بسم الله الرحمن الرحيم

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Antifungal Effect of Selected Native Plant Extracts on *Botrytis cinerea* the Causal Agent of Gray Mold

By

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List of Abbreviations

Abb.	Item	Abb.	Item
Ap	*Anthemis palestina*	PDA	Potato Dextrose Agar
Ah	*Artemisia herba-alba*	PDB	Potato Dextrose Broth
Cc	*Coridothymus capitatus*	*B. cinerea*	*Botrytis cinerea*
Iv	*Inula viscosa*	P.Bc	Palestinian *Botrytis cinerea* isolate
Ms	*Majorana syriaca*	CFU	Colony Forming Unit
Mc	*Marticria chamomilla*	RH	Relative Humidity
Mp	*Mintha piperita*	VPD	Vapor Pressure Deficit
Ob	*Ocimmum basilicum*	gm	Gram
Ov	*Origanum valgare*	cm	Centimeter
Pa	*Paronychia argentea*	ml	Milliliter
Pr	*Phagnalon rupestre*	µl	Microliter
Ro	*Rosemarinus officinalis*	CK	Control
So	*Salvia officinalis*	rpm	Round Per Minute
Sa	*Sinapis alba*	W/V	Weight /Volium
Sd	*Stachys distans*	µg	Microgram
Tp	*Teucrium polium*	UV	Ultraviolet
Tv	*Thymus vulgaris*	nUV	Near Ultraviolet
Vi	*Varthemia iphionoides*	MDR	Multidrug Resistant

ABSTRACT

The fungus *Botrytis cinerea* is an opportunistic pathogen on a wide variety of crops, causing a disease known as gray mold. The disease can be controlled by several means including cultural practices and fungicides. The development of resistance to several fungicides in *B. cinerea* was observed and several alternatives were suggested including biological control and plant extracts. The effect of eighteen native Palestinian selected plant extracts: *Anthemis palestina*, *Artemisia herba-alba*, *Coridothymus capitatus*, *Inula viscosa*, *Majorana syriaca*, *Marticria chamomilla*, *Mintha piperita*, *Ocimmum basilicum*, *Origanum valgare*, *Paronychia argentea*, *Phagnalon rupestre*, *Rosemarinus officinalis*, *Salvia officinalis*, *Sinapis alba,* *Stachys distans*, *Teucrium polium*, *Thymus vulgaris*, and *Varthemia iphionoides*, were evaluated against the pathogen and disease *in vitro* and *in vivo*.

The results showed potent inhibitory effects on mycelial growth rate of *B. cinerea* isolates by all selected plant extracts tested. The strongest antifungal activity was observed with the extracts of *I. viscosa*, *M. syriaca*, *S. officinalis*, *T. vulgaris* and *V. iphionoides*, which inhibited the Mycelial growth rate of *B. cinerea* isolates by 76 - 100% at the concentration of 4%, significant variation in *B. cinerea* isolates sensitivity was observed. The strongest antifungal activity against *B. cinerea* conidial germination was observed with the extract of *I. viscosa* which inhibited the conidial germination of *B. cinerea* isolates completely. However, at low concentration (EC_{50}) of *I. viscosa* extract, a significant variation in *B. cinerea* isolates sensitivity was observed; the inhibition of conidial germination ranged between 11.5% and 100% at the EC_{50}, and between 51.5% and 100% at the EC_{90}. In the integrated control study, when plant extracts were combined with the fungicide Rovral®,

significant reduction in the disease severity (94%) was observed. The application of *I. viscosa* extract, alone, at the concentration (3%), reduced the disease severity induced by *B. cinerea* isolates on bean plants by 31%. This study concludes an important potential of some selected (medicinal) plant extracts in controlling *B. cinerea*, especially when combined with reduced dose of fungicidal compound which gives this control method an integrated approach dimension that can aid in reducing the environmental and health hazards, potential fungicides in the full dose may cause. However, further studies in the field and on mode of action and stable formulation of these medicinal plant extracts are needed before reaching the stage of wide-scale application and conclusions in the field of gray mold disease control.

Chapter one

1. General Introduction

1.1 *Botrytis cinerea*

1.1.1 Importance and host range

The fungus *Botrytis cinerea* Pers.:Fr, the anamorph of *Botryotinia fuckeliana* (De Barry) Whetzel is pathogenic on a wide variety of crop plants (Schoonbeek *et al.,* 2001).

The asexual stage of *B. cinerea* is classified in the genus *Botrytis*, which belongs to the family *Moniliaceae.* All pathogenic *Botrytis* species are necrotrophic, since plant cells are actively killed during pathogenesis (Prins *et al*., 2000).

B. cinerea causes Gray mold rot or Botrytis blight that affects nearly all species of dicotyledons, including most vegetable and fruit crops, flowers, woody ornamentals, and greenhouse-grown crops. The fungus uses a wide range of infection strategies that allow it to directly penetrate mature-to-senescent leaves and other tender tissues, such as seedlings, floral organs, and mature fruits. The fungus generally infects host tissues in cool damp weather (10 to 25°C) in water droplets (Prins *et al*. 2000), but it also can germinate at high humidity in the absence of water droplets (Williamson *et al*. 1995).

1.1.2 Life cycle and epidemiology

The disease induced by the fungus *B. cinerea* is mainly described as gray mold and causes serious economic losses (Schoonbeek *et al.*, 2001). The most favorable conditions for growth and development of the fungus are warm temperatures and high humidity levels.

1.1.2.1 Survival

The disease cycles of *Botrytis* species and the growth habit and phenologies of their host plants are often inextricably linked. Dormant or metabolically inactive fungal structures play a central role in each of these disease cycles. Each part of the fungus thallus can serve as a survival structure.

All species of *Botrytis* form sclerotia which may, depending on isolate and cultural conditions, differ in size and shape. Sclerotia are generally considered to be the most important structures involved in the survival of *Botrytis* species. Sclerotia can survive adverse environmental conditions, can produce apothecia after a sexual process and possess a considerable capacity for producing successive crops of conidia in many *Botrytis* species (Coley-Smith, 1980). Under laboratory conditions. *B. cinerea* sclerotia continue to sporulate for about 12 weeks after the production of the first crop of conidia (Nair and Nadtotchei, 1987). Formation of sclerotia in the field is generally associated with plant tissues. Louis *et al.* (1996) demonstrated the ability of the vinegar fly, *Drosophila melanogaster,* to serve in survival and dispersal of *B. cinerea.* Long-term *D. melanogaster/B. cinerea* relationships were found during the life cycle of the insect. Conidia germinated in the insect foregut, developed into mycelium, and differentiated into microsclerotia, which can be carried by the flies. Since the fly overwinters as an adult, it was concluded that it could play a role in winter conservation of *B. cinerea* inoculum (Holz *et al.*, 2004).

Chlamydospores have been found in *B. cinerea, B. anthophila* and *B. fabae* (Coley- Smith 1980). The chlamydospores of *B. cinerea* are hyaline cells of extremely variable form and size (Holz *et al.*, 2004). They are generally found in ageing cultures and commonly occur in the stromatic sectors of cultures of the fungus which are contaminated by other

organisms, and in association with sclerotia. Clamydospores are formed as terminal or intercalary cells by transformation of vegetative mycelium parts and are liberated by hyphal disintegration. They were observed on and in tissue of naturally and artificially infected tomato and *Fuchsia hybrida* leaves and their numbers increased in older lesions (Holz *et al.*, 2004). Under moist conditions and without added nutrients, the chlamydospores germinated on the leaves by microconidia which remained dormant. When fresh nutrients were supplied to the chlamydospores, they germinated with hyphae penetrating the host, or they produced a new crop of macroconidia. On fruit of nectarine, plum and pear, germlings produced from dry airborne *B. cinerea* conidia formed chlamydospores on short germ tubes when fruits were subjected to intermittent dry periods, or were kept for 48 h at 5°C (Holz, 1999). Chlamydospores can therefore serve as short term survival structures which may help the fungus to overcome short unfavourable periods encountered on plant surfaces (Holz *et al.*, 2004).

Conidia of *Botrytis* are generally regarded as short-lived propagules in the field and their survival will largely be determined by temperature extremes, moisture availability, microbial activity and sunlight exposure. In the soil, *Botrytis* species are not particularly effective competitors and their conidia are subjected to fungistasis (Coley-Smith, 1980). Salinas *et al*. (1989) reported that conidia stored dry were able to survive at room temperature for up to 14 months. However, on the surface of Anjou pears, the viability of *B. cinerea* conidia after 7 weeks had declined to 10% germination (Sportts, 1985). When *B. cinerea* conidia were exposed to direct sunlight at midday in a Palestinian summer, survival was only for minutes (Rotem and Aust, 1991). The UV spectrum of sunlight appeared to be the most important environmental factor influencing mortality of conidia (Rotem and Aust, 1991; Seyb, 2003).

Microconidia, which occur in all *Botrytis* species, provide an alternative microscopic propagule for these fungi when subjected to adverse conditions. In general they are found in ageing cultures of the fungus or those which are contaminated by other organisms, and in association with sclerotia. Microconidia develop from germ tubes produced by macroconidia, more mature hyphae, inside empty hyphal cells, and from appressoria and sclerotia (Jarvis, 1980a).

The survival of mycelium of *Botrytis* species under natural conditions has hardly been investigated and, it is often difficult to decide whether survival is by mycelium or whether microsclerotia or chlamydospores are involved. There is some evidence that the mycelium of certain *Botrytis* species, and especially those more specialized in their parasitism, can survive for considerable periods in bulbs, seeds and other vegetative plant parts (Coley-Smith, 1980).

1.1.2.2 Inoculum production and dispersal

It is generally assumed that for *B. cinerea,* inoculum is always present in the field and that production, liberation and dispersal of inoculum is an ongoing process (Jarvis, 1980b). This is clearly not always the case in all crops (Sosa-Alvarez *et al*., 1995; Seyb, 2003). There are various factors essential for high propagule numbers in the air: a viable, productive inoculum source, conditions favourable for propagule production, and for their dispersal at the source site. Correlations have been found between dispersal and conditions favourable for sporulation (usually surface wetness with moderate temperature) in many *Botrytis* species (Jarvis, 1980b). The frequency and duration of wetness events, and temperature, vary greatly during a growing season. It is anticipated that interrupted wetness periods, and temperature fluctuations, will affect the number of propagules produced (Rotem *et al*., 1978). A complicated relationship

thus exists in the field between environmental conditions and propagule production and dispersal.

Conidia, which are dry and predominantly wind-dispersed, are generally considered to be the most important dispersal propagule of *Botrytis* species. Wind dispersal of conidia has three highly interdependent, yet distinct phases; liberation, transport and deposition (Aylor, 1990). Release of conidia in *Botrytis* species is caused by a twisting of the conidiophore which is brought about by changes in the relative humidity (Fitt *et al.*, 1985) and their ejection by a mechanical shock. This mechanical shock has been considered to be caused by two forces, wind and splash (Fitt *et al.*, 1985).

The last phase in the dispersal of wind-borne conidia, deposition, is composed of two main processes, sedimentation and impaction (Aylor, 1978), both of which are influenced by wind strength. On table grapes for example, at any time during the growing season it is most destructive after a rainfall period accompanied by warm temperatures either at flowering or late in the growing season.

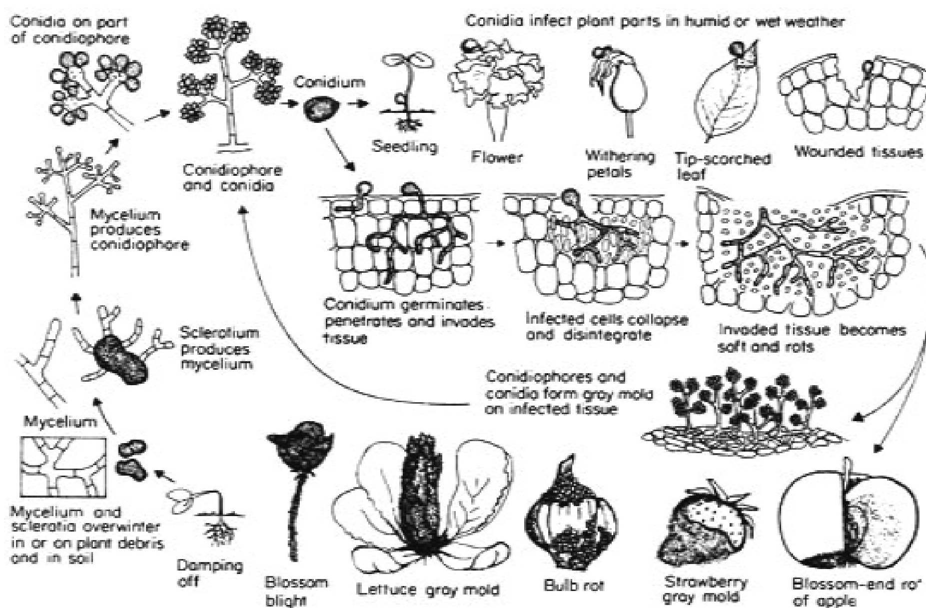

Fig. 1: Overview of the life cycle of *Botrytis cinerea*, adapted from Agrios (1997).

Conidia of *Botrytis* species are also insect-dispersed. The conidia of *B. cinerea* are trapped in the ornamentations of segments, cuticle, body hairs and sculptured areas of the vinegar fly *(D. melanogaster)* (Louis *et al*., 1996), the grape berry moth *(Lobesia botrana)* (Fermaud and Le Menn, 1989), the New Zealand flower thrips *(Thrips obscuratus)* (Fermaud and Gaunt, 1995) and the Mediterranean fruit fly *(Ceratitis capitata)* (Engelbrecht, 2002). Ingested conidia also remain viable inside faeces of these insects. In the case of the Mediterranean fruit fly, digital photography and visual observations of grape berries showed that the flies initially preferred to feed on the macerated tissue of the lesions that served as inoculum. However, they tended to feed on the sporulating colonies on the lesions (Engelbrecht, 2002). This was evident by the distinctive 'feeding paths' that appeared in the colonies as a result of their activities, and the disappearance of *B. cinerea* conidia from the colonies. Fluorescence microscopy revealed that flies deposited conidia singularly, in feeding packages and in faeces on the surface of unblemished grape berries (Engelbrecht, 2002).

In some cases conidia seem of less importance than saprophytically-based Mycelial inocula in establishing infections (Holz *et al*., 2004). It may well be that ascospores are more important than generally assumed; apothecia are easily overlooked in the field. In addition and due to the ability of chlamydospores to germinate, they also represent dispersal units which can function as structures of infection. Holz *et al*. (2004) noted that in moist conditions the protective covering around microconidia aggregates became sticky and speculated that this may aid microconidia to adhere to surfaces of plants and insect vectors, which is indicative of their potential role in the survival and dispersal of the fungus.

1.1.2.3 Factors that influence *B.* cinerea-incited epidemics in greenhouse crops.

1.1.2.3.1 Greenhouse climate

B. cinerea can thrive under a range of temperatures between 2 and 30°C (Elad and Yunis, 1993; Yunis *et al*, 1994). The optimum temperatures for the different growth phases are summarised by Jarvis (1992) and range from 12-30°C. *B. cinerea* will therefore always be a potential threat in greenhouse crops. Spore dispersal is stimulated by rising or falling humidity, and therefore atmospheres of quite constant humidity should be the objective. Temperature and relative humidity (RH), both have a direct effect on *B. cinerea* epidemics, but in greenhouses the effect of climate generally constitutes a combined effect of the two. The two important parameters associated with outbreaks of *B. cinerea*-incited epidemics in vegetables in non-heated greenhouses were duration of leaf wetness (threshold 7 h per day) and duration of night temperatures between 9 and 21°C (threshold 9.5 h per day) (Elad *et al.*, 1992; Yunis *et al*, 1994).

1.1.2.3.2 Light

Light, especially near UV (nUV) light, increases sporulation in *B. cinerea* , both on agar plates and on different crops. About a 54-fold increase in sporulation was found when colonies were irradiated with nUV light plus white light, compared to white light only when testing 83 isolates (West *et al.*, 2000). Different types of polyethylene used for tunnels influence the sporulation of *B. cinerea* (Reuveni *et al.*, 1989). In a field trial using polyethylene tunnels the incidence of infection of primula and strawberries over two seasons was reduced by 50% and 26%, respectively, when nUV blocking film (up to 405 nm) was used compared to a standard film (West *et al.*, 2000).

1.1.2.3.3 Carbon dioxide enrichment

Enrichment of greenhouse atmospheres with carbon dioxide is common practice in commercial greenhouses, usually combined with decreased ventilation to attain the target CO_2 levels. *B. cinerea* is inhibited by carbon dioxide levels of 4-5% (Svircev *et al.*, 1989; Elad, 1993), but unfortunately these levels are not reached in normal commercial greenhouses. Decreased ventilation and CO_2 enrichment may enhance foliage density and humidity, thus stimulating *B. cinerea*, especially when CO_2 burners are used which produce additional water vapour (Dik and Wubben, 2004).
Other factors like, sanitation, cultivar, plant spacing, cropping methods, fertilizer, and the irrigation regime, were discussed by (Dik and Wubben, 2004).

1.1.3 Disease control of *B.cinerea*

1.1.3.1 Plant breeding

Susceptibility and resistance to crop plants to *B. cinerea* appears to be a polygenic trait. This implies a considerable variation in *Botrytis* susceptibility amongst different varieties, which cannot be attributed to a single characteristic. A common strategy to breed for resistance is to cross susceptible crop plants with resistant relatives, to introduce quantitative trait loci associated with *B. cinerea* resistance. Another approach is the generation of transgenic crops that overproduce phytoalexins with a known activity against *B. cinerea*. Transgenic tobacco expressing the grapevine stilbene synthase produced resveratrol and displayed increased resistance (Hain *et al.*, 1993). This approach may also be valid for other crops , although transgenic expression of this gene in tomato did increase resistance to *Phytophtora infestans* but not to *B. cinerea* (Schoonbeek, 2004). Breeding for resistance has not been as

successful as hoped for but significant, *B. cinerea/*cultivar interactions indicate that greater emphasis should be placed on cultivars with morphological, anatomical and inherent host defence mechanisms which reduce susceptibility (Mlikota Gabler *et al.*, 2003).

1.1.3.2 Cultural methods

Good agricultural practices are very important in control of gray mold. A common practice is sanitation to reduce sources of inoculum. This can be achieved by starting with clean material and keeping/ remaining pruned plant material away from the crop. This practice is particularly useful in greenhouses. Another important practice is to reduce the length of leaf wetness periods, which is essential for spore germination and penetration. This can be achieved by increasing plant distance, trimming of the canopy, ventilation, thereby controlling of temperature and humidity (Schoonbeek, 2004).

According to Hayashi (2003), the main strategies to reduce gray mold by cultural methods under greenhouse and field conditions are: i. reduction of the humidity by ventilation, lowering of the water supply, and temperature control; ii. decrease of inoculum by removing dead, decayed, or infected materials; iii. reduction of wounding by birds, insects, fungal infection, hail, and frost; iv. reduction of crop density in order to create an unfavourable microclimate for *B. cinerea*; v. limited nutrient conditions, and vi. the use of UV films in greenhouse to prevent induction of conidia formation. Consequently, in many crops cultural practices cannot provide sufficient disease control.

1.1. 3.3 Biological control and induced resistance

Biological control is based on the application of competitive or parasitic micro-organisms which may compete for space or nutrients may produce

antagonistic antibiotics or may hyperparasitize mycelium (Jakab *et al*., 2001). For example, in practice successful results have been obtained with *Ulocladium atrum* which antagonize *B. cinerea* by competing for nutrients. The micro-organisms can also exert their function indirectly by stimulation of plant responses.

Several biocontrol agents are reportedly effective against *B. cinerea* (Elad *et al*., 1994), among which are isolates of the filamentous fungus *Trichoderma harzianum* Rifai. A commercial preparation developed from the isolate T39 of *T. harzianum* (Trichodex) has been registered for agricultural use in several countries. Barakat, and Al-Masri, 2005, reported that *T. harzianum* Jn14 isolated from Palestinian soils, significantly reduced gray mold disease severity on tomato and bean plants.

Various signaling pathways are involved in the activation of induced resistance, such as systemic acquired resistance or induced systemic resistance. These pathways depend to different degrees on signaling molecules such as salicylic acid, ethylene, and jasmonic acid. The induction of systemic resistance can also be triggered by application of salicylic acid or its homologue, benzothiadiazole and ß- aminobutyric acid (Jakab *et al*., 2001). Many microbial species have been investigated for biological control of *B. cinerea* . Although there are many successful reports, biological control is not yet a reliable method for *B. cinerea* control in practice. A general drawback of microbial biocontrol agents is that they do not maintain their activity in crops under field conditions that favour *B. cinerea*.

1.1.3.4 Chemical control

B. cinerea is one of the first recorded targets of chemical disease control ever. Nicot *et al.,* 2000, mentioned that the Romans already

applied elemental sulphur to control gray mold and mildew diseases in grapes. Fungicides are the main strategy for chemical control of gray mold (Nicot *et al.,* 2000). In 1950 non-systemic fungicides were introduced, but their specificity was low. From 1995 onwards, novel classes of fungicides with specificity against *B. cinerea* were commercialized. Resistance development to fungicides is due to the emergence of fungicide resistant mutants in wild-type populations upon selection pressure of fungicides in space and time (Schoonbeek, 2004). Chemical control is therefore of utmost importance, but have suffered in different crops due to the development of fungicide resistance in *B. cinerea* (Schoonbeek *et al.,* 2001). Several chemical classes of novel botryticides, with specificity against *B. cinerea* have been developed since the 1950's when conventional fungicides such as aromatic hydrocarbons and dithiocarbons and dithiocarbamates, were introduced. These fungicides have a specific mode of action and have no systemic activity in plants (Hayashi, 2003). Although conventional fungicides such as chlorothalonil, dichlofluanid, or thiram are still used to control *B. cinerea*, most of these compounds are weak botryticides. Systemic fungicides such as benzimidazoles and dicarboximides have also been used for gray mold control since the 1970's. During the last decade anilinopyrimidines, fenhexamid, fluazinam, phenylpyrroles, and strobilurins were introduced as new botryticides. However, their efficacy against *B. cinerea* is hampered by rapid emergence of resistance (Hayashi, 2003).

Chemical fungicides either kill the fungus itself (fungicidal products) or stop its growth (fungistatic products). In both cases, the fungicide attacks the biological structure (for example, the cell wall) or biological function (for example, protein synthesis) of the fungus. Over time, natural selection frequently occurs, with resistant strains of the fungus surviving

and eventually replacing the strains that are susceptible to the fungicide. The fungicides become progressively less effective and must eventually be either modified or replaced by an entirely new fungicide (Nicot *et al.,* 2000).

Historically, chemical fungicides have proved to be non-specific and therefore can act on organisms other than the target fungus, including other naturally occurring beneficial agents. Because of their chemical nature, they may also be toxic and non-biodegradable. Chemical residues can build up in the soil and throughout the food chain. Consumers worldwide are becoming increasingly conscious of the potential environment and health problems associated with the build - up of toxic chemicals, particularly in food products. This has resulted in a growing consumer pressure to reduce the use of chemical pesticides. As a result, “organic” products or those resulting from sustainable production programmes- produced without the aid of chemicals- are increasingly perceived as more-healthy, more desirable and of premium value (Built and Dubos, 1988).

Fungicide mixtures are widely used in commercial products. The main advantages of mixture are that they can extend the antifungal spectrum of the single products and delay resistance development to the individual component. Fungicide mixtures may also display a synergistic interaction by which the amount of active ingredients can be reduced (De Waard, 1987). If a synergist could annul the mechanism of resistance to a particular fungicide, the synergistic activity of a mixture would be limited to the fungicide-resistant subpopulation of a pathogen. Such synergists need not necessarily be fungitoxic by themselves and could be useful as an anti-resistance strategy (Hayashi, 2003). Deployment of mixtures of a multi-site inhibitor with a site-specific fungicide, are probably the best

strategy to lower the risk of resistance development and widen the fungicidal spectrum (Hayashi, 2003).

1.1.3.5 Multidrug resistance in *B. cinerea*

It is very well documented that micro-organisms have proven to become resistant against antimicrobial compounds (Marchetti *et al.*, 2000). One of the major modes by which pathogens develop resistance is through the development or enhancement of methods for the removal of antibiotics that have entered the cells of the organism. Thus, resistant microorganisms posses efficient systems known generally as multidrug resistant pumps (MDR-pumps) (Morel *et al.*, 2003).

Survival of micro-organisms in natural environments is favored by the capacity to produce compounds toxic to competing organisms, and the ability to resist the effects of such toxic compounds. Both factors contribute to a competitive advantage of organisms in ecosystems. Most organisms have evolved active transport mechanisms by which endogenous toxicants can be secreted. Two major classes of transport proteins are the ATP -binding cassette (AP.BC) and the major facilitator superfamily (MFS) transporters. Members of both families can be regarded as “first line defence barrier” in survival mechanisms. In plant pathogens, these transporters can play an essential role in protection against plant defence compounds during pathogenesis. AP.BC and MFS transporters can play a major role in fungicide sensitivity and resistance (Schoonbeek *et al.*, 2000b).

Another mechanism, which may play a big role in resistance to fungicides in *B. cinerea* , is decreased accumulation of the compound in Mycelial cells due to energy-dependant efflux. Subsequently the driving force behind the energy –dependant efflux of the fungicides can be the ATP - binding cassette (AP.BC) transporters. Members of the major facilitator

superfamily (MFS) transporters may have similar functions. They are membrane bound proteins; these transporters moderate the activity of several classes of toxic compounds. Some of these transporters can be regarded as fungicide pumps, which may account for the multi-drug resistance of *B. cinerea* (Schoonbeek *et al.*, 2001). AP.BC transporters use the energy of the ATP hydrolysis to transport compounds over membranes. They can move toxins from the inner leaflet of the membrane to the outer environment of cells thereby reducing accumulation of the compound in the cells. MFS transporters prevent accumulation of toxic compounds in cells through the process of the "proton motive force" over membranes (Hayashi *et al.*, 2002). Therefore the transporters in filamentous fungi play a role in the protection of *B. cinerea* to fungitoxic compounds (Schoonbeek *et al.*, 2000a).

1.1.4 Plant defence mechanism against *B. cinerea*

There are two types of antimicrobial metabolites: phytoalexins and phytoanticipins (VanEtten *et al.*, 1994). Phytoanticipins are preformed, while phytoalexins are induced by pathogen infection. Several of the plant hosts of *B. cinerea* produce defence compounds belonging to various chemical classes, for example, phytoalexins, that act as constitutive or inducible chemical barriers. Phytoalexins are antimicrobial compounds of low molecular weight that are both synthesised by and accumulated in plant parts after exposure to microorganisms (Paxton, 1981). Considerable evidence supports the view that accumulation of phytoalexins at the site of attempted infection is one mechanism by which plants resist disease (Darvill and Albersheim, 1984).

Baarlen *et al*. (2004) reviewed and discussed the antimicrobial secondary metabolites of plants, which act as defence compounds against *Botrytis* infection.

1.1.5 *B. cinerea* response to plant defence compounds

B. cinerea is able to withstand toxic effects of plant defence compounds with varying structures and mechanisms. The broad host range of *B. cinerea* most likely implies that the fungus possesses an arsenal of complementary infection factors. These include toxins, other hydrolytic enzymes, and mechanisms to cope with plant defence mechanisms (Prins *et al.* 2000). Phytotoxic compounds that play a role in pathogenesis are oxalic acid and botrydial (Colmenares *et al.* 2002). Fungal enzymes that may be involved in infection and tissue colonization are cell wall degrading enzymes such as endo- and exopolygalacturonases, endo- and exopectate lyases, pectin lyases, pectin methylesterases, and, furthermore, proteases and phenoloxidases.

The broad host specificity of *B. cinerea* may relate to the presence of multiple, degenerate gene families encoding some of these enzymes. The presence of multiple functional homologues could allow breakdown of cell wall tissues from various hosts under different conditions (Ten Have *et al.,* 1998). In contrast to many (hemi) biotrophic pathogens, cell death incited by *B. cinerea* might facilitate growth of the pathogen, for example by release of nutrients (Govrin and Levine, 2000). This strategy is especially effective since it operates in concert with enzymes involved in protection of the pathogen against oxidative stress such as peroxidase, laccases, catalases, gluthatione-S-transferase, glutathione peroxidase, and superoxidase dismutase (Gil-ad *et al*., 2000).

The ability to withstand toxic effects of plant defence compounds that act as constitutive or inducible chemical barriers can also contribute to the virulence of fungi (Osbourn, 1999). *B. cinerea* posseses specific enzymes that degrade particular toxic plant compounds, such as a-tomatinase, that inactivates tomatin from tomato leaves and a laccase-like enzyme that inactivates resveratrol from grapevine (Adrian *et al.,* 1998). The pathogen

also possesses non-specific detoxification mechanisms such as glutathione-S-transferases and efflux pumps that can prevent the accumulation of multiple plant defence compounds in cells of the pathogen (Del Sorbo *et al.,* 2000). These non-specific mechanisms may be of particular relevance to *B. cinerea* since it has to cope with many, chemically unrelated, plant defence compounds in a broad host range (Schoonbeek, 2004).

1.1.6 Major groups of antimicrobial compounds from plants

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Cowan, 1999). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defence mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigments. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food, yield useful medicinal compounds.

Useful antimicrobial phytochemicals can be divided into several groups, described below:

1.1.6.1 Phenolics and Polyphenols: simple phenols and phenolic acids

Phenolic acids are phenolic compounds that have an aromatic ring to which a carboxylic acid group is attached. They are common in the plant either free or combined into esters or glycosides. Phenolic acids are antibacterial and antifungal (Wiart *et al*., 2000). Phenolic compounds including simple phenols and phenolic acids, hydroxycinnamic acids

derivatives and flavonoids are bioactive substances occurring widely in food plants. Many phenolic compounds in plants are good sources of natural antioxidants. According to Pratt and Hudson (1992), phenolic compounds were found abundantly in all parts of the plant, such as wood, bark, stems, leaves, fruit, roots, flowers, pollen and seeds. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds which are in the highest oxidation state**.** The common herb thyme contain caffeic acid, which is effective against viruses (Cowan, 1999), bacteria (Brantner, *et al.***,** 1996), and fungi (Duke, 1985). Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two -OH groups, and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Scalbert, 1991). In addition, Scalbert, 1991 found that more highly oxidized phenols are more inhibitory.The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with proteins (Mason and Wasserman, 1987).

Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial as well (Cowan, 1999).

1.1.6.2 Quinones

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds, being colored, are responsible for the browning reaction in

cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin (Fessenden and Fessenden, 1982). Their presence in henna gives that material its dyeing properties. Hydroxylated amino acids may be made into quinones in the presence of suitable enzymes, such as a polyphenoloxidase (Vamos-Vigyazo, 1981).

In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern *et al*, 1996.), often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism (Cowan, 1999).

1.1.6.3 Flavones, flavonoids, and flavonols

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol (Fessenden and Fessenden, 1982). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983), it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms.

Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996).

Delineation of the possible mechanism of action of flavones and flavonoids is hampered by conflicting findings. Flavonoids lacking

hydroxyl groups on their b-rings are more active against microorganisms than are those with the 2OH groups (Chabot *et al*., 1992); this finding supports the idea that their microbial target is the membrane.

1.1.6.4 Tannins

"Tannin" is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3,000 (Haslam, 1996), and they are found in almost every plant part: bark, wood, leaves, fruits, and roots (Scalbert, 1991). They are divided into two groups, hydrolyzable and condensed tannins. Hydrolyzable tannins are based on gallic acid, usually as multiple esters with D-glucose, while the more numerous condensed tannins (often called proanthocyanidins) are derived from flavonoid monomers. Tannins may be formed by condensations of flavan derivatives which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinone units (Serafini *et al*., 1994). This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of ills (Serafini *et al*., 1994).

1.1.6.5 Coumarins

Coumarins are phenolic substances made of fused benzene and a-pyrone rings . They are responsible for the characteristic odor of hay. As of 1996, at least 1,300 had been identified (Hoult and Paya, 1996). Their fame has come mainly from their antithrombotic (Thastrup *et al*., 1985), anti-inflammatory (Piller, 1975), and vasodilatory activities (Namba *et al*., 1988).

1.1.6.6 Terpenoids and Essential Oils

The fragrance of plants is carried in the so called quinta essentia, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is C10H16, and they occur as diterpenes, triterpenes, and tetraterpenes (C20, C30, and C40), as well as hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain additional elements, usually oxygen, they are termed terpenoids (Cowan, 1999).

Plant secondary metabolites, such as essential oils and plant extracts (Tepe *et al*., 2004), are studied for their antimicrobial activities and most essential oils derived from plants are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activities (Faleiro *et al*., 1999). Therefore, they are intensely screened and applied in the fields of pharmacology, pharmaceutical botany, medicinal and clinical microbiology, phytopathology and food preservation (Daferera *et al*., 2000).

1.2 Selected Native Wild Plants

1.2.1 *Artemisia herba-alba*

Artemisia herba-alba Asso, (family Compositae), common name (Herba-alba), found throughout the northern half of the world (Marco and Barbera, 1990). In addition, Herba-alba is a medicinal and aromatic dwarf shrub that grows wild in arid areas of the Mediterranean basin, extending into northern Himalayas (Vernin *et al*., 1995). This species has a vegetative growth in autumn (large leaves) and then at the end of winter to spring (small leaves). It is traditionally known for its essential oils. It has a very pronounced purgative effect and play a major role in the control of intestinal worms (Idris *et al*., 1982). Extracts from *A. herba-*

alba have antidiabetic effect (Al-Waili, 1986; Al Khazraji *et al*., 1993; Al-Shamaony *et al*., 1994; Jouad *et al*., 2001) and strong antibacterial activities (Hatimi *et al*., 2001; Neerman, 2003). It also shows an allelopathic role against some other plants (Escudero *et al*., 2000). In addition, flavonoids from this plant have a neurological action (Medhat Salah and Jäger, 2005).

1.2.2 *Anthemis palestina*

Anthemis palestina Reuter, (family Compositae), common name (Palestine Chamomile) an annual herb, 15-30 cm long, showy, with many spreading, basal branches. Leaves dark green, dissected into narrow segments. Heads 2-3 cm in diameter, with bright white, spreading, marginal, ray florets and yellow disc florets. It is common in fields and waste grounds, especially mountainous regions (Al-Eisawi, 1998).

1.2.3 *Coridothymus capitatus*

Coridothymus capitatus (L.) Reichenb. fil. [syn. *Thymus capitatus* (L.) Hoffmanns. & Link], (family Labiatae (Lamiaceae), common name (conehead thyme) a perennial, herbaceous shrub commonly used as a spicy herb. Until recently, Thymus essential oils have been studied, mostly from the viewpoint of their flavour and fragrance chemistry, only for flavouring foods (Karousou, 1995). Nowadays, however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Sawamura, 2000; Ormancey *et al*., 2001). Many authors have reported antimicrobial, antifungal, antioxidant and radical scavenging properties of essential oils. Thyme essential oils were reported to have antimicrobial activities (Bhaskara *et al*., 1998), most of which are mediated by thymol and

carvacrol, as the phenolic components of the oil. Spasmolytic as well as antioxidant activities (Miguel *et al.*, 2004; Sacchetti *et al.*, 2005) were also reported for the phenolic oil extract of the plant. In addition, there is some evidence that minor components have a critical part to play in biological activities, possibly by producing a synergistic effect between other components. Several studies have focused on the antimicrobial activity of the essential oils of thyme in order to identify the responsible compounds (Bounatirou, *et al.*, 2007). Phenols seem to play an outstanding role; these terpene phenols join to the amine and hydroxylamine groups of the proteins of the bacterial membrane altering their permeability and resulting in the death of the bacteria (Juven *et al.*, 1994). Although this holds true for the majority of *C. capitatus* oils studied, scattered publications reported thymol or both phenols as main oil constituents (Ravid and Putievsky, 1983; Cosentino *et al.*, 1999; Go¨ren *et al.*, 2003).

1.2.4 *Inula viscosa*

Inula viscosa (L.) Aiton (syn. *Cupularia viscosa* G. et G., *Dittrichia viscosa* Greuter) (family Compositae), common name "Clammy Inula" is a perennial weed, native to the Mediterranean. It grows on hillslopes, damp habitats, and roadsides. In folklore medicine, this plant is used for therapeutic purposes, such as a diuretic, topical anti-inflammatic, and haemostatic (Lev and Amar, 2000). It is also well documented to have antiulcerogenic effects (Alkofahi and Atta, 1999), to cause abortion (Farnsworth *et al* ., 1975; Al-Dissi *et al.*, 2001) or inhibition of zygote implantation in mammals (Al-Dissi *et al* ., 2001), to inhibit growth of pathogenic fungi (Maoz and Neeman, 2000), to be a strong anti-oxidant (Schinella *et al* ., 2002) and to be a hypoglycaemic agent to treat diabetes

(Zohara *et al* .,1987). For these reasons, this species is of major interest for many pharmaceutical industries.

Aqueous extracts of *I. viscosa* were shown to exhibit antifungal activity *in vitro* (Qasem *et al*., 1995; Maoz, and Neeman, 1998), and organic solvent extracts were shown to be antibacterial (Debat, 1981). Cohen *et al.*, 2002, provided evidence for the antifungal activity *in planta* of extracts made with organic solvents, including methanol, ethanol, ethylacetate, acetone, chloroform, and nhexane (Maoz and Neeman, 1998).

1.2.5 *Majorana syriaca*

Majorana syriaca (L.), (family Labiatae (Lamiaceae), common name (thyme) is traditionally used in the Middle East as a food-flavouring ingredient. The plant is commonly named in Arabic as “sa’atar” or “za’atar” and there are several wild and cultivated populations in Palestine (Ravid and Putievsky, 1983, Putievsky *et al*, 1996). According to Putievsky *et al*. 1996, two chemotypes of *M. syriaca* are distinguished: one rich in carvacrol and the other rich in thymol. Ishac and Kharoub (1985) mentioned that the Palestinian variety is characterized by the high consistency of carvacrol in its essential oil. In addition to carvacrol and thymol, the other most common compounds of the essential oil of *M. syriaca* are the two monoterpene hydrocarbons, g-terpinene and *p*-cymene, which are the biogenetic precursors of the two phenolic terpenes, carvacrol and thymol (Putievsky *et al*, 1996).

Thus, Shimoni *et al.*, (1993) found that the whole essential oils from *M. syriaca*, exhibited *in vitro* activity against a number of phytopathogenic fungi. In the search for novel, environmentally benign approaches to plant disease control, essential oils may be useful in their own right or as useful leads for the development of specific antifungal agents.

1.2.6 *Marticria chamomilla*

Marticria chamomilla (L.), (family Compositae), common name (chamomile), it is aromatic, annual herb,5-20 cm long, usually with main stem, few lateral branches. Leaves reatedly dissected into narrow to filiform. Heads yellow, globular, 5-8 mm in diameter, made only of tubular flowers, surrounded by narrow, green, soft involucres. It grows in marginal and desert places (Al-Eisawi, 1998). Essential oils of *M. chamommilla*, *M. piperita*, *M. spicata*, *Lavandula angusti* folia, *O. basilicum*, *T. vulgaris*, *O. vulgare*, *S. officinalis*, *Citrus limon* and *C. aurantium* and their components: linalyl acetate, linalool, limonene, α-pinene, β-pinene, 1,8-cineole, camphor, carvacrol, thymol and menthol were assayed for inhibitory activity against the three major pathogens of the button mushroom, *Agaricus bisporus*, i.e. the fungi *Verticillium fungicola* and *Trichoderma harzianum* and the bacterium *Pseudomonas tolaasii* (Sokovic *et al.*, 2006), all of these components exhibited *in vitro* activity against these pathogens.

1.2.7 *Mintha piperita*

Mentha piperita L. (family Labiatae), common name peppermint is a perennial herb native to Europe, naturalized in the northern USA and Canada, and cultivated in many parts of the world. The chemical components of peppermint leaves and oil vary with plant maturity, variety, geographical region and processing conditions (Clark and Menary, 1981; Maffei and Scannerini, 1992; Rohloff, 1999; Gherma *et al.*, 2000; Blanco *et al.*, 2002; Pino *et al.*, 2002; Ruiz del Castillo *et al.*, 2003; Xu *et al.*, 2003). The fatty acid composition of the non-polar lipid fraction of peppermint leaves is dominated by palmitic (16:0), linoleic (18:2) and linolenic (18:3) acids (Maffei and Scannerini, 1992). The main volatile components identified in the essential oil of peppermint are

menthol (33–60%), menthone (15–32%), isomenthone (2–8%), 1,8-cineole (eucalyptol) (5–13%), menthyl acetate (2–11%), menthofuran (1–10%), limonene (1–7%), β-myrcene (0.1–1.7%), β-caryophyllene (2–4%), pulegone (0.5– 1.6%) and carvone (1%) (Clark and Menary, 1981; Sang, 1982; Pittler and Ernst, 1998; Dimandja *et al.*, 2000; Gherman *et al.*, 2000). The leaves contain 1.2– 3.9% (v/w) essential oil (Picuric-Jovanovic *et al.*, 1997; Blumenthal *et al.*, 1998) (0.38% yield from fresh leaves) (Kaul *et al.*, 2001), while an infusion of dried leaves is reported to contain 21% of the original oil (25 mg/L).

1.2.8 *Ocimum basilicum*

Ocimum basilicum (L.) (family Labiatae), common name (Basil) is a medicinal plant originating in Asia and it has been widely investigated in different countries (Margarida *et al.*, 1990). Its antiinflammatory (Singh, 1999), antibacterial (Thoppil *et al.*, 1998) and insecticidal (Umerie *et al.*, 1998) activities have been ascertained in a number of studies.
Production of essential oils by plants is believed to be predominantly a defence mechanism against pathogens and pests (Oxenham, 2003) and indeed, essential oils have been shown to possess antimicrobial and fungicidal properties. Previous work has shown that the volatile components of the essential oil of basil, *O. basilicum*, possess *in vitro* activity against a number of plant pathogenic fungi (Reuveni *et al.*, 1984).

1.2.9 *Origanum valgare*

Origanum vulgare (L.) (family Labiatae), common name (oregano) is one of the many plants studied extensively for its antimicrobial activity. The major component of commercial essential oils of oregano is carvacrol, which was found to be as high as 60–75% (Fleisher and Sneer, 1982). The other major component found in origano is thymol. These two

phenolic compounds, thymol and carvacrol, are generally reported in ratios of 1:10–1:20 (Salzer, 1977). The reported antimicrobial properties of carvacrol and thymol are 1.5 and 20.0 times that of phenol, respectively (Aeschbach *et al.*, 1994). Many studies indicated the antimicrobial activity of oregano and two of its major components, carvacrol and thymol (Sokovic *et al*, 2006).

1.2.10 *Paronychia argentea*

Paronychia argentea Lam. (family Caryophyllaceae) common name (Silvery Whitlow-wort) a perennial herb, with stems spreading on the ground. Leaves 0.5-1 cm long, oblong-elleptic, with sharp ends and rough marmargins, surrounded by dense, hyaline stipules. Flowers usually minute, arranged in heads of 1 cm in diameter, and surrounded by hyaline bracts. It grows in waste places of mountains (Al-Eisawi, 1998).

1.2.11 *Phagnalon rupestre*

Phagnalon rupestre (L.) DC. (family Asteraceae) common name (Phagnalon). In previous studies by (Go´ngora *et al*, 2002, Go´ngora *et al* , 2001), they described the isolation and identification of seven phenolic compounds from phagnalon: three hydroquinone glucosides, 1-O hglucopyranosyl- 1,4-dihydroxy-2-(3V,3V-dimethylallyl) benzene **(1)**, 1-O-h-glucopyranosyl-1,4-dihydroxy- 2-(3V-hydroxymethyl-3V methylallyl) benzene **(2)**, 1-O-(4''-O-caffeoyl)-h-glucopyranosyl-1,4-dihydroxy- 2-(3V,3V-dimethylallyl) benzene **(3)**; and four dicaffeoylquinic derivatives, 3,5-di-O-caffeoylquinic acid methyl ester **(4)**, 4,5-di-O-caffeoylquinic acid methyl ester **(5)**, 3,5-di-O caffeoylquinic acid **(6)** and 4,5- di-O-caffeoylquinic acid **(7).** They have also described the effects of these compounds on a contact hypersensitivity model (Go´ngora *et al* , 2001), and recently evaluated their antioxidant activities on different models such as enzymatic and non-enzymatic lipid

peroxidation, radical scavenger activity and inhibition of xanthine oxidase enzyme (Go´ngora *et al* , 2002).

1.2.12 *Rosemarinus officinalis*

Rosmarinus officinalis (L.) (family Labiatae), common name (Rosemary) widely used in folk medicine, cosmetics, phytocosmetics (Pintore *et al.*, 2002) and the flavouring of food products. Many studies have shown that the essential oils of rosemary are among the most active in this respect against a number of food spoilage and pathogen microorganisms (Smith-Palmer, *et al.*, 1998; Hammer *et al.*, 1999; Mangena and Muyima, 1999). The compositions of essential oils from a particular species of plant can differ between harvesting seasons (McGimpsey and Douglas, 1994) and between geographical locations (Juliano *et al.*, 2000). Plant secondary metabolites, such as essential oils and plant extracts (Tepe *et al.*, 2004), are studied for their antimicrobial activities and most essential oils derived from plants are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxic activities (Faleiro *et al.*, 1999). Therefore, they are intensely screened and applied in the fields of pharmacology, pharmaceutical botany, medicinal and clinical microbiology, phytopathology and food preservation (Daferera *et al.*, 2000). Recently, many studies have focussed on the biological and antimicrobial properties of the essential oils derived from *R. officinalis* species and their main constituents (Daferera *et al.*, 2000; Faleiro *et al.*, 1999; Koschier and Sedy, 2003; Ohno *et al.*, 2003).

1.2.13 *Salvia officinalis*

Salvia officinalis L., family Labiatae, common sage is the most representative species within the genus of Labiatae. The plant is mostly spread in the Mediterranean Basin, in South East Africa and in Central

and South America, where it is largely cultivated for culinary and medicinal purposes. Curative properties of sage are particularly recognized since earliest times and its use as a tonic, stimulant, carminative, antiseptic and antihydrotic is reported (Avato *et al*, 2005). More recently, sage antioxidant effects have also been demonstrated (Deans and Simpson, 2000). Several phytochemical investigations have been carried out on *S. officinalis* and two major chemical classes of secondary metabolites have been identified as typical products of the plant: terpenoids and phenolics,(Lu and Foo, 2001). Among the terpenoids, the volatile oil produced in the aerial parts of the plant has especially acquired interest for the broad range of applications in aromatherapy and in food industry (Barnes *et al*., 2002). *S. officinalis* is considered to have the highest amount of essential oil compared to the other species and it has been shown that secretion of the oil is associated with the presence of four different types of specialized glandular trichomes on the leaves of the plant, as evidenced by morphological studies (Venkatachalam *et al*., 1985).

1.2.14 *Sinapis alba*

Sinapis alba L, (family Cruciferae), common name is white mustard, is an annual herb, many branched, and usually covered with stiff spreading hairs. Basal leaves are petiolate, lyrate, lobed, and hairy; upper leaves are sessile, and lobed. Flowers are yellow, and arranged remotely on a terminal raceme. Fruits are petiolate, with 3-4 constricted seeds and flattened, dugger-like beak,and hairy (Al-Eisawi, 1998).

1.2.15 *Stachys distans*

Stachys distans (Benth.) (family Labiatae), and common name (Woundwort), is a perennial herb, which is distributed in the

Mediterranean woodlands and shrublands. It grows in hard rock outcrops (Ori *et al*. 1999), and is used in traditional medicine.

1.2.16 *Teucrium polium*

Teucrium polium L. (family Labiatae) common name (Mountain genander). It is a perennial herb, which has a wide distribution in Iran and is used in traditional medicine (Passmore and Eastwood, 1986).

1.2.17 *Thymus vulgaris*

Thymus vulgaris L., (family Labiatae) commonly known as thyme (*Za'tar* in Arabic). Plants belonging to the genus *Thymus* have been thoroughly investigated chemically and pharmacologically (Moleyar and Narasimhan, 1986). Several studies have shown that thyme oils, particularly those of *Thymus vulgaris* and *Thymus zygis* (Bruneton, 1999; Pina-Vaz et al., 2004; Stahl-Biskup & Sa´ ez, 2002), possess antimicrobial activity, those of the phenol type being the most active. The limited occurrence of these phenols in nature is one of the reasons why Thymus oils containing thymol and carvacrol have been of great interest for some time.

In recent years, studies on the antifungal activity of essential oil components have been reported by numerous investigators (Moleyar and Narasimhan, 1986; Kurita *et al.,* 1981). Further essential oils derived from many aromatic plants are known to possess insecticidal (Konstantopoulou *et al*., 1992), and antimicrobial activities. Among them, thyme essential phenolic oil has been reported to have antibacterial, antimycotic, antioxidative and food preservative properties (Hudaib *et al*., 2002). Of the 50 plant essential oils examined by Deans and Ritchie (1987), thyme oil was the most inhibitory against 25 genera of bacteria.

1.2.18 *Varthemia iphionoides*

Varthemia iphionoides Boiss and Blanche in Boiss, syn. *Chiliadenus iphionoides* (Boiss and Blanche in Boiss) Brullo, (family Compositae), common name Varthemia is endemic of the eastern Mediterranean area, spread in the Arabian Sahara and Irano-Turanian regions (Feinbrun-Dothan, 1977). The plant, which belongs to the family Compositae, grows wild in Jordan, where it is traditionally used as a decoction or infusion agent for its spasmolitic activity and for the treatment of gastrointestinal disorders. Previous studies on *V. iphionoides* (Afifi *et al.*, 1990, Afifi, 1991) have dealt mainly with the characterization of its flavonoid constituents. A series of flavonoids, new for the genus, have been in fact isolated from the plant and their biological activity determined (Afifi *et al.*, 1990, Afifi *et al.*, 1991), some of them showed antifungal effects. A previous preliminary screening (Al-Douri *et al.*, 2000) of the essential oil obtained from this plant, in combination with a pharmacological study on the *in vitro* relaxant effect of the extract on the isolated ileum of rabbits, only led to a partial identification of the oil constituents.

1.3 Study Objectives

1) Evaluate the effect of eighteen medicinal plants against the phytopathogenic fungus *B. cinerea in vitro* and *in vivo*.
2) Evaluate the variation in *B. cinerea* response to plant extract application.

Chapter Two

2. Materials and Methods

2.1 Fungal Isolates

Twenty six *B. cinerea* isolates used were provided from the collection of the Department of Plant Production and Protection, Faculty of Agriculture, Hebron University, as shown in (Table 1). The well known isolate Bo5.10 was as well used.

Table 1: *Botrytis cinerea* Isolates collected from twenty six greenhouses cultivated with tomato, cucumber, bean, gourd, squash, strawberry and eggplant during January and February 2007.

Area	No. of greenhouse	Isolates	Plants
Hebron	1	P.Bc2,	Cucumber
Jericho and Jordan Valley	3	P.Bc10,	Eggplant
		P.Bc12	Strawberry
		P.Bc13	Tomato
Jenin	15	P.Bc26, P.Bc31, P.Bc92	Gourd and Squash
		P.Bc64, P.Bc66, P.Bc72, P.Bc88	Tomato
		P.Bc29, P.Bc32, P.Bc 33, P.Bc 61, P.Bc62, P.Bc67, P.Bc 68,	Cucumber
		P.Bc19,	Bean
Tulkarem and Qalqelia	7	P.Bc 52, P.Bc 54, P.Bc 56, P.Bc 59, P.Bc99, P.Bc100	Cucumber
		P.Bc55	Tomato
Total	**26**		

2.2 Collection of medicinal plants

Eighteen Palestinian medicinal plants that are used in traditional medicine for the treatment of several diseases were collected from different areas in the Hebron District between May and August, 2007 (Table 2).

Table 2: Palestinian selected plants list.

No.	Common name	Arabic name	Scientific name
1.	Palestine chamomile	أقحوان فلسطين	*Anthemis palestina*
2.	Herba-alba	الشيح	*Artemisia herba-alba*
3.	Conhead thyme	الزحيف	*Coridothymus capitatus*
4.	Clammy Inula	الطيون	*Inula viscosa*
5.	Thyme	زعتر	*Majorana syriaca*
6.	Chamomile	البابونج	*Marticria chamomilla*
7.	Peppermint	نعنع	*Mintha piperita*
8.	Basil	ريحان,حبق	*Ocimmum basilicum*
9.	Oregano	مردقوش	*Origanum valgare*
10.	Silvery Whitwo-wort	رجل الحمامة,عصاة الراعي	*Paronychia argentea*
11.	Rock Phangalon	الصوفان,قديح	*Phagnalon rupestre*
12.	Rosemary	حصاالبان,إكليل الجبل	*Rosemarinus officinalis*
13.	Sage	مريمية	*Salvia officinalis*
14.	White Mustard	الخردل	*Sinapis alba*
15.	Woundwort	قرنية,فلية	*Stachys distans*
16.	Mountain genander	جعدة	*Teucrium polium*

17.	Thyme	زعتر غزال	*Thymus vulgaris*
18.	Varthemia	اكتيلا	*Varthemia iphionoides*

2.3 Preparation of plant extracts

Samples were air-dried, grinded, and stored in dark colored jars (1000ml) at 4°C.

Ground air-dried plant material from *A. palestina*, *A. herba-alba*, *C. capitatus*, *I. viscosa*, *M. syriaca*, *M. chamomilla*, *M. piperita*, *O. basilicum*, *O. valgare*, *P. argentea*, *P. rupestre*, *R. officinalis*, *S.officinalis*, *S. alba, S. distans*, *T. polium***,** *T. vulgaris*, and *V. iphionoides*, were extracted with distilled water by adding 400ml boiling distilled water to 40g of each plant. After ten minutes, the extract was filtered through 0.35 µm sieves and centrifuged at 5000 rpm for five minuets. Stock solution of 10% was autoclaved; concentrations were prepared as shown in Table 3.

Table 3: Preparation of plant extracts concentrations.

Concentration	Stock (10%) (ml)	Distilled water (ml)
1%	10	90
2%	20	80
3%	30	70
4%	40	60
5%	50	50
8%	80	20

2.4.1 Mycelial growth rate (MGR) - Experiment I

Mycelium growth rate of *Botrytis* isolates (P.Bc2, P.Bc30, P.Bc99,and Bo5.10) was assessed on plates containing potato dextrose agar (PDA) amended with extracts of the eighteen native plants at the concentrations of (0%, 1%, 2%, 3%, 4%) according to the following procedure: three Petri dishes (90mm diameter) containing PDA amended with plant extracts of each concentration were centrally inoculated with mycelial disk (5mm) of each isolate taken from 5-day-old culture to determine the average mycelial growth rate (MGR) for each isolate on each concentration. Plates were then incubated at 25°C under continuous light and inspected daily for three consecutive days. mycelial growth rate was recorded every 24 hours during this period. The colony diameter was measured as the mean of two perpendicular diameters measured at the third day minus the diameter at the first day. Mycelium growth rate (MGR) was calculated using the formula documented by (Elad et al., 1981):

MGR cm^2/day = (((D2-D1)2/400)*3.14)) (T2-T1)(day)

Where D2: Diameter in the second evaluation (mm), D1: Diameter in the first evaluation (mm), T2: Time of the second evaluation (day) and T1: Time of the first evaluation (day). The experimental design used was completely randomized with three replicates (plates) for each treatment.

2.4.2 Mycelial growth rate (MGR) - Experiment II

The first experiment was repeated in the same manner with the most promising plant extracts of (*I. viscosa* , *M. syriaca* , *S. officinalis T. vulgaris*, *V. iphionoides*) at the concentrations (0%, 2%) on *B.cinerea* isolates (P.Bc10, P.Bc13, P.Bc26, P.Bc29, P.Bc32, P.Bc52, P.Bc54,

P.Bc55, P.Bc56, P.Bc61, P.Bc62, P.Bc64, P.Bc67, P.Bc68, P.Bc72, P.Bc88, P.Bc92, P.Bc100).

2.5 Fungal mass weight (FMW)

Potato dextrose broth (PDB) was prepared by adding 1000ml distilled water to 24g (PDB) and 0.5g chloramfinicole. The mixture was shaken well and autoclaved. Twenty ml PDB amended with plant extracts of *I. viscosa* and *V. iphionoides*, at the concentrations of 0%, 2%, 3%, 5%, and 8% were placed in 50 ml glass flasks and covered with aluminum foil. In addition, twenty ml PDB amended with plant extracts of *A. palestina*, *A. herba-alba*, *C. capitatus*, *M.syriaca*, *M. chamomilla*, *M. piperita*, *O. basilicum*, *O. valgare*, *P. argentea*, *P. rupestre*, *R. officinalis*, *S. officinalis*, *S. alba, S. distans*, *T. polium***,** and *T. vulgaris*, at the concentrations of 0%, 1%, 2%, and 3% were placed in 50 ml glass flasks and covered with aluminum foil as above. The flasks were autoclaved and inoculated with Mycelial disks (5mm) of *B.cinerea* isolates P.BC99 and Bo5.10 taken from 5-day-old culture, and incubated with shaking at 25°C under continuous light for ten days; three replicates for each treatment were incorporated. The mycelium mass was filtered, air dried and weighed; the mass weight was calculated as the mean of three replicates.

2.6.1 Germination – Experiment I

B. cinerea isolates (P.BC99, Bo5.10) were grown on Petri dishes (50mm diameter) containing PDA. Plates were incubated at 25°C under continuous light for fourteen days. Conidia was then harvested by adding 5ml autoclaved distilled water, and filtered with conidia filter; filtered conidial concentration was determined by haemacytometer and adjusted at $2.5*10^5$/ml (CFU). The experiment

was completely randomized. Fructose (1M) was prepared and autoclaved before adding it to the eighteen native plant extracts with concentrations of (0%, 1%, 2%, 3%, 4%); 470µl of each concentration was placed in wells of 24 wells cell culture plates with three replicates for each concentration. Spore suspension (30 µl) of each isolate was added to plates which were incubated at 25°C under continuous light for 24 hours. The germination percentage of conidia was assessed under Olympus- CKX41 microscope. The conidia were considered germinated when the germ tube length was double the diameter of the conidium. Under each replicate (well), four conidial fields were observed; the mean of three replicates was calculated.

2.6.2 Germination – Experiment II

The first experiment was repeated in the same manner with the most promising plant extract (*I. viscosa*). EC_{50} (=2.7%) and EC_{90} (=4.8%) were determined by applying the formula {$y = -27.32x + 124.47$}, ($R^2 = 0.9042$) (Fig.23). *B.cinerea* isolates (P.Bc2, P.Bc10, P.Bc13, P.Bc19, P.Bc26, P.Bc29, P.Bc31, P.Bc33, P.Bc49, P.Bc52, P.Bc54, P.Bc55, P.Bc56, P.Bc61, P.Bc62, P.Bc64, P.Bc67, P.Bc68, P.Bc72, P.Bc88, P.Bc92, P.Bc96, and P.Bc100) were used in this assay.

2.7 Integrated control study

Bean (*Phasolius volgaris*) seeds were grown in 15cm diameter pots in greenhouse for forty days to study the effect of a combination of *I. viscosa* plant extract with low dose of the fungicide Rovral® on disease severity. Forty days bean plants were sprayed with Iv extract at the concentration of 3%; after thirty minutes, plants were sprayed with low dose (EC_{50} :effective concentration that are able to reduce disease by 50% = 320µg/ml) of the fungicide Rovral®. Thirty minutes

later, the plants were sprayed with *B. cinerea* isolates (P.BC2 and Bo5.10) spore suspension at the concentration of $2.5*10^5$ CFU. The control treated plants were sprayed with distilled water and *B. cinerea* spore suspension. Accordingly the experiment involved the following treatments:

(1) Distilled water and *B. cinerea* spore suspension (Control) **(2)** *I. viscosa* extract alone **(3)** Fungicide alone **(4)** Combination of *I. viscosa* extract and fungicide. Treated plants were then covered with transparent polyethylene bags with five replicates (plants) for each treatment and incubated in the growth chamber at 25°C. Disease severity was evaluated eight days after incubation as disease percentage (%) according to a disease severity scale designed for this purpose, Table (4). The experimental design was completely randomized and repeated twice.

Table 4: Disease severity scale

Disease (%)	Scale
0-20%	1-20% of the leaves are infected
20-40%	21-40% of the leaves are infected and lesions start to appear on stems
40-60%	41-60% of the leaves and 1-10% of the stems are infected
60-80%	61-80% of the leaves and 11-30% of the stems and the flowering buds are infected
80-100%	81-100% of the leaves, stems and the flowering buds are infected

Chapter Three

Results

3.1 Effect of medicinal plant extracts on *Botrytis cinerea* isolates *in vitro*

3.1.1 Mycelial growth rate (MGR) – Experiment I

In general, the mycelium growth rate of *B. cinerea* decreased with increasing plant extract concentrations.

All the medicinal plant extract concentrations inhibited significantly ($p \leq 0.05$) the MGR of *B. cinerea* isolates (P.Bc2, P.Bc30, P.Bc99 and Bo5.10) and showed antifungal effects with different levels. Figures (2-7) shows the effect each at a time of the eighteen medicinal plant extracts on mycelium growth rate of *B. cinerea* isolates (P.Bc2, P.Bc33, P.Bc99 and Bo5.10) at the concentrations (0%, 1%, 2%, 3%, and 4%) on PDA plates. Figure 8 shows the significant inhibitory effect of thirteen medicinal plant extracts against the well known *B.cinerea* isolate Bo5.10 at the concentrations of 0% and 4%, when compared to the control; results showed significant differences between these medicinal plants in their inhibitory effect against *B.cinerea*.

The plant extracts of *I. viscosa*, *M. syriaca*, *S. officinalis* , *T. vulgaris* , and *V. iphionoides* , showed the highest inhibitory effects against *B. cinerea* isolates (Fig.9); mycelial growth rates were reduced by 97%, 92%, 76%, 97%, and 99%, respectively, compared to the control at the concentration of 4%. Furthermore, variation between *B. cinerea* isolates was significant at all concentrations used.

Total mycelium growth inhibition (100%) was observed on *B. cinerea* (P.Bc30) when *I. viscosa* extract was applied at the concentration of 4%, and on (P.Bc2) when *T. vulgaris* and *V. iphionoides* extracts were applied at the same concentration (Fig.3&7).

Few plant extracts performed well at low concentrations (1%). *I. viscosa*, *T. vulgaris*, and *V. iphionoides* reduced mycelial growth rate by 88 %, 68%, and 93%, respectively compared to the control (Fig.3&7). *S. officinalis* reduced mycelium growth by 75% at the concentration of 2%, but growth was not further reduced at higher concentrations of plant extract (Fig.6). Figure 9 shows the comparative inhibitory effect of the most promising five medicinal plant extracts against the well known *B.cinerea* isolate (Bo5.10) at the concentrations (0% and 4%); significant differences were observed between plant extracts at the highest concentration (4%) except for *I. viscosa* and *V. iphionoides*.

The plant extracts of other medicinal plants tested in this study showed a moderate inhibitory effect against *B. cinerea in vitro* in the range of 35% for the extract of *O. basilicum*, and 74% for *R. officinalis* extract, at the highest concentration (4%), compared to the controls (Fig.2-7, and Fig.8).

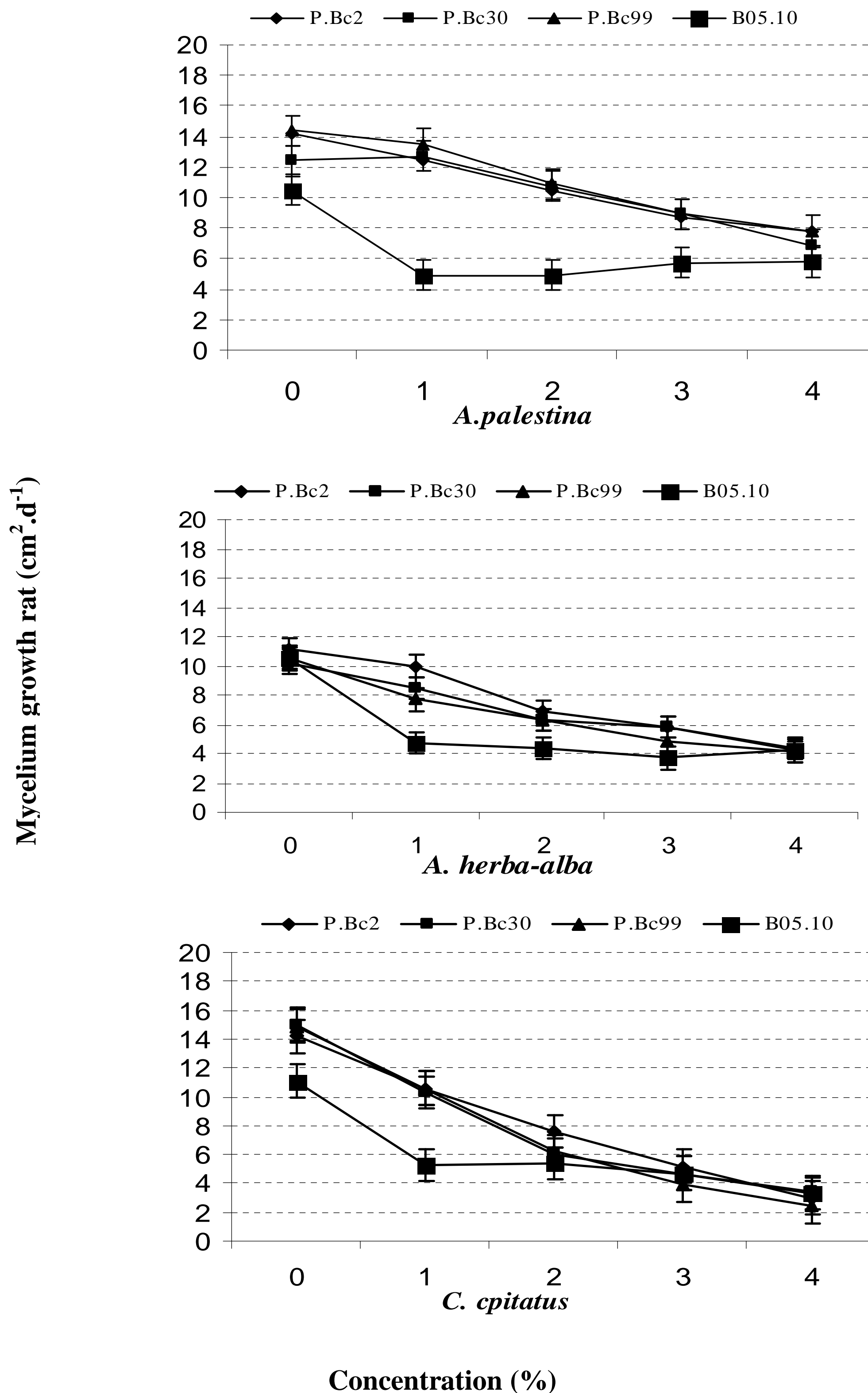

Fig. 2: Effect of plant extracts (*A. palestina*, *A .herba-alba*, *C. capitatus*) on *B. cinerea* isolates (P.Bc2, P.Bc30, P.Bc99, Bo5.10) mycelium growth rate (cm^2\day) grown on PDA amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) is: Ap =0.99, Ah =0.75, Cc=1.14.

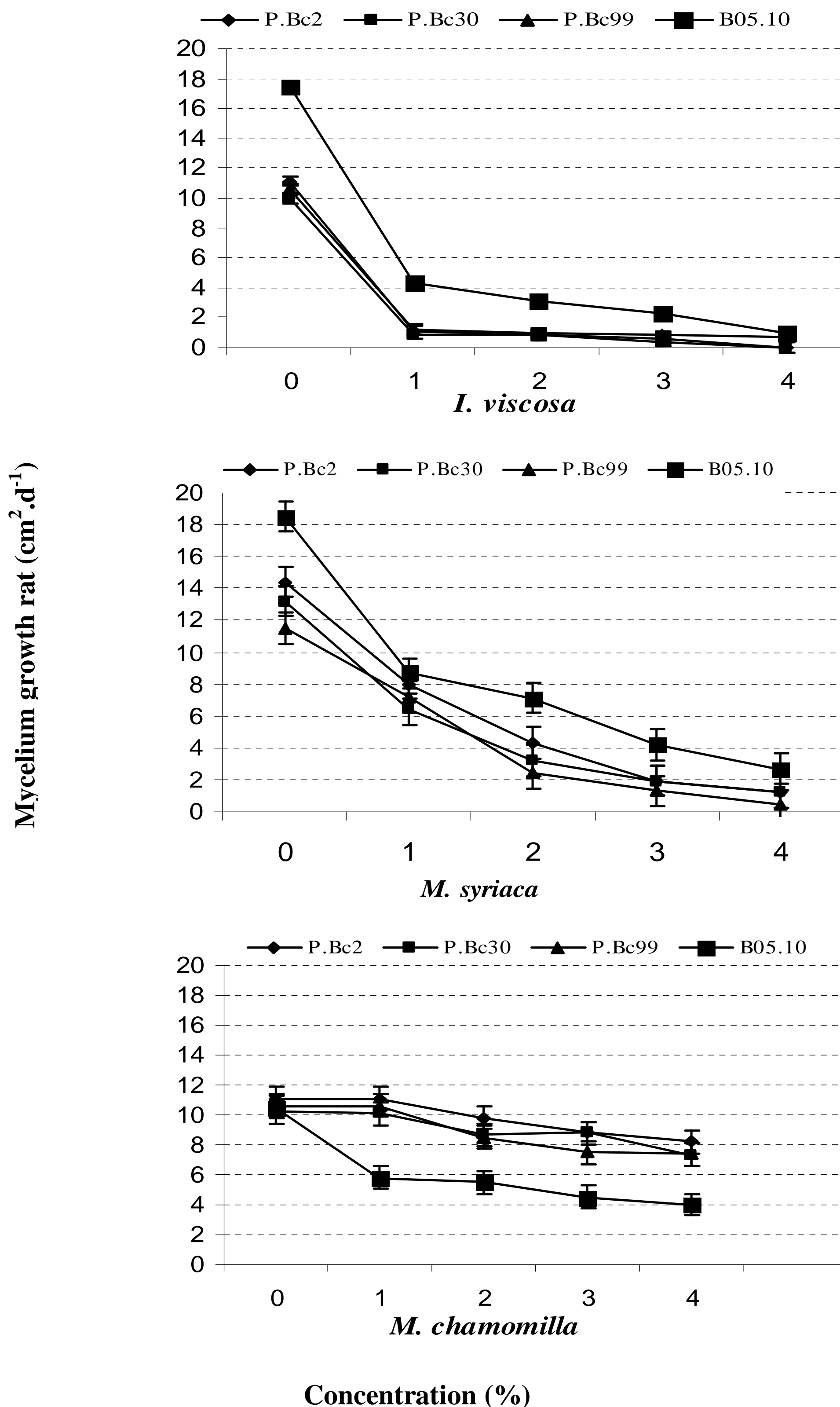

Fig. 3: Effect of plant extracts (*I. viscosa*, *M. syriaca*, *M. chamomilla*) on *B. cinerea* isolates (P.Bc2, P.Bc30, P.Bc99, Bo5.10) mycelium growth rate (cm^2)\day grown on PDA amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) is: Iv =0.31, Ms=0.96, and Mc =0.76

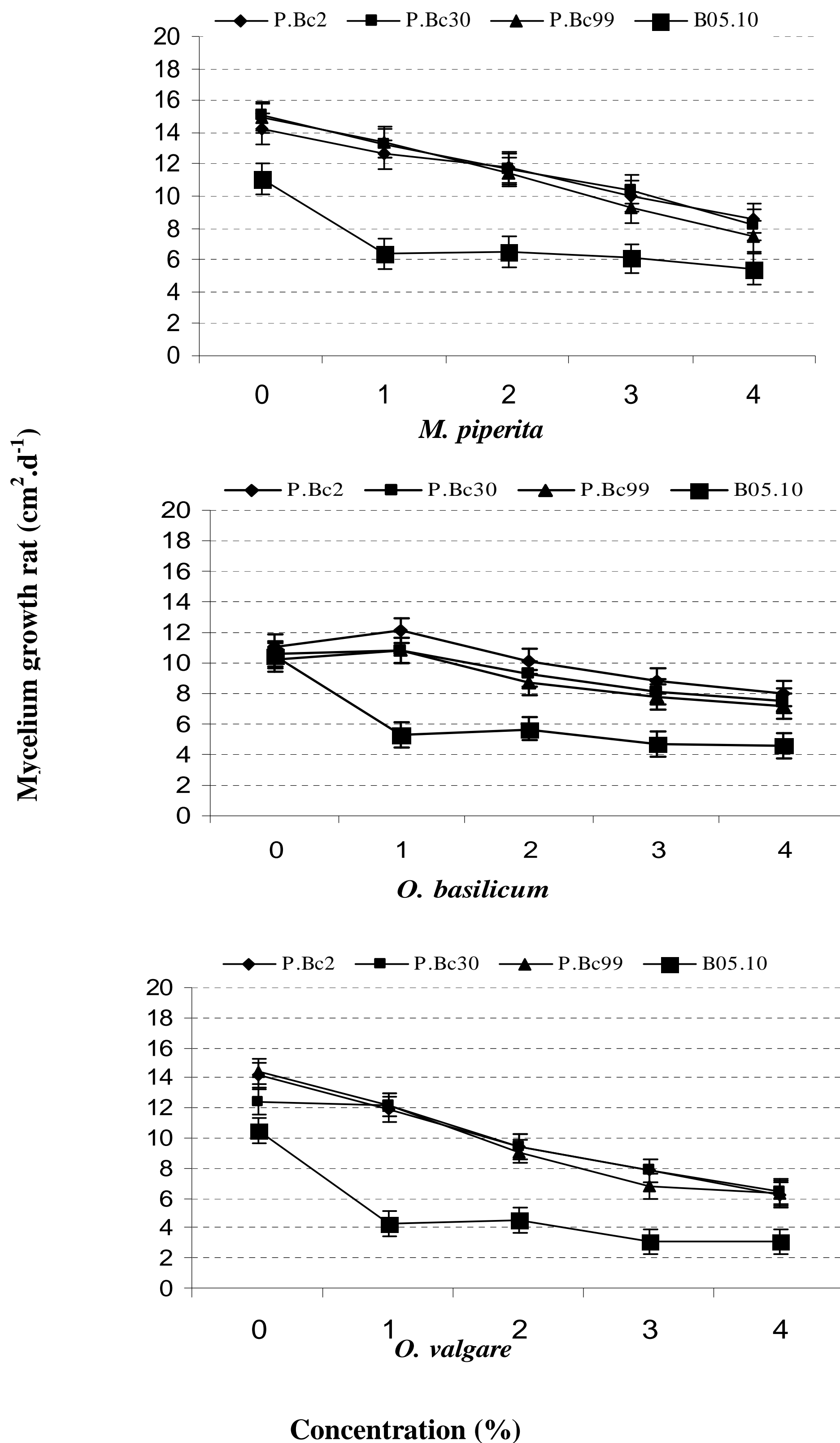

Fig. 4: Effect of plant extracts (*M. piperita*, *O. basilicum*, *O. valgare*) on *B. cinerea* isolates (P.Bc2, P.Bc30, P.Bc99, Bo5.10) mycelium growth rate (cm^2)\day grown on PDA amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) is: Mp =0.94,Ob =0.8, and Ov =0.82.

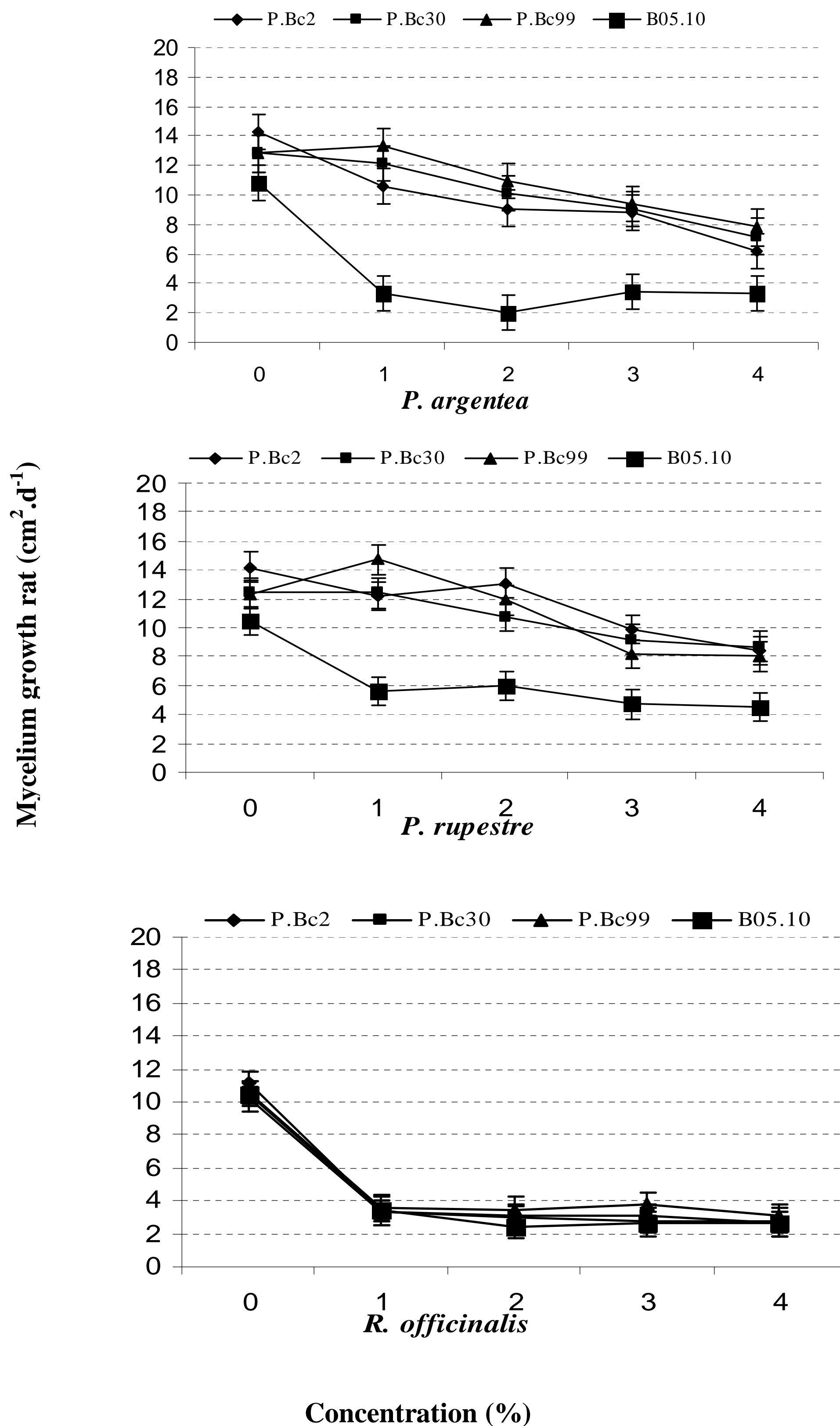

Fig. 5: Effect of plant extracts (*P. argentea*, *P. rupestre*, *R. officinalis*) on *B. cinerea* isolates (P.Bc2, P.Bc30, P.Bc99, Bo5.10) mycelium growth rate (cm^2\day) grown on PDA amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) is: Pa =1.2, Pr =1, and Ro =0.72.

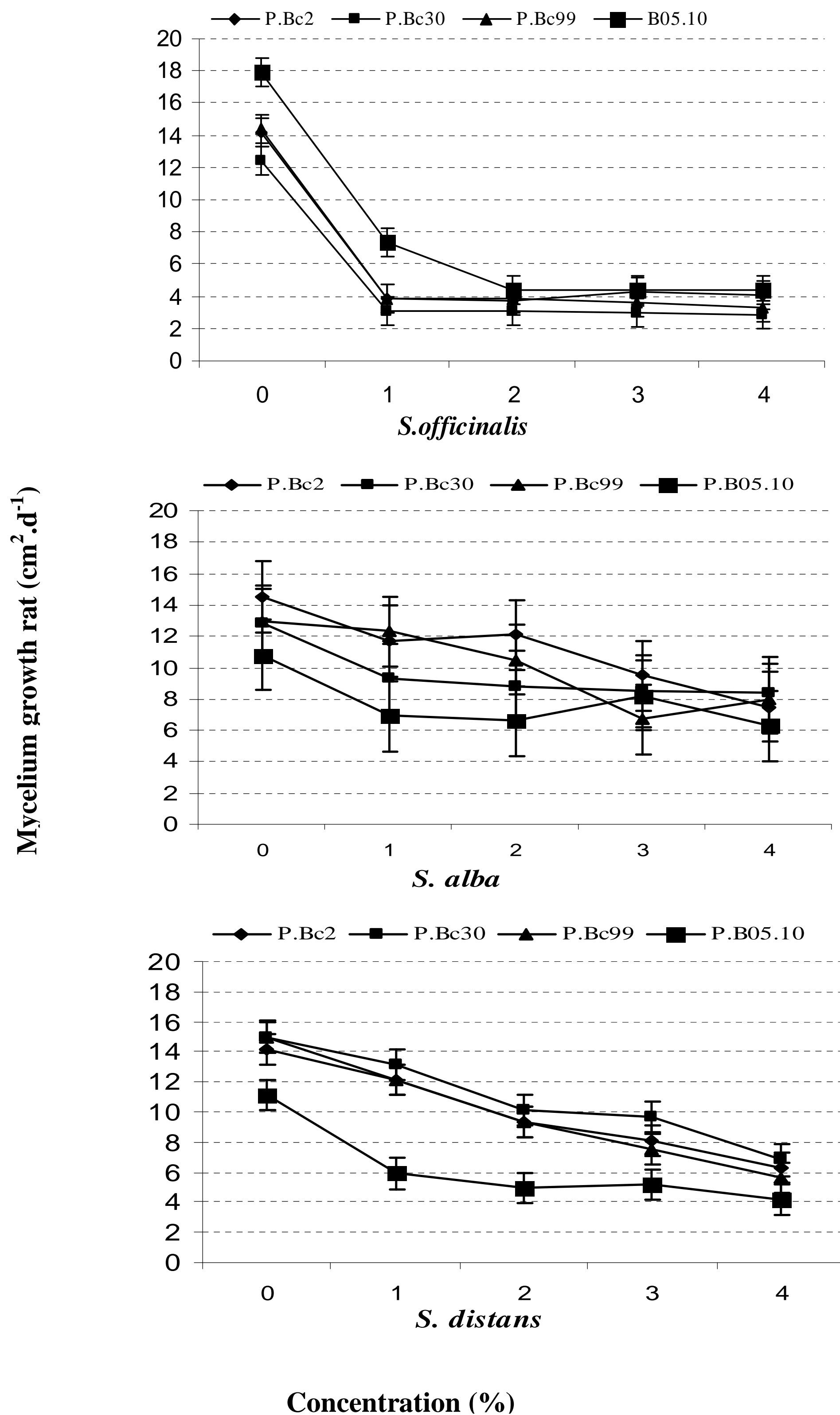

Fig. 6: Effect of plant extracts (*R. officinalis*, *S. officinalis*, *S. alba, S. distans*) on *B. cinerea* isolates (P.Bc2, P.Bc30, P.Bc99, Bo5.10) mycelium growth rate (cm^2\day) grown on PDA amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) is: So =0.88, Sa =2.24, and Sd =1.02.

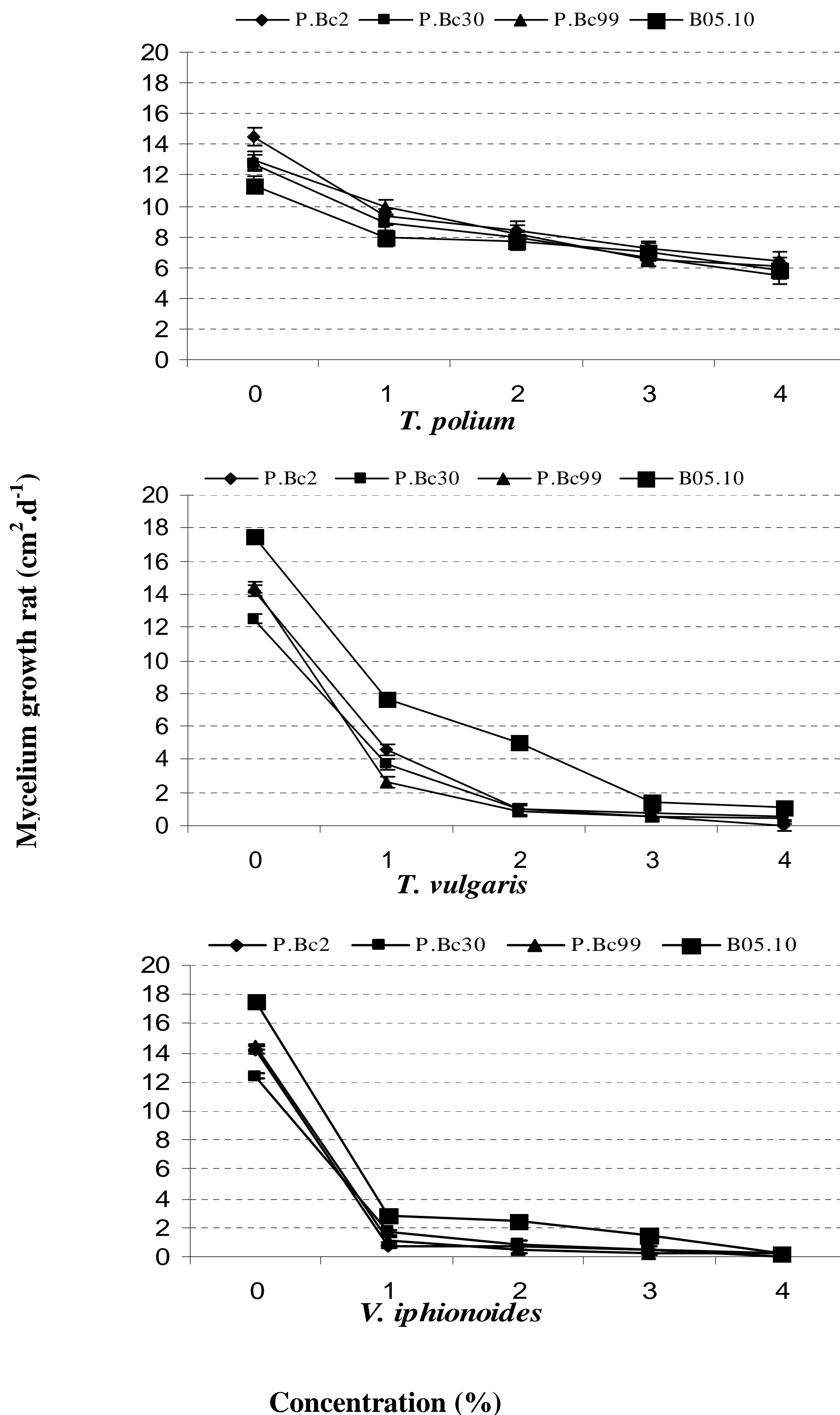

Fig. 7: Effect of plant extracts (*T. polium*, *T. vulgaris*, *V. iphionoides*) on *B. cinerea* isolates (P.Bc2, P.Bc30, P.Bc99, Bo5.10) mycelium growth rate (cm^2\day) grown on PDA amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) is: Tp =0.56, Tv =0.31, and Vi =0.2.

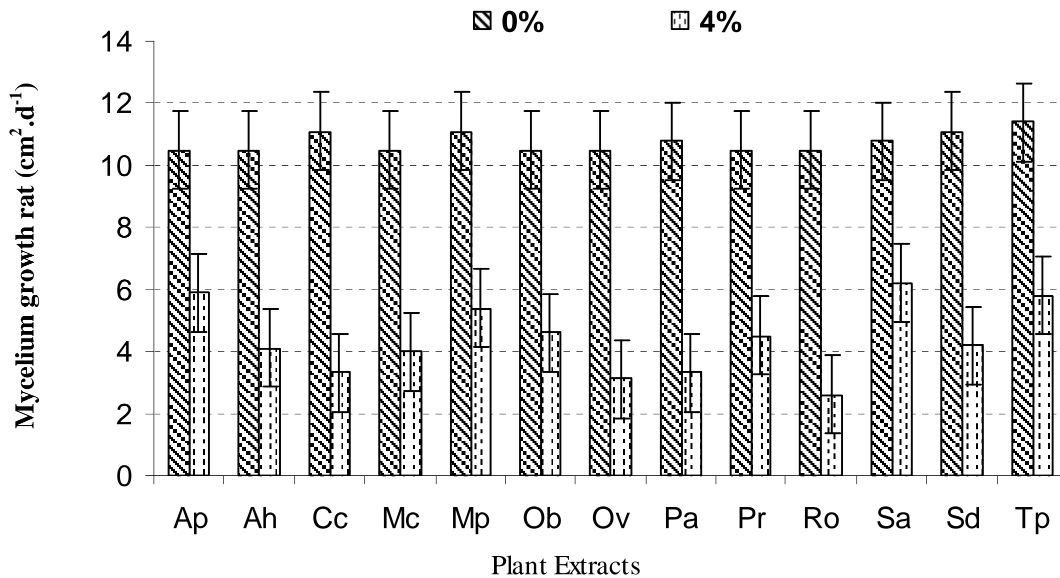

Fig. 8: Effect of 13 plant extracts (*A. palestina*, *A. herba-alba*, *C. capitatus*, *M. chamomilla*, *M. piperita*, *O. basilicum*, *O. valgare*, *P. argentea*, *P. rupestre*, *R. officinalis*, *S. alba, S. distans*, and *T. polium*) on *B. cinerea* isolate (Bo5.10) mycelium growth rate (cm^2\day) grown on PDA amended with plant extracts at two concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) = 1.26

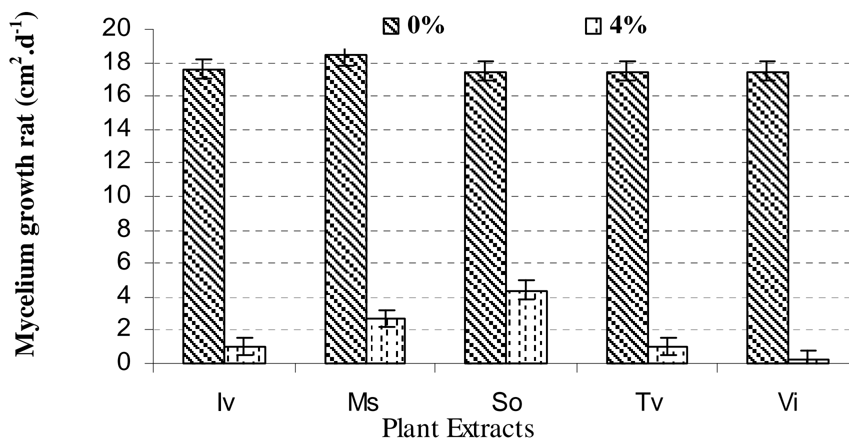

Fig. 9: Effect of the most promising 5 plant extracts (*I. viscosa*, *M. syriaca*, *S. officinalis* , *T. vulgaris* , and *V. iphionoides*) on *B. cinerea* isolate (Bo5.10) mycelium growth rate (cm^2\day) grown on PDA amended with plant extracts at two concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) = 0.55

3.1.2 Mycelial growth rate (MGR) – Experiment II

The medicinal plant extracts of *I. viscosa* , *M. syriaca* , *S. officinalis* ,*T. vulgaris* , and *V. iphionoides* , which gave the best inhibitory effects against *B. cinerea* isolates in the preliminary experiment (Fig. 9), were used to evaluate the variation in *B. cinerea* isolates response to plant extract application. The variation between the isolates was significant, as well as the variation between the five medicinal plants for each isolate. There was obvious and substantial variation between *B.cinerea* isolates in respect to their sensitivity to medicinal plants extracts. In addition, the five medicinal plants almost followed the same trend in reducing the mycelial growth rate for *B.cinerea* at the concentration of 2% as was in the preliminary experiment.

The *B.cinerea* isolate (P.Bc29) showed high sensitivity to plant extracts; mycelial growth of the isolate was reduced almost by all plant extracts in the range (33-94%), compared to the control. On the other hand, the isolates (P.Bc32 & P.Bc68) showed higher tolerance to plant extracts; Mycelial growth rates were reduced in the range 14-51% for the isolate P.Bc32 and in the range 0-31% for the isolate P.Bc68, compared to the control (Figs. 10-14).

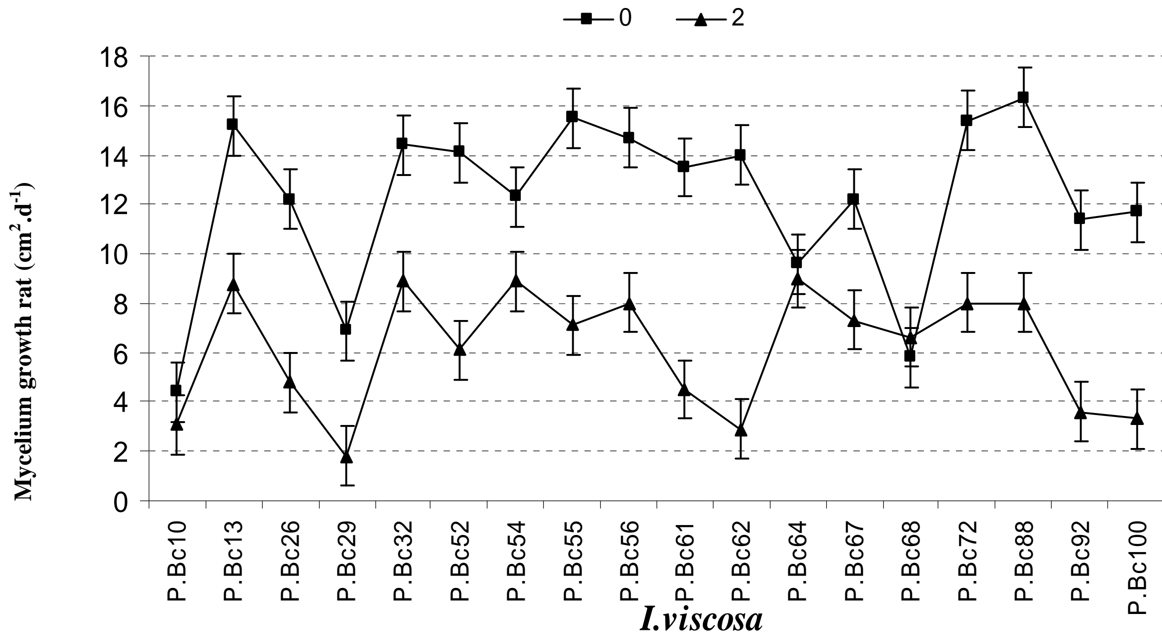

Fig. 10: Effect of *I. viscose* extract on mycelium growth rate (cm^2\day) of *B.cinerea* isolates grown on PDA at two concentrations (LSD=0.88, P≤ 0.05).

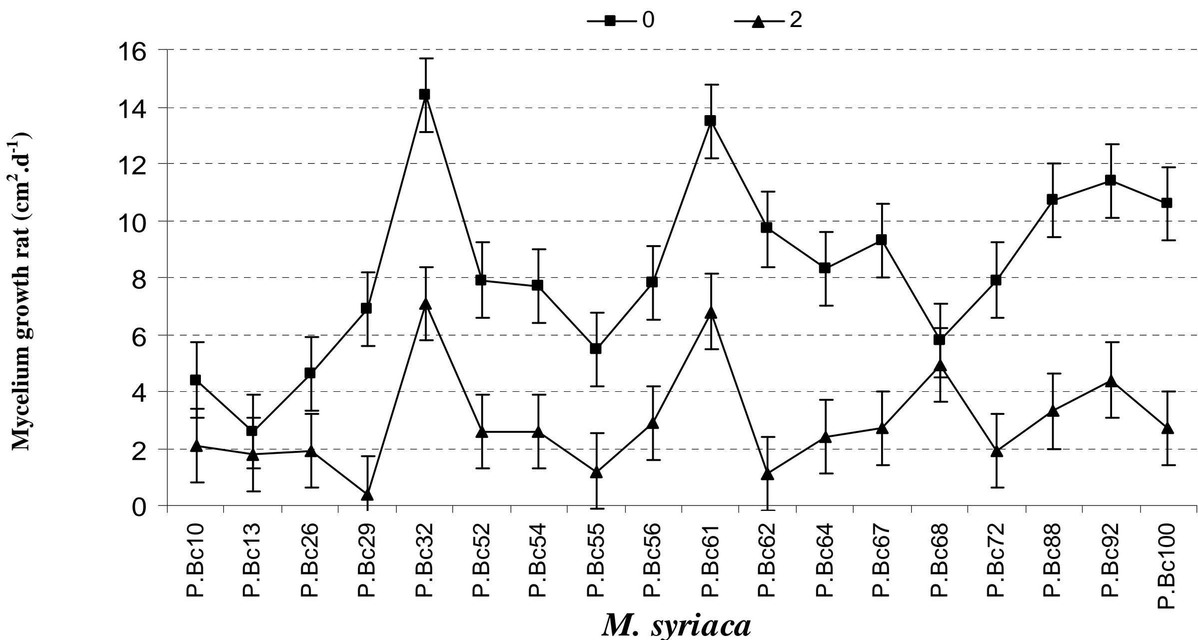

Fig. 11: Effect of *M. syriaca* extract on mycelium growth rate (cm^2\day) of *B.cinerea* isolates grown on PDA at two concentrations (LSD=0.88, P≤ 0.05).

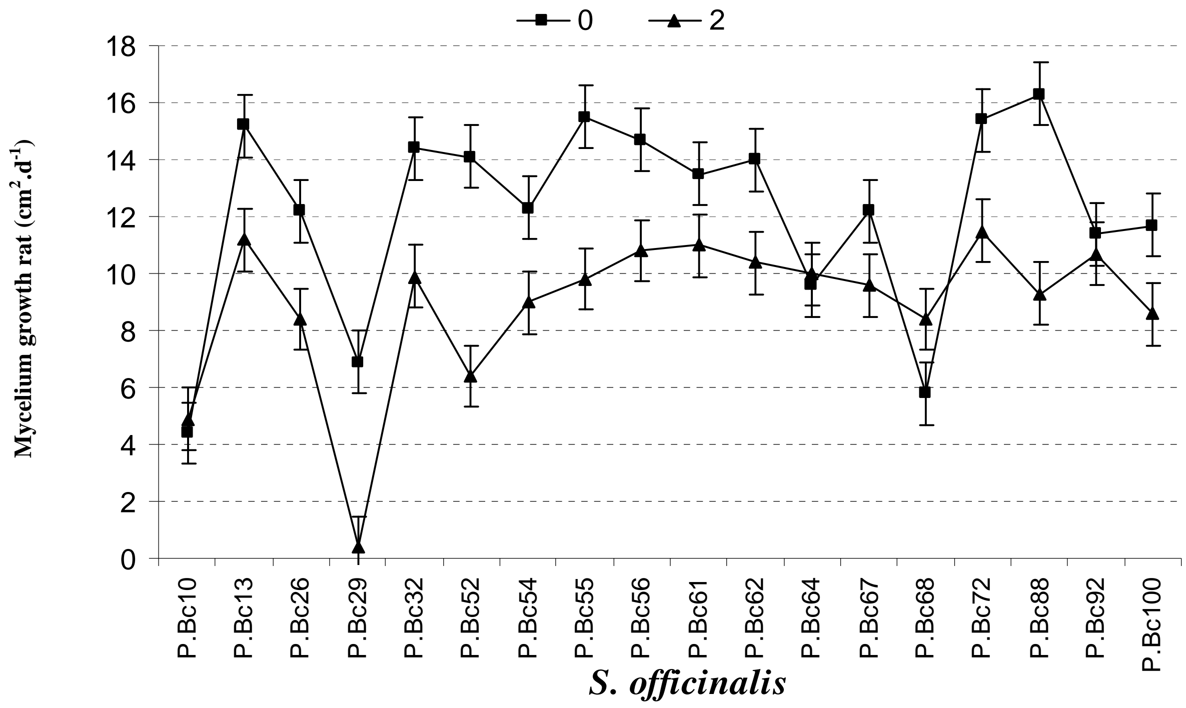

Fig. 12: Effect of *S. officinalis* extract on mycelium growth rate (cm^2\day) of *B.cinerea* isolates grown on PDA at two concentrations (LSD=0.88, P≤ 0.05).

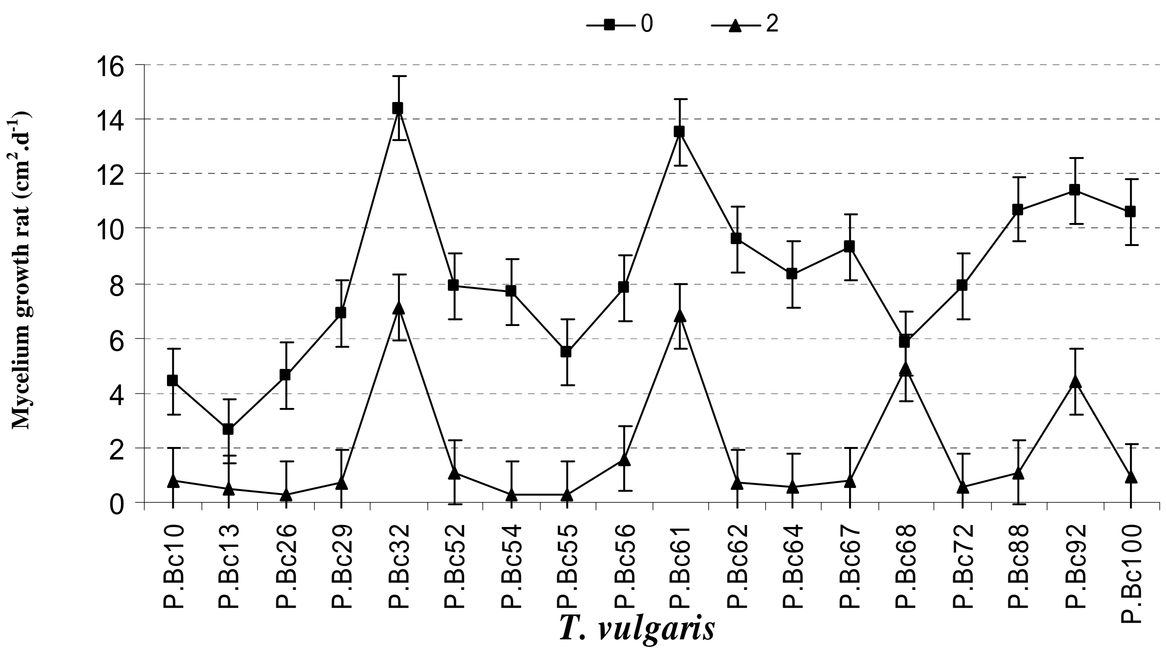

Fig. 13: Effect of *T. vulgaris* extract on mycelium growth rate (cm^2\day) of *B.cinerea* isolates grown on PDA at concentrations (0%, 2%) (LSD=0.88, P≤ 0.05).

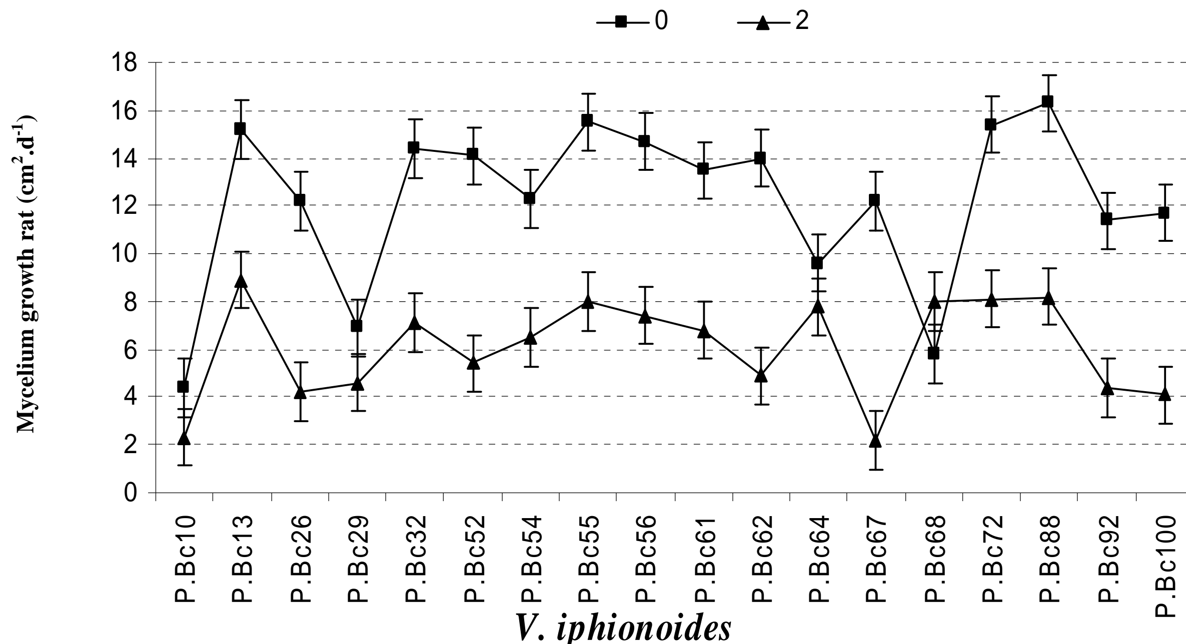

Fig. 14: Effect of *V. iphionoides* extract on mycelium growth rate (cm^2\day) of *B.cinerea* isolates grown on PDA at two concentrations (LSD=0.88, P≤ 0.05).

3.1.3 Fungal mass weight (FMW)

Fifteen medicinal plant extracts out of eighteen reduced significantly (p≤0.05) the fungal mass weight (FMW) of *B.cinerea* isolates (P.Bc99 and Bo5.10). *I. viscosa*, and *V. iphionoides* extracts showed a strong antifungal activity against *B.cinerea* isolates; a total reduction (100%) of fugal mass weight (FMW) was shown at the concentrations (3 and 5 %). At lower concentration (2%) of *I. viscose*, the FMW was reduced by 97%, and by 90% for *V. iphionoides*. *S. officinalis* extract at the concentrations (2% and 3%) reduced the FMW by 53% and 81%, while *M. syriaca* extract did not significantly reduce the FMW.

A. herba-alba, *C. capitatus*, *R. officinalis*, *M. piperita*, *O. basilicum*, *O. valgare*, *P. argentea*, *P. rupestre* , *S. alba*, *S. distans*, *T. polium*, and *T. vulgaris* extracts showed moderate antifungal activity against *B. cinerea* isolates *in vitro* by reducing the fungal mass weight by (72, 43, 68, 40,

56, 75, 77, 64, 76, 70, 54,and 24%) at the highest concentration (3%), respectively, (Fig.15-19).

Figure (20) shows the effect of those medicinal plants with significant difference compared to the control (*A. herba-alba*, *I. viscosa*, *M. piperita*, *P. argentea*, *P. rupestre*, *R. officinalis* , *S. officinalis* , *S. alba* , *S. distans*, and *V. iphionoides*). *I. viscosa* and *V. iphionoides* showed the strongest effect at the concentration 3%; FMW of *B. cinerea* was reduced by 98% and 93%, respectively, compared to the control.

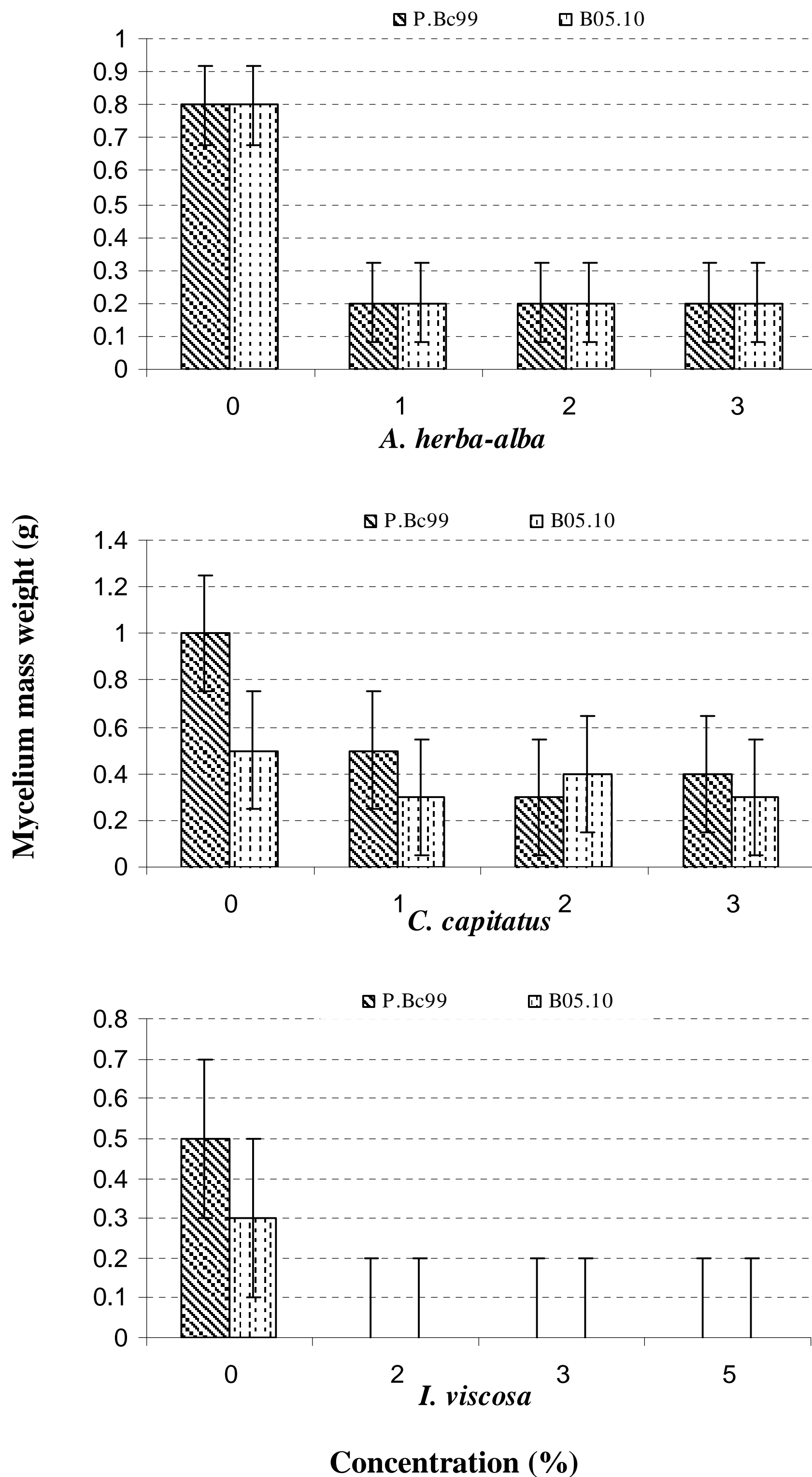

Fig. 15: Effect of plant extracts (*A. herba-alba*, *C. capitatus, I. viscosa*) on *B. cinerea* isolates (P.BC99 and Bo5.10) mycelium mass weight grown on PDB amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$): Ah =0.12, Cc =0.25, Iv =0.02.

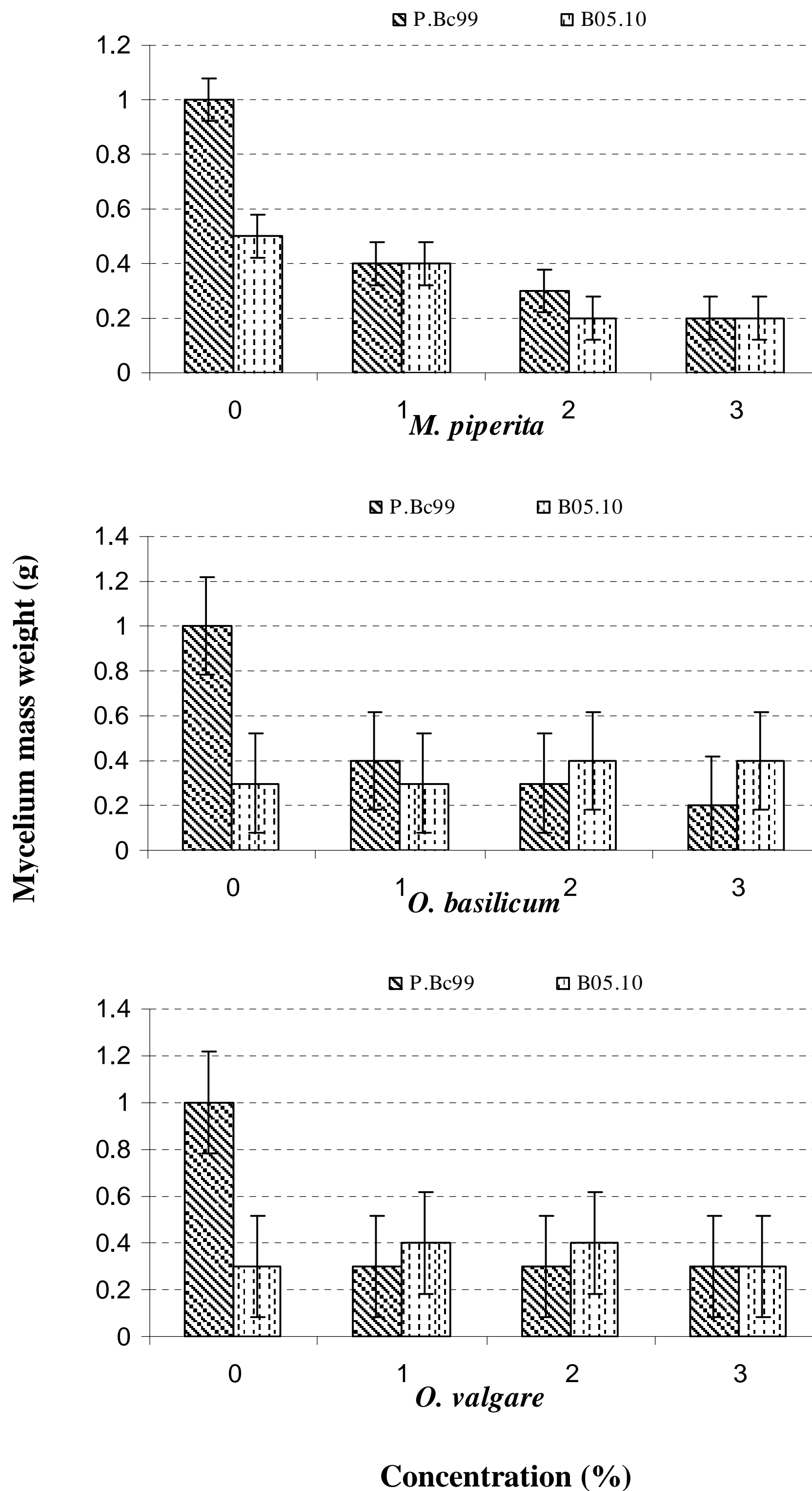

Fig. 16: Effect of plant extracts (*M. piperita*, *O. basilicum*, *O. valgare*) on *B. cinerea* isolates (P.Bc99 and Bo5.10) mycelium mass weight grown on PDB amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$): Mp =0.08, Ob=0.24, Ov=0.22.

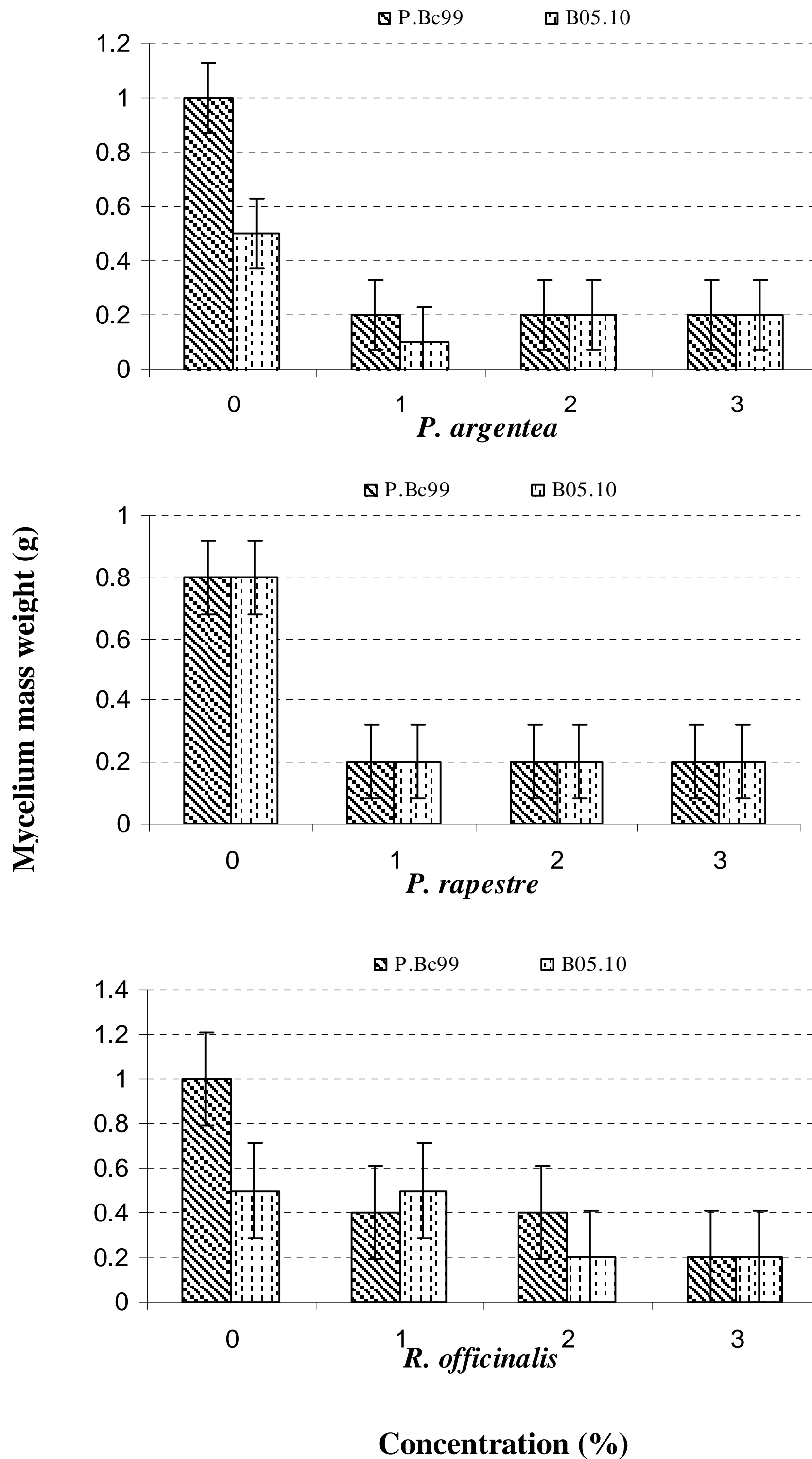

Fig. 17: Effect of plant extracts (*P. argentea*, *P. rupestre*, *R. officinalis*) on *B. cinerea* isolates (P.BC99 and Bo5.10) mycelium mass weight grown on PDB amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$): Pa=0.13, Pr=0.12, Ro=0.21.

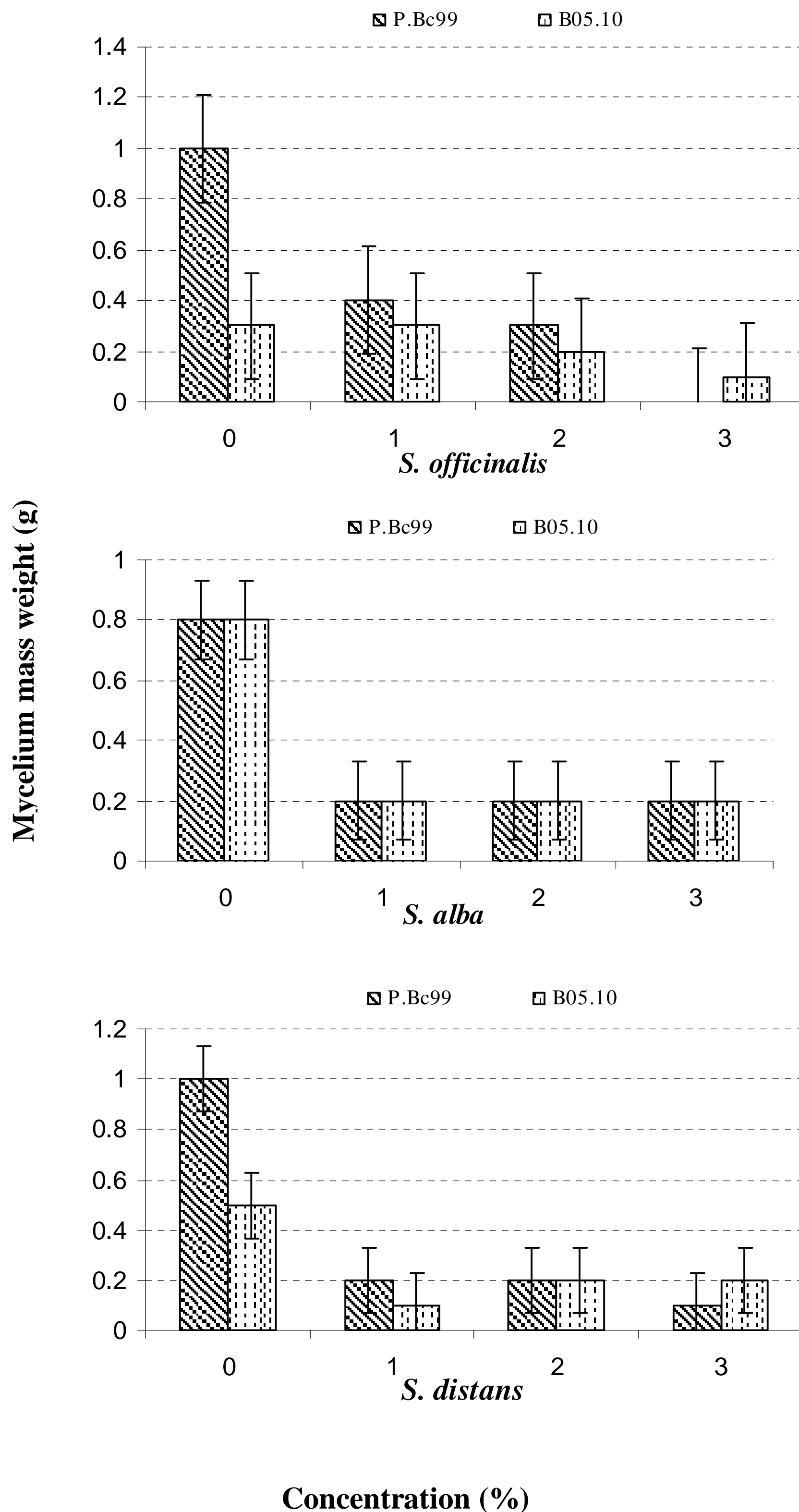

Fig. 18: Effect of plant extracts (*S. officinalis*, *S. alba*, *S. distans*) on *B. cinerea* isolates (P.BC99 and Bo5.10) mycelium mass weight grown on PDB amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$): So=0.21, Sa =0.13, Sd =0.13.

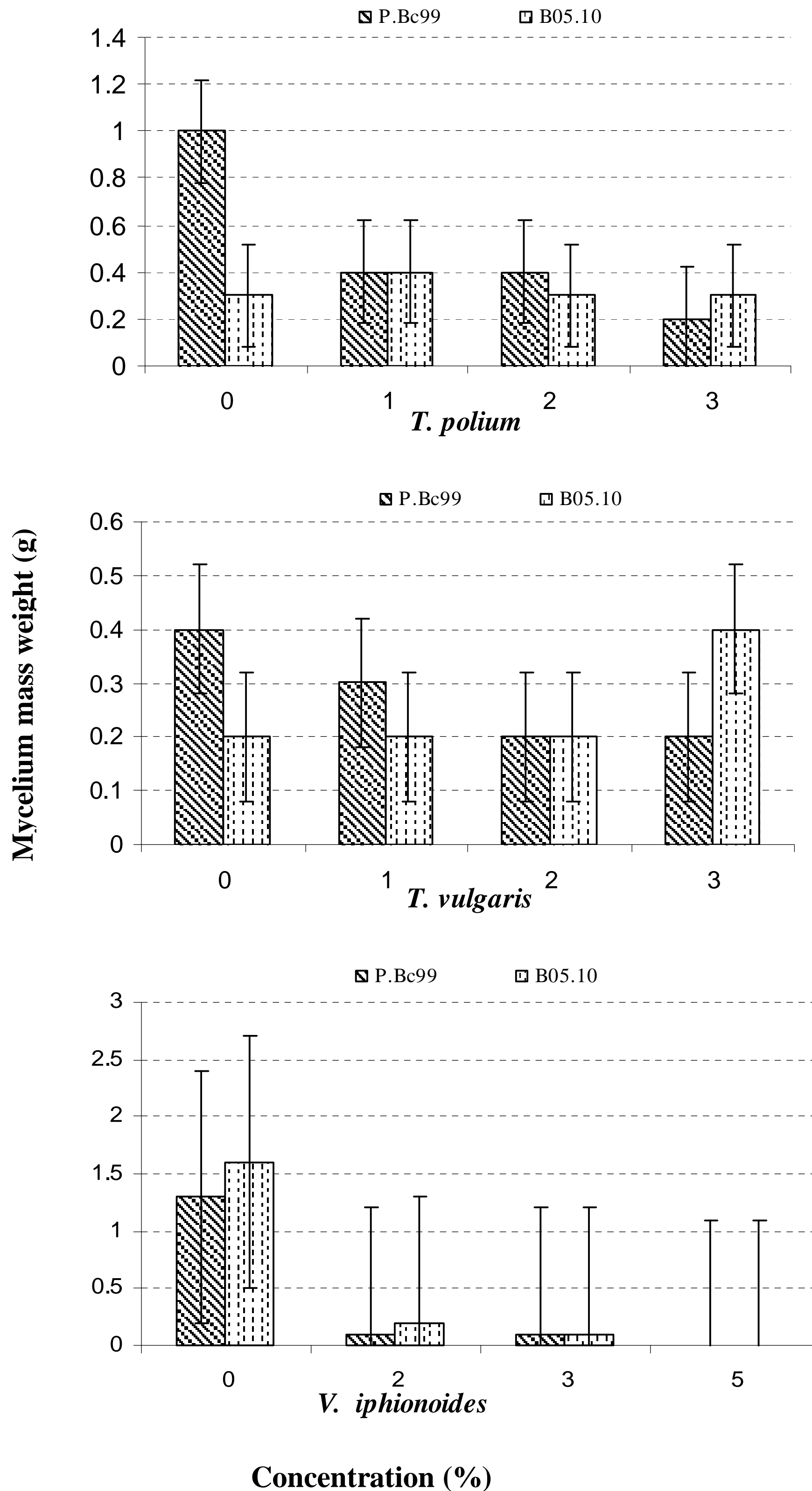

Fig. 19: Effect of plant extracts (*T. polium*, *T. vulgaris*, *V. iphionoides*) on *B. cinerea* isolates (P.BC99 and Bo5.10) mycelium mass weight grown on PDB amended with plant extracts at various concentrations. Values are means of three replicates; Fisher LSD ($p \leq 0.05$): Tp=0.22, Tv=0.12, Vi=1.1

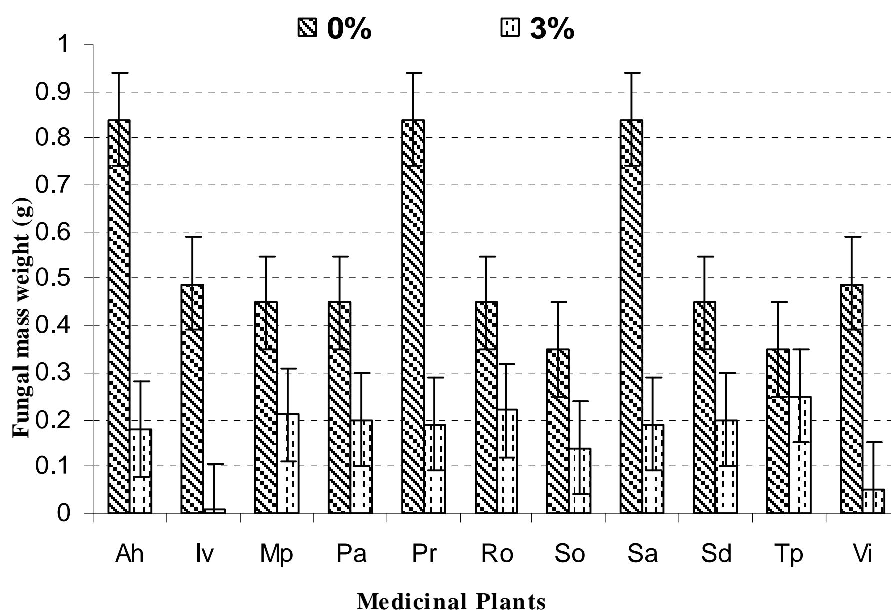

Fig. 20: Effect of plant extracts (*A. herba-alba*, *I. viscosa*, *M. piperita*, *P. argentea*, *P. rupestre*, *R. officinalis* , *S. officinalis* , *S. alba* , *S. distans*, and *V. iphionoides*) on *B. cinerea* isolate Bo5.10 fungal mass weight (g) grown on PDB amended with plant extracts. Values are means of three replicates; Fisher LSD ($p \leq 0.05$) = 0.1

3.1.4 Germination- Experiment I

The conidial germination of *B. cinerea* isolates (P.Bc99 and Bo5.10) was significantly ($p \leq 0.05$) inhibited by the fifteen medicinal plant extracts, but in lower rates than those of mycelium growth rate inhibition (Fig.21).

I. vicosa (Iv), *O. basilicum*(Ob), *O.valgare* (Ov), and *V. iphionoides* (Vi), at the highest concentration (4%), inhibited the conidial germination of *B. cinerea* isolate Bo5.10 by (100%, 19.4%, 19.4%, and 16%), respectively.

In general, conidial germination was inhibited in different rates at the plant extract concentration of 4%. *M. syriaca* strangely, have not affected significantly ($P \leq 0.05$) conidial germination. In addition, *O. basilicum* and *O.valgare* reduced germination in the same rate (19.4%).

I. viscosa extract showed a very strong inhibitory effect even at the low concentration (2%). Conidial germination decreased with increasing *I. viscosa* extract concentration reaching total inhibition (100%) at the concentration of 4%. *B. cinerea* isolate Bo5.10 was more sensitive to *I. viscosa* than the Palestinian isolate P. P.Bc99 (Fig.22and Fig. 23).

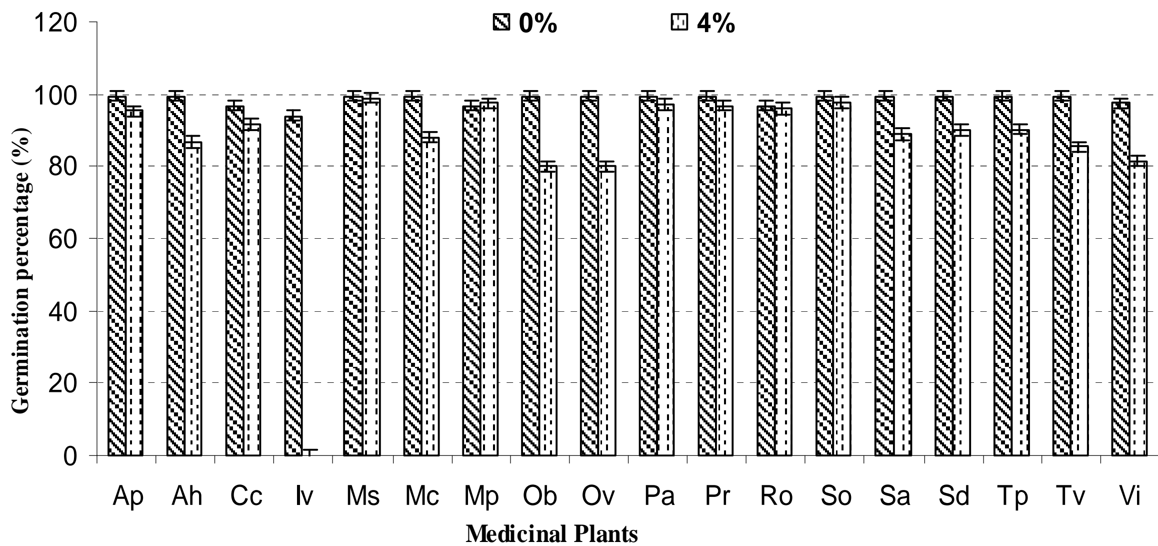

Fig. 21: Effect of the eighteen plant extracts on conidial germination of *B. cinerea* isolate Bo5.10 in sugar solution amended with plant extracts at two concentrations. Values are means of three replicates; Fisher LSD(P≤ 0.05)=1.47.

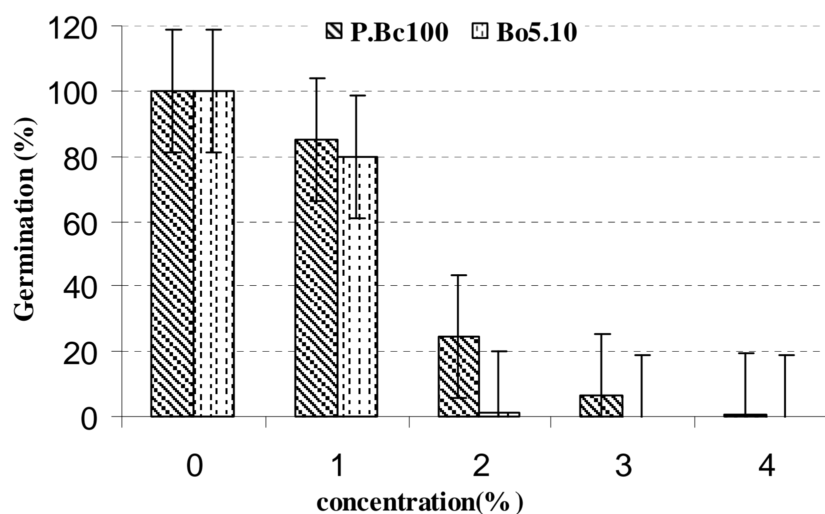

Fig. 22: Effect of *I. viscosa* on conidial germination of *B. cinerea* isolates (P.BC99, Bo5.10) in sugar solution amended with plant extract at different concentrations. (LSD=18.8, P≤ 0.05).

3.1.5 Germination- Experiment II

*I. viscosa***,** which gave the highest inhibitory effects against *B. cinerea* isolates, was further used to evaluate the variation in *B. cinerea* response in germination to plant extract application (Fig.23).Variation between isolates was significant ($p \leq 0.05$) at the two concentrations tested (Fig. 24). In addition, conidial germination of *B. cinerea* decreased with increasing plant extract concentration.

At the EC_{50} of plant extract, conidial germination of the isolates (P.Bc29 and P.Bc61) were completely (100%) inhibited. The lowest inhibition was observed with P.Bc49 (11.5%), and P.Bc67 (18.5%) at the EC_{50}. A complete inhibition (100%) at the EC_{90} was observed for the isolates P.Bc2, P.Bc13, P.Bc29, P.Bc61 and P.Bc96, and more than 99% inhibition was observed with the isolates P.Bc19 (99.6%), P.Bc33 (99%), P.Bc54 (99.6%), P.Bc59 (99.3%) and P.Bc64 (99.2%), (Fig.24).

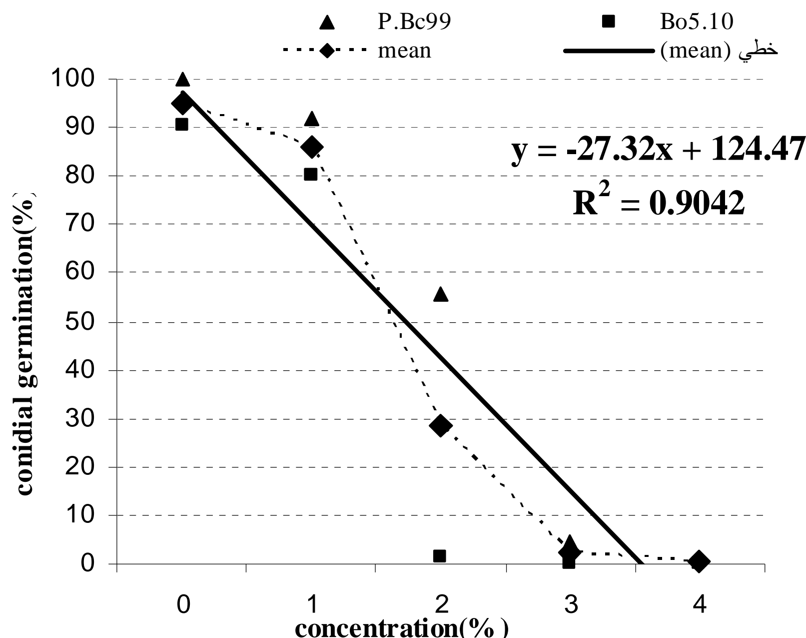

Fig. 23: Effect of *I. viscosa* on conidial germination of *B. cinerea* isolates (P.Bc99 and Bo5.10) in sugar solution amended with plant extract at various concentrations. (LSD=18.8, P≤ 0.05).

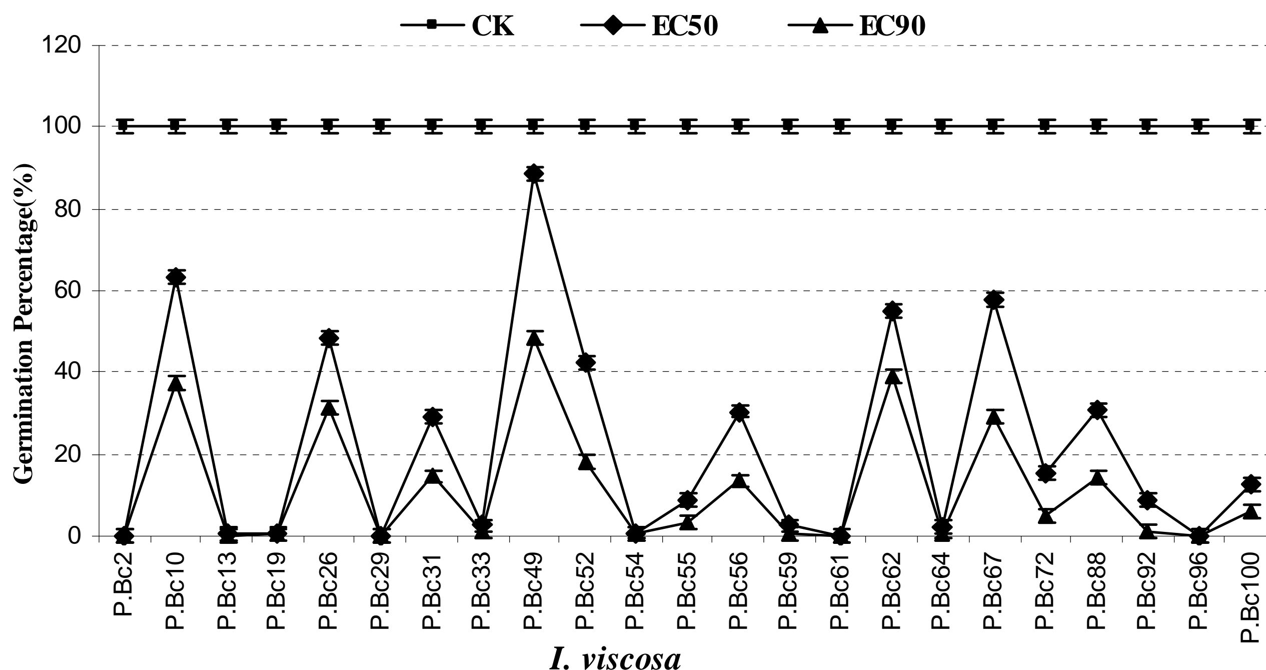

Fig. 24: Effect of *I.viscose* on conidial germination of *B. cinerea* isolates in sugar solution amended with plant extract at (EC_{50}&EC_{90}).(LSD=1.6, $P \leq 0.05$).

3.4 Integrated control studies

A combination of *I. viscosa* plant extract and Rovral® was able to reduce disease severity of gray mold by 94% (Fig.25).

I. viscosa extract alone reduced disease severity only by 31%, while Rovral® alone by 81%. There was no significant differences between *B. cinerea* isolates, however, under all treatments tested.

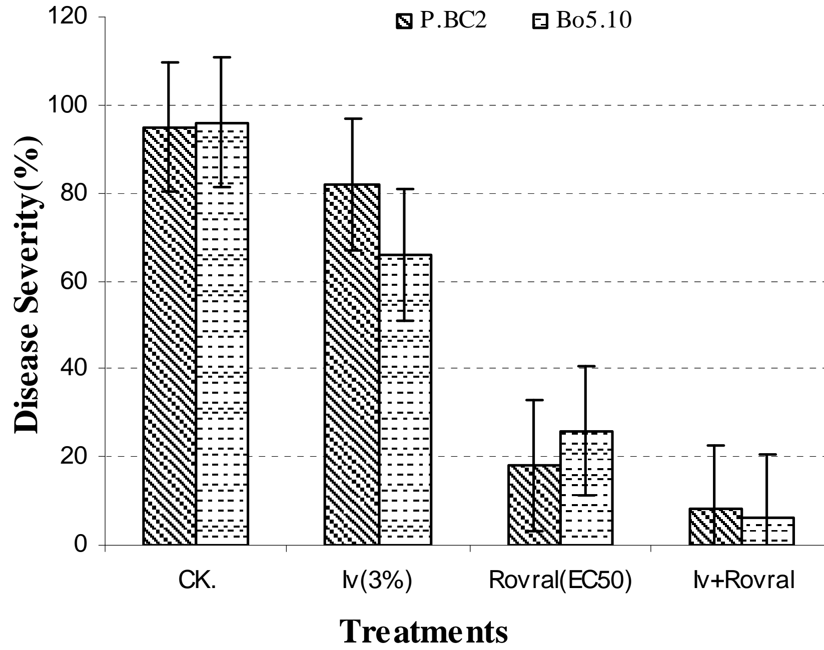

Fig. 25: Effect of *I. viscosa* extract (3%), Rovral® (EC_{50}),and a combination of Iv (3%) and Rovral® (EC_{50}) on disease severity induced by *B.cinerea* isolates (P.P.Bc2 and Bbo510) on bean plants. (LSD=14.8, P≤ 0.05).

Chapter Four

Discussion

The medicinal plant extracts of *I. viscosa*, *M. syriaca*, *S. officinalis* ,*T. vulgaris*, and *V. iphionoides* , showed the strongest antifungal activity *in vitro* against *B. cinerea* by reducing the mycelial growth rate; the activity increase as the concentration increases. Mycelial growth rate inhibition ranged between 76% and 100% at the concentration of 4%; at the lowest concentration (1%), the mycelial growth rate inhibition by *I. viscosa* , *T. vulgaris* , and *V. iphionoides* , ranged between 68% and 89%.

B. cinerea isolates response to plant extracts of *I. viscosa*, *M. syriaca, S. officinalis*, *T. vulgaris*, and *V. iphionoides*, which are Palestinian medicinal plants, was diverse at the concentration of 2% and ranged between 0% and 94 %. Similar results were observed by Bhaskara, *et al* 1997; the essential oils of *T. vulgaris* at the highest concentration (200 ppm) inhibited the MGR of *B. cinerea* by 90.5 %. Oil extracts from thyme (*C. capitatus*) (Arras *et al.* 1995), and sage (*S. officinalis*) (Carta *et al.,* 1996) inhibited *in vitro* mycelial growth of *B. cinerea.* Boyarz, and Ozcan, 2006, showed that the plant extract of *Satureja hortensis* completely (100%) inhibited the mycelial growth of *B. cinerea*. While, Knowls, (2005), in her search for antifungal activity in South African medicinal plants, showed that all of the plant extracts she selected inhibited the mycelial growth of *B. cinerea* below 70%. In addition the study showed that the mycelium growth of *B. cinerea* decreased with increased plant extract concentration.

The mycelial dry mass weight of *B. cinerea* isolates was reduced by 78-100% when the extract of *I. viscosa*, *S. officinalis* and *V. iphionoides* was applied at the concentration of 3%, and by 53-97% at the concentration of

2%. There were no similar studies for the fungal mass weight in the literature.

The conidial germination of *B. cinerea* isolates was less sensitive to extracts than mycelial growth rate. *I. viscosa*, *O. basilicum*, *O. valgare* and *V. iphionoides* reduced conidial germination by 16-100% at the highest concentration used (4%). *I. viscosa* was very effective even at the concentration of 2% with 89% reduction in conidial germination of *B. cinerea* isolate Bo5.10; conidial germination decreased with increasing concentration of plant extract. Similar results were observed by Antonov, *et al*. 1997, where conidial germination was highly (up to 100%) reduced by the oil extract of several plants. Wilson *et al*, (1997), reported that 13 plant extracts and 4 essential oils inhibited completely the conidial germination of *B. cinerea*.

The strongest antifungal activity in the germination test was observed with *I. viscosa*. However, at low concentration (EC_{50}) of *I. viscosa* extract a significant variation in *B. cinerea* isolates sensitivity was observed; the inhibition of conidial germination ranged between 11.5% and 100% at the EC_{50}, and between 51.5% and 100% at the EC_{90}. Similarly, Ziv 1996, showed that aqueous extracts obtained by boiling of old leaves of *I. viscosa* were inhibitory to fungi *in vitro* and against *B. cinerea* on fruits of grapes and tomato.

Extensive studies, has been conducted however, to elucidate the nature or biological activity of plant's water extracts, essential oils, and whole extracts in organic solvents. Many studies disclosed the presence of phenolics, flavonoids, terpenoids, sesquiterpene acids, sesquiterpene lactones, and other compounds in *I. viscosa* leaves (Shtacher and Kashman 1970, Debat, 1981, Grande, 1992, Muller-Riebau *et al*, 1997, Ali-Shtayeh *et al*, 1998, Ali-Shtayeh and Abu-Ghdeib 1999, Cohen *et al*, 2000, Yegen *et al*, 1992).

Furthermore, Shimoni *et al*, (1993) found that the whole essential oils from *M. syriaca*, exhibited *in vitro* activity against a number of phytopathogenic fungi. In recent years, studies on the antifungal activity of essential oil components have been reported by numerous investigators (Kurita *et al,* 1981, Moleyar, and Narasimhan 1986,). Deans and Ritchie (1987) reported that of the 50 plant essential oils examined, thyme oil from *T. vulgaris* was the most inhibitory against 25 genera of bacteria.

On the other hand Afifi *et al*, (1990) have dealt mainly with the characterization of *V. iphionoides* flavonoid constituents. A series of flavonoids, new for the genus, have been in fact isolated from the plant and their biological activity determined (Afifi *et al*, 1990, and Afifi *et al*, 1991) some of which showed antifungal effects.

A moderate antifungal activity against *B. cinerea* isolates, *in vitro*, was observed with the rest of the plant extracts tested (*A. palestina*, *A. herba-alba*, *C. capitatus*, *M. chamomilla*, *M. piperita*, *O.basilicum*, *O. valgare*, *P. argentea*, *P. rupestre* , *R. officinalis*, *S. alba*, *S. distans* , and *T. polium*); the reduction of mycelial growth rate ranged between 35% and 75%. Many authors have reported antimicrobial and antifungal, properties of essential oils; thyme essential oils were reported to have antimicrobial activities (Bhaskara *et al*, 1998), most of which are mediated by thymol and carvacrol, as the phenolic components of the oil. Several studies have focused on the antimicrobial activity of the essential oils of thyme in order to identify the responsible compounds. Although this holds true for the majority of *C. capitatus* oils studied, scattered publications reportfd thymol or both phenols as main oil constituents (Ravid and Putievsky, 1983; Cosentino *et al.*, 1999; Go¨ren *et al.*, 2003). In the same direction, oil extracts from thyme (*C. capitatus*) (Arras *et al.* 1995), and sage (*S. officinalis*) (Carta *et al.,* 1996) inhibited *in vitro* Mycelial growth of *B. cinerea.* Recently, many studies have focused on the biological and

antimicrobial properties of essential oils derived from *R. officinalis* species and their main constituents (Faleiro *et al*., 1999; Daferera *et al*., 2000; Koschier and Sedy, 2003; Ohno *et al*., 2003). Therefore, they were intensely screened and applied in the fields of pharmacology, pharmaceutical botany, medicinal and clinical microbiology, phytopathology and food preservation (Daferera *et al*., 2000). Furthermore, oil extracts from peppermint (*M. piperita*), (Cutler *et al*., 1996), inhibited Mycelial growth of *B. cinerea in vitro.*

Sokovic *et al*, 2006 studied the essential oils of *M. chamommilla*, *M. piperita*, *O. basilicum*, *T. vulgaris*, *O. vulgare*, and *S. officinalis*, and their components: linalyl acetate, linalool, limonene, α-pinene, β-pinene, 1,8-cineole, camphor, carvacrol, thymol and menthol. These were assayed for inhibitory activity against the three major pathogens of the button mushroom, *Agaricus bisporus*, i.e. the fungi *Verticillium fungicola* and *Trichoderma harzianum* and the bacterium *Pseudomonas tolaasii.* Further more, Hatimi *et al*., 2001, and Neerman, 2003 reported that extracts from *A. herba-alba* showed strong antibacterial activities.

Studies by (Go´ngora *et al* , 2001, Go´ngora *et al*, 2002), described the isolation and identification of seven phenolic compounds of *P. rupestre* . Ali-Shtayeh and Abu Ghdeib, 1999, reported the antifungal activity of some plant extracts including *P. rupestre* against dermatophytes.

In this study, water extracts *I. viscosa* exhibited the strongest antifungal properties against *B. cinerea* in the *in vivo* bioassays as well. When plant extracts were combined with the fungicide Rovral®, significant reduction in the disease severity (94%) was observed. The application of *I. viscosa* extract, alone, at the concentration (3%), reduced the disease severity induced by *B. cinerea* isolates on bean plants by 31%. Similarly, in a study held by Wang *et al,* (2004a), it was found that leaf extracts of *I. viscosa* were highly effective in controlling downy mildew of grapevine,

caused by *Plasmopara viticola.* Wang *et al,* (2004b), showed that extracts made from leaves of *I. viscosa* possess broad-spectrum activity against foliar diseases of crop plants. They were effective not only against grape downy mildew caused by *Plasmopara viticola*, but also against cucumber downy mildew caused by *Pseudoperonospora cubensis*, late blight in potato and tomato caused by *Phytophthora infestans*, wheat powdery mildew caused by *B. graminis*, and sunflower rust caused by *Puccinia helianthi*.

In addition, low concentrations of the plant extracts when combined with a low dose (EC_{50}) of Rovral®, a synergistic and additive interactions were observed; disease severity was reduced by 94% with the combination. This shows that a low dose of a fungicide and low concentrations of plant extracts act in synergy, and can be applied in an IPM context, which gives this control method an integrated approach dimension that can aid in reducing the environmental and health hazards, potential fungicides in the full dose may cause.

The idea behind the integrated disease management approach is to use a variety of control measures instead of relying solely on chemical pesticides. Fungicides should only be applied when absolutely necessary. Lowering the amount of fungicide applied in the environment reduces the selection pressure for pathogen resistance to develop. Fungicide residues have been found on food for human consumption, mostly from post-harvest treatments, and some of them are dangerous to human health (e.g: toxic and carcinogenic), especially when misused and abused.

Plants produce an enormous array of secondary metabolites, and it is commonly accepted that a significant part of this chemical diversity serves to protect plants against plant pathogens (Ark and Thompson, 1959; Fawcett and Spencer, 1970, Wilson *et a*l., 1997; Morrissey and Osbourne, 1999; Dixon, 2001). A problem with plant-produced

compounds as potential fungicides is that in the natural state, they are generally weakly active compared to commercial fungicides.

This study showed that Palestinian selected plants expressed strong to moderate antifungal activity against *B. cinerea* isolates *in vitro*.

Fungicide combinations and the novel regulatory functions of natural plant compounds offer the opportunity to find and validate disease control strategies with high biological activity, with low dose rate application, to overcome pathogen resistance. In this respect, it might be important to indicate that the application of plant extracts may enhance the efficacy of the synthetic fungicides, as shown in the *in vivo* assay against *B. cinerea*.

However, further detailed studies are needed before reaching the stage of a wide-scale application and conclusions in the field of gray mold disease control.

Conclusions

The study showed that some native Palestinian plants can be potential source of antifungal compounds against *B. cinerea*. *In vitro* studies showed that the medicinal plants with antifungal potential are *I. viscosa*, *M. syriaca*, *S. officinalis*, *T. vulgaris* and *V. iphionoides*. The antifungal potential increases as the plant extract concentration increase. The strongest antifungal potential in the germination test was observed with the extract of *I. viscosa* which reduced or even completely inhibited the conidial germination of *B. cinerea* isolates. *B. cinerea* isolates showed significant variation in their sensitivity to medicinal plant extracts (isolate dependent).

The integrated control study showed that the combination of plant extract (*I. viscosa*) with reduced dose of commercial fungicides can be potentially important in gray mold disease control.

Further studies are needed to confirm and shed more light on the identification of active ingredients in plants, mode of actions and stable formulations in the field, in addition to more field trials.

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Appendices

Appendix A

Fig. 26: ***Inula viscose***

Fig. 27 : ***Varthemia iphionoides***

Fig. 28: ***Thymus vulgaris***

Fig. 29: ***Majorana syriaca***

Fig. 30: ***Salvia officinalis***

Fig. 31: ***Coridothymus capitatus***

Fig. 32: ***Rosemarinus officinalis***

Fig. 33: ***Stachys distans***

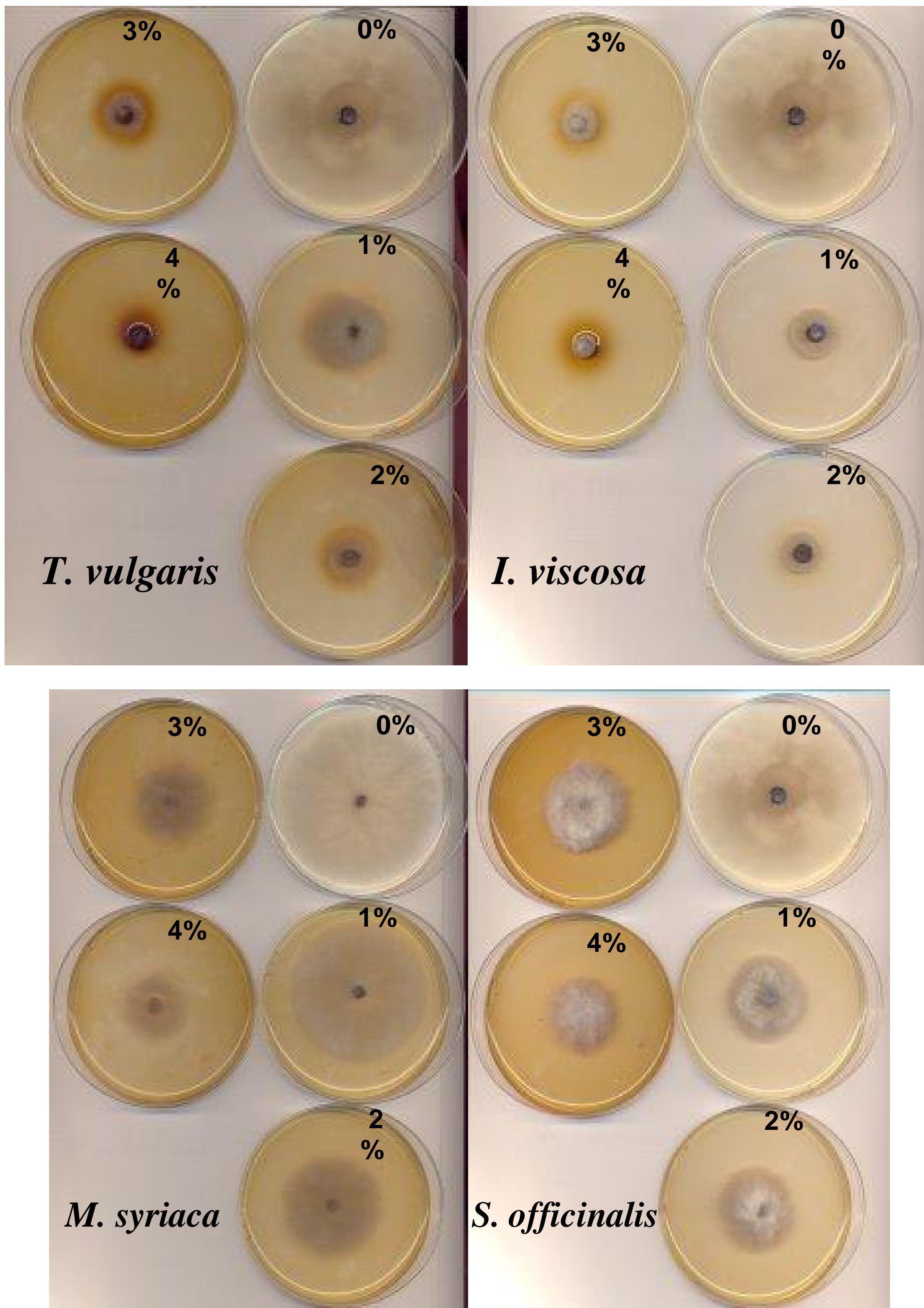

Fig. 34: Mycelium growth rate (MGR) of *B. cinerea* grown on PDA amended with plant extracts at various concentrations.

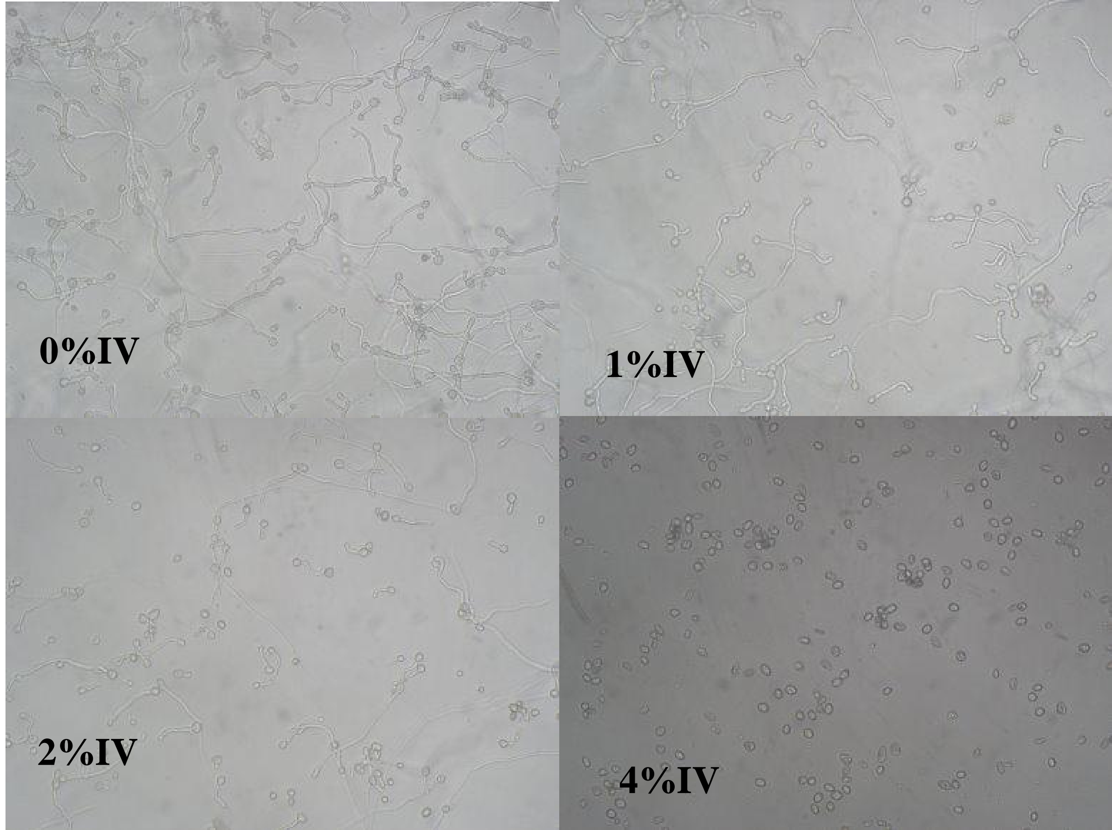

Fig. 35: Effect of *Inula viscose* on conidial germination of *B. cinerea* in sugar amended with plant extract at various concentrations.

Fig. 36: ***B.cinerea*** **mycelium growing on PDA**

Fig. 37: ***B.cinerea*** **mycelium growing on PDB (with shaking)**

Fig. 38: Effect of *I. viscosa* (Iv) extract (3%), Rovral® (EC50), combination of Iv (3%) and Rovral® (EC50) on disease severity of *B. cinerea* on bean plants.

Fig. 39: Symptoms of gray mold on bean plants.

Fig. 40: Symptoms of gray mold on fruits of various crops.

Appendix B: ANOVA Tables

Mycelial growth rate (MGR) - Experiment I

Plant	DF, treat	DF, Resiual	DF, total	SS, treat	SS, Residual	SS, total	MS, treat	MS, residual	F	LSD
Anthemis palestina	19	40	59	533.49	14.35	547.84	28.08	0.36	78.3	0.99
	19	40	59							
Artemisia herba-alba	19	40	59	366.18	8.1.7	366.18	19.27	0.2	94.4	0.75
Coridothymus capitatus	19	40	59	969.58	10.61	980.19	51.03	0.27	192	1.14
Inula viscose	19	40	59	1345.1	1.39	1346.5	70.8	0.03	2042	0.31
Majorana syriaca	19	40	59	1486.8	7.61	1494.4	78.35	0.19	411	0.96
Marticria chamomilla	19	40	59	265.39	8.49	273.88	13.97	0.21	65.8	0.76
Mintha piperita	19	40	59	527.13	13.01	540.14	27.74	0.33	85.3	0.94
Ocimum basilicum	19	40	59	285.15	9.43	294.58	15.01	0.24	63.7	0.8
Origanum valgare	19	40	59	709.59	9.88	719.47	37.35	0.25	151	0.82
Paronychia argentea	19	40	59	765.05	21.17	786.22	39.79	0.53	75.2	1.2
Phagnalon rupestre	19	40	59	584.82	14.55	599.37	30.78	0.36	84.6	1
Rosemarinus officinalis	19	40	59	550.99	7.59	558.58	29	0.19	153	0.72
Salvia officinalis	19	40	59	911.97	11.36	923.33	48	0.28	169	0.88
Sinapis alba	19	40	59	341.06	73.92	414.98	17.95	1.85	9.71	2.24
Stachys distans	19	40	59	687.38	15.21	702.59	36.18	0.38	95.1	1.02
Teucrium polium	19	40	59	833.03	4.75	837.78	43.84	0.12	369	0.57
Thymus vulgaris	19	40	59	1792	1.41	1793.4	94.31	0.04	2669	0.31
Varthemia iphionoides	19	40	59	1879.7	0.59	1880.3	98.93	0.02	6745	0.2

Mycelial growth rate (MGR) - Experiment II

Plant	DF, treat	DF, Resiual	DF, total	SS, treat	SS, Residual	SS, total	MS, treat	MS, residual	F	LSD
Inula viscose	35	72	107	1905.4	40.11	1945.5	54.44	0.56	97.73	1.22
Majorana syriaca	35	72	107	1437.26	43.67	1480.9	41.07	0.61	67.71	1.3
Salvia officinalis	35	72	107	1277.63	35.23	1312.9	36.5	0.49	74.6	1.14
Thymus vulgaris	35	72	107	1877.23	40.83	1918.1	53.64	0.57	94.59	1.23
Varthemia iphionoides	35	72	107	1852.25	40.19	1892.4	52.92	0.56	94.8	1.22

Experiment	DF, treat	DF, Resiual	DF, total	SS, treat	SS, Residual	SS, total	MS, treat	MS, residual	F	LSD
Germination- Exp. II	47	48	95	52064.4	30.64	52095.07	1107.75		1735	1.61
Integrated control study	7	32	39	53599.4	4220	57819.38	7657.05	131.88	58.1	14.8

Fungal mass weight (FMW)

Plant	DF, treat	DF, Resiual	DF, total	SS, treat	SS, Residual	SS, total	MS, treat	MS, residual	F	LSD
Artemisia herba-alba	7	15	22	0.05	0.03	0.08	0.01	0.002	2.96	0.12
Coridothymus capitatus	7	10	17	0.78	0.12	0.9	0.01	0.002	8.96	0.25
Inula viscose	9	19	28	0.55	0.001	0.551	0.11	0.01	749.8	0.02
Majorana syriaca							0.61	0		
Mintha piperita	7	14	21	1.13	0.02	1.15	0.16	0.001	115.1	0.08
Ocimum basilicum	7	13	20	1.16	0.23	1.39	0.17	0.02	9.22	0.24
Origanum valgare	7	15	22	1.21	0.24	1.45	0.17	0.02	10.63	0.22
Paronychia argentea	7	14	21	1.28	0.06	1.34	0.18	0.005	39.65	0.13
Phagnalon rupestre	7	16	23	1.7	0.08	1.78	0.24	0.005	51.22	0.12
Rosemarinus officinalis	7	14	21	1.07	0.16	1.23	0.15	0.01	13.22	0.21
Salvia officinalis	7	12	19	1.61	0.17	1.78	0.23	0.01	16.68	0.21
Sinapis alba	7	16	23	1.6	0.09	1.69	0.23	0.006	39.79	0.13
Stachys distans	7	14	21	1.28	0.06	1.34	0.18	0.005	39.65	0.13
Teucrium polium	7	15	22	1.18	0.25	1.43	0.17	0.02	10.09	0.22
Thymus vulgaris	7	15	22	0.14	0.07	0.21	0.02	0.004	4.55	0.12
Varthemia iphionoides	9	20	29	9.46	8.41	17.87	1.05	0.42	2.5	1.1

Germination – Experiment I

Plant	DF, treat	DF, Resiual	DF, total	SS, treat	SS, Residual	SS, total	MS, treat	MS, residual	F	LSD
Anthemis palestina	9	40	49	153.6	18.4	172	17.1	0.5	37.1	0.87
Artemisia herba-alba	9	40	49	788.5	25.1	813.6	87.6	0.6	139.6	1
Coridothymus capitatus	9	30	39	752.1	137.2	889.3	83.6	4.6	18.3	3.1
Inula viscose	9	30	39	72476	16.6	72492	8052.9	0.55	14591	1.1
Majorana syriaca	9	30	39	9617	122.9	9739.9	1068.6	4.1	2609	2.9
Marticria chamomilla	9	30		69.6	122.9					
Mintha piperita	9	40	49	69.6	23.6	93.2	7.7	0.79	9.8	1.3
Ocimum basilicum	9	40	49	3237.2	9.9	3247.1	359.7	0.25	1453	0.6
Origanum valgare	9	40	49	3293.6	9.2	3302.8	365.9	0.23	1591	0.6
Paronychia argentea	9	30	39	63.6	9.6	73.2	7.1	0.24	295.5	0.6
Rosemarinus officinalis	9	40	49	77.5	58.9	136.4	8.6	1.9	4.4	2
Salvia officinalis	9	40	49	24.2	13.9	38.1	2.7	0.3	7.8	0.75
Sinapis alba	9	40	49	550.5	18	568.5	61.2	0.5	135.9	0.86
Stachys distans	9	40	49	1171	45.7	1216.7	130.1	1.1	113.9	1.4
Teucrium polium	9	40	49	531.6	12.4	544	59.1	0.3	190.5	0.7
Thymus vulgaris	9	40	49	1963.6	11	1974.6	218.2	0.3	793.4	0.7
Varthemia iphionoides	9	30	39	25664	83.1	25747	2851.5	2.8	1029	2.4

التأثير المضاد للفطريات لمستخلصات نباتات محلية مختارة ضد فطر *Botrytis cinerea* المسبب لمرض العفن الرمادي

الملخص

الفطر *Botrytis cinerea* هو ممرض نباتي انتهازي يتطفل على العديد من المحاصيل الزراعية مسببا مرض العفن الرمادي، ويمكن مكافحة هـا المرض بعدة طرق منها استخدام المبيدات الفطرية و العديد من الممارسات الزراعية الصحيحة.

وقد طور هذا الفطر مقاومة للعديد من المبيدات الفطرية مما ادى الى البحث عن عدة بدائل علاجية منها المكافحة الحيوية و مستخلصات النباتات. وفي هذه الدراسة فقد تم دراسة تاثير مستخلصات ثمانية عشر نباتا محليا فلسطينيا و هي:

Anthemis palestina, *Artemisia herba-alba*, *Coridothymus capitatus*, *Inula viscosa*, *Majorana syriaca*, *Marticria chamomilla*, *Mintha piperita*, *Ocimmum basilicum*, *Origanum valgare*, *Paronychia argentea*, *Phagnalon rupestre*, *Rosemarinus officinalis*, *Salvia officinalis*, *Sinapis alba, Stachys distans*, *Teucrium polium*, ***Thymus vulgaris***, and *Varthemia iphionoides*، حيث تم تقييمها لمكافحة الفطر في المختبر والمرض المسبب له على نبات الفاصولياء.

أظهرت النتائج مقدرة عالية لجميع مستخلصات النباتات المذكورة على تثبيط معدل نمو الميسيليوم لعزلات فطر *B. cinerea* المستخدمة في هذه الدراسة. وان اعلى نشاط مضاد للفطريات لوحظ في مستخلصات كل من الطيون *I. viscose*، الزعتر *M. syriaca*، المريمية *S. officinalis*، زعتر غزال *T. vulgaris*، و الكتيلا *V. iphionoides*، والتي ثبطت معدل نمو الميسيليوم لعزلات فطر *B. cinerea* بنسبة 76-100% عند استخدامها بتركيز 4%، حيث لوحظ تفاوتاً معنوياً في حساسية عزلات الفطر.

أما أقوى تأثير مضاد للفطريات ضد انبات ابواغ الفطر فكان باستخدام مستخلص نبات الطيون *I. Viscosa*، والذي منع انبات أبواغ عزلات *B. cinerea* كلياً. وقد لوحظ تفاوتاً معنوياً في حساسية عزلات *B. cinerea* بتركيز (EC_{50}) من مستخلص الطيون حيث تراوحت نسبة تثبيط انبات ابواغ عزلات الفطر بين (% 100-11.5)، وبين (% 100-51.1) عند استخدام المستخلص بتركيز (EC_{90}) .

وفي دارسة فعالية المكافحة المتكاملة ضد المرض كان هناك انخفاضاً معنوياً في شدة المرض عند خلط مستخلص الطيون *I. viscosa* مع المبيد الفطري $Rovral^{®}$ بنسبة 94% بينما مستخلص الطيون لوحده بتركيز 3% خفض شدة المرض الناتج عن *B. cinerea* على نبات الفاصولياء بنسبة 31% .

ويستنتج من هذه الدراسة أن هناك مقدرة هامة لمستخلصات بعض النباتات الفلسطينية المختارة في مكافحة *B. cinerea* وخاصة عند دمجها مع جرعة مخفضة من المبيدات الفطرية و التي تعطي هذه الطريقة في المكافحة بعداً بيئياً هاماً ، و التي من الممكن ان تسهم في تقليل الاخطار الصحية و البيئية التي قد تتسبب في حال استخدام الجرعة الكاملة من المبيد الفطري.
بيد انه من الضروري إجراء المزيد من الدراسات حول استخدام المستخلصات النباتية في الحقل، وآلية عملها، و التركيب الثابت للمستخلصات قبل الوصول الى استنتاجات جازمة حول أهلية وكفاءة هذه المعاملات في مكافحة مرض العفن الرمادي حقلياً.