PATHOLOGICAL STUDIES ON DAHLIA TUBER ROTS

By

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B.Sc. Agricultural Sciences (Plant Pathology),
Fac. Agric. Moshtohor, Zagazig Univ., 1998

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CONTENTS

INTRODUCTION

Dahlia (*Dahlia pinnata* L.) is one of the most popular and attractive cut flower plants around the world. Many specialists and consumers consider it the queen of summer flowers. Dahlia flowers could be exported as cut flowers where they have a long vase life and could be used also as pot plants or as bedding plants for landscaping, especially in form of single variety group **(Bradley, 1993)**. Dahlia is propagated by different methods like seeds, divided tubers or by stem cuttings. Large number of stem cuttings can be taken from dahlia tubers where, they rooted easily in greenhouse then planted normally in roundly form into pot or planted in the field in rows **(Protich, 1988)**.

Many fungal and bacterial diseases are attacking dahlia plants in the field and during storage or during propagation by stem cuttings and divided tubers. The mostly fungal diseases caused by *Sclerotium rolfsii*, *Fusarium solani* and *Macrophomina phaseoli*. Sclerotium disease (*S. rolfsii*) is the most important and destructive one, it causes heavy annual losses to flowers and tuber production. Bacterial soft rot disease caused by *Erwinia carotovora* pv. *carotovora*, *E. carotovora* pv. *chrysanthemi*, *Pseudomonas cichorii* and *Bacillus polymyxa* were the most common pathogenic bacteria attacking and destroying dahlia tubers under ground in the field or in stores **(Lelliott and Stead, 1987)**.

This work aimed to isolate and identify the prevalent fungi and pathogenic bacteria attacking dahlia plants under Egyptian conditions. Studying the optimum conditions of these pathogens to grow and infect dahlia. Studying the

abilities of these pathogens to secrete cell wall degrading enzymes in order to understand the behavior of these pathogens in inducing tuber rots. Evaluation the effect of some fungicides and antibiotics in controlling these pathogens.

REVIEW OF LITERATURE

1. The causal microorganisms:

1.1. Fungi

Chu *et al.* (1961) recorded *Sclerotium rolfsii* as one of the most pathogenic fungi attacking sugar beet, causing damping-off and root rot.

Aycock (1966) stated that *S. rolfsii* was found on more than 500 species of plants in about 100 families and causes economically important disease in warm, moist climates throughout the world.

Brooks (1981) mentioned that *S. rolfsii* is a virulent parasite of many crop plants, e.g. beans, maize, potatoes, tomatoes, and cabbage attacking them from the soil near the ground level in warm countries.

Fahim *et al.* (1981) mentioned that *S. rolfsii*, *S. bataticola* [*Macrophomina phaseolina*], *Fusarium oxysporum* f.sp. *conglutinans* and *Pythium* sp. isolated from mature root tissues of sugar beet showing severe rotting, were pathogenic to all tested sugar beet cultivars. *S. rolfsii* was the most virulent and *M. phaseolina* was the least.

Bhargava *et al.* (1983) stated for the first time that *Fusarium equiseti* cause a severe foot and root rot of high yielding potato cultivars in India. Disease incidence varied from 10 to 30% .

Naiki *et al.* (1985) isolated sclerotia of *Rhizoctonia solani* from rotted roots of sugar beet in Hokkaido.

Papavizas and Collins (1990) mentioned that *S. rolfsii* causes extensive damage to plant in more than 100 families in warm regions of the world.

Manici and Cerato (1994) isolated 8 *F. oxysporum* f.sp. *tuberosi* isolates, 6 from rotten potato tubers and 2 from weak, but not wilted, potato plants in seed-growing areas of northern Italy. The pathogenicity and potential spread of the fungus within the plant were assessed in greenhouse and lab tests. All isolates caused dry rot on inoculated tubers. It is suggested that *F. oxysporum* f.sp. *tuberosi* may become a potential danger during potato storage, as propagules increase under favourable conditions although disease may not be evident in the field.

Abada (1994) isolated the following fungi: *Alternaria* spp.; *Mucor* spp.; *Fusarium* spp., *F. conglutinans*, *F. solani*; *Pleospora betae*; *Pythium debaryanum*; *R. solani*; *S. bataticola* [*Macrophomina phaseolina*]; *S. rolfsii* [*Corticium rolfsii*] and *Trichoderma harzianum* from rotted sugar beet roots collected in Egypt (Kafr El-Shikh governorate). All the isolated fungi were pathogenic except the antagonistic fungus *T. harzianum*.

Osai *et al.* (1996) investigated the response of yam (Dioscorea) cultivars to artificial and natural infections with minisett rot pathogens. None of the yam cultivars were resistant to *S. rolfsii*.

Ray *et al.* (1996) isolated several fungi and bacteria from decaying sweet potato tubers under ambient temperature (30° ± 2°C) and relative humidity (70-95%). These species were common storage fungi belonging to the genera *Rhizopus*, *Aspergillus*, *Fusarium*, *Penicillium* and *Botryodiplodia* and 2

bacteria. However, *Aspergillus* (*A. niger*, *A. oryzae* and *A. flavus*) were mostly secondary invaders and could not repeat the decay when inoculated into fresh and healthy tubers. *Rhizopus* decay (soft-rot caused by *R. oryzae*) was prolific and more prevalent following kharif (RH, 80-95%) than in rabi season (RH 70-85%). *B. theobromae* was the common storage fungus causing java black rot and was severe in both kharif and rabi. *Fusarium* rot was moderate intensity and the causal agent was *F. oxysporum*. Pathogenicity tests confirmed the susceptibility of sweet potatoes to these pathogens. Further, these storage fungi had wide host specificity ranging from *Amorphophallus*, cassava and *Colocasia* to white yams.

Ugwuanyi and Obeta (1997) reported that *Corticium rolfsii* isolated from cocoyam (*Colocasia* spp.) corms and cormels showing spilage symptoms from many stores in the Nsukka locality, Japan, caused cocoyam rot in pathogenicity tests. The rot due to *C. rolfsii* was extensive resulting in complete maceration of cocoyam tissue.

Gomaa and Mohamed (2000) isolated *S. rolfsii* from naturally rotted dahlia tubers in Egypt and studied its effects on vegetative, flowering characteristics and tubers formation of dahlia (*Dahlia pinnata*).

Okigbo and Ikediugwu (2000) mentioned that the fungi *A. niger*, *B. theobromae*, *Penicillium oxalicum*, *Rhizopus* sp. and *Rhizoctonia* sp. infected the white yam cultivars. The establishment of *Rhizoctonia* sp. as a postharvest pathogen in Nigeria was also discussed.

Okigbo (2003) isolated *A. niger*, *B. theobromae*, *F. solani*, *Thanatephorus cucumeris*, *Choanephora cucurbitarum*, *P. oxalicum*, *Rhizopus* sp., and *Trichoderma* sp. from the yam tuber surface of all cultivars (*Discorea rotundata* cultivars Omi, Iyawo, and Ikale, and a *D. alata* cultivar) immediately after harvest in Agbor, Nigeria. *B. theobromae* was most frequently isolated, followed by *A. niger*, *Trichoderma* sp., *Rhizopus* sp., and *F. solani*. *Aspergillus niger* was frequently isolated throughout the year except in *D. alata* on which it was relatively sparse, while *P. oxalicum* was recorded only during the early months of storage. The surface mycoflora of *D. alata* was particularly high in *Rhizopus* sp. and *Trichoderma* sp. Fungal density was highest on the head region of tubers.

1.2. Bacteria

Dowson (1957) stated that *Erwinia carotovora* subsp. *carotovora*, *Pseudomonas marginalis* and *Bacillus* spp. were among the most predominant rotting organisms into post harvest decay of vegetables and fruits.

Hoitink and Daft (1972) isolated *E. carotovora* var. *chrysanthemi* from the tissues but not from the surface of stunted plants of chrysanthemum, shasta daisy (*Chrysanthemum maximum*) *Syngonium podophyllum* and dahlia (*Dahlia pinnata*).

Boesewinkel (1975) mentioned that *E. chrysanthemi* is a serious disease on dahlia and carnation plants in North Island, New Zealand where it was recently discovered. It also affects many other ornamentals.

Alivizatos (1979) reported that *E. chrysanthemi* infected tomato, potato, tobacco, maize, chrysanthemum, tagetes, zinnia, hydrangea, canna and dahlia plants.

Jump *et al.* (1983) isolated *E. carotovora* from cactus plants showing soft rot symptoms.

McGuire and Kelman (1983) tested the susceptibility of nine potato cultivars grown on two soils to *Erwinia* soft rot. Tubers of Butte, Russet Burbank and Norgold Russet were consistently resistant to *E. carotovora* on both silt loam and fine sandy loam soils.

Piplani *et al.* (1983) isolated *Bacillus polymyxa* from rotten tuber tissues, the vascular tissues of apparently healthy tubers and healthy aerial stems. Also, observed frequently necrosis of leaf veins and apical buds during June-August in glasshouse-grown potato plants. All the cultures of *B. polymyxa* caused soft rot of tuber tissues and necrosis of aerial parts.

Peltzer and Sivasithamparam (1985) found that all isolates of *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* from Western Australia produced stem rots in potato when provided with their specific environmental requirements. The pathogenicity of *E. chrysanthemi* was highly variable. A high inoculum level (3×10^7 cfu/ml) was required for rapid rotting by *E. chrysanthemi*.

Bhattacharya and Mukherjee (1986) concluded that the soft rot of stored tissues could be due to some uncommon bacteria. Genera such as *Bacillus* and *Pseudomonas* as well as *Erwinia* were associated with soft rots of vegetables, fruits and other stored tissues. The bacteria caused rotting at high temp.

(40°C) and 100% RH or under water-soaked conditions. Some showed preference for different tissues although all caused equally extensive rotting of potato, carrot, pineapple and onion .

Dopke *et al.* (1987) studied susceptibility to potato blackleg and tuber soft rot, both caused by *E. carotovora* subsp. *atroseptica*, in 7 cultivars. Only Sieglinde and Hansa showed a positive correlation between stem and tuber responses. Resistance to *E. carotovora* subsp. *carotovora* and subsp. *chrysanthemi* [*E. chrysanthemi* pv. *chrysanthemi*] increased considerably as development progressed. Marked virulence on stems by some subsp. *chrysanthemi* strains was associated with high *in vitro* production of polygalacturonase.

Romeiro *et al.* (1988) stated that *E. carotovora* is the soft rot pathogen of Peruvian carrot in Minas Gerais State, Brazil, either under field conditions or after harvested roots have been stored.

Ramesh *et al.* (1989) observed a severe outbreak of carrot soft rot During Mar.-Apr. 1986 and 1987 in Bangalore, Karnataka, India, and described symptoms of the disease. The causal organism was isolated and identified as *E. carotovora* subsp. *carotovora* and its pathogenicity was confirmed. This is the first report of soft rot of carrot caused by *E. carotovora* subsp. *carotovora* in the field in India.

Khan *et al.* (1990) collected potato tubers with soft rot symptoms from various sources in Bangladesh. Bacteria isolated were rods of different sizes, Gram negative and non-spore forming. Pathogenicity of the isolates was confirmed by *in vitro*

tuber inoculation. Biochemical and physiological characteristics confirmed the isolates to be *Erwinia* and *Pseudomonas*.

Kozak (1990) evaluated about 50 carrot cultivars and breeding lines grown during 1988 and 1989 in Poland for their reaction to *E. carotovora* and for the effect of duration of root storage on disease incidence. No resistant cultivars were found but Rola cv. was the least susceptible in both years. The duration of root storage had no effect on susceptibility.

Togashi *et al.* (1995) detected *Erwinia* spp in tissues of periodically collected apparently healthy vegetables left in fields. Of the 17 species of vegetable collected, *Erwinia* bacteria were detected in 9 (spinach, turnips, cabbages, Jerusalem artichokes, potatoes, radishes, carrots and Chinese cabbages).

Clark *et al.* (1998) showed that isolation from sweet potato roots that displayed typical bacterial root rot yielded bacteria with similarity of 94.5% to *E. carotovora* subsp. *carotovora* using the Biolog GN Bacterial Identification System. This isolate (Ecc-LH) differed from isolates of *E. chrysanthemi* (Ech) from sweet potato and other hosts in that it was insensitive to erythromycin, did not produce phosphatase or lecithinase and did not produce gas from glucose. Ecc-LH differed from known strains of *E. carotovora* subsp. *atroseptica* in that it did not produce reducing substances from sucrose or acid from palatinose.

El-Hendawy *et al.* (2002) isolated thirty soft rot *Erwinia* strains from diseased carrot (*Daucus carota*) roots and pepper (*Capsicum annuum*) fruits. Pathogenicity tests showed that these strains are able to induce soft-rot disease symptoms in different

vegetable species (maize, cucumber, melon, pepper and tomato) indicating a wide host range.

2. Factors affecting growth and sclerotial formation of *S. rolfsii in vitro*

Higgins (1927) reported that mycelium of *S. rolfsii* grew at temperature range of 8-40°C and grew well at 20-30°C. The fungus grew in beef extract-peptone broth culture initially adjusted to a series of levels over the range of pH 1.4 to 8.8.

Johnson and Joham (1954) reported that xylose was more readily metabolizable sugars by *S. rolfsii* than arabinose. Of the hexoses, galactose and glucose were superior to mannose and fructose while lactose proved superior. Growth on maltose, raffinsoe, dextrin, starch and inulin was interior to that on glucose. They suggested that *S. rolfsii* was able to secrete a considerable array of exogenous carbohydrates which may play an important part in the parasitic action of the pathogen.

Hernandez (1986) isolated *S. rolfsii* from bean (*Phaseolus vulgaris*) and grown it on a medium based on leaves supplemented with dextrose. Optimum pH was 4-6 and growth was inhibited at pH 2 and pH 13.

Nwufo and Fajola (1986) mentioned that *S. rolfsii* grew best with dextrin, at pH 5 and 30°C. Sclerotial production was maximum at 30°C, with light and darkness alternating for 12 hours each.

Prasada *et al.* (1987) found that mycelial growth of *S. rolfsii* was best at 30°C and pH 5, while most sclerotia formed at

25°C and pH 7. Sucrose was the best carbon source for both processes.

Palakshappa *et al.* (1989) found that *S. rolfsii* grew well at 25-30°C and no growth occurred above 50°C.

Yuan *et al.* (1990) reported that *S. rolfsii* isolates grew well at 28-32°C, relative humidity 92%, pH 4-6, in darkness and under aerobic conditions.

Hari *et al.* (1991) reported that groundnut shoot extract was the best liquid medium for growth and sclerotial production by *S.* [*Corticium*] *rolfsii*. Growth was best at 30°C, pH 6 and with starch and dextrin as carbon sources and peptone and potassium nitrate as nitrogen sources. Sclerotial production was favoured at 25°C and by starch, dextrin and peptone.

Dey *et al.* (1992) showed that mycelial growth of *S.* [*Corticium*] *rolfsii* was best on cornmeal agar, taro (*Colocasia esculenta*) leaf extract agar, malt extract agar and PDA, while the most sclerotia were obtained in cultures on PDA. Mycelia growth and sclerotia formation occurred best over the temperature range 25-30°C and pH 4-7.

Jyoti-Mishra *et al.* (1996) reported that maximum growth and sclerotial formation of 2 isolates of *S. rolfsii* (SR1 & SR2) were recorded on potato dextrose agar medium at 30 ± 2°C and pH 6.5. Glucose and potassium nitrate were the best sources of carbon and nitrogen, respectively.

3. Relation between rot and biochemical composition of infected plants

Abdel-Aal *et al.* (1987) studied the chemical composition of potato tubers of 5 varieties inoculated with *E. carotovora* in the laboratory. Cardinal cv. was most susceptible to soft rot, followed by Diamant, Arran Banner and Mirka. The less susceptible varieties had higher contents of phenols, total sugars and dry matter and lower dehydrogenase activity.

Prasada *et al.* (1987) found that total, reducing and non-reducing sugars, glucose and fructose, total amino acids and phenols increased in both green and ripe tomato fruits during rotting by *S.* [*Corticium*] *rolfsii*.

Abo Ellil *et al.* (1998) showed that healthy tomato roots contained more free and conjugated phenols than (*Capsicum annuum*) or (*Phaseolus vulgaris*) roots. There was a positive correlation between levels of phenols and root rot infection caused by two isolates of *S. rolfsii*, *R. solani* and *F. solani*. Total phenols increased with infection in most systems except for *F. solani*-tomatoes. Generally, pathogen infection increased polyphenoloxidase activity in roots.

Somani *et al.* (2000) found that soft rot and dry rot and all rots put together were positively correlated with reducing sugar and phenolic contents in tubers.

Chowdhury (2003) investigated the control of sclerotium blight of groundnut by plant growth substances, i.e., indole acetic acid, cycocel, 2,4-dichloroacetic acid and 2,4,5-trichloroacetic acid. Biochemical studies revealed that treated

plants showed appreciable increases in the contents of total phenol, o-dihydroxy phenol and calcium and an increase in the activity of polyphenoloxidase activity [catechol oxidase] compared with untreated plants. The activity of pectolytic enzyme, on the other hand, was significantly reduced.

4. Enzymatic activity of rot pathogens

Scala and Zoina (1983) recorded that *S. rolfsii* produced large amounts of polygalacturonases in liquid cultures with pectin or autoclaved bean hypocotyls as carbon source and in infected bean and squash tissues. They found a basal polygalacturonase activity in cultures with glucose as sole carbon source. In culture there was a single peak of activity at pH 5.23, while infected tissues had 2 peaks at pH 5.23 and 5.95. Activity of all isoenzymes was highest at pH 4. Each peak exhibited both endo- and exo-polygalacturonase activities.

McGuire and Kelman (1983) found that the increased susceptibility of potato tubers to maceration, either by *E. carotovora* or by pectic enzymes prepared from the fungus, was correlated with a decreased of Ca content of medullar tissue.

Bock *et al.* (1984) found that *E. carotovora* produced pectate lyase-containing pectolytic enzyme that macerated potatoes and vegetables tissues.

Kararah *et al.* (1985) reported that *B. subtilis* showed the greatest cellulase (Cx) activity, followed by *E. carotovora* subsp. *carotovora* and *B. pumilus in vivo* or *in vitro* but all 3 isolates exhibited polygalacturonase (PG) activity *in vitro*. A positive correlation existed between the pathogenicity and activity of PG and Cx of the 3 bacterial isolates within the clove tissues.

Lei *et al.* (1985) purified polygalacturonase (PG) from *E. carotovora*. They identified a hybrid cosmid, pSH711, that encodes PG activity but not pectate lyase activity, from an *E. carotovora* genomic library by an immunological screening method. A cell extract of *Escherichia coli* cells containing pSH711 was able to produce plant tissue maceration when spotted on carrot, potato or turnip slices. In addition, the *E. coli* str. containing this plasmid was able to macerate carrot, potato and turnip slices. These results suggest that PG plays an important role in soft rot disease .

Punja *et al.* (1985) found that 5 isolates of *S. rolfsii* differed in the amounts of endo-polygalacturonase (PG), endopectinmethylgalactu-ronase and cellulase produced adaptively in culture. High activities of these enzymes also were detected in diseased tissue. Pathogenic of *S. rolfsii* isolates correlated greatly with their ability to produce sufficient quantities of oxalic acid and endo-PG and to grow rapidly, whereas virulence was highly correlated with endo-PG production.

Satyabrata Maiti *et al.* (1986) observed that pectin and sodium polypectate (NaPP) induced maximum polygalact-uronase activity; CMC-Na induced high cellulase and low PG. Optimum PG secretion was noted in 1% pectin + 0.25% CMC-Na or 1% CMC-Na + 0.5% pectin, while induction of Cx was best in 1% CMC-Na and was reduced by addition of pectin. PG and Cx induction decreased with increasing concentration of glucose and starch irrespective of the inducer.

Dopke *et al.* (1987) recorded that the high virulent *E. chrysanthemi* pv. *chrysanthemi* caused blackleg infection on 7 potato cultivars was associated with high *in vitro* production of polygalacturonase .

Darmwal and Gaur (1989) found that filter paper cellulase (FPCase) and carboxymethyl cellulase (CMCase) activities of *S.* [*Corticium*] *rolfsii* were maximum at pH 4.8 and 3.6, respectively, and at 55°C. Both activities increased 3-fold when the concentration of filter paper and carboxymethyl cellulose were increased by 6- and 5-fold, respectively. Enzyme activities decreased with increasing incubation period though sugar formation increased.

El-Abyad *et al.* (1992) found that *R. solani* was more efficient than *C. rolfsii* in producing cell wall-degrading enzymes in infected hypocotyls of sugarbeet cultivar Kaumera, with xylanase and galactanase the most effective. The rate of cell wall degradation by *R. solani* was nearly 2.5 times that of *C. rolfsii* when cell walls of healthy hypocotyls were used as the sole carbon source for the crude enzymes *in vitro*.

Ohazurike and Arinze (1992) stated that polygalact-uronase (PG) enzymes of *S.* [*Corticium*] *rolfsii* macerated tissue slices of taro, cocoyam, carrot and potato tubers to varying degrees *in vivo* and *in vitro*, while the macerating effect was more marked *in vitro*.

Saleh (1995) reported that *E. carotovora* subsp. *betavasculorum* produced extracellular pectic enzymes when tested on a pectin solution at pH 5 and 8 and showed transeliminase activity *in vivo* and *in vitro* with a higher

specificity towards sodium pectate than pectin when measured at 230 and 235 nm, suggesting that it also produces polygalact-uronate trans-eliminase.

Ohazurike and Arinze (1996) observed that crude aqueous extracts from the peripheral rot zone of cocoyam tubers (*Colocasia esculenta* and *Xanthosoma sagittifolium*) were shown to be inhibitory to dialysed *in vivo* polygalacturonase (PG) of *S. rolfsii* [*Corticium rolfsii*]. This inhibitory activity and the phenol and peroxidase activities were higher in *X. sagittifolium* than in *C. esculenta* extracts. The levels of these enzymes decreased by increasing the postharvest age of the tubers.

Wegener and Jansen (1996) reported that plant pathogenic *E. carotovora* bacteria secrete a mixture of enzymes including pectinases, cellulases and proteases to degrade the cell walls of host plant tissue.

Gubitz *et al.* (1997) found that xylanase from *Thermomyces lanuginosus* and a mannanase from *S. rolfsii* [*Corticium rolfsii*] acted synergistically on the pulp solubilizing 50% more mannan and 11% more xylan than did the individual enzymes.

Ugwuanyi and Obeta (1997) stated that *A. niger*, *B. theobromae*, *C. rolfsii*, *Geotrichum candidum*, *F. oxysporum* and *F. solani* isolated from rotten cocoyams (*Colocasia antiquorum*) produced high levels of hydrolase, lyase and pectinesterase in cocoyam tissue medium as well as lyase and pectinesterase in pectin medium. Maximum pectinesterase activity were obtained from *B. theobromae* and *C. rolfsii* in pectin medium. All the tested isolates produced cellulase in a medium containing

carboxymethyl cellulose. *C. rolfsii* showed significantly high activity followed by *F. oxysporum* and *A. niger*.

Nabanita *et al.* (1999) discussed the role of pectinolytic (polygalacturonase and pectin methyl esterase) and cellulolytic (Cx) enzymes produced by *F. oxysporum* f.sp. *ciceri*, *S. rolfsii* [*Corticium rolfsii*] and *M. phaseolina* in pathogenesis of the respective diseases on chickpeas. Enzyme production was highest by *S. rolfsii* followed by *M. phaseolina*.

Abd El-Khair and Nofal (2001) reported that polygalacturonase (PG) activity of *Pseudomonas syringae* pv. *delphinii* was greater than *E. carotovora* subsp. *carotovora*. The cellulytic (Cx) enzyme were produced by the bacterial pathogens. It is clear that *E. carotovora* subsp. *carotovora* produced a high of Cx enzyme comparing with *Ps. syringae* pv. *delphinii* isolates.

El-Hendawy *et al.* (2002) detected pectin methyl esterase [pectinesterase] and polygalacturonase activities in the extracts of carrot roots and pepper fruits that infected with three strains of *Erwinia* and were not detected in culture supernatants.

5. Chemical control of rot pathogens:

5.1. Using fungicides

Sivaprakasam and Kandaswamy (1983) obtained control for collar rot of sweet potato caused by *S. rolfsii* in fields by soil drenches with Vitavax [carboxin] or Plantvax [oxycarboxin].

Motikhaye (1983) examined the effects of 6 fungicides (Bordeaux mixture, Dithane Z-78 [zineb], Vitavax [carboxin],

Bavistin [carbendazim], BAS 70 F and aureofungin) on the germination and growth of *Colletotrichum capsici*, *C. indicum*, *Helminthosporium sativum* [*Cochliobolus sativus*], *Macrophomina phaseoli* [*M. phaseolina*] and *S.* [*Corticum*] *rolfsii*. The best results were given by aureofungin and carboxin.

Fahim *et al.* (1984) found that growth of *S. rolfsii* the causal agent of damping-off on sugar beet (*Beta vulgaris*) was completely inhibited on solid and liquid media by Homai 80 (thiophanate-methyl + thiram) at 25 ppm, Orthocide 75 (captan) at 10 ppm, Vitavax (carboxin) + Captan at 0.5 ppm and by Carboxin + Thiram at 1 ppm. Meanwhile, when these products were used as seed treatments at 3 and 6 g/kg seed by dusting or glueing (modification of pelleting method) pre-emergence damping off in infested soil was greatly reduced only by the glue treatment.

Sannegowda and Pandurangegowda (1984) tested 9 fungicides against wilt or foot rot disease caused by *S. rolfsii* (Sacc.), Bayleton (triadimefon) was highly effective *in vitro*, followed by Sicarol [pyracarbolid] at 1000 ppm. Daconil (2787) [chlorothalonil], Fytolan (copper oxychloride) and Difolatan [captafol] were also effective at 2000 ppm.

Csinos (1985) compared Tolclofos-methyl with PCNB [quintozene] in lab and field tests against the groundnut pathogens. There was little difference in their efficacy against *S. rolfsii* but tolclofos reduced radial growth of *R. solani* more than PCNB at 0.1, and 1 µg/ml. Tolclofos-methyl at 5.6 kg a.i./ha or less was more effective in the field.

Neweigy *et al.* (1985) applied 6 fungicides as seed dressing, Bavistin [carbendazim], Topsin M [thiophanate-methyl] and Tecto [thiabendazole] were the most effective in increasing fresh and dry wt, root and shoot length and nitrogen content in pea and soyabean plants. They also improved seed germination and plant survival under soil infestation with a mixture of *R. solani*, *F. solani* and *S.* [*Corticium*] *rolfsii* but showed poor protection against *C. rolfsii* alone using Vitavax, Captan and Vitavax 75%.

Srikant Kulkarni *et al.* (1986) tested 19 fungicides against *S. rolfsii in vitro.* Vitavax [carboxin] performed best completely inhibiting growth at 50 ppm followed by Bayton (Baytan [triadimenol + fuberidazole]) and benodanil.

Abou-Zeid *et al.* (1987) tested the inhibitory effect of 3 fungicides on the growth of *F. solani*, *M. phaseoli* [*M. phaseolina*], *R. solani* and *S.* [*Corticium*] *rolfsii in vitro* to evaluate their effectiveness against damping off of soybeans. The results varied depending on the fungus, fungicide and fungicide concn. Rovral (iprodione) was most effective, followed by Vitavax 300 (carboxin + captan) while Botec [dicloran + captan] was the least effective in this respect. Seed dressing before sowing decreased the incidence of damping off.

Amr *et al.* (1987) recorded that 10 fungicides inhibited mycelial growth of *R. solani*, *S.* [*Corticium*] *rolfsii* and *Pythium ultimum* to different degrees *in vitro.* Monceren (pencycuron), Monceren + Captan, Rizolex (tolclofos-methyl) and Vitavax (carboxin) + Captan were the most effective against *R. solani*;

while, Rizolex, Quinolate v4x (copper oxyquinolate + carboxin) and Monceren + Captan gave best control of *C. rolfsii*.

Dalvi and Raut (1987) evaluated 6 fungicides *in vitro,* Vitavax [carboxin], Hexathir [thiram], Hexacap [captan] and Emisan-6 gave effective control of *S.* [*Corticium*] *rolfsii* in pot experiments when used as seed dressings.

Radwan *et al.* (1987) reported that Vitavax [carboxin] + Captan inhibited five tested *S. rolfsii* isolates from groundnut collected from different localities in Egypt.

Sahu *et al.* (1990) found that Delsan-30 [2-thiocyanomethylthio benzothiazole], Vitavax-75SD [carboxin], Thiram and Thiram + Bavistin [carbendazim] completely inhibited *in vitro* growth of *S.* [*Corticium*] *rolfsii* and inhibition zones were observed around groundnut seeds treated with these fungicides. Benlate [benomyl] and Captaf [captan] were moderately effective, while Jkstein 50 WP and Topsin M [thiophanate-methyl] were ineffective. In pot trials, pre-emergence mortality was reduced to 16.5% following treatment with Bavistin + Thiram, compared with an untreated control with 62.5% pre-emergence mortality.

Deshpande and Tiwari (1991) tested Rizolex-S-3349 (tolclofos-methyl) as dust and wettable powder, Foltaf 80 WP [captafol], Bordeaux mixture and palash [*Butea monosperma*] water-soaked root extract *in vitro* and *in vivo* against *S.* [*Corticium*] *rolfsii*. Rizolex in both forms completely controlled infection of piper beetle by *C. rolfsii* but less a.i./ha was required of the dust form than the wettable powder. Captafol, Bordeaux mixture and *B. monosperma* extract were almost ineffective.

Henriquez and Montealegre (1992) tested 11 fungicide *in vitro* for controlling *S.* [*Corticium*] *rolfsii*, carboxin + thiram (as Vitavax T), mepronil (as Basitac), oxycarboxin (as Plantvax), tolclofos-methyl (as Rizolex), triadimefon (as Bayleton) and triadimenol (as Baytan) were effective.

Rahman *et al.* (1994) tested the effect of Vitavax-200 [carboxin], Apron-TZ [metalaxyl], Dithane M-45 [mancozeb], thiram, captan and Baytan 10DS [triadimenol] on foot and root rot disease (*C. rolfsii*) on cowpea (*Vigna unguiculata*) over 3 years. Vitavax-200 was the best in this respect. Crop yield was also significantly increased due to seed treatment to control seedling damping off.

Ahmed *et al.* (1994) found that *in vivo* sunflower seed dressing with the fungicide Rhizolex [tolclofos-methyl] (3 g/kg seeds) gave the best results in controlling the damping-off and reducing the percentage of infected plants with *S. rolfsii*.

Srikanta Das *et al.* (1995) tested 6 fungicides and their combinations for controlling root rot of sugarbeet caused by *S. rolfsii* [*Corticium rolfsii*]. Carboxin (Vitavax) and tolclofos-methyl (Rizolex) applied as a soil drench at 5 kg/ha at 70 and 100 days gave good control of the disease (75%), increasing the root yield and sugar content by up to 350%. Carboxin + captan (50% each), carboxin + carbendazim (50% each) and carboxin + thiram (50% each) at 5 kg/ha also reduced disease infection by 40-55% whereas thiram + captan, captan + carbendazim and thiram + carbendazim combinations reduced disease intensity by 25-30%.

Das and Panda (1997) evaluated six fungicides *in vitro*, Tilt (propiconazole), Opus (epoxiconazole), Blitox-50 (copper oxychloride), Calixin (tridemorph), Indofil M-45 (mancozeb) and Bavistin (carbendazim), at 5 concentrations against *S. rolfsii* [*Corticium rolfsii*], causal agent of collar rot of tuberose (*Polyanthes tuberosa*). Epoxiconazole at 50 µg/ml and propiconazole and mancozeb at 100 µg/ml completely inhibited mycelial growth. Carbendazim and copper oxychloride were moderately effective at 500 µg/ml.

Sabet *et al.* (2000) tested *in vitro* and greenhouse experiments the effect of Tolclofos-methyl, thiophanate-methyl and metalaxyl + copper oxychloride on the growth of pathogenic fungi (*F. solani, R. solani* and *S. rolfsii* [*Corticium rolfsii*]). They found that metalaxyl + copper oxychloride in combination effectively reduced damping off and root rot disease caused by *F. solani*, *R. solani* and *C. rolfsii*.

El-Habbaa *et al.* (2002) evaluated 7 fungicides i.e., Topsin-M, Rizolex-T, Benlate, Vitavax-T, Monceren, Maxim and Apron on the growth of *R. solani, S. rolfsii* [*Athelia rolfsii*], *F. solani, M. phaseolina* and *B. theobromae in vitro*. They found that Maxim was the most effective as it prevented the *in vitro* growth of *F. solani, M. phaseolina, B. theobromae, S. rolfsii* and *R. solani* at 1-5 ppm, followed by Benlate at (10-800 ppm), Vitavax-T (25-200 ppm) and Rizolex-T (200-800 ppm). Apron and Monceren had no or little effect and failed to produce a considerable reduction in growth of all tested fungi even at 800 ppm.

Bhoraniya *et al.* (2003) evaluated the efficacy of carbendazim (0.050%), thiophanate-methyl (0.075%), copper oxychloride (0.200%), carboxin (0.075%), mancozeb (0.225%) and captan (0.225%) against stem rot disease (caused by *S. rolfsii* [*Corticium rolfsii*]) in chilli cv. Reshampatta. Thiophanate-methyl resulted in the highest disease control percentage (84.56%), followed by carboxin (84.26%).

5.2. Using bactericides

Farag *et al.* (1986) found that *E. carotovora* subsp. *atroseptica* was round in refrigerated potato stocks stored under improper ventilation conditions. The bacteria proved sensitive to ampicillin, streptomycin and, to a lesser extent, chloramphenicol and penicillin. Although the application of ampicillin and chloramphenicol at 25 ppm gave good control of tuber rot, chloramphenicol at 50 ppm completely inhibited rot development.

Tanaka and Saito (1985) found that streptomycin sprays were ineffective in controlling of bacterial soft rot on onions caused by *E. carotovora.*

Banerjee *et al.* (1990) reported that blue copper, captan, thiram, ferrous sulfate, potassium permanganate and zinc sulfate inhibited growth of *E. carotovora* subsp. *cartovora in vitro*. The antibiotices comycin, ampicillin and althrocen were also inhibitory, even at 1 ppm.

Lewocz (1992) reported that Copper oxychloride + cymoxanil (as Curzate Cu), copper oxychloride + cymoxanil + oxadixyl (as Kipost Cu), copper oxychloride + metalaxyl (as Ridomil plus 45) and copper oxychloride + oxadixyl (as

Sandofan Copper) inhibited development of *E. carotovora* subsp. *atroseptica in vitro*. Copper oxychloride + metalaxyl, metiram (as Polyram Combi) and copper oxychloride + cymoxanil reduced infection in field experiments.

Abd El-Khair (1993) proved that the mixture of diluted ingredients of streptomycin and copper compounds were highly effective against *E. amylovora*. Laboratory studies on excised pear fruits confirmed this finding.

Almeida *et al.* (1994) studied the sensitivity of *E. carotovora* subsp. *carotovora* to some chemicals *in vitro*, and the best results were obtained with copper oxychloride + mancozeb, oxytetracycline, oxytetracycline + streptomycin and copper sulfate + oxytetracycline. These products were also tested *in vivo* under field conditions and the oxytetracycline + streptomycin showed the best control.

Alice and Sivaprakasam (1995) tested four antibiotics against *E. carotovora pv. carotovora*, the causal agent of soft rot of onion. Streptomycin sulfate (90%) and tetracycline hydrochloride (10%) (streptocycline) recorded the maximum inhibition zone of 27.66 mm. In enzyme studies, the maximum inhibition of pectinlyase (PL), polygalacturonase (PG) and protopectinase production was recorded by the same antibiotic. The antibiotics had a significant influence on the production and activity of cell wall degrading enzymes produced by plant pathogenic microorganisms.

Das *et al.* (1995) revealed that bacterial wilt caused by *Ps. solanacearum* [*Ralstonia solanacearum*] was the most important disease of aubergines in Assam, India. The ability of

14 antibiotics to control growth of *R. solanacearum in vitro* was determined. The results indicated that *R. solanacearum* was sensitive to tetracycline, streptomycin, chloramphenicol, rifampicin, spiramycin and vancomycin. Tetracycline was the most effective.

Ahiawat *et al.* (1997) found that ampicillin, neomycin, streptomycin, streptocycline and tetracycline (50 µg/ml medium) restricted the growth of 3 *Bacillus* isolates which were isolated from contaminated mushroom in India without affecting the mycelial growth of the 3 mushroom strains tested. Antibiotics alone were not effective in controlling bacterial growth. Autoclaving + antibiotics were effective; the best results were obtained when antibiotics were added after autoclaving. Among the antibiotics tested, ampicillin, streptocycline, streptomycin and tetracycline were the most effective.

Panic and Antonijevic (1997) studied the bacteriostatic (inhibitory) effects of 12 antibiotics (antimicrobial compounds) and 18 antibiotic remedies (agents) on the growth of 6 strains of *E. amylovora* under *in vitro* conditions, on nutrient agar (NA) at 22°C. The most effective degree of inhibitory action on growth of the bacterial strains on the medium was induced by the remedy Visiren (ofloxacin), much more than the standard, streptomycin sulfate. Geomycin (oxytetracycline), Longacef (cephalosporin), gentamicin and Doxycycline (tetracycline) showed similar effects to the standard. All other compounds showed weaker antibiotic effects. The strains most susceptible to the antibiotics were MS-1, JAB-1, DU-1 and GL-2, while KS-3a and KS-4a were least susceptible. It was recommended that

Visiren, Longacef and gentamicin be investigated for use for the chemical control of *E. amylovora* on fruit trees and ornamental shrubs.

Barakat *et al.* (1999) found that the mixtures of strepto-mancoper (50:300 and 50:400ppm) could protect potato tubers from decay caused by *E. carotovora* subsp. *carotovora.* Also, they obtained the same results when used Mancoper at 200ppm. This finding has proven that strepto-copper compound mixture could be recommended for protection of vegetables from bacterial soft rot.

Chao Yungchun *et al.* (1999) reported that lincomycin (1000 ppm) and streptomycin (125 ppm) inhibited the growth of *E. chrysanthemi* in the *in vitro* paper disc test.

Chen and Lin (2000) reported that among 8 bactericides and 12 microbial pesticides screened, 3 including 10% streptomycin + tetracycline, 63% copper oxychloride + mancozeb and 12.5% streptomycin showed high efficacy inhibition of the growth of soft rot bacterium. Application of chemical mixture C-mix 1 (a mixture of 12.5% streptomycin 500x, 10% streptomycin + tetracycline 500x and 40% oxinecopper + copper hydroxide 500x) stimulated tuber budding and reduced disease incidence of soft rot.

Singh *et al.* (2000) evaluated five antibiotics *in vitro* and *in vivo* against *Ps. solanacearum* (*Ralstonia solanacearum*). Streptomycin and streptopenicillin were superior over the other antibiotics against the pathogen under both conditions. *In vitro*, 500 and 1000 ppm concentrations of streptomycin and streptopenicillin were most effective in controlling the

bacterium. Penicillin G, tetracycline and plantomycin did not inhibit the bacterial wilt at any of the concentrations tested.

Abd El-Khair and Nofal (2001) tested different bactericides, i.e., chloramphenicol, neomycin, tetracycline and streptomycin sulfate in 30 µg/disc *in vitro* against *Ps. syringae* pv. *delphinii* and *E. carotovora* subsp. *carotovora* the pathogens of necrotic and soft rot tissue disease of flowers. Tetracycline gave an excellent effect against the two bacterial pathogens, while chloramphenicol was effective against *Ps. syringae* pv. *delphinii* while, both of neomycin and streptomycin sulfate were not effective.

MATERIALS AND METHODS

1. Isolation of fungi and bacteria associated to rotted dahlia tubers:

1.1. Isolation and purification of fungi

Samples of naturally infected dahlia plants (*Dahlia pinnata* L.) were collected from three different farms (Fac. Agric., Moshtohor, El-Dair and El-Manashy) located in Kalubia Governorate. Infected tubers were thoroughly washed using tap water to remove adherent soil and then air-dried. Small pieces (1cm thick) were surface sterilized by dipping in 2% sodium hypochlorite solution for 2 min. and rinsed several times in sterilized distilled water then dried within sterilized filter papers. These sterilized pieces were then transferred to ready PDA plates and incubated at 25°C for 5-7 days. Observations were daily recorded and any emerged fungus was picked up and cultured onto fresh PDA plates. All isolated fungi were purified using either the single spore method and/or the hyphal tip technique **(Hawker, 1950).**

The purified, isolated fungi were identified according to their morphological features using the description of **Gilman (1957)** and the genus description of **Barnett and Hunter (1972).** Stock cultures were maintained on PDA slants and kept in refrigerator at 5°C and subcultured every 30 days.

1.2. Isolation and purification of bacteria

For the isolation and purification of the pathogenic bacteria; small pieces (1cm thick) from the previously prepared samples for isolation of fungi were used. These sterilized pieces

were then transferred to ready nutrient agar (NA) medium and incubated at 30°C for 48 hrs. Observations were daily recorded and any emerged colony was picked up and cultured onto fresh NA plates. All picked colonies were purified using the single colony technique.

2. Pathogenicity test of isolated fungi

Three fungal isolates, namely *Sclerotium rolfsii*, *Fusarium* spp. and *Rhizoctonia solani* were chosen and tested for their pathogenicity on dahlia tubers.

Apparently healthy tubers were thoroughly washed using tap water to remove adherent soil, then rinsed several times in sterilized distilled water, dried between sterilized filter paper and then surface sterilized in 95% ethyl alcohol for 2 min. After drying, tubers were cut into slices (approx. 5 cm ϕ and about 5 mm thick) using sterilized knife. Each slice was transferred to sterilized Petri plate under septic conditions. The slices were inoculated with fungal disc (6 mm ϕ) taken from 7-days old cultures of the three tested fungi on the center of each slice. Slices without inoculation served as control. Four slices were used as replicates for each particular treatment. Inoculated and non inoculated tuber slices were incubated at 28°C for 4 days. Rot incidence was measured as the percentage of rotted tissue area, mean of infected area (IA) and percentage of infected area (IA). Re-isolation of the same fungi from artificially inoculated tuber slices was undertaken and the resulted fungi were compared with the original isolates.

3. Physiological studies of pathogenic fungi

S. rolfsii isolated from rotten dahlia tubers was used in the following physiological trials according to its pathogenic potentialities. In this respect, the effect of different media, temperature, relative humidity, carbon and nitrogen sources, pH values and light colors on the linear growth and sclerotial formation of the tested fungus were studied. The plates were inoculated with an equal disc (6 mm ϕ) of 4-days old PDA culture of the tested fungus. Three replicates were used in these experiments, all plates were incubated at 30±1°C.

In all physiological experiments, the mean of linear growth was measured after 2 and 4 days post inoculation. While, sclerotia formation was estimated after 10 days post inoculation.

- **Used media:**

1- Dahlia tuber extract agar: 200g of dahlia tubers were boiled in one liter of distilled water, filtered using cheese cloth then 20g dextrose and 20g agar were added to the filtered extract and made up to 1000ml with distilled water.

2- Peptone dextrose agar **(Johanson, 1957):**

5g peptone, 10g dextrose, 1g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, 20g agar, 1ml/100ml medium of 1/30000 rose bengal dilution and distilled water up to 1000ml.

3- Potato dextrose agar PDA **(Riker and Riker, 1936):**

Filtrates of boiled 200g potato slices in one liter of distilled water, then 20g dextrose and 20g agar were added to the filtered extract and made up to 1000ml with distilled water.

4- Plain agar medium: 20g agar and distilled water up to 100ml.

5- Barnes agar **(Rangaswami, 1984):**

1g NH_4NO_3, 1g glucose, 1g KNO_3, 1g K_3PO_4, 20g agar and distilled water up to 1000ml.

6- Brown's agar medium **(Rangaswami, 1984):**

2.0g glucose, 2.0g asparagine, 1.25g K_3PO_4, 0.75g $MgSO_4.7H_2O$, 20g agar/L and distilled water up to 1000ml

7- Conn's agar medium **(Bilgrami and Verma, 1980):**

2.0g KNO_3, 1.23g $MgSO_4.7H_2O$, 2.72g KH_2PO_4, 7.2g maltose, 10.0g potato starch, 15.0g agar and distilled water up to 1000 ml.

8- Czapek's agar medium **(Raper and Thom, 1949):**

30g sucrose, 0.5g KCl, 0.5g $MgSO_4.7H_2O$, 2.0g $NaNO_3$, 1.0g K_2HPO_4, 0.01g $FeSO_4.5H_2O$, 15.0g agar/L and distilled water up to 1000 ml.

3.1. Effect of different media

Eight different media i.e., dahlia tuber extract, plain agar (natural media), potato dextrose agar and peptone dextrose agar (semi synthetic media), Barnes, Brown's, Conn's, and Czapek's agar media (synthetic media) were used to study their effect on both linear growth and sclerotia number of *S. rolfsii*. The acidity of all tested media were adjusted at pH 6.8 and incubated at 30±1°C for 10 days.

3.2. Effect of different temperature degrees

PDA medium was used to study the effect of different temperature degrees on growth and sclerotia formation of *S. rolfsii*. Seven-temperature degree i.e., 10, 15, 20, 25, 30, 35 and 40°C were tested. Three replicates of Petri dishes were used for each particular treatment. These plates were inoculated with equal discs (6mm ϕ) of 4 days-old culture. The linear growth and sclerotia number were recorded as mentioned before.

3.3. Effect of different levels of relative humidity

In this experiment, eight different levels of relative humidity (RH), i.e., 14, 50, 74, 80, 85, 90, 95 and 100% were prepared according to **Solomen (1951)**. Petri dishes containing PDA were inoculated with equal discs (6mm ϕ) of 4 days-old culture of the tested fungus. 10 ml of the prepared solution was poured in each lid of inverted dishes to obtain the desired RH level. The lid was tighten to the dish with the aid of adhesive tape (cellotape). Linear growth and sclerotia number recorded were as mentioned before.

3.4. Effect of different pH values

A series of buffer solutions of 5 different pH values, i.e., 4, 5, 6, 7 and 8 were prepared from two stock solutions according to **Ju-Luric (1978)** as follows:

Solution A: was prepared by dissolving 9.4639g of $Na_2HPO_4.2H_2O$ in one liter of distilled water.

Solution B: was prepared by dissolving 9.0739g of KH_2PO_4 in one liter of distilled water. The solutions were mixed at the

following proportions to obtain 100 ml of the specified pH values.

Table (1) show preparation of pH values

pH values	Solution A (ml)	Solution B (ml)
4	0.07	99.93
5	0.93	99.07
6	12.10	87.90
7	61.20	38.80
8	96.90	3.10

A modified Czapek's medium (each 100ml contains: 3.0g sucrose, 0.05g KCl, 0.05g $MgSO_4.7H_2O$, 0.2g $NaNO_3$, 0.001g $FeSO_4.5H_2O$ and 1.5g agar) in phosphate buffer at different pH values were prepared and autoclaved in the usual way. The medium pH was checked after sterilization. It was found necessary to add aseptically 0.1N hydrochloric acid to Czapek's medium (not buffered) to bring down its pH value to pH 4 as well as 0.1N of NaOH was added to the same medium to raise its pH value to 8. The medium were poured in plates then inoculated with equal discs (6mm ϕ) of 4 days-old cultures of the tested fungus. The plates were incubated at 30±1°C for 10 days. Three replicates were made for each particular treatment. The linear growth and sclerotia numbers were recorded as usual.

3.5. & 3.6. Utilization of different carbon and nitrogen sources

Eight different carbon sources, i.e., sucrose, glycerol, glucose, starch, maltose, lactose, dextrin and arabinose as well as 9 different nitrogen sources, i.e., casein, peptone, beef extract, yeast extract, asparagine, gelatin, ammonium nitrate, sodium nitrate and urea were individually substituted with equivalent weight of carbon or nitrogen in Czapek's medium to test their effect on growth and sclerotia production of the tested fungus. Media containing carbon or nitrogen sources were sterilized filtration using sintered glass funnel. Control without any carbon or nitrogen source was used. Linear growth and sclerotia number were estimated as mentioned before.

3.7. Effect of light colors

Plates containing sterilized Czapek's agar medium were inoculated with equal discs (6 mm ϕ) of the test fungus. The inoculated plates were enveloped with different thin transparent cellophane paper i.e., hyaline, red, blue, yellow and green colors. Another group of plates was enveloped with black paper. The plates were incubated under continuously illuminated 60 cm long white florescent lamp, hanged 30 cm high above the incubated plates at 30±1°C. Linear growth and sclerotia number were determined as mentioned before.

4. Factors affecting disease severity

S. rolfsii isolate was chosen as highly virulent pathogen from the three tested fungi which, causing tuber rot of dahlia for studying some factors affecting disease incidence like levels of wounding and inoculum density.

4.1. Effect of wounding

Five levels of wounds were made on dahlia tubers which were previously washed and sterilized as mentioned before, i.e., superficial wound, needle wound, cyclic deep wound (3mm diameter), long deep wound (5mm long) and long deep wound (10mm long) and not wounded tuber. Unwounded tubers were used as a control. All treatments were inoculated with equal disc (6 mm ϕ) of *S. rolfsii* (4 days old culture) and incubated in a plastic moist chamber at 30±1°C **(El-Habbaa, 1995)**. Mean linear of infected area and weight loss of infected tubers were estimated after 3, 5, 7, 9 and 11days post inoculation according to **Zhou and Reeleader (1990)**.

$$\% \text{ Reduction weight} = \frac{R_1 - R_2}{R_1} \text{ x } 100$$

Where, R_1= tuber weight before inoculation

R_2= tuber weight after inoculation

4.2. Effect of inoculum density

4.2.1. On dahlia tubers under *in vitro* conditions

S. rolfsii sclerotia were added individually at different rates i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 sclerotia to only long deep wound (10mm long) of dahlia tubers which, were washed, sterilized and prepared previously for this purpose. Wounded tubers without inoculation were used as control. Three replicates for each treatment. All treatments were incubated in a plastic moist chamber at 30±1°C. Mean linear of infected area and weight loss in infected tubers was estimated as mentioned previously.

4.2.2. As soil treatment under greenhouse conditions

In this experiment, *S. rolfsii* isolated from infected dahlia tubers in Moshtohor farm was cultured onto sterilized corn meal sand medium **(Riker and Riker, 1936)** in 500cc bottles (each containing 200g corn meal sand medium) and incubated at 30±1°C for 15 days. Pots (25 cm ϕ) were potted with soil (3 kg) then, each pot was infested with tested isolate inoculum separately at five different rates i.e., 1, 2, 3, 4, 5% (w:w) soil. Potted soil infested with uninoculated media served as control. Surface sterilized healthy tubers were sown in the infested and un-infested pots. The disease incidence expressed as dead plants were recorded after 21 days.

- **Disease Assessment:**

Disease assessment was carried out as percentage of dead plants and survived plants. The readings were taken after 2 and 7 weeks respectively as follows:

$$\text{Dead plants \%} = \frac{\text{No. of dead plants}}{\text{Total cultivated plants}} \times 100$$

$$\text{Survived plants \%} = \frac{\text{No. of survived plants}}{\text{Total cultivated plants}} \times 100$$

5. Pathogenicity and host range of isolated bacteria

5.1. Pathogenicity test

Dahlia and potato tubers were surface disinfected by flaming, then cutted using sterile knife into 1cm thick slices with almost equal diameter. Each slice was placed on the

surface of moistened sterilized filter paper into Petri-dish. Bacterial dilution was prepared from each one of isolated bacteria at rate 1.3×10^8 cfu/ml, then 0.5ml of bacterial dilution was used for artificial inoculation by pipetting at the center of each slice. Three slices were used for each tested bacterial isolate as well as control. All treatments were incubated at 30°C for 4 days, then examined daily.

Disease assessment of rotted tubers were expressed according to **Lelliott and Dickey (1984)** as follow:

(-): Negative infection

(+): Low positive infection

(++): Moderate infection

(+++): Highly infection

5.2. Host range

Tubers, fruits, bulbs and corms of different plant hosts were inoculated with each of seven tested bacterial isolates for their pathogenicity (belong to different families as revealed by identification) to determine whether they are capable for attacking other hosts. Tubers of potato (*Solanum tuberosum*), sweet potato (*Ipomoea batats*), carrot (*Dacus carrot*), fruits of squash (*Cucurbita pepo*), dahlia tubers (*Dahlia pinnata*), sugar beet (*Beta vulgaris*), cucumber fruits (*Cucumis sativus*), onion bulbs (*Allium cepae*) as well as taro corms (*Colocasia antiquorum*) were used.

Bacterial dilution (0.5 ml at the rate of 1.3×10^8 cfu/ml) of each isolate was pipetted individually at the center

of each slice. Three slices were used for each of tested isolates as well as control. All treatments were incubated at 30°C for 4 days then examined. Disease readings were expressed as mentioned above.

6. Identification of pathogenic bacteria

Identification of the bacterial isolates were conducted on the bases of their morphological, nutritional and physiological characteristics according to schemes suggested by **Schaad, (1980)**; **Fahy and Persley (1983)**; **Krieg and Holt (1984)**; **Leliott and Stead (1987)**. This identification test was carried out only for the pathogenic bacteria, which verified their abilities on infection as mentioned above as following:

6.1. Morphological characteristics

Different morphological characteristics of the subjected bacterial isolates i.e. cell shape, Gram stain and spore formation was carried out.

6.2. Cultural characteristics

Various cultural properties of the examined isolates, i.e. the growth colony shape on different media, oxygen requirements and growth at different temperatures were also studied.

6.3. Physiological and biochemical characteristics

The following physiological characters and biochemical activities were used as bases for bacterial classifications:

1. Acid production from sucrose.
2. Reducing compounds from sucrose.

3. Degradation of macromolecules:
    i- Gelatin hydrolysis test.
    ii- Starch hydrolysis test.
4. Other tests:

- Catalase test.
- Salt tolerance test.
- Phosphates test.
- Pigment production.
- Soft rot symptoms on potato slices.
- Relation to free O_2.
- Hydrogen sulfide production (H_2S).
- Levan formation.
- Reducing compounds from sucrose.
- **Acid production from carbohydrates.**
- Potato dextrose agar (PDA).
- Yeast extract dextrose-$CaCO_2$ (YDC).
- Nutrient-broth yeast extract agar (NBY).
- King's medium B agar (KB).
- Miller-Schroth (MS) medium.
- **Gas from d-glucose.**
- Peptone yeast extract agar (PYEA).
- Pectate degradation.

6.4. Protein pattern and electrophoresis analysis

For emphasizing identification of pathogenic bacteria, bacterial cell suspensions of seven bacterial isolates, i.e., *Erwinia*

carotovora (4&12), *Bacillus polymyxa* (7&9) and *Pseudomonas cepacia* (10,11&14) were used for extracting the bacterial proteins. Fractionalization of bacterial protein was achieved using sodium dodecyle sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique as described by **Laemmli (1970)** at the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.

In this study, the tested bacterial isolates were left to grow at 30°C for 48 h in Erlenmeyer flasks (100 ml) where, each one contained 50 ml of Luria broth medium (LB). The LB medium contained 10g bacto tryptone, 10g NaCl, 5g yeast extract, 1000 ml distilled water and the pH value adjusted at pH 7).

Preparation of protein extracts:

Protein extracts was prepared according to the method of **Miniatis *et al.* (1989)** by the following way; one ml of each one of cultured bacteria was centrifuged at 12000 rpm for 30 seconds in a microfuge. The supernatant medium was removed and the precipitated protein pellets were resuspended by vortexing in 0.5 ml of ice-cold 50 mM Tris.Cl (pH 7.4). Then centrifuged again at 12000 rpm for 30 seconds at 0°C in a microfuge. The supernatant was removed and the precipitated protein pellets were left to dry as possible. The extracted protein pellets were resuspended again by vortexing in 25 µl of H_2O. As soon as, the pellet is dispersed, 25 µl of SDS gel-loading buffer is added and vortexing is continued for 20 seconds. The extracted protein samples containing soluble protein were stored at -20°C until usage.

Electrophoresis of native protein

The thawed protein-extract supernatant was mixed with an equal volume of a solution containing 20% glycerol (v/v) and 0.1% bromphenol blue (v/v) in 0.15 M Tris–HCl, pH 6.8. Twenty microliters of the resulting suspension (40 μg of protein) was subjected to electrophoresis was conducted at room temperature (approximately 20-25°C), for 9 hrs on an 15% polyacrylamide gel with a 6% stacking gel at 10 and 20 mA, respectively, until the dye reached the bottom of the separating gel. Electrophoresis was performed in a vertical slab mold (20 × 18 × 0.2 cm). Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, Mo.) prepared in a 1: 4: 6 mixture of 90% acetic acid, 70% methanol and water then re-stained for 6 hrs with a similar mixture of acetic acid, methanol and water **(Laemmli, 1970 and Latorre *et al.*, 1995).**

Densitometer scanning

In the electrophoresis studies, Jacard index (I) of any pair densitometer tracing of protein patterns was computed by a computerized program and the resulting matrix of correlation coefficient was used for evaluating the level of similarity between any pair of isolates **(Hadacova, *et al*., 1980)**.

$$\text{Jacard index (I)} = \frac{C}{A + B - C}$$

Where:

C = No. of similar band between the two taxon to be compared.

A = No. of bands present in on taxon.

B = No. of bands present in the compared taxon.

Cluster analysis

Electrophoretic protein patterns of the seven pathogenic bacterial isolates were clustered **(Joseph *et al.*, 1992)** by the average linked technique (un-weighed pair-group method). The results were expressed as phenograms. Cluster analysis was performed with a computerized program.

7. Biochemical changes in dahlia tubers:

Biochemical changes in sugar content and phenolic compounds were studied in relation to infection with *Sclerotium rolfsii* in diseased dahlia tubers as follows:

Extraction:

Samples of 1g were taken from artificially inoculated tubers 4 days post inoculation. Healthy tubers from un-infested samples were taken as control for comparison. All taken samples were cut to small portions, transferred to 50 ml of 95% ethanol in brown bottles and kept in darkness at room temperature for one month then homogenized in sterile mortar as recommended by **Bozarth and Diener (1963)**. The ethanolic extracts were air dried at room temperature till near dryness and then were quantitatively transferred to 10ml 50% isopropanol and stored in vials at 1°C. The obtained extracts were used for the following determinations:

7.1. Determination of sugars

Reducing, non-reducing and total sugars were colormetrically determined by the picric acid method described by **Thomas and Dutcher (1924)**. Sugar content was determined

as mg glucose/g fresh matter calculated from standard curve prepared for glucose.

To determine the total soluble and reducing sugars, two solutions were used:

(i) Picric acid solution, prepared by dissolving 36g of picric acid in 500ml of 1% NaOH. 400ml of boiled distilled water were added and the mixture was left for cooling, the solution was completed to one liter with distilled water.

(ii) Sodium carbonate solution, prepared by dissolving 16g in 100ml distilled water.

To determine the total soluble sugars, 1ml of each alcoholic extract was transferred to a glass tube (70ml). 5ml of distilled water and 4ml picric acid were added. This mixture was boiled for 10 min. in a water bath before transferring to ice bath for cooling. 4ml of sodium carbonate were added to each tube, boiled for 10 min. and left to cool. Tubes were then completed up to 50ml with distilled water. The optical density of the developed color was measured using colorimeter (Spectronic 20-D) at 540 nm. To determine the reducing sugars content, picric acid and Na_2CO_3 were added at the same time to the alcoholic extract and boiled only for 10 min. Non-reducing sugars content was calculated by subtracting reducing sugars from total soluble sugars.

7.2. Determination of phenols

Free, conjugated and total phenols were colorimetrically determined using the “Folin and Ciocalteu” reagent as described by **Snell and Snell (1953)**.

The reagent was prepared by adding 100g sodium tungestate and 25g sodium molybdate to 700ml water in conical flask then 50ml phosphoric acid (85%) and 100ml HCl were added. Flask containing this mixture was attached to reflective condenser. The mixture was left to boil gently in a water bath for 10 hrs, then it was left to cool, 150g lithium sulphate and 50ml distilled water were also added. Few drops of bromine were also added and the mixture was heated again to remove access bromine. Finally, the mixture was completed to 1000ml with distilled water.

Free, conjugated and total phenols were determined in previously prepared extracts of dahlia tuber samples. Determination was calculated as catechol in terms of mg phenols per 1-g fresh matter. To determine the free phenols, 1ml of sample extraction was put in a sterilized test tube with 1ml distilled water, 5ml Folin-Ciocalteu reagent and 15ml Na_2CO_3 (20% w/v). The mixture was completed to 50ml with distilled water and color density was recorded at 520 nm. The total phenols were determined by treating 1ml of extracted sample with 0.25ml HCl and boiled in a water bath for 10 min then cooled. Ten ml of Folin-Ciocalteu and 25ml of Na_2CO_3 were added. The mixture was completed to 100ml with distilled water and the values of color density were measured at 520 nm using the same apparatus. Conjugated

phenols were determined by subtracting the free phenols from the total phenols.

8. Enzymatic studies:

Sample preparation:

(A) From fungal and bacterial cultures of synthetic media

Different carbon sources at 1% of citrus pectin (Roth) or carboxy methyl cellulose (CMC) and 3% sucrorse (Serva) were added to Czapek's medium for enzyme production (2g $NaNO_3$, 1g K_2HPO_4, 0.5g $MgSO_4.7H_2O$, 0.5g KCl, 0.01g $FeSO_4.7H_2O$, 1ml 1% (w/v) $ZnSO_4$, 1ml 0.5% (w/v) $CuSO_4$ and supplemented with 0.01% yeast extract) made up to 1 liter with distilled water and adjusted at pH 6.8 **(Valsangiacomo and Gessler, 1992)**.

One hundred ml conical flasks containing 25 ml culture media with the different carbon sources, were inoculated with 6 mm disks from the tested fungus. In case of bacteria, the flasks were inoculated using 200 µl cell suspension (1 ml contains 1.3 $\times 10^8$ cfu.). All cultures were incubated at 30 ± 1°C for 4 days in the dark. Then, the cultured media were filtered and centrifuged at 12000 rpm for l0 min at 4°C. The supernatant was dialyzed then the samples were centrifuged at 12000 rpm for l0 min and stored at -18°C **(Turner and Ogundana, 1983)**.

(B) From infected dahlia tubers

Dahlia tubers were surface sterilized with ethanol 70 %. Plugs (5 cm ϕ) were cut into discs about 5 mm thick. Dahlia discs were transferred to sterile Petri dishes then inoculated with standard fungal discs (6mm diameter) or inoculated using 200 µl

bacterial cell suspension (1ml contains 1.3×10^8 cfu.), and incubated at 30±1°C for 4 days. Un-inoculated dahlia tubers were used as control. Inoculated and control samples were homogenized separately in 20 ml distilled water containing 0.6g poly clar AT(BDH) to adsorbed phenols using a virtis blender **(Ogundana *et al.*, 1971)**. The homogenous samples were centrifuged, dialyzed and stored as mentioned before.

8.1. Estimation of cellulase and xylanase activity

This quantitative colorimetric assay of the investigated enzymes, i.e., cellulase and xylanase is based on the preceptibility of the blue colored, non-degraded, highly polymerized substrates of CM-Cellulose-RBB and CM-Xylan-RBB 4 mg/ml, respectively **(obtained from Loewe-Biochem., Co. Germany)** by HCl after incubating any of these substrates together with the specific pure or crude enzyme. The precipitated non-degraded substrate and was removed by centrifugation and absorbancy of the supernatant was plotted as a function of incubation time to measure enzyme activity. Reaction mixture contained 0.1 ml aqueous blue substrate solution, 0.2 ml 0.1 M citrate buffer (Mc-Lavine buffer) and 0.1 ml of the above prepared *in vitro* and *in vivo* crude enzymes, incubated fro 1 h at 30°C, within Eppendorf tube. The Reaction was stopped by 0.1 ml 1N HCl and the Eppendorf tubes were cooled in ice for 10 min., centrifuged for 5 min. at 12.000 r.p.m. The obtained supernatants colors were measured colorimetrically at 620 nm. **(Schlösser *et al.*, 1997)**. Using a standard curve of cellulase TC

(Cellulase Cs 0.7 μ/mg from *Trichoderma reesi* EC 3.2.1.4. Serva Biochem. Co. Germany) and xylanase (xylanase Xs from *Trichoderma viride* EC 3.2.1.8 Sigma Chem. Co. U.S.A.), the activity of an enzyme (absorbance) was changed to units per ml of crude enzyme preparation (μ/ml) where the unit is the amount of enzyme forming 1μmole of its final product in 1 h. at 30°C.

8.2. Determination of polygalacturonase (PG) activity

Agar diffusion assay was used according to the method described by **Dingel *et al.* (1953)**. The assay medium contains 1% sodium salt of polygalacturonic acid (PGA) as a substrate (Serva), 0.7% ammonium oxalate, 0.01% salicylanilide and 2% agar. The medium was prepared in 0.2 M phosphate buffer, pH 5.2. Cut holes were filled with 0.2 ml of the test solution, which had been adjusted to 4.5. After 24-48 h. at 25°C, the plates were sprayed with 5N HCl. The appearance of white rings indicated the presence of PG. The enzyme activity in units was calculated using a standard curve with commercial polygalacturonase (CPG) (Pectinase Ps from *Aspergillus niger* 0.15-0.3 units/mg, Serva) where 1 unit is the amount of enzyme forming 1μmole of product in 1 h. at 30°C.

9. Evaluation of some different fungicides on growth of *Sclerotium rolfsii:*

9.1. *In vitro*

Seven different fungicides as shown in **Table (2)** were used to test their fungicidal effect on growth of *S. rolfsii* on PDA medium according to the method of **Kuli and Tims (1960)**. In this respect, Topsin-M70, Vitavax-200, Rizolex-T50, Sanlight,

Tachigaren, Galbin-Cu and Copper oxychloride were used at concentrations from 50 to 750 ppm.

The calculated amount of each fungicide was added to the medium after sterilization and directly before pouring the plates. The inhibitory effect of each fungicide was tested by measuring the diameter of linear growth of the tested fungus after 2 and 4 days post inoculation. Medium free from any fungicides was used as control. The plates of *S. rolfsii* were incubated 30±1°C for 4 days.

Table (2): List of tested fungicides, their active ingredients, recommended dose and manufactures

Active constituent	**Usage rate**
$3Cu(OH)_2.CuCl_2$	2.5g/L
Methyl N-phenylacetyl-N- 2,6-xylyl-DL-alanineate.	1.5g/L
• 20% Rizolex-T: tolclofos-(ethyluro-o- dimethyl)-o-(2,6 dichloro-4-methyl phenyl) o,o-dimethylphosphorothioate. • 30% Thiram (TMTD): bis (dimethyl-thiocarbamoyl) disulphide.	2g/L
(RS)-2- (4-flurophenyl)-1- (1H-1,2,4 triazol-1-yl)-3-trimethylsilylpropan-2-ol.	1g/L
3-hydroxy-5-methylis-o-oxazole; 5-methyl-isoxazole-3-ol.	1ml/L
Dimethyl [(1,2 phenylene) bis (iminocarbonothiole)] bis [carbonate]; dimethyl 4,4'-o-phynelenebis [3-thioallophanate].	1.5g/L
5,6- dihydro-2- methyl-N-phenyl-1,4-oxathim-3-cdarboximide (CAS).	1g/L

Trade name	Common name & formula
Copper oxychloride El-Nasr Co. (Egypt)	Copper oxychloride (WP)
Galben copper K.Z. (Egypt)	Benalaxyl- Cu (WP)
Rizolex-T50 Sumitomo Chem. Ltd. (Japan)	(Tolclofos-methyl +Thiram) 50% (WP)
Sanlight Sankyo Co. (Japan)	Semiconazole (WP)
Tachigaren Sankyo Co. (Japan)	Hymexazol 30% (SL)
Topsin-M70 Nippon-Soda (Japan)	Thiophanate-methyl 70% (WP)
Vitavax-200 Royal (USA)	Carboxin 19.5% + Thiram 19.5% (WP)

9.2. *In vivo:*

This experiment was achieved during 2001/2002 and 2002/2003 seasons on dahlia tubers in greenhouse of Plant Pathology Dept., Fac. Agric., Moshtohor.

Seven fungicides, i.e., Topsin-M70, Vitiavax-200, Rizolex-T, Sanlight, Tachigaren, Galben-Cu and Copper Oxychloride were used to test their effect in controlling dahlia tuber rot caused by *S. rolfsii* as follows:

9.2.1. Soil treatment

Infested pots (25 cm ϕ and 3kg soil) with *S. rolfsii* in form of 30g corn meal medium/pot were treated with the following fungicides, i.e., Rizolex-T, Vitavax-200, Topsin-M70, Galben-Cu, Copper oxychloride and Sanlight at rate 3g/pot. Meanwhile, Tachigaren fungicide was added to each pot at rate 3 ml from the commercial product. Dahlia tuber cuttings were sown at rate of

two tubers/pot then irrigated with sterilized water and left to grow for eight weeks.

9.2.2. Tuber treatment

The same tested fungicides were used in this experiment, then the concentration of all tested fungicides was adjusted at 750 ppm. Dahlia tuber cuttings which, prepared previously for sowing were dipped in fungicide solutions for five-min then transferred to ready inoculated pots with the tested fungus. One-gram of adhesive dispersed material (Tween-80) was added to all fungicide preparations. The pots were left to grow for 8 weeks as mentioned before.

10. Evaluation of bactericides on growth of pathogenic bacteria:

10.1. *In vitro*

As shown in **Table (3)**, five antibiotics namely, streptomycin, penicillin, ampicillin, tetracycline, erythromycin, as well as two fungicides i.e., galben-Cu and copper oxychloride, were used at five different concentrations (250, 500, 1000, 1500 and 2000ppm). Nutrient yeast dextrose agar media were inoculated at rate 1ml/100 ml media from pathogenic bacteria dilutions (1.3 x 10^8 cfu) at 45°C before pouring in Petri-plates. Saturated filter paper disks (5mm) of each antibiotic or fungicide at different concentrations were placed on the surface of inoculated plates. Disks without any treatment were used as control. Four disks/plate and 4 plates for each treatment were done then the plates were incubated at 30°C for 48 hrs. Effect

of tested antibiotics and fungicides were measured in form of inhibition zone surrounding the disks according to **Loo *et al.* (1945) and Thornberry (1960)**.

10.2. *In vivo*

Five antibiotics namely, streptomycin, penicillin, ampicillin, tetracycline, erythromycin, as well as two fungicides i.e., Galben-Cu and Copper oxychloride, were used at 200 ppm under greenhouse conditions.

Pots (25cm ϕ and 3 kg soil/pot) were infested individually with 50ml of the tested pathogenic bacteria (1.3×10^8 cfu/ml), then irrigated with sterilized water and left for one week to activate the bacteria. The prepared and sterilized dahlia tubers were dipped in tested antibiotics and fungicides solution at rate 200 ppm individually and then cultivated in infested pots. Copper oxychloride was mixed with five antibiotics, i.e., streptomycin, penicillin, ampicillin, tetracycline and erythromycin and the final concentration of the mixture was adjusted at 100 ppm **(Haggag and Abo Sadera, 2000)**.

Disease assessment was carried out by scoring the percentage of dead plant and survived plants. The readings were taken after 2 and 7 weeks respectively as mentioned before.

Table (3): List of tested antibiotics, their active ingredients and chemical formula.

Trade name & molecular weight	Common name (%)	Chemical formula
Ampecillin (M.W. 371.39)	D(-)- α - aminobenzylpenicillin (83.3%)	$C_{16}H_{19}N_3O_4S$
Erythromycin (M.W. 733.94)	Erythromycin (80%)	$C_{37}H_{67}NO_{13}$
Tetracycline (M.W. 480.90)	Tetracycline (83.3%)	$C_{22}H_{24}N_2O_8.XH_2O$
Penicillin (M.W. 356.37)	Penicillin (83.3%)	$C_{16}H_{17}N_2O_4SNa$
Streptomycin (M.W. 1457.38)	Streptomycin (80%)	$(C_{21}H_{39}N_7O_{12})_2.3H_2SO_4$

RESULTS

1. Isolation of fungi and bacteria associated to rotted dahlia tubers

Sum of 107 fungal and 55 bacterial isolates were obtained from naturally infected dahlia tubers in three locations i.e. Moshtohor, El-Dair and El-Manashy in Kalubia Governorate. Data shown in **Table (4)** indicate that the isolated fungi belong to 6 genera and 7 species. Fungi were identified as: *Sclerotium rolfsii*, *Fusarium* sp., *Rhizoctonia solani*, *Aspergillus niger*, *Aspergillus flavus*, *Penecillium digitatum* and *Rhizopus* sp.

S. rolfsii was more occurrence in all tested samples comparing to the other isolated fungi. The highest isolation number and frequency % of *S. rolfsii* was recorded in El-Dair location (70.0%) followed by El-Manashy (60.0%) and Moshtohor (54.1%) locations. The other fungi were isolated within small numbers and frequency % affecting location. On the other hand, the isolated bacteria from rotten dahlia tubers were identified and discussed for their pathogenic abilities in further part. The highest frequency% was recorded in El-Manashy followed by Moshtohor and El-Dair locations i.e. 42.2%, 32.7% and 27.3% respectively.

2. Pathogenicity test of fungal pathogens

Data in **Table (5)** show that *S. rolfsii* is the most pathogenic of tested fungi in inducing rotting of dahlia tubers where the infection area increased gradually to decay 17 mm after the first day from infestation, this area reached 50mm after three days of infestation with 34-100 infection % respectively.

Meanwhile, *R. solani* followed by *Fusarium* spp. revealed infection % ranged between 22-28 and 20-22%, for both fungi after the first and third day, respectively.

Table (4): **Frequency (%) and percentage of fungi and bacteria isolated from dahlia tubers represent three different locations in Kalubia governorate.**

Isolated microorganisms	Location of isolation						Total
	Moshtohor		El-Dair		El-Manashy		
	Fre.	%	Fre.	%	Fre.	%	
Fungi:	37	**67.27**	**40**	**72.73**	**30**	**57.69**	**107**
rotium rolfsii	**20**	**36.36**	**28**	**50.91**	**18**	**34.62**	**66**
***Fusarium* spp.**	**0**	**0.00**	**1**	**1.82**	**0**	**0.00**	**1**
octonia solani	**5**	**9.09**	**0**	**0.00**	**2**	**3.85**	**7**
rgillus niger	**3**	**5.45**	**2**	**3.64**	**2**	**3.85**	**7**
rgillus flavus	2	**3.63**	**2**	**3.64**	2	**3.85**	**6**
cillium digitatum	3	**5.45**	**3**	**5.45**	**3**	**5.77**	**9**
opus sp.	4	**7.27**	**4**	**7.27**	**3**	**5.77**	**11**
Bacteria	18	**32.70**	**15**	**27.27**	**22**	**42.31**	**55**
TOTAL	55		**55**		**52**		**162**

Table (5): Pathogenic abilities of three chosen fungi isolated from rotted dahlia tubers.

Pathogenic fungi	1 day		3 days	
	*IA (mm)	%	IA (mm)	%
Sclerotium rolfsii	17	34.0	50	100.0
***Fusarium* spp.**	10	20.0	11	22.0
Rhizoctonia solani	11	22.0	14	28.0

*IA = infected area (mm)

3. Physical factors affecting growth and sclerotial formation of *S. rolfsii:*

3.1. Different media

Data presented in Table (6) **clearly show that *S. rolfsii* was able to grow on most of the tested media. The highest average of linear growth was obtained on PDA where the fungus filled the plate after 4-days post inoculation, followed by dahlia tuber agar, Brown's media, while the lowest average of linear growth occurred on Czapek's medium.**

Concerning sclerotial formation, media of PDA followed by dahlia tuber and peptone were the most favourable media for producing sclerotia of *S. rolfsii*. Sclerotia were also produced on the other tested media but with small numbers ranged between 5-11 sclerotia/plate. On the other hand, it is clear that the highest weight of five sclerotia (g) was obtained on Brown's medium, while the least weight was in cases of peptone and dahlia tuber media.

The first sclerotium formed after 3 days on Brown's and Baren's media, while it formed after 9 days in case plain agar medium. Also, the first sclerotium was formed on PDA, dahlia tuber and peptone media after 7 and 8 days within the highest numbers.

Table (6): Effect of different media on the growth and sclerotium formation of *S. rolfsii*.

Media	**Growth (mm) after days**		**No. of sclerotia/ plate**	**Weight of 5 sclerotia (g)**	**The first sclerotium formed after (days)**
	2	**4**			
PDA	39	90	168	0.006	7
Dahlia tuber	36	84	67	0.003	7
Peptone	33	60	55	0.003	8
Czapek's	25	44	7	0.008	4
Brown	37	62	10	0.019	3
Baren's	27	56	11	0.009	3
Conne	36	61	6	0.011	8
Plain agar	34	56	5	0.013	9

3.2. Temperature

Seven temperature degrees were studied for their effect on the growth, sclerotia yield and formation of the first sclerotium of *S. rolfsii*. Data in **Table (7)** show that *S. rolfsii* could be grow within 15 - 40°C, while, no growth was recorded below 15°C. Maximum growth of the fungus was obtained at 30°C whereas, minimum growth was at 15°C.

The highest number of sclerotia was formed at 30°C (263 sclerotium/plate) and at 25°C (243 sclerotium/plate), while, the least number was at 40°C (94 sclerotium/plate). Also, no sclerotia were formed below 15°C. Maximum weight of 5 sclerotia obtained at 30 and 35°C (0.009 g) and 0.008 g at 25°C. The minimum weight of 5 sclerotia was 0.007 g at 40°C. Therefore, the first sclerotium formed after 4 days at 40°C, but 15°C was the least favourable temperature degree tested for formation of sclerotia where, the first sclerotia appeared after 11 days.

Table (7): Effect of different temperature degrees on the growth and sclerotium formation of *S. rolfsii*.

Temperature (°C)	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
10	0	0	0	0.0	0
15	15	37	108	0.009	11
20	18	42	162	0.007	7
25	25	83	243	0.008	7
30	37	90	263	0.009	5
35	39	60	112	0.009	5
40	27	50	94	0.007	4

3.3. Relative humidity

Results in **Table (8)** indicate that *S. rolfsii* could be grow at RH values ranged between 14-100%, however, the average linear growth has gradually increased by increasing RH values from 14 to 100% after four days incubation. The maximum

growth was recorded between 80 -100% RH, resulting 67-63 mm after 4 days incubation. Meanwhile, 90% RH gave the best linear growth (71 mm). On the other hand 14 and 50% RH were not favourable for growth of *S. rolfsii*. In this respect also, all tested RH values were not suitable for sclerotial formation.

Table (8): Effect of different levels of relative humidity on the growth of *S. rolfsii.*

RH (%)	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
100	48	63	0.0	0.0	0.0
95	47	66	0.0	0.0	0.0
90	49	71	0.0	0.0	0.0
85	41	65	0.0	0.0	0.0
80	46	67	0.0	0.0	0.0
74	41	62	0.0	0.0	0.0
50	34	53	0.0	0.0	0.0
14	29	46	0.0	0.0	0.0
Control	39	90	168	0.006	7

3.4. pH values

Data in **Table (9)** show that, acidity and alkalinity of the culture media play an important role on growth and sclerotial formation of *S. rolfsii*. In this respect, the optimum pH for growth of the fungus was pH 5 where the maximum growth scored after 6 days incubation (90 mm). Meanwhile, the lowest growth was obtained at pH 4 and 8 after 6 days incubation. Also,

the results indicate that *S. rolfsii* could be grow at different pH values ranging from pH 3 to 8.

Concerning sclerotial formation, the best pH values for producing the highest number of sclerotia were pH 5 and 6 (172 and 120 sclerotium/plate), respectively. Meanwhile, the least number was scored at pH 8.0.

Moreover, the highest weight of 5 sclerotia was occurred when the fungus grown at pH 5 and 4 where the weight were 0.024 and 0.021 g, respectively, while, the least weight was at pH 7. Additionally, the first sclerotium formed after 6 days in medium adjusted at pH 8, followed after 7 days at pH 5.

Table (9): Effect of different pH values on the growth and sclerotial formation of *S. rolfsii*.

pH value	Growth (mm) after days			No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4	6			
4	28	47	70	112	0.021	8
5	31	55	90	172	0.024	7
6	31	53	75	120	0.017	8
7	21	41	59	97	0.016	8
8	12	19	28	55	0.019	6

3.5. Carbon sources

Data presented in **Table (10)** show that *S. rolfsii* isolate use many different carbon sources in nutrition such as glycerol, sucrose, glucose, starch, maltose, lactose, dextrin and arabinose. Glucose and lactose were the best carbon sources for growth of the fungus where, its growth was 90 and 83 mm, respectively

after 6 days post inoculation. Glycerol and dextrin were the lowest favourable in this respect. It is clear also that starch, arabinose, maltose and sucrose were moderately affected growth of *S. rolfsii*. On the other hand, glucose, lactose and starch were the best carbon sources for sclerotial formation where, the formed sclerotia were 140, 134 and 72 sclerotium/plate, respectively. Therefore, the highest weight of 5 sclerotia was in case of arabinose followed by glucose and sucrose respectively. Also, the first sclerotium formed on media containing dextrin after 2 days, while in presence of maltose or arabinose the first sclerotium appeared after 9 days post inoculation.

Table (10): Effect of different carbon sources on the growth and sclerotium formation of *S. rolfsii.*

Carbon Sources	**Growth (mm) after days**			**No. of sclerotia/ plate**	**Weight of 5 sclerotia (g)**	**The first sclerotium formed after (days)**
	2	**4**	**6**			
Glycerol	24	31	38	15	0.016	8
Sucrose	24	36	46	13	0.018	5
Glucose	38	65	90	140	0.019	6
Starch	30	54	68	72	0.013	7
Maltose	24	44	56	56	0.006	9
Lactose	35	61	83	134	0.018	6
Dextrin	18	25	28	10	0.013	2
Arabinose	28	41	61	41	0.020	9
Without C	19	20	20	9	0.008	2

3.6. Nitrogen sources

Results in **Table (11)** indicate that yeast extract, peptone, asparagine and gelatin were the most favourable nitrogen sources

for growth of *S. rolfsii* where its growth was 90, 55, 52 and 45 mm, respectively, after four days post inoculation. Meanwhile, media containing casein, urea, sodium nitrate were not favourable for growth of the fungus. Media containing yeast extract, peptone and asparagine as sole nitrogen sources were the best for sclerotial formation of *S. rolfsii,* respectively. While the lowest sclerotial numbers were produced on media containing ammonium nitrate, urea, sodium nitrate. On the other hand, the highest weight of five sclerotia was in case of yeast extract, peptone, asparagine, beef extract and gelatin as sole nitrogen source, respectively. While, the least weight was in case of urea and sodium nitrate. Additionally, the first sclerotium formed after 4 days post inoculation when used ammonium nitrate, urea, peptone and sodium nitrate, while it formed after 6 days in cases of casein and gelatin as nitrogen sources.

Table (11): Effect of different nitrogen sources on the growth and sclerotium formation of *S. rolfsii.*

Nitrogen Sources	**Growth (mm) after days**		**No. of sclerotia/ plate**	**Weight of 5 sclerotia (g)**	**The first sclerotium formed after (days)**
	2	**4**			
Casein	25	30	27	0.024	6
Ammonium nitrate	30	42	23	0.027	4
Beef extract	27	42	45	0.021	5
Urea	0	18	12	0.009	4
Yeast extract	43	90	232	0.007	5
Peptone	28	55	75	0.013	4
Asparagin	30	52	68	0.014	5
Gelatin	31	45	30	0.022	6
Sodium nitrate	22	38	12	0.006	4
Without N	31	47	5	0.013	4

3.7. Light colors

Six different light colors were tested for their effect on growth and sclerotial formation of *S. rolfsii*. In this respect, results in **Table (12)** revealed clear differences between the different colors in their effect on growth of *S. rolfsii*. The highest linear growth was obtained on black color, while the lowest growth was recorded on green color.

Also, black, blue and yellow colors were the best for sclerotial formation (184, 91 and 62 sclerotia/plate, respectively).

The highest weight of five sclerotia was obtained in case of red, green and black colors which, were 0.019, 0.018 and 0.013 g). While, the minimum weight was in case of yellow and

blue colors. In addition the first sclerotium formed with using black color after 5 days, followed by white and yellow colors after 6 days, while it formed after 8 days with blue and red colors.

Table (12): Effect of different light colors on the growth and sclerotium formation of *S. rolfsii*.

Light colors	Growth (mm) after days		No. of sclerotia/ plate	Weight of 5 sclerotia (g)	The first sclerotium formed after (days)
	2	4			
White	34	66	50	0.019	6
Green	33	63	53	0.018	7
Blue	35	79	91	0.010	8
Yellow	36	72	62	0.010	6
Red	37	77	44	0.019	8
Black	45	90	184	0.013	5

4. Factors affecting disease incidence on dahlia tubers infected with *S. rolfsii*:

4.1. Effect of wounding

Six wound levels were made into dahlia tubers before inoculation with an equal disc (6 mm) of *S. rolfsii* to test their relations with infection. In this respect, data in **Table (13)** show that there are positive relation between wounding and appearance of infection with *S. rolfsii* on dahlia tubers. The

infected area was increase gradually by increasing the wound levels from needle wound level till 10 mm cyclic wound at all incubation days from the 3rd – 11th. The highest infection area were 94mm and 91mm which were recorded onto dahlia tubers wounded at levels 10 and 5 mm cyclic wounds respectively after 11 days incubation. The infected area in case of needle wound onto dahlia tubers reached 64 mm after 11 days incubation. The results indicate that the fungus was able to infect dahlia tuber naturally wounded and its infection increased gradually to reach 61mm after 11 days incubation.

The results clearly show that infection with *S. rolfsii* reduced the weight of dahlia tubers and these reduction were affected by incubation period and wound level where the reduction in infected dahlia tubers increase gradually from the 3rd till 11th. The highest reduction in infected dahlia weight was 22.3% at 10 mm cyclic wound after 11 days incubation, while, the least reduction in weight of infected dahlia tubers was 14.9% after 11 days in case of needle wound comparing with control (non-wounded/non-inoculated).

Table (13): **Effect of wounding dahlia tubers on infection with *S. rolfsii* under *in vitro* conditions.**

Depth of wound	Days post inoculation with *S. rolfsii*									
	3		5		7		9		11	
	*IA	*RW	IA	RW	IA	RW	IA	RW	IA	RW
Needle	13	2.8	25	5.9	34	8.9	50	12.7	64	14.9
Superficial	19	3.6	32	7.3	48	9.8	62	12.9	76	15.3
3mm-depth cyclic	23	4.1	46	7.5	55	10.5	65	13.1	81	16.5
5mm-depth cyclic	28	4.5	51	8.6	62	10.7	77	13.5	91	16.8
10mm-depth cyclic	33	5.1	59	9.1	71	13.2	82	16.8	94	22.3
Naturally wounded	10	2.5	23	5.6	31	7.2	43	10.4	61	13.5
Control	0.0	1.5	0.0	2.3	0.0	3.0	0.0	3.5	0.0	4.6

***IA= infected area (mm)**

RW= reduction in weight of dahlia tuber infected with *S. rolfsii* (g).

4.2. Effect of inoculum density:

4.2.1. On dahlia tubers under *in vitro* conditions.

Data in **Table (14)** reveal that all used inoculum density of *S. rolfsii* (1-10 sclerotia/wound) had the ability to induce infection on wounded dahlia starting from the 5th day. This infection was developed gradually by increasing incubation period. The highest infected area reached 92 mm after 11 days using inoculum density 10 sclerotia/wound, meanwhile, the least infected area was 46 mm after the same time using one sclerotia/wound.

Also, this infection reduced the weight of infected dahlia gradually in correlation to the used inoculum density, where the highest reduction percentage was 10.2% after 11 days by using 10 sclerotia/wound. On the other hand, the least reduction

percentage in weight was 6.4% using one sclerotia/wound. The reduction % values between the tested 1-10 sclerotia/wound were gradually increased affected by sclerotia number and incubation period.

Table (14): **Relationship between inoculum density of *S. rolfsii* and disease incidence on wounded dahlia tubers (10-mm cyclic wound) under *in vitro* conditions.**

No. of Sclerotia/ wound	Period after inoculation with sclerotia of *S. rolfsii* (days)									
	3		5		7		9		11	
	*IA	RW	IA	RW	IA	RW	IA	RW	IA	RW
1	0	0.0	13	2.7	23	3.8	33	5.2	46	6.4
2	0	0.0	19	2.8	30	3.9	43	5.3	52	6.5
3	0	0.0	21	3.0	33	4.3	45	5.5	55	7.3
4	0	0.0	24	3.2	35	5.0	47	6.3	59	7.9
5	0	0.0	26	3.5	40	5.3	50	6.5	64	8.4
6	0	0.0	28	3.7	45	5.5	63	6.8	73	8.7
7	0	0.0	30	3.8	48	5.8	66	7.2	77	9.0
8	0	0.0	32	4.0	51	6.1	68	7.6	81	9.3
9	0	0.0	35	4.2	53	6.3	70	8.1	89	10.1
10	0	0.0	39	4.5	59	6.5	74	8.3	92	10.2
Control	0	0.0	0.0	2.5	0.0	3.0	0.0	3.7	0.0	4.5

***IA= infected area (mm)**

RW= reduction in weight of dahlia tuber infected with *S. rolfsii* (g).

4.2.2. As soil treatment under greenhouse conditions.

Data in **Table (15)** indicate that, infesting soil with different inoculum levels of *S. rolfsii* has a great effect on surviving of dahlia plants. In this respect, increasing of

inoculum potential from 1 –5 % increased gradually the dead plants where 5% inoculum potential gave the highest death percentage of dahlia plants which was 87.5%, followed by 4% inoculum were 62.5% whereas, 1% gave the least percentage of dead plants of dahlia.

Table (15): Effect of inoculum potential (IP) of *S. rolfsii* on disease incidence in dahlia tubers.

*IP (%)	2000/2001 season	
	Dead Plant (%)	Survived Plant (%)
1	16.6	83.4
2	37.5	62.5
3	54.1	45.9
4	62.5	37.5
5	87.5	12.5

*IP= inoculum potential

5. Pathogenicity and host range of isolated bacteria

Results in **Table (4)** show that bacterial isolation trial from rotted dahlia tubers resulted in 55 bacterial isolates. Out of these, 7 isolates (No. 4, 7, 9, 10, 11, 12 and 14) were only pathogenic and caused soft rot on dahlia tubers, meanwhile, the remained isolates were not pathogenic and showed no symptoms on dahlia tubers when tested for their pathogenicity. The pathogenic ability of bacteria isolates (7 isolates) was tested on different plant hosts as shown in **Table (16)**. All these tested isolates were highly pathogenic on dahlia and potato tubers and never affected sugar beet and taro. On the other hand, isolate No. 12 was highly pathogenic on all tested host plants except sugar beet roots and taro-(corms). While, isolate 4 was highly

pathogenic only on potato, squash, dahlia and cucumber, meanwhile, it was moderately affected sweet potato and carrot. Also, isolate No. 7 was highly pathogenic on potato, carrot and dahlia and moderately affected sweet potato, squash, cucumber and onion. Meanwhile, isolate No. 9 was highly pathogenic on potato, carrot, dahlia and low affected squash, cucumber and onion, but moderately affected only sweet potato. Isolate No. 10 revealed highly pathogenic ability on potato, carrot, squash and dahlia, while, it was moderately pathogenic on sweet potato, cucumber and onion. Moreover, isolate No. 11 was highly pathogenic only on potato and dahlia but it was moderately pathogenic on sweet potato, carrot, squash and onion and low affecting cucumber. The last isolate (No. 14) was highly affected potato, squash, dahlia and moderately affected carrot and onion, while it was low affected sweet potato and cucumber.

Table (16): Reaction of different plant hosts to inoculation by the pathogenic bacteria isolates of dahlia tuber rot.

Plant – (organ)	**Pathogenic bacterial isolates**						
	4	**7**	**9**	**10**	**11**	**12**	**14**
Potato – (tuber)	H	H	H	H	H	H	H
Sweet potato- (tuber root)	M	M	M	M	M	H	L
Carrot – (root)	M	H	H	H	M	H	M
Squash – (fruit)	H	M	L	H	M	H	H
Dahlia – (tuber)	H	H	H	H	H	H	H
Sugar beet – (root)	-	-	-	-	-	-	-
Cucumber – (fruit)	H	M	L	M	L	H	L
Onion – (bulb)	M	M	L	M	M	H	M
Taro – (corm)	-	-	-	-	-	-	-

- = Non pathogenic, H= highly pathogenic, M = moderate pathogenic and L = low pathogenic.

6. Identification and classification of pathogenic bacterial isolates

6.1. Traditional techniques

Out of 55 bacterial isolates, 7 isolates only were pathogenic. These pathogenic isolates were identified and classified based on their morphological and physiological properties. In the first scheme **(Table, 17)**, the pathogenic bacterial isolates were classified in three groups based on Gram stain and spore formation. The first group included two (2) isolates which are long rod shaped, Gram positive (G+) and spore formers, growing under aerobic condition. The aforementioned properties indicate that these two isolates belong to the genus Bacillus. The second group consisted two isolates which were short rod shaped Gram negative (G-), white colonies, no diffusible pigment, non-spore former, so it may belong either to the genus *Erwinia* or *Pseudomonas*. The second scheme **(Table, 18)**, classified the Bacillus isolates for two species according to their reactivity with different bio tests. The isolates No. 7 and 9 belonged to the species *Bacillus polymyxa.* While the third scheme **(Table, 19)** show that both isolate No. 4 and 12 were short rod shape, Gram negative (G-), positive for potato soft rot incidence, gelatin liquefaction and producing acid from lactose, so they were classified as *E. carotovora*. Fourth scheme **(Table, 20)** indicated that the isolates No. 10, 11 and 14 might be identified as *Ps. cepacia*. On the basis of host plant

reaction and source of isolation (dahlia tuber), this isolate was identified as *Ps. cepacia*.

Table (17): Classification of pathogenic bacterial isolates (Scheme-1*).

Identification Tests	Isolate No.						
	4	**7**	**9**	**10**	**11**	**12**	**14**
Gram reaction	-	+	+	-	-	-	-
Growth on common media	+	+	+	+	+	+	+
Size	Short	Long	Long	Short	Short	Short	Short
Spore production	-	+	+	-	-	-	-
Pigment K.B.	-	-	-	-	-	-	-
Starch hydrolysis	-	+	+	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+	+
Yeast extract dextrose $CaCO_3$	+	+	+	+	+	+	+
Pectate degradation	+	+	+	+	+	+	+
Growth on peptone yeast extract agar (PYEA)	+	+	+	+	+	+	+
Growth on MS medium	+	+	+	+	+	+	+
Fried egg on PDA	+	-	-	-	-	+	-
Tolerance to NaCl 5%	H	H	M	M	M	H	H
Tolerance to NaCl 7%	M	M	L	L	L	M	M
Reducing substance from sucrose after:							
2 min.	+	+	+	-	-	+	-
10 min.	-	+	+	-	+	-	-
Acid production from glucose	-	+	+	-	+	-	+
Acid production from lactose	+	+	+	+	+	+	+
Gas production from lactose	-	-	-	-	-	-	-
Anaeorbic production of gas from glucose	+	-	-	-	-	+	-
Relation to O_2	F.	A.	A.	A.	A.	An.	A.
Catalase activity	+	+	+	+	+	+	+
****Bacterial genera**	***E.***	***B.***	***B.***	***P.***	***P.***	***E.***	***P.***

[*] Based on classification tests suggested by **Schaad (1980)**, **Fahy and Persley (1983)** and **Lelliott and Stead (1987)**.

[**] ***E.= Erwinia, B.= Bacillus* and *P.= Pseudomonas*.**

Table (18): Classification of the rod shape, Gram positive (G^+) and spore former bacterial isolates (*Bacillus* classification) Scheme-2.

Identification Tests	Isolate No.	
	7	**9**
Gram reaction	+	+
Shape	rod	rod
Spore production	+	+
Pigments K.B.	-	-
Starch hydrolysis	+	+
Catalase activity	+	+
Gelatin liquefaction	+	+
Tolerance to NaCl 5%	+	+
Tolerance to NaCl 7%	+	+
Anaerobic production of gas from glucose	-	-
Growth at 50°C	-	-
Bacterial isolate identified	***Bacillus polymyxa***	

Table (19): Classification of the rod shape and Gram negative (G^-) bacterial isolates (*Erwinia* classification) Scheme-3[*].

Identification Tests	Isolate No.	
	4	**12**
Potato soft rot	+	+
Potato soft rot	+	+
Gelatin liquefaction	+	+
Starch hydrolysis	-	-
Catalase activity	+	+
Pectate degradation	+	+
Fried egg on PDA	+	+
Growth on MS medium	+	+
Yeast extract dextrose $CaCO_3$	+	+
Tolerance to NaCl 5%	+	+
Reducing substance from sucrose after 10min.	-	-
Anaerobic production of gas from glucose	+	+
Acid production from lactose	+	+
Relation to O_2	F.	An.
Bacterial isolate identified	***E. carotovora***	***E. carotovora***

* Based on classification tests suggested by **Schaad (1980)**, **Fahy and Persley (1983)** and **Lelliott and Stead (1987)**.

Table (20): *Pseudomonas* classification, the non fluorescent group, short rod shape and Gram negative (G^-) Scheme-4*.

Identification Tests	Isolate No.		
	10	**11**	**14**
Pigments diffusible	Non	Non	Non
Non diffusible pigments	-	-	-
Starch hydrolysis	-	-	-
Gelatin liquefaction	+	+	+
Pectate degradation	+	+	+
Peptone yeast extract agar (PYEA)	+	+	+
Fried egg on PDA	-	-	-
Tolerance to NaCl 5%	M	M	H
Tolerance to NaCl 7%	L	L	M
Potato soft rot	+	+	+
H_2S production	-	-	-
Aerobiosis	A.	A.	A.
Reducing substance from sucrose	+	+	+
Hypersensitivity reaction	-	-	-
Maximum temp. for growth (°C)	35	35	35
pH of glucose nutrient broth (after 72)	7.0	7.0	6.9
Growth on KB Medium	-	-	-
Pectate gel liquefaction	+	+	+
Levan type	+	+	+
Bacterial isolate identified	***Pseudomonas cepacia***	***Pseudomonas cepacia***	***Pseudomonas cepacia***

* Based on classification tests suggested by **Schaad (1980)** and **Fahy and Persley (1983)** .

6.2. Electrophoretic analysis of protein patterns

Data in **Fig. (1)** and **Tables (21 & 22)** show that protein bands derived from the electrophoretic gel of the soluble proteins of the seven pathogenic bacterial isolates which infected dahlia tubers show that the two bacterial isolates *E. carotovora*-4 (Lane 1) and *E. carotovora*-12 (Lane 2) which, identified previously by the traditional techniques are similar to each other in 10 molecular weights, i.e., 189.6, 166.5, 158.8, 143.4, 95.4, 73.3, 65.6, 56.1, 53.03 and 51.3 KDa with similarity coefficient 0.374. Meanwhile, *E. carotovora*-4 (Lane 1) is found high similar to the bacterial isolate (Lane 6) which previously identified as *Ps. cepacia*-10 by traditional techniques where the similarity coefficient is 0.401. On the other hand, bacterial isolates which previously identified as *B. polymyxa*-7 & -9 (Lanes 3 & 4) by the traditional techniques were found similar to each other in 5 molecular weights, i.e., 151.1, 143.4, 107.9, 86.5 and 78.4 KDa with similarity coefficient 0.227. On the other hand, the last 3 isolates which, identified previously as *Ps. cepacia*-10, -11 & -14 (Lanes 5, 6 &7) are similar somewhat to each other in some molecular weights. In this respect, *Ps. cepacia*-10 (Lane 5) is similar *to Ps. cepacia*-11 (Lane 6) in molecular weights, i.e., 128.1, 112.9, 75.8, 63.05, 57.6 and 49.55 with similarity % 0.194, while, the similarity coefficient of *Ps. cepacia*-10 (Lane 5) and *Ps. cepacia*-14 (Lane 7) was 0.230. Meanwhile, it is clear from the results that the similarity coefficient between *Ps. cepacia*-11 (Lane 6) and *Ps. cepacia*-14 (Lane 7) was low (0.107) where the two isolates are similar in only 4 molecular weights of total resulted protein bands.

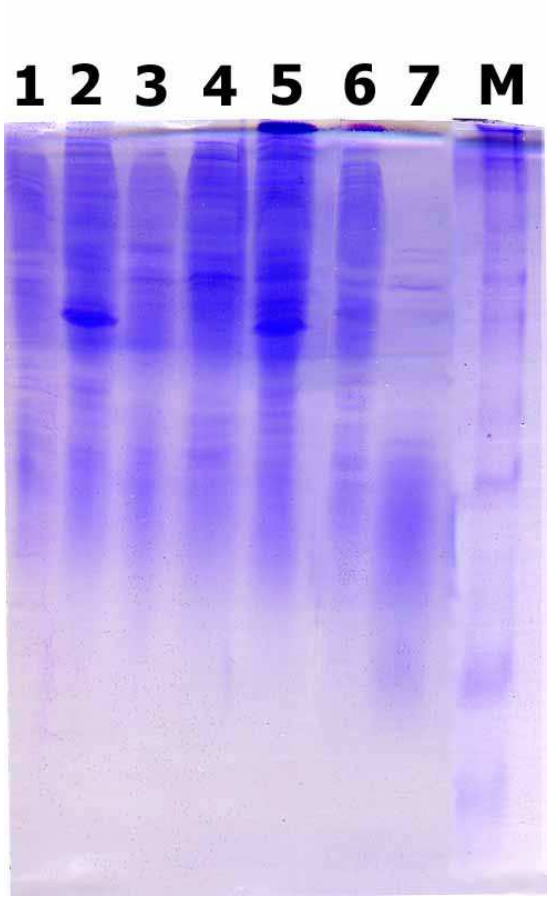

Fig. (1): SDS-PAGE protein pattern of 7 bacterial isolates infected dahlia tubers.

Lane 1 =*Erwinia carotovora*-4
Lane 2 =*Erwinia carotovora*-12
Lane 3 = *Bacillus polymyxa*-7
Lane 4 = *Bacillus polymyxa*-9
Lane 5 = *Pseudomonas cepacia*-10
Lane 6 = *Pseudomonas cepacia*-11
Lane 7 = *Pseudomonas cepacia*-14

Table (21): Molecular weights of the seven tested pathogenic bacterial isolates infected dahlia tubers.

Molecular weights	**Lane 1*	*Lane 2*	**Lane 3**	**Lane 4**	**Lane 5**	**Lane 6**	**Lane 7**
212.7	0	0	1	0	0	0	0
205.0	0	1	0	0	1	0	0
189.6	1	1	0	0	0	0	0
181.9	0	1	1	0	0	1	0
174.2	0	0	0	1	1	0	0
166.5	1	1	0	0	0	1	0
158.8	1	1	0	0	1	0	0
151.1	0	0	1	1	0	1	0
143.4	1	1	1	1	0	1	0
128.1	1	0	0	1	1	1	0
112.9	0	1	0	0	1	1	0
107.9	0	0	1	1	0	0	0
101.5	1	0	1	0	0	0	0
99.3	0	0	0	0	1	0	1
95.4	1	1	0	1	0	1	1
91.5	0	1	0	0	1	0	0
90.3	0	0	0	1	1	0	0
86.5	1	0	1	1	0	1	0
80.9	1	0	0	0	1	0	1
78.4	0	0	1	1	1	0	0
75.8	0	0	0	0	1	1	0
73.3	1	1	0	1	1	0	1
65.6	1	1	1	0	0	1	0
63.05	1	0	0	0	1	1	1
62.2	1	0	0	0	0	0	0
57.6	1	0	0	0	1	1	1
56.1	1	1	0	0	0	1	0
54.6	0	1	0	0	1	0	0
53.03	1	1	0	0	0	1	0
52.2	0	0	0	0	1	0	1
51.3	1	1	0	1	0	0	0

Table (21): Molecular weights of the seven tested pathogenic bacterial isolates infected dahlia tubers (continued).

Molecular weights	*Lane 1	*Lane 2*	**Lane 3**	**Lane 4**	**Lane 5**	**Lane 6**	**Lane 7**
49.55	0	0	0	0	1	1	0
48.8	0	0	0	1	1	0	0
48.1	1	0	0	0	0	0	1
46.7	0	1	0	1	0	0	0
45.8	0	0	0	0	0	1	0
44.03	1	0	0	0	0	0	1
42.3	0	0	1	0	0	1	0
37.98	0	1	1	0	0	0	1
37.2	0	0	1	0	0	0	0
35.9	0	0	1	0	1	0	0
34.15	1	0	0	0	0	1	0
32.84	0	0	1	0	0	0	1
30.35	0	0	0	0	0	0	1
28.32	0	0	0	0	0	0	1
Total Bands	**20**	**17**	**14**	**13**	**19**	**18**	**13**

* Lane 1 =*Erwinia carotovora*-4 Lane 2 =*Erwinia carotovora*-12

Lane 3 = *Bacillus po;ymyxa*-7 Lane 4 = *Bacillus polymyxa*-9

Lane 5 = *Pseudomonas cepacia*-10 Lane 6 = *Pseudomonas cepacia*-11

Lane 7 = *Pseudomonas cepacia*-14

Table (22): Similarity index matrix among seven bacterial isolates based on SDS-PAGE.

	1	2	3	4	5	6	7
1	1.0	0.374	0.097	0.179	0.182	0.401	0.269
2		1.0	0.148	0.200	0.161	0.296	0.111
3			1.0	0.227	0.065	0.231	0.038
4				1.0	0.185	0.192	0.083
5					1.0	0.194	0.230
6						1.0	0.107
7							1.0

7. Biochemical changes in infected dahlia tubers:

In this experiment, biochemical changes in infected dahlia tubers and control was carried out to determine sugars contents (total, reducing and non-reducing) and phenols (total, conjugated and free) in order to explain their role in infection.

7.1. Sugars content

Data in **Table (23)** reveal that sugar contents was affected as a result for infection with bacterial pathogens and *S. rolfsii*. In this respect its clear that the highest amount of total sugars in infected dahlia tubers was 79.8 mg/g fresh weight with *S. rolfsii*, followed by *Ps. cepacia* 51.7 and 42.7 mg/g with *E. carotovora*-12, meanwhile the least amount of total sugars was 30.2 mg/g (f.w.) in case of *E. carotovora*-4. Also, its clear from results that all pathogenic bacteria did not have a great effect on reducing sugars, while the fungus *S. rolfsii* reduced sugars in infected dahlia to high level where the reduction reached 58.8 mg/g (f.w.) comparing with control (un infected tubers) and bacterial

inoculated tubers. On the other hand, all non-reducing sugars were determined in low amounts in all infected dahlia tubers with bacterial pathogenic and *S. rolfsii* comparing to control treatment. The same trend was recorded during replaying this experiment and gave similar results although the readings were different to somewhat.

Table (23): Effect of artificial inoculation with tuber rot pathogens on sugar content (mg/g fresh weight) of tubers incubated *in vitro.*

Pathogens	Total sugars	Reducing sugars	Non-reducing sugars
Experiment I			
***Erwinia carotovora*- 4**	30.2	16.7	13.5
***Erwinia carotovora*-12**	42.7	14.0	28.7
Pseudomonas cepacia	51.7	23.5	28.2
Sclerotium rolfsii	79.9	58.8	21.1
Control	85.9	26.7	59.2
Experiment II			
***Erwinia carotovora*-4**	42.3	9.1	33.2
***Erwinia carotovora*-12**	56.3	13.4	42.9
Pseudomonas cepacia	51.2	11.8	39.4
Sclerotium rolfsii	73.5	39.4	34.1
Control	78.4	11.3	67.1

7.2. Phenols content

Data in **Table (24)** indicate that infection with the fungus *S. rolfsii* increased total and conjugated phenols in dahlia tubers to high extent comparing with bacterial infection during the two separately experiments. In this respect, total and conjugated phenols were 29.4 and 8.4 mg/g fresh weight in infected dahlia tubers with the fungus followed by 33.5 and 10.9 mg/g (f.w.)

during the first and second experiment, respectively. On the other hand, all conjugated phenols determined in infected dahlia tubers with pathogenic bacteria and *S. rolfsii* were high comparing to control (un-inoculated) during the two experiments. Free phenols were high also in infected dahlia tubers with *S. rolfsii* comparing to control treatment and inoculated tubers with pathogenic bacteria.

Table (24): Effect of artificial inoculation with tuber rot pathogens on phenols content (mg/g fresh weight) of tubers incubated *in vitro.*

Pathogens	Total phenols	Conjugated phenols	Free phenols
Experiment I			
***Erwinia carotovora*-4**	14.6	6.1	8.5
***Erwinia carotovora*-12**	20.5	5.4	15.1
Pseudomonas cepacia	21.1	4.4	16.7
Sclerotium rolfsii	29.4	8.4	21.0
Control	18.7	1.8	16.9
Experiment II			
***Erwinia carotovora*-4**	21.2	5.7	15.5
***Erwinia carotovora*-12**	17.7	6.3	11.4
Pseudomonas cepacia	22.3	6.4	15.9
Sclerotium rolfsii	33.4	10.9	22.5
Control	25.2	4.8	20.4

8. Enzymatic studies:

8.1. In synthetic media

Data presented in **Table (25**) show that the fungus *S. rolfsii* and different tested pathogenic bacteria were not able to produce cellulase enzyme in Czapek's media containing sucrose, pectin and CMC. Meanwhile, these tested isolates which mentioned previously their pathogenic ability on dahlia tubers

were able to produce xylanase enzyme in Czapek's media containing sucrose, pectin and CMC. In this respect the highest activity of xylanase was produce in media containing CMC (0.2125 unit/ml) that inoculated with *E. carotovora*-4, followed by media containing sucrose which was inoculated with *E. carotovora* 12 (0.1903 unit/ml). It is clear from these results also, that all pathogenic bacteria were able to produce xylanase enzyme more than the fungus on media containing sucrose, pectin and CMC. Production of xylanase by *E. carotovora*-4 and -12 in Czapek's media containing pectin was less than in case of sucrose and CMC. Meanwhile, *S. rolfsii* and *Ps. cepacia* were not able to produce xylanase in media containing pectin.

Concerning polygalacturonase (PG) enzyme, the fungus *S. rolfsii* and other tested bacterial pathogens had the ability to produce PG in Czapek's media containing sucrose, pectin and CMC, but with different quantities ranged between 0.008-2.2 u/ml. *S. rolfsii* was the highest PG producer in media containing CMC and pectin as a carbon sources compared with other tested bacterial pathogens. Meanwhile, *Ps. cepacia* produced the highest activity of PG in media containing sucrose more than *E. carotovora* (4&12) and *S. rolfsii* grown at the same conditions.

Table (25): Cell wall degrading enzymes (cellulase, xylanase and PG) produced in synthetic media (unit/ml).

Pathogens	Cell wall degrading enzyme produced in media containing								
	Cellulase			xylanase			PG		
	Sucrose	Pectin	CMC	Sucrose	Pectin	CMC	Sucrose	Pectin	CMC
S. rolfsii	-	-	-	0.057	-	0.009	0.012	0.800	2.200
***E. carotovora*-4**	-	-	-	0.187	0.025	0.213	0.128	0.035	0.008
***E. carotovora*-12**	-	-	-	0.190	0.145	0.160	0.128	0.170	0.500
Ps. cepacia	-	-	-	0.016	-	0.080	1.250	0.016	0.170

8.2. On dahlia tubers

Data in **Table (26)** reveal that the fungus *S. rolfsii* and other tested bacterial pathogens were not able to produce cellulase in infected tissue of dahlia tubers where no activities of this enzyme were detected in this case. On the other hand, xylanase activity was detected in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora* (4 & 12 isolates) only. The highest activity of xylanase was recorded in case of *S. rolfsii* infection followed by *E. carotovora*-12. The highest activity of PG enzyme was 15.5 u/ml, followed by 10.5 u/ml in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora*-4. *E. carotovora*-12 and *Ps. cepacia* produced also considerable activities in infected dahlia tubers but so little comparing to the other mentioned pathogens.

Table (26): Cell wall degrading enzyme (cellulase, xylanase and PG) enzymes produced on dahlia tubers (unit/ml).

Pathogens	**Cell wall degrading enzyme (u/ml) produced in media containing**		
	Cellulase	**Xylanase**	**PG**
S. rolfsii	-	0.224	15.50
***E. carotovora*-4**	-	0.074	10.50
***E. carotovora*-12**	-	0.198	2.20
Ps. cepacia	-	-	1.25

9. Evaluation of different fungicides against *S. rolfsii*:

9.1. On culture plates (*In vitro*)

In this experiment seven different fungicides belonging to different chemical groups (systemic and non-systemic) **(Table 2)** at five concentration (50, 100, 250, 500, 750 ppm) were tested *in vitro* for their effect on growth of *S. rolfsii* the causal agent of dahlia tuber rot. Data in **Table (27)** show that all tested fungicides had the ability to inhibit or reduce the growth of *S. rolfsii* depending on tested concentrations and time of incubation. In this respect, Vitavax-200 and Topsin-M70 were completely inhibited the growth of *S. rolfsii* at concentrations starting from 100 - 750 ppm, while, visual growth of the fungus was remarkable at concentration 50 ppm only for the two fungicides, where the measured growth was 14-23 mm and 16-32 mm after 2 and 4 days post inoculation with the fungus, respectively. On the other hand, Sanlight, Tachigaren and Rizolex-T50 completely inhibited the growth of *S. rolfsii* at

concentrations starting from 250-750 ppm. Meanwhile, concentrations 50 and 100 ppm could not inhibit the growth, where there was a remarkable growth at 2 and 4 days of the fungus. Also, Galben copper and Copper oxychloride inhibited the growth of the fungus only at the high concentration (750 ppm). Although, the fungus grew on some concentrations for all tested fungicides (systemic and non-systemic), this growth was slight and less than control (without fungicide).

Table (27): Effect of different concentrations of some systemic fungicides on linear growth (mm) of *S. rolfsii 'in vitro'*.

Fungicide	Conc. (ppm)	Linear growth (mm) after 2 and 4 days	
		2	4
Tachigaren	**50**	23	35
	100	13	23
	250	0	0
	500	0	0
	750	0	0
Vitavax-200	**50**	14	23
	100	0	0
	250	0	0
	500	0	0
	750	0	0
Sanlight	**50**	12	45
	100	0	17
	250	0	0
	500	0	0
	750	0	0
Topsin-M70	**50**	16	32
	100	0	0
	250	0	0
	500	0	0
	750	0	0
Rizolex-T50	**50**	18	39
	100	16	31
	250	0	0
	500	0	0

	750	0	0
Galben copper	**50**	26	41
	100	24	39
	250	21	31
	500	13	20
	750	0	0
Copper oxychloride	**50**	32	49
	100	26	39
	250	21	31
	500	18	25
	750	0	0
Control		47	90

9.2. Under greenhouse conditions (*In vivo):*

9.2.1. Soil treatment

Data in **Table (28)** reveal that treating soil with fungicides control effectively *S. rolfsii* infection on dahlia tubers comparing with untreated soil during two seasons. In this respect, treating soil with Rizolex-T50 and Vitavax-200 completely control the infection of *S. rolfsii* during the first season, where the survived dahlia plants were 100%. As well as, the same fungicides were also the highest effective ones during the second season comparing with other fungicides. Copper oxychloride and Galben copper were the least effective fungicides during the two seasons. It is obvious that the effect of Topsin-M70 and Sanlight was raised during the second season more than the first season.

Table (28): Effect of soil application by some fungicides on disease incidence caused by *Sclerotium rolfsii* in dahlia tuber-roots during 2001/2002 and 2002/2003 seasons in greenhouse.

Fungicides (3 g/pot)	2001/2002 season		2002/2003 season	
	Dead Plants (%)	Survived plants (%)	Dead Plants (%)	Survived plants (%)
Tachgaren	50.0	50.0	37.5	62.5
Topsin M70	37.5	62.5	25.0	75.0
Rizolex T50	0.0	100.0	12.5	87.5
Sanlight	37.5	62.5	25.0	75.0
Vitavax 200	0.0	100.0	20.8	79.2
Copper oxycloride	75.0	25.0	62.5	37.5
Galben copper	62.5	37.5	58.3	41.7
Control	75.0	25.0	83.3	16.7

9.2.2. Tuber treatment

The previously tested fungicides *in vitro* were tested again in this experiment as tuber treatment under greenhouse conditions using concentration 750 ppm for their effect on infection development by *S. rolfsii.* Data in **Table (29)** show that dipping dahlia tubers in Rizolex-T50 and Vitavax-200 suspension before sowing gave the best control for *S. rolfsii* infection during the first and second seasons, respectively, where the survived plant for the first fungicide was 83.3% at the first season, while, 79.2% for Vitavax-200 at the second season comparing with control treatment. Also, dipping dahlia tubers in Topsin-M70, Sanlight and Vitavax-200 gave good control with 25% dead plants, whereas it was 75% in control treatment

(inoculated with pathogen) during the first season 2001/2002. In addition, treating dahlia tubers with Topsin-M70, Rizolex-T50 and Sanlight gave a good disease control where the dead plants were 25% for the first two fungicides and 37.5% for the last fungicide when compared with control treatment at the second season. On the other hand, treating dahlia tubers with Tachigaren followed by Copper oxychloride and Galben copper were the least effective fungicides in controlling *S. rolfsii* infection during the two seasons.

Table (29): Effect of tuber dipping in some fungicides on disease incidence caused by *Sclerotium rolfsii* in dahlia tuber during 2001/2002 and 2002/2003 seasons under greenhouse condition.

Fungicides **(750 ppm)**	**2001/2002 season**		**2002/2003 season**	
	Dead plants (%)	**Survived plants (%)**	**Dead plants (%)**	**Survived plants (%)**
Tachigaren	50.0	50.0	45.8	54.2
Topsin-M70	25.0	75.0	25.0	75.0
Rizolex-T50	16.7	83.3	25.0	75.0
Sanlight	25.0	75.0	37.5	62.5
Vitavax-200	25.0	75.0	20.8	79.2
Copper oxychloride	54.2	45.8	62.5	37.5
Galben copper	54.2	45.8	50.0	50.0
Control	75.0	25.0	83.3	16.7

10. Evaluation of different bactericides against bacterial pathogens:

10.1. On culture plates (*In vitro*)

In this experiment five antibiotics, i.e, ampicillin, erythromycin, streptomycin, tetracycline and penicillin as well as Galben copper and Copper oxychloride “as bactericides” were tested for their inhibition effect against pathogenic bacteria infecting dahlia tubers *in vitro*. Data in Table (30) show that all tested antibiotics and bactericides inhibited the growth of bacterial pathogens depending on antibiotic kind and used concentrations because increasing the concentration of tested materials whether the antibiotics or other bactericides from 25-200 ppm increase gradually the inhibited zone of pathogenic bacteria. Streptomycin was the first effective antibiotic against growth of tested bacteria where it gave high inhibition zone 33 mm against *Bacillus polymxa*-7 followed by 30.6 mm against *Ps. cepacia*-14. While, penicillin was the second effective antibiotic against *Ps. cepacia* -11 and *E. carotovora*- 4 where the inhibition zones were 30.6 and 29.2 mm, respectively. Meanwhile, erythromycin was also effective against *E. carotovora*-12. Copper oxychloride and Galben copper “as bactericides” were the least effective materials against growth of all tested bacterial pathogens.

Table (30): Effect of different concentrations of some antibiotics and copper compounds on growth of pathogenic bacteria isolates of dahlia tubers *'in vitro'*.

Bactericides	Concn. (ppm)	Pathogenic bacterial isolates									
		E. carotovora isolate No.			*B. polymyxa* isolate No.			*Ps. cepacia* isolate No.			
		4	**12**	**M**	**7**	**9**	**M**	**10**	**11**	**14**	**M**
Ampicillin	**25**	15	16	15.5	20	12	16.0	10	10	0	6.6
	50	24	19	21.5	21	24	22.5	15	14	12	13.6
	100	26	20	23.0	22	28	25.0	18	20	15	17.6
	150	28	24	26.0	24	30	27.0	19	30	20	23.0
	200	30	25	27.5	33	35	34.0	21	35	24	26.6
	M	24.6	20.8	22.7	24.0	25.8	24.9	16.6	21.8	14.2	17.35
Erythromycin	**25**	13	25	19.0	0	0	0.0	10	0	17	9.0
	50	16	26	21.0	0	10	5.0	13	11	20	14.6
	100	20	28	24.0	10	15	12.5	18	13	21	17.3
	150	21	30	25.5	15	18	16.5	19	18	25	20.6
	200	25	31	28.0	16	30	23.0	23	19	28	23.3
	M	19.0	28.0	23.5	8.2	14.6	11.4	16.6	12.2	22.2	17.0
Streptomycin	**25**	17	12	14.5	29	23	26.0	10	18	20	16.0
	50	20	16	18.0	30	25	27.5	15	22	22	19.6
	100	21	17	19.0	32	28	30.0	24	25	30	26.3
	150	25	24	24.5	36	29	32.5	27	28	37	30.6
	200	26	25	25.5	38	30	34.0	37	34	44	38.3
	M	21.8	18.8	20.3	33.0	27.0	30.0	22.6	25.4	30.6	26.2
Tetracycline	**25**	13	0	6.5	16	0	8.0	13	0	17	10.0
	50	20	10	15.0	22	11	16.5	18	10	20	16.0
	100	22	12	17.0	24	15	19.5	20	13	21	18.0
	150	24	13	18.5	31	18	24.5	21	14	23	19.3
	200	25	15	20.0	33	20	26.5	23	15	25	21.0
	M	20.8	10.0	15.4	25.2	12.8	19.0	19.0	10.4	21.2	16.9
Penicillin	**25**	17	19	18.0	0	0	0.0	18	20	0	12.6
	50	27	20	23.5	25	0	12.5	25	28	18	23.6
	100	30	22	26.0	28	18	23.0	28	30	20	26.0
	150	35	25	30.0	30	25	27.5	29	35	30	31.3
	200	37	30	33.5	35	33	34.0	30	40	40	36.3
	M	29.2	23.2	26.2	23.6	15.2	19.4	26.0	30.6	21.6	26.1
Galben copper	**25**	0	0	0.0	0	0	0.0	0	0	0	0.0
	50	0	0	0.0	9	9	9.0	10	0	7	5.6
	100	0	0	0.0	10	10	10.0	11	9	9	9.6
	150	7	10	8.5	11	11	11.0	12	10	10	10.6
	200	11	13	12.0	13	13	13.0	15	13	11	13.0
	M	3.6	4.6	4.1	8.6	8.6	8.6	9.6	6.4	7.4	7.8
Copper oxychloride	**25**	0	10	5.0	0	0	0.0	0	0	0	0.0
	50	0	16	8.0	10	12	11.0	12	9	13	11.3
	100	10	18	14.0	12	15	13.5	14	10	14	12.6
	150	13	20	16.5	15	18	16.5	15	13	16	14.6
	200	14	33	23.5	17	21	19.0	20	16	17	17.6
	M	7.4	19.4	13.4	10.8	13.2	12.0	14.6	9.6	12.0	12.1

10.2. Under greenhouse conditions (*In vivo*)

Data in **Table (31)** indicated that, dipping dahlia tubers in antibiotic solution at concentration 200 ppm before sowing in pots under greenhouse conditions was effective in controlling tested pathogenic bacteria. In this respect, during the first season, erythromycin followed by ampicillin were the best effective antibiotics in controlling *E. carotovora*-4 & -12 where the survival plants were 79.2 & 75.0 and 66.7 & 70.8%, respectively, comparing to other tested antibiotics and control treatment. Also, streptomycin and tetracycline were the best for controlling *Ps. cepacia* were the survival plants 75 and 70.8% comparing with other tested antibiotics and bactericides as well as control treatment. In the second season, the same trend was recorded for erythromycin and ampicillin as the best effective materials against *E. carotovora*-4 & -12. Meanwhile, tetracycline followed by streptomycin and ampicillin in controlling *Ps. cepacia* infection on dahlia tubers where the dead plants were only 25%, 29.2% and 29.2%, respectively. Tetracycline and penicillin were not effective for controlling *E. carotovora*-4 infection during the two seasons *in vivo*. On the other hand, Galben copper and copper oxychloride, as bactericides, were the least effective in this respect.

Data in **Table (32)** indicate that mixing antibiotics with copper oxychloride improved the efficacy of antibiotics against bacterial pathogens which rotted dahlia tubers during cultivation for two seasons. In this respect, the mixed erythromycin was the best against E. carotovora-4 & -12 where the survived plants were 87.5%, followed by streptomycin and mixed tetracycline

against E. carotovora-12, meanwhile, mixed ampicillin gave 25.0% dead plants in case of E. carotovora-4. On the other hand, mixed ampicillin followed by the mixed antibiotics (tetracycline, streptomycin and penicillin) was the best against Ps. cepacia respectively. The same trend was remarkable during the second season where the mixed erythromycin was more effective in controlling E. carotovora-4 & -12 than other mixed antibiotics. While, mixed streptomycin and ampicillin were the best for controlling Ps. cepacia. It is clear from the results that treating dahlia tubers with all mixed antibiotics improved effectively the survived dahlia plants comparing with un-treated dahlia tubers.

Table (31): Effect of some antibiotics and two copper compounds on growth of dahlia rot pathogen in greenhouse.

Bactericides (200 ppm)	Bacterial isolates					
	E. carotovora-4		*E. carotovora*-12		*Ps. cepacia*	
	Dead plants (%)	Survived plants (%)	Dead plants (%)	Survived plants (%)	Dead plants (%)	Survived plants (%)
Season 2001/2002						
Erythromycin	20.8	79.2	25.0	75.0	50.0	50.0
Penicillin	62.5	37.5	62.5	37.5	62.5	37.5
Streptomycin	50.0	50.0	50.0	50.0	25.0	75.0
Tetracycline	62.5	37.5	54.2	45.8	29.2	70.8
Ampecilline	33.3	66.7	29.2	70.8	37.5	62.5
Copper oxychloride	50.0	50.0	50.0	50.0	37.5	62.5
Galben copper	62.5	37.5	62.5	37.5	54.2	45.8
Control	100.0	0.0	75.0	25.0	87.5	12.5
Season 2002/2003						
Erythromycin	25.0	75.0	25.0	75.0	54.2	45.8
Penicillin	54.2	45.8	50.0	50.0	62.5	37.5
Streptomycin	45.8	54.2	62.5	37.5	29.2	70.8
Tetracycline	66.7	33.3	50.0	50.0	25.0	75.0
Ampecilline	37.5	62.5	25.0	75.0	29.2	70.8
Copper oxychloride	54.2	45.8	50.0	50.0	50.0	50.0
Galben copper	75.0	25.0	54.2	45.8	50.0	50.0
Control	100.0	0.0	79.2	20.8	79.2	20.8

Table (32): Evaluation of mixing antibiotics with copper oxychloride on bacterial pathogens infecting dahlia tubers under greenhouse conditions.

Bactericides	Bacterial isolates					
	***E. carotovora*-4**		***E. carotovora*-12**		***Ps. cepacia***	
	Dead plants (%)	**Survived plants (%)**	**Dead plants (%)**	**Survived plants (%)**	**Dead plants (%)**	**Survived plants (%)**
Season 2001/2002						
Erythromycin + copper	12.5	87.5	12.5	87.5	37.5	62.5
Penicillin + copper	33.3	66.7	33.3	66.7	12.5	87.5
Streptomycin + copper	37.5	62.5	12.5	87.5	12.5	87.5
Tetracycline + copper	50.0	50.0	20.8	79.2	12.5	87.5
Ampicillin + copper	25.0	75.0	33.3	66.7	0.0	100.0
Control	100.0	0.0	75.0	25.0	87.5	12.5
Season 2002/2003						
Erythromycin + copper	0.0	100.0	12.5	87.5	33.3	66.7
Penicillin + copper	37.5	62.5	37.5	62.5	20.8	79.2
Streptomycin + copper	37.5	62.5	25.0	75.0	16.7	83.3
Tetracycline + copper	37.5	62.5	25.0	75.0	25.0	75.0
Ampicillin + copper	37.5	62.5	33.3	66.7	16.7	83.3
Control	100.0	0.0	79.2	20.8	75.0	25.0

DISCUSSION

Dahlia (*Dahlia pinnata* L.) is one of the most popular and attractive cut flower plants around the world. Many fungi like *Sclerotium rolfsii*, *Fusarium solani* and *Macrophomina phaseolina* and bacteria like *Erwinia carotovora* pv. *carotovora*, *E. carotovora* pv. *chrysanthemi*, *Pseudomonas cichorii* and *Bacillus polymyxa* are attacking dahlia plants in the field, storage and during propagation by stem cuttings or divided tubers (**Lelliott and Stead 1987** and **Gomaa and Mohamed 2000)**.

Sum of 107 fungal and 55 bacterial isolates were obtained from naturally infected dahlia tubers in three locations i.e. Moshtohor, El-Dair and El-Manashy in Kalubia Governorate. The isolated fungi belong to 7 genera and 7 species. Fungi were identified as: *S. rolfsii*, *Fusarium* sp., *R. solani, A. niger, A. flavus, P. digitatum* and *Rhizopus* sp. *S. rolfsii* showed the highest frequency % and isolation number in all tested samples. These results are similar to those obtained by **Abada (1994)** who isolated *Alternaria* spp.; *Mucor* spp.; *Fusarium* spp., *F. solani*; *Pythium debaryanum*; *R. solani*; *M. phaseolina* and *S. rolfsii* from rotted sugar beet roots collected in Egypt. Also, **Gomaa and Mohamed (2000)** isolated *S. rolfsii* from naturally rotted dahlia tubers. The highest isolation number and frequency % of *S. rolfsii* was recorded in El-Dair location followed by Moshtohor and El-Manashy locations respectively. *S. rolfsii* is the most pathogenic fungus in inducing rotting of dahlia tubers where the infection area increased gradually after the first day from infestation. *R. solani* and *Fusarium* sp. were pathogenic also but lesser than *S. rolfsii* in inducing rotting of dahlia tubers.

In this respect, the results of **Papavizas and Collins (1990)** support our obtained results where they found that *S. rolfsii* causes extensive damage to plant in more than 100 families in warm regions of the world.

As for physical factors affecting growth of *S. rolfsii,* the fungus was able to grow on most of the tested media. PDA was the best followed by dahlia tuber agar and Brown's media. PDA followed by dahlia tuber and peptone were the most favourable media for producing sclerotia of *S. rolfsii*. Also, it is clear that kind of media affected sclerotial weight and appearance. *S. rolfsii* could use many different carbon and nitrogen sources in nutrition. Glucose and lactose were the best carbon sources as well as, yeast extract, peptone, asparagine and gelatin were the most favourable nitrogen sources for growth of *S. rolfsii*. Meanwhile, glucose, lactose and starch were the best carbon sources for sclerotial formation. Whereas, yeast extract, peptone and asparagine as sole nitrogen sources were the best for sclerotial formation of *S. rolfsii,* respectively. Also, the kind of carbon or nitrogen sources affected sclerotial weight and appearance. *S. rolfsii* could grow within 15 - 40°C, while, no growth was recorded below 15°C. Maximum growth of the fungus was obtained at 30°C whereas, minimum growth was at 15°C. The highest number of sclerotia was formed at 30°C and at 25°C. *S. rolfsii* could grow at RH values ranged between 14-100%. The maximum growth was recorded between 80 -100% RH. Meanwhile, 90% RH gave the best linear growth. Also, *S. rolfsii* could grow at different pH values ranging from pH 3

to 8. The optimum pH was pH5. It is pronounced that acidity and alkalinity affected sclerotial production, weight and appearance of sclerotia. In addition, growth, sclerotial formation, sclerotial weight and appearance of sclerotia of *S. rolfsii* were affected greatly by the kind of light colors. In this respect, black color was the best for growth while, black, blue and yellow colors were the best for sclerotial formation. These results are in harmony with the findings of Hernandez (1986), Nwufo and Fajola (1986) and Palakshappa *et al.* (1989) **whom found that *S. rolfsii* grew at temperature range from 8-40°C and grew well at 20-30°C. The fungus grew in beef extract-peptone broth culture adjusted at pH 1.4-8.8. Also,** Yuan *et al.* (1990) **reported that *S. rolfsii* isolates grew well at 28-32°C, relative humidity 92% and pH 4-6. Growth was best at 28-32°C, RH >92%, pH 4-6, in darkness and under aerobic conditions. Also,** Dey *et al.* (1992) **mentioned that the most sclerotia of *S. rolfsii* were obtained in cultures on PDA. While,** Hari *et al.* (1991) **and** Jyoti-Mishra *et al.* (1996) **reported that growth of *S. rolfsii* was best at 30°C, pH 6 and with PDA, starch and dextrin as carbon sources and peptone and potassium nitrate as nitrogen sources. Sclerotial production was favored at 25°C and by starch, dextrin and peptone.**

Concerning factors affecting rotting incidence on dahlia tubers, there was a positive relation between wounding and *S. rolfsii* infection on dahlia tubers, where, the infection area was increased gradually by increasing the wound levels from needle wound level till 10 mm cyclic wound at all incubation days from

the 3^{rd} – $11^{th.}$. Also, the fungus was able to infect dahlia tubers without wounding and its infection increased gradually to high level after 11 days incubation. Infection with *S. rolfsii* reduced the weight of dahlia tubers and these reductions were affected by incubation period and wound level. These findings could be confirmed by the results of **Vincelli and Burne (1989)** who found that wounded, inoculated sugar beet plants with a spore suspension of *Rhizopus arrhizus* ($7X10^6$/ml) and incubated at 35°C developed decay within 48 h.

All used inoculum densities of *S. rolfsii* (1-10 sclerotia/wound) induced infection and reduced the weight of wounded dahlia tubers starting from the 5^{th} day. This infection was developed gradually by increasing incubation period to reach its maximum after 11 days using inoculum density 10 sclerotia/wound under *in vitro* conditions. Furthermore, infesting soil with different inoculum levels of *S. rolfsii* affected infection and surviving of dahlia plants. Increasing inoculum potential from 1–5 % increased gradually the dead plants where 5% inoculum potential gave the highest death percentage of dahlia plants. These results are similar to those obtained by **Hiremath (1992)** who mentioned that addition of 2% inoculum to the soil was sufficient to produce high disease levels of *S.* collar rot on sunflower.

Bacterial isolation trial from rotten dahlia tubers resulted in 55 bacterial isolates. Out of these, 7 isolates (No. 4, 7, 9, 10, 11, 12 and 14) were highly pathogenic and caused soft rot onto potato and dahlia tubers but not affected sugar beet and taro. All tested bacterial isolates were differed in their pathogenic abilities

according to the difference of the host kind. Identification and classification of these pathogenic isolates based on their morphological and physiological properties according to **Schaad** (**1980**), **Fahy** and **Persley**, (**1983**), **Lelliott** and **Stead**, (**1987**) and **Collins** and **Lyne's** (**1995**) revealed that two of them were *B. polymyxa* (7 &9), two others were classified as *E. carotovora* (4&12) and the late three isolates were identified as *Ps. cepacia* (10,11&14). In this respect, **Jump *et al.* (1983)** isolated *E. carotovora* from, cactus plants showing soft rot symptoms. While, **Piplani *et al.* (1983)** isolated *B. polymyxa* from rotten potato tuber tissues, the vascular tissues of apparently healthy tubers and healthy aerial stems. Also, they verified the pathogenic ability of *B. polymyxa* on tested tuber tissues. Also, **Bhattacharya and Mukherjee (1986)** attributed the soft rot of stored tissues to some uncommon bacteria such as *Bacillus* and *Pseudomonas* as well as *Erwinia* which associated with soft rots of vegetables, fruits and other stored tissues. On the other hand, all of **Khan *et al.* (1990), Togashi *et al.* (1995)** and **El-Hendawy *et al.* (2002)** verified also our obtained results. On the other hand, emphasizing identification using SDS-PAGE at protein level was found not enough to ensure the identification of the seven bacterial isolates which isolated from dahlia tubers where the similarity % between the previously identified isolates by the traditional techniques were low and confused. For this purpose, the identification at DNA level may be necessary. In this respect, **Ab-El-Khair *et al.* (2003)** differentiate between ten bacterial isolates of *Erwinia amylovora* isolated from Behera, Gharabiya Nobaria and Giza Governorates using SDS-PAGE technique and found that protein band of 42 KDa was a common

protein band in all isolates as well as the similarity coefficient among *E. amylovora* protein profiles ranged from 0.50 to 0.80. They concluded that the protein band of 42 KDa could be used for the identification of *E. amylovora* isolates, in addition to successful pathogenicity tests on pear immature fruitlets and hypersensitive reaction (HR). They added also that the elctrophoretic technique of cellular proteins of different Egyptian isolates showed that the *E. amylovora* profiles were not similar.

As for biochemical changes in infected dahlia tubers and control, sugar contents and phenols were affected as a result for infection with bacterial pathogens and *S. rolfsii*. In this respect, it is clear that the highest amount of total sugars in infected dahlia tubers was with *S. rolfsii*, followed by *Ps. cepacia* and *E. carotovora*-12. Meanwhile, all pathogenic bacteria did not have a great effect on reducing sugars. Furthermore, all non-reducing sugars were in low amounts in all infected dahlia tubers with bacterial pathogens and *S. rolfsii* comparing with control treatment. On the other hand, *S. rolfsii* increased total and conjugated phenols in dahlia tubers to high extent comparing with bacterial infection. Free phenols were high also in infected dahlia tubers with *S. rolfsii* comparing to control treatment and inoculated tubers with bacterial pathogens. These results could be interpret in light the findings of **Abo Ellil *et al.* (1998)** who showed that there was positive correlation between levels of phenols and root rot infection caused by *S. rolfsii*, *R. solani* and *Fusarium solani*. Where, total phenols increased with infection. Also, **Somani *et al.* (2000)** found that soft rot, dry rot and all rots

put together were positively correlated with reducing sugar and phenolic contents in tubers.

Concerning cell wall enzymes, *S. rolfsii* and tested pathogenic bacteria were not able to produce cellulase enzyme in Czapek's media containing sucrose, pectin and CMC. Meanwhile, these isolates were able to produce xylanase enzyme in Czapek's media containing sucrose, pectin and CMC. The highest activity of xylanase was in media containing CMC and inoculated with *E. carotovora*-4, followed by media containing sucrose inoculated with *E. carotovora*-12. All pathogenic bacteria were able to produce xylanase enzyme more than the fungus in media containing sucrose, pectin and CMC. Moreover, *S. rolfsii* and other tested bacterial pathogens had the ability to produce PG in Czapek's media containing sucrose, pectin and CMC, but with different quantities. *S. rolfsii* was the highest PG producer in media containing CMC and pectin as sole carbon sources compared with other tested bacterial pathogens. Meanwhile, *Ps. cepacia* produced the highest activity of PG in media containing sucrose more than *E. carotovora* (4&12) and *S. rolfsii* grown at the same conditions. On the other hand, *S. rolfsii* and other tested bacterial pathogens were not able to produce cellulase in infected tissue of dahlia tubers. Meanwhile, xylanase activity was detected in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora* (4&12,) only. The highest activity of PG enzyme was detected in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora*-4. These results are in agreement with the findings of **Scala** and **Zoina (1983)** who, mentioned that *S. rolfsii* produced large amounts of polygalacturonases in

liquid cultures with pectin or autoclaved bean hypocotyls as carbon source and in infected bean and squash tissues. Also, **Bock *et al.* (1984)** found that *E. carotovora* produced pectate lyase-containing pectolytic enzyme that macerated potatoes and vegetables tissues. In this respect also, **Kararah *et al.* (1985), Satyabrata Maiti *et al.* (1986), Ohazurike and Arinze (1992), Wegner and Jansen (1996) Abd El-Khair and Nofal (2001)** and **El-Hendawy *et al.* (2002)** verified our results where all of them confirmed the abilities of *S. rolfsii, E. carotovora* and others of pathogenic bacteria and fungi for producing cell wall degrading enzymes as well as they correlated the infection with enzymes production.

As for chemical control, all tested fungicides had the ability to inhibit or reduce the growth of *S. rolfsii* depending on tested concentrations and time of incubation. In this respect, out of 7 fungicides, Vitavax-200 and Topsin-M70 were completely inhibited the growth of *S. rolfsii* at concentrations starting from 100-750 ppm. Meanwhile, Sanlight, Tachigaren and Rizolex-T50 completely inhibited the growth of *S. rolfsii* at concentrations starting from 250-750 ppm. Also, Galben copper and Copper oxychloride inhibited the growth of the fungus at the high concentration 750 ppm. On the other hand, dipping dahlia tubers with Rizolex-T50 and Vitavax-200 before sowing gave the best control of *S. rolfsii* infection during two seasons. While, dipping dahlia tubers in Topsin-M70, Sanlight and Vitavax-200 gave good control during the first season only. As well as, treating soil with fungicides controlled effectively *S. rolfsii* infection on dahlia tubers comparing with untreated soil during two seasons. Rizolex-T50 and Vitavax-200 controlled

completely the infection of *S. rolfsii* during the first season. Also, the same fungicides were effective during the second season comparing to other fungicides. These results agreed with the findings of **Motikhaye (1983), Fahim *et al.* (1984), Abou-Zeid *et al.* (1987), Sahu *et al.* (1990) Henriquez and Montealegre (1992), Ahmed *et al.* (1994), Sabet *et al.* (2000), El-Habbaa *et al.* (2002)** and **Bhoraniya *et al.* (2003**) whom verified the ability of Rizolex-T50, Vitavax-200, Topsin-M70 and other fungicides on controlling *S. rolfsii* and other pathogenic fungi *in vitro* and *in vivo* on different plant hosts.

All tested antibiotics and bactericides inhibited the growth of bacterial pathogens depending on antibiotic kind and used concentration, because increasing the concentration of tested materials whether the antibiotics or other bactericides from 25-200 ppm increase gradually the inhibited zone of pathogenic bacteria. Streptomycin was the first effective antibiotic against growth of *Bacillus polymxa*-7 and *Pseudomonas cepacia*-14. While, penicillin was the second effective antibiotic against *Pseudomonas cepacia*-11 and *E. carotovora*-4. Meanwhile, erythromycin was also effective against *E. carotovora*-12. Copper oxychloride and Galben copper "as bactericides" were the least effective materials against growth of all tested bacterial pathogens.

Dipping dahlia tubers in antibiotic solutions at concentration 200 ppm before sowing in pots under greenhouse conditions was effective in controlling tested pathogenic bacteria. Erythromycin followed by ampicillin were the best effective antibiotics in controlling *E. carotovora*-4 & -12 comparing to other tested antibiotics and control treatment

during the first season. Also, streptomycin and tetracycline were the best for controlling *Pseudomonas* comparing with other tested antibiotics and bactericides as well as control. Therefore, mixing antibiotics with copper oxychloride improved the efficacy of antibiotics against bacterial pathogens, which rotted dahlia tubers during cultivation. The mixed erythromycin was the best against *E. carotovora*-4 & -12 followed by mixed streptomycin and mixed tetracycline against *E. carotovora*-12. On the other hand, mixed ampicillin followed by the other mixed antibiotics (tetracycline, streptomycin and penicillin) was the best against *Ps. cepacia*, respectively. These results could be interpret in light the findings of **Farag *et al.* (1986)** who mentioned that *E. carotovora* subsp. *atroseptica* was sensitive to ampicillin, and streptomycin. Also, **Banerjee**, ***et al.* (1990), Lewocz (1992),** and **Alice and Sivaprakasam (1995)** confirmed these results, while, **Ahiawat *et al.* (1997)** reported that ampicillin, neomycin, streptomycin, streptocycline and tetracycline restricted the growth of 3 *Bacillus* isolates. In addition, **Karwasra and Parashar (1998**) stated that treating potato tubers pre-sowing with streptocycline was effective for preventing soft rot caused by *E. carotovora*, improved sprouting and reduced weight loss of potato tubers. On the other hand, **Chen and Lin (2000), Singh *et al.* (2000)** and **Abd El-Khair and Nofal (2001)** had similar results which support our results *in vitro* and *in vivo*. Regarding mixing antibiotics and copper oxychloride, the results of **Abd El-Khair (1993)** support our obtained results where he reported that mixture of diluted ingredients of streptomycin and copper compounds were highly effective against *E. amylovora*. While, **Almeida *et al.* (1994)** found that copper oxychloride + mancozeb, oxytetracycline,

oxytetracycline + streptomycin and copper sulfate + oxytetracycline were effective against *E. carotovora* subsp. *carotovora in vitro*.

SUMMARY

Dahlia (*Dahlia pinnata* L.) is one of the most popular and attractive cut flower plants around the world. Many fungi like *Sclerotium rolfsii*, *Fusarium solani* and *Macrophomina phaseoli* and bacteria like *Erwinia carotovora* pv. *carotovora*, *E. carotovora* pv. *chrysanthemi*, *Pseudomonas cichorii* and *Bacillus polymyxa* are attacking dahlia plants in the field, storage and during propagation by stem or divided cuttings.

The obtained results of the present study could be summarized as follows:

1. Sum of 107 fungal and 55 bacterial isolates were obtained from naturally infected dahlia tubers in three locations i.e. Moshtohor, El-Dair and El-Manashy in Kalubia Governorate. The isolated fungi belong to 6 genera and 7 species. Fungi were identified as: *S. rolfsii*, *Fusarium* sp., *R. solani*, *A. niger*, *A. flavus*, *P. digitatum* and *Rhizopus* sp. *S. rolfsii* was more occurrence in all tested samples. The highest isolation number and frequency % of *S. rolfsii* was recorded in El-Dair location followed by Moshtohor and El-Manashy locations respectively.
2. *S. rolfsii* was the most pathogenic fungus in inducing rotting of dahlia tubers followed by *R. solani* and *Fusarium* sp.
3. As for physical factors affecting growth of *S. rolfsii*, The fungus was able to grow on most of the tested media. PDA was the best followed by dahlia tuber agar and Brown's media, while the lowest average of linear growth occurred on Baren's and Czapek's media. Regarding sclerotial formation, PDA followed by dahlia tuber and peptone were the most

favourable media for producing sclerotia of *S. rolfsii*. Moreover, the highest weight of five sclerotia (g) was obtained on dahlia tuber medium. The first sclerotium formed after 3 days on Brown's and Baren's media, while it was formed after 9 days in case of plain agar medium.

4. The results indicated that *S. rolfsii* could grow within 15-40°C, while, no growth was recorded below 15°C. Maximum growth of the fungus was at 30°C whereas, minimum growth was at 15°C. Meanwhile, the highest number of sclerotia was formed at 30°C and at 25°C. Maximum weight of 5 sclerotia was obtained at 35°C, 30 °C and 25°C.
5. *S. rolfsii* could grow at RH values ranged between 14-100%, however, the average linear growth has gradually increased by increasing RH values from 14 to 100% after four days incubation. The maximum growth was recorded between 80 - 100% RH. Meanwhile, 90% RH gave the best linear growth.
6. The results indicated also that *S. rolfsii* could grow at different pH values ranging from pH 3 to 8. The best pH values for producing the highest number of sclerotia were pH 5. The highest weight of 5 sclerotia was occurred when the fungus grown at pH 5 and 4.
7. The results cleared that *S. rolfsii* isolate could use many different carbon sources in nutrition such as glycerol, sucrose, glucose, starch, maltose, lactose, dextrin and arabinose. Glucose and lactose were the best carbon sources for growth of the fungus. Starch, arabinose, maltose and sucrose were moderately affected growth of *S. rolfsii*. Also, glucose, lactose and starch were the best carbon sources for

sclerotial formation. Therefore, the highest weight of 5 sclerotia was in case of arabinose followed by glucose and sucrose respectively. Also, the first sclerotium formed on media containing dextrine after 2 days, while in presence of maltose or arabinose the first sclerotium appeared after 9 days post inoculation.

8. As for nitrogen sources, yeast extract, peptone, asparagine and gelatin were the most favourable for growth of *S. rolfsii*. Meanwhile, media containing casein, urea, sodium nitrate were not favourable for growth of the fungus. In addition, media containing yeast extract, peptone and asparagine as sole nitrogen sources were the best for sclerotial formation of *S. rolfsii,* respectively. While, the lowest sclerotial numbers were produced on media containing ammonium nitrate, urea, sodium nitrate. In addition, the highest weight of five sclerotia was in case of yeast extract, peptone, asparagine, beef extract and gelatin as sole nitrogen source, respectively.
9. The results revealed clear differences between the different colors in their effect on growth of *S. rolfsii*. The highest linear growth was obtained on black color. Also, black, blue and yellow colors were the best for sclerotial formation. The highest weight of five sclerotia was obtained in case of red, green and black colors. Also, the first sclerotium formed with using black color after 5 days, followed by white color after 6 days.
10. Concerning factors affecting rotting incidence on dahlia tubers, there was a positive correlation between wounding and appearance of infection with *S. rolfsii* on dahlia tubers.

The infected area was increased gradually by increasing the wound levels from needle wound level till 10 mm cyclic wound at all incubation days from the 3^{rd}–$11^{th.}$. The highest infection area was recorded onto dahlia tubers wounded at levels 10 and 5 mm cyclic wounds after 11 days incubation. The fungus was able to infect dahlia tubers without wounding and its infection increased gradually to high level after 11 days incubation. Infection with *S. rolfsii* reduced the weight of dahlia tubers and these reductions were affected by incubation period and wound level. The highest reduction in infected dahlia weight was at 10 mm cyclic wound after 11 days incubation.

11. All used inoculum density of *S. rolfsii* (1-10 sclerotia/wound) had the ability to induce infection on wounded dahlia starting from the 5^{th} day. This infection was developed gradually by increasing incubation period. The highest infected area was recorded after 11 days using inoculum density 10 sclerotia/wound. Also, this infection reduced the weight of infected dahlia gradually in correlation to the used inoculum density.
12. Bacterial isolation trial from rotted dahlia tubers resulted in 55 bacterial isolates. Out of these, 7 isolates (No. 4, 7, 9, 10, 11, 12 and 14) were highly pathogenic and caused soft rot onto potato and dahlia tubers, while these isolates can not affected sugar beet and taro. All tested bacterial isolates were differed in their pathogenic abilities according to the difference of the host kind.

13. Identification and classification of these pathogenic isolates based on their morphological and physiological properties revealed that two of them were *Bacillus polymyxa* (7 &9), two others were classified as *Erwinia carotovora* (4&12) and the last three isolates were identified as *Pseudomonas cepacia* (10,11&14).
14. Emphasizing identification using SDS-PAGE at protein level was found not enough to ensure the identification of the seven bacterial isolates which isolated from dahlia tubers where the similarity % between them were low and confused.
15. As for biochemical changes in infected dahlia tubers and control, sugar contents and phenols were affected as a result for infection with bacterial pathogens and *S. rolfsii*. In this respect, it is clear that the highest amount of total sugars in infected dahlia tubers was with *S. rolfsii*, followed by *Ps. cepacia* and *E. carotovora*-12. Meanwhile, all pathogenic bacteria did not have a great effect on reducing sugars. Furthermore, all non-reducing sugars were in low amounts in all infected dahlia tubers with bacterial pathogens and *S. rolfsii* comparing with control treatment. On the other hand, *S. rolfsii* increased total and conjugated phenols in dahlia tubers to high extent comparing with bacterial infection. Free phenols were high also in infected dahlia tubers with *S. rolfsii* comparing to control treatment and inoculated tubers with bacterial pathogens.
16. Concerning cell wall degrading enzymes, *S. rolfsii* and tested pathogenic bacteria were not able to produce cellulase

enzyme in Czapek's media containing sucrose, pectin and CMC. Meanwhile, these isolates were able to produce xylanase enzyme in Czapek's media containing sucrose, pectin and CMC. The highest activity of xylanase was in media containing CMC and inoculated with *E. carotovora*-4, followed by media containing sucrose inoculated with *E. carotovora*-12. All pathogenic bacteria were able to produce xylanase enzyme more than the fungus in media containing sucrose, pectin and CMC.

17. Moreover, *S. rolfsii* and other tested bacterial pathogens had the ability to produce PG in Czapek's media containing sucrose, pectin and CMC, but with different quantities. *Sclerotium rolfsii* was the highest PG producer in media containing CMC and pectin as sole carbon sources compared with other tested bacterial pathogens. Meanwhile, *Ps. cepacia* produced the highest activity of PG in media containing sucrose more than *E. carotovora* (4&12) and *S. rolfsii* grown at the same conditions.
18. On the other hand, *S. rolfsii* and other tested bacterial pathogens were not able to produce cellulase in infected tissue of dahlia tubers. Meanwhile, xylanase activity was detected in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora* (4&12,) only. The highest activity of PG enzyme was detected in infected tissues of dahlia tubers with *S. rolfsii* and *E. carotovora*-4.
19. As for chemical control, all tested fungicides inhibited or reduced the growth of *S. rolfsii* depending on tested concentrations and time of incubation. Out of 7 fungicides,

Vitavax-200 and Topsin-M70 were completely inhibited the growth of *S. rolfsii* at concentrations starting from 100-750 ppm. Meanwhile, Sanlight, Tachigaren and Rizolex-T50 completely inhibited the growth of *S. rolfsii* at concentrations starting from 250-750 ppm. Also, Galben copper and Copper oxychloride inhibited the growth of the fungus at the high concentration 750 ppm.

20. On the other hand, dipping dahlia tubers in Rizolex-T and Vitavax-200 before sowing gave the best control of *S. rolfsii* infection during two seasons. While, dipping dahlia tubers in Topsin-M70, Sanlight and Vitavax-200 gave good control during the first season only.
21. Moreover, treating soil with fungicides controlled effectively *S. rolfsii* infection on dahlia tubers comparing with untreated soil during two seasons. Rizolex-T50 and Vitavax-200 controlled completely the infection of *S. rolfsii* during the first season. Also, the same fungicides were effective during the second season comparing to other fungicides.
22. All tested antibiotics and bactericides inhibited the growth of bacterial pathogens depending on antibiotic kind and used concentration, because, increasing the concentration of tested materials whether the antibiotics or other bactericides from 25-200 ppm increased gradually the inhibited zone of pathogenic bacteria. Streptomycin was the first effective antibiotic against growth of *B. polymxa*-7 and *Ps. cepacia*-14. While, penicillin was the second effective antibiotic against *Ps. cepacia*-11 and *E. carotovora*-4. Meanwhile, erythromycin was also effective against *E. carotovora*-12.

Copper oxychloride and Galben copper “as bactericides” were the least effective materials against growth of all tested bacterial pathogens.

23. Dipping dahlia tubers in antibiotic solutions at concentration 200 ppm before sowing in pots under greenhouse conditions was effective in controlling tested pathogenic bacteria. Erythromycin followed by ampicillin were the best effective antibiotics in controlling *E. carotovora*-4 & -12 comparing to other tested antibiotics and control treatment during the first season. Also, streptomycin and tetracycline were the best for controlling *Pseudomonas* comparing with other tested antibiotics and bactericides as well as control.

24. On the other hand, mixing antibiotics with copper oxychloride improved the efficacy of antibiotics against bacterial pathogens, resulting in increasing survived plants. The mixed erythromycin was the best against *E. carotovora*-4 & -12 followed by mixed streptomycin and mixed tetracycline against *E. carotovora*-12. Therefore, mixed ampicillin followed by the other mixed antibiotics (tetracycline, streptomycin and penicillin) was the best against *Ps. cepacia* respectively.

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الملخص العربي

دراسات مرضية علي أعفان درنات نباتات الداليا

تعتبر الداليا أحد أهم زهور القطف شعبية وجاذبية في العالم حيث يعتبرها العديد من المتخصصين والمستهلكين ملكة الزهور الصيفية. ويمكن تصدير زهور الداليا لما تتمتع به من طول عمر فى الزهرية، كما يمكن إستخدامها كنباتات أصص أو في تجميل الحدائق العامة فى شكل مجموعة فردية لنوع واحد. وهناك العديد من الأمراض الفطرية والبكتيرية التي تهاجم نباتات الداليا في الحقل وأثناء التخزين وكذلك أثناء تجهيز الدرنات للزراعة (تفصيص الدرنات). ومن أكثر الفطريات الممرضة للداليا فطريات سكليروشيوم رولفسياي وفيوزاريوم وماكروفومينا فاسيولينا. ويعد مرض الأسكلريوشيوم المتسبب عن الفطر سكليروشيوم رولفسياي واحد من أهم الأمراض المهلكة لنباتات الداليا مسببا نقصا في محصول الأزهار والدرنات الناتجة. كما يعتبر العفن الطري البكتيري الناتج عن بكتريا إرونيا كاروتوفورا (طراز مرضى كاروتوفورا) و إرونيا كاروتوفورا (طراز مرضى كريزانثيمى) وزيدوموناس سيكورياى وباسيلس بوليمكسا هى أكثر الأمراض البكتيرية شيوعا حيث تهاجم وتتلف تحت ظروف التربة والمخزن. وقد تناولت هذه الدراسة عزل وتعريف الفطريات والبكتريا التي تهاجم درنات الداليا تحت الظروف المصرية، دراسة أفضل الظروف البيئية التي تناسب نمو تلك الكائنات الممرضة وتساعدها علي إحداث الإصابة علي نباتات الداليا.دراسة بعض التغيرات الكيماوية فى الفينولات والسكريات وربطها بالإصابة ، كذلك دراسة مقدرة هذه الكائنات الممرضة (فطرية وبكتيرية) علي إفراز الانزيمات المحللة لجدر الخلايا وذلك لفهم ميكانيكية فعل هذه الكائنات الممرضة علي جذور نباتات الداليا وأخيرا تقييم بعض المبيدات الفطرية والمضادات الحيوية وتأثيرها علي مقاومة هذا المرض.

والنتائج المتحصل عليها يمكن تلخيصها كالتالي:

1- أمكن الحصول على 107 عزلة فطرية و 55 عزلة بكتيرية من درنات الداليا المصابة طبيعيا من ثلاث مناطق هى مشتهر والدير والمناشى فى محافظة القليوبية، وقد وجد أن الفطريات المعزولة تتبع سبع أنواع وهى: سكليروشيوم رولفسياى و فيوزاريوم و رايزوكتونيا سولانى وأسبرجلس نيجر و أسبرجلس فلافس و بنسيليوم ديجيتاتم و ريزوبس. وقد كان فطر سكليروشيوم رولفسياى هو الأعلى تكرارا عددا ونسبة فى كل العينات المختبرة. كما كان أعلى نسبة تكرار فى منطقة الدير متبوعة بمنطقتى مشتهر والمناشى على التوالى.

2- كان فطر سكليروشيوم رولفسياى هو الأكثر مرضية فى إحداث عفن درنات الداليا حيث إزدادت منطقة العدوى تدريجيا بعد اليوم الأول من

تلويثها بالفطر وكان فطرى رايزوكتونيا سولانى و فيوزاريوم ممرضين أيضا واكن بدرجة أقل من الفطر الأول، وقد تمت الدراسة على فطر سكليروشيوم رولفسياى الأكثر قدرة مرضية على درنات الداليا.

3- وفيما يتعلق بالعوامل الطبيعية المؤثرة على نمو فطر سكليروشيوم رولفسياى فقد اتضح أن الفطر كان قادرا على النمو على معظم البيئات المختبرة. وكانت بيئة آجار البطاطس دكستروز هى الأفضل يليها بيئة آجار درنات الداليا وبيئة براون بينما كانت أقل درجة نمو على بيئة بيرنس وكزابكس. وفيما يتعلق بتكوين الأجسام الحجرية فإن بيئة آجار البطاطس دكستروز متبوعة ببيئتى درنات الداليا والببتون كانت هى الأكثر ملائمة لإنتاج الأجسام الحجرية لفطر سكليروشيوم رولفسياى، فضلا عن ذلك كان أعلى وزن لخمس أجسام حجرية على بيئة براون وقد تكون أول جسم حجرى بعد ثلاثة أيام على بيئتى براون وبيرنس فى الوقت الذى تكون أول جسم حجرى على بيئة الآجار المائى بعد 9 أيام وقد تكون أيضا أول جسم حجرى على بيئات البطاطس دكستروز آجار ودرنات الداليا والببتون بعد 7 أيام وبأعداد كبيرة.

4- تشير النتائج إلى أن فطر سكليروشيوم رولفسياى يمكنه النمو على درجات حرارة تتراوح بين 15-40°م ولم يسجل أى نمو على درجة حرارة أقل من 15°م وأعلى من 40°م وقد سجل أعلى نمو للفطر على درجة حرارة 30°م فى حين سجلت أقل درجة نمو على 15°م. فى نفس الوقت تكون أكبر عدد من الأجسام الحجرية على 30 ، 25°م على التوالى وكان أعلى وزن لخمس أجسام حجرية على 35 ، 30 ، 25°م.

5- إستطاع الفطر سكليروشيوم رولفسياى النمو على درجات رطوبة نسبية تراوحت بين 14-100% ومع ذلك إزداد معدل نمو الفطر تدريجيا بزيادة درجة الرطوبة من 14 إلى 100% بعد أربعة أيام من التحضين. وقد سجل أعلى نمو على درجات رطوبة تراوحت بين 80-100%. فى الوقت نفسه سجل أفضل نمو على درجة رطوبة نسبية 90% والجدير بالذكر أن كل درجات الرطوبة النسبية المختبرة لم تكن مناسبة لتكوين الأجسام الحجرية.

6- أشارت النتائج إلى أن فطر سكليروشيوم رولفسياى أمكنه النمو على درجات مختلفة من الحموضة تتراوح ما بين 4-8، وكانت أفضل درجة حموضة لإنتاج أكبر عدد من الأجسام الحجرية وأعلى وزن لخمس أجسام حجرية هى درجة حموضة 5.

7- أوضحت النتائج أن عزلة فطر سكليروشيوم رولفسياى يمكنها إستخدام مصادر مختلفة من الكربون فى التغذية وهى الجلسرول والسكروز والجلوكوز والنشا والمالتوز واللاكتوز والدكسترين والأرابينوز. وكان

الجلوكوز واللاكتوز هما أفضل المصادر الكربونية لنمو الفطر كما كان النشا والأرابينوز والمالتوز والسكروز متوسطى التأثير على نمو الفطر. وكان الجلوكوز واللاكتوز والنشا هم أفضل المصادر الكربونية لتكوين الأجسام الحجرية. علاوة على ذلك كان أعلى وزن لخمس أجسام حجرية فى حالة سكر الأرابينوز متبوعا بالجلوكوز والسكروز على التوالى. كما تكون أيضا أول جسم حجرى على البيئة المحتوية على الدكسترين بعد يومين بينما ظهر أول جسم حجرى بعد 9 أيام فى حالة وجود المالتوز أو الأرابينوز.

8- بالنسبة لمصادر النيتروجين كان مستخلص الخميرة والببتون والأسباراجين والجيلاتين هم الأكثر ملائمة لنمو فطر سكليروشيوم رولفسياى. فى نفس الوقت كانت البيئات المحتوية على الكازين واليوريا ونترات الصوديوم غير ملائمة لنمو الفطر. و كانت البيئات المحتوية على مستخلص الخميرة والببتون والأسباراجين كمصادر فردية للنيتروجين هى الأفضل لتكوين الأجسام الحجرية للفطر على التوالى. بينما أنتجت البيئات المحتوية على نترات الأمونيوم واليوريا ونترات الصوديوم أقل الأعداد من الأجسام الحجرية المتكونة. بالإضافة إلى ذلك كان أعلى وزن لخمس أجسام حجرية فى حالة إستخدام مستخلص الخميرة والببتون والأسباراجين ومستخلص اللحم والجيلاتين كمصادر فردية للنيتروجين على التوالى.

9- أظهرت النتائج أيضا إختلافات واضحة فى تأثير الألوان المختلفة على نمو فطر سكليروشيوم رولفسياى وقد تحصل على أعلى درجة نمو مع اللون الأسود وكانت أيضا ألوان الأسود والأزرق والأصفر هى الأفضل لتكوين الأجسام الحجرية، وكان أعلى وزن لخمس أجسام حجرية فى حالة ألوان الأحمر والأخضر والأسود كما تكون أول جسم حجرى مع اللون الأسود بعد 5 أيام متبوعا باللون الأبيض بعد 6 أيام.

10- فيما يتعلق بالعوامل المؤثرة على حدوث العفن على درنات الداليا، كانت هناك علاقة موجبة بين الجروح و ظهور العدوى بفطر سكليروشيوم رولفسياى على درنات الداليا. وقد تزايدت منطقة الإصابة تدريجيا بزيادة مستويات الجروح من مستوى جرح الإبرة إلى مستوى جرح دائرى قطره 10 مم عند كل أيام التحضين من اليوم الثالث حتى اليوم الحادى عشر. وقد سجلت أكبر منطقة إصابة على درنات الداليا المجروحة عند مستويات 5، 10 مم لجروح دائرية بعد 11 يوم تحضين. وقد كان الفطر أيضا قادرا على عدوى درنات الداليا بدون جروح كما تزايدت العدوى تدريجيا إلى أعلى مستوى لها بعد 11 يوم تحضين. وقد خفضت العدوى بفطر سكليروشيوم رولفسياى من وزن درنات الداليا كما تأثر هذا الفقد فى الوزن بفترة التحضين ومستوى الجرح وكان أكبر إنخفاض فى وزن درنات الداليا المصابة عند مستوى جرحى 10 مم بعد 11 يوم من التحضين.

11- كانت لكل مستويات الطاقة اللقاحية لفطر سكليروشيوم رولفسياى (1-10 جسم حجرى/ جرح) القدرة على إحداث العدوى على درنات الداليا المجروحة بداية من اليوم الخامس وقد تطورت هذه العدوى تدريجيا بزيادة فترة الحضانة.كما كانت أكبر منطقة مصابة بعد 11 يوم فى حالة إستخدام المستوى اللقاحى 10 جسم حجرى/ جرح كما خفضت هذه الإصابة من وزن درنات الداليا المعداة تدريجيا ومرتبطة بمستوى اللقاح المستخدم.

12- أمكن عزل 55 عزلة بكتيرية من درنات الداليا المتعفنة كان من بينها 7 عزلات فقط أخذت أرقام 4، 7، 9، 10، 11، 12، 14 هى الأعلى قدرة مرضية و سببت عفن طرى على درنات البطاطس والداليا ولم تؤثر هذه العزلات على بنجر السكر والقلقاس. وقد اتضح أيضا أن كل العزلات البكتيرية المختبرة قد إختلفت فى قدراتها المرضية تبعا لإختلاف نوع العائل النباتى.

13- أظهر تعريف وتصنيف هذه العزلات البكتيرية الممرضة بناء على خصائصها الفسيولوجية والمورفولوجية أن عزلتين منهم (7، 9) هما باسيلس بوليميكسا، وعزلتين آخرتين (4، 12) صنفا على أنهما إروينيا كاروتوفورا والثلاث عزلات المتبقية (10، 11، 14) عرفوا على أنهم زيدوموناس سباسيا.

14- لم يكن استخدام تكنيك الـ SDS-PAGE على مستوى البروتين كافيا لتأكيد التعريف السابق للعزلات السبعة التى ثبت أنها ممرضة لدرنات الداليا حيث كانت درجة التشابه منخفضة بين العزلات بهذه الطريقة.

15- بالنسبة للتغيرات البيوكيماوية فى درنات الداليا المصابة والسليمة، إتضح أن محتوى السكريات والفينولات تأثر نتيجة الإصابة بالمسببات البكتيرية وفطر سكليروشيوم رولفسياى. فقد إتضح أن أعلى كمية للسكريات الكلية كان فى درنات الداليا المصابة بفطر سكليروشيوم رولفسياى متبوعة ببكتيريا زيدوموناس و إروينيا كاروتوفورا-12. فى الوقت نفسه لم يكن لكل البكتيريا الممرضة تأثيرا كبيرا على السكريات المختزلة، وعلاوة على ذلك كل السكريات الغير مختزلة كانت بكميات منخفضة فى درنات الداليا المصابة بالمسببات البكتيرية وفطر سكليروشيوم رولفسياى مقارنة بمعاملة الكنترول. على الجانب الآخر رفعت الإصابة بفطر سكليروشيوم رولفسياى مستويات الفينولات الكلية والمرتبطة فى درنات الداليا إلى أعلى مستوى مقارنة بالعدوى البكتيرية وكان مستوى الفينولات الحرة عاليا أيضا فى درنات الداليا المعدية بفطر سكليروشيوم رولفسياى مقارنة بمعاملة الكنترول ودرنات الداليا المعدية بالمسببات البكتيرية

16- فيما يتعلق بالإنزيمات المحللة للجدر الخلوية، لم يكن لفطر سكليروشيوم رولفسياى والبكتيريا الممرضة المختبرة أى قدرة على إنتاج إنزيم سليوليز

فى بيئة كزابكس المحتوية على السكروز والبكتين والكربوكسى ميثايل سليولوز. فى الوقت نفسه كانت تلك العزلات قادرة على إنتاج إنزيم زيلينيز فى بيئة كزابكس المحتوية على السكروز والبكتين والكربوكسى ميثايل سليولوز والملقحة ببكتيريا إروينيا كاروتوفورا-4 متبوعة بالبيئة المحتوية على السكروز والملقحة ببكتيريا إروينيا كاروتوفورا-12. وكانت كل عزلات البكتيريا قادرة على إفراز إنزيم الزيلينيز بدرجة أكبر من الفطر فى البيئة المحتوية على السكروز أو البكتين أو الكربوكسى ميثايل سليولوز.

17- فضلا عن ذلك، كان لفطر سكليروشيوم رولفسياى والمسببات البكتيرية المختبرة القدرة على إنتاج إنزيم البولى جالاكتورونيز فى بيئة كزابكس المحتوية على السكروز والبكتين والكربوكسى ميثايل سليولوز ولكن بكميات متباينة. وكان فطر سكليروشيوم رولفسياى هو الأعلى إنتاجا للبولى جالاكتورونيز فى البيئة المحتوية على الكربوكسى ميثايل سليولوز والبكتين كمصادر كربونية فردية مقارنة بالمسببات الممرضة البكتيرية الأخرى المختبرة. فى الوقت نفسه كانت عزلة زيدوموناس هى الأعلى إنتاجا لإنزيم البولى جالاكتورونيز فى البيئة المحتوية على السكروز.

18- على الجانب الآخر لم يكن لفطر سكليروشيوم رولفسياى والمسببات الممرضة المختبرة القدرة على إنتاج إنزيم سليوليز فى أنسجة درنات الداليا المعدية. فى الوقت نفسه إكتشف نشاط إنزيم زيلينيز فى أنسجة الدرنات المصابة بفطر سكليروشيوم رولفسياى وإروينا كاروتوفورا (4، 12) فقط. كما إكتشف أعلى نشاط لإنزيم بولى جالاكتورونيز فى أنسجة درنات الداليا المصابة بفطر سكليروشيوم رولفسياى و إروينيا كاروتوفورا-4.

19- بالنسبة للمقاومة الكيماوية، ثبطت كل المبيدات الفطرية المختبرة نمو فطر سكليروشيوم رولفسياى معتمدة على التركيزات المختبرة وزمن التحضين. ومن بين 7 مبيدات فطرية مختبرة ثبط مبيدى فيتافاكس-200 و توبسين م بدرجة كاملة نمو فطر سكليروشيوم رولفسياى عند تركيزات بدأت من 100-750 جزء فى المليون. فى الوقت نفسه ثبطت مبيدات سان لايت، تشجارين، ريزولكس ت بدرجة كاملة نمو الفطر سكليروشيوم رولفسياى بتركيزات بدأت من 250 إلى 750 جزء فى المليون، كما ثبطت أيضا مبيدات جالبين نحاس وأكسى كلورو النحاس نمو الفطر عند التركيز العالى فقط (750 جزء فى المليون).

20- على الجانب الآخر أعطى نقع درنات الداليا فى مبيد ريزولكس ت وفيتافاكس-200 قبل لزراعة مقاومة ممتازة ضد الإصابة بفطر سكليروشيوم رولفسياى خلال موسمين من الزراعة. كما أعطى نقع درنات الداليا فى مبيد توبسين م، سان لايت، وفيتافاكس 200 نتيجة جيدة خلال الموسم الأول فقط.

21- قاومت معاملة التربة بالمبيدات الفطرية بكفاءة إصابة درنات الداليا بفطر سكليروشيوم رولفسياى مقارنة بالتربة الغير معاملة خلال موسمى الزراعة. فى هذا الشأن إستطاعت مبيدات ريزولكس ت و فيتافاكس-200 أن تقاوم بدرجة تامة العدوى بفطر سكليروشيوم رولفسياى خلال الموسم الأول وأيضا نفس المبيدات كانت مقاومة خلال الموسم الثانى ولكن بدرجة أقل من الموسم الأول عند مقارنتها بالمبيدات الأخرى.

22- ثبطت كل المضادات الحيوية المختبرة نمو المسببات البكتيرية معتمدة على نوع المضاد الحيوى والتركيز المستخدم حيث أدى زيادة التركيز فى المضاد الحيوى أو المبيد البكتيرى من 25-200 جزء فى المليون إلى زيادة تدريجية فى المنطقة المثبطة للبكتيريا الممرضة.

23- كان نقع درنات الداليا فى محاليل المضاد الحيوى عند تركيزات 200 جزء فى المليون قبل الزراعة فى الأصص تحت ظروف الصوبة فعالا فى مقاومة البكتيريا الممرضة المختبرة. وكان الإريثروميسين متبوعا بالأمبيسيللن هما أكثر المبيدات الحيوية كفاءة فى مقاومة إروينيا كاروتوفورا (4، 12) مقارنة بالمضادات الحيوية الأخرى المختبرة خلال الموسم الأول، وكان أيضا الإستربتوميسين والتتراسيكلين هما الأفضل لمقاومة زيدوموناس مقارنة بالمضادات الحيوية الأخرى والمبيدات البكتيرية والكنترول.

24- على الجانب الآخر فقد رفع خلط المضادات الحيوية مع مبيد أكسى كلورو النحاس من كفاءة المضادات الحيوية ضد المسببات البكتيرية الممرضة والتى تصيب درنات الداليا بالعفن أثناء الزراعة. وكان الإريثروميسين المخلوط هو الأفضل ضد بكتيريا إروينيا كاروتوفورا (4، 12) متبوعا بالإستربتوميسين المخلوط والتتراسيكلين المخلوط ضد إروينيا كاروتوفورا-12. علاوة على ذلك فقد كان مخلوط الأمبيسيللين متبوعا بمخاليط المضادات الحيوية الأخرى (تتراسيكلين، إستربتوميسين، بنسيللين) هم الأفضل ضد بكتيريا زيدوموناس.