4.07	7.66	4.00
4	10.50	5.33	11.00	5.50
5	13.30	7.93	13.30	7.93
Mean	8.49	4.41	7.86	4.32
L.S.D at 0.5%		0.121		0.083
		0.077		0.053
		0.850		0.582

Regardless pathogens, DS recorded 3.5, 4.45, 5.79, 7.92 and 10.62% on cucumber fruits and 1.25, 4.5, 5.83, 8.25 and 10.62% on squash fruits aged 1, 2, 3, 4 and 5 days, respectively. Whatever, DS at any fruit age, with only one exception, was significantly higher on fruits inoculated with *B. cinerea* than those inoculated with *F. solani* either on the cucumber or squash fruits. However, DS caused by *B. cinerea* or *F. solani* on 1-day old squash fruits were significantly equal, recording 1.33 and 1.17% for both pathogens, respectively. These findings suggested that, harvesting cucumber or squash fruits every 2 or 3 days might be successful practical procedure to minimize the incidence of fruit rot diseases caused by *B. cinerea* or *F. solani.*

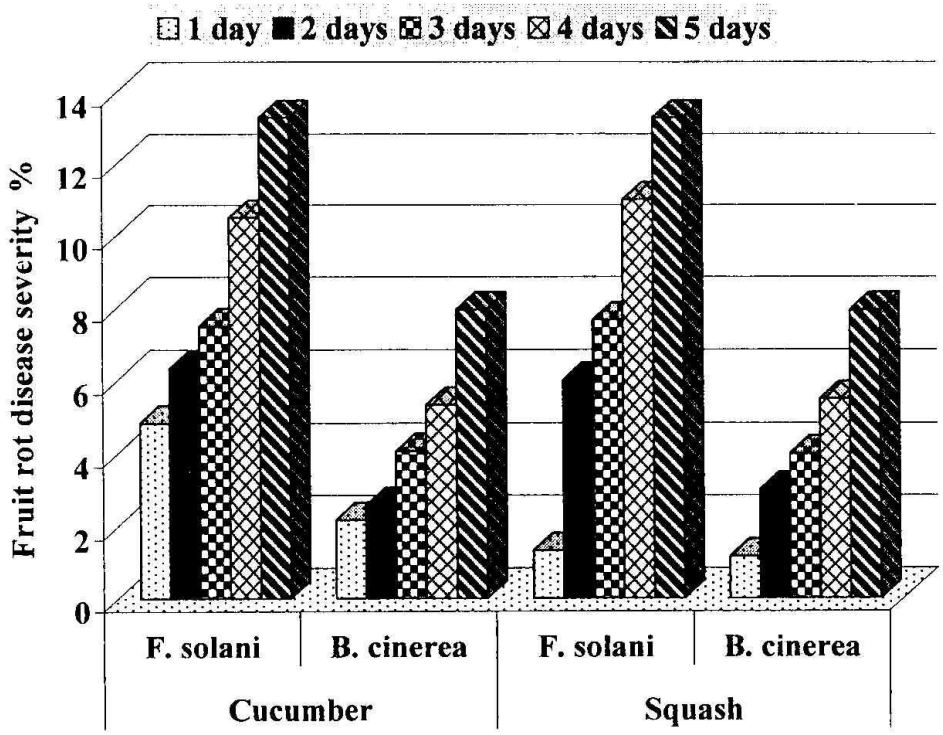


Fig. (7): Effect of fruit age on the cucumber and squash fruit rot disease severity caused by *B. cinerea* and *F. solani*

6.3. Effect of storage temperature and storage period:

In this study, 4 days old of cucumber and squash fruits inoculated with spore suspensions of either *B. cinerea* or *F. solani*. The inoculated fruits were stored at different temperatures *i.e.* 2, 5, 7 and 24°C then DS was determined after 4 and 8 days. Data in **Table (9a)** and **Fig. (8a)** show that DS on the cucumber fruits was significantly affected by storage period, and storage temperature. It is clear that, DS was significantly higher on fruits stored for 8 than those stored for 4 days. Average DS recorded 4.14 and 11.73% (*B. cinerea*) and 1.48 and 3.83% (*F. solani*) after storage for 4 and 8 days, respectively.

Table (9a): Effect of storage cucumber fruits for 4 and 8 days at different temperature regimes on development of fruit rot DS caused by *B. cinerea* and *F. solani*.

Temperature °C	*B. cinerea*		Mean	*F. solani*		Mean
	4 days	8 days		4 days	8 days	
2 °C	1.3	4.9	3.08	0.0	0.0	0.00
5 °C	1.6	5.8	3.72	0.0	1.7	0.84
7 °C	2.8	9.1	5.94	0.0	1.8	0.92
24 °C	10.9	27.1	18.99	5.9	11.8	8.85
Mean	4.14	11.73		1.48	3.83	
L.S.D. at 5%						
Days (D)			0.098			0.056
Temperature (T)			0.062			0.036
D x T			0.686			0.393

Disease severity was significantly increased as storage temperature increased. It recorded 3.08, 3.72, 5.94, 18.99% (*B. cinerea*) and 0.0, 0.84, 0.92, 8.85% (*F. solani*) at storage temperatures 2, 5, 7 and 24°C, respectively. As for days/temperature interaction, the obtained results revealed that the development of DS occurred more slowly on the cucumber fruits stored at lower temperature regimes (2, 5 and 7°C) than those stored at room temperature (24°C). This was more conspicuous, particularly on cucumber fruits inoculated with *F. solani*.

Disease severity of cucumber fruits inoculated with *F. solani* was completely stopped until storage for 4 days at 2, 5 and 7°C, but recorded 0.0, 1.7 and 1.8% after 8 days storage at the same temperatures. Whereas, DS caused by *F. solani* recorded 5.9 and 11.8% after storage at 24°C for 4 and 8 days, respectively. This was the case also on cucumber fruits inoculated with *B. cinerea*,

although DS was still occurred (1.3%) on the fruits stored for 4 days even at the lowest storage temperature (2°C). By increasing storage period up to 8 days at 24°C DS caused by *B. cinerea* developed more quickly than that caused by *F. solani*.

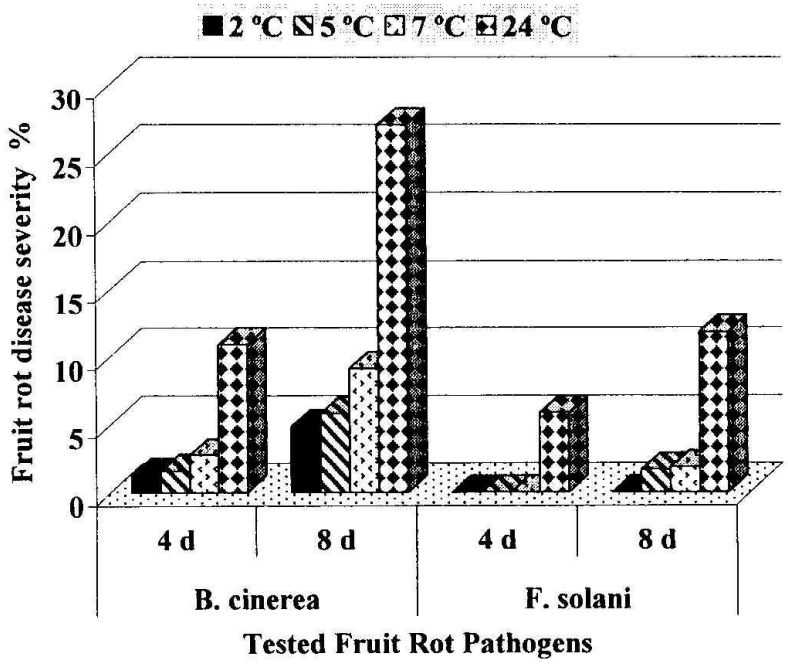


Fig. (8a): Effect of storage cucumber fruits at different temperature regimes on DS caused by *B. cinerea* and *F. solani* after storage for 4 and 8 days.

Regarding squash fruits, data in **Table (9b)** and **Fig. (8b)** show that, the FRDS, regardless pathogens, recorded 2.39 and 5.87% (*B. cinerea*) and 1.17 and 5.39% (*F. solani*) after storage for 4 and 8 days, respectively. Disease severity was significantly increased as storage temperature increased. It recorded 1.89, 3.5, 4.75, and 6.39% (*B. cinerea*) and 1.2, 1.94, 4.62 and 5.37% (*F. solani*) on squash fruits stored at temperatures of 2, 5, 7 and 24°C, respectively. As for days/temperature interaction, it is clear that DS

was significantly lower after 4 than 8 days storage periods at any tested temperature regime.

Table (9b): Effect of storage squash fruits for 4 and 8 days at different temperature regimes on the development of fruit rot DS caused by *B. cinerea* and *F. solani*.

Temperature °C	*B. cinerea*		Mean	*F. solani*		Mean
	4 days	8 days		4 days	8 days	
2 °C	1.2	2.6	1.89	0.7	1.7	1.20
5 °C	1.9	5.1	3.50	1.1	2.8	1.94
7 °C	2.4	7.1	4.75	1.3	7.9	4.62
24 °C	4.1	8.7	6.39	1.6	9.2	5.37
Mean	2.39	5.87		1.17	5.39	3.28
L.S.D. at 5%						
Days (D)			0.097			0.086
Temperature (T)			0.062			0.055
D x T			0.682			0.605

Under different temperature regimes, DS caused by *B. cinerea* was relatively higher than that caused by *F. solani* after storage for 4 days but the rate of disease development in case of the pathogen (*F. solani*) was more quickly and increased several times after storage for 8 days comparing with the rate of increase in the disease caused by the pathogen (*B. cinerea*). For example, DS caused by *B. cinerea* at 7°C recorded 2.4% after 4 days and 7.1% after 8 days, this means that DS after 8 days increased by 2.95 folds compared with DS after 4 days. In case of *F. solani*, DS under the same conditions increased by approximately 6 folds on fruits stored for 8 days compared with those stored only for 4 days. Similar comparison was also observed on fruits stored at 24°C.

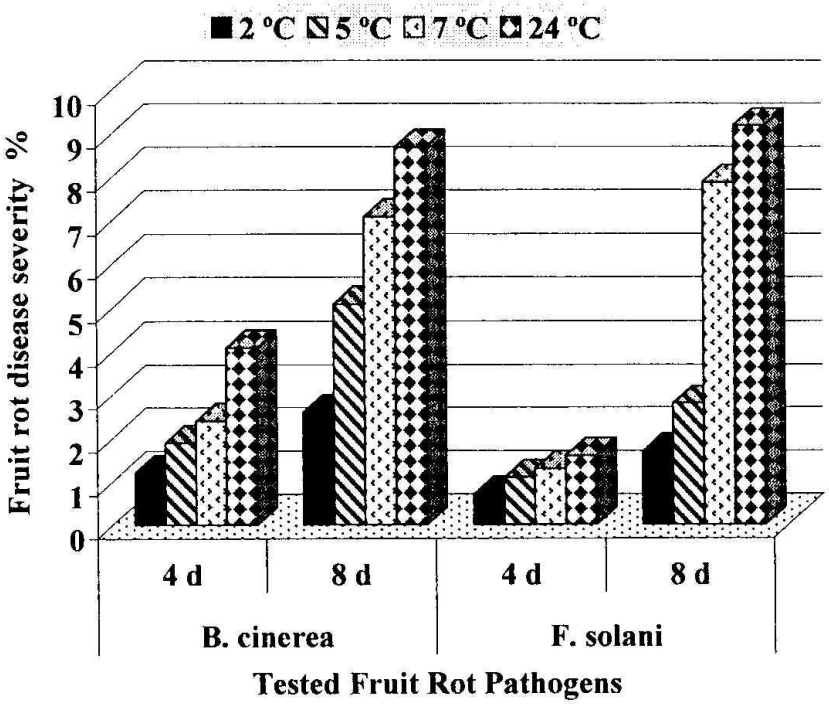


Fig. (8b): Effect of storage squash fruits at different temperature regimes on DS caused by *B. cinerea* and *F. solani* after 4 and 8 days.

6.4. Effect of modified atmosphere (using CO_2):

In this study, cucumber fruits were inoculated with *B. cinerea* or *F. solani* and stored for 10, 15 and 21 days in polyethylene bags containing different concentrations of carbon dioxide (CO_2) to investigate the effect of these treatments on FRDS. The obtained results **(Table, 10)** and **Fig. (9)** show that DS was significantly affected by CO_2 concentration, storage period and the interaction between them. With regard to storage periods, *B. cinerea* recorded DS of 23.8, 37.4 and 58.3% while *F. solani* recorded 27.5, 38.3 and 47.9% DS after storage periods of 10, 15 and 21 days, respectively. Regarding CO_2 concentrations, DS recorded 53.3, 22.7 and 10.6 (*B. cinerea*) and 33.9, 21.7 and 17.2% (*F. solani*) at CO_2 concentrations 20, 15 and 10%, respectively comparing with

72.8 and 78.9% for the control (untreated) of both pathogens, respectively.

Concerning interactions between CO_2 treatments and storage periods, data revealed that, the lowest DS on cucumber fruits inoculated with *B. cinerea* or *F. solani* after 10 days of storage under conditions containing 10% CO_2 concentration. It is interested to state that the DS recorded by *B. cinerea* (5.0%) or *F. solani* (15.0%) after storage for 10 days at 10% CO_2 concentrations was increased over than 3.5 and 1.5 folds after storage for 21 days at the same CO_2 concentration.

Table (10): Effect of storage cucumber fruits under different concentrations of CO_2 and 20°C on the fruit rot caused by *B. cinerea* and *F. solani*.

CO_2%	*B. cinerea*				*F. solani*			
	10 d	15 d	21 d	Mean	10 d	15 d	21 d	Mean
20 %	30.0	51.6	78.3	53.3	25.0	28.3	48.3	33.9
15 %	10.0	18.0	40.0	22.7	20.0	20.0	25.0	21.7
10 %	5.0	10.0	16.7	10.6	15.0	15.0	21.7	17.2
Control	50.0	70.0	98.3	72.8	50.0	90.0	96.7	78.9
Mean	23.8	37.4	58.3		27.5	38.3	47.9	
L.S.D at 0.5%								
CO_2 % (C)				0.494				0.436
Days (D)				0.315				0.278
C x D				3.460				3.054

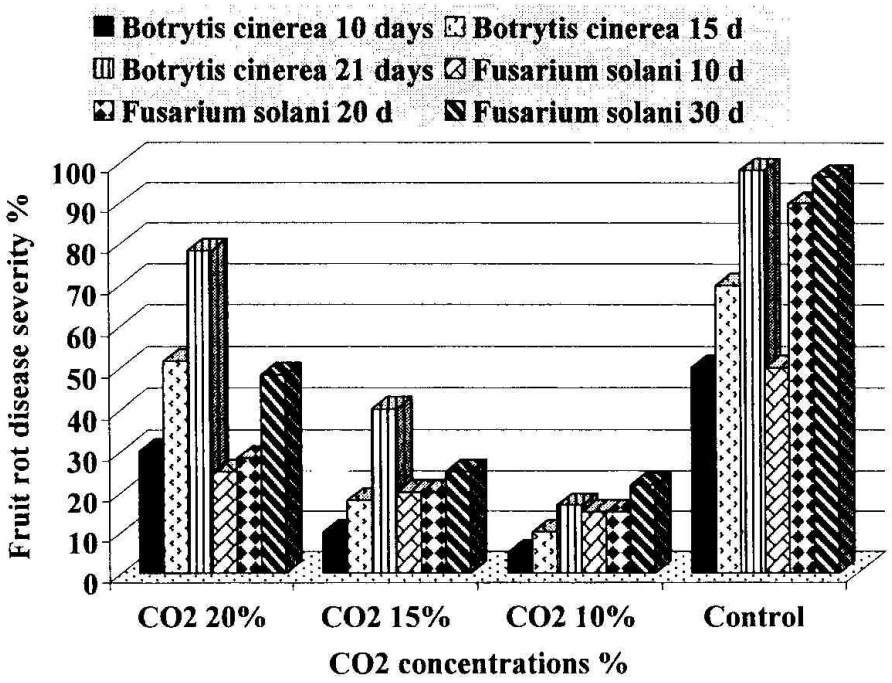


Fig. (9): Disease severity caused by *B. cinerea* and *F. solani* on cucumber fruits stored under different concentrations of CO_2

6.5. Effect of UV radiation:

In this study, DS was determined on cucumber fruits inoculated with either *B. cinerea* or *F. solani* and exposed to UV wavelengths at 280 or 320 nm for 1 or 2 hours comparing with the inoculated untreated control. Disease severity was estimated 21 days after treatment.

Data in **Table (11)** and **Fig. (10)** declared that, DS was significantly affected by UV wavelengths, and the exposure time. Exposing fruits to UV-treatments resulted in significant decreases in DS but the recorded data still high and proved that the UV-exposure, under conditions of the present study, could not be satisfactory practice for suppressing DS of cucumber fruit rot diseases.

Table (11): Effect of UV exposure treatments on the fruit rot severity caused by *B. cinerea* and *F. solani* on cucumber fruits.

UV exposure treatments		% DS caused by		
Wave length	Time	*B. cinerae*	*F. solani*	Mean
280w	1hr	50.00	65.00	57.50
280w	2hr	31.66	55.00	43.33
320w	1hr	47.00	55.00	51.00
320w	2hr	27.00	40.00	33.50
Control		93.30	91.66	92.48
Mean		49.79	61.33	55.56

L.S.D. at 0.05

Fungi (F)	0.488
UV Treatment (T)	0.311
F x T	3.416

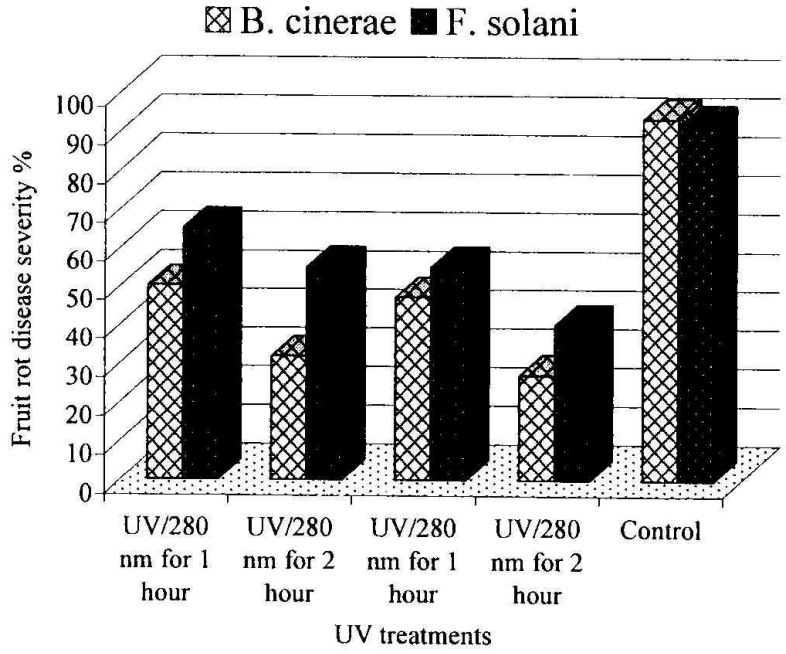


Fig. (10): Effect of exposing cucumber fruits inoculated with *B. cinerea* and *F. solani* for 1 and 2 hours to different UV treatments on the FRDS.

Regardless pathogens, DS was significantly decreased from 92.48% in the untreated control to 57.5, 43.3, 51.0 and 33.5% on cucumber fruits exposed for 1 or 2 hours to each UV wavelengths 280 and 320 nm, respectively. However, the lowest significant decreases in DS *i.e.* 27.0% (*B. cinerea*) and 40.0% (*F. solani*) was recorded by exposing the inoculated to UV/320 nm for two hours comparing with 93.3 and 91.66% in the untreated controls of both pathogens, respectively.

7. Effect of fungicides:

Data in **Table (12a)** indicate that all tested fungicides were effective in controlling the tested fruit rot pathogens *in vitro*. It is clear also that all of these tested fungicides varied significantly in their inhibitory effects against growth of the fruit rot pathogenic fungi *i.e.*, *B. cinerea* and *F. solani.* In this respect, among the tested fungicides, Ronilan was the best effective one where it completely inhibited the growth of *B. cinerea* at 50ppm followed by Sumisclex fungicide at 100 ppm. On the other hand, Rovral came in the third rank where it completely inhabited the growth of the tested *B. cinerea* at 150ppm. Meanwhile, the least effective ones of the tested fungicides were Ridomil, Teledor, Copral and Tecto where they inhibited the growth of *B. cinerea* at the high tested doses (400 and 500ppm). Also, the obtained results cleared that increasing the concentration of the tested fungicides increased gradually their effects against pathogens *in vitro*.

Table (12a): Effect of some fungicides at different concentrations on the *in vitro* growth of *B. cinerea*.

Fungicides (PPM)	Growth in mm at different concentrations										Mean
	Control	25	50	100	150	200	250	300	400	500	
Ronilan	90	17	0	0	0	0	0	0	0	0	10.7
Sumisclex	90	19	6	0	0	0	0	0	0	0	11.5
Rovral	90	37	22	8	0	0	0	0	0	0	15.7
Tecto	90	57	47	21	18	13	10	5	0	0	26.1
Teledor	90	50	30	25	20	18	15	7	0	0	25.5
Copral	90	48	33	25	22	15	9	5	0	0	24.7
Ridomil	90	60	50	30	30	25	15	10	0	0	31.0
Mean	90	41.1	26.9	15.6	12.9	10.1	7.0	3.9	0.0	0.0	20.7

Table (12b): Effect of some tested fungicides at different concentrations on the *in vitro* growth of *F. solani*.

Fungicides (PPM)	Growth in mm at different concentrations										Mean
	Control	25	50	100	150	200	250	300	400	500	
Ronilan	90	16	8	5	5	0	0	0	0	0	12.4
Sumisclex	90	13	7	6	0	0	0	0	0	0	11.6
Rovral	90	10	0	0	0	0	0	0	0	0	10.0
Tecto	90	52	33	8	5	0	0	0	0	0	18.8
Teledor	90	55	40	30	25	25	15	10	0	0	29.0
Copral	90	50	35	30	25	20	22	15	0	0	28.7
Ridomil	90	60	50	41	35	30	23	20	0	0	34.9
Mean	90	36.6	24.7	17.1	13.6	10.7	8.6	6.4	0.0	0.0	20.8

As for the effect of tested fungicides on growth of *F. solani*, data in **Table (12b)** indicate also that all tested fungicides were highly effective in controlling growth of *F. solani* the causal agent of cucurbits fruit rots *in vitro* just at 25ppm. It is clear also that increasing the concentration of the tested fungicides increased gradually their effects in inhibiting the growth of *F. solani*; *in vitro*. In this respect, Rovral, Sumisclex Ronilan, and Tecto were the best effective ones, respectively. On the other hand, Copral, Teledor and Ridomil were the least effective ones.

7.1. Effect of pre-harvest fungicidal treatments on controlling fruit rot-diseases:

As for cucumber, data in **Table (13a)** illustrated that, the cucumber fruit rot disease severity (DS) caused by *B. cineria* and *F. solani* were significantly affected by fungicides, concentrations and by the interaction between them. Regarding fruit rot caused by *B. cinerea*, the pre-spraying with fungicides, *i.e.,* Copral and Teledor were highly effective in controlling the fruit rot disease caused by *B. cineria* on the harvested cucumber fruits. Ridomil and Rovral came in the second rank where they were moderately effective in controlling cucumber fruit rot infection. Sumisclex, Tecto and Ronilan were the least effective ones in this respect. It is clear from the obtained results that increasing fungicide concentration decreased gradually the recorded DS on infected cucumber fruits. In this respect, the least DS were recorded at 750 ppm followed by 500 ppm with all tested fungicides. In case of *F. solani* infection on cucumber, data of recorded DS indicate that all the pre-spraying fungicidal treatments significantly decreased the infection with fruit rot of cucumber caused by *F. solani*. In this respect, the fungicides Copral, Ridomil and Teledor were the best effective comparing with the other tested fungicides and check treatments. Rovral was moderately effective in this respect. On the other hand, Sumisclex, Tecto and Ronilan were the least effective ones in controlling cucumber fruit rot caused by *F. solani.*

Table (13a): Effect of pre-harvest fungicidal treatment against cucumber fruit rots caused by *B. cinerea* and *F. solani.*

Fungicides	*B. cinerea*			Mean	*F. solani*			Mean
	250 ppm	500 ppm	750 ppm		250 ppm	500 ppm	750 ppm	
Ronilan	36.0	17.7	6.8	20.17	49.5	27.6	12.4	29.83
Sumisclex	46.7	23.7	8.3	26.23	53.4	24.9	10.2	29.50
Rovral	27.5	10.3	2.7	13.50	36.5	12.3	6.3	18.37
Tecto	35.7	25.2	15.2	25.37	46.3	25.2	10.8	27.43
Copral	1.0	0.7	0.5	0.73	12.5	8.3	6.5	9.10
Ridomil	15.0	12.0	8.0	11.67	16.5	13.3	10.5	13.43
Teledor	1.8	0.8	0.5	1.03	20.5	16.7	5.5	14.23
Control	70.2	70.2	70.2	70.20	65.3	65.3	65.3	65.30
Mean	29.24	20.08	14.03	21.11	37.56	24.20	15.94	25.90
L.S.D. 5%								
Fungicides			1.65				1.39	
Concentrations			1.64				1.41	
Interaction			3.29				2.80	

Concerning squash fruit rot, data in **Table (13b)** illustrate that, fruit rot DS caused by *B. cineria* or *F. solani* were significantly affected by fungicides, concentrations as well as by the interaction between them. The highest significant decrease in DS caused by *B. cineria* was induced by the fungicides Teledor (3.3%) and Copral (13.3%). On the other hand, Copral and Teledor were the best among all tested fungicides in decreasing DS caused by *F. solani* respectively. Also, increasing the concentration decreased gradually the determined DS caused by *B. cinerea* or *F. solani* where the lowest DS was recorded at 750ppm followed by 500ppm while the highest DS were recorded at 250ppm. As for interactions between the fungicides and concentrations, the obtained results show that, Ridomil was the least effective fungicide followed by Sumisclex and Ronilan

against *B. cinerea*. Meanwhile, Ronilan, Rovral and Sumisclex were the least effective fungicides against *F. solani* fruit rot infection on squash.

Table (13b): Effect of post-harvest fungicidal treatment against squash fruit rots caused by *B. cinerea* and *F. solani*.

Fungicides	*B. cinerea*			Mean	*F. solani*			Mean
	250 ppm	500 ppm	750 ppm		250 ppm	500 ppm	750 ppm	
Ronilan	40.7	27.0	3.2	23.63	56.4	26.3	12.6	31.77
Sumisclex	45.8	16.7	11.4	24.63	51.4	19.7	12.2	27.77
Rovral	37.2	19.2	5.6	20.67	46.7	30.3	13.7	30.23
Tecto	31.1	16.5	11.4	19.67	30.5	15.2	5.7	17.13
Copral	20.5	14.0	5.5	13.33	10.5	7.83	4.5	7.61
Ridomil	30.0	25.0	20	25.00	20.5	15.7	10.2	15.47
Teledor	6.5	3.0	0.5	3.33	15.5	9.7	4.5	9.90
Control	70.2	70.2	70.2	70.20	65.3	65.3	65.3	65.30
Mean	35.25	23.95	15.98	25.06	37.10	23.75	16.09	25.65
L.S.D. 5%								
Fungicides			1.65				1.39	
Concentrations			1.64				1.41	
Interaction			3.29				2.80	

7.2. Effect of pre-spraying cucumber and squash plants with some chemicals and salts:

7.2.1. Effect on fruit rot infections after harvesting:

Cucumber and squash plants were sprayed 7 days after sowing with aqueous solutions of different chemicals and salts as inducer treatments *i.e.* SA, OA, $CaCl_2$, $MgSO_4$, $LiSO_4$, K_2HPO_4, Na_2SO_4, Bion, Ethephon and Catechol at the concentrations of 250, 500 and 1000 ppm for each particular chemical inducer. After 45 days from

sowing, cucumber and squash fruits were harvested, surfaced sterilized, dipped for 5 minutes in an aqueous solution of the above mentioned chemical inducers then inoculated as above mentioned with fungi *B. cineria* or *F. solani*. Fruit rot disease severity was determined as described above 10 days after inoculation comparing with control treatment (treated with sterilized distilled water).

As for cucumber fruits, data in Table (14a) and Fig. (11a) revealed that, DS caused by *B. cinerea* or *F. solani* was significantly decreased by all tested chemical inducer treatments comparing to their untreated controls. In this regard, the lowest significant DS caused by *B. cineria* was recorded by KH_2PO_4 (0.5%), $CaCl_2$ and Na_2SO_4 (0.7%), SA (0.8%) and OA (1.1%) without significant differences between them followed by $LiSO_4$ (1.9%), Bion (4.4%), Esephone (5.3%), Catechol (7.0%) and $MgSO_4$ (7.4%) comparing with the untreated control (76.8%), respectively. Disease severity was successively and significantly decreased as concentrations of the tested chemical inducers increased. Disease severity caused by *B. cineria* recorded 10.8, 9.95 and 8.26% at the concentrations of 250, 500 and 1000 ppm respectively.

Concerning interactions between tested resistance inducers and their concentrations, it is clear that increasing the concentration decreased gradually the cucumber fruit rot infections caused by *B. cinerea* and *F. solani*. In this respect the least DS was 0.3% with KH_2PO_4 at 500 and 1000ppm followed by 0.4% with $CaCl_2$ and 0.5% with SA and OA at 1000ppm. On the other hand,

the highest recorded DS were obtained with all tested resistance inducers at low tested concentration (250ppm) in case of *B. cinerea* infection. Meanwhile, the highest recorded value of DS was achieved with $MgSO_4$ at all tested concentrations. Similar trends were noticed concerning DS of cucumber caused by *F. solani*. Allover interactions, OA and KH_2PO_4 (at 250, 500 & 1000 ppm), SA at 500 and 1000 ppm were the best for controlling cucumber fruit rot caused by *F. solani*.

Table (14a): Effect of some chemical inducer treatments on severity of cucumber fruit rots caused by *B. cinerea* and *F. solani*.

Chemical inducers	DS of cucumber fruit rots caused by							
	B. cinerea				*F. solani*			
PPM	250	500	1000	Mean	250	500	1000	Mean
SA	1.1	0.8	0.5	0.8	1.6	0.6	0.4	0.9
OA	2.0	0.7	0.5	1.1	0.8	0.6	0.5	0.6
$CaCl_2$	1.1	0.7	0.4	0.7	7.5	6.5	2.2	5.4
$MgSO_4$	9.4	7.9	4.8	7.4	10.4	9.3	7.5	9.1
$LiSO_4$	3.0	1.8	0.8	1.9	4.7	2.3	1.5	2.8
KH_2PO_4	0.8	0.3	0.3	0.5	0.3	0.2	0.3	0.3
Na_2SO_4	1.1	0.7	0.4	0.7	7.5	6.5	2.2	5.4
Bion	6.7	5.8	0.7	4.4	20.5	25.0	0.8	15.4
Ethephon	8.3	6.5	1.2	5.3	2.5	1.5	1.2	1.7
Catechol	9.0	7.5	4.5	7.0	9.0	6.5	5.2	6.9
Control	76.8	76.8	76.8	76.8	57.3	57.3	57.3	57.3
Mean	10.85	9.95	8. 26		11.1	10.57	7.42	
L.S.D at 0.5% for								
Treatment (T)				1.31				1.37
Concentration (C)				0.72				0.75
T x C.				2.27				2.37

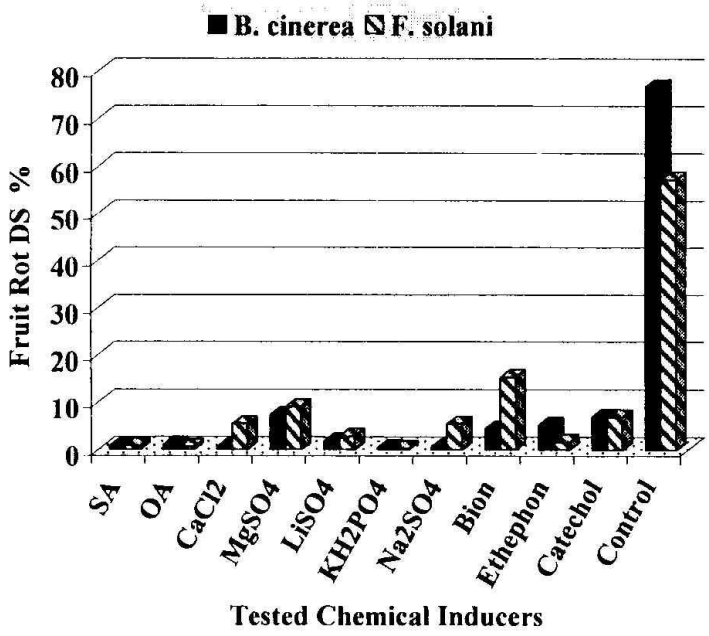


Fig. (11a): Effect of some chemical inducer treatments on the averages of severity of cucumber fruit rots caused by *B. cinerea* and *F. solani.*

Table (14b): Effect of some chemical inducer treatments on severity of squash fruit rots caused by *B. cinerea* and *F. solani.*

Resistance inducers	DS of cucumber fruit rots caused by							
	B. cinerea				*F. solani*			
PPM	250	500	1000	Mean	250	500	1000	Mean
SA	3.0	2.0	0.5	1.9	3.6	2.7	1.2	2.5
OA	3.5	2.2	0.8	2.2	0.8	0.6	0.3	0.6
$CaCl_2$	6.0	3.0	1.3	3.4	11.3	5.4	3.6	6.8
$MgSO_4$	11.5	9.7	8.9	10.0	12.2	10.6	9.6	10.8
$LiSO_4$	5.2	2.6	1.6	3.1	7.9	3.4	1.6	4.3
KH_2PO_4	3.4	1.8	0.9	2.0	2.7	0.9	0.7	1.4
Na_2SO_4	6.0	3.0	1.3	3.4	11.3	5.4	3.6	6.8
Bion	3.5	2.2	2.0	2.6	13.3	10.5	1.5	8.4
Ethephon	11.7	9.5	4.5	8.6	4.8	2.5	2.0	3.1
Catechol	9.2	7.5	3.5	6.7	4.8	2.5	2.0	3.1
Control	65.1	65.1	65.1	65.1	47.9	47.9	47.9	47.9
Mean	11.7	9.9	8.2		11.0	8.4	6.7	
L.S.D at 0.5% for								
Treatment (T)				1.45				1.40
Concentration (C)				0.79				0.77
T x C.				2.51				2.43

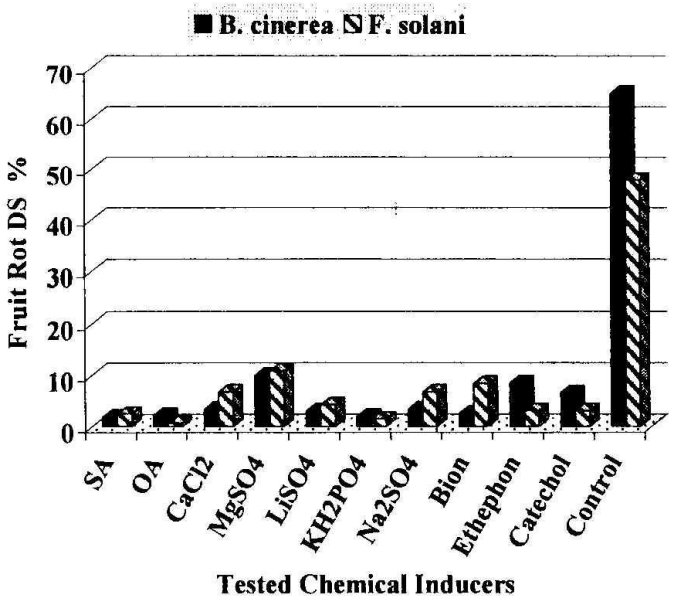


Fig. (11b): Effect of some chemical inducer treatments on the averages of severity of squash fruit rots caused by *B. cinerea* and *F. solani.*

In case of squash fruits, data in **Table (14b)** and **Fig. (11b)** clear that, DS caused by *B. cinerea* or *F. solani* was significantly decreased also by all tested chemical inducer treatments comparing to their untreated controls. In this regard, the lowest significant DS caused by *B. cineria* was recorded by SA (1.9%) followed by KH_2PO_4 (2.0%), OA (2.2%), $LiSO_4$ (3.1%), Na_2SO_4 (3.4%), $MgSO_4$ (10.0%) comparing with the untreated control (65.1%).

7.2.2. Effect on changes in protein patterns in leaves of cucumber and squash plants:

The changes in the soluble protein in leaves of cucumber and squash plants previously sprayed with some resistance inducers i.e. Bion, SA, OA, KH_2PO_4, $CaCl_2$ were determined using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

technique. Data in **Table (15)** illustrated the detected protein bands based on their molecular weights (MW) whereas **Fig. (12)** showed a hand drawing diagram for protein bands detected in the different tested treatments in comparison with the untreated control. In most cases, the number of detected protein bands was higher in any tested resistance inducer than the untreated control. This was true either in leaves of cucumber or squash plants. In this regard, the resistance inducers Bion, SA, OA, KH_2PO_4 and $CaCl_2$ recorded 16, 16, 11, 14 and 10 bands in case of cucumber leaves and 17, 18, 17, 14 and 19 bands in squash leaves compared with 8 and 14 bands in the untreated control of both crops, respectively. It is clear that a total number of 27 protein bands with MW ranged between 51 and 39 kDa were appeared in cucumber leaves treated the tested resistance inducers but not in the untreated cucumber leaves (control). Similarly, 22 bands with MW ranged between 55 and 47 kDa were detected only in squash leaves treated with some tested resistance inducer treatments but not in the untreated control. In general, the highest number of the new protein bands were detected in cucumber or squash leaves treated with Bion or salicylic acid (SA).

Table (15): Protein bands detected in leaves of cucumber and squash plants sprayed with Bion (L 1), SA (L 2), OA (L 3), KH_2PO_4 (L 4), $CaCl_2$ (L 5) and untreated leaves (L 6).

Marker	Cucumber						Squash					
	Molecular weight of bands detected in different lanes						Molecular weight of bands detected in different lanes					
	L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6
130							+					
121							+	+				
116												
115					+						+	
109												
107		+	+	+		+	+		+		+	+
106												
105												
104	+							+		+		
										+		
96											+	
93												
92	+	+	+	+	+	+		+	+		+	+
91												
90							+					
86							+					
								+				
85											+	
83												
	+	+	+	+	+	+	+	+	+		+	+
										+		
75		+										
74	+		+	+	+	+	+	+	+	+	+	+
73												
											+	+
66												
65	+	+	+	+	+			+		+	+	+
64						+	+		+			
62												+
60	+	+	+								+	+
59				+	+	+			+	+		
58							+				+	
56	+	+										+
55			+	+	+	+	+	+	+	+		

Table (15): Continue

Marker	Cucumber						Squash					
	Molecular weight of bands detected in different lanes						Molecular weight of bands detected in different lanes					
	L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6
53	+	+	+	+	+	+			+			
52							+	+		+	+	
51	+				+		+	+	+		+	
50	+	+								+		
49								+	+		+	
48		+					+	+				
47								+		+	+	
46	+			+			+	+		+	+	+
45		+		+					+			
44	+	+	+	+			+	+	+	+	+	+
43	+	+						+	+			+
42	+	+						+	+	+	+	+
41	+	+	+	+	+		+	+	+	+	+	+
40	+	+		+			+		+			
39			+	+					+			
No.	17	16	11	15	10	8	17	18	18	14	19	14

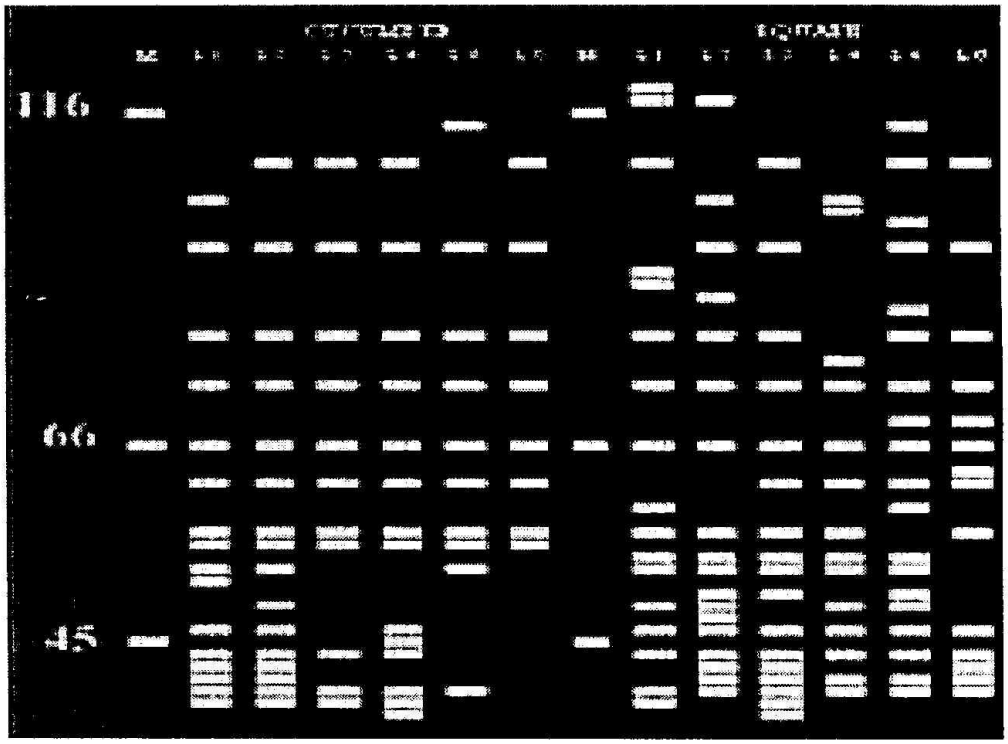


Fig. (12): Diagram showing protein bands in leaves of cucumber and squash plants sprayed with Bion (Lane 1), SA (Lane 2), OA (Lane 3), KH_2PO_4 (Lane 4), $CaCl_2$ (Lane 5) compared to the untreated plants (Lane 6).

7.2.3. Effect on changes in analysis of amino acids in the cucumber and squash leaves:

The obtained results indicate that the amounts of different amino acids detected in the cucumber leaves **(Table, 16a)** and squash leaves **(Table, 16b)** were affected considerably by spraying plants with the different tested salts and chemical inducers. The tested treatments increased some amino acids and decreased others in the cucumber fruits comparing with the untreated control. The rate of increase ranged between 1.9 to 8000.0% dipinding on the tested treatment and type of amino acis. In this respect, SA treatment increased only the Glu (26.5%). However, Bion treatment increased Asp (1.9%), Thr (660.7%), Pro (3500.0%), Ile (19.5%), His (300.0%), and Arg (54.2%). Oxalic acid treatment increased Ser (114.8%), Pro (8000.0%), Valin (20.8%), Leu (42.4%), Phe (24.6%), and His (27.3%). Calcium chloride treatment increased Asp by (36.2%), Gly (61.8%), Ala (10.1%), Ile (10.9%), Phe (13.1%), His (81.8%) and Arg (29.5%). Potassium phosphate (KH_2PO_4) treatment increased Asp (1.9%), Thr (7.1%), Ser (29.6%), Glu (25.3%), Leu (6.1%) and His (27.3%). It is of interesting to state that, the amino acid cysteine was detected neither in any tested treatments nor in the untreated control. On the other hand the amino acid methionine which not detected in the untreated control was detected only in the OA treatment (0.24 g/100g dry weight).

Table (16a): Effect of some tested resistance inducers on changes of free amino acids assayed in cucumber leaves

Amino Acid *		Tested treatments of resistance inducers					Control
		Bion	SA	OA	$CaCl_2$	KH_2PO_4	
Amino acids (g/100g dry weight)	Asp	0.107	0.083	0.087	0.143	0.107	0.105
	Thr	0.213	0.017	0.019	- **	0.030	0.028
	Ser	-	0.016	0.058	-	0.035	0.027
	Glu	0.062	0.215	0.025	0.126	0.213	0.170
	Pro	0.036	0.001	0.081	-	-	0.001
	Gly	0.025	0.040	0.013	0.110	0.060	0.068
	Ala	-	0.146	-	0.186	0.143	0.169
	Cys	-	-	-	-	-	-
	Val	0.034	0.058	0.116	0.070	0.068	0.096
	Met	-	-	0.024	-	-	-
	Ile	0.055	0.031	0.038	0.051	0.040	0.046
	Leu	0.034	0.073	0.141	0.021	0.105	0.099
	Tyr	0.071	0.012	0.074	0.079	0.033	0.083
	Phe	0.022	0.040	0.076	0.069	0.038	0.061
	His	0.044	0.010	0.014	0.020	0.014	0.011
	Lys	0.046	0.058	0.047	0.029	0.067	0.111
	Arg	0.350	0.101	0.187	0.294	0.145	0.227
Change % compared to control	Asp	1.9	-21.0	-17.1	36.2	1.9	0.0
	Thr	660.7	-39.3	-32.1	-	7.1	0.0
	Ser	-	-40.7	114.8	-	29.6	0.0
	Glu	-63.5	26.5	-85.3	-25.9	25.3	0.0
	Pro	3500.0	0.0	8000.0	-	-	0.0
	Gly	-63.2	-41.2	-80.9	61.8	-11.8	0.0
	Ala	-	-13.6	-	10.1	-15.4	0.0
	Cys	-	-	-	-	-	-
	Val	-64.6	-39.6	20.8	-27.1	-29.2	0.0
	Met	-	-	-	-	-	-
	Ile	19.6	-32.6	-17.4	10.9	-13.0	0.0
	Leu	-65.7	-26.3	42.4	-78.8	6.1	0.0
	Tyr	-14.5	-85.5	-10.8	-4.8	-60.2	0.0
	Phe	-63.9	-34.4	24.6	13.1	-37.7	0.0
	His	300.0	-9.1	27.3	81.8	27.3	0.0
	Lys	-58.6	-47.8	-57.7	-73.9	-39.6	0.0
	Arg	54.2	-55.5	-17.6	29.5	-36.1	0.0

- * Asp (asparatic acid); Thr (threonine); Ser (serine); Glu (glutamic acid); Pro (proline); Gly (glycine); Ala (alanine); Cys (cysteine); Val (valin); Met (methionine); Ile (isoleucine); Leu (leucine); Tyr (tyrosine); Phe (phenylalanine); His (histidine); Lys (lysine) and Arg (arginine)
- ** - Not detected

Table (16b): Effect of some tested resistance inducers on changes of free amino acid of squash leaves treated with fruit rot pathogens.

Amino Acid *		Tested treatments of resistance inducers					Control
		Bion	SA	OA	$CaCl_2$	KH_2PO_4	
Amino acids (g/100g dry weight)	Asp	0.048	0.082	0.115	0.078	0.079	0.079
	Thr	0.010	-	-	0.221	-	-
	Ser	-	-	-	-	-	-
	Glu	0.030	0.369	0.386	-	0.169	0.294
	Pro	-	0.004	0.002	0.001	0.002	0.004
	Gly	0.019	0.036	0.056	0.018	0.038	0.043
	Ala	0.030	0.236	0.302	0.313	0.200	0.424
	Cys	-	-	-	-	-	-
	Val	0.028	0.091	0.097	0.124	0.075	0.140
	Met	-	-	-	-	-	-
	Ile	0.010	0.044	0.051	0.057	0.023	0.060
	Leu	0.027	0.085	0.086	0.098	0.052	0.106
	Tyr	0.018	0.159	0.199	0.090	0.102	0.164
	Phe	-	0.009	0.038	0.021	-	0.016
	His	-	0.008	0.007	0.005	-	0.007
	Lys	-	0.012	0.033	0.009	-	0.014
	Arg	0.579	0.165	0.129	0.064	0.262	0.148
Change % compared to control	Asp	-39.2	3.8	45.6	-1.3	0.0	0.0
	Thr	-	-	-	-	-	-
	Ser	-	-	-	-	-	-
	Glu	-89.8	25.5	31.3	-	-42.5	0.0
	Pro	-	0.0	-50.0	-75.0	-50.0	0.0
	Gly	-55.8	-16.3	30.2	-58.1	-11.6	0.0
	Ala	-92.9	-44.3	-28.8	-26.2	-52.8	0.0
	Cys	-	-	-	-	-	-
	Val	-80.0	-35.0	-30.7	-11.4	-46.4	0.0
	Met	-	-	-	-	-	-
	Ile	-83.3	-26.7	-15.0	-5.0	-61.7	0.0
	Leu	-74.5	-19.8	-18.9	-7.6	-50.9	0.0
	Tyr	-89.0	-3.1	21.3	-45.1	-37.8	0.0
	Phe	-	-43.8	137.5	31.3	-	0.0
	His	-	14.3	0.0	-28.6	-	0.0
	Lys	-	-14.3	135.7	-35.7	-	0.0
	Arg	291.2	11.5	-12.8	-56.8	77.0	0.0

- * Asp (asparatic acid); Thr (threonine); Ser (serine); Glu (glutamic acid); Pro (proline); Gly (glycine); Ala (alanine); Cys (cysteine); Val (valin); Met (methionine); Ile (isoleucine); Leu (leucine); Tyr (tyrosine); Phe (phenylalanine); His (histidine); Lys (lysine) and Arg (arginine)
- ** - Not detected

As for squash leaves, two amino acids *i.e.* Ser and Cysteine were not detected in all tested treatments including the untreated control one. However, the amino acid Thr which was found at the rates of 0.1 g/100 g dry weight (in Bion treatment) and 0.221 g/100 g dry weight (in $CaCl_2$ treatment), was never detected either in the untreated control or in the SA, OA KH_2PO_4 treatments. In addition, Ser, Cys and Met were not detected in any of the tested treatments. The tested treatments increased some amino acids and decreased others in squash fruits comparing with the untreated control. The rate of increase was ranged from 3.8 to 291.2% dipinding on the tested treatment and type of amino acid. In this respect, Bion treatment increased only the amino acid Arg (291.2%) while it decreased Asp (39.2%), Glu (89.8%), Gly (55.8%), Ala (92.9%), valin (80.0%), Ile (83.3%), Leu (74.5%), and tyrosine (89.0%) and prevented synthesis of serine, Pro , Cys, Met, Phe, His and Lys which were completely absent in this treatment. In case of SA treatment, abreciable increases were found in Asp (3.8%), Glu (25.5%), His (14.3%) and Arg (11.5%). In OA treatment, the threonine, serine, cysteine and methionine were not detected whereas His was not changed when compared with the untreated control. In the same treatment (OA), Asp, Glu, Gly, Tyr, Phe and Lys were increased by 45.6, 31.3, 30.2, 21.3, 137.5 and 135.7%, respectively whereas the rest of amino acids were decreased (12.8-50.0%) compared with the untreated control. Similarly, $CaCl_2$ treatment increased Asp (36.2%), Gly (61.8%), Ala (10.1%), Ile

(10.9%), Phe (13.1%), His (81.8%) and Arg (29.5%), while it decreased Glu (25.9%), Val (27.1%), Leu (78.8%), Tyr (4.8%) and Lys (73.9%) and the remained amino acids (Thr, Ser, Pro, Cys and Met) were not detected compared with the untreated control. As for KH_2PO_4 treatment, Arg only was increased by 77.0% whereas Glu, Pro, Gly, Ala, Val, Ile, Leu and Tyr were decreased (11.6-61.7%). In the same treatment (KH_2PO_4), Asp was not changed whereas Phe, His and Lys were not detected comparing with the untreated control.

7.2.4. Effect biochemical components in cucumber and squash fruits inoculated with fruit rot pathogens:

7.2.4.1. Changes in phenolic compounds:

Data in **Table (17a)** indicate that most of the tested inducers and fungicides increased the quantities of total and free phenols in treated and infected fruits with *B. cinerea* and/or *F. solani* comparing to control treatment. It is clear from the obtained results that all tested treatments reduced the conjugated phenols where the determined quantities of conjugated phenols were always lesser than free phenols. Also, Catechol followed by Bion and Ethephon were the best effective treatments in increasing quantities of total and free phenols in treated and infected fruits with *B. cinerea*. On the other hand, Teledor, Copral and Catechol were the best effective treatments in increasing the quantities of total and free phenols in cucumber fruits infected with *F. solani*. The least

quantity of conjugated phenols was recorded with Ethephon treatment in case of *F. solani* infection.

Table (17a): Phenol contents (mg/g fresh weight) in cucumber fruits inoculated with *B. cinerea* and *F. solani* 15 days post inoculation.

Treatments	*B. cinerea*			*F. solani*		
	Free	Conjugated	Total	Free	Conjugated	Total
Bion	0. 240	0.191	0.431	0.250	0.109	0.359
Calcium chloride	0.162	0.011	0.173	0.297	0.075	0.372
Catechol	0.292	0.140	0.432	0.250	0.171	0.421
Ethephon	0.240	0.180	0.420	0.132	0.007	0.139
Copral	0286	0.109	0.395	0.255	0.198	0.453
Teledor	0.175	0.150	0.325	0.273	0.211	0.484
Control	0.150	0.120	0.270	0.210	0.190	0.400

Data in **Table (17b)** indicate that pre-spraying squash plants with the tested resistance inducers and fungicides affected greatly the content of phenols in their harvested fruits treated with either tested fruit rot pathogens. In this respect, all quantities of free phenols were more than conjugated phenols in all infected tissues of harvested fruits which inoculated with *B. cinerea* and/or *F. solani*. The highest recorded quantity of free phenols was recorded with Catechol treatment while the highest quantity of total phenols was recorded with Copral fungicide treatment in case of *B. cinerea* infection. In case of *F. solani* infection, the highest quantities of free phenols were recorded with Ethephon and Teledor treatments. Similar trends were recorded with the determined quantities of total phenols. On the other hand, it is clear from the obtained results that free, conjugated and total phenols were high in control

treatment than other treatments of tested plant extracts and fungicides with both *B. cinerea* and *F. solani* infection.

Table (17b): Phenol contents (mg/g fresh weight) in inoculated squash fruits with *B. cinerea* and *F. solani,* 15 days post inoculation.

Treatments	*B. cinerea*			*F. solani*		
	Free	Conjugated	Total	Free	Conjugated	Total
Bion	0.129	0.011	0.140	0.238	0.002	0.240
Calcium chloride	0.182	0.040	0.222	0.122	0.101	0.223
Catechol	0.203	0.020	0.223	0.262	0.086	0.348
Ethephon	0.121	0.096	0.217	0.448	0.174	0.614
Copral	0.139	0.115	0.254	0.231	0.077	0.318
Teledor	0.111	0.101	0.212	0.102	0.338	0.440
Control	0.180	0.120	0.300	0.200	0.180	0.380

7.2.4.2. Changes in total amino acids:

Data in **Table (18)** indicate that pre-spraying cucumber and squash plants with some resistance inducers and fungicides affected the total amino acids content of harvested fruits which inoculated with *B. cinerea* and/or *F. solani*. In this respect, Teledor and Catechol were the best effective treatments in increasing the amino acids in inoculated cucumber fruits with *F. solani*. While calcium chloride was the best treatment in case of inoculated squash fruits with *F. solani*. On the other hand, Catechol followed by Teledor, Copral and Ethephon treatments were the best effective treatments in increasing total amino acids content in inoculated cucumber fruits with *B. cinerea*. Also, Teledor and Catechol were the best effective treatments in increasing total amino acids content in squash fruits inoculated with *B. cinerea*. It

is clear from the obtained results that Bion and Copral treatments were not effective in increasing total content of amino acids in inoculated cucumber fruits with *F. solani* where their quantities were lesser than in control treatment. Meanwhile, Bion, Copral and Teledor treatments were lesser effective on squash fruits in this respect. In case of *B. cinerea* infection, Bion, Calcium chloride, were the least effective treatments on the total amino acids content in inoculated cucumber fruits with *B. cinerea*. While, Bion, Ethephon and Calcium chloride were the least effective treatments on total amino acids contents in inoculated squash fruits with *B. cinerea*.

Table (18): Total amino acids contents (mg/g fresh weight) in inoculated cucumber and squash rotted fruits caused by *B. cinerea* and *F. solani*, 15 days post inoculation.

Treatment	*F. solani*		*B. cinerea*	
	Cucumber	Squash	Cucumber	Squash
Bion	0.348	0.130	0.314	0.237
Calcium chloride	0.731	0.604	0.415	0.311
Catechol	0.828	0.471	1.095	0.833
Ethephon	0.777	0.467	0.886	0.257
Copral	0.623	0.171	0. 960	0.591
Teledor	0.847	0.233	1.047	0.849
Control	0.629	0.322	0.629	0.322

7.3. Effect of pre-spraing cucumber and squash plants with plant extracts and kombucha tea preparations:

7.3.1. Effect on the infection with the fruit rot pathogens (*B. cinerea* or *F. solani*):

In this study, cucumber and squash plants were sprayed during growth under greenhouse conditions with 10 or 20% concentration of a particular tested preparations **(Table, 1c)**.

As for cucumber fruit rot, data in **Table (19a)** and **Fig. (13a)** proved that, DS % caused by either *B. cinerea* or *F. solani* was significantly decreased by all tested preparations comparing to the untreated control. Disease severity caused by both pathogens was significantly lower at 20% than at 10% concentration. Regardless concentrations, marjoram preparation was the most effective as it completely suppressed DS caused by *B. cinerea* (0.0%) followed by kombucha tea (0.13%), ginger & garlic (0.33%), henna (0.52%) and nigella (0.6%) without significant differences between all of them. Among all preparations, thyme (6.32%) and carnation (8.5%) were the lowest effective comparing to the untreated control (75.33%). Concerning interactions between preparations and concen-trations, it is clear that, the latter two preparations *i.e.* thyme and carnation only were significantly more effective at 20% than at 10% concentration, whereas, no significant differences were found between the two concentrations of marjoram, kombucha tea, ginger, garlic, henna and nigella preparations.

However, the nigella, henna, marjoram and ginger preparations were the most effective where they decreased DS

caused by *F. solani* to 0.88%, 0.97%, 1.08% and 1.8%, followed by thyme (4.95%), kombusha tea (5.73%), garlic (6.77%) and carnation (7.9%), respectively comparing with the untreated control treatment (57.62%). Disease severity caused by *F. solani* was not significantly varied at 20% and 10% concentrations of kombusha tea, carnation, henna, marjoram and nigella but it was significantly lower at 20% than 10% concentration of garlic, ginger and thyme preparations. In general, henna, marjoram and nigella were the most effective for controlling DS caused by *F. solani* particularly when used at 20% concentration.

Table (19a): Effect of pre-harvest spraying cucumber plants with preparations of plant extracts and Kombucha on the fruit rot DS % caused by *B. cinerea* and *F. solani*.

Preparations	*B. cinerea*			*F. solani*		
Conc.	10%	20%	Mean	10%	20%	Mean
Carnation	12.16	4.83	8.50	7.93	7.86	7.90
Garlic	0.33	0.33	0.33	10.83	2.70	6.77
Ginger	0.33	0.33	0.33	3.26	0.33	1.80
Henna	1.03	0.00	0.52	1.33	0.60	0.97
Kombucha	0.26	0.00	0.13	5.73	5.73	5.73
Marjoram	0.00	0.00	0.00	1.83	0.33	1.08
Nigella	1.20	0.00	0.60	1.33	0.43	0.88
Thyme	12.23	0.41	6.32	6.40	3.50	4.95
Control	75.33	75.33	75.33	57.62	57.62	57.62
Mean	11.43	9.03	10.23	10.70	8.79	9.74
L.S.D at 0.5%						
Extracts (E)			2.79			1.41
Concentration (C)			1.71			0.86
E x C			4.84			2.44

As for squash fruit rot, data in **Table (19b)** and **Fig. (13b)** declared that, preparations of henna, garlic, carnation and ginger

were the most effective for decreasing DS caused by *B. cinerea* (1.0-2.3%) followed by nigella, kombusha tea and marjoram (3.3-4.8%) whereas thyme preparation induced the lowest significant decrease in DS caused by that pathogen (22.5%) compared with the untreated control (70.2%). However, garlic, henna, ginger, nigella carnation and thyme preparations were the most effective against fruit rot caused by *F. solani*, recording DS ranging from 1.3-2.5% followed by marjoram (3.0%) and kombusha tea (5.1%) comparing with the untreated control (65.3%).

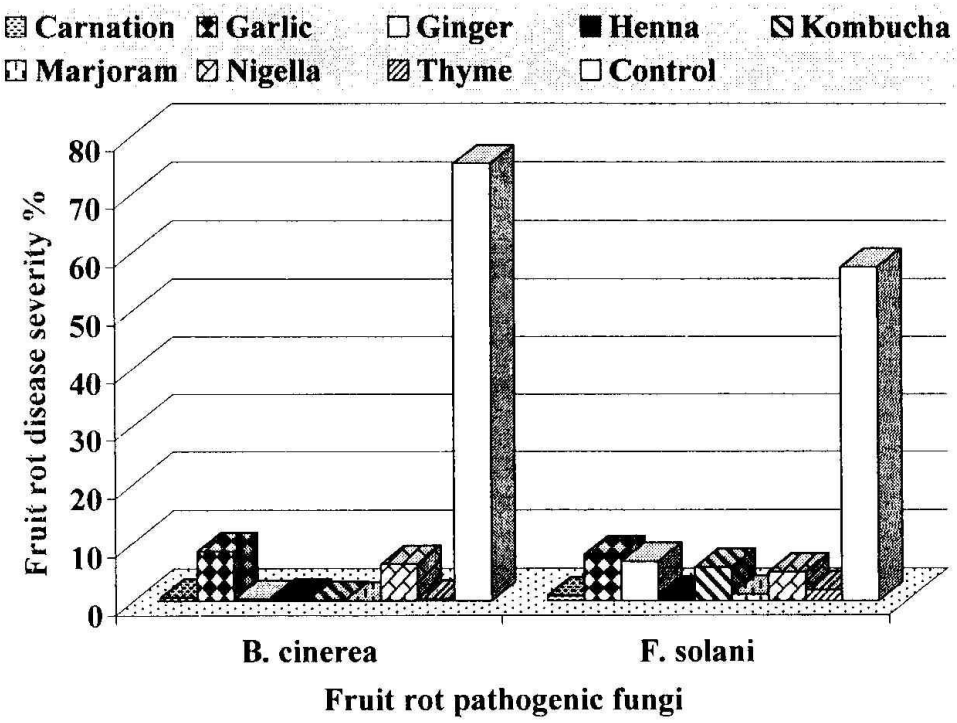


Fig. (13a): Effect of pre-harvest spraying cucumber plants with different plant extracts and kombusha on the fruit rots DS (average) caused by *B. cinerea* and *F. solani.*

Generally, decreases in DS due to applying most preparations either at 10 or 20% concentrations were significantly equal

although DS was apparently lower at 20% than at 10% concentration. Only thyme, marjoram and kombusha tea preparations were more effective at 20% than at 10% concentration when used against *B. cinerea* or *F. solani*. Applying kombusha tea, marjoram, and thyme preparations against *B. cinerea* recorded DS of 2.5, 3.0 and 15.0% (at 20% conc.) compared with 5.5, 6.6 and 30.0% (at 10% conc.) meanwhile, applying them against *F. solani* recorded 3.5, 2.0 and 1.5% (at 20% conc.) and 6.6, 4.1 and 3.5% (at 10% conc.), respectively.

Table (19b): Effect of pre-harvest spraying cucumber plants with preparations of plant extracts and kombucha on the fruit rot DS % caused by *B. cinerea* and *F. solani* .

Preparations	*B. cinerea*			*F. solani*		
Conc.	10%	20%	Mean	10%	20%	Mean
Carnation	3.0	1.3	2.1	2.5	1.5	2.0
Garlic	2.0	1.1	1.6	1.5	1.0	1.3
Ginger	3.0	1.5	2.3	2.0	1.0	1.5
Henna	1.5	0.5	1.0	1.7	1.0	1.4
Kombucha	5.5	2.5	4.0	6.6	3.5	5.1
Marjoram	6.6	3.0	4.8	4.1	2.0	3.0
Nigella	4.0	2.5	3.3	2.2	1.0	1.6
Thyme	30.0	15.0	22.5	3.5	1.5	2.5
Control	70.2	70.2	70.2	65.3	65.3	65.3
Mean	14.0	10.8	12.4	9.9	8.6	9.3
L.S.D at 0.5%						
Extracts (E)			1.34			1.38
Concentration (C)			0.63			0.65
E x C			1.89			1.95

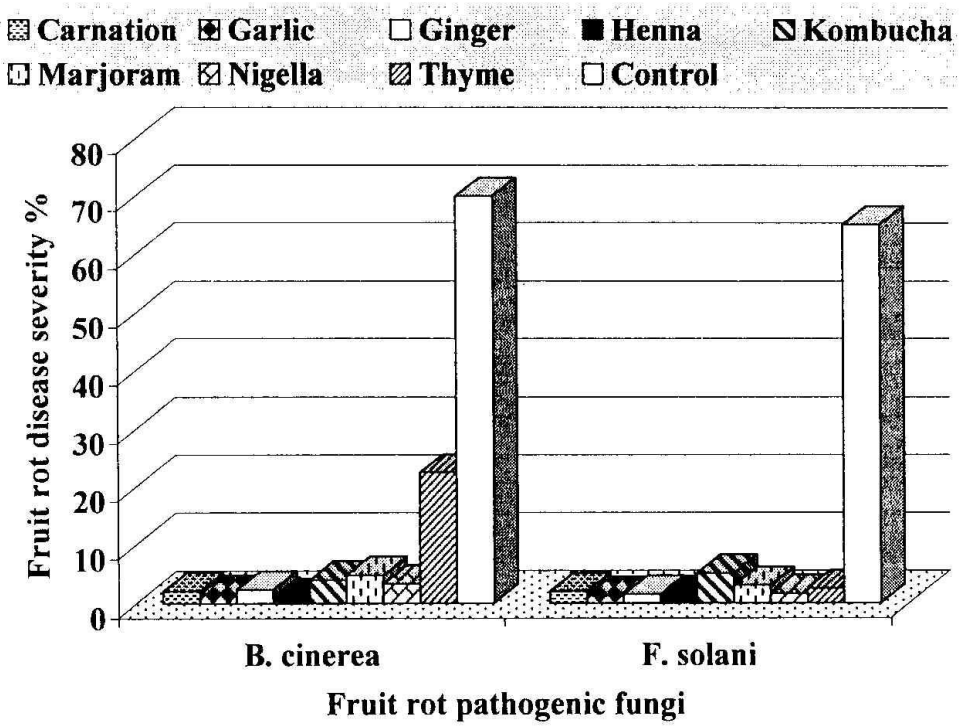


Fig. (13b): Effect of pre-harvest spraying cucumber plants with different plant extracts and kombusha on the fruit rots DS (average) caused by *B. cinerea* and *F. solani.*

7.3.2. Effect on enzymes activity in fruits inoculated with rot pathogens (*B. cinerea* or *F. solani*):

7.3.2.1. Activities of the pectolytic enzymes.

A. Pectin methyl esterase (PME) activity:

Data in **Table (20a)** revealed that, the pre-spraying cucumber plants with any of the tested preparations (plant extract or kombusha) greatly decreased the activity of PME enzyme in cucumber fruits inoculated with *B. cinerea* or *F. solani* comparing to the fruits harvested from the untreated plants. The reduction in PME activity was more pronounced in fruits harvested from plants sprayed with 20% than 10% concentration of any one of the tested preparations. In fruits inoculated with *B. cinerea*, ginger extract

was the most effective for reducing activity of PME enzyme (85.71 & 96.24% reduction) followed by marjoram extract (83.46 & 90.23%), nigella extract (74.44 & 88.72%), garlic extract (71.43 & 87.97%), kombucha (71.43 & 85.71%), carnation extract (61.05 & 74.44%), henna extract (45.11 & 57.89%) and thyme extract 24.81 & 47.37%) at 10 and 20% concentrations, respectively compared with the untreated control. In fruits inoculated with *F. solani*, ginger extract still the most effective for decreasing PME activity followed by nigella, marjoram, kombucha, garlic, thyme, carnation and henna, respectively.

Table (20a): Effect of some plant extracts and Kombusha preparations used at 10 and 20% conc. on PME activity (NaOH (ml)/g FW) in cucumber fruits inoculated with *B. cinerea* or *F. solani*.

	B. cinerea				*F. solani*			
	NaOH (ml)/ g		Efficiency %		NaOH (ml)/ g		Efficiency %	
Conc. Preparations	10%	20%	10%	20%	10%	20%	10%	20%
Carnation	5.18	3.4	61.05	74.44	4.0	2.3	45.21	68.49
Garlic	3.8	1.6	71.43	87.97	2.5	1.0	65.75	86.30
Ginger	1.9	0.5	85.71	96.24	1.6	0.4	78.08	94.52
Henna	7.3	5.6	45.11	57.89	5.6	3.7	23.29	49.32
Kombucha	3.8	1.9	71.43	85.71	2.8	0.9	61.64	87.67
Marjoram	2.2	1.3	83.46	90.23	1.5	0.8	79.45	89.04
Nigella	3.4	1.5	74.44	88.72	1.8	0.6	75.34	91.78
Thyme	10.0	7.0	24.81	47.37	3.0	1.2	58.90	83.56
Control	13.3	13.3	0.00	0.00	7.3	7.3	0.00	0.00

Also, the pre-spraying of squash plants with any of the tested preparations greatly decreased activity of PME enzyme in squash fruits inoculated with any tested pathogens (*B. cinerea* or *F. solani*) comparing to fruits harvested from the untreated plants **(Table, 20b)**.

Table (20b): Effect of some plant extract and kombusha preparations used at 10 and 20% conc. on PME activity (ml NaOH/g FW) in squash fruits inoculated with *B. cinerea* and *F. solani*.

	B. cinerea				*F. solani*			
	NaOH (ml)/g		Efficiency%		NaOH (ml)/g		Efficiency%	
Conc. Preparations	10%	20%	10%	20%	10%	20%	10%	20%
Carnation	4.6	2.5	59.29	77.88	3.2	1.2	34.69	75.51
Garlic	3.5	1.5	69.03	86.73	1.8	0.4	63.27	91.84
Ginger	1.5	0.75	86.73	93.36	1.4	0.5	71.43	89.80
Henna	6.2	4.0	45.13	64.60	1.5	0.7	69.39	85.71
Kombucha	2.0	0.5	82.30	95.58	1.9	0.5	61.22	89.80
Marjoram	1.0	0.7	91.15	93.81	1.2	0.7	75.51	85.71
Nigella	1.9	0.5	83.19	95.58	1.0	0.0	79.59	100.00
Thyme	6.18	4.8	45.31	57.52	2.0	0.0	59.18	100.00
Control	11.3	11.3	0.00	0.00	4.9	4.9	0.00	0.00

In most cases, the reduction in PME activity was obviously higher in fruits from plants sprayed with 20% than those sprayed with 10% concentration of any tested preparations. In fruits inoculated with *B. cinerea*, Kombucha tea and Nigella extracts were the most effective for reducing activity of PME enzyme followed by Marjoram extract, ginger extract, garlic extract, carnation extract, henna and thyme extracts particularly at 20% concentrations, comparing to the untreated control. However, in fruits inoculated with *F. solani*, nigella and thyme extracts used at 20% concentration were the most effective and completely suppressed PME activity followed by garlic, Kombucha, ginger, Marjoram, henna and carnation, respectively.

B. Polygalacturonase (PG) activity:

Data in **Table (21a)** and **Fig. (14a)** indicate that, pre-spraying cucumber plants with any of the tested preparation (plant extract or kombusha) greatly decreased the activity of PG enzyme in cucumber fruits either inoculated with *B. cinerea* or *F. solani* comparing to untreated plants. The reduction in the viscosity of PG substrate was more pronounced in fruits harvested from plants sprayed with 20% than 10% concentration of any tested preparation. In fruits inoculated with *B. cinerea*, garlic extract caused the highest reduction in viscosity (17.91 & 9.45%) followed by kombucha (22.89 & 13.33%), marjoram extract (26.57 & 18.21%), nigella extract (34.78 & 22.75%), carnation extract (27.82 & 23.37%), henna extract (32.5 & 23.5%), thyme extract (44.78 & 24.92%) and ginger (46.15 & 33.73%) at 50 and 100% concentrations, respectively comparing with the untreated control (73.39%).

All tested preparations, particularly when used at 20% concentration, seemed to be more effective for decreasing PG activity in fruits inoculated with *F. solani* than in those inoculated with *B. cinerea*. In fruits inoculated with *F. solani*, thyme extract at 100% concentration caused the highest decrease in PG activity (3.14%), followed by nigella extract (4.35%), kombucha (4.94%), henna (5.33%), marjoram (7.02%), ginger (8.7%), garlic (9.3%) and carnation (13.33%), respectively comparing to the untreated

control (73.68). This trend was slightly varied when these preparations were used at 10% concentration.

Table (21a): Effect of spraying plants with the tested plant extracts and kombucha on PG activity in cucumber fruits inoculated with *B. cinerea* and *F. solani*.

Conc. / Preparations	*B. cinerea*			*F. solani*		
	50%	100%	Mean	50%	100%	Mean
Carnation	27.82	23.37	25.60	21.05	13.33	17.19
Garlic	17.91	9.45	13.68	16.27	9.30	12.79
Ginger	46.15	33.73	39.94	28.57	8.70	18.64
Henna	32.0	23.50	27.75	16.81	5.33	11.07
Kombucha	22.89	13.33	18.11	17.39	4.94	11.17
Marjoram	26.57	18.21	22.39	18.67	7.02	12.85
Nigella	34.78	22.75	28.77	24.24	4.35	14.30
Thyme	44.78	24.92	34.85	21.97	3.14	12.56
Control	73.39		73.39	73.68		73.68

Concerning squash fruits, data in **Table (21b)** stated that, pre-spraying of squash plants with any of the tested preparations either at 10 or at 20% concentrations caused considerable reduction in PG activity subsequent harvested fruits inoculated with *B. cinerea* and/or *F. solani*.

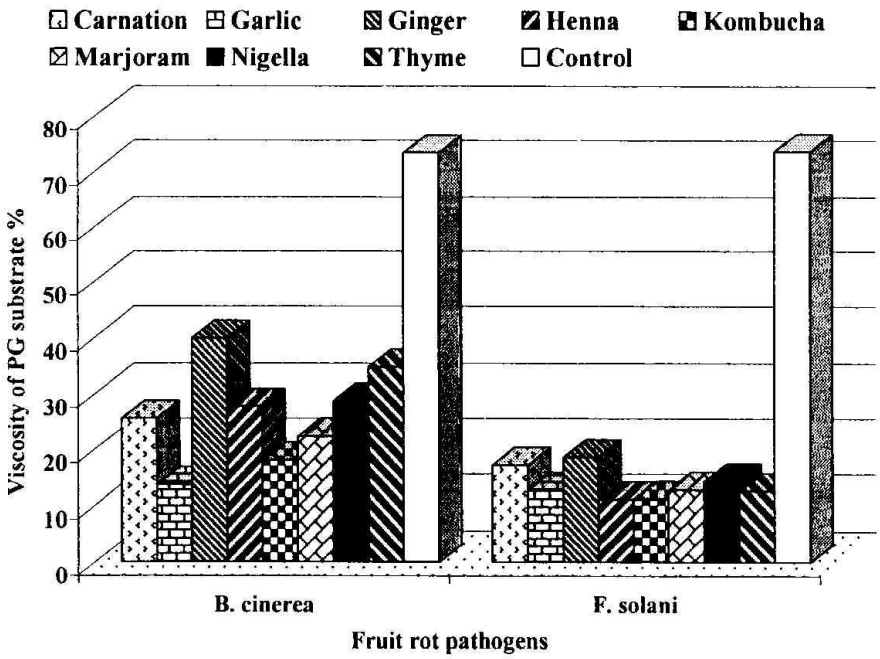


Fig. (14a): Effect of spraying plants with the tested plant extracts and kombucha on mean of the PG activity in cucumber fruits inoculated with *B. cinerea* and *F. solani*.

Table (21b): Effect of spraying plants with the tested plant extracts and kombucha on the PG activity in inoculated squash fruits with *B. cinerea* and *F. solani* (expressed as % substrate viscosity).

Conc. / Preparations	*B. cinerea*			*F. solani*		
	10%	20%	Mean	10%	20%	Mean
Carnation	19.51	16.50	18.01	17.10	15.60	16.35
Garlic	11.88	9.00	10.44	8.30	6.50	7.40
Ginger	32.20	22.10	27.15	27.20	24.70	25.95
Henna	19.93	16.50	18.22	16.01	14.50	15.26
Kombucha	12.37	10.50	11.44	10.11	7.90	9.01
Marjoram	17.17	14.80	15.99	15.00	13.40	14.20
Nigella	21.05	15.50	18.28	19.00	17.80	18.40
Thyme	27.39	19.20	23.30	25.00	22.67	23.84
Control	64.06		64.06	60.00		60.00

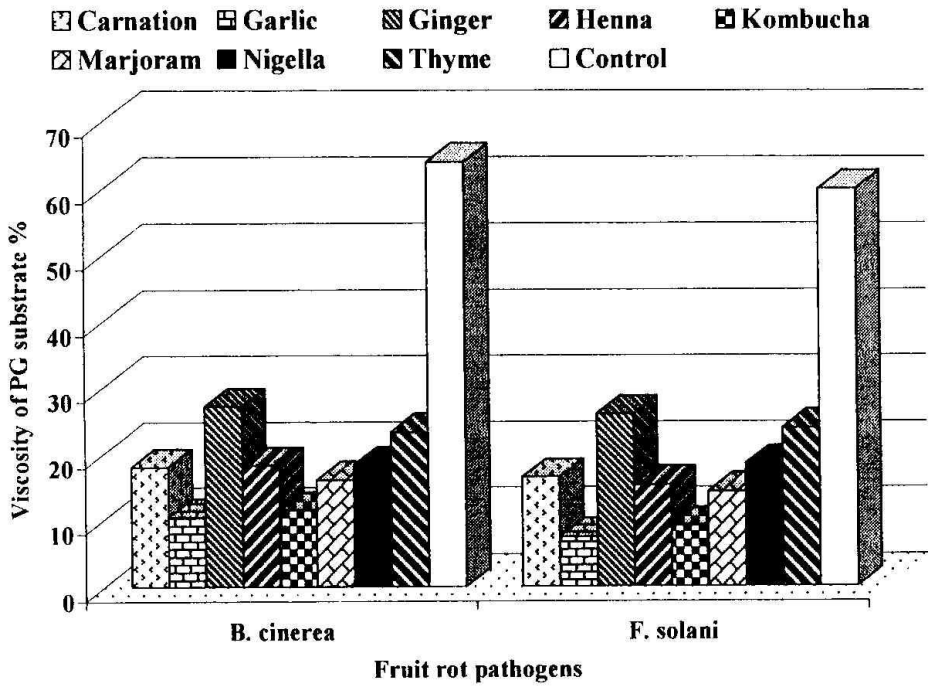


Fig. (14b): Effect of spraying plants with the tested plant extracts and kombucha on mean of PG activity in squash fruits inoculated with *B. cinerea* and *F. solani*.

In case of *B. cinerea*, garlic extract was the most effective, preparation that reduced PG activity to 11.88 & 9.0% at 10 and 20% concentrations, respectively followed by kombucha (12.37 & 10.5%), marjoram (17.17& 14.8%), nigella extract (21.05 & 15.5%), carnation (19.51 & 16.5%), henna extract (19.93 & 16.5%), thyme extract (27.39 &19.2%) and ginger extract (32.2 & 22.1%), respectively compared with the untreated control (64.06%). Similar trend was noticed in squash fruits inoculated with *F. solani*.

7.3.2.2. Activity of the oxidative enzymes.

A. Poly Phenyl Oxidase (PPO) Enzyme:

Data in **Table (22a)** revealed that, the pre-spraying cucumber plants with any of the tested preparations (plant extracts or kombusha) greatly affected the activity of PPO enzyme in the harvested fruits. In this respect, the activities of PPO enzyme were greatly higher in all treatments of tested plant extracts and kombusha at both tested concentrations comparing to control treatment (un-treated). On the other hand, the determined activities and their efficiency% were higher at 20% concentration than at 10% concentration for both tested fruit rot pathogens (*B. cinerea* and *F. solani*). Also, the highest enzyme activity and efficiency% were recorded with nigella extract at 10 and 20% concentrations in case of *B. cinerea*. Meanwhile the situation was different in case of *F. solani* where the highest activity and efficiency of PPO enzyme were recorded with carnation extract followed by marjoram, thyme and garlic extracts respectively at 20% concentration while the highest recorded activity of PPO enzyme at 10% concentration of the tested plant extracts was recorded with marjoram and Nigella extracts. On the other hand, the lowest activity of PPO enzyme was recorded with garlic extract at 10% concentration and with carnation extracts in case of *B. cinerea* infection while, it was recorded with ginger extract at 10% concentration and with kombucha filterate at 20% concentration in case of *F. solani* infection.

Table (22a): Effect of some plant extracts on polyphenoloxidase (PPO) enzyme activity (OD/min/g fresh weight) in cucumber fruits artificially inoculated with *B. cinerea* and *F. solani.*

	B. cinerea				*F. solani*			
	OD at 495 nm		Efficiency (folds)		OD at 495 nm		Efficiency (folds)	
Conc. Preparations	10%	20%	10%	20%	10%	20%	10%	20%
Carnation	0.172	0.338	2.37	5.63	0.166	0.642	3.15	15.05
Garlic	0.164	0.348	2.22	5.82	0.163	0.562	3.08	13.05
Ginger	0.175	0.404	2.43	6.92	0.132	0.453	2.30	10.33
Henna	0.189	0.401	2.71	6.86	0.166	0.555	3.15	12.88
Kombucha	0.173	0.397	2.39	6.78	0.154	0.329	2.85	7.23
Marjoram	0.192	0.368	2.76	6.22	0.172	0.592	3.30	13.80
Nigella	0.257	0.522	4.04	9.24	0.176	0.528	3.40	12.20
Thyme	0.196	0.382	2.84	6.49	0.170	0.584	3.25	13.60
Control	0.051	0.051	0.00	0.00	0.04	0.04	0.00	0.00

As for PPO enzyme activities in squash fruits sprayed previously with plant extracts and kombusha, data in **Table (20b)** revealed that the same trend was recorded where all determined PPO activities were high in fruits of sprayed squash plants with all tested extracts and kombusha at 10 and 20% concentrations comparing to control treatment (un-treated with any tested fruit rot pathogen infections, *B. cinerea* and *F. solani.* The highest activity of PPO enzyme was recorded with nigella extract at 10% concentration while, it was recorded with kombusha followed by thyme, henna and carnation extracts respectively at 20% concentration with similar trends in the estimated efficiency% in case of *B. cinerea* infections.

Table (22b): Effect of some plant extracts on activity of polyphenoloxidase (PPO) enzyme (OD/min/g fresh weight) in squash fruits artificially inoculated with *B. cinerea* and *F. solani.*

	B. cinerea				*F. solani*			
	OD at 495 nm		Efficiency (Folds)		OD at 495 nm		Efficiency (Folds)	
Conc. Preparations	10%	20%	10%	20%	10%	20%	10%	20%
Carnation	0.161	0.624	4.37	19.80	0.158	0.632	14.80	62.20
Garlic	0.154	0.553	4.13	17.43	0.135	0.528	12.50	51.80
Ginger	0.174	0.598	4.80	18.93	0.126	0.389	11.60	37.90
Henna	0.163	0.636	4.43	20.20	0.159	0.364	14.90	35.40
Kombucha	0.165	0.678	4.50	21.60	0.141	0.354	13.10	34.40
Marjoram	0.179	0.586	4.97	18.53	0.166	0.499	15.60	48.90
Nigella	0.186	0.543	5.20	17.10	0.139	0.582	12.90	57.20
Thyme	0.159	0.658	4.30	20.93	0.158	0.451	14.80	44.10
Control	0.03	0.03	0.00	0.00	0.01	0.01	0.00	0.00

On the other hand, the highest PPO activities in case of *F. solani* infections was recorded with marjoram followed by henna and carnation extracts at 10% concentration with similar trends in estimated efficiency%. Meanwhile, the highest PPO activities and efficiency% at 20% concentration were recorded with carnation followed by nigella and garlic extracts respectively.

B. Peroxidase (PRO) Enzyme:

Data in **Table (23a)** revealed that, the pre-spraying cucumber plants with any of the tested preparations (plant extract or kombusha) greatly affected the activities of peroxidase (PRO) enzyme in the harvested fruits. In this respect, all determined PRO activities were higher in fruits of pre-sprayed cucumber plants with 10 and 20% concentrations of all tested plant extracts and

kombusha with either of the fruit rot pathogens (*B. cinerea* or *F. solani*). In case of *B. cinerea* infection, the highest PRO activity was recorded with henna extract followed by garlic extract at 10% while, the highest PRO activity at 20% concentration of the tested plant extracts was recorded with henna followed by ginger and kombusha tea respectively with similar trends in estimated efficiency%. The lowest PRO activity was recorded with thyme followed by marjoram and ginger extracts at 10% concentration comparing to other tested extracts it was recorded with carnation followed by garlic and thyme extracts respectively at 10% concentration. In case of *F. solani* infection, the highest PRO activity obtained at 10% concentration which recorded with henna and marjoram respectively while, it was recorded with henna and kombusha treatments followed by marjoram extract respectively at 20% concentration with similar trends in estimated efficiency%.

As for PRO activities in fruits harvested from squash plants pre sprayed with plant extracts and kombusha tea, data in **Table (23b)** indicate that PRO activities were higher in fruits of pre-sprayed squash plants with 10 and/or 20% of the different tested extracts than control treatment (untreated). In case of *B. cinerea* infection, the highest PRO activity was recorded with henna followed by garlic extracts at 10% concentration. While, the lowest PRO activity was recorded with ginger extract at the same tested concentration. However, the highest PRO activities were recorded at 20% concentration of henna and kombusha tea preparations.

While, the lowest PRO activity at 20% concentration was recorded also with ginger extract.

Table (23a): Effect of some plant extracts on peroxidase activity expressed (optical density/min/g fresh weight) on artificial inoculated cucumber fruits with *B. cinerea* and *F. solani*.

	B. cinerea				*F. solani*			
	OD at 425 nm		Efficiency (Folds)		OD at 425 nm		Efficiency (Folds)	
Conc. Preparations	10%	20%	10%	20%	10%	20%	10%	20%
Carnation	0.26	0.50	0.86	2.57	0.38	0.59	1.53	2.93
Garlic	0.38	0.51	1.71	2.64	0.41	0.50	1.73	2.33
Ginger	0.21	1.05	0.50	6.50	0.24	0.22	0.60	0.47
Henna	0.47	1.15	2.36	7.21	0.84	1.39	4.60	8.27
Kombucha	0.31	1.01	1.21	6.21	0.52	1.39	2.47	8.27
Marjoram	0.21	0.81	0.50	4.79	0.70	1.02	3.67	5.80
Nigella	0.27	0.67	0.93	3.79	0.36	0.91	1.40	5.07
Thyme	0.16	0.51	0.14	2.64	0.21	0.72	0.40	3.80
Control	0.14	0.14	0.00	0.00	0.15	0.15	0.00	0.00

In case of *F. solani* infection, the lowest PRO activities were recorded with kombusha and thyme treatments respectively at 10% concentration. Meanwhile the highest PRO activity at the same tested concentration was recorded with nigella, carnation, garlic, henna and ginger respectively. At 20% concentration, the highest PRO activity was recorded with nigella extract followed by henna extract while the lowest value of PRO activity was recorded with thyme extract. It is clear also that increasing the concentration of the tested extracts increased gradually the efficiency% of the determined PRO enzyme where there were a great correlation between values of PRO enzyme and their efficiency%.

Table (23b): Effect of some plant extract on peroxidase activity expressed (optical density/min/g fresh weight) on artificial inoculated squash fruits with *B. cinerea* and *F. solani*.

	B. cinerea				*F. solani*			
	OD at 425 nm		Efficiency (Folds)		OD at 425 nm		Efficiency (Folds)	
Conc. Preparations	10%	20%	10%	20%	10%	20%	10%	20%
Carnation	0.43	0.82	2.07	4.86	0.57	0.65	3.75	4.42
Garlic	0.56	0.73	3.00	4.21	0.53	0.53	3.42	3.42
Ginger	0.15	0.48	0.07	2.43	0.50	0.50	3.17	3.17
Henna	0.67	1.26	3.79	8.00	0.53	0.98	3.42	7.17
Kombucha	0.52	1.25	2.71	7.93	0.15	0.56	0.25	3.67
Marjoram	0.23	0.35	0.64	1.50	0.35	0.53	1.92	3.42
Nigella	0.36	0.87	1.57	5.21	0.65	1.23	4.42	9.25
Thyme	0.25	0.73	0.79	4.21	0.21	0.35	0.75	1.92
Control	0.14	0.14	0.00	0.00	0.12	0.12	0.00	0.00

DISCUSSION

DISCUSSION

Cucurbits are considered one of the most important and widely distributed vegetable crops in Egypt and all over the world. Several fungal diseases like powdery and downy mildews, grey mould, white mould, Fusarium wilt, stem and fruit rots anthracnose and root rot are usually attack cucurbits, *i.e.* cucumber, melon, watermelon, and squash during different growing stages in relation to successive cropping periods resulting great yield losses (**Bedlan, 1986** and **1992; Siviero and Motton, 2000**).

Fruit rots disease severity (DS) on cucumber and squash fruits under greenhouse conditions were significantly varied between tested locations and seasons. Mostly, it was higher in 2003 than 2004 seasons and in Beheira than other locations (Qalubiya, Dakahliya, Kafr El-Sheikh, and Sharkiya). The variations in the environmental and culture conditions at different locations might be responsible for incidence and severity of fruit rot diseases on the cucurbits fruits. **Park *et al.* (1996)** reported that, the incidence of grey mould was related to culture conditions such as density of plant stand and ventilation. Also, the variations in pathogenic abilities of the the fungi associated with rotted cucumber and squash fruits might play an important role in severity of fruit rot disease severity at different locations. In our study, isolates of *B. cinerea* isolates were more pathogenic than isolates of *F. solani* on cucumber and squash fruits. *B. cinerea* and

F. solani isolated from Beheira recorded the highest average of DS followed by those from Ismaelia, Kafr El-Sheikh, Sharkia whereas isolates of Dakahliya were the least pathogenic ones on cucumber fruits. **Cotoras and Silva (2005)** reported that, the ability of *B. cinerea* strains to colonize tomato leaves also differs between the isolated strains obtained from grapes and tomato. Strains isolated from tomato were more virulent on tomato leaves than strains isolated from grapes.

The pathogenic abilities of the tested isolates were increased progressively as their inoculum potential increased. The fruit rot DS on cucumber or squash fruits were significantly varied according to the tested pathogen, inocula concentration and the interaction between them. *Botrytis cinerea* was more pathogenic than *F. solani* either on cucumber or on the squash fruits. Increasing the concentration of tested inocula from 1000-7000 spores/ml increased progressively DS on cucumber or squash fruits in most cases. The highest significant increase of DS was recorded when inocula concentration increased from 6000 to 7000 spores/ml of *B. cinerea* on cucumber and squash fruits and *F. solani* on cucumber fruits. In case of *F. solani* on squash fruits, the highest significant difference in DS occurred when inocula concentration increased from 5000 to 6000 spores/ml. These results emphasized that DS was affected significantly by the interactions between pathogens and inocula concentration on either cucumber or squash fruits. These results are in harmony with **Choi *et al.* (1990)** and

Maklad (2004). **Choi *et al.* (1990)** mentioned that disease severity of grey mould on cucumber caused by *B. cinerea* was increased by increasing of spore concentration of the inocula where reached 70% of infected leaf area with $5x10^6$ spores/ml. They also mentioned that spore age had no effect on disease severity.

The responses of different cucumber cultivars to the tested fruit rot causal fungi were significantly varied. Cucumber fruits cv. Heikal seemed to be the most resistant where it recorded the lowest DS followed by cvs. Delta Star, Shams, New Star, Fysal, Sinai I and Samar. The lowest DS was recorded on cucumber fruits of Heikal cultivar either infected with *B. cinerea* or *F. solani* whereas fruits of cucumber cv. Samar recorded the highest significant DS for both pathogens. The variations in responses of different cucumber cultivars against infection with fruit rot pathogenic fungi were reported also by **Dautel (1978)**, **McCall and Willumsen (1991)** and **Maklad (2004)**. The infection with *B. cinerea* on cucumber fruits was significantly higher than that caused by *F. solani* meanwhile, *B. cinerea* or *F. solani* infection was significantly equal on squash fruits. Also, DS of both tested pathogens increased as plant age increased and it was significantly higher on the wounded than the unwounded cucumber or squash fruits. Similarly, **Granke and Hausbeck (2010)** recorded that, wounding increases the susceptibility of cucumbers to the fruit rot disease caused by *P. capsici.*

Fruit age of the harvested cucumber or squash fruits might play an important role in the incidence of the fruit rot infections where, DS was gradually and significantly increased as fruit age increased. These findings suggested that, harvesting of cucumber or squash fruits every 2 or 3 days might be successful practical way to minimize incidence of the fruit rot diseases caused by *B. cinerea* or *F. solani.* These results are in disagreement with **Gevens *et al.* (2006)** who found that fruits ≥14 days postpollination old were usually symptom-free, and fruits ≤10 days postpollination old displayed both water-soaked lesions and pathogen sporulation. This resistance could also be defined using size as a surrogate for age. In fact, fruit age is an important factor for infection and sporangial production of *Phytophthora capsici* on cucumbers **(Gevens *et al.*, 2006)** and peppers **(Biles *et al.*, 1993)**.

Increasing storage period affected positively the fruit rot infection where DS was significantly higher on fruits stored for 8 days than those stored for 4 days. Also, DS was significantly increased as storage temperature increased. The development of DS occurred more slowly on the cucumber fruits stored at lower temperature regimes (2, 5 and 7°C) than those stored at room temperature (24°C). This was more conspicuous, particularly on cucumber fruits inoculated with *F. solani* where its infection completely stopped at 2, 5 and 7°C, respectively when stored for 4 and 8 days. This was the case also on cucumber fruits inoculated with *B. cinerea*, although DS was still occurred on the fruits stored

for 4 days even at the lowest storage temperature (2°C). These results could be expected becuase lower temperature slowed down and might be stopped the growth and vital activity of the fungi responsible for fruit rot infection.

The obtained results showed that DS was significantly affected by CO_2 concentration in the storage atmosphere. The lowest DS was recorded on cucumber fruits inoculated with *B. cinerea* or *F. solani* after 10 days at 10% CO_2 concentration. It is pronounced that the DS recorded by *B. cinerea* or *F. solani* after storage for 10 days at 10% CO_2 concentrations was increased over than 3.5 and 1.5 folds after storage for 21 days at the same CO_2 concentration. In this respect, **Hakan *et al.* (2005)** reported that storage of cucumbers to be processed to pickle could be possible for less than 10 days at 7°C temperature and 90–95% RH under normal atmosphere (NA). However, physical and chemical analyses showed that storage period of fresh pickling cucumbers could be prolonged up to 30 days under the same storage conditions, if suitable atmosphere combinations are created. Nevertheless, it was concluded that restricting the storage period of fresh pickling cucumbers to 20 days could give better results after processing to pickle.

Also, exposing fruits to UV-treatments at 280 and 320 nm resulted in significant decreases in DS but the recorded data proved that UV-exposure under conditions of the present study could not be

satisfactory practice for suppressing DS on the cucumber fruits. The lowest significant decrease in DS was recorded by exposing the inoculated cucumber fruits to UV/320 nm for two hours comparing with the untreated controls of both pathogens, respectively. Although ultraviolet light has a lethal effect on bacteria and fungi that are exposed to the direct rays, there is no evidence that it reduces decay of packaged fruits and vegetables **(Hardenburg *et al.*, 1986).** The low doses of ultraviolet light irradiation (254 nm UV-C) reduced postharvest brown rot of peaches **(Stevens *et al.*, 1998)**. In this case, the low dose ultraviolet light treatments had two effects on brown rot development; reduction in the inoculum of the pathogen and induced resistance in the host. However, it has not become a practical postharvest treatment as yet and requires more research **(http://www.ba.ars.usda.gov/hb66/022 pathology.pdf)**.

Concerning of using fungicides, results revealed that, all tested fungicides were effective in controlling the tested fruit rot pathogens *in vitro*. Among these tested fungicides, Ronilan was the best effective one against *B. cinerea* followed by Sumisclex and Rovral. Meanwhile, Ridomil, Teledor, Copral and Tecto were the least effective ones against *B. cinerea*. On the other hand, Rovral, Sumisclex, Ronilan, and Tecto were the best effective fungicides against *F. solani* respectively while Copral, Teledor and Ridomil were the least effective ones. Also, increasing the concentration of the tested fungicides increased gradually their effects against the pathogens *in vitro*. *In vivo* studies, results confirmed that treating

cucumber or squash fruits with either of the tested fungicides affected significantly DS caused by *B. cinerea* and *F. solani*. In this respect, Copral and Teledor were the highly effective fungicides in controlling the fruit rot disease caused by *B. cinerea* on cucumber fruits followed by Ridomil and Rovral which were moderately effective ones. Meanwhile, Sumisclex, Tecto and Ronilan were the least effective ones in this respect. On the other hand, Copral, Ridomil and Teledor were the best effective fungicides in controlling *F. solani* infections on cucumber fruits while, Sumisclex, Tecto and Ronilan were the least effective ones.

Among all tested fungicides on squash fruits, Copral and Teledor were the best fungicides in decreasing DS caused by *B. cineria* and *F. solani*. Meanwhile, Ridomil was the least effective fungicide followed by Sumisclex and Ronilan against *B. cinerea*. Meanwhile, Ronilan, Rovral and Sumisclex were the least effective fungicides against *F. solani* fruit rot infection on squash. It is clear from the obtained results that increasing the concentration of any fungicide decreased gradually the recorded DS on infected cucumber or squash fruits. In this respect, the least DS were recorded at 750 ppm followed by 500 ppm with all tested fungicides. These obtained results are logic and in harmony with those obtained by **Park and Yu (1998), Markoglou *et al.* (2006), Shi *et al.* (2007)** and **Nasreen and Ghaffar (2010)** who studied the effect of fungicides, microbial antagonists and oilcakes in the control of *F. solani* the cause of seed rot, seedling and root

infection on bottle gourd, bitter gourd and cucumber *in vitro* and *in vivo*. They observed complete inhibition of colony growth of *F. solani* when fungicides *viz*., Aliette, Benlate and Carbendazim at 100 ppm were used. Carbendazim completely eradicated seed borne infection of *F. solani* in bitter gourd and gave maximum reduction in cucumber and bottle gourd. Also, **Takagaki *et al.* (2010)** reported that Pyribencarb methyl {2-chloro-5-[(E)-1-(6-methyl-2-pyridyl-methoxyimino) ethyl] benzyl} carbamate, is a novel fungicide having excellent activity against a wide range of plant pathogenic fungi, especially grey mold diseases caused by *B. cinerea*. Pyribencarb exhibited not only a preventive effect but also a curative effect. When spraying was performed 48 hr after inoculation (after visible symptoms appeared), pyribencarb also showed strong inhibitory activity against lesion development by cucumber grey mold that was significantly superior to its preventive activity.

Spraying cucumber and squash plants with some resistance inducers reduced significantly the fruit rot infections after harvesting where the determined DS of cucumber fruit rot caused by *B. cinerea* or *F. solani* was significantly decreased by all tested resistance inducer treatments comparing to their untreated controls. The lowest DS caused by *B. cineria* was recorded by KH_2PO_4 (at 500 and 1000ppm), $CaCl_2$ (at 1000ppm) and SA, OA (1000ppm) followed by Bion, respectively comparing with the untreated control. Meanwhile, OA and KH_2PO_4 (at 250, 500 & 1000 ppm),

SA at 500 and 1000 ppm were the best for controlling cucumber fruit rot caused by *F. solani*. Disease severity was successively and significantly decreased as concentrations of the tested chemical inducers were increased. In case of squash fruits, the lowest significant DS caused by *B. cineria* was recorded by SA followed by KH_2PO_4 and OA at 1000 ppm comparing with the untreated control. However, OA, KH_2PO_4 and SA were the most effective for decreasing squash fruit rot caused by *F. solani*. Similar results were obtained by **Saber *et al.* (2003)** who stated that, the tested salts and antioxidants generally, depressed strawberry fruit rots. Salicylic acid and ascorbic acid gave the best effects as they decreased the incidence and severity of fruit rots and increased fruit yield. The least effective one was mannitol. Oxalic acid was moderately effective in this respect.

After resistance induction due to spraying cucumber and squash plants with tested resistance inducers (Bion, SA, OA, KH2PO4 and CaCl2), changes in protein patterns in leaves were detected by using SDS-PAGE technique. In general, the highest number of the new protein bands was detected in cucumber or squash leaves treated with Bion or salicylic acid (SA). The newly differentiated protein bands may be act as inhibitors against fungal infection. In this regard, **Morrissey and Osbourn (1999)** recorded that, many plants produce low-molecular-weight compounds which inhibit the growth of phytopathogenic fungi in vitro. These compounds may be preformed inhibitors that are present constitutively in healthy plants (also known

as phytoanticipins), or they may be synthesized in response to pathogen attack (phytoalexins). **Rauscher *et al.* (1999)** reported that, treatment of broad bean leaves with salicylic acid (SA) or 2, 6-dichloro-isonicotinic acid (DCINA) induces resistance against the rust fungus *Uromyces fabae*. Heterologous antibodies were used to study changes in the extracellular pathogenesis-related (PR) protein pattern after resistance induction. Western blots indicated that chitinases and β-1,3-glucanases were present in both induced and control plants. In contrast, PR-1 proteins were newly synthesized in response to SA or DCINA application. The major induced PR-1 protein was purified and exhibited strong differentiation-inhibiting activity towards *U. fabae* infection structures. We conclude that the inhibition of rust infection hyphae in acquired resistant broad bean plants is mainly due to the anti-fungal activity of this induced basic PR-1 protein.

Spraying plants with the different tested resistance inducers considerably affected amounts of different amino acids detected in the cucumber leaves and thus might transferred in to the harvested fruits. Some amino acids increased by tested treatments while others were decreased. The rate of increase depended on the tested treatment and type of amino acids. In cucumber leaves, SA treatment increased only Glu while Bion treatment increased Asp, Thr, Pro, Ile, His, and Arg. The OA treatment increased Ser, Pro, Valin, Leu, Phe, and His. The $CaCl_2$ treatment increased Asp, Gly, Ala, Ile, Phe, His and Arg whereas, KH_2PO_4 treatment increased

Asp, Thr, Ser, Glu, Leu and His. In squash leaves, the rate of increase in amino acids depended on the tested treatment and type of amino acids. Bion treatment increased only Arg. However SA treatment increased Asp, Glu, His and Arg. With OA treatment, the amino acids *i.e.*, Asp, Glu, Gly, Tyr, Phe and Lys were increased compared with the untreated control. Similarly, $CaCl_2$ treatment increased Asp, Gly, Ala, Ile, Phe, His and Arg. Potassium phosphate (KH_2PO_4) increased Arg only whereas it decreased Glu, Pro, Gly, Ala, Val, Ile, Leu and Tyr.

In fact, some amino acids and their combinations in plants might play an important role in plant defenses and induce resistance against certain plant pathogens. RPS2 is a resistance gene of *Arabidopsis thaliana* that confers resistance against *Pseudomonas syringae* bacteria that express a virulence gene avrRpt2. The derived amino acid sequence of RPS2 contains leucine-rich repeat, membrane-spanning, leucine zipper, and P loop domains. The function of the RPS2 gene product in defense signal transduction is postulated to involve nucleotide triphosphate binding and protein-protein interactions and may also involve the reception of an elicitor produced by the avirulent pathogen **(Bent *et al.*, 1994)**. Also, *Pto* gene encodes a serine/threonine kinase was found to be confers resistance to bacterial speck disease (*P. syringae* pv. *tabaci*) in tomato **(Zhou *et al.* 1995)**. Proline (Pro) accumulation is a common physiological response in many plants in response to a wide range of biotic and abiotic stresses

(Verbruggen and Hermans, 2008). Raj *et al.* (2009) evaluated the amino acid proline for its efficiency to elicit resistance in pearl millet (*Pennisetum glaucum*) against downy mildew disease caused by *Sclerospora graminicola* both under greenhouse and field conditions. Apart from disease protection, proline was also found effective in enhancing vegetative and reproductive growth of the plants, as evidenced by the increase in height, fresh weight, leaf area, tillering capacity, 1000-seed weight and grain yield in comparison with the control plants.

L-Arginine is the precursor of nitric oxide (NO). In order to examine the influence of L-arginine on tomato fruit resistance **Zheng *et al.* (2011)** treated the preharvest green mature tomato fruits (*Solanum lycopersicum* cv. No. 4 Zhongshu) with 0.5, 1, and 5 mM L-arginine. The authors found that induced resistance increased and reached the highest level at 3–6 days after treatment. Endogenous NO concentrations were positively correlated with PAL, PPO, CHI, and GLU activities after treatment with Pearson coefficients of 0.71, 0.94, 0.97 and 0.87, respectively. These results indicate that arginine induces disease resistance via its effects on NO biosynthesis and defensive enzyme activity.

Pre-spraying the grown cucumber and squash plants under greenhouse conditions several times with 10 or 20% concentrations of different plant extracts (carnation, garlic, ginger, henna, marjoram, nigella, thyme extracts in addition to kombucha preparation) gave promising results in controlling the fruit rot

diseases caused by *B. cinerea* or *F. solani* under lab conditions on harvested and inoculated fruits. Disease severity was significantly lower at 20% than at 10% concentrations. Marjoram extract was the best among all tested extracts in controlling *B. cinerea*-infection whereas henna, marjoram and nigella were the most effective for controlling *F. solani*-infection in cucumber fruits. As for squash fruit rot, the henna, garlic, carnation and ginger extracts were the most effective for decreasing DS caused by *B. cinerea*-infection while, garlic, henna, ginger, nigella carnation and thyme extracts were the most effective against *F. solani*-infection. These results could be interpreting in light the findings of **Bautista-Baños *et al.* (2002)** who tested the aqueous extracts of leaves and stems of *Achras sapota*, *Annona reticulata*, *Bromelia hemisphaerica*, *Carica papaya*, *Citrus limon*, *Chrysophylum cainito*, *Dyospiros ebenaster*, *Mangifera indica*, *Persea americana*, *Pouteria sapota*, *Spondias purpurea*, and *Tamarindus indicus* from the state of Morelos, Mexico for their antifungal activity against *Colletotrichum gloeosporioides in vitro* and *in vivo* experiments. They reported that leaf extracts from *C. limon* and *P. americana* totally inhibited growth of *C. gloeosporioides in vitro*. Leaf extracts of *C. papaya* completely inhibited postharvest rots of papaya, while leaf and stem extracts of *D. ebenaster* had an adequate fungicidal effect when applied to mango. These results are in harmony with **Abou-Jawdah *et al.* (2004)** who reported that, the wild marjoram (*Origanum syriacum*) extract showed the

highest and widest range of activity against eight phytopathogenic fungi. It resulted in complete inhibition of mycelial growth of six of eight fungi tested and also gave nearly complete inhibition of spore germination of the six fungi included *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. *melonis*, and *Verticillium dahlia*. Also, **Aba AlKhail (2005)** revealed that, *Allium sativum, Carum carvi* and *Eugenia caryophyllus* were the most effective plant extracts having antifungal activity against *Fusarium oxysporum* f.sp. *lycopersici, Botrytis cinerea* and *Rhizoctonia solani*. Also, **Sipailiene *et al.* (2006)** reported that, thyme and marjoram oils have antimicrobial activity against the growth of food poisoning and spoilage organisms. Similar findings also were obtained by **El-Mougy and Abdel-Kader (2007)** who observed high significant inhibitory effect on radial fungal growth of various soil-borne fungi causing damping-off disease of faba bean for different concentrations of carnation (*Dianthus caryophyllus*), cinnamon (*Cinnamomum burmannil*), garlic (*Allium sativum*) and thyme (*Thymus vulgaris*). Meanwhile, fennel (*Foeniculum vulgare*), marjoram (*Origanum majorana*) and chamomile (*Matricaria hamomilla*) showed a low inhibitory effect on the tested fungi. Kombucha which known as metabolic of different microorganisms such as yeasts, fungi and bacteria. **Hasan (2011)** recorded that the kombucha filtrate was better than mentha oil, clove oil, ascorbic acid, salicylic acid for inhibition of *Xanthomonas campistris* particularly isolates 2 and 3.

Pre-spraying cucumber and squash plants with some plant extracts or kombusha tea greatly increased the activities of polyphenoloxidase (PPO) enzyme in the harvested fruits at both tested concentrations comparing to control treatment (un-treated). On the other hand, the determined activities and their efficiency% were higher at 20% concentration than at 10% concentration for both tested fruit rot pathogens (*Botrytis cinerea* and *Fusarium solani*). Also, the highest enzyme activity and efficiency% were recorded with nigella extract at 10 and 20% concentrations in case of *Botrytis cinerea*. Meanwhile, the highest activity and efficiency of PPO enzyme were recorded with carnation extract followed by marjoram, thyme and garlic extracts respectively at 20% concentration in case of *Fusarium solani* while the highest recorded activity of PPO enzyme at 10% concentration of the tested plant extracts was recorded with marjoram and nigella extracts. Also, PPO activities were high in fruits of sprayed squash plants with all tested extracts and kombusha at 10 and 20% concentrations comparing to control treatment (untreated with both tested fruit rot pathogens (*B. cinerea* and *F. solani*).

The highest activity of PPO enzyme was recorded with nigella extract at 10% concentration and with kombusha followed by thyme, henna and carnation extracts respectively at 20% concentration in case of *B. cinerea* infections. On the other hand, the highest PPO activity in case of *F. solani* infections was recorded with marjoram followed by henna and carnation extracts

at 10% concentration. Meanwhile, the highest PPO activities at 20% concentration were recorded with carnation followed by nigella and garlic extracts respectively. Also, PRO activities were higher in fruits of pre-sprayed cucumber plants with 10 and 20% concentrations of all tested plant extracts and kombusha with either of the fruit rot pathogens (*Botrytis cinerea* or *Fusarium solani*). In case of *B. cinerea* infection, the highest PRO activity was recorded with henna extract followed by garlic extract at 10% while, the highest PRO activity at 20% concentration of the tested plant extracts was recorded with henna followed by ginger and kombusha tea respectively.

The lowest PRO activity was recorded with thyme followed by marjoram and ginger extracts at 10% while, it was recorded with carnation followed by garlic and thyme extracts, respectively at 20% concentration. In case of *F. solani* infection, the highest PRO activity at 10% concentration was recorded with henna and marjoram respectively, while it recorded with henna and kombusha treatments followed by marjoram extracts respectively at 20% concentration.

Also, PRO activities were higher in fruits of pre-sprayed squash plants with 10 and 20% of the different tested extracts than control treatment (un-treated) with either of tested pathogens. In case of *B. cinerea* infection, the highest PRO activity was recorded with henna followed by garlic extracts at 10% concentration. While, the lowest PRO activity was recorded with ginger extract at

the same tested concentration. Whereas, the highest PRO activities were recorded with henna and kombusha tea, respectively at 20% concentration.

In case of *F. solani* infection, the lowest PRO activities were recorded with kombusha and thyme treatments respectively at 10% concentration. Meanwhile, the highest PRO activity at the same tested concentration was recorded with nigella, carnation, garlic, henna and ginger, respectively. At 20% concentration, the highest PRO activity was recorded with nigella extract followed by henna. It is clear also that increasing the concentration of the tested extracts increased gradually the efficiency% of the determined PRO enzyme. The obtained results are in harmony with those obtained by **Turner (1965)** who, showed that secretion of polyphenoloxidase enzyme was considered an indicator of resistance in a number of plants to fungal pathogens. Also, **Yurina *et al.* (1993)** reported that peroxidase activity was related to resistant and to tolerance against powdery and downy mildew of cucurbits. While, **Abd El-Megeed and Khafagi (1998)** reported that some biochemical related resistance such as plasma membrane, lipid peroxidation and accumulation of phenolic compounds were increased with enhancing resistance when used some plant extracts *i.e.* guava, lemon, scented and white mulberry, as seed treatment to control root rot and wilt diseases of watermelon cv. Giza-1 under greenhouse and field conditions.

On the other hand, **Shang *et al.* (2007)** showed that chitosan decreased disease grey mould of *Botrytis cinerea* index of cucumber seedlings by 39.4%, increased phenylalanine ammonia lyase (PAL), peroxidase (PO) and polyphenoloxidase (PPO) activities, enhanced total polyphenol, flavonoid and lignin content. Chitosan played an important role in inducing disease resistance of cucumber seedlings with the optimum concentration at 200 mg. Also, the obtained results could be interpret in light the findings of **Galeotti *et al.* (2008)** who isolated flavonoid glycoside, kaempferol 3-*O*-β-D-glucopyranosyl (1→2)-*O*-β-D-glucopyranosyl (1→2)-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (1), along with two known C- and *O*-flavonoid glycosides (2 and 3, respectively), were isolated from carnation (*Dianthus caryophyllus*). The isolated compounds and other flavonoid glycoside analogues exhibited antifungal activity against different *Fusarium oxysporum* f.sp. *dianthi* pathotypes.

Cucumber and squash fruits harvested from plants pre-sprayed with most of the tested plant extracts or kombusha tea preparations showed obvious decrease in activity of the pectolytic enzymes (pectin methyl esterase "PME" and polygalacturonase "PG") when these fruits were inoculated with *B. cinerea* or *F. solani* comparing to the inoculated fruits harvested from the unsprayed plants. The reduction in activity of the enzymes was high in harvested fruits from pre-sprayed plants with 20% than 10% concentration of any tested preparation. Ginger and garlic extracts were the most effective for reducing activity of PME and PG enzymes, respectively in cucumber or squash fruits inoculated

with either *B. cinerea* or *F. solani* compared with the untreated control. These obtained results could be interpreting in light the findings of **Rha *et al.* (2001), El-Habbaa (2003), Veronesi *et al.* (2009)** and **Ahmed (2010)** who, found that *B. cinerea* was more active in producing PG enzyme than *S. sclerotiorum*. Also, *B. cinerea* was less active in producing Cx than *S. sclerotiorum* at 15 days of incubation *in vivo*.

Most of the tested resistance inducers and fungicides increased quantities of total and free phenols in treated and infected fruits with *B. cinerea* and *F. solani* compared with control treatment. Also, all tested treatments reduced the conjugated phenols where the quantities of conjugated phenols were always lesser than the free phenols. Also, Catechol followed by Bion and Ethephon were the best effective treatments in increasing the quantities of total and free phenols in treated and infected fruits with *B. cinerea*. Meanwhile, Teledor, Copral and Catechol were the best effective treatments in increasing the quantities of total and free phenols in cucumber fruits treated and infected with *F. solani*. The least quantity of conjugated phenols was recorded with Ethephon treatment in case of *F. solani* infection. Also, pre-spraying squash plants with the tested resistance inducers and fungicides affected greatly the content of phenols in their harvested fruits treated with either of tested rot pathogens. All quantities of free phenols were more than conjugated phenols in all infected tissues of harvested fruits, which inoculated with *B. cinerea* and/or

F. solani. The highest quantity of free phenols was recorded with Catechol treatment while the highest quantity of total phenols was recorded with Copral fungicide treatment in case of *B. cinerea* infection. In case of *F. solani* infection, the highest quantities of free phenols were recorded with Ethephon and Teledor treatments. These results could be interpreting in light the findings of **Turner (1965), Yurina *et al.* (1993), Abd El-Megeed and Khafagi (1998), Tripathi and Dubey (2004), El-Mougy and Abdel-Kader (2007), Shang *et al.* (2007)** and **Faccoli and Schlyter (2007)** who found that phenols are important in conifer resistance to fungi associated with bark beetles and as markers for resistance to beetle mass-attacks. Also, **Hassan *et al.* (2007)** revaled that, citric and benzoic acids were the most effective ones, since they recorded the lowest percentages of disease severity of *Botrytis fabae* and/or *B. cinerea* on faba bean plants and the highest levels of peroxidase activities. Moreover, pretreated faba bean plants showed some new isozymes and increment in the density of original isozymes, especially in infected plants.

Pre-spraying cucumber and squash plants with some resistance inducers and fungicides affected the total amino acids content of harvested fruits, which inoculated with *B. cinerea* and *F. solani*. Teledor and Catechol were the best effective treatments in increasing the total amino acids in inoculated cucumber fruits with *F. solani*. While, Calcium chloride was the best treatment in case of inoculated squash fruits with *F. solani*. On the other hand,

Catechol followed by Teledor, Copral and Ethephon treatments were the best effective treatments in increasing the total amino acids content in inoculated cucumber fruits with *B. cinerea.* Also, Teledor and Catechol were the best effective treatments in increasing the total amino acids content in squash fruits inoculated with *B. cinerea*. Bion and Copral treatments were not effective in increasing the total content of amino acids in inoculated cucumber fruits with *F. solani* where their quantities were lesser than in control treatment. Meanwhile, Bion, Copral and Teledor treatments were lesser effective on squash fruits in this respect. In case of *B. cinerea* infection, Bion and Calcium chloride were the least effective treatments on the total amino acids content in inoculated cucumber fruits with *B. cinerea*. While, Bion, Ethephon and Calcium chloride were the least effective treatments on the total amino acids contents in inoculated squash fruits with *B. cinerea*. These obtained results could be interpreting in light that phytoalexins are antimicrobial, low-molecular-weight secondary metabolites that are both synthesized by and accumulated in plant cells as a result of the interaction between the metabolic systems of the host and a fungal parasite, and that require *de novo* expression of the enzymes involved in their biosynthetic pathway **(Müller, 1956** and **Paxton, 1980)**. Also, the genes encoding L-phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and chalcone reductase (CHR) were the most rapidly activated, within 10-20 min after the elicitation of the isoflavonoids

biosynthetic pathway **(Dixon *et al.*, 1995** and **Salles *et al.*, 2002)**. Phytoalexins are substances not detectable before infection, and are considered to inhibit the further development of most attacking pathogens of the species under consideration. In this respect, **Zimmerli *et al.* (2001)** found that the non-protein amino acid β-aminobutyric acid (BABA) protects numerous plants against various pathogens. Protection of Arabidopsis plants against virulent pathogens involves the potentiation of pathogen-specific defense responses. BABA-treated Arabidopsis were found to be less sensitive to two different strains of the gray mold fungus *B. cinerea*. Treatments with benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester, a functional analog of salicylic acid (SA), also markedly reduced the level of infection. Also, **Wayne and Benny (2010)** reported that separation and quantitation of amino acids derivatives is reliable tool to examine the inter play of genetic potential and growing conditions on the levels of physiologic amino acid pools in cucurbits.

SUMMARY

SUMMARY

Our study was designed to combat rot the fruits of cucurbits. Surveying the natural disease incidence of the most cucumber and squash fruit rots occurrence in many locations of seven governorates (*i.e.* Beheira, Kafr El-Shikh, Qalubiya, Ismailia, Sharkiya, Dakahliya and Giza) was performed. All laboratory, greenhouse and field experiments were carried out during autumn and spring of 2003 and 2004 seasons.

The obtained results could be summariezed as following:

1- Surveying of cucumber and squash fruit diseases under greenhouse conditions indicated that, the fruit rot disease severity (DS) was obviously higher in 2003 than 2004 seasons with great variation between seasons and tested locations.

2- Isolation trials showed that 152 and 81 fungal isolates were isolated from rotted fruits of cucumber and squash respectively. These fungal isolates included *Alternaria* spp., *B. cinerea, F. solani, Mucor* spp., *Penicillium* spp., *Pythium* spp. and *Sclerotinia sclerotiorum.*

3- On cucumber fruits, the highest DS was incited by *B. cinerea* isolated from Ismaelia and Beheira and *F. solani* isolated from Beheira, Kafr El-Sheikh and Ismaelia whereas, *B. cinerea* isolated from Sharkia, Ismaelia and *F. solani* isolated from Ismaelia recorded the highest DS on the squash fruits.

4- Disease severity on cucumber or squash fruits were increased with increasing inocula concentrations of the tested fruit rot pathogens from 1000 to 7000 spores/ml. The highest significant increase of DS was recorded when inocula concentration increased from 6000 to 7000 spores/ml (*B. cinerea* on cucumber and squash fruits) and *F. solani* (on cucumber fruits) mean while increasing inocula concentration of *F. solani* from 5000 to 6000 spores/ml caused the highest significant increase in DS comparing with any other rots.

5- Fruits of cucumber Heikal cultivar seemed to be the most resistant against infection with *B. cinerea* and *F. solani* followed by Delta Star, Shams, New Star, Fysal, Sinai I and Samar cultivars.

6- Disease severity caused by *B. cinerea* on cucumber fruits was significantly higher than DS caused by *F. solani* meanwhile, DS caused by both pathogens was significantly equal on squash fruits. Also, DS of both tested pathogens were increased as plant age and fruit age increased and it was significantly higher on the wounded than the unwounded cucumber or squash fruits.

7- Disease severity was significantly higher on fruits stored for 8 than those stored for 4 days. It was noticed that DS developed more slowly on cucumber fruits stored at lower temperature regimes (2, 5 and 7°C) than those stored at room temperature

(24°C). At 24°C, DS caused by *B. cinerea* developed more quickly than that caused by *F. solani*.

8- Disease severity was significantly affected by CO_2 concentrations and storage period. The lowest DS on cucumber fruits inoculated with *B. cinerea* or *F. solani* was recorded after 10 days of storage under conditions containing 10% CO_2 concentration.

9- Exposing fruits to the UV-treatments at 280 and 320 nm resulted in significant decreases in DS. Applying the UV-exposure under conditions of the present study could not be satisfactory practice for suppressing DS on the cucumber fruits.

10- All tested fungicides were effective in suppressing the growth of the tested fruit rot pathogens *in vitro*. Ronilan fungicide was the most effective one where it completely inhibited the growth of *B. cinerea* followed by Sumisclex fungicide. However, the fungicides Rovral, Sumisclex, Ronilan, and Tecto were the most effective ones respectively for inhibiting growth of *F. solani*. Inhibition of fungal growth (both tested pathoges) increased as the concentration of the tested fungicides increased.

11- Spraying cucumber or squash plants with any tested fungicide significantly decreased the fruit rot DS caused by *B. cinerea* or *F. solani*. The fungicides Copral and Teledor were the highly effective in controlling DS caused by *B. cinerea*

whereas, Copral, Ridomil and Teledor were the best effective fungicides in controlling DS caused by *F. solani* on cucumber fruits. Also, Copral and Teledor were the best fungicides in decreasing DS caused by *B. cinerea* and *F. solani* on squah fruits. Increasing the fungicdal concentration decreased gradually the recorded DS on infected cucumber or squash fruits.

12- Spraying cucumber and squash plants with some resistance inducers significantly reduced the fruit rot DS on the harvested cucumber and squash fruits. On cucumber fruits, the lowest DS caused by *B. cineria* was recorded on fruits harvested from plants previously sprayed with KH_2PO_4, $CaCl_2$ and Na_2SO_4, SA, OA Meanwhile, OA, KH_2PO_4 and SA were the best for controlling cucumber fruit rot DS caused by *F. solani*. In case of squash fruits, the lowest significant DS caused by *B. cineria* was recorded by SA followed by KH_2PO_4, OA, $LiSO_4$, and Na_2SO_4 comparing with the untreated control. Also, the least DS of *F. solani* infections on squash fruits was recorded with OA followed by KH_2PO_4.

13- Spraying of cucumber and squash plants with some resistance inducers (Bion, SA, OA, KH_2PO_4 and $CaCl_2$) induced changes in the electrophoresis of the soluble proteins in plant leaves. The number of protein bands was higher in leaves of treated plants than the untreated control. Comparing to the untreated cucumber leaves (control), 27 new protein

bands with MW ranged between 51 and 39 kDa were appeared in leaves of treated cucumber plants. Similarly, 22 new bands with MW ranged between 55 and 47 kDa were detected in squash leaves. In general, the highest number of the new protein bands was detected in cucumber or squash leaves treated with Bion or salicylic acid (SA).

14- Spraying cucumber and squash plants with the most tested resistance inducers and fungicides resulted in conspicuous increases in the quantities of total and free phenols in fruits which inoculated with *B. cinerea* and *F. solani* comparing to fruits harvested from untreated plants (control treatment). In case of cucumber fruits, all tested treatments reduced the conjugated phenols while, Catechol, Bion and Ethephon (*B. cinerea*), Teledor, Copral and Catechol (*F. solani*) recorded the highest increases in quantities of total and free phenols in treated and infected cucumber fruits comparing with the untreated control. Similar trend was recorded concerning squash fruits. Spraying squash plants with Catechol recorded the highest quantity of free phenols while the highest quantity of total phenols was recorded with Copral fungicide treatment in case of *B. cinerea* infection. In case of *F. solani* infection, the highest quantities of free phenols were recorded with Ethephon and Teledor treatments.

15- Spraying cucumber and squash plants with the tested resistance inducers and fungicides affected the total amino

acids content of harvested fruits, which inoculated with *B. cinerea* and/or *F. solani*. The highest increase in total amino acids in cucumber fruits harvested from plants sprayed with Teledor and Catechol and squash fruits harvested from plants sprayed with Calcium chloride (*F. solani*). On the other hand, spraying cucumber plants with Catechol, Teledor, Copral or Ethephon recorded the highest amounts of total amino acids in cucumber fruits inoculated with *B. cinerea* whereas, Teledor and Catechol were the best effective treatments in increasing total amino acids content in squash fruits inoculated with *B. cinerea*.

16- Spraying cucumber and squash plants with plant extracts or kombucha tea preparation significantly decreased DS of fruit rots caused by *B. cinerea* or *F. solani*. On cucumber fruits, marjoram extract was the most effective as it completely suppressed DS caused by *B. cinerea* followed by kombucha tea, ginger and garlic, henna and nigella. However, nigella, henna, marjoram and ginger preparations were the most effective where they decreased DS caused by *F. solani*, followed by thyme, kombusha tea, garlic and carnation, respectively comparing with the untreated control. As for squash fruits, henna, garlic, carnation and ginger preparations were the most effective for decreasing DS caused by *B. cinerea* followed by nigella, kombusha tea and marjoram whereas, the preparations of garlic, henna, ginger, nigella,

carnation and thyme were the most effective against fruit rot caused by *F. solani*.

17- Using any of the tested plant extracts or kombusha tea greatly decreased the activity of PME enzyme in cucumber fruits inoculated with *B. cinerea* or *F. solani* comparing to the untreated control. In cucumber fruits inoculated with *B. cinerea*, ginger extract was the most effective for reducing PME activity followed by marjoram, nigella, garlic, kombucha, carnation, henna and thyme, respectively whereas, ginger extract still the most effective for decreasing PME activity in fruits inoculated with *F. solani*, followed by nigella, marjoram, kombucha, garlic, thyme, carnation and henna, respectively. In squash fruits inoculated with *B. cinerea*, kombucha tea and nigella were the most effective for reducing PME activity followed by marjoram, ginger, garlic, carnation, henna and thyme particularly comparing to the untreated control. However, in fruits inoculated with *F. solani*, nigella and thyme extracts were the most effective and completely suppressed PME activity followed by garlic, kombucha, ginger, marjoram, henna and carnation, respectively.

18- Spraying cucumber and squash plants with any of the tested preparation (plant extracts or kombusha) decreased activity of the polygalacturonase (PG) enzyme in their fruits inoculated with *B. cinerea* or *F. solani* comparing to the inoculated fruits harvested from the untreated plants. In general, garlic extract

caused the highest reduction in activity of PG followed by kombucha, marjoram, nigella, carnation, henna, thyme and ginger extracts, respectively in fruits inoculated with *B. cinerea*. However, thyme extract caused the highest decrease in PG activity in inoculated fruits with *F. solani* followed by nigella extract, kombucha, henna, marjoram, ginger, garlic and carnation, respectively.

19- The activity of polyphenoloxidase (PPO) enzyme was higher in cucumber and squash fruits harvested fron plants previously sprayed with any of the tested plant extracts or kombusha tea after inoculation with *B. cinerea* or *F. solani* comparing to inoculated fruits harvested from control treatment (untreated). In fruits of cucumber, the highest PPO enzyme activity was recorded with pre-harvest treatment with nigella extract (*B. cinerea*) and marjoram and nigella extracts (*F. solani*). As for squash fruits, the highest activity of PPO enzyme was recorded in fruits harvested from plants formerly sprayed with with nigella extract and kombusha followed by thyme, henna and carnation extracts respectively (*B. cinerea*) and marjoram, henna and carnation extracts (*F. solani*).

20- As for the activity of peroxidase (PRO) enzyme in cucumber fruits under stress of infection with the tested fruit rot pathogens, the highest PRO activity was recorded in fruits harvested from cucumber plants previously sprayed with henna followed by ginger and kombusha tea respectively (*B. cinerea*)

and henna, kombusha and marjoram extract, respectively (*F. solani*). In squash fruits inoculated with *B. cinerea*, the activity of PRO enzyme was higher in fruits harvested from plants that were pre-sprayed with henna and kombusha tea, respectively. In case of squash fruits inoculated with *F. solani*, the highest PRO activity was recorded in fruits harvested from plants treated with nigella extract followed by henna.

SUMMARY

Our study was designed to combat rot the fruits of cucurbits. Surveying the natural disease incidence of the most cucumber and squash fruit rots occurrence in many locations of seven governorates (*i.e.* Beheira, Kafr El-Shikh, Qalubiya, Ismailia, Sharkiya, Dakahliya and Giza) was performed. All laboratory, greenhouse and field experiments were carried out during autumn and spring of 2003 and 2004 seasons.

The obtained results could be summariezed as following:

1- Surveying of cucumber and squash fruit diseases under greenhouse conditions indicated that, the fruit rot disease severity (DS) was obviously higher in 2003 than 2004 seasons with great variation between seasons and tested locations.

2- Isolation trials showed that 152 and 81 fungal isolates were isolated from rotted fruits of cucumber and squash respectively. These fungal isolates included *Alternaria* spp., *B. cinerea, F. solani, Mucor* spp., *Penicillium* spp., *Pythium* spp. and *Sclerotinia sclerotiorum*.

3- On cucumber fruits, the highest DS was incited by *B. cinerea* isolated from Ismaelia and Beheira and *F. solani* isolated from Beheira, Kafr El-Sheikh and Ismaelia whereas, *B. cinerea* isolated from Sharkia, Ismaelia and *F. solani* isolated from Ismaelia recorded the highest DS on the squash fruits.

4- Disease severity on cucumber or squash fruits were increased with increasing inocula concentrations of the tested fruit rot pathogens from 1000 to 7000 spores/ml. The highest significant increase of DS was recorded when inocula concentration increased from 6000 to 7000 spores/ml (*B. cinerea* on cucumber and squash fruits) and *F. solani* (on cucumber fruits) mean while increasing inocula concentration of *F. solani* from 5000 to 6000 spores/ml caused the highest significant increase in DS comparing with any other rots.

5- Fruits of cucumber Heikal cultivar seemed to be the most resistant against infection with *B. cinerea* and *F. solani* followed by Delta Star, Shams, New Star, Fysal, Sinai I and Samar cultivars.

6- Disease severity caused by *B. cinerea* on cucumber fruits was significantly higher than DS caused by *F. solani* meanwhile, DS caused by both pathogens was significantly equal on squash fruits. Also, DS of both tested pathogens were increased as plant age and fruit age increased and it was significantly higher on the wounded than the unwounded cucumber or squash fruits.

7- Disease severity was significantly higher on fruits stored for 8 than those stored for 4 days. It was noticed that DS developed more slowly on cucumber fruits stored at lower temperature regimes (2, 5 and 7°C) than those stored at room temperature

(24°C). At 24°C, DS caused by *B. cinerea* developed more quickly than that caused by *F. solani*.

8- Disease severity was significantly affected by CO_2 concentrations and storage period. The lowest DS on cucumber fruits inoculated with *B. cinerea* or *F. solani* was recorded after 10 days of storage under conditions containing 10% CO_2 concentration.

9- Exposing fruits to the UV-treatments at 280 and 320 nm resulted in significant decreases in DS. Applying the UV-exposure under conditions of the present study could not be satisfactory practice for suppressing DS on the cucumber fruits.

10- All tested fungicides were effective in suppressing the growth of the tested fruit rot pathogens *in vitro*. Ronilan fungicide was the most effective one where it completely inhibited the growth of *B. cinerea* followed by Sumisclex fungicide. However, the fungicides Rovral, Sumisclex, Ronilan, and Tecto were the most effective ones respectively for inhibiting growth of *F. solani*. Inhibition of fungal growth (both tested pathoges) increased as the concentration of the tested fungicides increased.

11- Spraying cucumber or squash plants with any tested fungicide significantly decreased the fruit rot DS caused by *B. cinerea* or *F. solani*. The fungicides Copral and Teledor were the highly effective in controlling DS caused by *B. cinerea*

whereas, Copral, Ridomil and Teledor were the best effective fungicides in controlling DS caused by *F. solani* on cucumber fruits. Also, Copral and Teledor were the best fungicides in decreasing DS caused by *B. cinerea* and *F. solani* on squah fruits. Increasing the fungicdal concentration decreased gradually the recorded DS on infected cucumber or squash fruits.

12- Spraying cucumber and squash plants with some resistance inducers significantly reduced the fruit rot DS on the harvested cucumber and squash fruits. On cucumber fruits, the lowest DS caused by *B. cineria* was recorded on fruits harvested from plants previously sprayed with KH_2PO_4, $CaCl_2$ and Na_2SO_4, SA, OA Meanwhile, OA, KH_2PO_4 and SA were the best for controlling cucumber fruit rot DS caused by *F. solani*. In case of squash fruits, the lowest significant DS caused by *B. cineria* was recorded by SA followed by KH_2PO_4, OA, $LiSO_4$, and Na_2SO_4 comparing with the untreated control. Also, the least DS of *F. solani* infections on squash fruits was recorded with OA followed by KH_2PO_4.

13- Spraying of cucumber and squash plants with some resistance inducers (Bion, SA, OA, KH_2PO_4 and $CaCl_2$) induced changes in the electrophoresis of the soluble proteins in plant leaves. The number of protein bands was higher in leaves of treated plants than the untreated control. Comparing to the untreated cucumber leaves (control), 27 new protein

bands with MW ranged between 51 and 39 kDa were appeared in leaves of treated cucumber plants. Similarly, 22 new bands with MW ranged between 55 and 47 kDa were detected in squash leaves. In general, the highest number of the new protein bands was detected in cucumber or squash leaves treated with Bion or salicylic acid (SA).

14- Spraying cucumber and squash plants with the most tested resistance inducers and fungicides resulted in conspicuous increases in the quantities of total and free phenols in fruits which inoculated with *B. cinerea* and *F. solani* comparing to fruits harvested from untreated plants (control treatment). In case of cucumber fruits, all tested treatments reduced the conjugated phenols while, Catechol, Bion and Ethephon (*B. cinerea*), Teledor, Copral and Catechol (*F. solani*) recorded the highest increases in quantities of total and free phenols in treated and infected cucumber fruits comparing with the untreated control. Similar trend was recorded concerning squash fruits. Spraying squash plants with Catechol recorded the highest quantity of free phenols while the highest quantity of total phenols was recorded with Copral fungicide treatment in case of *B. cinerea* infection. In case of *F. solani* infection, the highest quantities of free phenols were recorded with Ethephon and Teledor treatments.

15- Spraying cucumber and squash plants with the tested resistance inducers and fungicides affected the total amino

acids content of harvested fruits, which inoculated with *B. cinerea* and/or *F. solani*. The highest increase in total amino acids in cucumber fruits harvested from plants sprayed with Teledor and Catechol and squash fruits harvested from plants sprayed with Calcium chloride (*F. solani*). On the other hand, spraying cucumber plants with Catechol, Teledor, Copral or Ethephon recorded the highest amounts of total amino acids in cucumber fruits inoculated with *B. cinerea* whereas, Teledor and Catechol were the best effective treatments in increasing total amino acids content in squash fruits inoculated with *B. cinerea*.

16- Spraying cucumber and squash plants with plant extracts or kombucha tea preparation significantly decreased DS of fruit rots caused by *B. cinerea* or *F. solani*. On cucumber fruits, marjoram extract was the most effective as it completely suppressed DS caused by *B. cinerea* followed by kombucha tea, ginger and garlic, henna and nigella. However, nigella, henna, marjoram and ginger preparations were the most effective where they decreased DS caused by *F. solani*, followed by thyme, kombusha tea, garlic and carnation, respectively comparing with the untreated control. As for squash fruits, henna, garlic, carnation and ginger preparations were the most effective for decreasing DS caused by *B. cinerea* followed by nigella, kombusha tea and marjoram whereas, the preparations of garlic, henna, ginger, nigella,

carnation and thyme were the most effective against fruit rot caused by *F. solani*.

17- Using any of the tested plant extracts or kombusha tea greatly decreased the activity of PME enzyme in cucumber fruits inoculated with *B. cinerea* or *F. solani* comparing to the untreated control. In cucumber fruits inoculated with *B. cinerea*, ginger extract was the most effective for reducing PME activity followed by marjoram, nigella, garlic, kombucha, carnation, henna and thyme, respectively whereas, ginger extract still the most effective for decreasing PME activity in fruits inoculated with *F. solani*, followed by nigella, marjoram, kombucha, garlic, thyme, carnation and henna, respectively. In squash fruits inoculated with *B. cinerea*, kombucha tea and nigella were the most effective for reducing PME activity followed by marjoram, ginger, garlic, carnation, henna and thyme particularly comparing to the untreated control. However, in fruits inoculated with *F. solani*, nigella and thyme extracts were the most effective and completely suppressed PME activity followed by garlic, kombucha, ginger, marjoram, henna and carnation, respectively.

18- Spraying cucumber and squash plants with any of the tested preparation (plant extracts or kombusha) decreased activity of the polygalacturonase (PG) enzyme in their fruits inoculated with *B. cinerea* or *F. solani* comparing to the inoculated fruits harvested from the untreated plants. In general, garlic extract

caused the highest reduction in activity of PG followed by kombucha, marjoram, nigella, carnation, henna, thyme and ginger extracts, respectively in fruits inoculated with *B. cinerea*. However, thyme extract caused the highest decrease in PG activity in inoculated fruits with *F. solani* followed by nigella extract, kombucha, henna, marjoram, ginger, garlic and carnation, respectively.

19- The activity of polyphenoloxidase (PPO) enzyme was higher in cucumber and squash fruits harvested fron plants previously sprayed with any of the tested plant extracts or kombusha tea after inoculation with *B. cinerea* or *F. solani* comparing to inoculated fruits harvested from control treatment (untreated). In fruits of cucumber, the highest PPO enzyme activity was recorded with pre-harvest treatment with nigella extract (*B. cinerea*) and marjoram and nigella extracts (*F. solani*). As for squash fruits, the highest activity of PPO enzyme was recorded in fruits harvested from plants formerly sprayed with with nigella extract and kombusha followed by thyme, henna and carnation extracts respectively (*B. cinerea*) and marjoram, henna and carnation extracts (*F. solani*).

20- As for the activity of peroxidase (PRO) enzyme in cucumber fruits under stress of infection with the tested fruit rot pathogens, the highest PRO activity was recorded in fruits harvested from cucumber plants previously sprayed with henna followed by ginger and kombusha tea respectively (*B. cinerea*)

and henna, kombusha and marjoram extract, respectively (*F. solani*). In squash fruits inoculated with *B. cinerea*, the activity of PRO enzyme was higher in fruits harvested from plants that were pre-sprayed with henna and kombusha tea, respectively. In case of squash fruits inoculated with *F. solani*, the highest PRO activity was recorded in fruits harvested from plants treated with nigella extract followed by henna.

REFERENCES

REFERENCES

Aba AlKhail, A.A. (2005): Antifungal activity of some extracts against some plant pathogenic fungi. Pakistan J. Biol. Sci., **8**(3): 413-417.

Abd El-Megeed, H.A. and Khafagi, Y.S. (1998): Effect of some plant extracts, bioproducts and fungicides on growth, productivity and resistance of root diseases on watermelon cv. Giza.1. Menofiya J. Agric. Res., **23**(3): 703-723.

Abd El-Moneim, M. (2001): Evaluations of some non chemical methods to control of some soil borne fungi and Foliage diseas of cucumber. Ph.D. Thesis, Fac. Agric., Zagazig Univ., p121.

Abd-El-Rehim, M.A.; Abou-Taleb, E.M.; Al-Menoufi, O.A.; Raffat, F.M.; Tohamy, A. (1987): The efficiency of seed treatment with calcium compounds in controlling damping-off disease of certain vegetable crops. Alexandria Journal of Agricultural Research, **32**(1): 333-344.

Abou-Jawdah, Y.; Rania Wardan; Hana Sobh; Abdu Salameh (2004): Antifungal activities of extracts from selected Lebanese wild plants against plant pathogenic fungi. Phytopathologia Mediterranea, **43**(3): 377-386.

Ahmed, F.A. (2010): Studies methods of controlling postharvest bean and pea diseases. M.Sc. Thesis, Fac. Agric., Benha Univ. 137 pp.

Akutsu, K.; Takatsu, Y.; Sakiyama, H. and Okuyama, S. (1987): Stimulative effect of potassium phosphate on infection of cucumber leaves by conidia of *Botrytis cinerea*. Ann. Phytopathol. Soc. Japan, **53**(2): 175-181.

Albornett, N.; Sanabria, Y.J. and De Albarracin, N.H. (1994): Diagnosis of fungal diseases in fruits of pawpaw (*Carica papaya*) and melon (*Cucumis melo*) for exportation. Revista de la Facultad de Agronomia, Universidad Central de Venezuela, **20**(1-2): 13-20.

Allam, A.I. and Hollis, J.P. (1972): Sulfide inhibition of oxidases in rice roots. Phytopathology, **62**(5): 634-639.

Armengol, J.; Jose, C.M.; Moya, M.J.; Sales, R.; Vicent, A. and Garcia-Jimenez, J. (2000): *F. solani* f. sp. *cucurbitae* race 1, a potential pathogen of grafted watermelon production in Spain. Bulletin OEPP, **30**(2): 179-183.

Arul, J. (1994): Emerging technologies for the control of postharvest diseases of fresh fruits and vegetables. In: Biological Control of Postharvest Diseases- Theory and Practice, Wilson, C.L. & Wisniewski, M.E., pp.1-10, CRC Press, Boca Raton, FL, USA, pp.182.

Assawah, M.W.; Al-Zarari, A.J. and Ahmed, K.A.J. (1984): Identification and study of fungi causing diseases and post-harvest rots of squash in Ninevah province, Iraq. Iraqi Journal of Agricultural Sciences "ZANCO", **2**(3): 67-75.

Barnett, H.L. and Hunter, B.B. (1998): Illustrated Genera of Imperfect Fungi, 4th ed., 218pp., APS Press, St. Paul, MN., USA.

Bautista-Baños, S.; Barrera-Necha, L.L.; Bravo-Luna, L. and Bermudez-Torres, K. (2002): Antifungal activity of leaf and stem extracts from various plant species on the incidence of *Colletotrichum gloeosporioides* of papaya and mango fruit after storage. Revista Mexicana de Fitopatologia **20**:8-12.

Bautista-Baños, S.; Barrera-Necha, L.L.; Ganesh, S.; Cheah, L. and Long, P.G. (2001): Inoculum variables affecting pathogenicity of *Botrytis cinerea* infection of kiwifruit. Revista Mexicana de Fitopatologia (Mexican Journal of Phytopathology), **19**(2): 161-167.

Bedlan, G. (1986): The most important fungal diseases of Cucumber. Pflanzenschutz, **9**: 8-11.

Bedlan, G. (1992): Cucumber diseases. Pflanzenschutz, (Wien), **2**: 4-7.

Bell, T.A.; Etchells, J.L. and Jones, I.D. (1955): A method for testing cucumber salt-stock brine for softening activity. USDA, ARS, 72- 75, 15 pp.

Bent, A.F.; Kunkel, B.N.; Dahlbeck, D.; Brown, K.L.; Schmidt, R.; Giraudat, J.; Leung, J. and Staskawicz, B.J. (1994): RPS2 of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. **Science, 265**: 1856-1860.

Biles, C.L.; Wall, M.M.; Waugh, M. and Palmer, H. (1993): Relationship of Phytophthora fruit rot to fruit maturation and cuticle thickness of New Mexican-type peppers. Phytopathology, **83**: 607-611.

Block, R.J.; Durrum, E.L. and Zweig, G. (1958): A Manual of Paper Chromatography and Paper Electrophoresis, 2nd ed. Academic Press Inc., New York.

Boughalleb, N.; Armengol, J. and El-Mahjoub, M. (2005): Detection of races 1 and 2 of *Fusarium solani* f.sp. *cucurbitae* and their distribution in watermelon fields in Tunisia. Journal of Phytopathology, **153**(3): 162-168.

Bourbos, V.A.; Skoudridakis, M.T.; Darakis, G.A. and Koulizakis, M. (1997): Calcium cyanamide and soil solarization for the control of *F. solani* f.sp. *cucurbitae* in greenhouse cucumber. Crop Protection, **16**(4): 383-386.

Broglie, K.E.; Gaynor, J. and Broglie, R.M. (1986): Ethylene regulated gene expression Molecular cloning of the gene encoding on endochitinase from Phaseolus vulgaris. Proc. Natl. Acad. Sci. U.S.A, **83**: 6820-6824.

Castroagudin, V.L.; Correll, J.C. and Cartwright, R.D. (2009): First report of fruit rot of pumpkin caused by *Fusarium solani* f.sp. *cucurbitae* in Arkansas. Plant Disease, **93**(6): p 669.

Chardonnet, C. and Doneche, B. (1995): Influence of calcium pretreatment on pectic substance evolution in cucumber fruit (*Cucumis sativus*) during *Botrytis cinerea* infection. Phytoparasitica, **23** (4): 335-344.

Choi, G.J.; Chung, Y.R. and Cho, K.Y. (1990): Various factors affecting sporulation and growth of *Botrytis cinerea* and the severity of cucumber gray mold rot. Korean Journal of Plant Pathology, **6**(2): 186-192.

Correll, J.C.; Mitchelt, J.K. and Andersen, C.R. (1991): Fruit rot pumpkin in Arkansas caused by *Fusarium equiseti.* Plant Disease, **75** (7): 751.

Cotoras, M. and Silva, E. (2005): Differences in the initial events of infection of *B. cinerea* strains isolated from tomato and grape. Mycologia, **97**(2): 485-492.

Dautel, F. (1978): Cucumber cultivars in a 3 year test. Gemuse, **14**(8): 268-270.

Deverall, B.J. (1995): Plant protection using natural defense systems of plants. Adv. Pl. Pathol., **11**:211-228.

Dixon, R.A.; Harrison, M.J. and Paiva, N.L. (1995): The isoflavonoid phytoalexin pathway: from enzymes to genes to transcription factors. Physiol. Plantarum, **93**: 385-392.

Ekramoddoullah, A.K.M. and Davidson, J.J. (1995): A method for the determination of conifer foliage protein extracted using sodium dodecyl sulfate and mercaptoethanol. Phytochem. Anal. **6**: 20-24.

Elad, Y. (1992): The use of antioxidants (free radical scavengers) to control grey mould (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*) in various crops. Plant Pathology, **41**(4): 417-426.

Elad, Y. and Zimand, G. (1991): Experience in integrated chemical - biological control of grey mould (*Botrytis cinerea*). Bulletin SROP, **14**(5): 195-199.

Elad, Y.; Shtienberg, D.; Yunis, H. and Mahrer, Y. (1992a): Epidemiology of grey mould, caused by *Botrytis cinerea* in vegetable greenhouses. Recent advances in *Botrytis* research. Proceedings of the 10th. International *Botrytis* Symposium, Heraklion, Crete, Greece, 5-10 April 1992. 147-158pp.

Elad, Y.; Yunis, H. and Katan, T. (1992b): Multiple fungicide resistance to benzimidazoles, dicarboximides and diethofencarb in field isolates of *Botrytis cinerea* in Israel. Plant Pathology, **41**(1): 41-46.

El-Habbaa, G.M. (2003): Polygalacturonase isozymes and cell wall degrading enzymes secreted by .*Botrytis cinerea*, the causal of grey mould disease on vegetables. Egyptian Journal of Phytopathology **31**(1/2): 87-101.

El-Khadem, M.; Abdel-Sattar, M.A.; Baraka, M.A. and Ali, I.N. (1985): Reaction of cucumber varieties to powdery mildew in relation to activities of oxidative enzymes. Proc. 1st Conf. of Pests and Dis, of Veg. and Field Crops in Egypt. Ismailia, P. 766-778.

Elmer, W.H. (1996): *Fusarium* fruit rot of pumpkin in Connecticut. Plant Disease, **80**(2): 131-135.

El-Mougy, N.S. and Abdel-Kader, M.M. (2007): Antifungal effect of powdered spices and their extracts on growth and activity of some fungi in relation to damping-off disease control. Journal of Plant Protection Research **47**: No. 3

El-Toony, A.M.E. (1992): Studies on leaf spot fungi on some cucurbitaceous plants. Ph.D. Thesis, Faculty of Agric., Al-Azhar Univ., Cairo, pp. 157.

Faccoli, M. and Schlyter, F. (2007): Conifer phenolic resistance markers are bark beetle antifeedant semiochemicals. Agricultural and Forest Entomology, **9**(3): 237-245.

Fantino, M.G.; Fantuz, F. and Gennari, S. (1989): Study of squash rot caused by *Fusarium solani* f.sp. *cucurbitae*. La Difesa delle Piante. **12** (1-2): 147-154

Fernandez-Trujillo, J.P. and Martinez, J.A. (2006): Ultrastructure of the onset of chilling injury in cucumber fruit. Journal of Applied Botany and Food Quality, **80**(2): 100-110.

Galal, A.A.; Shaat, M.M.N. and El-Bana, A.A. (2000): Sensitivity of *Alternaria redicina* and *Alternaria tenuissima* to some antioxidant compounds. J. Agric. Sci., Mansoura Univ., **25**(3): 1553-1562.

Galeotti, F.; Barile, E.; Curir, P.; Dolci, M. and Lanzotti, V. (2008): Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. Phytochemistry Letters, **1**: 44–48

Galvis, A.C. and Morais, A.M.M.B. (2001): Effects of controlled atmosphere (CA) storage on pectinmethylesterase (PME) activity and texture of 'Rocha' pears. Journal of the Science of Food and Agriculture **82**(1): 143 - 145.

Galvis-Sánchez, A.C. and Morais, A.M.M. (2002): Effects of controlled atmosphere (CA) storage on pectinmethylesterase (PME) activity and texture of 'Rocha' pears. Journal of the Science of Food and Agriculture, **82**: 143–145.

Garcia-Jimenez, J.; Armengol, J.; Moya, M.J. and Sales, R., Jr (1997): First report of *Fusarium solani* f. sp. *cucurbitae* Race 1 in Spain. Plant Disease, **81**(10): 1216.

Gevens, A.J.; Lamour, O.K.; Grumet, K.H.R. and Hausbeck, M.K. (2006): A detached cucumber fruit method to screen for resistance

to *Phytophthora capsici* and effect of fruit age on susceptibility to infection. Plant Dis., **90**:1276-1282.

Granke, L.L. and Hausbeck, M.K. (2010): Effects of temperature, humidity, and wounding on development of Phytophthora rot of cucumber fruit. Plant Dis., **94**:1417-1424.

Hakan, O.M.; Akbudak, B.; Uylaser, V. and Tamer, E. (2005): The effect of controlled atmosphere storage on pickle production from pickling cucumbers cv. 'Troy'. European Food Research and Technology, **222,** (1-2): 118-129.

Hanan, J.J.; Holley, W.D. and Goldsberry, K.L. (1978): Greenhouse management. Springer-Verlag, Berlin, Heidelberg and New York, 530 pp.

Hardenburg, R.E.; Watada, A.E. and Wang, C.Y. (1986): The commercial storage of fruits and vegetables, and florist and nursery stocks. USDA Agric. Handbook No. 66.

Hasan (Eman), O.A. (2011): Biological and chemical control of bacterial diseases infecting tomato plants. Ph.D. Thesis, Fac. Agric. Moshtohor, Benha Univ. Egypt. 167p

Hassan, Maggie E.M.; Saieda S. Abd El-Rahman; El-Abbasi, I.H. and Mikhail, M.S. (2007): Changes in peroxidase activity due to resistance induced against faba bean chocolate spot disease. Egypt. J. Phytopathology, **35**(1): 35-48.

Hawthorne, B.T. (1988): Fungi causing storage rots on fruit of *Cucurbita* spp. New Zealand Journal of Experimental Agriculture, **16**(2): 151-157.

Hochstrasser, D.F.; Harrington, M.G.; Hochstrasser, A.C.; Miller, M.J. and Merrill, C.R. (1988): Improved separation and detection of proteins with retained background silver staining. Anal. Biochem., 173, 424-435.

Horst, R.K.; Israel, H.W. and Langhans, R.W. (1994): Bicarbonate and *Botrytis*: III- Effects on germination of *Botrytis cinerea.* Phytopathology, **84** (10): 1056.

Ishii, M. (1989): Film excluding ultra-violet rays in pest control. Agriculture and Horticulture, **64**(1): 189-193.

Jamiolkowska, A. (2011): Laboratory effect of azoxystrobin (Amistar 250 SC) and grapefruit extract (Biosept 33 SL) on growth of fungi

colonizing zucchini plants. Acta Scientiarum Polonorum-Hortorum Cultus. **10**(2): 245-257.

Jun, O.; Naosuke, O.; Atsuhiko, S. and Mitsuo, T. (1988): Primary structure of cucumber (*Cucumis sativus*) ascorbate oxidase deduced from cDNA sequence: Homology with blue copper protein and tissue-specific expression. Proc. Nall. Acad. Sci. USA, 86: 1239-1243.

Kalamarakis, A.E.; Petsikos-Panagiotarou, N.; Mavroidis, B. and Ziogas, B.N. (2000): Activity of fluazinam against strains of *Botrytis cinerea* resistant to benzimidazoles and/or dicarboximides and to a benzimidazole-phenylcarbamate mixture. Journal of Phytopathology, **148** (7/8): 449-455.

Kerssmann, H.; Staub, J.; Hofmann, C.; Matzk, T.; Herzog, J.; Ward, E.; Vknes, S. and Ryals, J. (1994): Induction of systemic acquired resistance in plants by chemical, Ann. Rev. Phytopathol., **32:**439-459.

Kertesz, Z.I. (1951): The pectic substances. Interscience Publishers. New York, 628pp.

Kim, B.S.; Park, E.W. and Cho, K.Y. (2001): Population dynamics and fitness comparison of sensitive and resistant phenotypes of *B. cinerea* to benzimidazole, dicarboximide and N.-phenylcarbamate fungicides. Plant Pathology Journal, **17**(3) 149-153.

Kim, H.T.; Chung, Y.R. and Cho, K.Y. (1990): Activity of prochloraz against *Botrytis cinerea* causing cucumber gray mold rot. Korean Journal of Plant Pathology, **6**(3): 333-337.

Kim, J.J.; Kim, J.W.; Lee, C.W. and Chung, Y.R. (1997): Relationships between pathogenicity and activities of polygalacturonase, laccase and β-glucosidase produced by *Botrytis cinerea*. Korean Journal of Plant Pathology, **13**(4): 225-231.

Kim, J.Y.; Yi, Y.K. and Song Y. (1998): Plant diseases on green-house crops in Kyeongbuk areas. Korean Journal of Plant Pathology, **14**(1): 41-45.

Krishna, H.; Stephens, C.; Carpenter, A.; Grant, N.; Galbraith, G.; Starr, M.; Lill, R. and Ryan, R.F. (1998): Envirosol technology - a new approach to fungicide application in greenhouses. Proceedings of the Fifty First New Zealand Plant Protection

Conference, Quality Hotel, Hamilton, New Zealand, 11-13 August, 152-156.

Kuć, J. (1982): Phytoalexins from the Solanaceae. In: Bailey J A, Mansfield J W, editors. Phytoalexins, New York, N.Y: John Wiley & Sons, Inc.; pp. 81–105.

Ma, Z.H.; Yan, L.Y.; Luo, Y. and Michailides, T.J. (2007): Sequence variation in the two-component histidine kinase gene of *B. cinerea* associated with resistance to dicarboximide fungicides. Pesticide Biochemistry and Physiology, **88**(3): 300-306.

Maklad, Amal H.M. (2004): Studies on Gray mould diseases of some cucurbit crops. M.Sc., Thesis, Fac. Agric., Suez Canal Univ., P. 110.

Markoglou, A.N.; Malandrakis, A.A.; Vitoratos, A.G.; and Ziogas, B.N. (2006): Characterization of laboratory mutants of *Botrytis cinerea* resistant to QoI fungicides. European Journal of Plant Pathology, **115** (2): 149-162.

Matsudaira, P. (1987): Sequence from picomle quantities of protein electroblotted onto polyvinylidene difluoride membranes. J. Bio. Chem. **262:**10035-10038.

Matta, A. and Dimond, A.F. (1963): Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology, **64:**574-578.

McCall, D. and Willumsen, J. (1991): Evaluation of glasshouse cucumber cultivars for whole season, spring and summer crops. Tidsskrift for Planteavl, **95**(1): 73-79.

Mehl, H.L. and Epstein, L. (2007): *Fusarium solani* species complex isolates conspecific with *Fusarium solani* f.sp. *cucurbitae*. race 2 from naturally infected human and plant tissue and environmental sources are equally virulent on plants, grow at 37 degrees C and are interfertile. Environmental Microbiology, **9**(9): 2189-2199.

Mohamed, S.A.; Basiony, A.M.; Awad, M.A. and El-Desouky, S.M. (1995): The nature of downy mildew resistance in cucumber and some factors affecting. Minufiya J. Agric. Res; **20** (4): 1403-1417.

Morrissey, J.P. and Osbourn, A.E. (1999): Fungal Resistance to Plant Antibiotics as a Mechanism of Pathogenesis. Microbiol Mol. Biol. Rev., **63**(3): 708–724.

Morsy, S.M.; Drgham, E.A. and Mohamed, G.M. (2009): Effect of garlic and onion extracts or their intercropping on suppressing damping-off and powdery mildew diseases and growth characteristics of Cucumber. Egyptian Journal of Phytopathology, **37**(1): 35-46.

Moyano, C.; Gomez, V. and Melgarejo, P. (2004): Resistance to pyrimethanil and other fungicides. In: *Botrytis cinerea* populations collected on vegetable crops in Spain. Journal of Phytopathology, **152**(8-9):449-528.

Müller, K.O. (1956): Some simple tests for the detection of phytoalexins. Phytopathol. Z., **27**: 237-254.

Muting, D. and Kaiser, E. (1963): Spectrophotometric method of determining of-amino-N in biological materials be means of the ninhydrin reaction. Hoppe-Seyler's Zschr. Physiol. Chem. 332, 276.

Nabil, A.A.A.C. (2000): Effects of chemicals and hot water treatments on seeds on squash infection by cucumber mosaic virus (CMV). Assiut J. Agric. Sci., **30**(4): 193-206.

Nada, M.C. (2002): Studies on antifungal activities of some Egyptian medicinal and aromatic plants. Ph.D. Thesis, Fac. Agric., Zagazig Univ., 189

Nasreen Sultana and Ghaffar, A. (2010): Effect of fungicides, microbial antagonists and oilcakes in the control of *F. solani*, the cause of seed rot, seedling and root infection of bottle gourd, bitter gourd and cucumber. Pakistan Journal of Botany, **42**(4): 2921-2934.

Ono, M.; Yamada, M. and Nakazawa, Y. (1997): Current status in sensitivity to fungicides of *Botrytis cinerea* on cucumber and grapevine in Yamanashi Prefecture. Proceedings of the Kanto-Tosan Plant Protection Society, **44**: 127-129.

Ooswtendrop, M.V.; Kessmann, H.; Fridrich, L.; Geissmann, A.J.; Gorpach, G.H; Nordmeyer, D.; Reist, R.; Ruess, W. and Staub, T. (1996): Influence of plant activator Bion and of Triazole-fungicides on plant defense mechanisms. Gesunde Pflanzen, **48**(7): 260-264. (c.f. CAB Abstracts, 1996-1998)

Palmer, C.L.; Horst, R.K.; Israel, H.W. and Lanphans, R.W. (1994b): Bicarbonate and Botrytis: IV- Additional concentration of

effects on germination of *Botrytis cinerea* conidia. Phytopathology, **84**: 1374.

Palmer, C.L.; Horst, R.K.; Lanphans, R.W. and Israel, H.W. (1994a): Bicarbonate and Botrytis: VII- Viability of fungal conidia. Phytopathology, **84**: 1204.

Park, S.; Kwon, T.Y.; Lim, Y.S,; Jung, K. and Choi, B.S. (1996): Disease survey in melon, watermelon, and cucumber with different successive cropping periods under vinylhouse conditions. Korean Journal of Plant Pathology, **12** (4): 428-431.

Park, Y.S. and Jung, S.T. (2005): Pre storage conditioning and CO_2 pretreatment for control of post harvest rot in kiwifruit inoculated with *Botrytis cinerea* and *Botryosphaeria dothidea*. Journal of the Korean Society for Horticultural Science, **46** (1): 49-54.

Park, Y.S. and Yu, S.H. (1998): Chemical resistance of gray mold caused by *Botrytis cinerea* of tomatoes in greenhouses. RDA Journal of Crop Protection, **40**(1): 44-49.

Paxton, J.D. (1980): A new working definition of the term "phytoalexin". Plant Dis., **64**: 734.

Petsikos-Panayotarou, N.; Markellou, E.; Kalamarakis, A.E.; Kyriakopoulou, D. and Malathrakis, N.E. (2003): *In vitro* and *in vivo* activity of cyprodinil and pyrimethanil on *Botrytis cinerea* isolates resistant to other botryticides and selection for resistance to pyrimethanil in a greenhouse population in Greece. European Journal of Plant Pathology, **109** (2): 173-182.

Pieterse, C.M.J.; Wees, S.C.M.; Hoffland, E.; Pelt, J.A. and Loon, C.L. (1996): Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis related gene expression. Plant Cell, **8**(8): 1225-1237.

Raj, S.N.; Shetty, N.P. and Shetty, H.S. (2009): Proline- An inducer of resistance against pearl millet downy mildew disease caused by *Sclerospora graminicola*. Phytoparasitica, **32** (5): 523-527.

Rampersad, S.N. (2009): First report of *Fusarium solani* fruit rot of pumpkin (*Cucurbita pepo*) in Trinidad. Plant Disease, **93**(5): 547.

Rath, G.C.; Mishra, D. and Nayak, N.C. (1990): A note on fungi causing rotting of cucurbits in Orissa markets. Orissa Journal of Agricultural Research, **3**(2): 161-162.

Raum, J. (1997): Bion- a plant activator also for vegetable gardening. Gemuse Munchen, **33**(3): 185-187.

Rauscher, M.; Adám, A.; Wirtz, S.; Guggenheim R.; Mendgen, K. and Deising, H.B. (1999): PR-1 protein inhibits the differentiation of rust infection hyphae in leaves of acquired resistant broad bean. Plant J., **19**(6): 625-633.

Reddy, P.B. and **Reddy, S.M. (1989):** Incidence of different species of *Fusarium* causing fruit-rot of cucurbitaceous vegetables. Indian Journal of Mycology and Plant Pathology, **18** (2): 193-194.

Reuveni, M.; Agapov, V. and Reuveni, R. (1995): Suppression of cucumber powdery mildew (*Sphaerotheca fuliginea*) by foliar sprays of phosphates and potassium salts. Plant Pathology, **44:** 31-39.

Reuveni, R. and Kue, J. (1989): Association of peroxidase with systemic resistance to *Cladosporium cucumerinum* induced by *Colletotricum lagenarium* in cucumber cultivars resistant or susceptible to *Cladosporium cucumerinum.* (Abstr.) Phytopathology, **79**: 1150.

Reuveni, R., Shimoni, M., Karchi, Z. and Kuc, J. (1992): Peroxidase activity as a biochemical marker for resistance of muskmelon (*Cucumis melo*) to *P. cubensis*. Phytopathology **82**: 749-753.

Rha, E.; Park, H.; Kim, M.O.; Chung, Y.R.; Lee, C.W. and Kim, J.W. (2001): Expression of exo-polygalacturonases in *Botrytis cinerea*. FEMS Microbiology Letters, **201** (1):105-109.

Ruess, W.K.; Mueffer, G.; Khauf, B. and Kunz, W. (1997): Plant activator Bion 50 WG an innovative approach for disease control in cereals. Progress in plant Protection, **37**(1): 36-41.

Saber, M.M.; Sabet, K.K.; Moustafa, S.M. and Iman, Khafagi, Y.S. (2003): Evaluation of Biological Products, Antioxidants and Salts for control of Strawberry Fruit Rot. Egypt. J. Phytopath., **31**: 31-43.

Salles, I.I.; Blount, J.W.; Dixon, R.A. and Schubert, K. (2002): Hytoalexin induction and β-1,3-glucanase activities in *Colletotrichum trifoliiL* infected leaves of alfalfa (*Medicago sativa* L.) Physiol. Mol. Plant Pathol. **61,** 89-101.

Schmitt, A. (2002): Induced responses by plant extracts from Reynoutria sachalinensis: a case study. Bulletin OILB/SROP. **25** (6): 83-88.

Shang, Q.M.; Zhang, Z.G.; Song, S.Q.; Li, X.F. and Dong, T. (2007): Induced resistance of cucumber seedlings to *Botrytis cinerea* Pers. by chitosan treatment. Acta Phytophylacica Sinica, **34** (2): 136-140.

Shi, Y.X.; Yuan, L.P.; Zhang, Y.B. and Li, B.J. (2007): Fungicidal activity of novel fungicide chlorphenomizole and efficacy in controlling main diseases on vegetable in Field. Chinese Journal of Pesticide Science, **9**(2): 126-130.

Sipailiene, A.; Venskutonis, P.R.; Baranauskiene; R. and Sarkinas, A. (2006): Antimicrobial activity of commercial samples of thyme and marjoram oils. J. Essent. Oil Res., **18**: 698-703.

Siviero, P. and Motton, M.S. (2000): The enemies of the marrow. Informatore Agrario, **56**(16): 39-49.

Smolinka, U. and Kowalska, B. (2006): The effectivity of plant extracts and antagonistic microorganisms on the growth inhibition of French bean pathogenic fungi. Vegetable Crops Research Bulletin, **64:** 67-76.

Snedecor, G.W. and Cochran, W.G. (1999): Statistical Methods. Iowa State University Press, 9th Edt., 503pp.

Snell, F.D. and Snell, C.T. (1953): Colorimetric methods of analysis including some turbidimetric and nephelometric methods. D. van Nostrand Company Inc. Toronto-New York London. Vol. 111, 606 pp.

Sommer, N.F.; Fortlog, R.J.; Mitchell, F.G. and Maxte, E.G. (1974): Reduction of post harvest losses of strawberry fruits from grey mould. J. Amer. Soc. Hort. Sci., **98:** 285 - 288.

Sonoda, R.M.; Paris, H.S.; Shubert, T.S. and El-Gholl, N.E. (1993): Fruit rot of squash cv. Orangetti in Florida. Plant Disease, **77**(8): 847.

Stevens, C.; Khan, V.A.; Lu, J.Y.; Wilson, C.L. ; Pusey, P.L.; Kabwe, M.K.; Igwegbe, E.C.K.; Chalutz, E. and Droby, S. (1998): The germicidal and hormetic effects of UV-C light on reducing brown rot disease and yeast microflora of peaches. Crop Protection, **17**:75-84.

Takagaki, M.; Kataoka, S.; Kida, K.; Miura, I.; Fukumoto, S. and Tamai, R. (2010): Disease-controlling effect of a novel fungicide pyribencarb against *Botrytis cinerea*. Journal of Pesticide Science, **35** (1): 10-14.

Takuo, S.S; Tatsuji, H.J. and Erick, V. (1993): Pectin, pectinase and protopectin properties and applications. Adv. Appl. Microbiol. **39**: 213 – 294.

Townsend, G.K. and Heuberger, T.W. (1943): Methods for estimating losses caused by diseases in fungicides experiments. Plant Dis. Reptr., **27**:340-343.

Tripathi, P. and Dubey, N.K. (2004): Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biol. Technol., **32**: 235-245.

Turner, P.D. (1965): Phenoloxidase activity in cacao section showing variable resistance to phytophthora root rot. Plant Dis. Reptr., **49**: 319.

Tuzun, S.; Rao, M.N.; Vogeli, U.; Schardl, C.L. and Kuc, J. (1989): Induced systemic resistance to blue mold early induction and accumulation of β-1,3-glucanases, chitinases, and other pathogenesis-related proteins (b-proteins) in immunized tobacco. Phytopathology, **79:** 979-983.

Verbruggen, N. and Hermans, C. (2008): Proline accumulation in plants: A Review of Amino Acids, **35**(4): 753-9

Veronesi, A.; Sigala, C. and Roberti, R. (2009): Identification and characterization of *Fusarium solani* f. sp. *cucurbitae* race 1 on zucchini in the growing area of Bologna. Micologia Italiana, **38** (2): 24-33.

Vozenilkova, B. and Zvara, J. (1990): New possibilities of greenhouse cucumber protection against fungal diseases. Sbornik-Vysoka Skola Zemedelska v Praze, Agronomicka Fakulta v Ceskych Budejovicich, Fytotechnicka Rada, **7** (2): 49-60.

Wasfy, E.H. (1967): Studies on certain diseases of cucurbitaceous. Ph.D., Agric., Alex. Univ., 114 pp.

Watada, A.E. (1997): Qi Ling Physiology, quality and microorganism population on fresh-cut products stored under low O_2 controlled

atmospheres. 14th International congress on plastics in agriculture, Tel Aviv, Israel, March 1997. 1998. 579-585.

Wayne, W.F. and Benny, D.B. (2010): Quantification of L-citrulline and other physiologic amino acids in watermelon and selected cucurbits. Cucurbitaceace 2010 Conference 14-18 November 2010, Charleston, South Carolina, USA.

Xu, J. (1994): Using carbendazol dust to control gray mold of cucumber (*Botrytis cinerea* Fries) in greenhouse. Bulletin of Agricultural Science and Technology, **3**: 28-29.

Yabuki, S.; Fukuhara, I.; Yamada, M. and Nakazawa, Y. (1997): Surveillance of fungicide sensitivity of *Botrytis cinerea* with a rapid sensitivity test using toothpick on protected fruit vegetables in Kanagawa Prefecture. Proceedings of the Kanto-Tosan Plant Protection Society, **44**: 121-125.

Yu, W.Y.; Zhang, S.S.; Du, X.R. and Zhang, X.F. (2009): Identification and investigation on occurrence regularity of the cucumber diseases in greenhouse of Fujian. Journal of Fujian Agriculture and Forestry University (Natural Science Edition), **38**(2): 119-123.

Yucel, S. (1994): Survey studies of fungal diseases of protected vegetable areas in Mediterranean region. Bitki Koruma Bulteni. **34**(1/2): 23-34.

Yunis, H.; Elad, Y. and Mahrer, Y. (1991): Influence of fungicidal control of cucumber and tomato grey mould (*B. cinerea*) on fruit yield. Pesticide Science, **31**(3): 325-335.

Yurina, O.V.; Yurina T.P. and Anikina, I.I. (1993): Peroxidase activity of the leaves in cucumber as a test for resistance to mildew. Sel'skhazyaistvennaya Biologiya, **1**: 113-117.

Zedan, A.M. (1993): Antifungal Properties of certain plant extracts with a special reference to the possibility of controlling onions white rot disease using *Eucalyptus robusta* leaves. Egypt. J. Appl. Sci., **8**(12): 574 -589.

Zheng, Y.; Sheng, J.; Zhao, R.; Zhang, J.; Lv, S.; Liu, L. and Shen, L. (2011): Pre harvest L-Arginine Treatment Induced Post harvest Disease Resistance to *Botrysis cinerea* in Tomato Fruits. J. Agric. Food Chem., **59** (12): 6543–6549.

Zhou, J.; Loh, Y.T.; Bressan, R.A. and Martin, Gregory B. (1995): The tomato gene Pti1 encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. Cell, **83**: 925-935.

Zhu, Z.N. and Zhang, D.Y. (1995): Post harvest diseases of marrow squash (*Cucurbita ovifera*) and its control. Acta Agriculturae Shanghai, **11** (2): 44-46.

Zhukovskaya, S.A.; Plyusnina, R.A.; Azbukina, Z. M. and Egorova, L. N. (1984): Main diseases of cucumbers and tomatoes in the Primor'e glasshouse collective farm and measures to control them. Sistematiko Floristicheskie Issledovaniya Sporovykh Rastenii Dal'nego Vostoka, **1984**: 107-113.

Zimmerli, L.; Métraux, J.P. and Mauch-Mani, B. (2001): β-Aminobutyric Acid-Induced Protection of Arabidopsis against the Necrotrophic Fungus *Botrytis cinerea*. Plant Physiology, **126**(2): 517-523.

ARABIC SUMMARY

الملخص العربى

تعتبر القرعيات التى تضم محاصيل هامة اقتصاديا مثل الخيار والكوسة والشمام والبطيخ من أهم محاصيل الخضر التى تنتشر زراعتها على نطاق واسع في مصر وجميع أنحاء العالم. وتصاب القرعيات خلال مراحل النمو المختلفة بالعديد من الأمراض الفطرية مثل أمراض البياض الدقيقى والزغبى والعفن الرمادى والعفن الأبيض والإنثراكنوز وذبول الفيوزاريوم وأعفان الجذور والساق والثمار مما يتسبب عنه خسائر كبيرة فى المحصول الناتج. وفى هذه الدراسة تم التعرض بالدراسة للنقاط التالية: حصر انتشار مرض تعفن الثمار على الخيار والكوسة في مواقع مختلفة في مصر، عزل وتحديد الفطريات المرتبطة بهذه الأمراض من ثمار تم جمعها من مختلف المناطق المزروعة بالخيار والكوسة في مصر ، دراسة القدرة الإمراضية للعزلات المختلفة المتحصل عليها من ثمار الخيار والكوسة ، دراسة مدى مقاومة الأصناف المختلفة من الخيار للإصابة بأعفان الثمار ، تأثير معاملة النباتات النامية تحت ظروف الصوبة ببعض المعاملات مثل المبيدات والمواد المحفزة للمقاومة والمستخلصات النباتية على حدوث تعفن ثمار الخيار والكوسة والنشاط الأنزيمي فى الثمار المحقونة لفطريات العفن المختبرة وتأثير بعض محفزات المقاومة على إحداث تغيرات في أنماط البروتين ، ومحتوى الفينولات والأحماض الأمينية. ويمكن تلخيص أهم نتائج تلك الدراسة على النحو التالي :

١- أظهرت الدراسة أن معدل الإصابة بعفن ثمار الخيار والكوسة كان أعلى فى موسم ٢٠٠٣ مقارنة بموسم ٢٠٠٤ كما أظهرت الدراسة أيضا تفاوتا فى شدة الإصابة بين المواقع التى شملتها الدراسة.

٢- أمكن الحصول على عدد ١٥٢ ، ٨١ عزلة فطرية من ثمار خيار وكوسة مصابة بالعفن على التوالي وقد اشتملت هذه العزلات الفطرية على أنواع من فطريات ألترناريا ، بوطرريتس سينيريا ، فيوزاريوم سولانى ، ميوكر ، بنيسيليوم ، بيثيوم ، سكليوتينيا سكليروتيورم.

٣- كانت أعلى إصابة على ثمار الخيار مصاحبة للفطر بوطريتس سينيريا المعزول من محافظات الإسماعيلية والبحيرة والفطر فيوزاريوم سولانى المعزول من محافظات البحيرة وكفر الشيخ والإسماعيلية بينما سجل فطر بوطريتس المعزول من محافظات الشرقية والاسماعيلية والفطر فيوزاريوم سولاني المعزول من الاسماعيلية أعلى شدة مرضية على ثمار الكوسة.

٤- حدثت زيادة مضطردة فى شدة المرض على ثمار الخيار والكوسة بزيادة تركيز لقاح الفطريات المسببة لعفن الثمار من ١٠٠٠ الى ٧٠٠٠ جرثومة/ملل. وقد أدت زيادة لقاح الفطر بوطريتس سينيريا من ٦٠٠٠ الى ٧٠٠٠ جرثومة/ملل (على ثمار الكوسة والخيار) وكذلك الفطر فيوزاريوم سولانى (على ثمار الكوسة) إلى أعلى زيادة معنوية فى شدة للإصابة بالعفن بينما أدت زيادة لقاح الفطر الأخير من ٥٠٠٠ الى ٦٠٠٠ جرثومة/ملل إلى أعلى زيادة فى شدة الإصابة مقارنة بالتركيزات الأخرى المستخدمة من اللقاح.

٥- كانت ثمار الخيار صنف هيكل الأقل اصابة بفطريات عفن الثمار تحت الدراسة يليه الأصناف دلتا ستار، نيوستار، فيصل ، سيناء- ١ ثم الصنف سمر.

٦- كانت شدة الإصابة بالفطر بوطريتس سينيريا أعلا معنويا على ثمار الخيار مقارنة بالفطر فيوزاريوم سولانى بينما تساوت شدة الإصابة بكلا الفطرين معنويا على ثمار الكوسة. لوحظت ايضا زيادة شدة الإصابة بعفن الثمار معنويا كلما زاد عمر النبات أو عمر الثمار كما كانت الإصابة أعلى معنويا على الثمار المجروحة مقارنة بغير المجروحة.

٧- زادت شدة الإصابة معنويا بتخزين الثمار المحقونة لمدة ٨ أيام مقارنة بتخزينها لمدة ٤ يوم. كما كان تطور المرض أكثر بطئا عندما خزنت ثمار الخيار علي درجة حرارة منخفضة (٢ ، ٥ أو ٧ درجات مئوية) مقارنة بتخزينها علي درجة حرارة

الغرفة (٢٤ درجة مئوية) وكان تطور الإصابة بالفطر بوطريتس سينيريا بسرعة أكبر من الإصابة بالفطر فيوزاريوم سولانى.

٨- تأثرت شدة الإصابة على الثمار معنويا بتركيز ثانى أكسيد الكربون ، وفترة التخزين وكذلك التفاعل بينهما وقد سجلت أدنى شدة إصابة على ثمار الخيار (المحقونة بالفطريات بوطريتس أو فيوزاريوم) المخزنة لمدة ١٠ يوم عند تركيز ١٠٪ من ثانى أكسيد الكربون.

٩- حدث انخفاض واضح فى شدة إصابة الثمار بفطريات العفن تحت الدراسة عند تعريض تلك الثمار للأشعة فوق البنفسجية عند ٢٨٠ أو ٣٢٠ نانومتر غير أن النتائج (في ظل ظروف هذه الدراسة) أظهرت أن الأشعة فوق البنفسجية لا تعتبر إجراءً عمليا لقمع شدة الإصابة بفطريات عفن الثمار على ثمار الخيار.

١٠- كانت المبيدات الفطرية المختبرة فعالة في قمع نمو مسببات عفن الثمار فى المعمل وكان المبيد رونيلان أكثرها فعالية فى قمع نمو الفطر بوطريتس سينيريا حيث أدى الى منع نموه تماما يليه فى ذلك المبيد سوميسكلكس بينما كانت المبيدات روفرال ، سوميسكلكس ، رونيلان ، تكتو على التوالى الأشد فعالية فى قمع نمو الفطر فيوزاريوم سولانى فى المعمل وقد زاد معدل تثبيط نمو هذه الفطريات بشكل عام كلما زاد تركيز المبيدات المستخدمة.

١١- أدى رش نباتات الخيار والكوسة بأى من المبيدات الفطرية المستخدمة فى تلك الدراسة إلى انخفاض معنوى فى شدة إصابة الثمار بفطريات عفن الثمار وكان المبيد كوبرال ثم تيلدور (فى حالة الفطر بوطريتس) والمبيد كوبرال ثم ريدوميل (فى حالة الفطر فيوزاريوم) الأشد فعالية فى قمع شدة إصابة ثمار الخيار والكوسة بهذه الفطريات. وقد لوحظت زيادة تدريجية فى فعالية هذه المبيدات على قمع شدة الإصابة بفطريات عفن الثمار.

١٢- أدى رش نباتات الخيار والكوسة ببعض محفزات المقاومة إلى خفض كبير فى شدة الإصابة بأعفان الثمار بعد الحصاد. وقد تم تسجيل أدنى إصابة بالفطر بوطريتس سينيريا على ثمار الخيار المقطوفة من نباتات تم رشها بفوسفات البوتاسيوم ، كلوريد الكالسيوم ، كبريتات الصوديوم ، حمض السليسيليك أو حمض الأوكساليك بينما سجلت النباتات المرشوشة بحمض الأوكساليك ، فوسفات البوتاسيوم ، حمض السليسيليك أدنى شدة إصابة على الثمار المحقونة بالفطر فيوزاريوم سولانى. أما على ثمار الكوسة فقد سجل حمض السليسيليك أدنى إصابة بالفطر بوطريتس يليه فوسفات البوتاسيوم ، حمض الأوكساليك ، كبيريتات الليثيوم ثم كبريتات الصوديوم ومن ناحية أخرى سجل حمض الأوكساليك وفوسفات البوتاسيوم أقل إصابة بالفطر فيوزاريوم سولانى.

١٣- أظهرت أوراق نباتات الخيار والكوسة التى تم رشها ببعض محفزات المقاومة (بيون ، حمض ساليسيليك ، حمض أوكساليك ، فوسفات بوتاسيوم ، كلوريد كالسيوم) تغيرات واضحة فى البروتينات القابلة للذوبان حيث أظهر الفصل الكهربي أن عدد حزم البروتين كان أعلى في أوراق النباتات المعاملة، ومقارنة بالكنترول (غير المعامل) أظهر الفصل الكهربى وجود عدد ٢٧ حزمة بروتين جديدة أوزانها الجزيئية تتراوح بين ٥١ و ٣٩ كيلو دالتون في أوراق نباتات الخيار المعاملة وعدد ٢٢ حزمة بروتين جديدة أوزانها الجزيئية تتراوح بين ٥٥ و ٤٧ كيلو دالتون في أوراق الكوسة. وبشكل عام ، فقد لوحظ أن المعاملة بالبيون وحمض الساليسيليك قد سببا أكبر عدد من الحزم الجديدة للبروتين فى أوراق كل من الخيار والقرع.

١٤- أدى رش نباتات الخيار والكوسة ببعض المركبات السابقة والمحفزة للمقاومة وبعض المبيدات إلى زيادات واضحة في كمية الفينولات الكلية والحرة فى ثمار تلك النباتات عند حقنها بالفطريات بوطريتس أو فيوزاريوم مقارنة بالثمار المقطوفة من نباتات الكنترول. في حالة من ثمار الخيار قللت جميع المعاملات من كمية الفينولات الكلية بينما سجلت المركبات كاتيكول و بيون (عند الحقن

بفطر بوطريتس) و تيلدور و كوبرال و كاتيكول (عند الحقن بالفطر فيوزاريوم) أعلى كمية من الفينولات الكلية والحرة مقارنة بثمار نباتات الكنترول. وقد لوحظ اتجاها مماثلا بشأن ثمار الكوسة حيث أدى رش النباتات بمركب كاتيكول أعلى كمية من الفينولات الحرة في حين سجلت المبيد كوبرال أعلى كمية من الفينولات الكلية عند حقن الثمار بالفطر بوطريتس بينما سجلت الثمار المقطوفة من نباتات سبق رشها بالمركبات أيثيفون أو تيلدور أعلى كميات من الفينولات الحرة عند حقن تلك الثمار بالفطر فيوزاريوم.

١٥- أدى رش نباتات الخيار والكوسة بمحفزات المقاومة السابقة إلى حدوث زيادات واضحة فى الأحماض الأمينية الكلية فى ثمار تلك النباتات عند حقنها بمسببات أعفان الثمار تحت الإختبار. وفى هذا الخصوص تم تسجيل أعلى زيادة فى ثمار الخيار المقطوفة من نباتات عوملت بال تيلدور أو ال كاتيكول (عن حقن الثمار بالفطر بوطريتس) وثمار الكوسة المقطوفة من نباتات عوملت بكلوريد الكالسيوم (عن حقن الثمار بالفطر فيوزاريوم). من ناحية أخرى ، سجل رش نباتات الخيار بأحد المركبات كاتيكول ، تيلدور ، إيثيفون أو كوبرال أعلى كمية من الأحماض الأمينية الكلية في الثمار المحقونة بالفطر بوطريتس بينما سجلت المركبات تيلدور ، كاتيكول أكبر كمية من محتوى الأحماض الأمينية الكلية فى ثمار الكوسة المحقونة بالفطر فيوزاريوم.

١٦- أدى رش نباتات الخيار والكوسة بالمستخلصات النباتية المستخدمة إلى انخفاض معنوى فى شدة الإصابة بأعفان الثمار. وبالنسبة لإصابة ثمار الخيار بالفطر بوطريتس سينيريا كان مستخلص البردقوش الأكثر فعالية حيث أدى إلى منع الإصابة تماما يليه شاى الكومبوشا ، الزنجبيل ، الثوم ، الحناء وحبة البركة بينما كانت مستخلصات حبة البركة ، والحناء ، البردقوش والزنجبيل الأكثر فعالية فى قمع الإصابة بالفطر فيوزاريوم سولانى يليها الزعتر وشاى الكومبوشا على التوالي. .أما بالنسبة لثمار الكوسة فقد كانت مستخلصات الحناء والثوم والقرنفل والزنجبيل الأكثر فعالية فى خفض إصابتها بالفطر بوطريتس

سينيريا يليها مستخلصات حبة البركة ، شاى الكومبوشا ، والبردقوش في حين كانت مستخلصات الثوم ، والحناء ، والزنجبيل ، وحبة البركة ، القرنفل والزعتر الأكثر فعالية ضد عفن الثمار الذى يسببه الفطر فيوزاريوم سولانى.

١٧- لوحظ انخفاض كبير فى نشاط انزيم بكتين ميثيل استريز (PME) في ثمار الخيار (المحقونة بالفطريات المسببة لأعفان الثمار) والتى تم حصادها من نباتات سبق رشها بأي من المستخلصات النباتية المختبرة أو شاي الكومبوشا مقارنة بثمار النباتات غير المعاملة. وقد كانت مستخلص الزنجبيل الأكثر فعالية لخفض نشاط هذا الإنزيم فى ثمار الخيار المحقونة بالفطر بوطريتس سينيريا يليه مستخلصات البردقوش ، حبة البركة ، الثوم ، شاى الكومبوشا ، القرنفل ، الحناء ، والزعتر، على التوالي. أيضا كان مستخلص الزنجبيل الأكثر فعالية لتقليص نشاط هذا الإنزيم فى الثمار المحقونة بالفطر فيوزاريوم سولانى يليه مستخلصات حبة البركة ، البردقوش ، شاى الكومبوشا ، الثوم ، الزعتر ، القرنفل والحناء ، على التوالي. أما في حالة ثمار الكوسة المحقونة بالفطر بوطريتس فقد كان شاى الكومبوشا ومستخلص حبة البركة هما الأكثر فعالية للحد من نشاط هذا الإنزيم يليهما، البردقوش والزنجبيل ، الثوم ، القرنفل ، الحناء والزعتر على التوالى مقارنة بمعاملة الكنترول. أما فى الثمار الملقحة بالفطر فيوزاريوم فقد سببت مستخلصات حبة البركة والزعتر قمعا تاما لنشاط هذا الإنزيم يليهما مستخلص الثوم ، ثم شاى الكومبوشا ، الزنجبيل ، البردقوش ، الحناء والقرنفل، على التوالي.

١٨- أدى رش نباتات الخيار أو الكوسة بأي من المستخلصات المختبرة إلى انخفاض كبير فى نشاط انزيم بولي جالاكتورونيز (PG) فى ثمارها المحقونة بفطريات عفن الثمار تحت الإختبار مقارنة بثمار النباتات غير المرشوشة (الكنترول). وبشكل عام سبب مستخلص الثوم فى الثمار المحقونة بفطر بوطريتس أعلى انخفاض في نشاط هذا الإنزيم يليه شاى الكومبوشا ثم مستخلص البردقوش ، حبة البركة ، القرنفل ، الحناء ، الزعتر ثم الزنجبيل على التوالي أما فى الثمار المحقونة بفطر

فيوزاريوم فقد كان مستخلص الزعتر الأكثر فعالية فى هذا الخصوص يليه مستخلص حبة البركة ، شاى الكومبوشا ، الحناء ، البردقوش ، الزنجبيل ، الثوم ثم القرنفل، على التوالي.

١٩- كان نشاط الإنزيم بوليفينول أوكسيديز أعلى في ثمار الخيار والكوسة التى تم حصادها من نباتات تم رشها بأي من المستخلصات النباتية المختبرة أو شاي الكومبوشا وذلك عند حقنها بفطريات عفن الثمار تحت الإختبار مقارنة بثمار معاملة الكنترول. وقد تم تسجيل أعلى نشاط لهذا الانزيم في ثمار الخيار التى تم حصادها من نباتات عوملت بمستخلص حبة البركة عند حقن هذه الثمار بالفطر بوطريتس ، أو مستخلصات البردقوش وحبة البركة عند حقنها بالفطر فيوزاريوم. أما فى ثمار الكوسة فقد سجلت مستخلصات حبة البركة ، شاى الكومبوشا ، الزعتر ، الحناء ثم القرنفل على التوالي أعلا نشاط لهذا الإنزيم عند حقن الثمار بالفطر بوطريتس ومستخلصات البردقوش والحناء والقرنفل عند حقنها بالفطر فيوزاريوم.

٢٠- تم تسجيل أعلا نشاط لإنزيم البيراوكسيديز في ثمار الخيار المقطوفة من نباتات سبق رشها بمستخلصات الحناء أو الزنجبيل أو شاي الكومبوشا على التوالى عند حقن الثمار بفطر بوطريتس أو مستخلصات الحناء ، شاى الكومبوشا أو البردقوش على التوالى عند حقنها بالفطر فيوزاريوم) وبالنسبة للكوسة فقد تم تسجيل أعلا نشاط لهذا الإنزيم فى الثمار المقطوفة من نباتات سبق رشها بمستخلصات الحناء أو شاى الكومبوشا عند حقن هذه الثمار بالفطر بوطريتس مستخلصات حبة البركة أو الحناء عند حقنها بالفطر فيوزاريوم.

صفحة الموافقة علي الرسالة

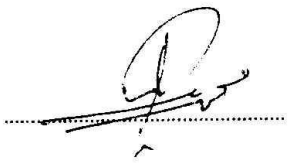
دراسات مرضية على أعفان ثمار القرعيات

رسالة مقدمة من

صفـــــاء السيد علـــوان

بكالوريوس العلوم الزراعية (أمراض نبات) ١٩٩٢
ماجستير العلوم الزراعية (أمراض نبات) ١٩٩٩
كلية الزراعة – جامعة الزقازيق

وقد تمت الموافقة على الرسالة ومناقشتها:
اللجنـــة:



أ.د. محمد محمد عمـــار

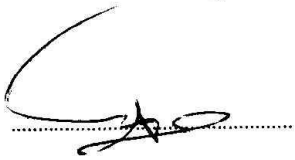
أستاذ أمراض النبات – كلية الزراعة

جامعة المنوفية (رئيساً)

أ.د. عبد المنعم إبراهيم إسماعيل الفقي

أستاذ أمراض النبات – كلية الزراعة

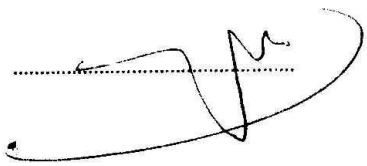
جامعة بنها (مشرفاً)



أ.د. جهاد محمد دسوقي الهباء

أستاذ أمراض النبات – كلية الزراعة

جامعة بنها (مشرفاً)



أ.د. فتحي جاد محمد عبد الجواد

أستاذ أمراض النبات – كلية الزراعة

جامعة بنها (ممتحناً)

تاريخ المناقشة: ٦ / ٢ /٢٠١٢م

لجنة الإشراف

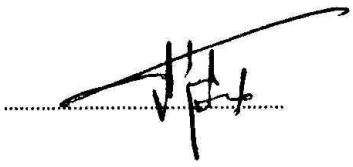
دراسات مرضية على أعفان ثمار القرعيات

رسالة مقدمة من

صفـــــــاء السيد علـــوان

بكالوريوس العلوم الزراعية (أمراض نبات) ١٩٩٢
ماجستير العلوم الزراعية (أمراض نبات) ١٩٩٩
كلية الزراعة ـجامعة الزقازيق

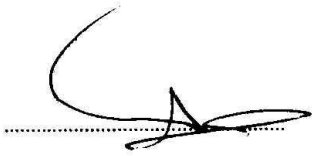
لجنة الإشراف:



أ.د. عبد المنعم إبراهيم إسماعيل الفقي

أستاذ أمراض النبات – كلية الزراعة

جامعة بنها



أ.د. جهاد محمد دسوقي الهباء

أستاذ أمراض النبات – كلية الزراعة

جامعة بنها

د. فاطمة سيد أحمد .....

باحث أول أمراض النبات -معهد بحوث أمراض النبات

مركز البحوث الزراعية - الجيزة

قسم النبات الزراعي

فرع أمراض النبات

كلية الزراعة بمشتهر

جامعة بنها

دراسات مرضية على أعفان ثمار القرعيات

رسالة مقدمة من

صفـــــاء السيد علـــوان

بكالوريوس العلوم الزراعية (أمراض نبات) ١٩٩٢

ماجستير العلوم الزراعية (أمراض نبات) ١٩٩٩

كلية زراعة –جامعة الزقازيق

لاستيفاء متطلبات الحصول على درجة

دكتوراة الفلسفة في العلوم الزراعية

(أمراض النبات)

قسم النبات الزراعي

كلية الزراعـــة

جامعة بنها

٢٠١٢