Mansoura University

Faculty of Science at Damietta

Botany Department

Studies on the Biological Control of Brown Rot Disease of Potato

A Thesis submitted for the degree of Doctor of Philosophy

In Botany (Microbiology)

By

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Declaration

Thesis submitted for fulfillment of requirements for the degree of Doctor of Philosophy in Botany (Microbiology). This work was carried out during the period from 2003 to 2005.

I declare that this thesis has been composed by me and that work of which has been done by me. It has not been submitted for a degree of this or any or University.

Signature

Zeiad Moussa Abd El-Moati Ahmed

Dedication to

My Parents

My Wife

and

My Two Sons

"Tarek and Maged"

First and foremost, all praise to **Allah** (**Almighty**), the Beneficent, without whose mercy and guidance this work would never, had been neither started nor completed. I praise Him (**Almighty**) as much as the heavens and earth and what are in between or behind.

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INTRODUCTION AND AIM OF THE WORK

Potato (*Solanum tuberosum L.*) is one of the most important vegetable crops in all over the world. In Egypt, potato is important crops not only for local consumption but also for exportation.

Brown rot disease of potato is worldwide disease that causes damage of potato plantation. It leads to economic loss of potato production, where in some cases the yield loss may reach up to 50 – 75 %. This disease causes rejection of potato exportation in the quarantine.

The causal agent of this disease is a gram-negative rod shaped bacterium called *Ralstonia solanacearum*. This pathogen has a wide host range on other economic plants (such as tomato and tobacco). *R. solanacearum* survives in soil for long period and may also exist in water of irrigation. Furthermore, it can infect many weeds acting as reservoirs of this pathogen.

Chemical control of plant diseases causes frightful pollution leading to destruction of the environmental balance. Moreover, the chemical control leads to emergence of resistant strains of pathogens and damage of natural beneficial enemies of pathogens. In addition, numerous chemical pesticides are carcinogenic and/ or causative agent of many diseases of human being (*e.g.* hepatic failure, liver fibrosis …etc). Therefore, the chemical pesticides represent a problem for human being and biologists must offer another way for control pests.

Integrated pest management (IPM) and non-chemical control of pests is the suitable alternative way to control the plant pests. IPM includes; biological control, crop rotation, irrigation, amendments … etc.

The aim of the work:

The present study is an endeavor to find out a successful and safe biological control measure for the brown rot of potato.

The plane of this investigation includes the following:

1- Isolation of the causative agent of brown rot disease of potato (*R. solanacearum*) from infected tubers.
2- Isolation and identification of phages active against the virulent strain of *R. solanacearum*.
3- Isolation of actinomycetes from the root zone of potato plants and identification of the isolates that inhibit the growth of *R. solanacearum*.
4- Testing the ability of some essential oils to inhibit the growth of *R. solanacearum.*
5- Studying the ability of some mushroom wastes and some plant wastes to inhibit the growth of *R. solanacearum.*
6- Testing the antagonistic activity of some isolates of basidomycetes against *R. solanacearum*.
7- Application of the most active biocontrol agents under greenhouse conditions singly and in combinations.
8- Studying the effect of cocktail of phages and the most active actinomycete as biocontrol agents under field conditions.

II. REVIEW OF LITERATURE

II.1. Biological control:

The first definition of biological control was “the suppression of insect populations by the actions of their native or introduced enemies”. The scope of this definition was narrow; therefore, many scientists developed it. In the earlier definition of biological control, **Baker and Cook (1974)** described it as “the reduction of inoculums potential or disease producing activities of a pathogen or a parasitism in its active or dormant state, by one or more organisms accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists”.

Van Driesche and Bellows (1996) proposed a wider definition, “the use of parasitoid, predator, pathogen, antagonism or competitor populations to suppress the pest population makes it less abundant and thus less damaging than it would otherwise be”. **Shurtleff and Averre (1997)** define biological control as "disease or pest control through counter balance of microorganisms and other natural component of environment.", while, **Agrios (1997)** referred to biological control as "the total or partial destruction of pathogen populations by other organisms".

Gnanamanicham (2002) mentioned that the U.S. National Academy of Sciences defined biological control as "the use of natural or modified organisms, genes or gene products to reduce the effects of undesirable organisms to favour desirable organisms such as crops, beneficial insects and microorganisms".

II.2. Brown rot and wilt disease and causal agent:

II.2.1. Brown rot and wilt disease of potatoes is worldwide problem:

The brown rot of potato may have originated in the temperate highland regions in Peru and Bolivia **(Van der Wolf and Perombelon, 1997 and Lemay *et al.*, 2003)**. Now, it is worldwide disease present in EPPO regions, Africa, South America, Central America and Caribbean and Australia **(CABI/EPPO, 1999 and Lemay *et al.*, 2003)**

This disease is economic serious disease, where it causes loss of potato production **(Elphinstone, 1989, Toth *et al.*, 1997)**. **Gunawan (1987)** found that the loss of the potato production depend on the potato variety. **Elphinstone (1989)** found linear relationship between the yield loss and disease intensity as well as plant wilt and tuber rot intensities. **Toth *et al.* (1997)** stated that this disease caused yield loss up to 50 % in Burundi and up to 75% in Florida.

II.2.2. Symptoms of the disease:

Above ground, symptoms are wilting, stunting and yellowing of the foliage (so, it is also termed as bacterial wilt disease). A cross section through a young diseased potato stem shows brown discoloration of the vascular system. Upon slight pressure, a milky slime may exude. In a longitudinal section, the vascular system may show dark, narrow stripes beneath the epidermis **(Martin and French, 1997, Stevenson *et al.* 2001)**.

Below ground, External symptoms are not always visible on infected tubers. In case of severe infection, bacterial ooze collects at tuber eye or stolon end, causing soil to adhere. A cut tuber often shows brownish discoloration of the vascular ring. Slight squeezing forces the typical pus-like slime out of the ring, or it oozes out naturally. The vascular ring, or the whole tuber, may disintegrate completely at more

advanced stages of disease development **(Martin and French, 1997; Stevenson *et. al.* 2001)**.

Tubers may also harbour latent infection **(Hayward, 1991 and Toth *et al*., 1997)**. Latent infection can be detected by incubation of the tubers for 3 – 4 weeks at 30 ˚C **(Priou and Aley, 1999)**.

Symptoms of brown rot disease may confuse with those of ring rot disease. A major difference is that brown rot causes direct collapse of green plants, whereas, ring rot wilting is usually associated with chlorosis, yellowing, and necrosis of foliage. In the laboratory, the pathogen of brown rot is gram-negative bacteria, whereas, the pathogen of the other disease is gram-positive bacteria **(Martin and French, 1997)**.

II.2.3. Infection:

Infection commonly occur in infested soil where bacteria enter the root system of the plant via wound sites (e.g. caused by nematode activity or soil particle abrasion), natural openings from which secondary roots emerge, or via infected mother tuber **(Hayward, 1991** and **Shinohara *et al.*, 2004)**. The pathogen enters the vascular system, under favorable conditions, cell numbers increase, and spread up stem and to daughter tubers (**Hayward, 1991 and Martin and French, 1997)**.

II.2.4. The pathogen:

Smith (1896 and 1914) isolated the pathogen in pure culture and tested its ability to induce the disease. He identified this bacterium as *Bacillus solanacearum* in 1896, then as *Pseudomonas solanacearum* in 1914. Recently, this pathogen is identified as *Burkholderia solanacearum* (**Yabuuchi *et al*., 1992)** and finally as *Ralstonia solanacearum* **(Yabuuchi *et al*., 1995)**.

Ralstonia solanacearum is a strictly aerobic, non-spore forming, gram-negative, and non-capsulated bacterium **(Hayward, 1991** and **Stevenson *et al.*, 2001)**. The bacterium has rod shape and measuring approximately (0.5 – 0.7) X (1.5 – 2.5) μm. The bacterium highly motile, bear 1 – 4 polar flagella **(Kelman and Hruschka, 1973)**. This bacterium is active between 24 – 35 °C (optimal temperature 27°C) and decrease when temperature exceeds 35 °C or fall below 10 °C **(Stansbury *et al.*, 2001** and **Lemay *et al.*, 2003)**.

Kelman (1954) and Adhikari (1993) studied the morphology of the colony on triphenyl tetrazolium chloride (TZC) agar medium. They found two strains of the pathogen; virulent and avirulent strains. Virulent strain (wild type) had fluidal colonies that are slightly raised, slimy and appear entirely white or white with a light central red area. In addition, the virulent colonies are irregular in shape. Avirulent strain had truly round, butyrous in texture and deep red with a very narrow light bluish border.

R. solanacearum has a very wide host range. It infects more than 200 plant species belong to more than 50 plants **(Poussier *et al.*, 1999; Salanoubat *et al.*, 2002** and **Guo *et al.*, 2004)**. It infects important economic plants such as potato, tomato, tobacco, pepper, eggplant, groundnut and banana. Moreover, it can infect ornamental plants and weeds, which can act as reservoirs of infection **(Toth *et al.*, 1997**).

II.2.5. Taxonomic position of *Ralstonia solanacearum* (Lemay *et al.*, 2003):

Kingdom: Proteobacteria

Class: Neisseriae

Order: Burkholderiales

Family: Burkholderiaceae

II.2.6. Races and biovars of *R. solanacearum*:

R. solanacearum had been classified into five races according to host range **(Buddenhagen *et al*., 1962** and **Buddenhagen, 1986)** and five biovars according to their ability to oxidize three hexose alcohol and three disaccharides (**Hayward, 1991)**. Race 1 (biovars 1, 3 and 4) infects a broad host-range solanaceous plants and is restricted to tropical areas. Race 2 infects banana and heliconia. Race 3 (equivalent to biovar 2) has a narrow host-range, infecting potato, tomato, eggplant and some solanaceous weeds such as woody night shade (*Solanum dulcamara*) and black nightshade (*S. nigrum*). Race 3 has a lower optimum temperature than race 1 and occur in cool and subtropical areas, it responsible for presence of brown rot disease in Europe and North Africa **(Hayward, 1995)**.

French *et al.* (1995) showed the briefly classification of *R. solanacearum* into five biovars and four races in two tables as the following:

Table 1: Classification of *R. solanacearum* into biovars based on the ability to utilize disaccharides and oxidize hexose alcholols producing acid when positive (+).

Physiological tests		Biovars				
		1	2	3	4	5
Utilization of disaccharides	**Cellobiose**	—	+	+	—	+
	Lactose	—	+	+	—	+
	Maltose	—	+	+	—	+
Oxidation of alcohols	**Dulcitol**	—	—	+	+	—
	Mannitol	—	—	+	+	+
	Sorbitol	—	—	+	+	—

Table 2: Definition of races of *R. solanacearum* by host range and biovars determined in each.

Race	Natural hosts	Biovars
1	Many Solanaceae, some diploid banas, numerous other crops and weeds in many families.	1, 3 or 4
2	Triploid bananas, certain heliconias	1 or 3
3	Potato, tomato and rarely, a few other hosts	2
4	Mulberry	5

II.2.7. Epidemiology:

The source of inocula of *Ralstonia solanacearum* may be the infected seed tubers, infested soil or irrigation water.

The infected seed tubers are the most common source of inocula; especially latent infection cause problems. Seed tubers produced in cool climate may not show any symptoms when planted at warmer locations, however, disease development may be severe. *R. solanacearum* may be carried out over long distance through infected seed tubers **(Martin and French, 1997)**.

R. solanacearum is soil born pathogen that can persist in wet soils, deep soil layers (75 cm) and reservoir plants. Its distribution in potato fields can be spotty, and is commonly found in areas that have poor drainage. It is adapted to low temperature, however its survival in very cold temperature is reduced **(Lemay *et al.*, 2003)**. In addition, survival of this bacterium is influenced by humidity, physical and chemical factors. In certain soils, it may survive for many years, in others it may disappear from one growing season to the next **(Martin and French, 1997)**.

Moreover, soil amendments effect the survival of *R. solanacearum* (**Michel and Mew, 1998)**.

R. solanacearum was detected in waterways and can survive in waterways and in weed hosts. *R. solanacearum* can survive over several years in roots of the aquatic weed host, from which it leaches into the water and infects other plants (**Elphinstone *et al*., 1998).** The densities of this bacterium have seasonal variation in waterways; its density is relatively high and the organism actively multiplies but its density is low in winter **(Caruso *et al.,* 2005)**. The symptoms of bacterial wilt disease (brown rot disease) were observed on susceptible plants after irrigation by contaminated water **(Janse *et al.,* 1998** and **Caruso *et al.,* 2005)**. Moreover, **Farag *et al*. (1999)** reported that irrigation water could play a role in epidemiology of the brown rot disease in Egypt.

II.2.8. Virulence of the pathogen:

The virulent strain R. *solanacearum* produces large amount of extracellular polysaccharides slime (EPSs) that are most virulence factor and play an important role in wilting and killing of host plant **(Buddenhagen and Kelman, 1964, Toth *et al*. 1997 and Chapman and Koa, 1998)**. EPSs enable bacteria to evade plant immunity **(D'Haeze and Holsters, 2004)**. EPSs might interfere with water transport in plant by plugging the xylem vessels leading to wilt **(Husain and Kelman, 1958 and Chapman and Koa, 1998)**. In addition, the ESP help to aggregate bacterial cell causing occlusion of transport vessels within the plant stem **(Toth *et al*., 1997)**.

In addition, extracellular proteins (EXPs) play an important role in the virulence of *R. solanacearum* **(Kang *et al*., 1994; Shell *et al*. 1994, Shell, 1996)**. EXPs may be required for infection via roots, as well as wilting and killing of the host plants.

Kersten *et al.* (2001) suggested that swimming motility has a most important contribution to bacterial wilt virulence in the early stages of host plant invasion and colonization.

Complex network, of *R. solanacearum*, control virulence factors are EPSs, EXPs and motility. The purpose of this complex network may be to allow this phytopathogen to both co-ordinately or independently regulate diverse virulence factors in order to cope with dynamic situations and conditions encountered during interaction with plants **(Huang *et al.*, 1995, Shell, 1996 and Clough *et al.*, 1997)**.

This complex regulatory network contains many genes. The gene *vsrB* is responsible for production of EXPs **(Huang *et al.*, 1993)**. Another virulence factor, *vsrA*, is required for normal production of EPSs, some EXPs (**Shell *et al.*, 1994)**. Other virulence gene *espR* controls the production of EPSs **(Chapman and Kao, 1998)**. The *hrp* genes of *R.solanacearum* are pathogenicity determinants; they encode a type III protein secretion. These *hrp* genes are under the control of *hrpB* regulatory gene **(Aldon *et al.,* 2000).** The virulence regulator gene *phcR* controls the expression of *hrp* genes **(Genin *et al.,* 2005)**.

II.2.9. Phenotypic conversion:

R. solanacearum undergoes spontaneous phenotype shift from fluidal wild type (virulent strain) to non-fluidal avirulent or weakly virulent phenotype when stored in water, grown for 7 days or more days in unshaked broth or grown in agar plates **(Kelman and Hruschka, 1973)**. **Morals and Sequeira (1985) and Shekhawat *et al.* (1992)** studied the phenomenon of conversion of virulent strain of this pathogen to avirulent strain and termed this phenomenon as “phenotypic conversion”.

II.2.10. Control of brown rot pathogen:

Kishore *et al.* (1996) indicated that *R. solanacearum* can be controlled by cultural practice. They revealed that the use of disease free seed tubers and ploughing the field after harvest caused reduction of the disease severity. **Farag *et al.* (1999)** suggested that the use of artesian water in desert and avoidance of re-use of irrigation water reduce the disease severity.

Furthermore, *R. solanacearum* can be inhibited by antibiotic(s). **Abo El-Dahab (1957)** indicated that chlortetracycline, tetracycline, erythromycin and streptomycin inhibited this pathogen. The different strains varied in their response to chloramphicol. He also clarified that this bacterium was very sensitive to streptomycin and erythromycin. Moreover, **El-Helaly *et al.* (1963)** found that streptomycin tetracycline and agromycin were the most effective antibiotic for inhibition (*in vitro*) of the growth of Egyptian isolates of this bacterium. While, **Farag *et al.* (1986)** revealed that *R. solanacearum* was not sensitive to chloramphenicol or penicillin. Both virulent and avirulent strains were sensitive to streptomycin and dihydrostreptomycin. Economically, the use of antibiotic(s) to control plant pathogen(s) is not practical due to the expensive price of the antibiotic(s).

Moreover, *R. solanacearum* can be inhibited by antagonistic effect of some bacterial strains. **Wagle *et al.* (1983)** showed that the *China flava* (isolated from soil of Pune in India) produced antibiotic substances (*in vitro*) that antagonized *R. solanacearum*. In addition, **Sunaina *et al.* (1997)** indicated that three *Bacillus* spp (*B. subtilis* S1 & B5 and *B. cerrus* B4) exhibited antagonistic effect against bacterial wilt pathogen under culture and greenhouse conditions.

Averre and Kelman (1964) observed that the avirulent strain of *R. solanacearum* could reduce the disease severity in tobacco, tomato or eggplant when inoculated as a mixture of virulent and avirulent strains. Furthermore, **Abo El-Dahab and Goorani (1969)** showed that the growth of virulent strain of *R. solanacearum* was inhibited in the presence of another virulent or avirulent strain, but the avirulent strain was not. They suggested that this inhibitory effect might be due to production of inhibitory substances. In addition, **Wagih (1991)** reported that an avirulent strain of *R. solanacearum* inhibited growth of its virulent strain (*in vitro*) but the nature of inhibitory agent was unknown.

II.3. Study of Phages as biocontrol agent:

II.3.1. Using phages for the control of the plant pathogens:

Phages (Bacterial viruses or Bacteriophages) are viruses that kill bacteria (**Adams, 1959**). They are very specific for target bacteria and non-toxic to workers and non-target bacteria. Phages were reported as potential agents for control of plant diseases as early as 1926 **(Moore, 1926)** and successfully used for control of Stefwart's disease in corn in 1935 **(Thomas, 1935)**.

In addition, phages were used for the control of *R. solanacearum*. **Tanaka *et al.* (1990)** used avirulent strain M4 of *R. solanacearum* and its phage for control the bacterial wilt disease of tobacco. They reported that the co-application of avirulent strain and its phages against the virulent strain of *R. solanacearum* against its virulent strain was more effective than the use of only avirulent strain. The ratio of wilted plants was reduced from 95.8 % for untreated control to 39.5 % for following treatments with avirulent strain alone and 17.6 % for avirulent strain and its phage.

McKenna *et al.* (2001) used a highly virulent and polyvalent *Streptomyces* phage to control scab disease of potato. They treated tubers with phage by placing tubers into the inner trough of the bath of circulating prepared phage suspension at 28 °C for 24 hours. They revealed that the progeny of phage treated plants reduced levels of surface lesions of scab (1.2 %) compared with tubers harvested from non-treated tubers (23 %). The number of scab lesions was significantly reduced from 44.23 lesion/ tuber for control plants to 3.82 lesion/ tuber for treated plants. While, no significant differences were recorded in weight, size or number of harvested tubers in both treatments.

II.3.2. *R. solanacearum* phages:

Singh *et al.* (1986) isolated two phages (Psp13 and Psp14) active against *Pseudomonas* (*Ralstonia*) *solanacearum*. In addition, **Tanaka *et al.* (1990)** reported that treatment with both avirulent strain M4 of *Pseudomonas* (*Ralstonia*) *solanacearum* and its bacteriophage was more effective than either treatment alone. They showed that use of avirulent strain of *R. solanacearum* enhanced biological control of bacterial wilt and rot disease of potato.

In addition, **Toyoda *et al.* (1991)** isolated a virulent phage PK-101 from soil infested with strain K-101 of *Pseudomonas* (*Ralstonia*) *solanacearum*. **Kakutani *et al.* (1994)** isolated two virulent phages active against *R. solanacearum*. **Ozawa *et al.* (2001)** isolated P4282 active against *R. solanacearum.*

II.3.3.Effect of exopolysaccharides on phage adsorption:

Defives *et al.* (1996) reported that the exopolysaccharides produced by *Rhizobium meliloti* M11S inhibited non-specifically the adsorption of phage NM8 by coating the cells but lipopolysaccharides (LPS) had specific inhibitory effect. Only the polysaccharide moiety of LPS (composed of glucose, glucosamine, galactose, 3-deoxy-D-mono-

octulosonic acid and large amount of sialic acid) inhibited phage adsorption; neither the lipid A moiety nor a cellular glucan was involved. *Rhizobium* strains lacking sialic acids did not bind phage NM8. Inhibition of phage binding by lectin specific for N-acetylneuraminic acid demonstrated that phage NM8 bound to sialic acids. Preincubation of the phage with monosaccharides showed that inactivation of phage was very stereospecific for N-acetylneuraminic acid. Phage adsorption was strongly inhibited by N-acetylglucosamine, which was not present in the LPS. Therefore, the receptors of phage NM8 appeared to be a saccharide site, probably involving the acetyl group of sialic acids.

Forde and Fitzgerald (1999) found that *Lactococcus lactis* subsp. *cremoris* MG1363 harbouring PCI658, a 58 Kb plasmid originating in *L. lactis* subsp. *cremoris* HO2, adsorbed phages Φ712 and ΦC2 less efficiently than the plasmid-free phage sensitive strain. In addition, this strain (containing PC1658) possessed a more hydrophilic cell surface. Furthermore, electron micrographs also illustrated significant plasmid mediated alteration of the cell surface HPLC analysis of the loosely associated extracellular material revealed that galactose and glucuronic acid is its major components. It was concluded that PC1658 encodes the production of a hydrophilic exopolysaccharides that masks cell surface receptors causing a decrease in bacteriophage adsorption.

On the other hand, some bacteriophages penetrated exopolysaccharides due to presence of enzymes degrading exopolysaccharides. The source of these enzymes may be the phage or the bacterial host itself. **Hughes *et al.* (1998 a)** reported that bacteriophage for three representative strains of Gram-negative biofilm bacteria had proved to be of wide spread occurrence. Lytic bacteriophage has been isolated from local sewage for the bacterium, an

exopolysaccharide (EPS) producing *Pseudomonas* found originally as a component of biofilms in Local River, and for two *Enterobacter* agglomerans strains from industrial biofilms. The bacteriophage was similar to other viruses for EPS-producing bacteria in inducing the synthesis of enzymes degrading the polymers that occlude the bacterial cell surface. The soluble phage enzymes degrade their substrate by acting as endo-glycanohydrolases.

Hughes *et al.* (1998 b) studied biofilm susceptibility of bacterial strains *Enterobacter agglomerans* 53b and *Serratia marcescens* serr**,** which were isolated from a food processing factory to phage attack. A bacteriophage (SF153b), which could infect and lyse strain 53b, was isolated from sewage. This has been shown to possess a polysaccharide depolymerase enzyme specific for the exopolysaccharide (EPS) of strain 53b. It was observed that phage SF153b degraded the EPS of a mono-species biofilm (strain 53b) and infected the cells. The disruption of the biofilm by phage was a combination of EPS degradation by depolymerase and infection and subsequent cell lysis by the phage. Strain serr (*Serratia marcescens* serr) biofilms were not susceptible to the phage and the biofilm EPS was not degraded by the phage glycanse, with the result that the biofilm was unaffected by the addition of SF153b phage. Scanning electron microscopy confirmed that specific phage could extensively degrade susceptible biofilms and continue to infect biofilm bacteria whilst EPS degradation was occurring.

Hanlon *et al.* (2001) showed that to cause infection, bacteriophages must penetrate the alginate exopolysaccharide of *Pseudomonas aeruginosa* to reach the bacterial surface. They suggested that bacteriophage migration through *P. aeruginosa* biofilms may be

facilitated by a reduction in alginate viscosity brought about by enzymatic degradation and that the source of the enzyme may be bacterial host itself.

II.3.4. Characterization of phages:

II.3.4.1. Morphological characterization:

II.3. 4.1.i. Plaque morphology:

Plaques are the clear areas (or holes) which formed in the bacterial lawn (confluent layer of bacteria on the surface of a nutrient agar plate) due to the lytic action of the virulent phage **(Stansfield *et al*., 1996)**.

Atkins (1973) observed that phages in one structural group formed plaques of similar morphology and size on indicator bacteria (structural group A gave clear plaques while group B produced turbid ones). **Barnet and Humphery (1975)** studied two phages of *Rhizobium trifolii*, which produced plaques, surrounded by "halo". Moreover; they reported that these phages induced enzymes able to depolymerize exopolysaccharides of *R. trifolii and R. leguminosarum*, but not for other rhizobia species.

Prathuangwong and Janekittivong (1985) indicated that there was one phage growing on *Xanthomonas campestris* pv. *phaseoli*. However; this phage had three plaque types of variable width, large (2.5 – 3 mm diameter), medium (1.5 – 2 mm) and small (<1.5 mm) in size. This phage has been isolated from soyabean leaves and soils beneath diseased plants.

El-Sayed *et al.* (2001) isolated two phages (ΦS and ΦL) attacking *Streptomyces scabies*. They found that the two phages had clear plaques which were different in diameter, (2 – 2.5 mm diameter) for ΦS plaques and (1 – 1.5 mm diameter) for ΦL plaques.

El-Helali (2001) isolated three phages (Φ1C2, Φ3A and Φ4C) growing on *Rhizobium leguminosarum* and found that both plaques of the two phages Φ1C2 and Φ3A appeared after 24 hours, while the plaques of

Φ4C appeared after 72 hours. Φ1C2 plaques were clear with sharp edges and their diameters measuring about 2 mm, Φ3A plaques were pinpoint diameter and Φ4C plaques were very minute.

O'Flynn *et al.* (2004) studied the plaque morphology of three phages infecting *Escherichi coli* O157:H7 strain P1432. They reported that the two phages e11/2 and pp01 formed pinpoint plaques (0.5 mm), whereas e4/1c formed medium-sized plaques (3 mm diameter).

II.3. 4.1.ii. Electron microscopy:

Pfankuch and Kousche (1940) were the pioneer who applied the electron microscopy to study phages. Most of the phages examined resemble tadpoles, with long tails attached to spherical, cylindrical or polyhedral heads. Some phages, at first, thought to be tailless spheres revealed on closes examination polyhedral shapes and a rudimentary tail.

Bradley (1967) classified bacteriophages into six basic types according to three main criteria, *viz* nucleic acid, morphology and host range. The scheme of bacteriophages classification was as follow:

I-Double-stranded DNA:

1- Group A: had contractile tails.

2- Group B: had long non-contractile tails.

3- Group C: had short non-contractile tails.

П- Single-stranded DNA:

4- Group D: were tail-less and had large capsomer.

5- Group E: were filamentous phages.

Ш- Single-stranded RNA:

6- Group F: were tail-less and had small capsomeres.

Mathews (1982) classified bacteriophages into 10 families according to the electron microscopy, DNA fingerprint, protein analysis, replication and host range. He reported that there are three families with

tails, which are *Myoviridae*, *Styloviridae* (*Siphoviridae)* and *Podoviridae*. Where, *Myoviridae* family has phages with long contractile tails (80 – 455 nm) consisting of a central tube and contractile sheath separated from the head by a neck. While, *Styloviridae* (*Siphoviridae)* family has phages that have long (64 – 539 nm) non-contractile tails. Also, *Podoviridae* family has phages having short (about 20 nm) non-contractile tails.

Prathuangwong and **Janekittivong (1985)** studied the phage of *Xanthomonas campestris* pv. *phaseoli*. They reported that this phage had a polyhedral head (70 X 55 nm) and rod-shaped tail (15 X 18 nm). While, **Singh *et al.* (1986)** studied the electron microscopy of two phages, PSP13 and PSP14 specific for *Pseudomonas* (*Ralstonia*) *solanacearum*; PSP13 was 60 nm in diameter with a rudimentary tail and PSP14 was 54 nm in diameter and without tail.

Kakutani *et al.* (1994) studied two virulent phages isolated from *Pseudomonas (Ralstonia) solanacearum* infested soil. They revealed that one phage had a polygonal head with three tails while the other phage had a smaller head with two tails.

El-Didamony (1995) studied 17 phages active against *Rhizobium leguminosarum* isolated from soil samples cultivated with collected a field *Vicia faba* in Al-Ibrahimia, Sharkia, Egypt. Isolated phages had isometric heads and tails and were classified into different groups and families. These phages were 2 phages with contractile tails (RLz_1 & RLz_2) belonging to group A family *Myoviridae*, 7 phages with long non-contractile tails (RLz_3 to RLz_9) belonging to group B family, *Styloviridae* (*Siphoviridae*) and 8 phages with very short tails (RLz_{10} to RLz_{17}) belonging to group C, family *Podoviridae.*

Tremblay and Moineau (1999) revealed that phage DT1 (of *Streptococcus thermophilus*) had an isometric head of 60 nm and a non-

contractile tail of 260 X 8 nm. Moreover, **Ashelford *et al.* (1999)** indicated that phage ΦCP6-4 is belonging to *Podoviridae* family, where it had a polyhedral head with short tail (12.35 ± 3.07 nm).

El-Sayed *et al.* (2001) reported that both two phages ΦS and ΦL (active againist *Streptomyces scabies*) had a polyhedral head and flexible non-contractile tail. Phage ΦS had a head measuring (100 X 170 nm) and a tail of (250 X 80 nm), while phage ΦL had a larger head of (212 X 162 nm) and tail of (187 X 18 nm). Therefore, these two phages were classified as group B phage belong to family *Styloviridae* (*Siphoviridae*).

El-Helali (2001) studied three phages active against *Rhizobium leguminosarum.* He revealed that phage Φ1C2 had a polyhedral head, non-contractile tail and base plate. Phage Φ3A had an icosahedral head with non-contractile tail, while phage Φ4C had a spherical head with non-contractile tail. In addition, these three phages had base plates with different measurements.

Scholl *et al.* (2001) revealed that the virion particle of phage ΦK1-5 (infecting *E. coli*) consists of icosahedral head of 60 nm in diameter and small tuft of short tail fibers. Therefore, this phage belongs to *Podoviridae* family.

Ackermann (2003) clarified that phages are classified into one order and 13 families. Over 5100 phages have been examined with electron microscope since 1959; at least 4950 phages (96 %) are tailed. They constitute the order *Caudovirales* and three families. *Siphoviridae* or phages with long, non-contractile tails predominate (61 % of tailed phages). Polyhedral, filamentous and polymorphic phages comprise less than 4 % of bacterial viruses. Tailed phages are the oldest known group.

O'Flynn *et al.* (2004) studied the electron micrscopy of the three phages e11/2, pp01 and e4/1c growing on *Escherchia coli* O157:H7. They indicated that phages e11/2 and pp01 had a contractile long rigid

and relatively thick tail. Therefore, these two phages had the A2 morphotype and classified into *Myoviridae* family. Phage e4/1c had a relatively thin, long, non-contractile and flexible tail, with an isometric head. Thus, this phage was classified into *Siphoviridae* family.

Quiberoni *et al.* (2004) studied three phages (YAB, Ib3 and BYM) active against *Lactobacillus delbrueckii* subsp. *bulgaricus*. They reported that all the three phages had long non-contractile tails; therefore, they belonged to *Siphoviridae* family (morph-type B1).

Chibani-Chennoufi *et al.* (2004) studied electron microscopy of *E. coli* phages isolated from the stool of pediatric diarrhea in Bangladesh. They found that these phages belonged into *Myoviridae* and *Siphoviridae*. From these phages, there were eight *Siphoviridae* phages (designated as JS77.1) had a characteristic trait; a fork like base plate.

II.3.5. Host range (Specificity of bacterial viruses):

Host range is the range of a phage measured in term of the varieties of bacteria in which it can grow. Host range is often, but not always, determined by success or failure of adsorption **(Adams, 1959)**. The lytic activity of bacteriophages isolated from soil is either specific (monovalent) phage lyses only a single strain of bacteria or polyvalent phage lyses numerous bacterial strains.

Early studies of bacteriophage host range showed contradictory results. **Hitchner (1930)** isolated a bacteriophage from the root nodule of red clover, which was specific only for its own homologous strain. On the other hand, **Laird (1932)** revealed that bacteriophage isolated from a stock culture of *Rhizobium trifolii* (218) was active against unrelated strains of *R. trifolii*, *R. meliloti* and *Bradyrhizobium japonicum*.

Adams (1959) indicated that some bacteria are able to adsorb phage very well but fail to serve as host cell for phage multiplication.

Lysogenic bacteria, for example, usually adsorb the phage and carry it in the latent (prophage) state, but are not affected by it. Lysogenic bacteria seemed therefore to be immune to super-infection and susceptible to lyses by other phage.

Singh *et al.* (1986) found that two phages (PSP13 and PSP14) were specific for *Pseudomonas (Ralstonia) solanacearum*. In addition, **Supriadi and Green (1989)** studied phages that lysed indicator strains of the Sumatra disease bacteria (SDB) of cloves that were isolated from cultures of *Pseudomonas* (*Ralstonia*) *solanacearum* and from banana blood disease bacterium (BDB) on bananas. Of these phages, three phages (P3, P5 and P6) lysed 20 isolates of SDB and 1of 10 isolates of BDB, but failed to lyse 8 isolates of *P. solanacearum* one isolate of *Xanthomonas campestris* pv *celebensis* and one isolate of *X. campestris* pv. *manihotis*.

El-Sawi (1998) found that the rhizophages T, V, M and J isolated from Egyptian soils had a wide host range on the 16 strains of *Rhizobium* and *Bradyrhizobium* used for the isolation of phages. The tested strains of *Rhizobium* showed variable sensitivity against the phages. Among the tested rhizobial strains, 6 strains were lysed by phages T and V (*R. Leguminosarum* biovar *trifolii* and biovar *vicia*), while phage J lysed 7 strains of *Bradyrhizobium japonicum*. On the other hand, the phage M did not lysed more than 5 strains.

El-Sayed *et al.* (2001) indicated that the two phages ΦS and ΦL (infective to *Streptomyces scabies*) were polyvalent phages. By testing using 20 different isolates of pathogenic and non-pathogenic *Streptomyces* belong to nine species, it was found that phage ΦS had a relatively narrow host-range (attacking only 9 isolates of 20), while phage ΦL had a wide host range (14 hosts).

El-Helali (2001) indicated that the three rhizophages Φ1C2, Φ13A and Φ4C had a high degree of specificity against *Rhizobium leguminosarum* strains. Phage Φ1C2 was specific for *R. leguminosarum* biovar *vicia* strain S, while two phages Φ13A and Φ4C were specific for *R. leguminosarum* biovar *vicia* strain E.

O'Flynn *et al.* (2004) showed that all their three phages (e11/2, pp01 and e4/1c) were monovalent; specific for *Escherichia coli* O157:H7. All of these phages infected two non-toxigenic *Escherichia coli* strains and ten toxigenic *E. coli,* while they failed to lyse other *E. coli* strains and other gram-negative bacteria.

II.3.6. The phage-soil interaction:

Most of the time, the soil is partially hydrated in most crop environments and can adsorb phage on its particles. Therefore, it can be speculated that the soil-phage interactions may help or hinder the phages in their search for a suitable host. On the other hand, adsorption to clays might have a protective effect by holding phage within a hydrated environment. If the soil itself is disseminated, become fully hydrated, or is well mixed, then associated phages whether free or bound, may be disseminated. Therefore, soil-adsorbed phage could serve as a viable infectivity pool that is tapped only as bacteria grow, diffuse or swim into the phage vicinity, or if the soil particle itself is transferred into or onto a bacterium-containing environment **(Gill and Abedon, 2003)**.

II.4. Actinomycetes and their biocontrol potential:

The actinomycetes comprise an ubiquitous order of bacteria (actinobacteria) which exhibit wide physiological and morphological diversity. They can be defined as gram-positive, unicellular filamentous bacteria, about 1 um in diameter, which monopodial more rarely dictiomous, branching and producing colonies of radiating structure

(Waksman, 1967). Reproduction is by almost total fragmentation of the hypha or by production of spores in specialized areas of the mycelium. Most species are chemoorganotrophic, aerobic, and mesophilic and grow optimally at pH near neutrality **(William and Cross, 1971** and **Mansour and Mashaly 1985).**

Actinomycetes are the most widely distributed groups of microorganisms in nature. They are attractive, bodacious and charming filamentous gram-positive bacteria. They make up in many cases, especially under dry alkaline conditions, a large part of the microbial population of the soil **(Athalyle *et al.*, 1981; Mansour and Mashaly, 1985; Lacey, 1997 and Oskay *et al.*, 2004)**. Generally, actinomycetes may be separated into two large categories; the oxidative forms that are very numerous and are soil inhabitants and the fermentative type that are primarily found in the natural cavities of human beings and animals **(Lechvalier and Pine, 1977** and **Mansour *et al.* 1992a & b)**.

Based on several studies among bacteria, the actinomycetes are the noteworthy as antibiotic producers, making three quarters of all known products; members of *Streptomyces* are especially prolific and occupy the leading position **(Lacey, 1973; Mansour, 1979; Saadoun and Gharaibeh, 2003 and Oskay *et al.*, 2004)**.

II.4.1. *Streptomyces*:

The genus *Streptomyces* was coined by **Waksman and Henrici (1943)** to designate certain aerobic saprophytic aerial mycelium-producing actinomycetes from the rest genera of the order Actinomycetales coined the genus *Streptomyces*. The taxonomic position of the family Streptomycetaceae in the order actinomycetales (based on biochemical criterion) and the classification of the family to the genus

level (based on the gross morphology) were discussed by **Cummins and Harris (1958)**, **Goodfellow and Alderson (1979) and Mansour (1979)**.

Streptomyces are catalase positive and generally reduce nitrate to nitrite and degrade adenine, esculin, casein, gelatin, hypoxanthine, starch, and L-tyrosine **(Mansour and Mashaly, 1985)**. In addition, they are capable of utilizing a wide range of organic compounds as sole source of carbon for energy and growth **(Waksman, 1967 and Alexander, 1991)**.

The colonies of *Streptomyces* are small (1 – 10 um) in diameter discrete and lecheroid, leathery or butyrous; initially relatively smooth surfaced but late develop a wide variety of aerial mycelia that may appear granular, powdery, velvety or floccose. All species have a unique earthy odour caused by production of *Streptomyces* metabolite called geusmins **(Waksman, 1967; Mansour 1977** and **Mansour *et al.*, 1992 a &b)**.

Streptomycetes are known to produce a wide range of pigments that are responsible for the different colourfull appearance of the vegetative (aerial) and substrate mycelium. Thus, the colour of mature sporulated aerial mycelium should be white, yellow, red, grey, green or blue; a phenomenon that is used as a reliable taxonomic criterion in differentiation among *Streptomyces* species **(Shirling and Gottlieb, 1946, 1968 a & b, 1969, 1972 and Mansour *et al.*, 1992 a & b)**.

Streptomyces spp are Gram-positive aerobic members of the order actinomycetales within the class *Actinobacteria* and have a DNA G + C content of 96 – 78 mol % **(Anderson and Wellington, 2001)**. They can be distinguished from other actinomycetes by their cell wall type which is characterized as Type I *sensu* (**Lechevalier and Lechevalier, 1970** a). The presence of LL-Diaminopimelic acid and glycin and the absence of characteristic sugars are typical of this wall type. In addition, the acyl

type of the muramamyl residues in the cell-wall peptidoglycans is acetyl (**Uchida and Seino, 1997,** and **Anderson and Wellington, 2001**).

II.4.2. Biological activities of *Streptomyces*:

II.4.2.i. Production of antibiotics:

It is now well authenticated that streptomycetes are producers for very valuable biologically active substances, of which the most important are antibiotics, vitamins, enzymes, growth regulators… etc **(Mansour, 1979; Mansour *et al.*, 1994 & 1996 and AlDesuqy *et al.*, 1998)**.

Streptomyces spp were the source of useful antibiotics compound that are used not only in the treatment of various human and animal diseases but also in agriculture and in biochemistry as metabolic poisons **(Waksman and Lechevalier, 1962 and Martin, 1982)**. Members of *Streptomyces* spp produced many antibiotics, which show a diversity of chemical structure and biosynthetic pathways (**Okanishi and Umezawa, 1978)**.

In Egypt, many investigators have demonstrated the ability of many *Streptomyces* spp to produce various antibacterial and antifungal antibiotics (**Mansour and Mashaly, 1985; Mansour *et al.*, 1992a, Mansour *et al.*, 1992b, Mansour, 2003**).

Actinomycetes synthesis wide range of antimicrobial compounds, which have different chemical structure, different metabolisms of action and varied antimicrobial spectrum. **Kurylowicz (1976)** estimated that 1530 antibiotics are produced by actinomycetes, of which 1467 originated from *Streptomyces* species.

The biological significance of this unique capacity for antibiotic-producing potential of these organisms remains to be elucidated.

II.4.2.ii. Antiviral activities of actinomycetes:

Actinomycetes can inhibit plant, animal and plant viruses. Preparations of some actinomycetes those were effective against bacterial viruses (phages) were inactive against animal and plant viruses **(Waksman, 1967)**. In addition, five *Streptomyces* strains inhibited tomato mosaic virus TMV. Theses *Streptomyces* strains were *S. guogeroti* (MF17), *S. olivochromogenenes* (MC-35), *S. rimosus* forma *mansourensis* (MA-4) and *S. rochei* (MC-31) **(Mansour *et al.*, 1988)**.

Progress in the isolation and development of antiviral agents has been slow in comparison with the rapid advances made with antimicrobial agents. The empirical approach successful with antibacterial or antifungal antibiotics has yielded only few useful substances. In general, no relation was found between antimicrobial and antiviral activity **(De Banchero *et al.*, 1965)**.

II.4.2.iii. Production of growth regulators:

Streptomyces spp produce growth regulators and hydrolases enzymes. **Mansour *et al.* (1994)** revealed that twenty-four strains belonging to genus *Streptomyces* had higher potentialities to produce growth regulators (i.e., auxins, gibberellins and cytokinins) and some hydrolases (e.g., α-amylases and proteinases) in shaken culture. Studying the role of microorganisms in the life of cultivated plants, **Koaze (1958)** reported that *Streptomyces* SP-S-580 accelerated seed germination and growth of rice, barely and oat.

AlDesuqy *et al.* (1998) revealed that grain priming with culture filtrates of *Streptomyces olivaceoviridis, S. rimosus* or *S. rochei* appeared to enhance growth vigor and crop yield of wheat plants. They indicated that these microbes possess a high capacity for the production of growth regulators. In addition, actinomycetes have been reported to stimulate

plant growth in the presence of plant pathogens due to their ability to produce growth regulators **(Turhan, 1981; Tahvonen and Avikainen, 1990** and **El-Tarabily *et al.* 1997)**

II.4.2.vi. Production of lytic enzymes:

Many investigators (**Lloyd *et al*., 1965; Beyer and Diekmann, 1984 and Okazaki** and **Tagawa, 1991**) reported the potentiality of actinomycetes for production of lytic enzymes. Lytic enzymes have mainly been studied with respect to biological control of plant pathogenic fungi or protoplast formation from yeast cell and the careful isolation of intracellular proteins (**Grigorova *et al*., 1997** and **Yoshida *et al*., 1997**). Lytic enzymes may have clinical significance in infectious diseases caused by low-toxic gram-negative bacteria such as *Serratia marcesens* **(Suzuki *et al*., 1985)**.

II.4.3. Using of *Streptomyces* spp in biological control of plant pathogens:

Many investigators used *Streptomyces* spp in biological control of plant pathogens. **Yuan and Crawford (1995)** found that *Streptomyces lydicus* WYEC108 showed strong antagonism against various fungal pathogens in plate assays by producing extracellular antifungal metabolites *in vitro*.

El-Abyad *et al.* (1996) isolated thirty-seven actinomycetes from fertile cultivated soils in Egypt. These actinomycetes isolates were screened for the production of antimicrobial compounds against a variety of test organisms. Most of the isolates exhibited activities against Gram-positive, Gram-negative, and acid fast bacteria, yeasts and filamentous fungi, with special attention to fungal and bacterial pathogens of tomato. On starch-nitrate agar, 14 strains were active against *Fusarium oxysporum* f. sp. *Lycopersici*, and 18 against *Alternaria solani*. In Liquid

media, 14 isolates antagonize *Pseudomonas (Ralstonia) solanacearum*, and 20 antagonized *Clavibacter michiganensis* spp. *michiganensis*. The most active antagonists of the pathogenic microorganisms studied were *Streptomyces pulcher,S. canescens* and *S. citreofluorescens*.

El-Tarabily *et al.* (1997) isolated forty-five Streptomycetes and non-Streptomycete actinomycete isolates from the carrot rhizosphere for screening their antagonism to *Pythium coloratum* Vaartaja, a causal agent of cavity-spot disease of carrots (*Daucus carota* L.) *in vitro* and *in vivo*. These isolates were screened *in vitro* antagonism in carrot bioassay. All of seven tested isolates produced non-volatile antifungal metabolites, but failed to produce inhibitory volatile compounds. All seven isolates significantly reduced the incidence of cavity spot in soil artificially infested with the pathogen in glasshouse. *Streptomyces junthinus* and *Streptosporangium albidum* were the most effective in reducing the disease in inoculated plants**.**

Youssef *et al.* (2001) investigated the biological control of root rot disease of white lupine (caused by *Plectosporium tabacinum*) by *Streptomyces* species. They isolated 70 rhizosphere actinomycetes. Three of these isolates were found to be strongly antagonistic against *P. tabacinum in vitro*. They also significantly reduced the incidence of white lupine root rot disease in soil infested with *P. tabacinum* in green house trials. The three actinomycetes isolates were identified as *Sterptomyces cynoviridis, S. murinus,* and *S. grisoeoplanus.*

Oskay *et al.* (2004) isolated a total 50 different actinomycetes strains from farming soil samples collected from Manisa and its surrounding, Turkey. These strains were assessed for their antibacterial activity against four phytopathogenic and six human pathogenic bacteria. They revealed that 34 % of all isolates (17 isolates) were active against of

the tested organisms. The four tested phytopathogenic bacteria were *Agrobacterium tumefaciens, Erwinia amylovora, Pseudomonas viridiflova* and *Clavibacter michganensis* subsp. *michganensis*. They reported that of both *C. michgansis* and P. *viridiflova* were inhibited by five isolates, *A. tumefaciens* was inhibited by four isolates and *E. amylovora* was inhibited by three isolates.

Badr (2006) isolated forty actinomycetes from the root zone of healthy potato plants growing on potato fields. The antagonistic activities of these isolate against *Erwinia carotovora* subsp. *carotovora* "the causal agent of soft rot disease of potato" were tested. Five isolates had the ability to inhibit the growth of the studied bacteria; all of these isolates belonged to *Streptomyces* spp. The most effective *Streptomyces* isolate against the studied pathogenic bacteria was *Streptomyces sioyaensis*. Treatment of potato tubers with *Streptomyces sioyaensis* perior to sowing showed high reduction in disease incidence at harvest time and after storage.

II.5. Use Essential oils in biological control:

Many plant species produce volatile essential oil compounds. These oils are considered to play a role in host defense mechanism against plant pathogens **(Mihaliak *et al.*, 1991, and Pradhanang *et al.*, 2003)**. Essential oils and their components, usually from medicinal plants, have been recognized as having fungicidal effects **(Wilson *et al.*, 1997)**.

Essential oils do not inhibit only microorganisms but inhibit other plant pests. **Tunç and Sahinkaya (1998)** reported that vapours of essential oils extracted from cumin, anise, oregano, and eucalyptus were found to be toxic to two greenhouse pests; the carmine spider mite and cotton aphid.

Abd El-Aziz (2002) studied the inhibitory effect of 11 commercial oils against the causal agent of brown rot disease of potato (*R. solanacearum*). By applying fumigation method, he found that four essential oils caused completely inhibition of *R. solanacearum* growth. These four essential oils were peppermint, fennel, thyme and citronella oils. In addition, he tested the inhibitory effect of these 11 essential by contact method (made a well in each seeded plate and put 0.1 ml tested oil in each well). The strongest antagonistic effect was recorded with Thyme oil (42 mm) then peppermint oil (35.33 mm).

Pradhanang *et al.*, (2003) investigated the effectiveness of plant essential oils as soil fumigants to manage bacterial wilt (caused by *R. solanacearum*) of tomato in greenhouse experiment. Potting mixture soil infested with *R. solanacearum* was treated with the essential oil at 400 mg or µl and 700 mg or µl per litter of soil in this experiment. *R. solanacearum* population densities were determined just before and 7 days after treatment. Populations declined to undetectable levels in thymol, plamarosa oil and lemongrass oil treatments at both concentrations, whereas tea tree oil had no effect. Tomato seedlings transplanted in soil treated with 700 mg/ litter of Thymol, 700 ml/ litter of palmarosa oil and 700 ml / litter of lemon grass oil were free from bacterial wilt and 100 % of plants were free from *R. solanacearum.*

Hamido (2003) studied the antimicrobial activities of essential oils extracted from different parts eleven plants. She selected two essential oils of two plants (*Artemisia monosperma* and *Pulicaria incisa*) and she studied the antimicrobial activity of them against gram-positive and gram-negative bacteria, fungi and yeasts. The minimal inhibition concentration (MIC) values of *A. monosperma* against tested

microorganisms were ranged from 0.032 – 20 µg/ ml and the corresponding values of *P. incise* were ranged from 0.8 – 20 µg/ ml.

Takarada *et al.* (2004) the antibacterial effect of essential oils against oral bacterial pathogens. They tested five essential oils (manuka, tea tree, eucalyptus, lavandula, romarinus) against the following oral bacteria: *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitanus, Streptococcus mutans* and *Streptococcus sobrinus*. They tested essential oils inhibited against these oral bacteria. Manuka oil had the best minimal inhibition concentration (MIC) that ranged from 0.03 to 0.25 % for different tested bacterial strain and tea tree oil was the second one; it had MIC values ranged from 0.06 to 1.0 %.

Anise, *Pimpinella anisum* L., is an annual aromatic crop belonging to family Apiaceae **(Davis, 1972)**. Anise is native to the Middle East and it has been known since the ancient Egypt **(Hemphill and Hemphill, 1988)**. Important anise producing countries are India, Mixico, Egypt, Italy, Spain, Syria, France, Bulgaria and Tunisia **(Reineccius, 1994).**

The Romans discovered that the anise and other aromatic species helped digestion and they used anise as an ingredient of special cake. They also used anise seed in perfumes. The people of Asia Minor and Greece used it for many medicinal applications **(Dwyer and Rattray, 1977 and Arslan *et al.*, 2004)**.

Pimpiella anisum is primarily for its fruits, commercially called seeds that are currently used for flavouring. In addition, the essential oil of anise is a valuable commodity in medicine and perfumery and especially its seeds have been used as an appetizer, diuretic and tranquilizer **(Gülcin *et al*., 2003**).

The essential oil content of anise seed was recorded between 2.1 % and 2.8 %, and important compounds were determined as *trans*-anethole,

methyl chavicol and anisaldehyde **(Bayram, 1992)**. **Arslan *et al.* (2004)** showed that anise essential oil levels varied from 1.3 % to 3.7 %. The major component of the essential oil was *trans*- anethole. This compound ranged from 78.63 % to 95.21 %.

II.6. Antimicrobial activities of basidiomycetes:

Cyathus is a widely distributed genus all over the world. It is found most often on decaying wood, small twigs, tree fern debris and sometimes on animal dung **(Alexopolos *et al.*, 1996** and **Lui and Zhang, 2004). Anke and Oberwinkler (1976)** isolated three crystalline antibiotics from the mycelium of the basidomycetes *Cyathus striatus* strain No.12. They named these antibiotics as striatins A, B and C. These striatins are highly active against fungi imperfecti and a variety of Gram-positive bacteria, as well as against some Gram-negative bacteria.

Heim and Anke (1988) isolated a new marasmane derivative, Pilatin, from fermentations of the cyphylloid fungus *Flagellosecypha pillatii* (a small astipitate cup shaped basidomycetes growing on grasses). Pilatin inhibits the growth of bacteria and fungi at concentrations of 5 – 50 µg/ ml.

Amoros *et al.* (1997) tested extracts from fresh fruiting bodies of 121 basidomycetes species for their antimicrobial activity against herpes simplex (type 1 and type 2) polio and vesicular stomatatitis viruses. 11 % of extracts tested were found to inhibit one or more of these viruses.

Hwang *et al.* (2000) isolated a chitin synthesis inhibitor (named as phellinsin) from the cultured broth of fungus *Phellinus* sp. PL3. This compound showed antifungal activity against *Colletotrichum lagenarium, Pyricularia oryzae, Rhizoctonia solani, Aspergillus fumigatuts and Trichophyton mentagrophytes*.

Lui and Zhang (2004) tested twelve selected *Cythus* spp. for their abilities to produce antimicrobial metabolites. Most of them were found to produce secondary exo-metabolites that could induce morphological abnormalities of pathogenic fungus *Pyricullaria oryza*. Some extracts from cultivated liquid obviously inhibited human pathogenic fungi *Aspergillus fumigatus, Candida albicans* and *Crypotococcus meoformans*. Activities against human pathogenic bacteria were also obtained from some of these extracts.

II.7. Organic amendments and plant residues:

Incorporating organic amendments and managing crop residues (type and quality) have a direct impact on plant health and crop productivity. Crop rotations consisting of wheat, beans or legumes followed by either a fallow period or a green manure were frequently used in the time of ancient Greece and Rome **(Karlen *et al.,* 1994** and **Bailey and Lazarovits, 2003)**. For centuries, farmers have manipulated the ecology of the soil by the addition or depletion of organic matter. Organic matter is known to affect soil aeration, structure, drainage, moisture holding capacity, nutrient availability, and microbial ecology **(Davey, 1996; Gök *et al.* 2002)**.

Agriculture practices such as incorporating organic amendments and managing the type and quality of crop residue have a direct impact on plant health and crop productivity. Soil management practices involving tillage, rotation and burning will impact the amount and the quality of organic matter that is returned to the soil. The application of organic amendments, manures and composts that are rich in nitrogen may reduce soil borne diseases by releasing allelochemicals generated during product storage or by subsequent microbial decomposition **(Bailey and Lazarovits, 2003)**.

Potential benefits of maintaining cover crop residue on soil surface include reduction in costs association with repeated intensive tillage, improvement in weed and insect control, improving in soil fertility and structure prevention of erosion and soil moisture retention. Residue on the soil surface decreases soil temperature and increase soil moisture **(Ferguson and Shew, 2001).**

The addition of organic matter from rotational or cover crops may directly or indirectly suppress disease by enhancing production of decomposition products by antagonistic microbial populations that inhibit pathogens and enhance growth **(Huang and Huang, 1993; Shetty, *et al.*, 2000 and Gamliel *et al.*, 2000)**. Plant may present physical barrier that reduces contact with the pathogen or the impacts of non-target chemicals **(Hau and Beutte, 1983 and Ferguson and Shew, 2001)**.

Carbon releases from crop residues contributes to increasing soil microbial activity and so increases the likelihood of competition effects in the soil. The placement of the residue in soil can lead to the disease displacement of the pathogen from it preferred niche diminishing the pathogen's ability to survive. The benefits of applying organic amendments for disease control are incremental, generally slower acting than chemical fumigants or fungicides, but may last longer and their effects can be cumulative **(Bailey and Lazarovits, 2003).**

Some organic matter can stimulate the production of lytic enzymes involved in the degradation of plant pathogens. Microbial degradation of plant residues can also produce secondary products with antimicrobial activities that inhibit the growth of plant pathogens **(Bardin *et al*., 2004)**.

III. MATERIALS AND METHODS

III.A. MATERIALS

III.A.1.Media:

III.A.1.1. Triphenyl tetrazolium Chloride (TZC) salts medium (Kelman, 1954):

This medium was used for differentiation and isolation of virulent and avirulent strains of *Ralstonia solanacearum*.

This medium consists of the following:

TZC stock solution: Dissolve 1 g of 2, 3, and 5 triphenyl tetrazolium chloride (TZC) in 100 ml of distilled water, place in light-proof capped bottle, and autoclave for only 8 min. or sterilize by filtration. Store the solution at refrigerator.

Basal medium:

Peptone	10.0 g
Casein hydrolysate	1.0 g
Dextrose	10 g (or 2.5 g)
Agar-agar	15.0 g
Distilled water	1 L

Adjust pH to 7.0 approximately.

Modification by reducing the amount of dextrose to 2.5 gm results in a better growth rate especially of potato strain (biovar 2 = race 3). Sucrose can be used as substitute **(French and Hebert, 1980** and **French *et al*., 1995)**.

The basal medium can be autoclaved and stored, then melted as needed. To each liter of melted basal medium, add 5ml of TZC stock solution to give final concentration 0.005 % (i.e. add 1 ml TZC stock solution to 200 ml molten basal medium).

III.A.1.2. Carbohydrate basal medium (for determination *R. solanacearum* biovar) (Hayward 1964 and 1976):

$NH_4H_2PO_4$	1.0 g
KCl	0.2 g
$MgSO_4 .7H_2O$	0.2 g
Peptone	1.0 g
Bromothymol blue	0.03 g
Agar-agar	8.0 g
Distilled water	1.0 L

III.A.1.3. Sucrose-peptone agar (SPA) medium (Jacobs and Gerstein, 1960):

Sucrose	20.0 g
Peptone	5.0 g
K_2HPO_4	0.5 g
$MgSO_4 .7H_2O$	0.25 g
Agar–agar	15 g
Distilled water	1 L

III.A.1.4. Nutrient agar medium:

Peptone	5 g
NaCl	5 g
Beef extract	3 g
Agar–agar	15 g
Distilled water	1 L

Adjust pH to 7.0 approximately.

III.A.1.5. Yeast extract mannitol (YEM) medium (Vincent, 1970):

This medium was used in host-range experiment for the growth of *Rhizobium leguminosarum* strains.

This medium consists of:

K_2HPO_4	5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
Mannitol	10.0 g
Yeast extract	0.5 g
$CaCl_2$ (anhydrous)	0.1 g
Agar-agar	15 g
Distilled water	1 L

Adjust pH to 6.8 – 7.2.

III.A.1.6. Starch-casein agar (Küster and Williams, 1964):

Starch	10.0 g
Casein	0.3 g
KNO3	2.0 g
NaCl	2.0 g
K_2HPO_4	2.0 g
$MgSO_4.7H_2O$	0.5 g
$CaCO_3$	0.2 g
Fe SO_4	0.01 g
Agar-agar	15 g
Distilled water	1L

Adjust pH to 7.0 – 7.2.

III.A.1.7. Starch-nitrate agar (Waksman, 1959):

Starch	20.0 g

KNO_3	2.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
NaCl	0.5 g
$CaCO_3$	3.0 g
Fe SO_4	0.01
Trace salt solution	1.0 ml
Agar-agar	15 g
Distilled water	1L

Adjust pH to 7.0 – 7.2.

Trace salt solution is adapted by Pridham *et al.* (1958) and **Mansour (1977)**. It is composed of:

$FeSO_4.7H_2O$	0.1 g
$MnCl_2.4H_2O$	0.1 g
$ZnSO_4.7H_2O$	0.1 g
Distilled water	1 L

III.A.1.8. Glycerol-asparagine agar (Pridham and Lyons, 1961):

Glycerol	10.0 g
L. Asparagine	1.0 g
K_2HPO_4	1.0 g
NaCl	0.5 g
Fe SO_4	0.5 g
Trace salt solution	1.0 m
Agar-agar	15 g
Distilled water	1 L

Adjust pH 6.8 – 7.2.

III.A.1.9. Starch-ammonium-sulphat agar (Küster, 1959)

Starch	20.0 g
$(NH_4)_2 SO_4$	2.0 g
NaCl	1.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	1.0 g
$CaCO_3$	2.0 g
Trace salt solution	1.0 ml
Agar-agar	15 g
Distilled water	1L

Adjust pH to 7.0 – 7.2.

III.A.1.10. Hutchinson medium (Crawford and Mc Coy, 1972):

Ca $(NO_3)_2$	2.5 g
K_2HPO_4	1.5 g
$CaCl_2$	0.1
$MgSO_4.7H_2O$	0.3 g
NaCl	0.1 g
$FeSO_4$	0.01 g
Distilled water	1.0 L

Filter paper strips 2 X 10 cm

Adjust pH to 6.8 – 7.2.

III.A.1.11. Oat meal agar (Küster, 1959):

Oat meal	20 g
Trace salt solution	1.0 ml
Distilled water	1.0 L
Agar-agar	15 g

Adjust pH to 7.0 – 7.2.

III.A.1.12. Yeast-Malt extract agar (Pridham *et al.*, 1956 – 1957):

Malt extract	100.0 ml
Yeast extract	4.0 g
Glucose	4.0 g
Distilled water	1.0 L
Agar-agar	15 g

Adjust pH to 7.0 – 7.2.

III.A.1.13. Czapek's solution agar:

Sucrose	20.0 g
$NaNO_3$	2.0 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4$	0.01 g
Distilled water	1.0 L
Agar-agar	15 g

Adjust pH to 6.8 – 7.2.

III.A.1.14. YM agar medium:

Yeast extract	3 g
Malt extract	3 g
Peptone	5 g
Glucose	10 g
Distilled water	1.0 L
Agar-agar	15 g

III.A.2. Microorganisms used in this study:

In this study, some microorganisms were collected from different origins for the study of host range and antimicrobial activities of selected

actinomycetes, selected fungus, selected mushroom waste and wheat straw. The origins of these microorganisms were indicated in the Table 2.

Table 3: The origins of microorganisms that used in host range of isolated phages antimicrobial activities during the current study:

Group	Microorganisms	Origin
Bacteria	*Ralstonia solanacearum* V1 (Yabuuchi *et al.,* 1995)	Current study
	R. solanacearum V4	
	R. solanacearum V6	
	R. solanacearum V9	
	R. solanacearum AV1	
	R. solanacearum AV 4	
	R. solanacearum AV 6	
	R. solanacearum AV9	
	Pseudomonas aeruginosa 15	Botany Department, Faculty of Science, Mansoura University
	Pseudomonas flourscens 40	
	Escherichia coli O157: H7	
	Escherichia coli 95	
	Pseudomonas flourscens 50	
	Erwinia carotovra carotonara 40	
	Erwinia carotovra carotonara 36	
	Rhizobium leguminosarum G	Genetics Department, Faculty of Agriculture, Mansoura University
	Erwinia amylovra (Burril, 1882 & Winslow *et al.,* 1920)	Plant disease Department, Faculty of Agriculture, Mansoura University
	Staphylococcus aureus	
	Bacillus subtilus (Ehrenberg, 1835)	
Fungi	*Rhizoctonia solani*	
	Fusarium oxysporum	
	Trichoderma viride	
Yeast	*Lipomyces starkeyi* (lodder and Kreger-Van Rij)	Microbiology Department, Faculty of Agriculture, Mansoura University
	Candida lipolytica	
	Saccharomyces cerevisiae El	

III.A.3. Essential oils:

Commercial essential oils were used in this study to select the most effective essential oil that antagonizes *Ralstonia solanacearum* (**Yabuuchi *et al.*, 1995)**.

Table 4: Tested essential oils for the control of *R. solanacearum*.

English name	Scientific name	Arabic name	Family
Anise	*Pimpinella anisum* L.	الينسون	Apiaceae
Thyme	*Thymis vulgaris* L.	الزعتر	Labiatae
Lettuce	*Lactuca sativa* L.	الخس	Asteraceae
Nigella	*Nigella sativa* L.	حبة البركة	Rumunculaceae
Spearmint	*Mentha piperita* L.	النعناع البلدي	Labiatae
Caraway	*Carum sativum* L.	الكراوية	Apiaceae
Rocket	*Eruca sativa* Mill.	الجرجير	Cruciferae
Onion	*Allium cepa* L.	البصل	Liliaceae
Garlic	*Allium sativum* L.	الثوم	Liliaceae
Eucalyptus	*Eucalyptus globules* L.	الكافور	Myrtaceae
Fenugreek	*Trigonella foenum -gracecum* L.	الحلبة	Leguminosae
Ground nut	*Arachis hypogaea* L.	الفول السوداني	Leguminosae
Fennel	*Foeniculum vulgare* Mill.	الشمر	Umbeliferae
Seasame	*Sesamum indicum* L.	السمسم	Pedoliceae
Dianthus	*Syzgium aromaticum* L.	القرنفل	Caryophylaceae
Marjoram	*Magarona hortensis* L.	البردقوش	Labiatae

III.B. METHODS:

III.B.1. Isolation, purification and identification of the causal pathogen (*Ralstonia solanacearum*, Yabuuchi *et al.*, 1995):

III.B.1.1. Isolation, purification and storage of *Ralstonia solanacearum*:

The pathogen was isolated from infected potato tubers (shown typical brown rot symptoms) that were collected from different locations at Dakahlia and Damietta Governorates. Tubers were washed in tap water and surface sterilized by soaking in 0.1 sodium hypochlorite solution for two minutes, then by rinsing twice in sterilized-distilled water. Tubers were sectioned and the white-slime bacterial ooze was obtained by pressing the sectioned potato tuber and it was suspended in sterilized-distilled water. The obtained bacterial suspension was streaked on TZC agar medium and the plates were incubated for 48 hours at 30 ºC **(Kelman, 1954** and **Adhikari, 1993)**. After the incubation pure culture of *R. solanacearum* can be obtained **(French *et al.*, 1993)**. Single colonies of virulent strain (white with red center "V" colonies) or avirulent strain (deep red color "AV" colonies) were picked up singly in sterilized distilled water (or tap water boiled to eliminate chlorine) in screw cap test tubes. Two loopfulls of 48 hrs old bacteria colonies grown on TZC agar medium are transferred into 5 ml sterile water. *R. solanacearum* was maintained at 4 ºC. It should be streaked on TZC agar every month **(French *et al.*, 1995)**.

III.B.1.2. Determination of biovar of *R. solanacearum*:

Determination of the biovar of *R. solanacearum* is based on the utilization of the disaccharides; cellobiose, lactose and maltose and the oxidation of the hexose alcohols; dulcitol, mannitol and sorbitol. Carbohydrate solutions of the used disaccharides and alcohols of

concentration 10 % were prepared. The used disaccharides are heat-liable, so they sterilized by filtration (through Millipore membrane). Whereas, the used hexose alcohols are relatively heat- stable, so they were sterilized by autoclaving at 110 ºC for 20 min. or by filtration.

To 90 ml molten basal medium cooled to about 60 ºC, 10 ml of carbohydrate solution was added in previously sterilized flask and was shaken well. Dispense 3 ml into previously sterilized test tubes. Inoculate 0.1 ml of virulent strain (48 hrs age in king medium, O.D. = 0.1 at 600 nm) into each tube. For each isolate, there are three replicate and control. Incubate the test tubes in 30 ºC and examine at 3, 7 and 14 days for change to acid pH (yellow colour) from the top downward. Hexose alcohol usually takes 3 – 5 days; disaccharides may take a few days longer **(French *et al.*, 1995)**.

III.B.1.3. Pathogenicity test by using indicator plant:

Inject the virulent strain of *R. solanacearum* suspension (48 hrs age in king medium, O.D. = 0.1 at 600 nm) into the stem of tomato seedlings (at the 3-leaf stage) just above the cotyledons (3 replicates for each *R. solanacearum* isolate). The absence of symptoms after growing the seedlings under controlled conditions for 4 weeks indicates the negative result **(Janse, 1988 and French *et al.*, 1993)**.

III.B.2. Phages:

III.B.2.1. Collection of soil samples:

Soil samples were collected from different fields of potato in El-Dakahlia and Damietta Governorates during the period of 15 December 2003 to 10 January 2004 according to procedure described by **Johnson *et al.* (1959)**. These soil samples were taken from the root zone of healthy potato plants (of age 60 – 80 days) at depth 15 – 20 cm depth (from bulk soil > 2 cm away from the potato root) directly into clean plastic bags.

Five samples (about 500 g each) were taken at random from each location. The samples were kept in a cool place during transportation to the laboratory. The soil samples from each location were brought together into one composite sample, thoroughly mixed and divided into several portions. These were used for isolation of actinomycetes and phages growing on virulent strain *R. solanacearum*.

III.B.2.2. Isolation of phages:

Soil samples (collected from root zone of healthy potato plants) were used for isolation of phages. An enriched procedure similar to that obtained by **Patel (1976)** was used in this study. Samples were grounded in a mortar with pestle and suspended in sucrose-peptone broth (SPB), kept in an incubator shaker at 30 ºC for one hour, and filtered through cotton cloth. Filtrate was inoculated with actively virulent *R. solanacearum* strain (that was grown in TZC medium which was incubated for 48 hrs at 30 ºC). Enriched mixture was freed from heavy particles by centrifugation at 4000 rpm for 30 minutes, and then filtered through millipore filter (0.450 µm). This cell-filterate was re-enriched for further two passages on the virulent strain of *R. solanacearum* culture (that was incubated for 48 hours). Then, the mixture was re-centrifuged (4000 rpm for 30 minutes). The supernatant was sterilized by filtration through millipore filter (0.450 µm). The presence or absence of phage(s) was detected by drop method technique **(Adams, 1959)**.

III.B.2.3. Detection of phages:

Two methods using a virulent strain of *R. solanacearum* (16 V) to detect phage(s):

III.B.2.3.i. Spot test method (drop method):

A volume of 0.1 ml from each filtrate was mixed with 0.1 ml of *R. solanacearum* (1V) (48 hrs age) and 3 ml of molten SPA agar (0.75 %) and the mixture was vortexed well. Mixture was overlaid on SPA agar plates and spotted with a drop of each phage suspension on the corresponding marked area. Plates were left to dry at room temperature with lid closed before incubation at 30 ºC for 24 hours. Sensitivity of *R. solanacearum* strains was scored according to **Anderson (1957)**. Positive plates were recorded and assayed by double layer agar technique.

III.B.2.3. ii. Plaque assay method:

This method was used for either detection and/ or titration of existing phages. Serial dilution technique was done for each filtrate that gives positive result in drop method technique. To 3 ml of molten soft (semisolid) SPA agar (kept at 45 ºC), 0.1 ml of 48 hrs broth cultures of *R. solanacearum* 1V were mixed together with 0.1 ml of each dilution, vortexed well together and poured onto basal SPA agar medium **(Adams, 1959).** After solidification of agar overlay, the plates were incubated inversely at 30 ºC for overnight or until development of plaques.

III.B.2.4. Phages purification:

Isolated phages were purified by six successive single passages. Phage stocks were obtained by infecting an exponentially growing plate culture of *R. solanacearum* 1V strain with sufficient phage to produce confluent lysis plate. Each phage was recovered by liquid medium suspension of the top agar layer and centrifuged at 4000 rpm for 30 minutes. Phage suspensions (lysates) were filtered through (0.450 μm) millipore filter membrane and stored in SPB (Sucrose-Peptone Broth) containing 0.5 % chloroform at 4 ºC. The phages were marked according

to their morphological appearance and dimensions that were determined by electron microscopy.

After five successive isolations of single plaques, phages were propagated in plates to give confluent lysis. The collected washes of these plates were treated with chloroform at a final concentration of 0.5 %, to give the phage stocks of high titers **(Jensen *et al*., 1998).**

III.B.2.5. Transmission Electron microscopy:

High concentration of phage suspensions (about 10^{12}) were spotted on carbon film picked up on copper grids, and then the phage particles were negatively stained by 2 % uranyl acetate. The grids were air dried for about 5 minutes. The phage particles were examined by transmission electron microscope (TEM) (Jeol Jem 1010 electron microscope, at Regional Centre of Mycology and Biotecnolgy, Al-Azhar University, Cairo, Egypt).

III.B.2.6. Host range of isolated phages:

Phage host range was established by using spot test method (drop method) **(Chopin *et al*., 1976)**. The plate inoculum has consisted of 3 ml of soft agar mixed with 100 µl of 48 hrs culture of the tested isolate. This mixture was briefly vortexed and spread onto the surface of solidified suitable agar plate; single drops of each phage lysate were spotted onto the inoculated agar plates and the plates were left for 30 minutes for drying, then they were incubated overnight at 30 ºC. Bacterial sensitivity to the phages was established by bacterial lysis at the spot where the phage lysate drop was deposited. Phage spotting was replicated three times for each phage and each bacterial strain. Positive spot test s were confirmed by plaque assay method using diluted phage preparation.

III.B.2.7. Isolation of lysogenic bacteria:

III.B.2.7. i. Spot method: Each phage stock was spotted on King medium plates of freshly seeded *R. solancearum* and incubated for 7 days at 30 ºC. Colonies developed on the lytic area were isolated and purified by colonial isolation. These isolated pure colonies were streaked onto the deferential medium (TZC) to confirm that these colonies were virulent *R. solanacearum*. Spot tests of these lysogens onto lawns of *R. solanacearum* V1 showed zones of lysis, indicating the presence of lysogenic bacteria. The immunity of these lysogens was tested against the four isolated phages.

III.B.2.7. ii. Plaque assay: The plaque assay method was applied for each phage lysate of suitable dilution by using king medium. The plates were incubated at 30 ºC for 7 days. The production of turbid plaque mutation suggested that the phage was temperate phage. The lysogens could be isolated from the center of these turbid plaques **(Jarell *et al.*, 1992).** These lysogens were streaked on TZC medium for purity and for detection of the presence of virulent strain of *R. solanacearum*. As mentioned before, spot tests of these lysogens onto lawns of *R. solanacearum* V1, and the immunity of these lysogens against the four isolated phages were applied.

III.B.3.Actinomycetes:

III.B.3.1. Isolation of actinomycetes:

Soil samples were usually air-dried at room temperature. The dried samples were gently powdered with mortar and stored in freezer until further use **(Grace, 1989)**. Soil suspensions were usually made by stirring 10 g of air-dried soil in 90 ml of sterilized saline solution. Serial dilutions were made to the supernatant after the suspension had been allowed to stand for a period of half hour.

Actinomycetes were generally isolated using dilution plate method, where 0.1 ml of 10^{-3}, 10^{-4} and 10^{-5} dilution was transferred to solidified starch-casein plates (having mycostane as antifungal agent) and were distributed by sterilized glass spreader. The plates were incubated at 28 ºC for 7 days. Colonies were isolated and purified by streak plate technique.

III.B.3.2. Investigation of antagonistic activity of Actinomycetes isolates against *R. solanacearum*:

The methods adapted by **Waksman (1967)** and **Mansour (1972 & 1979)** were used.

III.B.3.2.i. Double layer method:

This antagonism was done by double layer method. Only one loop of each actinomycete isolate was seeded in the centre of solidified sucrose-peptone agar (SPA) plates. These plates were incubated at 30 ºC for 5days. Virulent culture of *R. solanacearum* V1 isolate (incubated at 30 ºC for 48 hrs in SPA liquid medium, O.D. = 0.1 at 600 nm) was prepared. Aliquot of 0.1 ml of the previous bacterial suspension was inculcated in a test tube containing 3 ml molten semi-solid SPA medium. The inoculated tubes were vortexed well and poured onto the plates seeded by actinomycetes and rotated gently by palm to homogenous distribution of the soft agar onto the plate. Three replicates were used for each actinomycetes isolate. The plates were incubated at 30 ºC for 24 hrs. The presence of clear zone around the actinomycetes growth (colonies) indicates the positive antagonistic activity of the actinomycete isolate versus *R. solanacearum*. The diameters of clear zones and growth of actinomycetes were measured. The ratio between clear zone diameter and growth diameter of actinomycetes were calculated and considered as

a function for the magnitude of antagonistic activity of the various isolates.

II.B.3.2.ii. Disc agar method:

Each actinomycete isolate was cultured on solidified plates of starch casein agar medium and incubated at 28 ºC for 7 days. Discs of each actinomycete isolate of diameter 9 mm were prepared by sterilized cork borers. The actinomycetes discs were appropriately placed onto the centre of solidified SPA plates freshly seeded with *R. solanacearum*. Three replicates were prepared for each treatment. The plates were incubated at 28 ºC for 24 hours. The inhibition zones were measured (in millimeters); the width of the inhibition zones is considered as a function of the magnitude of antagonistic activity **(Mansour, 1979)**.

III.B.3.2.iii. Ability of actinomycetes to produce volatile compounds on agar (Fumigation method):

This experiment was applied as the method described by **El-Trabily *et al.* (1997)** with some modifications. The solidified SPA plates were seeded by *R. solanacearum* by using semisolid medium as mentioned before. Discs of 9 mm diameter of each tested actinomyctes (of 7 days age) were prepared. One disc of each actinomycetes was placed on the center of the lid of the plate and the Petri dish was converted. Each plate was taped by double layer of Parafilm (Amrican National Can TM, Greenwish, CT 06836, USA). The control plates were prepared in the same way except that the discs of actinomycetes were not placed on the lid of the Petri dish. All plates were incubated at 28 ºC for 24 hours. The inhibition zones, if present, were recorded.

III.B.3.3. Methods for characterization and identification of actinomycete isolates:

III.B.3.3.1. Cell wall analysis:

III.B.3.3.1.i. Determination of isomers of 2, 6 – diaminopimelic (DAP) acid:

The occurrence of LL-, DL- or hydroxy-DAP was ascertained by thin layer chromatography of whole cell hydrolyzates. The following method is a modification of that described by **Backer *et al*. (1964)** and **Lechvalier and Lechvalier (1970 a)**.

1 mg of dried cells of each organism was hydrolyzed with 1 ml 6 N HCL in a sealed pyrex tube held at 100 ºC for 18 hr. After cooling, each sample was filtered through filter paper and washed with 1ml H_2O. The filtrate is dried two or three times at 40 ºC to remove the most of the HCL. The residue was taken in 0.3 ml in H_2O and then 5 µl was spotted on the thin layer plate coated with cellulose. For separation of amino acids, the following solvent system is used; n-butanol: acetic acid: water (60: 15: 25 V/ V). Amino acids are detected by spraying the chromatograme with 0.1 % W/ V) ninhydrin solution in acetone followed by heating for 2 minutes at 100 ºC. DAP appeared as coloured spots.

III.B.3.3.1.ii. Whole cell analysis and whole cell sugar composition of actinomycetes isolates (modified method of Lechvalier and Lechvalier, 1970 b):

Dried cells (50 mg) are hydrolyzed in 1 ml of 2N H_2SO_4 in a sealed pyrex tube at 100 ºC for two hours. The pH of hydrolyzate was adjusted to pH 5.0 to 5.5 with saturated solution of $Ba(OH)_2$**.** The precipitate of $Ba(OH)_2$ is centrifuged at 6000 rpm and the supernatant fluid was removed by a Pasteur pipette and evaporated at 40 ºC. The residue was taken in 0.4 ml H_2O, and then 5µl were then spotted on a thin-aluminum

crades (0.2 mm thickness) for separation of sugars. The used solvent mixture was n-butanol: acetic acid: water (60: 15: 25 V/ V).

The plates were chromatographed two consecutive times with intermediate air drying. After the second run, the sugar are detected by spraying with aniline-phthalic acid reagent (9.2 ml of aniline, 10g phthalic acid, 490 ml n-butanol, 490 diethyl ether and 20ml of water) **(Harborne, 1973)**. The plates were air dried and then heated at 105 ºC. After 2 – 5 minutes, the monosaccharides appeared as coloured spots. Reference sugars were prepared at a concentration of 0.1 % for each sugar.

III.B.3.3.2. Morphological studies:

III.B.3.3.2.i. Morphology of spore chain:

The direct microscopic method of agar cultures described in "Methods Manual of the International Cooperative Project for Description and Deposition of Type Culture of Streptomyces **(Shirling and Gottelieb, 1966)** was applied for the studying the morphological features of sporophores. Spore suspensions of the tested isolates were streaked over the surface of starch-casein agar plates and incubated at 28 ºC. The cultures were microscopically examined after 7 and 14 days. Microphotographs were taken using microscope film camera device Karel-zeise-Jena.

III.B.3.3.2.ii. Electron microscopy of spore chain:

The spore-print technique **(Trenser *et. al.*, 1961)** was applied to prepare materials for electron microscopy. Grides with collodion films were generally pressed over the sporulation surface of culture of age about 5 days grown on starch-casein agar or starch-nitrate agar media. Grides were fixed with osmium oxide and the dehydrated through serial dilution of ethanol and finally in acetone. Grids were placed in a critical

point dryer model EMS 850 and then coated with gold using SPI Module™ Sputter coated model 11430. The spore surface was examined by electron microscope (Joel JSM 5500 LV scanning electron microscope, Regional Center of Mycology and Biotecnolgy, Al-Azhar University, Cairo, Egypt) at different magnifications from 3000 to 20000. Spore surfaces were classified according to **Trenser *et. al*. (1961)** and **Dietz and Mathews (1971)** as; smooth, warty, spiny, hairy or rugoses.

III.B.3.3.3. Cultural properties of the isolates on different media:

The cultural properties of the experimental isolates were studied on slant cultures of six media namely; starch-nitrate agar, starch-ammonium-sulphate agar, glycerol-asparagine agar, oat-meal agar, yeast-malt agar and Czapek's solution agar.

The cultural properties involved the determination of the colour of aerial mycelium (spore colour in mass), colour of the substrate mycelium (reverse side of colony) and colour of diffusible pigment (colour in media).

III.B.3.4. Physiological properties of the isolates:

III.B.3.4.1. Utilization of different carbon sources:

The ability of tested isolates to utilize different carbon sources (D-glucose, D-fructose, mannose, D-xylose, sucrose, mannitol, arabinose, rafinose, D-galactose, L-rhaminose, L-insitole, lactose, glycerol and starch). These carbon sources were studied on Gauzes No.1 as a basal medium. The tested carbon sources were added singly at a concentration of 20 g/L. Tested tubes containing 2 ml of these media were inoculated with spore suspension of the studied isolates and incubated at 28 ºC. Growth intensity that was recorded after 7 and 14 days of incubation was considered as a criterion of the ability of organisms to utilize the tested carbon sources.

III.B.3.4.2. Production of melanin pigments:

Actinomycete isolates were cultivated on glycerol-tyrosine agar, peptone-yeast-iron agar and tryptone-yeast broth and incubated at 28 ºC as described by **Shirling and Gottleib (1966)**. The production of brown or dark brown soluble pigments at early stages of growth indicated positive melanin production.

III.B.3.4.3. Cellulose decomposition:

Test tubes containing strips (2 X 10 cm) of Whatman filter paper No. 1 and 10 ml of Hutchinson liquid medium were inoculated with the studied isolates. The inoculation was carried out by transferring a loop of spore suspension to the surface of the filter paper, as near as possible to the level of the liquid medium. The filter paper strips were examined after 15, 21, 30 and 45 days of incubation at 28 ºC. The destruction or rupture of paper was considered as indicator of positive ability of the organism to decompose cellulose **(Mansour, 1977)**.

III.B.3.5. Antimicrobial activities of the selected four actinomycetes isolates:

Antimicrobial activities (antagonistic activities or antagonistic effects) of studied actinomycetes isolates were determined by "agar disc method". These isolates (A11, A36, A39 and A84) were seeded on solidified plates of starch casein agar medium and incubated at 28 ºC for 7 days. Discs of each actinomycete isolate of diameter 9 mm were prepared by sterilized cork borers. One disc was transferred into each solidified plate to study its antagonistic activity. The actinomycetes discs were placed on the center of solidified nutrient agar medium plates seeded by bacteria and on the center of solidified YM agar plates seeded by yeast (Table 2). Whereas, the actinomycetes discs were placed on apart from the periphery of solidified PDA agar medium plates by 10

mm, where, there was one disc of used fungus (0.9 mm in diameter that had been incubated at 25 ºC for 7 days) on the opposite direction (at the same axis) apart from the periphery of the solidified PDA agar plates by the same distance. Three replicates were prepared for each treatment.

The plates of bacteria and yeast were incubated at 28 ºC for 48 hours. Those of fungi were incubated at 25 ºC for 7 days. Finally, the inhibition zones were measured in millimeters.

III.B.4. Essential oils.

III.B.4.1 Evaluation of antimicrobial activity of some essential oils towards *R. solanacearum*.

III.B.4.1.i Filter paper disc method:

Members of 16 commercial essential oils (as listed in Table 3) (obtained from Badawaya Company for oils, Mansoura, Egypt) were tested for their antimicrobial action versus *R. solanacearum* using filter paper disc method **(Mansour, 1977 and Murray *et al*., 1995)**. Solidified SPA plates that were seeded by *R. solanacearum* 1V by mixing 0.1 ml of culture (incubated for 24 hours at 28 ºC, O.D. = 0.1 at 600 nm) with 3 ml liquefied semi-solid SPA, vortexing well, pour on the surface of the plate and distributing well. Filter paper discs (Whatman No. 1, 5 mm diameter containing 5 µL of essential oil) were applied to the surface of the previously prepared plates. The plates were incubated at 28 ºC for 24 hours. The diameters of inhibition zones were measured by millimeters.

III.B.4.1.ii. Fumigation method:

Solidified SPA plates were seeded by *R. solanacearum* by using 3 ml liquefied semisolid SPA (its temperature was about 45 ºC) inculcated by 0.1 ml *R. solanacearum* (its age was 24 hours; about 10^6 CFU/ ml). The seeded plates were left to dry for about 30 ºC. The plates were kept inverted and 0.1 ml of the tested essential oil was placed on the lid of

each plate. Each plate was taped by double layer of Parafilm® (American National Can TM, Greenwish, CT 06836, USA). The control plates were prepared in the same way except that the essential oil was not placed on the lid of the Petri dish. All plates were incubated at 28 ºC for 24 hours. The inhibition zones were recorded **(Reuveni *et al.*, 1984)**.

III.B.4.1.iii. Determination of minimal inhibitory concentration (MIC):

The minimum inhibitory concentrations for the best four essential oils were determined by using the agar dilution method **(Hammer *et al.*, 1999)** with some modifications. A final concentration of 0.5% (v/v) Tween-80 was incorporated into the agar after autoclaving to enhance oil solubility. A series of two folds dilutions of each essential oil, ranging from 2 % (v/v) to 0.25 % (v/v), was prepared in SPA medium with 0.5% (v/v) Tween-80. Plates were dried prior to inoculation with *R. solanacearum*. Inoculation was carried out by two methods;

1. Placing a 0.9 mm diameter disc of 24 hours age *R. solanacearum* growtn on SPA medium on the middle of the prepared plates.
2. Streaking *R. solanacearum* by sterilized loop from liquid culture onto the prepared plates.

Plates of SPA medium, with 0.5% (v/v) Tween-80 and without oil, was used as a positive growth control. Inoculated plates were incubated at 28 °C for 24 hrs. The MICs were determined as the lowest concentration of the oil inhibiting the visible growth of the bacteria on the agar plate. The presence of one or two colonies was disregarded.

III.B.4.2. Antimicrobial activities of selected essential oil:

Antimicrobial activities of the selected essential oil (having the lowest value of MIC) against some collected microbes (Table 2) were studied by filter paper disc method. The filter paper discs were placed on

the centre of plates seeded by bacteria and yeasts, while they placed apart from periphery of plates and on the opposite direction, at the same axis of plate, the disc of fungus (9 mm diameter, 7 days age) was placed. The diameter of inhibition zones were measured in millimeters after the same incubation periods and temperatures shown previously.

III.B.5. Effect of mushroom spent straws, raw wheat straw and rice straw on the growth of *R. solanacearum*:

III.B.5.1. Collection of mushroom wastes wheat straw and rice straw:

The mushroom wastes were kindly provided by Dr. Amira Ali El-Fallal, (Botany Department, Faculty of Science at Damietta, Mansours University) directly after the cropping of three *Pleurotus* species (*P. columbinus, P. pulnonarius and P. floridanus*) on wheat straw and rice straw. The superficial layers of *Pleurotus* wastes were removed and the samples were collected from 5 – 10 cm depth. Five samples were taken for each treatment. These samples were collected aseptically in clean plastic bags. The samples of each treatment were mixed together to form composite sample which divided into separate samples. The samples were stored in cool place.

The wheat straw and rice straw were obtained from the same straws used as mushroom bed of *Pleurotus* (i.e. straw before seeding mushroom). These were also kept in cool place in clean bags.

III.B.5.2. Preparation of water extracts of wastes and straws:

Samples were grounded well to be fine powder. Sterilized distilled water was added to the fine powder of each sample in a ratio 20: 1 (volume: weight) in a clean dry flask. The flasks were shaken at speed 200 rpm for 24 hours. The water solutions were filtered by double layer of clothes. The supernatants were centrifuged at 4000 rpm for 30 minutes. The supernatants were divided into two portions for each treatment. One

portion was sterilized by filtrations through fritted glass filter and the other portion was sterilized by autoclaving. The sterilized water extracts were kept at 4 °C.

III.B.5.3. Estimation the antibacterial activity of water extracts against *R. solanacearum*:

The antibacterial activity of water extracts ─ of *Pleurotus columbinus* spent wheat straw (WE-PCSWS) of and water extract of wheat straw (WE-WS) ─ against *R. solanacearum* was estimated by filter paper disc method as mentioned before with essential oils.

III.B.5.4. Antimicrobial activity of the water extracts having the best inhibitory effect against *R. solanacearum*:

The antimicrobial activity of selected water extracts were preformed by filter paper disc method against the same microbes used before by selected essential oil and isolated actinomycetes.

III.B.6. Basidiomycetes:

III.B.6.1. Antagonistic effect of some basidiomycetes against *R. solanacearum*:

The antagonistic effects of some species of basidiomycetes were tested by "agar disc method" as adapted and described by **Mansour *et al.* (1997)**. Nine basidiomycetes species were kindly provided by Dr. Amira Ali El-Fallal were used in this experiment. These basidiomycetes were *Cyathus stercoreus* Egyptian strain, *Agaricus campester* Egyptian strain, *Podaxis pistillaris* Egyptian strain, Corticium *vellerum* Egyptian strain, *Pleurotus columbinus*, *Volvariella volvaceae*, *Pleurotus sajor – caju*, *Letinus striatus*. These fungi were incubated at 25 °C for 14 days. Discs of diameter length 9 mm were prepared for each tested fungal species. Moreover, plates seeded by *R. solanacearum* were prepared. Discs of fungal species were placed at the middle of seeded plates of *R.*

solanacearum. Three replicates were prepared for each fungal species. These plates were incubated at 28 °C for 24 hours. The inhibition zone diameters were measured (if it was present) and the main value were calculated for each treatment. The fungal species having the longest diameter of inhibition zone was selected to further study.

III.B.6.2. Antimicrobial activity of the selected fungal species:

The antimicrobial activity of the selected fungal species was preformed by disc agar method. Discs of diameter 0.9 mm (prepared from plates incubated for 14 days at 25°C) were prepared. The discs of selected fungal species were placed in solidified plates of nutrient agar, YM agar and PDA to determine the inhibition zone diameters as described before with the antimicrobial activities of four selected actinomycetes in III.B.3.6.

III.B.7. Antagonism between Actinomycete A11 (*Streptomyces mutabilis*) and *Cyathus stercoreus* Egyptian strain:

This antagonism was tested as mentioned in III.B.3.6.1 in by agar disc method.

III.B.8. Greenhouse experiment:

This experiment was carried out at the farm of Faculty of Agriculture, Mansoura University, Egypt during the period of 3 February to 25 May 2005 at open air. The plastic sacs of 30 cm diameter were filled with 15 kg non-sterilized soil. This soil was mixture of clay and sand 1:1 (w: w). Six replicates were prepared for each treatment. This experiment was conducted according to complete randomized design.

III.B.8.1. Soil infestation:

Soils of each plastic sac were soaked with water and left to dry for 72 hours. The sacs were infested by *R. solanacearum* (about 10^6 CFF

O.D. 0.1 at 600 nm); 100 ml for each sac. The sacs were left for about 48 hours before sowing.

III.B.8.2. Preparation of biological control variants (treatments):

The used treatments were located under the following main categories:

1. **Phage cocktail (PhC)**: This cocktail was composed of mixture of the four isolated phage (RSP4, RSP5, RSP6 and RSP7) with a ratio 1: 1: 1: 1 (v: v: v: v), each phage suspension was prepared with a concentration of approximately 10^8. So, the final concentration of phage cocktail was about 10^{24}.
2. ***Streptomyces mutabilis* (SM)**: spore suspension was prepared by incubate *S. mytabilis* at 28 °C for 21 days and adjust the concentration to 10^9.
3. ***Cyathus stercoreus* Egyptian strain (CSE):** The fungus was inoculcated on solidified PDA plates in Petri dishes of 9 cm diameter and incubated at 28 °C for 14 days. Each plate was mixed with 1 litter sterilized tap water to prepare fungus suspension.
4. **Anise oil (AO):** Two concentrations (0.25 % and 0.5 %) were prepared and few drops of tween 80 were added for complete distribution of oil in water.
5. ***Pleurotus columbinus* spent wheat straw (PCSWS):** Fine powder of this waste was prepared. Moreover, water extract of 5 % concentration was prepared as mentioned before.
6. **Wheat straw (WS):** Fine powder and 5 % water extract was prepared as mentioned before.
7. **PhC + SM**: Suspensions of PhC and SM were mixed together by ratio 1: 1 (v: v).

8. **PhC + CSE**: Suspensions of PhC and CSE were mixed together by ratio 1: 1 (v: v).
9. **SM + CSE**: Suspensions of SM and CSE were mixed together by ratio 1: 1 (v: v).
10. **PhC + SM + CSE**: the three suspensions were mixeg by ratio 1: 1: 1 (v: v: v).

Potato pieces were soaked in PhC, SM, CSE and their combinations and also the two concentration of AO emulsion and filtrates of PCSWS and WS for 10 minutes before sowing. Also, the plants after 70 days of sowing were drenched by these suspensions, emulsion or filtrates; each treatment drenched by the corresponding one.

The fine powders of PCSWS and WS were used in two methods:

1. Coating the potato pieces at sowing time as thin film.
2. Putting 1g of the fin powder in the hole of sowing near the potato pieces.

After 70 days of sowing 1g of corresponding fine powder was put on the soil surface near the plants for both above mentioned treatments 1 and 2.

The treatments of this experiment were 31 treatments (as shown in results). Each treatment had 6 replicates.

III.B.8.3. Storage of potato tubers after harvest:

In order to show the latent infection of brown rot disease, after harvest, the non-infected tubers were stored at room temperature (20 – 26 ºC) for 60 days. The tubers were examined for the presence of the studied disease at the end of the storage period. The percentage of infected tubers was calculated for each treatment after storage period and also percentage of total infected tubers for each treatment was calculated.

III.B.9. Field experiment:

The experiment was carried out at the farm of Kfr-El-Baramon research station of, Mansoura Horticulture Station, El-Dakahlia, Egypt during the period between 29 January to 25 May 2005.

III.B.9.1. Detection of presence of *R. solanacearum* in the selected field:

The selected field had history of brown rot disease. To confirm the presence of *R. solanacearum* in this field, soil samples were taken from the field as described before. Serial dilutions were applied for the composite sample of this soil and 0.1 ml of 10^{-4} dilution was spotted and spread on the surface of TZC solidified plate. Three replicates were prepared. After about 48 and/or 72 hours number of colony of *R. solanacearum* was recorded for each replicate and the mean value were calculated.

III.B.9.2. Experiment design:

This experiment was conducted during the period between 29th February and 20th May 2005 in order to investigate the ability of phage cocktail (PhC) of lytic phages against *R. solanacearum* and *S. mytabilis* (SM) and their combination (PhC + SM) to control bacterial wilt disease of potato under field conditions.

PhC, SM and PhC + SM suspensions were prepared as mentioned before in greenhouse experiment. Also, these suspensions were used as the same manner at sowing time and after 70 days of sowing. The control plant had no treatments at sowing time or after 70 days of sowing.

Each treatment was replicated in three plots. Each plot consisted of three lines; each line had 5 meters length and 75 cm width. The distance between tubers was 25 cm and the number of tubers in each line was 20 tubers.

Moreover, the 30 tubers of progeny were stored after harvest at room temperature for 60 days. At the end of storage period, percentage of infected tubers for each treatment was calculated.

III.B.10. Statically analysis:

Data were analyzed with the Statistical Analysis System **(SAS Institute, 1988).** All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using least significant differences (LSD) at P =0.05 and Duncan's multiple range test **(Duncan, 1955).**

IV. RESULTS

IV. 1. Isolation of *R. solanacearum*:

Potato tubers, infected with brown rot disease, were collected from different field locations in Dakahlia and Damietta Governorates (Table 5). In Dakahlia Governorate, the infected potato tubers were collected from eight villages (El-Baklia, Salka, Batra, Manshaat-Badawy, El-Tawila, Diast, El-Mahmoudia and Manshaat-Abd-El-Rahman) that belong to four centres (Mansoura, Talkha, Dekernis and Aga). While, in Damietta Governorate the infected potato tubers were collected from two villages (Kafr El-Battikh and El-Tawfikia) that are locate in Kafr Saad centre. These tubers were belonging to three cultivars of potato (sponta, diamont and cara).

When these infected tubers were sectioned, the symptoms of brown rot were shown as a brown ring (Photo 1). After surface sterilization, sections of infected tubers were pressed for obtaining the bacterial ooze. The bacterial ooze was suspended and stored in sterilized distilled water in screw cap test tube at 4 ºC. These bacterial suspensions were streaked on TZC agar medium to differentiate between virulent (V) and avirulent (AV) strains of *R. solanacearum*. Virulent (V) strain gives white with red center colonies, while AV strain gives deep red colonies. Single colonies of each strain were picked up and re-streaked on TZC medium to obtain pure culture (Photo 2). Virulent (V) strain of each isolate were re-suspended in and stored in sterilized distilled water in screw cap test tube at 4 ºC.

These V isolates were tested for utilization of disaccharides and oxidation of alcohols to determine the biovar of *R. solanacearum*. All V isolates had the same ability to utilize the used disaccharides (maltose, lactose and cellobiose) whereas all of them did not oxidize

Table 5: Isolation and characterization of *Ralstonia solanacearum* strains from different locations of Dakahlia and Damietta Governorates.

Location	Governorate	Potato cultivar	virulent Isolate	Utilization of disaccharides			Oxidation of alcohols			Pathogenicity test
				Cellobiose	Lactose	Maltose	Dulcitol	Mannitol	Sorbitol	
El-Baklia – Mansoura	Dakahlia	Sponta	V1	+	+	+	—	—	—	+++
Salka – Mansoura	Dakahlia	Diamont	V2	+	+	+	—	—	—	+
Batra – Talkha	Dakahlia	Sponta	V3	+	+	+	—	—	—	++
Manshaat–Badway – Talkha	Dakahlia	Sponta	V4	+	+	+	—	—	—	+++
El-Tawella – Talkha	Dakahlia	Cara	V5	+	+	+	—	—	—	+
Diast – Talkha	Dakahlia	Sponta	V6	+	+	+	—	—	—	+++
El-Mahmodeia – Dekrnis	Dakahlia	Cara	V7	+	+	+	—	—	—	+
Manshaat-Abd-El-Rahman – Aga	Dakahlia	Diamont	V8	+	+	+	—	—	—	++
Kafr El-Battikh – Kafr-Saad	Damietta	Sponta	V9	+	+	+	—	—	—	+++
El-Tawfikia – Kafr-Saad	Damietta	Sponta	V10	+	+	+	—	—	—	++

Where; V: Virulent strain of *R. solanacearum*, the ability to utilize disaccharides and oxidize hexose alcholols producing acid when positive (+) and Pathogenicity test; +: Weak symptoms, ++: Moderate Symptoms, +++: acute symptoms

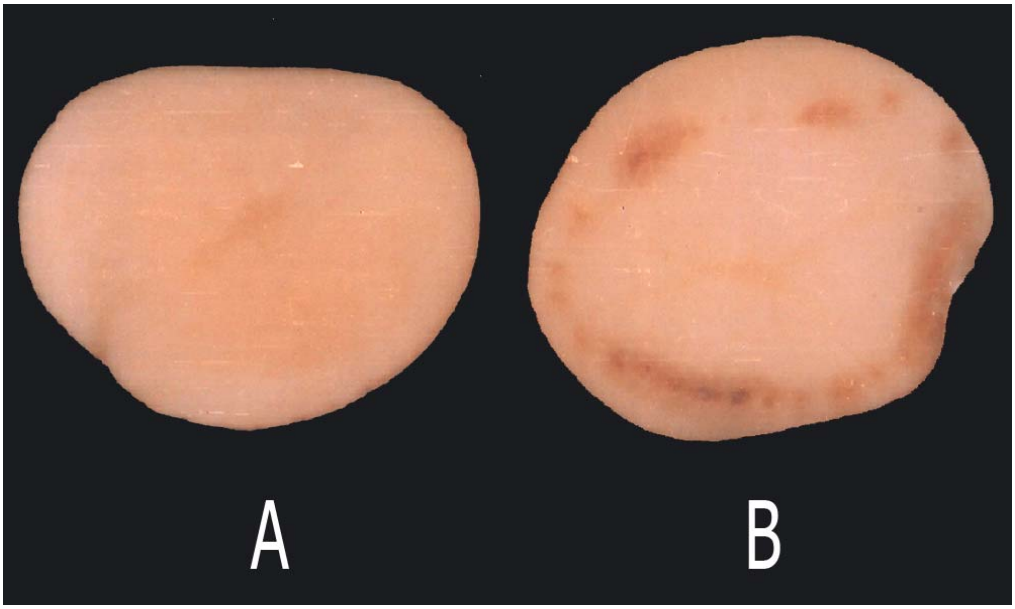

Photo1: Sectioned potato tubers showing brown rot disease; where A: Healthy sectioned potato tuber and B: Infected sectioned potato tuber shown brown ring.

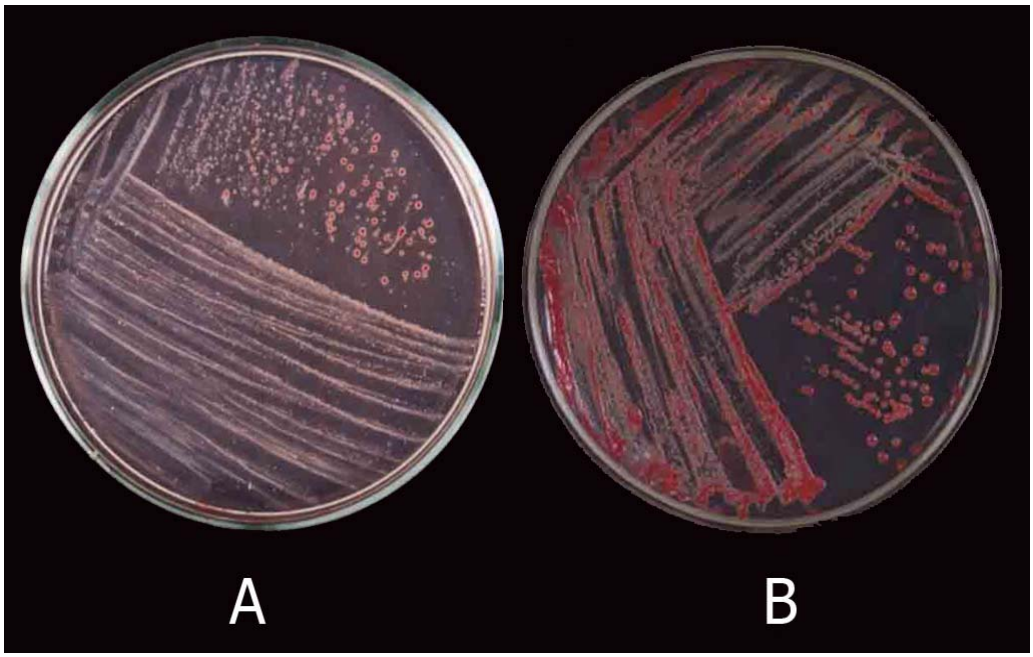

Photo 2: Appearance of *Ralstonia solanacearum* on TZC medium, where; A: Virulent (V) strain having white with red centre colonies. B: Avirulent (AV) strain having deep red colonies.

the used alcohols (sorbitol, mannitol and dolcitol). Therefore, all V isolates belong to biovar 2 which equivalent to race 3.

Injection the V isolates of *R. solanacearum* suspension (48 hrs age in king medium, O.D. = 0.1 at 600 nm) into the stem of tomato seedlings (at the 3-leaf stage) just above the cotyledons (3 replicates for each *R. solanacearum* isolate) resulted in presence of wilting symptoms. The degree of wilting symptoms was varied from one isolate to another. Weak symptoms resulted with three isolates (V2, V5 and V7), moderate symptoms resulted with three isolates (V3, V8 and V10), whereas, severe symptoms resulted with four isolates (V1, V4 and V6 and V9). Therefore, the last 4 isolates are the most aggressive isolates. V1 isolate was selected in the following experiments for further studies.

IV. 2. Phages:

IV. 2.1. Isolation of lytic phages growing on virulent strain of *R. solanacearum*:

Ten soil samples, collected from the soil of root zone of healthy potato plants from fields in Dakahlia and Damietta Governorates, were used for the isolation of phages that are active against virulent strain of *R. solanacearum* (Table 6). When the drop method had applied for the filtrates of these soil samples, the lytic areas had formed with only 4 of them. Lytic areas were formed with 2 filtrates of soil sample from Dakahlia Governorate (Salka, El-Mahmodeia) that belong to two centres (Mansoura and Dekernis). In addition, the lytic area had formed with the filtrate of soil sample of "Kafr El-Battikh" that belongs to "Kafr-Saad" centre, Damietta Governorate. Whereas, the other 6 soil samples did not form lytic area. Therefore, the four samples that formed lytic areas contained one or more lytic phage growing on virulent strain of *R. solanacearum*.

Table 6: Isolation of lytic phages, growing on the virulent strain of *R. solanacearum*, from the root zone of healthy potato plants.

Location	Governorate	Formation of lytic area in bacterial lawns	Phage
El-Baklia – Mansoura	Dakahlia	+	RSP4 & RSP5
Salka – Mansoura	Dakahlia	+	RSP4, RSP5 & RSP6
Batra – Talkha	Dakahlia	—	—
Manshaat–Badway – Talkha	Dakahlia	—	—
El-Tawella – Talkha	Dakahlia	—	—
Diast – Talkha	Dakahlia	—	—
El-Mahmodeia – Dekrnis	Dakahlia	—	RSP6 & RSP7
Manshaat-Abd-El-Rahman – Aga	Dakahlia	—	—
Kafr El-Battikh – Kafr-Saad	Damietta	+	RSP4& RSP6
El-Tawfikia – Kafr-Saad	Damietta	—	—

+: Presence of lytic areas. —: Absence of lytic areas

The application of plaque assay technique indicated that the occurrence of four phages (RSP4, RSP5, RSP6 and RSP7) active against selected virulent strain of *R. solanacearum* in the soil filtrates. The soil filtrates of three locations (El-Baklia, El-Mahmodia and Kafr El-Battikh) had two different phages (RSP4& RSP5, RSP6 & RSP7 and RSP4& RSP6 respectively), while only the filtrate of Salka location had three phages (RSP4, RSP5 & RSP6).

IV. 2.2. Plaques morphology of the four isolated phages:

Single plaque technique had been applied many times until obtained purified plaques. The plaques of the four isolated phages were different in the size and shape, but all of them are clear plaques (Photos 3 & 4 and Table 7). The plaques of RSP4 phage were the smallest plaques of the four tested phages; their plaque diameter 0.5 mm (pinpoint). On the other hand, the plaques of RSP6 phage were the largest plaques of the four tested phages; their diameters were 6 mm. Where, the diameters of plaques of RSP5 phage were medium plaques 4 mm and those of RSP7 phage were minute 2 mm. The shape of RSP7 phage was tetragonal (quadrate) whereas the other three phages had circular plaques.

IV. 2.3. Electron microscopy of the four isolated phages:

The examination of the four isolated phages indicated that all of them had tails (Photo 5 and Table 8); therefore they belong to the order *Caudovirales*. The phage RSP4 had short tail, so it belongs to the *Podoviridae* family or Baradly's group C. On the other hand, the other three phages had long flexible non-contractile tails, thus they can be classified into the *Siphoviridae* family or Baradly's group B.

All of the four-isolated phages had polygonal head. The head of RSP7 is the largest one (96.67 X 103.33 nm), whereas the dimensions of

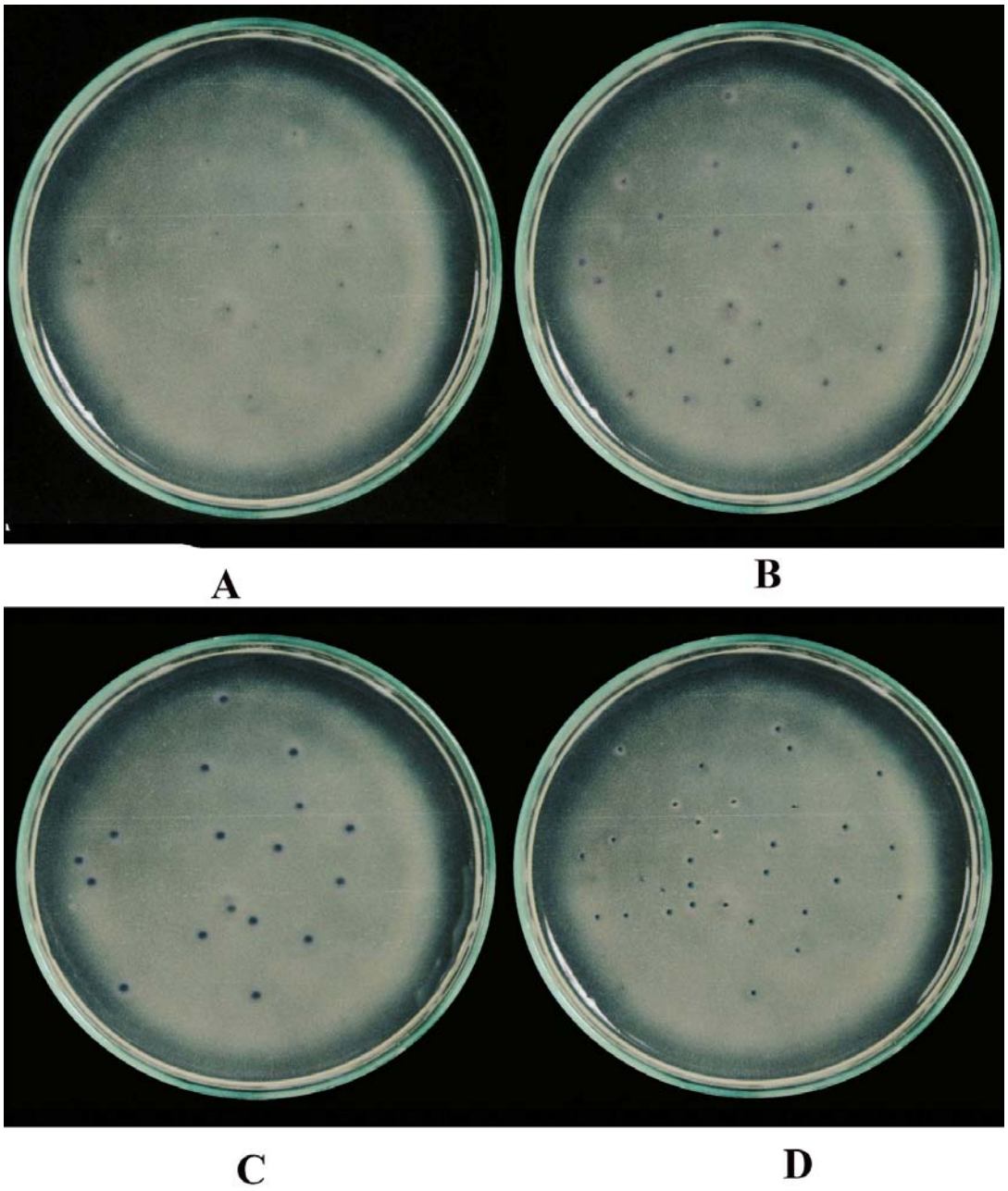

Photo 3: Plaques morphology of isolated phages; A: RSP4, B: RSP5, C: RSP6 and D: RSP7

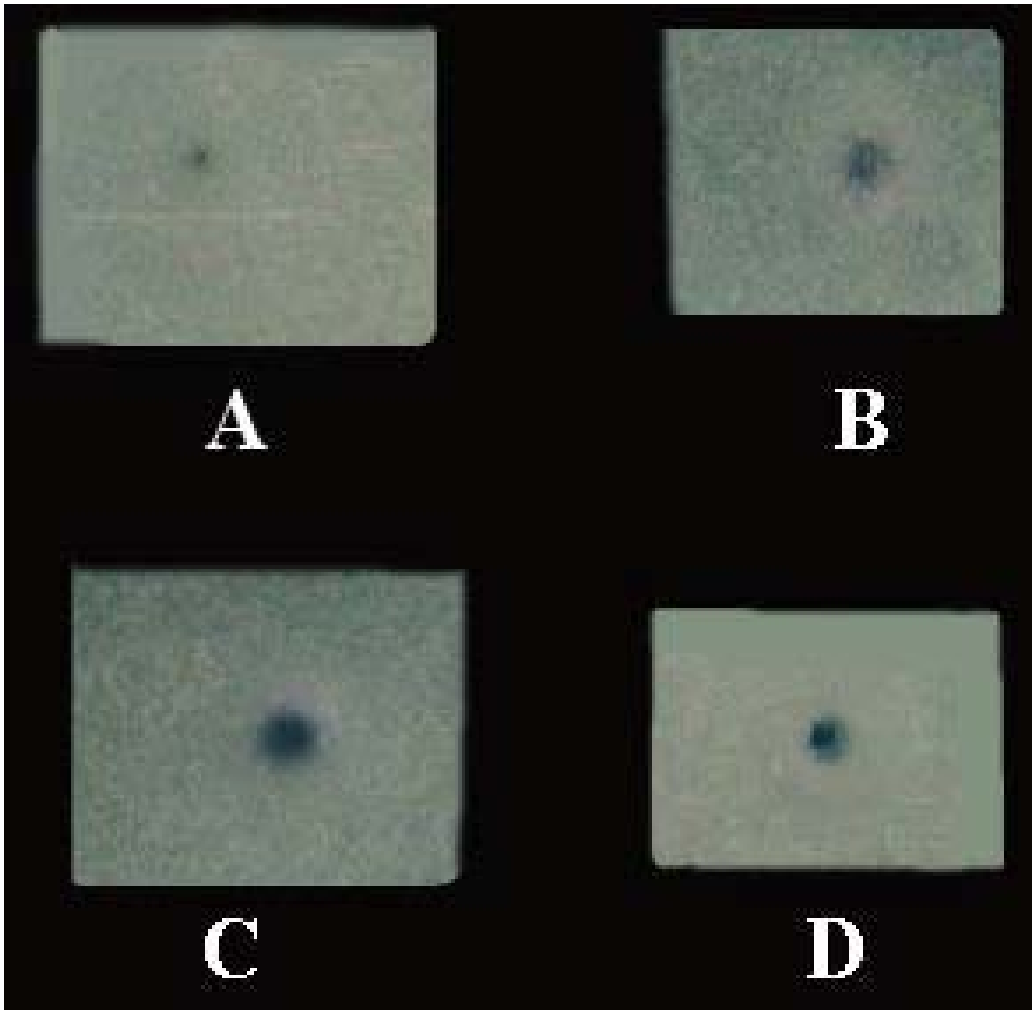

Photo 4: Magnified single plaque for each isolated phage, where; A: RSP4, B: RSP5, C: RSP6 and D: RSP7

Table 7: Plaques morphology of the four isolated lytic phages.

Character	RSP4	RSP5	RSP6	RSP7
Diameter*	Pinpoint (0.5 mm)	minute (2 mm)	large (6 mm)	medim (4 mm)
Transparence	Clear	Clear	Clear	Clear
Shape	Circular	Circular	Circular	Tetragonal

* The diameters of plaques were recorded directly from the plates.

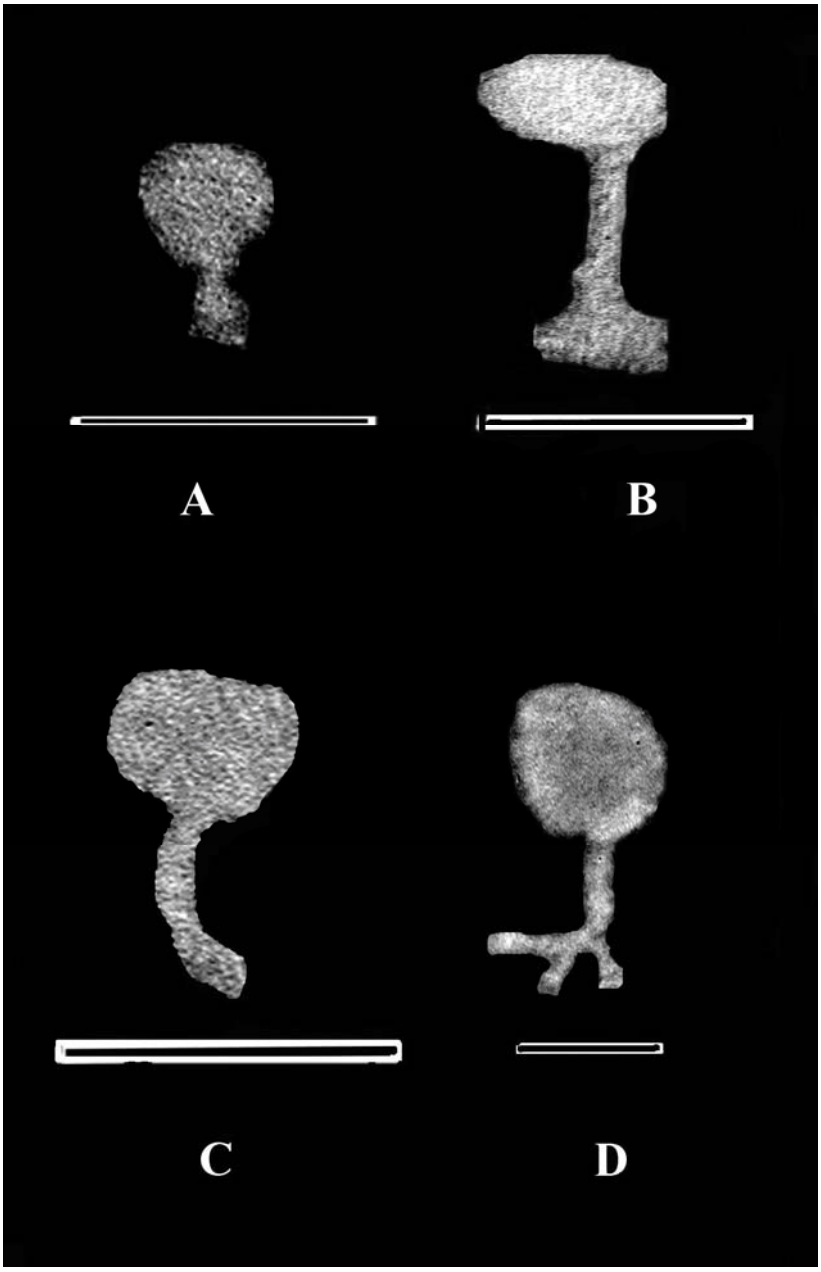

Photo 5: Electron microscopy of the isolated phages, where; A: RSP4, B: RSP5, C: RSP6 and D: RSP7; Bar = 100 nm and the magnification is 150,000.

Table 8: Electron microscopy of the isolated phages.

Phage	Family	Head		Tail		Base plate	Tail pins	Terminal bulbs
		Shape	Dimensions*	Type	Dimensions*			
RSP4	*Podoviridae*	Polygonal	41.67 X 46.67	N.C.	8.33 X 10.00	+	+	—
RSP5	*Siphoviridae*	Polygonal	34.54 X 69.09	N.C.	58.18 X 14.55	+	+	—
RSP6	*Siphoviridae*	Polygonal	36.23X 56.52	N.C.	50.72 X 11.59	+	—	+
RSP7	*Siphoviridae*	Polygonal	96.67 X 103.33	N.C.	66.67 X 23.33	+	—	+

*: Dimensions were (length X width) and measured by nm**.**

+/-: Presence or absence of tail fibers. N.C.: Non- contractile**.**

the other three virions had approximate values. The tail of RSP4 virion was short non-contractile tail (8.33 nm), while the tails of the other three phages were long tails (58.18, 50.72 and 66.67 nm for RSP5, RSP6 and RSP7, respectively). The width of the tails was different, the largest width was that of RSP7 (23.33 nm), while the smallest width was that of RSP4 (10.00 nm).

Base plate was observed in the four isolated phages. In three phages (RSP4, RSP5, RSP6) base plate was polyhedral, whereas, the base plate of RSP7 was fork-like possessing three branches. Tail pins presented in the end of base plate of RSP4 virion and RSP5 virion. In addition, terminal bulb was found in the end of base plate of tail of RSP6 particle. Two terminal bulbs were found at the end of two branches of fork-like base plate of RSP7 vrion.

IV. 2.4. Host range of isolated phages:

The four studied phages were spotted onto eight isolates of *R. solanacearum* (4 virulent isolates and 4 avirulent isolates) and 11 different bacterial isolates belong to 8 bacterial species (Table 9). These isolates were; one isolate of *Pseudomonas aeruginosa*, *Rhizobium leguminosarum*, *Erwinia amylovra*, *Staphylococcus aureus*, *Bacillus subtilus*, two isolates of *Pseudomonas fluorescens*, *Escherichia coli* and *Erwinia carotovra carotonara*. These bacterial isolates were collected from different sources and the origins of this isolates were demonstrated in Table 2. These isolates were used to study their susceptibility to either low or high titer of isolated phages using spot-test technique (drop method). Lytic areas were observed when the tested phages were spotted on virulent isolates (V1, V4, V6 and V9) of *R. solanacearum*, *Pseudomonas aeruginosa* and *Erwinia amylovra.* On the other hand, lytic areas were not observed with the other tested bacterial

Table 9: Host range of isolated phages.

Bacterial isolates	Phages			
	RSP4	**RSP5**	**RSP6**	**RSP7**
Ralstonia solanacearum V1	+	+	+	+
R. solanacearum V4	+	+	+	+
R. solanacearum V6	+	+	+	+
R. solanacearum V9	+	+	+	+
R. solanacearum AV1	—	—	—	—
R. solanacearum AV 4	—	—	—	—
R. solanacearum AV 6	—	—	—	—
R. solanacearum AV 9	—	—	—	—
Pseudomonas aeruginosa 15	+	+	+	+
Pseudomonas flourscens 40	—	—	—	—
Pseudomonas flourescens 50	—	—	—	—
Escherichia coli 55	—	—	—	—
Escherichia coli 95	—	—	—	—
Erwinia Carotovra Carotonara 36	—	—	—	—
Erwinia Carotovra Carotonara 40	—	—	—	—
Erwinia amylovora 19	+	+	+	+
Rhizobium leguminosarum 102	—	—	—	—
Staphylococcus aureus 49	—	—	—	—
Bacillus subtilus 64	—	—	—	—

+: Formation of lytic area, and —: Absence of lytic area

species. Therefore, these phages were polyvalent where they can infect more than one bacterial species. They can grow on virulent isolates of *R. solanacearum* (biovar 2), *Pseudomonas aeruginosa* and *Erwinia amylovra*.

IV. 2.5. Isolation of lysogens:

By using both spot test method and plaque assay technique using *R. solanacearum* V1 isolate, after 7 days of incubation at 30 ºC, there were no any lysogens could be detected for the four studied phages. Therefore, under the laboratory conditions (*in vitro*), these four phages were lytic (virulent) phages and there were no temperate (avirulent) phages. So, these four lytic phages could be used as biocontrol agents in fighting *R. solanacearum*.

IV. 3. Actinomycetes:

IV. 3.1. Isolation of actinomycetes:

A total 40 different actinomycetes strains were isolated from ten soil samples taken from the root zone of healthy potato plants; the same soil samples that used for isolation of phages. These actinomycetes isolates were isolated, purified and maintained on starch-casein agar medium.

IV. 3.2. Antagonism of actinomycetes against *R. solanacearum*:

By application of double layer method, (one loop of each isolate was seeded on SPA solidified plates and incubated at 28 ºC for 7 days, before layer of semi-solid SPA were inoculated by 0.1ml of *R. solanacearum* and seeded over actinomycete isolate), only four actinomycetes isolates (from the forty obtained isolates) inhibited the growth of *R. solanacearum*. These isolates were designed as A11, A36, A39 and A84 (Table 10 and Photo 6).

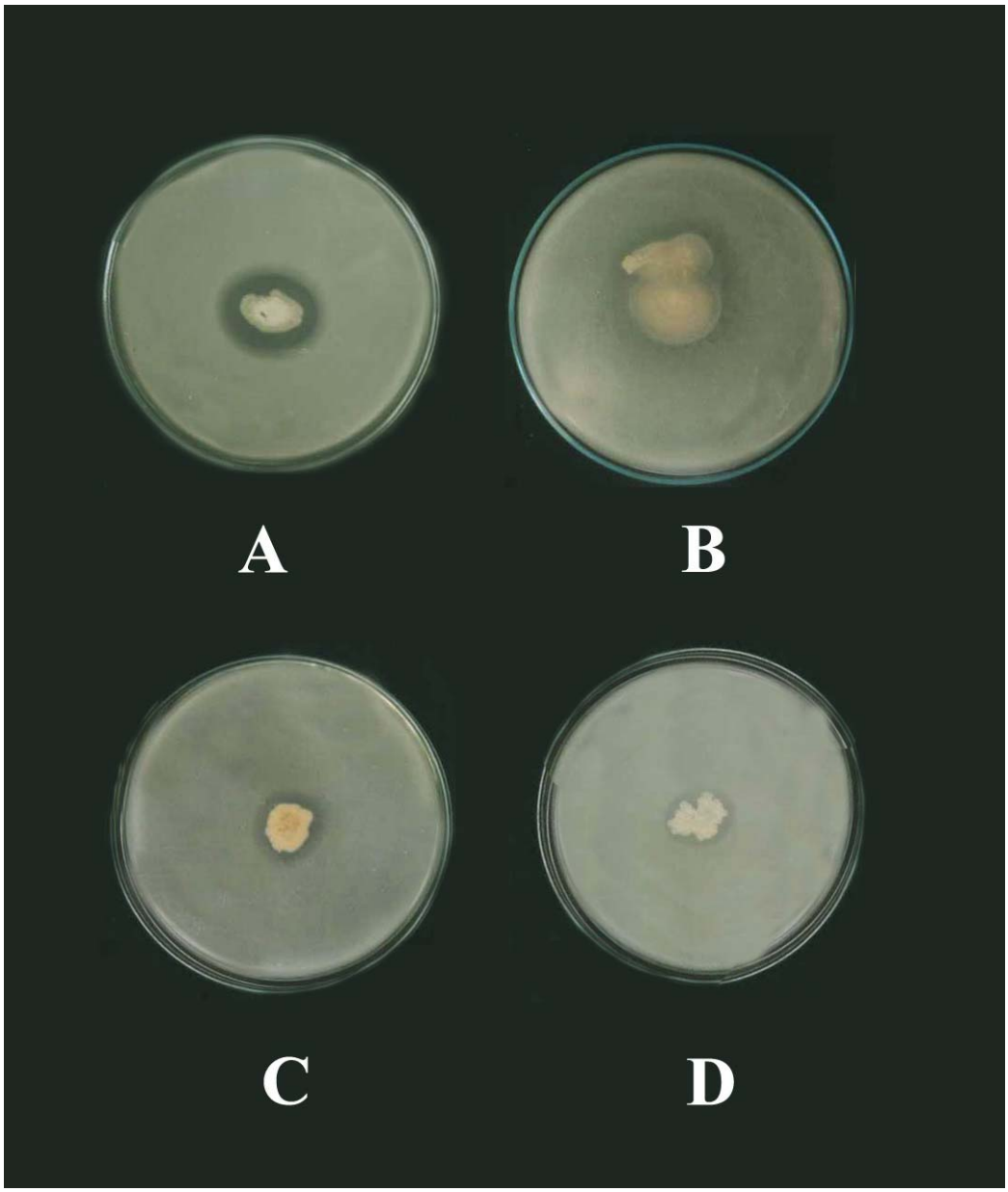

Photo 6: Antagonism of four effective actinomycetes isolates against *R. solanacearum* by application double layer method, where; A: A11, B: A36, C: A39 and D: A84.

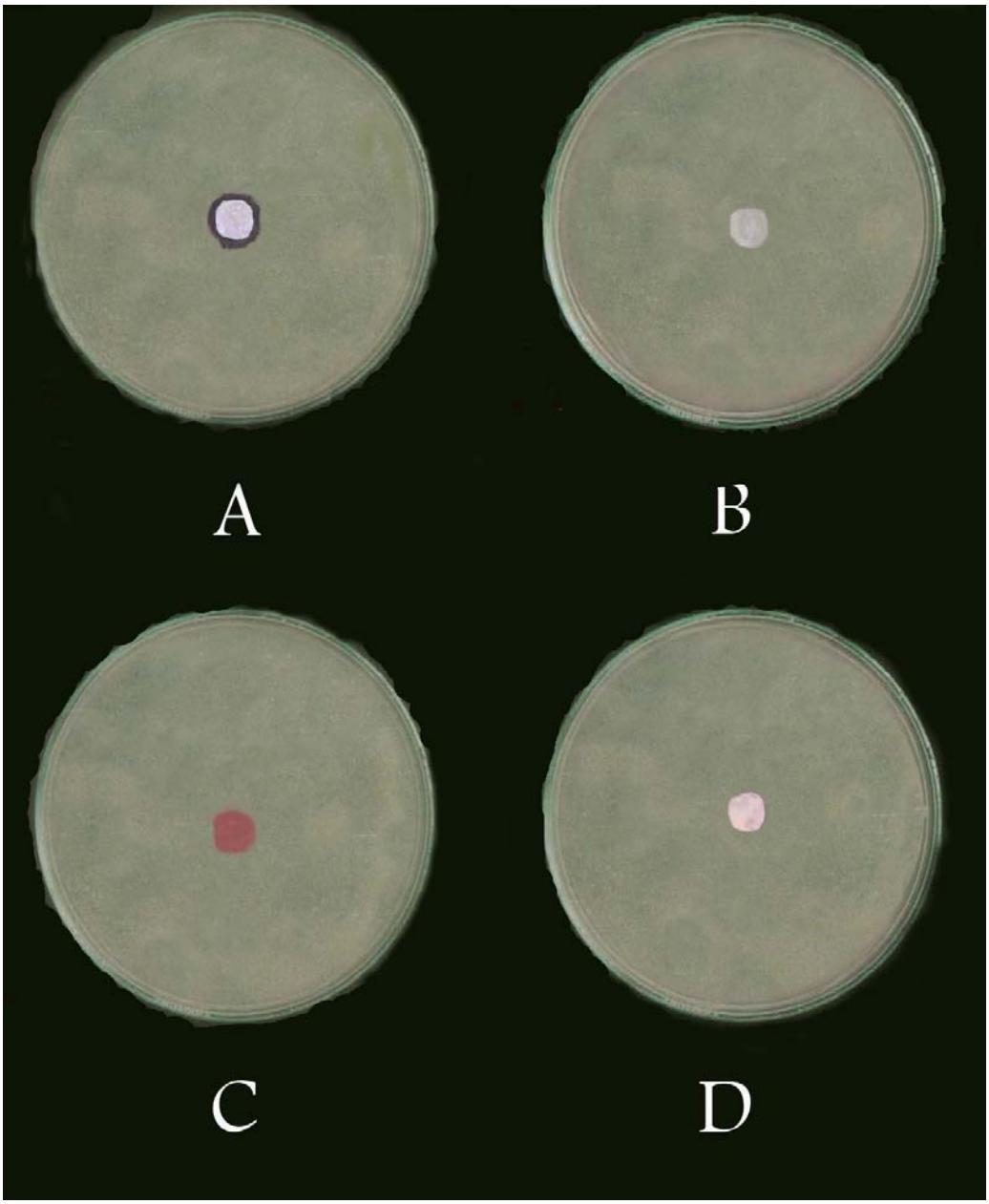

Photo 7: Antagonism between actinomycetes isolates and *R. solanacearum* seeded on SPA using agar-disc method where; A: Isolate A11, B: Isolate A36, C: Isolate A39, D: Isolate A84

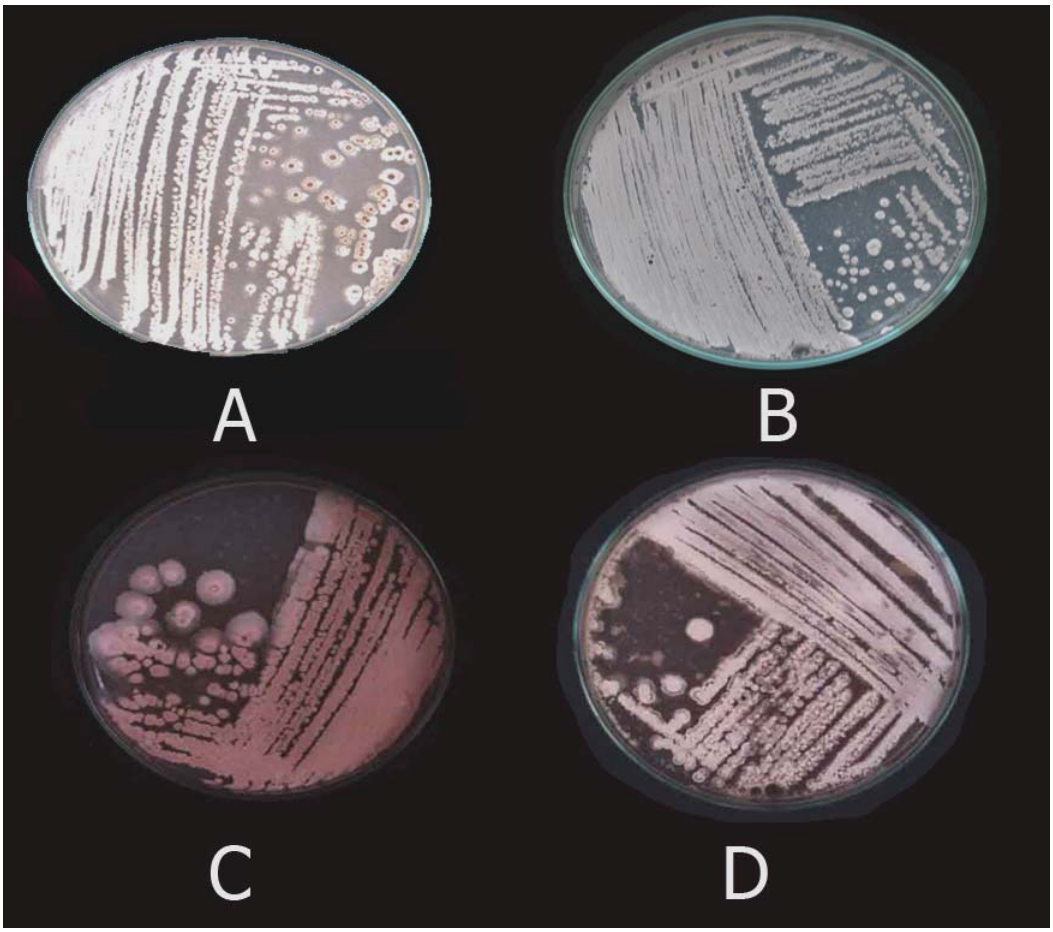

Photo 8: Substrate mycelium and single colonies of actinomycetes antagonized with *R. solanacearum* growing on starch-casein agar medium, where; A: Isolate A11, B: Isolate A36, C: Isolate A39, D: Isolate A84.

Table 10: Antagonism of actinomycetes isolate against *R. solanacearum*:

Isolate	Double layer method			Disc agar method (Inhibition zone mm)	Fumigation method
	Clear zone diameter (mm)*	Actinomycetes growth diameter (mm)*	Inhibition ratio % **		
11 A	24	10	240.00	12	–
36 A	30	26	115.38	–	–
39 A	21	15	140.00	–	–
84 A	21	14	150.00	–	–

*: The diameters were measured directly from the plate and the recorded value was the mean value of three replicates for each treatment

**: Antagonism ratio percentage = $\frac{\text{Clear zone diameter}}{\text{Actinomycete growth diameter}}$ X100

Antagonism (inhibition) ratio was varied from one actinomycetes isolate to another. The antagonism ratio of the A11 isolate (240%) was the highest value, whereas A39 and A84 values had proximate values (140 & 150 % respectively). On the other hand, A36 isolate had the lowest ratio (115.38 %).

When agar-disc method was applied for these four actinomyctes isolates, only one isolate (A11) inhibited the growth of *R. solanacearum* (Table 9 & Photo 7). The recorded inhibition zone was 12 mm. whereas the other three actinomycetes did not inhibit the growth of *R. solanacearum*.

The fumigation method was applied to study the ability of these actinomycetes to produce volatile compounds that can inhibit the growth of *R. solanacearum*. It was found that no inhibition was observed for *R. solanacearum* with the four tested actinomycetes isolates. So, there was no volatile compounds which produced by tested isolates that inhibited *R. solanacearum.*

The selected four isolates had different aerial mycelium (spore-colour in mass) on starch casein medium (Photo 8).

These four actinomycetes isolates were characterized to be classified to species level.

IV. 3.3. Cell wall analysis:

Cell wall analyses of the four studied actinomycetes indicate the same results, where:

- All of them had L-DAP and glycine.
- All of them had no characteristic sugars.

Therefore, all of them had cell wall of type I and they belonged to genus *Streptomyces*.

IV. 3.4. Characterization of the four active isolates:

IV. 3.4.1. *Streptomyces* No. A11 isolate:

IV. 3.4.1.i. Morphology:

1. **Spore chain**: Rectiflexible (Photo 9).
2. **Spore surface**: Smooth (Photo 11).

IV. 3.4.1.ii. Cultural properties

1. **Growth:** Growth of this isolate was poor on starch nitrate agar, starch ammonium sulphate agar, oat meal agar and Czapek's solution agar. Whereas, the growth was moderate on glycerol asparagine agar and yeast malt agar (Table 11).
2. **Colour of aerial mycelium**: This isolate belonged to white series the super colour was white. The aerial mycelium had white colour on yeast malt agar and yellowish white colour on starch ammonium sulphate agar, glycerol asparagine agar and oat meal agar. Brownish white colour presented on Starch nitrate agar and Czapek's solution agar
3. **Colour of substrate mycelium**: The main colour of substrate mycelium was yellow coulor. The coulor of substrate mycelium on starch nitrate agar and starch ammonium sulphate agar was brownish yellow. On oat meal agar and Czapek's solution agar substrate mycelium had coulor whitish yellow coulor. While, pale.

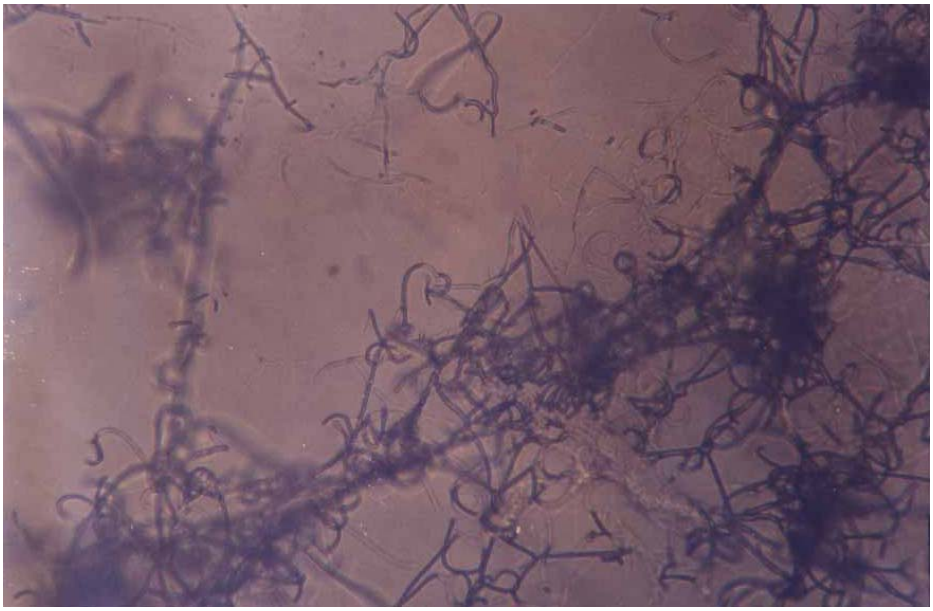

Photo 9: Micromorphology of spore chain of A11isolate.

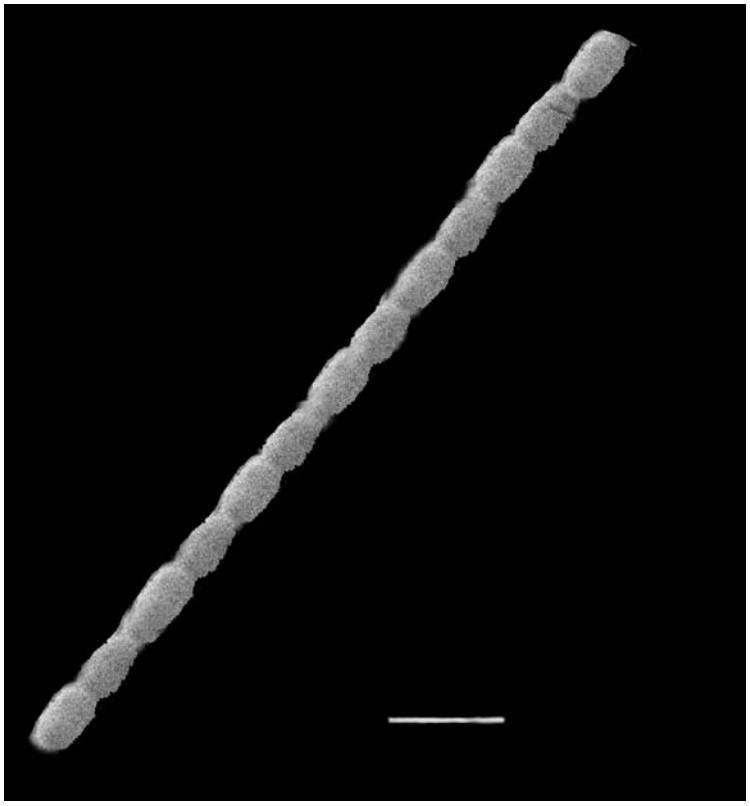

Photo 10: Electron micrograph of spore surface of A11isolate, bar = 1 um and magnification = X 18,000.

Table 11: Cultural properties of *Streptomyces* No. A11 isolate on the different media:

Media	Growth	Colour of		
		Aerial mycelium (Spore-colour in mass)	Substrate mycelium* (Reverse side of colony)	Soluble pigment (Diffusible pigment)
Starch nitrate agar	Poor	Brownish white	Non-distinctive	Non-pigmented
Starch ammonium sulphate agar	Poor	Yellowish white	Non-distinctive	Non-pigmented
Glycerol asparagine agar	Moderate	Yellow white	Non-distinctive	Non-pigmented
Oat meal agar	Poor	Yellowish white	Non-distinctive	Non-pigmented
Yeast malt agar	Moderate	White	Non-distinctive	Non-pigmented
Czapek's solution agar	Poor	Brownish white	Non-distinctive	Non-pigmented

*: The mycelium pigment was not pH sensitive when tested with 0.05 N NaOH or 0.05 N HCl.

yellow colour presented on glycerol asparagine agar and brown colour presented on yeast malt agar.

4. **Soluble pigment:** No soluble pigment was detected in different media.

IV. 3.4.1.iii. Physiological properties:

1. **Production of melanoid pigments:** *Streptomyces* isolate No. A11 did not produce the melanoid pigments (Table 15).
2. **Cellulose decomposition:** *Streptomyces* isolate No. A11 did not have the ability of cellulose decomposition after 15, 21 30 and 45 days (Table 16).
3. **Carbon utilization:** Arabinose, L-rhaminose, L-insitole, lactose, glycerol, starch, D-glucose, mannose and sucrose were utilized for the growth of *Streptomyces* No. A11 isolate, but no growth was observed with D-fructose, D-xylose, mannitol, rafinose and D-galactose (Table 17).

IV. 3.4.1.iv. Antimicrobial activities:

Streptomyces isolate No. A11 had the highest activities against the 12 tested microorganisms. It inhibited the growth of 7 microorganisms' species. It inhibited the growth of four bacterial species (*R. solanacearum, Staphylococcus aureus, Bacillus subtilus* and *Escherchia coli*), one fungus (*Trichoderma viride*) and two yeasts (*Lipomyces starkeyi* and *Saccharomyces cerevisiae* El) (Table 18).

IV.3.4.1.v. Taxonomic Identification of the Experimental Actinomycetes Isolates:

Summery of *Streptomyces* isolate No. A11 were shown in Table 19. According to the diagnostic key of Bergey's manual (1989) **(Williams *et al.*, 1989)** and surveying the literature on the description of *Streptomyces* spp in the articles of ISP (1966, 1968 a & b, 1969 and

1972) *Streptomyces* isolate A11 is *Streptomyces mutabilis* **(Preobrazhenskaya and Ryabova, 1957).**

IV. 3.4.2.Characterization of *Streptomyces* isolate No. A36:

IV. 3.4.2.i. Morphology:

1. **Spore chain**: Spiral (Photo 11).
2. **Spore surface**: Spiny (Photo 12).

IV. 3.4.2.ii. Cultural properties:

1. **Growth**: Moderate (Table 12).
2. **Colour of aerial mycelium**: The grey colour presented on all used media, but only on Yeast malt agar the colour was faint Grey.
3. **Colour of substrate mycelium**: The colour was Non distinctive.
4. **Soluble pigment**: There were no soluble pigments.

IV. 3.4.2.iii. Physiological properties:

1. **Production of melanoid pigments:** *Streptomyces* isolate No. A36 did not produce the melanoid pigments (Table 15).
2. **Cellulose decomposition:** *Streptomyces* isolate No. A36 did not have the ability of cellulose decomposition after 15, 21 30 and 45 days (Table 16).
3. **Carbon utilization:** D-glucose, D-fructose, mannose, D-xylose, sucrose, mannitol, arabinose, rafinose, D-galactose, L-rhaminose, L-insitole, lactose, glycerol and starch were utilized for the growth of *Streptomyces* isolate No. A36 (Table 17).

IV. 3.4.3.iv. Antimicrobial activities:

Streptomyces isolate No. A36 inhibited only three microbial species; *Staphylococcus aureus* (bacterium), *Lipomyces starkeyi* and *Saccharomyces cerevisiae* El (yeasts), whereas, it did not inhibit any of the studied fungal species (Table 18).

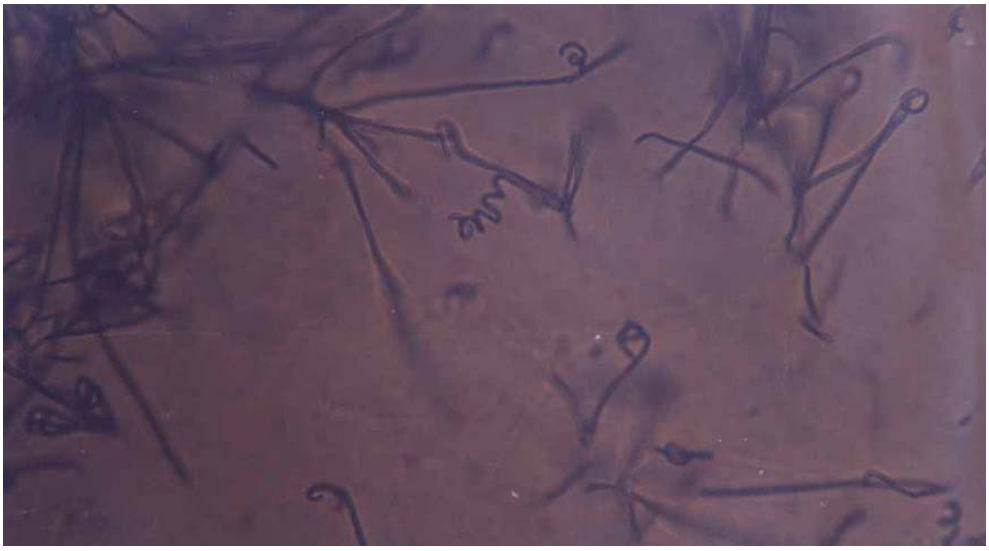

Photo 11: Micromorphology of spore chain of A36 isolate.

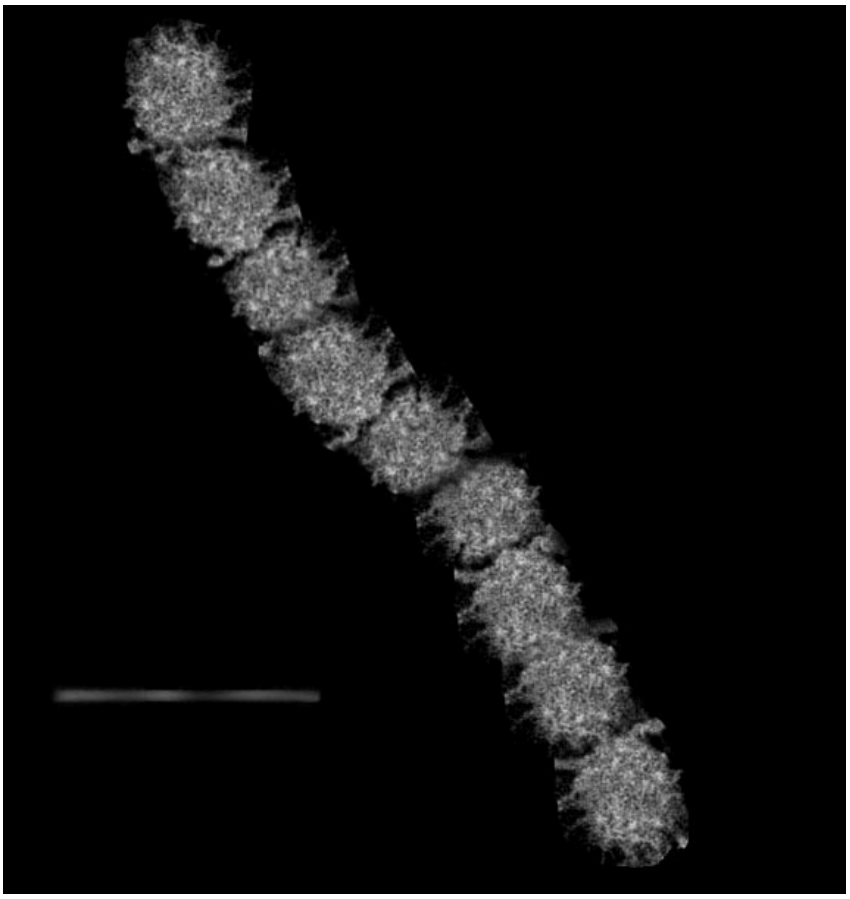

Photo 12: Electron micrograph of spore surface of A36 isolate, bar = 2um and magnification = X 9,000.

Table 12: Cultural properties of *Streptomyces* isolate No. A36 isolate on the different media:

Media	Growth	Colour of		
		Aerial mycelium	Substrate mycelium*	Soluble pigment
Starch nitrate agar	Moderate	Grey	Non-distinctive	Non-pigmented
Starch ammonium sulphate agar	Moderate	Grey	Non-distinctive	Non-pigmented
Glycerol asparagine agar	Moderate	Grey	Non-distinctive	Non-pigmented
Oat meal agar	Moderate	Grey	Non-distinctive	Non-pigmented
Yeast malt agar	Moderate	Faint grey	Non-distinctive	Non-pigmented
Czapek's solution agar	Moderate	Grey	Non-distinctive	Non-pigmented

*: The mycelium pigment was not pH sensitive when tested with 0.05 N NaOH or 0.05 N HCl.

IV. 3.4.3.v. Taxonomic Identification :

Summery of studied characters of *Streptomyces* isolate No. A36 were shown in Table 20. According to the diagnostic key of Bergey's manual (1989) **(Williams *et al.*, 1989)** and surveying the literature on the description of *Streptomyces* spp in the articles of ISP (1966, 1968 a & b, 1969 and 1972) *Streptomyces* isolate No. A36 was identified as: *S. sparogenes* **(Owen *et al.* 1962)**.

IV. 3.4.3.Characterization of *Streptomyces* isolate No. A39:

IV. 3.4.3.i. Morphology:

1. **Spore chain**: Rectiflexible (Photo 13).
2. **Spore surface**: Smooth (Photo 14).

IV. 3.4.3.ii. Cultural properties:

1. **Growth**: well spreading (Table 13).
2. **Colour of aerial mycelium**: This isolate belong to red series. The colour of spore mass on all used media (D-glucose, D-fructose, mannose, D-xylose, sucrose, mannitol, arabinose, rafinose, D-galactose, L-rhaminose, L-insitole, lactose, glycerol and starch). was brownish red.
3. **Colour of substrate mycelium:** Non-distinctive.
4. **Soluble pigments:** There were no soluble pigments.

IV. 3.4.3.iii. Physiological properties:

1. **Production of melanoid pigments:** *Streptomyces* isolate No. A39 had the ability to produce the melanoid pigments (Table 15).
2. **Cellulose decomposition:** *Streptomyces* isolate No. A39 did not have the ability of cellulose decomposition after 15, 21 30 and 45 days (Table 16).

Carbon utilization: D-glucose, mannose, sucrose and starch were utilized for the growth of *Streptomyces* isolate No. A39, but no growth was observed with D-fructose, D-xylose, mannitol, arabinose, rafinose

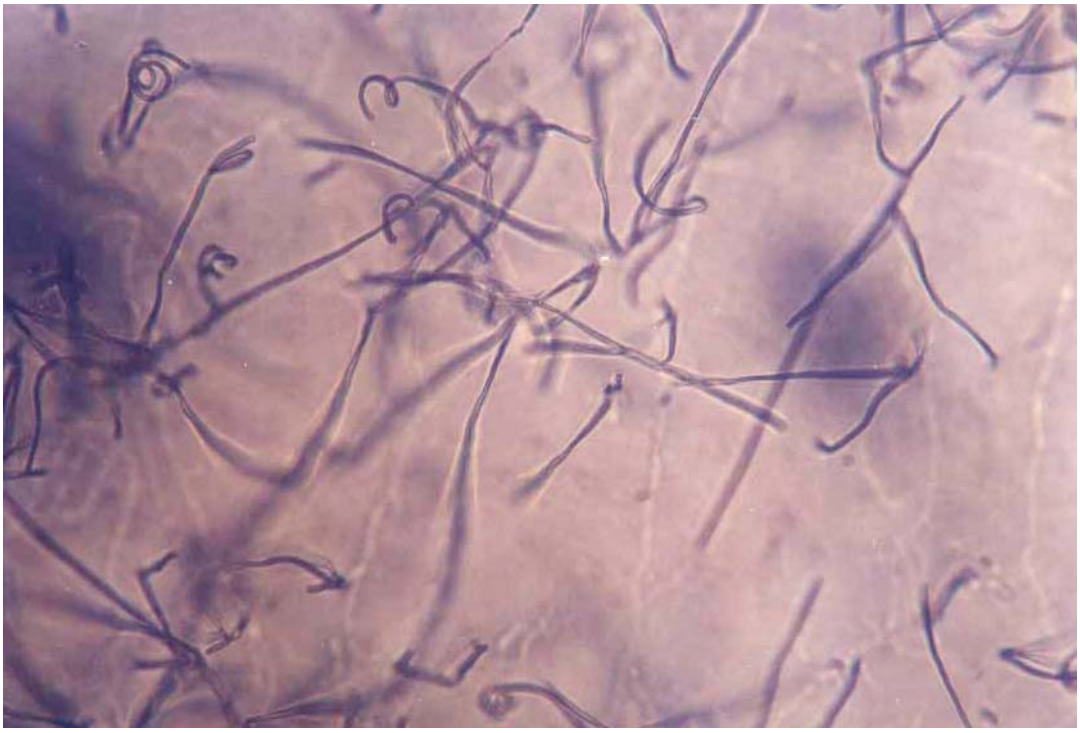

Photo 13: Micromorphology of spore chain of A39 isolate.

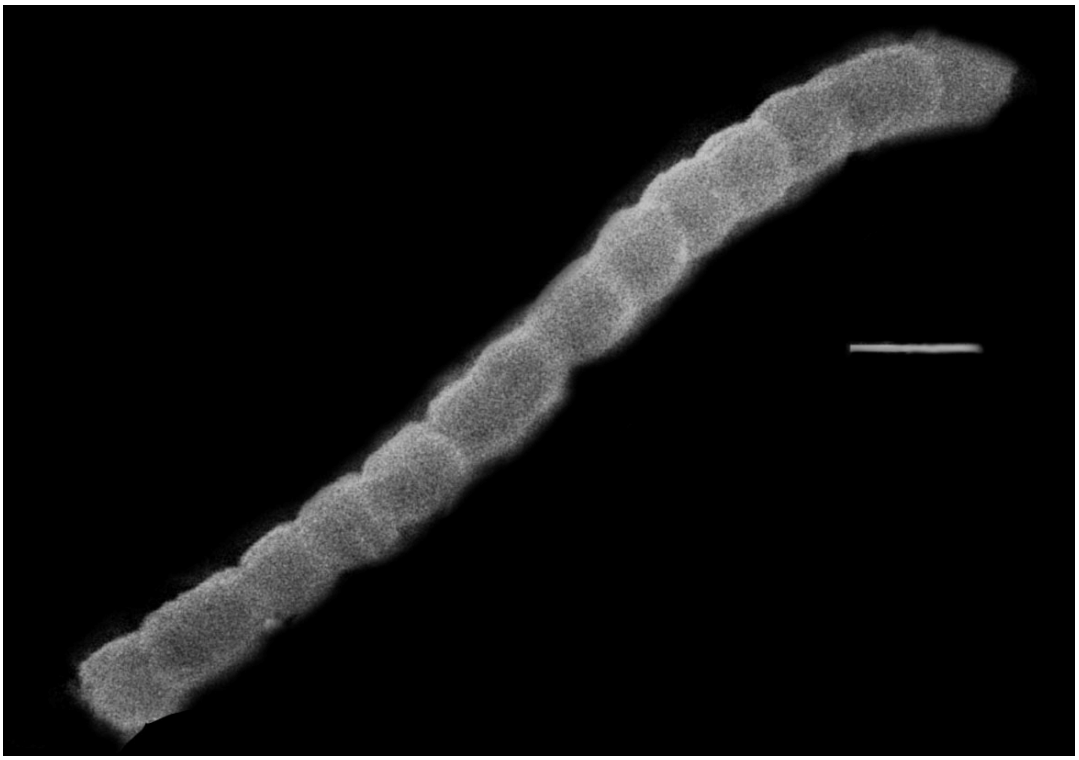

Photo 14: Electron micrograph of spore surface of A39 isolate, bar = 1 um and magnification = X 14,000.

Table 13: Cultural properties of *Streptomyces* isolate No. A39 on the different media:

Media	Growth	Colour of		
		Aerial mycelium	**Substrate mycelium***	**Soluble pigment**
Starch nitrate agar	Well spreading	Brownish red	Non-distinctive	Non-pigmented
Starch ammonium sulphate agar	Well spreading	Brownish red	Non-distinctive	Non-pigmented
Glycerol asparagine agar	Moderate	Brownish red	Non-distinctive	Non-pigmented
Oat meal agar	Well spreading	Brownish red	Non-distinctive	Non-pigmented
Yeast malt agar	Well spreading	Brownish red	Non-distinctive	Non-pigmented
Czapek's solution agar	Well spreading	Brownish red	Non-distinctive	Trace of brown

*: The mycelium pigment was not pH sensitive when tested with 0.05 N NaOH or 0.05 N HCl.

, D-galactose, L-rhaminose, L-insitole, lactose, and glycerol (Table 17).

IV. 3.4.3.iv. Antimicrobial activities:

Streptomyces isolate No. A39 had the ability to antagonize 5 tested microbial species; three bacteria species (*Staphylococcus aureus, Bacillus subtilus* and *Escherchia coli),* a fungus (*Rhizoctonia solani*) and yeast *(Candida lipolytica*) (Table 18).

IV. 3.4.3.v. Taxonomic Identification of:

Summery of studied characters of *Streptomyces* isolate No. A39 were shown in Table 21. According to the diagnostic key of Bergey's manual (1989) **(Williams *et al.*, 1989)** and surveying the literature on the description of *Streptomyces* spp. in the articles of ISP (1966, 1968 a & b, 1969 and 1972) *Streptomyces* isolate No. A39 was classified to species level as: *S. luridus* **(Krasilinkov *et al.*, 1957**).

IV. 3.4.4.Characterization of *Streptomyces* isolate No. A84:

IV. 3.4.4.i. Morphology:

1. **Spore chain**: Rectiflexible (Photo 15).
2. **Spore surface**: Smooth (Photo 16).

IV. 3.4.4.ii. Cultural properties:

1. **Growth:** Growth of this isolate was well spreading on starch nitrate agar, starch ammonium sulphate agar, oat meal agar and yeast malt agar. While, growth of this isolate was moderate on

Czapek's solution agar and it was poor on glycerol asparagine agar (Table 14).

2. **Colour of aerial mycelium**: The colour of spore mass was white to reddish white on starch nitrate agar, starch ammonium sulphate agar and yeast malt agar. Its colour was white on glycerol asparagine agar, oat meal agar and Czapek's solution agar**.**

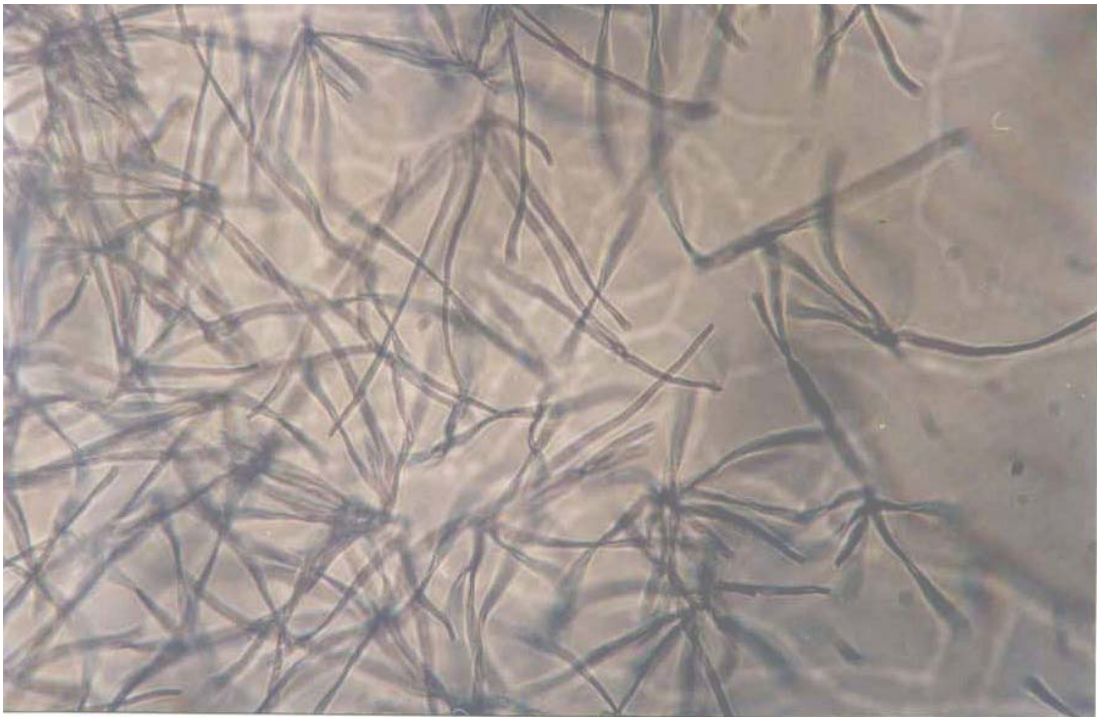

Photo 15: Micromorphology of spore chain of A84 isolate.

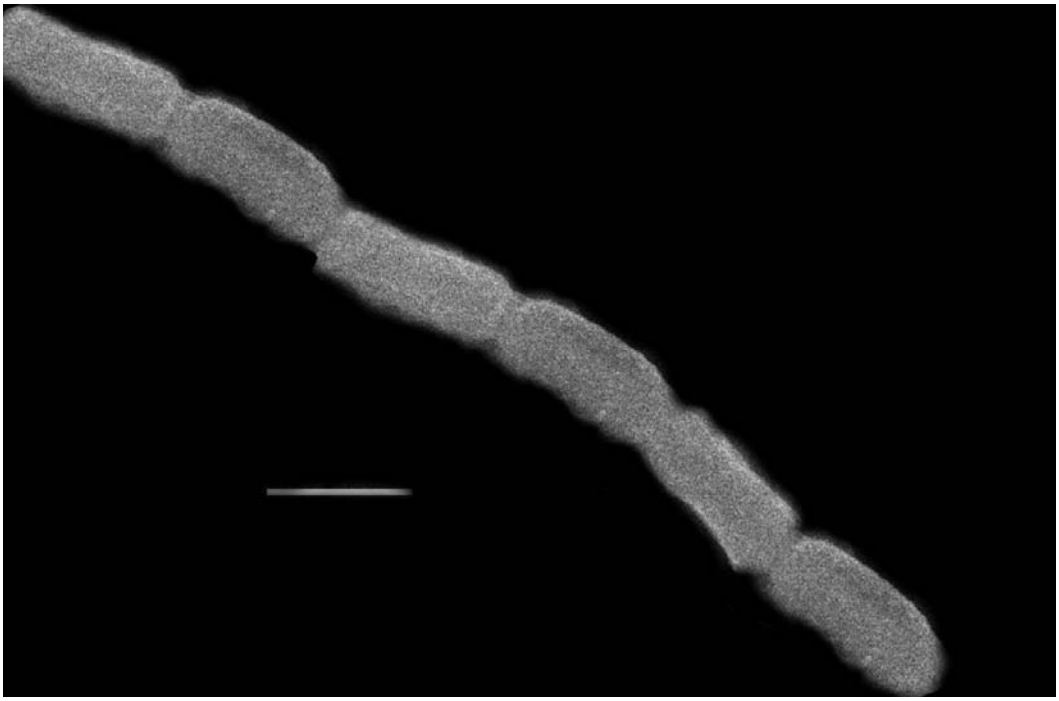

Photo 16: Electron micrograph of spore surface of A84 isolate, bar = 1 um and magnification = X 18,000.

Table 14: Cultural properties of *Streptomyces* isolate No. A84 on the different media:

Media	Growth	Colour of		
		Aerial mycelium	**Substrate mycelium***	**Soluble pigment**
Starch nitrate agar	Well spreading	Reddish white	Non distinctive	Yellow
Starch ammonium sulphate agar	Well spreading	Reddish White	Non distinctive	Non-pigmented
Glycerol asparagine agar	Poor	White	Non distinctive	Non-pigmented
Oat meal agar	Well spreading	White	Non distinctive	Non-pigmented
Yeast malt agar	Well spreading	Reddish white	Non distinctive	Non-pigmented
Czapek's solution agar	Moderate	White	Non distinctive	Non-pigmented

*: The mycelium pigment was not pH sensitive when tested with 0.05 N NaOH or 0.05 N HCl.

Therefore, isolate No. A84 is classified belong to white colour series

3. **Colour of substrate mycelium:** No-distinctive substrate mycelium pigment was observed on any of the medium used.
4. **Soluble pigment:** There were no soluble pigment was produced on starch-ammonium-sulphate agar, glycerol-asparagine agar, oat-meal agar, yeast-malt agar and Czapek's solution agar. However, a faint yellowish pigment was observed on starch-nitrate agar; this pigment was not pH sensitive.

IV. 3.4.4.iii. Physiological properties:

1. **Production of melanoid pigments:** *Streptomyces* isolate No. A84 did not produce the melanoid pigments (Table 15).
2. **Cellulose decomposition:** *Streptomyces* isolate No. A84 did not have the ability of cellulose decomposition after 15, 21 30 and 45 days (Table 16).
3. **Carbon utilization:** D-fructose, D-glucose, mannose, sucrose, mannitol, D-galactose, L-insitole, starch, rafinose and L-rhaminose were utilized for the growth of *Streptomyces* isolate No. A84, but no growth was observed with D-xylose, arabinose, rafinose, L-rhaminose, lactose and glycerol (Table 17).

IV. 3.4.4.iv. Antimicrobial activities of isolate A84:

Streptomyces isolate No. A84 had the antagonistic effect on only two tested microbes; Erwinia *carotovora carotovora (*bacterium) and *Candida lipolytica* (yeast). Whereas, it did not inhibit any used fungi (Table 18).

Taxonomic Identification:

Summery of studied characters of *Streptomyces* isolate No. A84 were shown in Table 22. According to the diagnostic key of Bergey's

Table 15: Production of melanoid pigments from the four studied *Streptomyces* isolates:

Media	Production of melanoid from the four studied isolates			
	A11	A36	A39	A84
Tyrosin agar	—	—	+	—
Peptone yeast agar	—	—	+	—
Trypton yeast broth	—	—	+	—

—: negative and +: positive

Table 16: The ability of the four studied *Streptomyces* isolates to decompose cellulose after different periods:

Period	Cellulose decomposition			
	A11	A36	A39	A84
15 days	—	—	—	—
21 days	—	—	—	—
30 days	—	—	—	—
45 days	—	—	—	—

—: negative.

Table 17: Utilization of different carbon sources of the four studied *Streptomyces* isolates.

Carbon source	Growth intensities of the studied isolates			
	A11	A36	A39	A84
D-glucose	+	+	+/—	+
D-fructose	+	+	—	+/—
Mannose	+	+	+	+
D-xylose	+	+	—	—
Sucrose	+	+	+	+
Mannitol	+	+	—	+
Arabinose	+/—	+	—	—
Rafinose	—	+	—	+
D-galactose	—	+	—	+
L-rhaminose	+/—	+	—	+
L-insitole	+/—	+	—	+
Lactose	+/—	+	—	—
Glycerol	+/—	+	—	—
Starch	+/—	+	+	+
No carbon source	—	—	—	—

—: no utilization; +/—: poorly utilization and +: positive utilization

Table 18: Antimicrobial activity of the four studied *Streptomyces* isolates:

Indicator		Inhibition zone (mm)*			
		A11	**A36**	**A39**	**A84**
Bacteria	***Ralstonia solanacearum***	12	—	—	—
	Escherchia coli	20	—	26	—
	Pseudomonas flourscens	—	—	—	—
	Erwinia carotovora carotovora	—	—	—	15
	Staphylococcus aureus	22	34	20	—
	Bacillus subtilus	27	—	26	—
Fungi	***Rhizoctonia solani***	—	—	30	—
	Fusarium oxysporum	—	—	—	—
	Trichoderma viride	30	—	—	—
Yeast	***Lipomyces starkeyi***	18	14	—	—
	Candida lipolytica	—	—	21	14
	***Saccharomyces cerevisiae* El**	18	14	—	—

— = negative activity (no inhibition zone detected); * : Inhibition zone measured directly from the plate, each treatment had three replicates; the diameter of used actinomycetes discs 0.9 mm.

Used media: nutrient agar medium used for bacteria; PDA medium used for fungi and used YM agar for yeast.

Temperature of incubation: 28 ºC for bacteria and yeast and 25 ºC for fungi.

Table 19: Summery of studied characters of *Streptomyces* isolate No. A11:

Character			**Result**
Cell wall type			Type 1
Morphological characters	**Spore chain**		Rectiflexibles
	Spore surface		Smooth
Cultural characters	**Colour of aerial mycelium**		White
	Colour of substrate mycelium		Non -distinctive
	Soluble pigment		Negative
Physiological characters	**Melanoid pigments production**		Negative
	Cellulose decomposition		Negative
	Carbon utilzation	**D-glucose**	Positive
		D-fructose	Positive
		Mannose	Positive
		D-xylose	Positive
		Sucrose	Positive
		Mannitol	Positive
		Arabinose	Positive
		Rafinose	Negative
		D-galactose	Negative
		L-rhaminose	Positive
		L-insitole	Positive
		Lactose	Positive
		Glycerol	Positive
		Starch	Positive
		No carbon source	Negative
Antimicrobial activities	***Ralstonia solanacearum***		Positive
	Escherchia coli		Positive
	Pseudomonas flourscence		Negative
	Erwinia carotovora carotovora		Negative
	Staphylococcus aureus		Positive
	Bacillus subtilus		Positive
	Rhizoctonia solani		Negative
	Fusarium oxysporum		Negative
	Trichoderma viride		Positive
	Lipomyces starkeyi		Positive
	Candida lipolytica		Negative
	***Saccharomyces cervesia* El**		Positive
Nomenclature	***Streptomyces mutabilis.***		

Table 20: Summery of studied characters of *Streptomyces* isolate No. A36:

Character			**Result**
Cell wall type			Type 1
Morphological characters	**Spore chain**		Spiral
	Spore surface		Spiny
Cultural characters	**Colour of aerial mycelium**		Gery
	Colour of substrate mycelium		Non -distinctive
	Soluble pigment		Negative
Physiological characters	**Melanoid pigments production**		Negative
	Cellulose decomposition		Negative
	Carbon utilzation	**D-glucose**	Positive
		D-fructose	Positive
		Mannose	Positive
		D-xylose	Positive
		Sucrose	Positive
		Mannitol	Positive
		Arabinose	Positive
		Rafinose	Positive
		D-galactose	Positive
		L-rhaminose	Positive
		L-insitole	Positive
		Lactose	Positive
		Glycerol	Positive
		Starch	Positive
		No carbon source	Negative
Antimicrobial activities	***Ralstonia solanacearum***		Negative
	Escherchia coli		Negative
	Pseudomonas flourscence		Negative
	Erwinia carotovora carotovora		Negative
	Staphylococcus aureus		Positive
	Bacillus subtilus		Negative
	Rhizoctonia solani		Negative
	Fusarium oxysporum		Negative
	Trichoderma viride		Positive
	Lipomyces starkeyi		Positive
	Candida lipolytica		Negative
	***Saccharomyces cervesia* El**		Positive
Nomencalture	***S. sparogenes***		

Table 21: Summery of studied characters of *Streptomyces* isolate No. A39:

Character			**Result**
Cell wall type			Type 1
Morphological characters	**Spore chain**		Rectiflexibles
	Spore surface		Smooth
Cultural characters	**Colour of aerial mycelium**		Red
	Colour of substrate mycelium		Non -distinctive
	Soluble pigment		Negative
Physiological characters	**Melanoid pigments production**		Positive
	Cellulose decomposition		Negative
	Carbon utilzation	**D-glucose**	Positive
		D-fructose	Negative
		Mannose	Positive
		D-xylose	Negative
		Sucrose	Positive
		Mannitol	Negative
		Arabinose	Negative
		Rafinose	Negative
		D-galactose	Negative
		L-rhaminose	Negative
		L-insitole	Negative
		Lactose	Negative
		Glycerol	Negative
		Starch	Positive
		No carbon source	Negative
Antimicrobial activities	***Ralstonia solanacearum***		Negative
	Escherchia coli		Positive
	Pseudomonas flourscence		Negative
	Erwinia carotovora carotovora		Negative
	Staphylococcus aureus		Positive
	Bacillus subtilus		Positive
	Rhizoctonia solani		Positive
	Fusarium oxysporum		Negative
	Trichoderma viride		Negative
	Lipomyces starkeyi		Negative
	Candida lipolytica		Positive
	***Saccharomyces cervesia* El**		Negative
Nomenclature	***S. luridus***		

Table 22: Summery of studied characters of *Streptomyces* isolate No. A84:

Character			**Result**
Cell wall type			Type 1
Morphological characters	**Spore chain**		Rectiflexibles
	Spore surface		Smooth
Cultural characters	**Colour of aerial mycelium**		White
	Colour of substrate mycelium		Non -distinctive
	Soluble pigment		Negative
Physiological characters	**Melanoid pigments production**		Negative
	Cellulose decomposition		Negative
	Carbon utilzation	**D-glucose**	Positive
		D-fructose	Positive
		Mannose	Positive
		D-xylose	Negative
		Sucrose	Positive
		Mannitol	Negative
		Arabinose	Positive
		Rafinose	Positive
		D-galactose	Positive
		L-rhaminose	Positive
		L-insitole	Positive
		Lactose	Negative
		Glycerol	Negative
		Starch	Positive
		No carbon source	Negative
Antimicrobial activities	***Ralstonia solanacearum***		Negative
	Escherchia coli		Negative
	Pseudomonas flourscence		Negative
	Erwinia carotovora carotovora		Positive
	Staphylococcus aureus		Negative
	Bacillus subtilus		Negative
	Rhizoctonia solani		Negative
	Fusarium oxysporum		Negative
	Trichoderma viride		Negative
	Lipomyces starkeyi		Negative
	Candida lipolytica		Positive
	***Saccharomyces cervesia* El**		Negative
Nomenclature	***S. pyridomyceticus***		

manual (1989) **(Williams *et al.*, 1989)** and surveying the literature on the description of *Streptomyces* spp. in the articles of ISP (1966, 1968 a & b, 1969 and 1972) the four isolates were classified to species level as: *S. pyridomyceticus* **(Okani and Unezawa, 1955)**.

IV. 4. Essential oils:

IV. 4.1. Screening of the antimicrobial activities of some commercial essential oils against *R. solanacearum*:

The antimicrobial activities of 16 commercial essential oils against *Ralstonia solanacearum* were tested by using filter paper disc method. After 24 hours incubation, the inhibition zones were measured by millimeters. The largest inhibition zones (≥ 90 mm) were obtained by three essential oils; anise, thyme and lettuce (Table 23 and Photo 17). Thus all of these three essential oils completely inhibited the growth of *R. solanacearum* where no growth of the bacteria was observed on the agar plates having 90 mm.

Inhibition zones of small diameters were obtained by nine essential oils. These essential oils were nigella, spearmint, caraway, rocket, onion, garlic, camphor, fennel and dianthus. On the contrast, four essential oils (fenugreek, ground nut, sesame and marjoram) did not cause any antagonistic effect against *R. solanacearum*.

Fumigation method was applied for the three essential oils that had the best results with the previous method. When 0.1 ml of anise, thyme or lettuce oil was placed on the lid of plates and the plates were taped by double layer of Parafilm, the growth of *R. solanacearum* was completely inhibited at the plates of 9 cm diameter.

Table 23: Inhibition of *R. Solanacearum* by essential oils.

No.	Essential Oil	Inhibition zone diameter (mm) *		Minimal inhibition concentration (MIC)	
		Filter paper disc method	Fumigation method	Agar Disc method	Striking method
1	Anise	≥ 90**	≥ 90**	≥ 0.25 %	0.5 %
2	Thyme	≥ 90**	≥ 90**	1 %	2 %
3	Lettuce	≥ 90**	≥ 90**	< 2%	< 2%
4	Nigella	7	N.D.	N.D.	N.D.
5	Spearmint	7	N.D.	N.D.	N.D.
6	Caraway	7	N.D.	N.D.	N.D.
7	Rocket	8	N.D.	N.D.	N.D.
8	Onion	8	N.D.	N.D.	N.D.
9	Garlic	8	N.D.	N.D.	N.D.
10	Camphor	6	N.D.	N.D.	N.D.
11	Fenugreek	—	N.D.	N.D.	N.D.
12	Ground nut	—	N.D.	N.D.	N.D.
13	Fennel	10	N.D.	N.D.	N.D.
14	Seasame	—	N.D.	N.D.	N.D.
15	Dianthus	10	N.D.	N.D.	N.D.
16	Marjoram	—	N.D.	N.D.	N.D.

*: The inhibition zone diameters were measured directly from the plate, the recorded numbers were the means of three replicates, —: No inhibition zone and N.D.: Not detected. **: The plates of inhibition zones ≥ 90 mm had complete inhibition of *R. Solanacearum.*

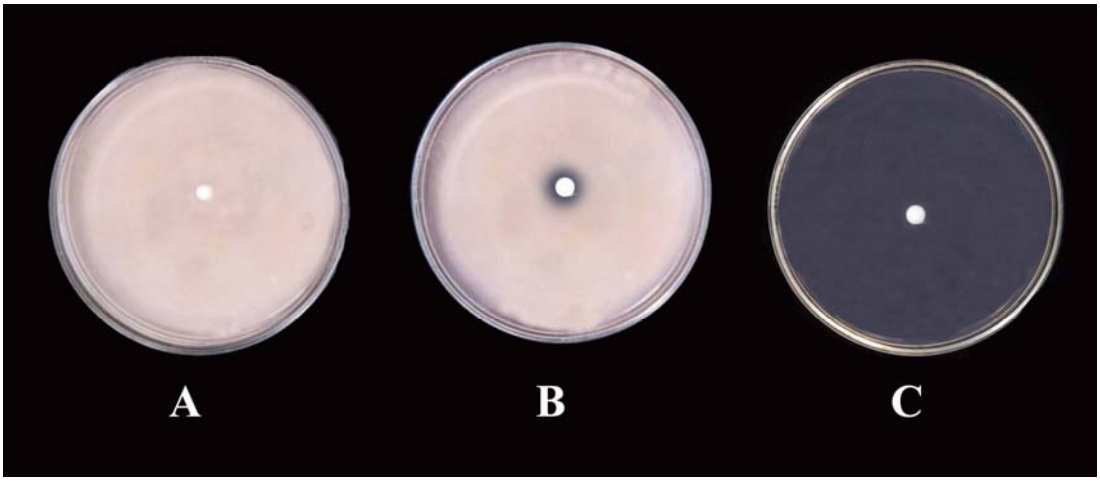

Photo 17: Antimicrobial activities of essential oils against *R. solanacearum*; A: control (no inhibition), B: small inhibition zone (with nigella oil), C: Complete inhibition (diameter of inhibition zone ≥ 90 mm, with anise oil).

IV.4.2. Determination of minimal inhibitory concentrations (MICs) of effective essential oils:

The MICs were determined only for the three essential oils (anise, thyme and lettuce) that caused inhibition zone ≥ 90 mm. The MICs were determined by two methods; disc method and striking (Table 23). Anise oil had the lowest values of MICs (≤ 0.25 % in disc method and 0.5 % in striking method). Thyme oil had middle values of MICs (1 % in disc method and 2 % in striking method). Lettuce oil had the highest value of MICs (> 2 % in both disc and striking methods), where, *R. solanacearum* did not inhibited with SPA of 2 % lettuce oil. It is clear that the MICs values of anise and thyme oils were lower with disc method from those of the same two oils with striking method.

IV.4.3. Antimicrobial effect of anise oil:

The antimicrobial activity of anise oil (that shows the highest antimicrobial action versus *R. solanacearum*) was investigated against bacteria, fungi and yeasts using filter disc method. The results depicted in Table 24 showed that anise oil has wide spectrum antimicrobial action. Anise oil causes complete inhibition for the studied plant pathogen *R. solanacearum*, pronounced antimicrobial action towards other gram positive bacteria (*Staphylococcus aureus, Bacillus subtilus*), weak activity against gram negative bacteria (*Escherchia coli*), strong activity against fungi (*Rhizoctonia solani* and *Fusarium oxysporum*) and moderate activity against yeast test organisms (*Lipomyces starkeyi, Candid lipolytica, Saccharomyces cerevisiae* El), but it was inactive against *Pseudomonas flourscens*, *Erwinia carotovora carotovora* and *Trichoderma viride*.

Table 24: Antimicrobial effect of anise oil.

Indicator		Inhibition zone (mm)*
Bacteria	***Ralstonia solanacearum***	≥ 90**
	Staphylococcus aureus	8
	Bacillus subtilus	9
	Escherchia coli	7
	Pseudomonas flourscens	—
	Erwinia carotovora carotovora	—
Fungi	***Rhizoctonia solani***	25
	Fusarium oxysporum	20
	Trichoderma viride	—
Yeast	***Lipomyces starkeyi***	10
	Candida lipolytica	8
	***Saccharomyces cerevisiae* El**	8

*: The diameter of disc filter paper is 0.5 mm; the diameters of inhibition zone were measured directly from the plate; and there were three replicates for each treatment, —: No inhibition zone. **: The plates of inhibition zones ≥ 90 mm had complete inhibition.

IV. 5. Effect of water extracts of mushroom spent wheat straw and mushroom spent rice straw on *R. solanacearum* growth:

IV. 5.1. Effect of water extracts of *Pleurotus* spp. spent wheat straw on *R. solanacearum* growth:

The filter paper disc method was applied to study the antagonistic effect of water extracts (WE) of three *Pleurotus* spp. spent wheat straw and the WE of raw wheat straw (WE-WS) against *R. solanacearum* in case of sterilization by filtration and autoclaving (Table 25 & Figure 1). All of the four tested water extracts inhibited the growth of *R. solanacearum* in case of their sterilization by filtration, but their sterilization by autoclaving resulted in that only water extract of *Pleurotus floridanus* spent wheat straw (SWS) did not inhibit the growth of *R. solanacearum*. Generally, the sterilized of water extracts of SWS and WS by filtration causes inhibition zones larger from the inhibition zones of the sterilization by autoclaving (Table 25 and Photo 18).

In case of sterilization by filtration, the largest recorded inhibition zone diameter was obtained with the WE of *P. columbinus* SWS (10 mm), which followed by *P. pulmonarius* (9 mm). While, the inhibition zone diameter of WE of *P. floridanus* SWS was equal to that of WE-WS (8 mm).

In case of sterilization by autoclaving, there were no remarkable differences between the three recorded inhibition zones. The largest inhibition zone diameter was obtained with both WE of *P. columbinus* SWS and WE-WS (7 mm). The diameter of inhibition zone in case of WE of *P. pulmonarius* SWS was only 6 mm.

Table 25: Inhibition of *R. solanacearum* growth by water extracts (WEs) of *Pleurotus* spp. spent wheat straw (SWS) and wheat straw (WS).

Treatment	Diameter of inhibition zone (mm)*	
	Filtration	Autoclaving
***Pleurotus columbinus* SWS**	10	7
***Pleurotus pulnonarius* SWS**	9	6
***Pleurotus floridanus* SWS**	8	—
Wheat straw (WS)	8	7

*: The diameter of filter paper disc is 0.5 mm, the diameters of inhibition zones were measured directly from the plates; the recorded values were the mean values of three replicates for each treatment.

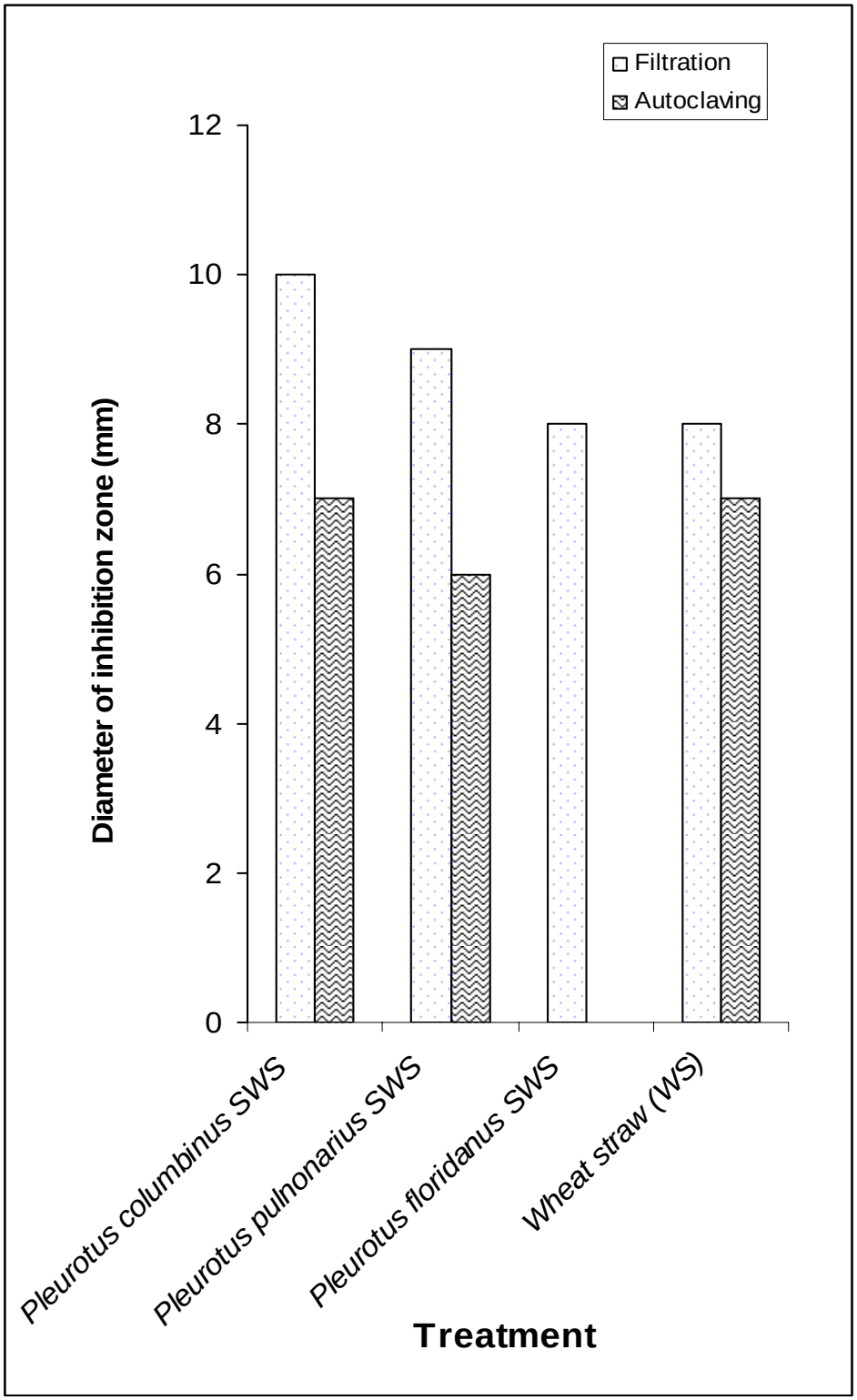

Figure 1: Inhibition of *R. solanacearum* growth by using water extracts of *Pleurotus* spp. Spent wheat straw and wheat straw.

IV. 5.2. Effect of water extracts of *Pleurotus* spp. spent rice straw on *R. solanacearum* growth:

The tested WE of *Pleurotus* spp. spent rice straw (SRS) and WE of rice straws (WE-RS), sterilized only by filtration, stimulated the growth of *R. solanacearum* (Table 26, Photo 18 and Figure 2). The diameters of stimulation zones were relatively small but larger than respective inhibition zone in the previous experiment. The stimulation zone caused by WE of *P. columbinus* SRS (20 mm) and that of WE of *P. floridanus* SRS (15 mm) was better than that caused by WE-RS (12 mm), while that caused by WE of *P. pulmonarius* SRS (14 mm) was not significantly different from that of WE-RS.

IV. 5.3. Antimicrobial activity of water extract (WE) of *P. columbinus* spent wheat straw (PCSWS) and wheat straw (WS):

Generally, the antimicrobial activities of WE of *P. columbinus* spent wheat straw (WE- PCSWS) were better than that of WEWS. WE-PCSWS inhibited the growth of three tested microorganisms (*Ralstonia solanacearum, Erwinia carotovora carotovora, Rhizoctonia solani* and *Saccharomyces cerevisiae* El**) (**Table 27). On the other hand, from the eleven tested microbes, WEWS inhibited growth of only (*Ralstonia solanacearum, Pseudomonas flourscens*).

The diameter of inhibition zone resulted from WE- PCSWS against *Rhizoctonia solani* was the largest one in this experiment (15 mm). The other three inhibition zones of this experiment had the same diameter (7 mm).

Table 26: Stimulation of *R. solanacearum* growth by using water extracts (WEs) of *Pleurotus* spp. spent rice straw (SRS) and rice straw (RS).

Treatment	Diameter of stimulation zone (mm) *
***Pleurotus columbinus* SRS**	20
***Pleurotus pulmonarius* SRS**	14
***Pleurotus floridanus* SRS**	15
Rice straw (RS)	12

*: The diameter of filter paper disc is 0.5 m; the diameters of stimulation zones were measured directly from the plate; there were three replicates for each treatment.

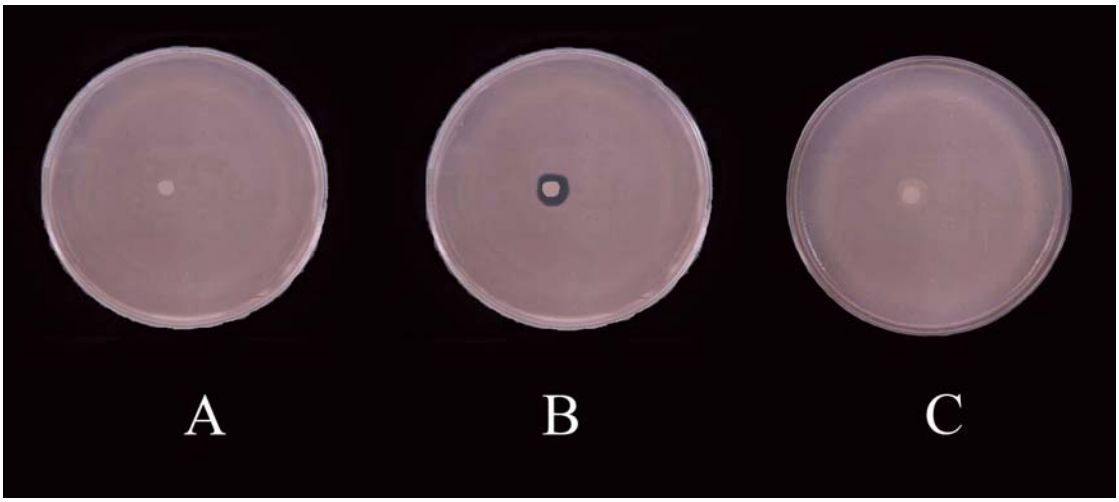

Photo 18: Effect of water extracts of wheat straw and rice straw on *R. solanacearum* after incubation at 28 °C for 24 hours: A: control, B: inhibition of *R. solanacearum* by water extract of spent mushroom wheat straw and C: stimulation of *R. solanacearum* water extract of spent mushroom rice straw.

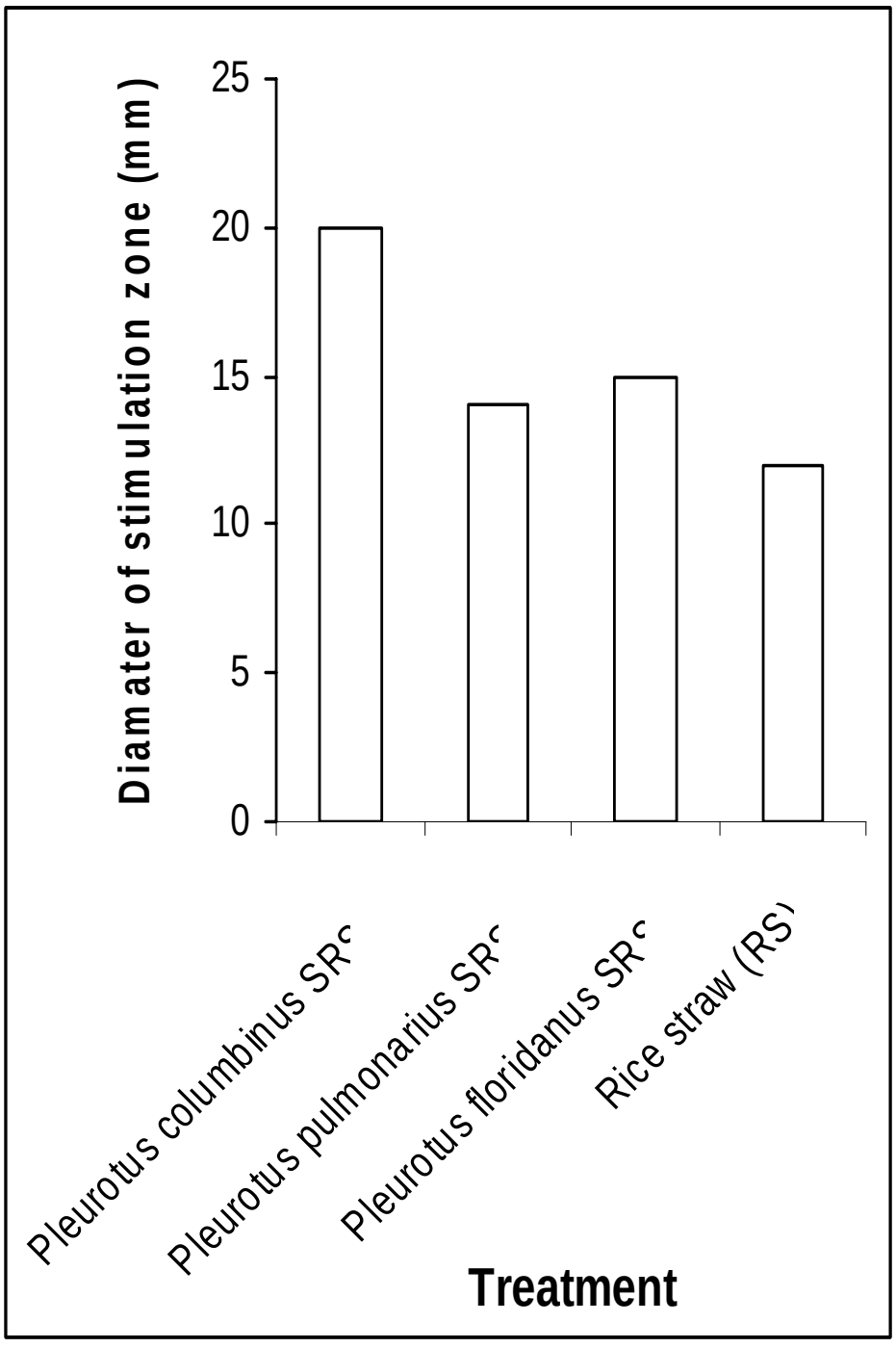

Figure 2: Stimulation of *R. solanacearum* growth by using water extracts of spent *Pleurotus* spp. rice straw and rice straw,

Table 27: Antimicrobial activity of water extract of *Pleurotus columbinus* spent wheat straw (WE-PCSWS) of and water extract of wheat straw (WE-WS):

Indicator		Inhibition zone (mm) *	
		WE-PCSWS	WE-WS
Bacteria	*Ralstonia solanacearum*	10	8
	Escherchia coli	—	—
	Pseudomonas flourscens	—	7
	Erwinia carotovora carotovora	7	—
	Staphylococcus aureus	—	—
	Bacillus subtilus	—	—
Fungi	*Rhizoctonia solani*	15	—
	Fusarium oxysporum	—	—
	Trichoderma viride	—	—
Yeast	*Lipomyces starkeyi*	—	—
	Candida lipolytica	—	—
	Saccharomyces cerevisiae El	7	—

*: The diameter of filter paper disc is 0.5 mm, —: No inhibition zone was detected, the diameters of inhibition zones were measured directly from the plates; the recorded values were the mean values of three replicates for each treatment.

IV. 6. Effect of some basidiomycetes on *R. solanacearum*:

IV. 6.1. Antagonistic effect of some basidiomycetes against *R. solanacearum*:

All tested eight basidiomycetes species had antagonistic effect against *R. solanacearum* (Table 28, Photo 19 and Figure 3). The largest recorded inhibition zone diameter (17 mm) was observed with *Cyathus stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain. In addition, the other recorded inhibition zone diameters were proximate. Therefore, the two strains (*Cyathus stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain had the best antagonistic effect against *R. solanacearum*.

Cyathus stercoreus Egyptian strain was selected for further studies during this investigation because *Cyathus* spp were recorded to uses as a bio agent and its fruiting body does not form in the soil in ordinary circumstances.

IV. 6.2. Antimicrobial activity of *Cyathus stercoreus* Egyptian strain:

The agar disc method was applied to investigate the antimicrobial activities of *C. stercoreus* Egyptian strain against the eleven tested microbes used before. It was observed that *C. stercoreus* Egyptian strain did not inhibit the growth of any tested microbe and no inhibition zones were formed. *C. stercoreus* Egyptian strain, so, had not antimicrobial activities against the tested microbes.

IV. 7. Antagonism between *Streptomyces mutabilis* and *Cyathus stercoreus* Egyptian strain:

By the application of agar disc method, after incubation of *C. stercoreus* Egyptian strain for 14 days at 25°C against *S. mutabilis*, inhibition zone of diameter 1.9 was observed and recorded (Photo 20). Therefore, *S. mutabilis* inhibit the growth of *C. stercoreus* Egyptian strain. So, it was expected that the application of combination of the two mentioned microbes in green house or in field had antagonistic effect.

Table 28: Antimicrobial activities of some basidiomycetes against *R. solanacearum*.

No.	Fungus	Diameter of inhibition zone (mm)*
1	*Cyathus stercoreus* (Schw.) de Toni Egyptian strain	17
2	*Agaricus campester (*L.) Fr. Egyptian strain	17
3	*Podaxis pistillaris* (Lin.Ex.Press) Egyptian strain	14
4	*Corticium vellereum* Ellis & Cragin Egyptian strain	13
5	*Pleurotus columbinus* Quel .ap .Bres	12
6	*Volvariella volvacea* (Bull.Ex Fr.)Sing.	13
7	*Pleurotus sajor – caju*(Fr.) Sing	13
8	*Lentinus striatuls* Le*'v*	12

*: The diameter of disc of the fungus is 0.9 mm, the diameters of inhibition zones were measured directly from the plates; the recorded values were the mean values of three replicates for each treatment.

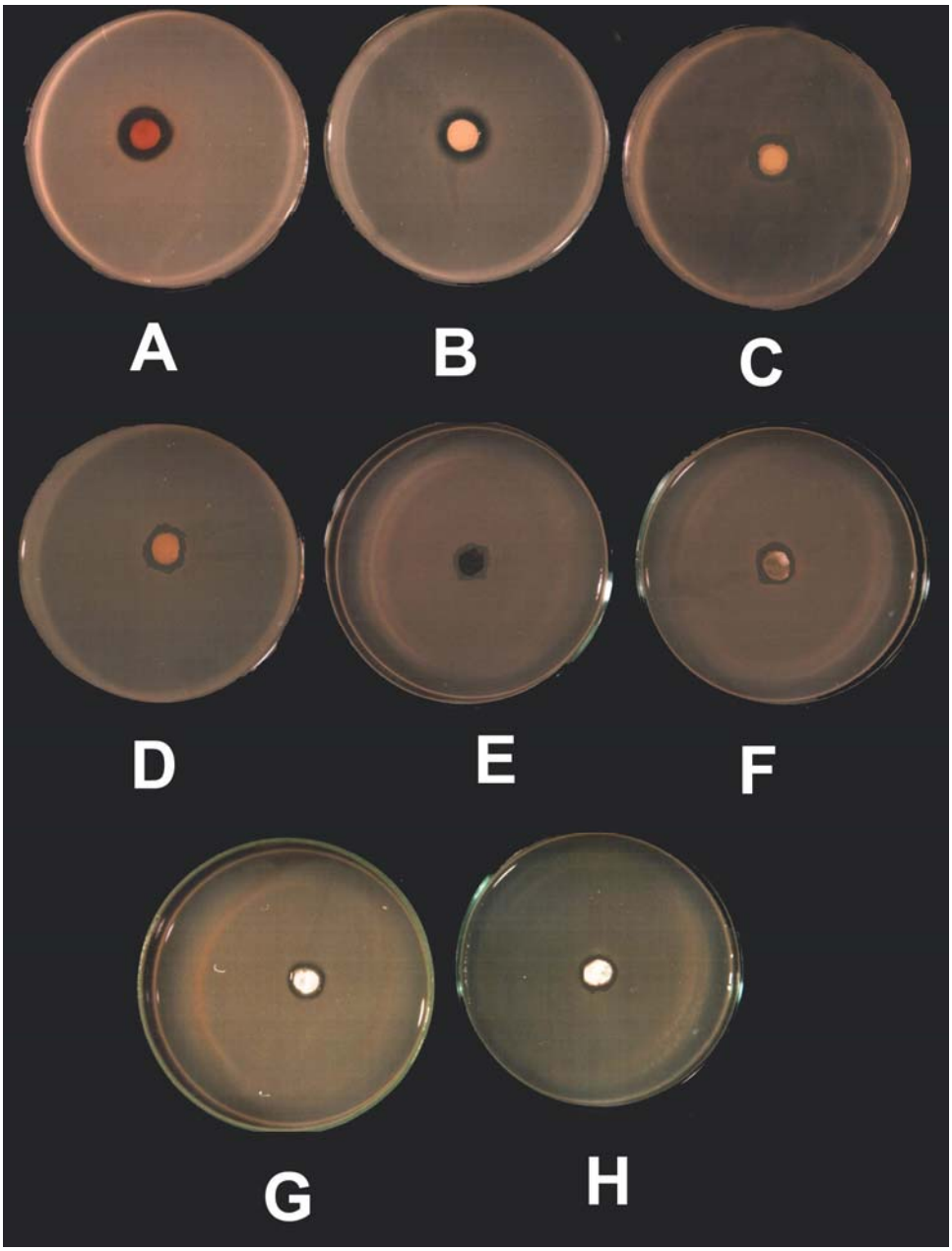

Photo 19: Inhibition of *R. solanacearum* by some basidiomycetes, where; A: *Cyathus stercoreus* Egyptian strain, B: *Agaricus campester* Egyptian strain, C: *Podaxis pistillaris* Egyptian strain, D: *Corticium vellerum* Egyptian strain, E: *Pleurotus columbinus, F*: *Volvariella volvaceae*, G: *Pleurotus sajor – caju*, H: *Letinus striatus*

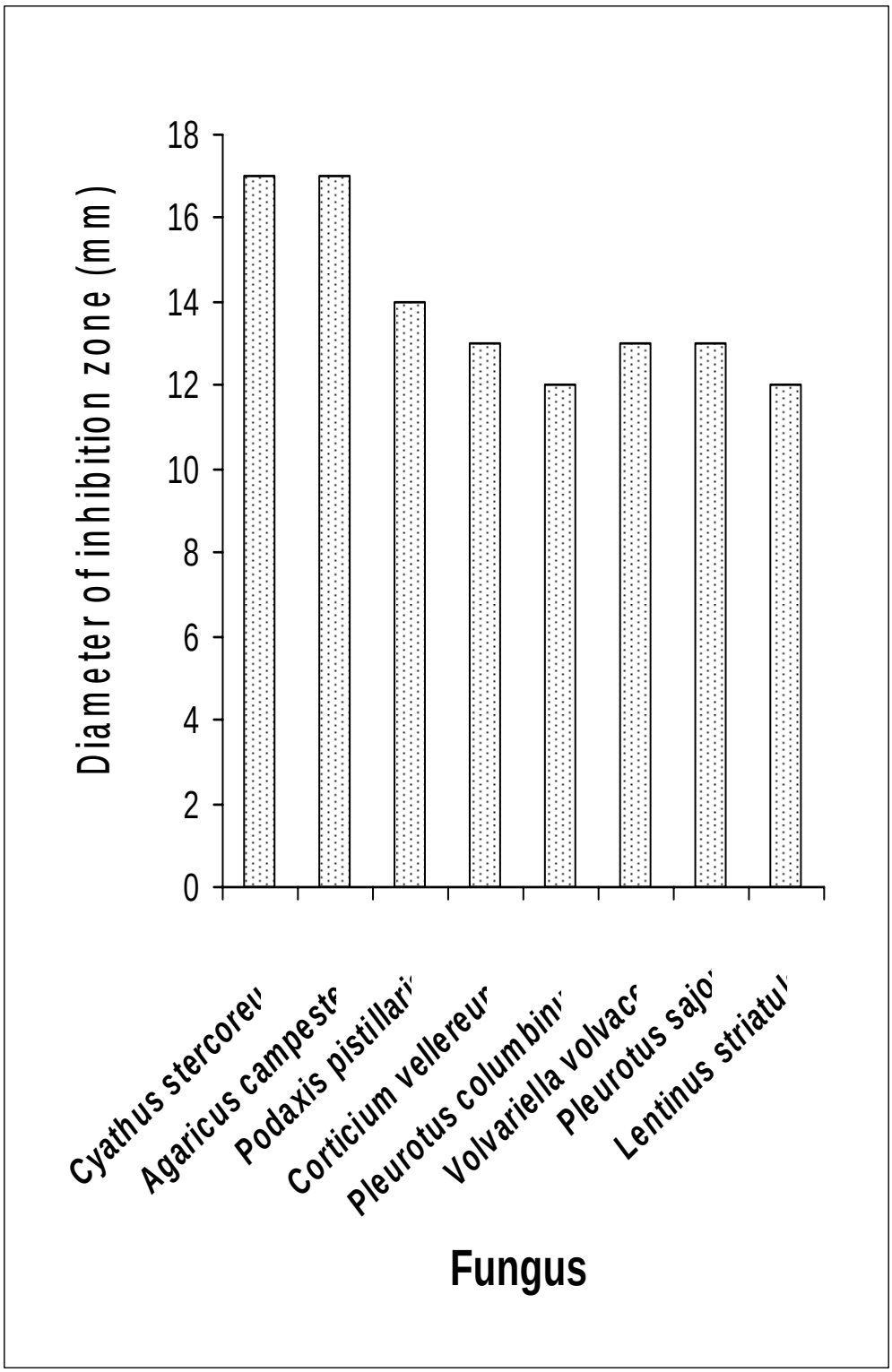

Figure 3: Inhibition of *R. solanacearum* by some basidiomycetes.

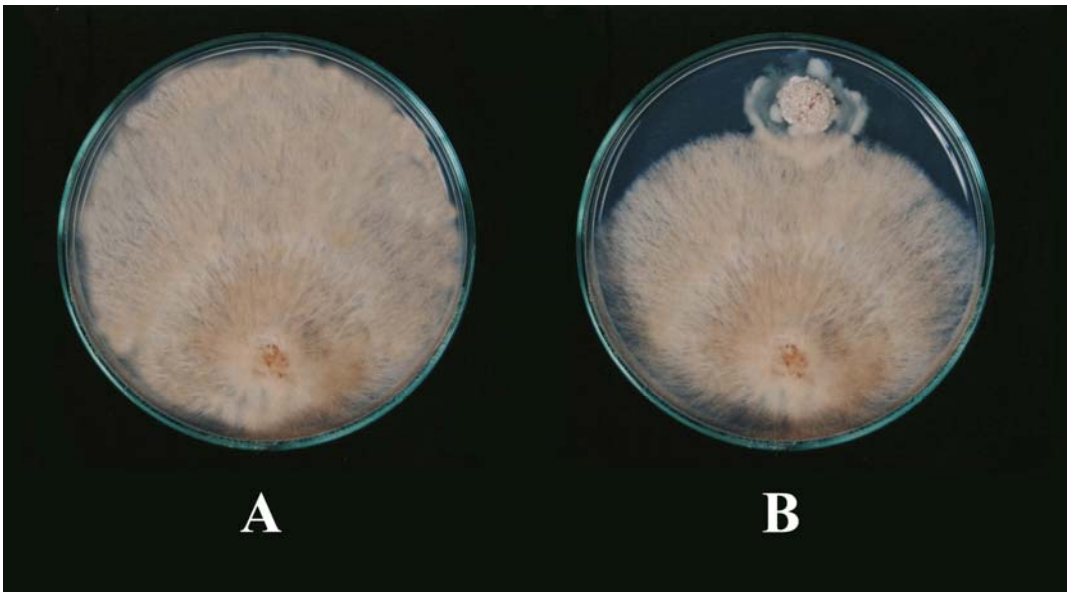

Photo 20: Inhibition of *Cyathus stercoreus* Egyptian strain by *Streptomyces mutabilis*, where, A: Control after 14 days, and B: antagonism after 14 days.

IV. 8. Green house experiment:

Data in Table 29 revealed that the best results were recorded with plants treated by phage cocktail and plants that treating by coating potato pieces seed tubers with wheat straw (WS) or *Pleurotus columbinus* spent wheat straw **(PCSWS);** they emerge the highest significant increase in plant height and mean weight of tubers for each pot and highest significant decrease in disease incidence and percentage of infected tubers. On the contrast, the worst results were recorded with plants treated by addition of 1 g of WS or **PCSWS** at planting time in planting hole. Moreover, soaking of potato pieces seed tubers in suspensions of *S. mutabilis*, *C. stercoreus* emulsion of anise oil recorded significant increase in plant height and mean weight of tubers for each pot and significant decreased in disease incidence and percentage of infected tubers.

The combinations between phage cocktail and *S. mutabilis* and/ or *C. stercoreus* significantly decreased the effect of phage cocktail in plant productivity and control of the experimental pathogen. While, the combinations between *S. mutabilis* and/ or *C. stercoreus* significantly increased the plant height and mean weight of tubers for each pot but did not significantly affect on the disease incidence and percentage of infected tubers.

The use of additional dose of the experimental bioagents significantly increased the mean weight of tubers for each pot but did not significantly affect the other studied parameters.

Table 29: Evaluation of the ability of the selected biocontrol agents to control brown rot disease of potato under greenhouse conditions.

No.	Treatment	Plant height (cm) after 80 days of sowing	Disease incidence after 80 days of sowing	Mean weight of tubers for each pot (g)	* % of infected tubers
1	**Control**	11.67m	83.00a	48.33q	100.00a
2	**PhC1**	26.50a	0.00d	101.67cd	8.33ij
3	**PhC1+ PhC2**	25.50ab	0.00d	110.00a	4.17j
4	**SM1**	17.00hij	16.67cd	66.67n	15.00hij
5	**SM1 + SM2**	18.33efg	16.67cd	80.00ij	9.44hij
6	**(AO 0.25)1**	19.67def	16.67cd	88.33g	27.78ghij
7	**(AO 0.25)1 + (AO 0.25)2**	19.67def	16.67cd	97.50f	11.39hij
8	**(AO 0.5)1**	20.17de	16.67cd	70.83klm	20.83hij
9	**(AO 0.5)1 + (AO 0.5)2**	18.00ghi	16.67cd	95.00f	14.17hij
10	**CSE 1**	18.50fgh	16.67cd	60.83o	27.28ghij
11	**CSE 1 + CSE 2**	19.00efg	16.67cd	80.00ij	15.00hij
12	**(WE-PCSWS)1**	18.50fgh	33.33bcd	72.33k	65.65bcd
13	**(WE-PCSWS)1 + (WE-PCSWS)2**	19.83def	33.33bcd	80.50ij	48.33defg
14	**(PCSWS -1g)1**	12.50m	66.67ab	67.67mn	64.39bcd
15	**(PCSWS -1g)1 + (PCSWS-1g)2**	14.83 l	50.00abc	83.33hi	47.22defg
16	**(PCSWS .coat.)1**	23.00 c	16.67cd	100.00de	24.17ghij
17	**(PCSWS–coat.)1 + (PCSWS -1g)2**	24.33bc	16.67cd	105.00bc	15.56hij
18	**(WE -WS)1**	19.00efg	33.33bcd	67.50 mn	86.94ab
19	**(WE -WS)1 + (WE-WS)2**	20.00def	33.33bcd	71.67kl	77.78abc
20	**(WS-1g)1**	12.67m	66.67ab	55.00 p	53.89cdef
21	**(WS-1g)1 + (WS-1g)2**	15.33kl	50.00abc	61.00o	59.72cde
22	**(WS-coat.)1**	22.83c	16.67cd	101.00cde	25.00ghij
23	**(WS-coat.)1+ (WS-1g)2**	23.67c	16.67cd	106.67ab	15.28hij
24	**(PhC + SM)1**	18.00ghi	16.67cd	76.67j	20.83hij
25	**(PhC + SM)1 + (PhC + SM)2**	20.67d	16.67cd	78.33j	19.44hij
26	**(PhC + CSE)1**	16.50ijk	16.67cd	58.33op	20.83hij
27	**(PhC + CSE)1 + (PhC + CSE)2**	15.50 ijk	16.67cd	76.67j	19.44hij
28	**(SM + CSE)1**	17.00hij	16.67cd	86.67gh	29.17fghij
29	**(SM + CSE)1 +(SM + CSE)2**	18.33efg	16.67cd	99.17de	15.00hij
30	**(PhC + SM + CSE)1**	17.00hij	16.67cd	85.00gh	34.72efgh
31	**(PhC + SM + CSE)1 + (PhC+ SM + CSE)2**	18.00ghi	16.67cd	85.83gh	30.55fghi

Values in the columns represent the means of 6 values for 6 potato plants. Means followed by different letter(s) within a column are significantly different according to Duncan's multiple range test (P=0.05).

PhC: Lysate of phage cocktail of the four isolated phages; **SM**: suspension of *Streptomyces mutabilis suspension;* **AO**: Anise oil**; CSE**: *Cyathus. stercoreus* Egyptian strain suspension; **PCSWS**: *Pleurotus columbinus* spent wheat straw; WS: wheat straw; WE: water extract, **1**: treatment before the planting and **2**: treatment after 70 days after planting. ***: % of infected tubers** = [(No. of infected tubers at harvesting time + No. of infected tubers after storage for 60 days)/ No. of total number of tubers] X 100.

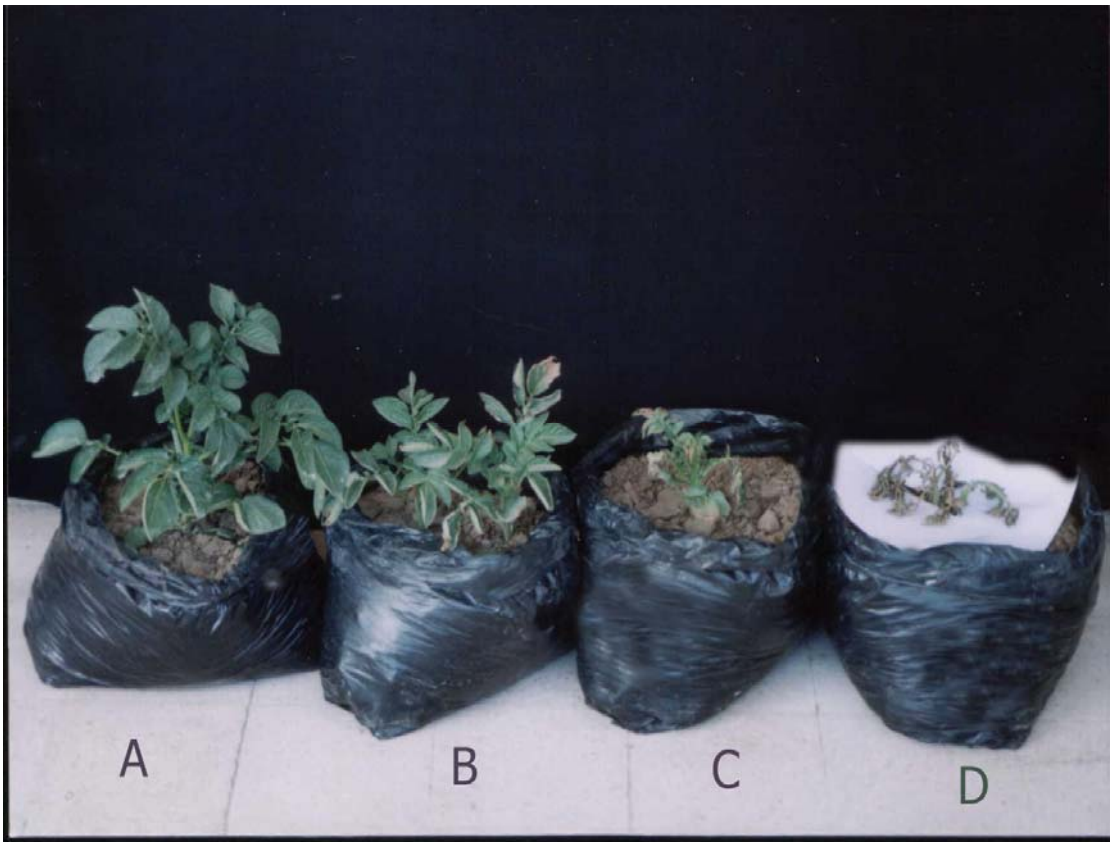

Photo 21: Potato plants in green house experiment at age of 80 days showing symptoms of brown rot disease, where; A: healthy plant, B: Slightly infected plant, C: Moderate infected plant and D: Highly infected plant (or dead plant).

IV.9. Field experiment:

Although, the field of this experiment had history of bacterial wilt disease of potato, the presence of *R. solanacearum* was confirmed by cultivation soil extract on TZC medium. The mean number of colonies of *R. solanacearum* in the soil was 1.83 X 10^6 CFU/ g soil. So, the soil of this field was surely naturally infested by *R. solanacearum*.

Data in Table 30 revealed that all experimental bioagents significantly increased in plant height and mean weight of tubers and significantly decreased percentage of non-marketable tubers at harvesting time and percentage of infected tubers by the studied pathogen. Except the treatment No. 3, there were no significant differences in mean numbers of tubers between the experimental treatments.

There were no significant differences between the reported values of the studied parameters due to using phage cocktail or *S. mutabilis*. The combination between the two aforementioned bioagents resulted only significantly negative effect on percentage of non-marketable tubers where it significantly increase the reported value of this parameter, whereas this combination had no significant effect on the other studied parameters.

Generally, the addition of additional dose of phage cocktail or *S. mutabilis* or the combination between them had no significant effect on studied parameters (except with the addition of phage cocktail significantly reduced percentage of the non-marketable tubers).

Table 30: Evaluation of the ability of phage cocktail, *Streptomyces mutabilis* and the combination between them to control brown rot disease of potato under field condition.

No.	Treatment	Plant height (cm) after 90 days of sowing	* Non-marketable tubers % at harvesting time	Mean number of tubers/ plot	Mean weight of tubers at harvesting time (ton/feddan)	** Infected tubers %
1	**Control**	39.33c	25.50a	154.33b	7.27c	8.89a
2	**PhC1**	48.67ab	17.86c	165.33ab	10.45ab	0.00b
3	**PhC1+ PhC2**	50.00a	13.85e	177.67a	12.13a	2.22b
4	**SM1**	47.17ab	15.96cd	161.33b	10.63ab	3.33b
5	**SM1 + SM2**	48.00ab	14.30de	169.00ab	11.56a	2.22b
6	**(PhC + SM)1**	45.67b	21.21b	160.67b	9.17bc	3.33b
7	**(PhC + SM)1 + (PhC + SM)2**	47.00ab	20.78b	161.67b	9.45b	2.22b

Values in the columns represent the means of 6 values for 6 potato plants. Means followed by different letter(s) within a column are significantly different according to Duncan's multiple range test (P=0.05).

PhC: Lysate of phage cocktail of the four isolated phages; **SM**: Suspension of *Streptomyces mutabilis***; 1**: treatment before the planting and **2**: treatment after 70 days after planting. *: Non-marketable tubers % = [No. of the infected tubers by other pests other than *R. solanacearum* or any tubers had any other quality disorder/ No. of total number of tubers] X 100. ****: % of infected tubers** = [(No. of infected tubers at harvesting time + No. of infected tubers after storage for 60 days)/ No. of total number of tubers] X 100.

V. DISCUSSION

Brown rot disease of potato incited by the bacterium *Ralstonia solanacearum* is a problem that threat potato plantation all over the world. In Egypt, this disease causes economic problem as it decreases the productivity of potato yield and hinders potato exportation to European Union. So, this study was a trial to find a solution for this problem and safe ways for environment and human being health.

In this investigation, the infected potato tubers, showing identical symptoms of brown rot disease of potato, were used to isolate *R. solanacearum* (the causative agent of this disease). These infected tubers were collected from different field locations in Dakahlia and Damietta Governorates. This bacterium was isolated from different potato cultivars (sponta, diamont and cara).

The bacterial ooze, from each infected potato tuber, was streaked on solidified TZC medium (differential medium). The virulent (V) isolates of this bacterium were tested to determine their biovar(s). The obtained results indicate that − according to **French *et al.* (1995)** − all studied isolates belong to biovar 2 (equivalent to race3). These results were in agreement with those reported by **Abd El-Ghafar *et al.* (1995)** who found that *R. Solanacearum* biovar 2 was widely distributed in Egypt. It may recalled here that **Hayward (1995) and Toth *et al.* (1997)** reported that race 3 (biovar 2) is responsible for the prevalence of potato brown rot disease in Europe and North Africa. Moreover, the present results are in confirmed with the results reported by **Farag *et al.* (1999)** who detected race 3 of *R. Solanacearum* in both irrigation and drainage canals near to the infected fields within infected three potato-growing areas in the Delta of Egypt.

It has been repeatedly shown that the bacterium *R. solanacearum* can survive in soil for a long period and most probably reach to irrigation

water and drainage systems. Thus it can infect numerous weeds that act as a reservoir for this pathogen **(Janse *et al.*, 1998; Farag *et al.*, 1999** and **Caruso *et al.*, 2005)**.

The current method of control of plant diseases is the application of some chemical pesticides that cause a serious environmental pollution and lead to the emergence of resistance strains of the plant pathogens **(Agrios 1997; Whipps, 2001** and **Gnanamanicham, 2002)**.

Henceforth, the problem has to be faced on a scientific basis. Thus, researches in recent years are directed to the biological control that has become an integral part of the broad field of pest management. This includes any type of disease reduction or decrease in inoculums potential of a pathogen brought about directly or indirectly by other agencies.

In the present study, several lines of trial have been preformed to find out a promising integrated biological control of the brown rot disease of potato, including virulent (lytic) phage (s), soil antagonists and essential oils.

Four active phages against virulent (V) strains of *R. solanacearum* were isolated from the root zone of healthy potato plants from two locations in Dakahlia Governorate (Salka and El-Mahmodeia) and one location in Damietta Governorate "Kafr El-Battikh". These phages were designated as "RSP4, RSP5, RSP6 and RSP7". These symbols were the first letters of "*Ralstonia solanacearum* phages".

It may be mentioned in this connection that *R. solanacearum* phages were previously isolated by **Singh *et al.* (1986**), **Tanaka *et al.* (1990)**, **Toyoda *et al.* (1991), Kakutani *et al.* (1994)** and **Ozawa *et al.* (2001)**. The four isolated phages were active only against V strains of *R. solanacearum* and they did not infect AV strains of this bacterium.

It had been demonstrated by several investigator that the virulent (V) strains of *R. solanacearum* produce exopolysaccharides (extracellular

polysaccharide slime) (EPSs)**;** an accumulated polymers of polysaccharide that is lacking in case of the avirulent strain of *R. solanacearum* **(Husain and Kelman, 1958; Hayward, 1991; Huang *et al*., 1993 and Toth *et al*., 1997)**. Therefore, the results reported in suggest that these EPSs may play important role in the susceptibility of the bacteria to phages infectivity. These EPSs may act as receptors for the four isolated phages. In addition, these phages may have the ability to produce enzymes that degrade EPSs or have the ability to induce the host cells themselves to produce these enzymes when the phages attached to their receptor sites. Whereas, all of these phages failed to infect the avirulent strains of *R. solanacearum* bacterium due to absence of EPSs (their receptor sites). In support of this explanation it may recall that **Defives *et al.* (1996)** have demonstrated that the receptors of phage NM8 is a polysaccharide site, probably involving the acetyl group of sialic acids. Also, **Hughes *et al.* (1998 a and b) and Hanlon *et al.* (2001)** indicated that some phages penetrated EPSs due to presence of enzymes degrading EPSs. The deduced that the source of these enzymes may be the phage(s) or the bacterial host itself.

On the other hand, the phage of **Tanaka *et al.* (1990)** did not infect V strains of *R. solanacearum* because EPSs covered the receptors of these phages that present on cell wall. This suggestion agreed with **Defives *et al.* (1996)** and **Forde and Fitzgerald (1999)**. Also these phages failed to produce enzymes degrading EPSs or induce cells to produce them **(Hughes *et al.* 1998 a &b and Hanlon *et al.* 2001)**.

Plaque morphology is one of the prime criteria that used for detection, identification and classification of phages. Therefore, the majority of phages exhibited well-defined plaque characteristics including size, outline, structure and transparency or turbidity. In the present investigation, all of the four isolated phages had clear plaques and all of

them had circular plaque edges except RSP7 that had tetragonal plaques. The plaques of these phages had different diameters. Therefore, the plaques of RSP4 phage resemble those of phage Φ3A of **El-Helali (2001)** and phages e11/2 and pp01 of **O'Flynn *et al.* (2004)**. The plaques, of the other phages, did not resemble any of the recorded plaques in the review of present study.

All of the four expermintal phages were tailed phages; therefore they belonged to the order *Caudovirales*. The phage RSP4 had short tail, so it was classified belong to the *Podoviridae* family or Bradley 's group C **(Bradley, 1967)**. Therefore, this phage was similar to phages RLz_{10} to RLz_{17} **(El-Didamony, 1995),** phage ΦCP6-4 **(Tremblay and Moineau, 1999)** and phage ΦK1-5 **(Scholl *et al.*, 2001)**.

On the other hand, the other three phages (RSP5, RSP6, RSP7) had long flexible non-contractile tails, so, they were belonged to the *Siphoviridae* family or Bardley's group B. Therefore, these phages resembled phages RLz_3 to RLz_9 **(El-Didamony, 1995)**, phage DT1 **(Tremblay and Moineau, 1999),** phages ΦS and ΦL **El-Sayed *et al.* (2001),** phages Φ1C2, Φ3A and Φ4C **(El-Helali, 2001),** phage e4/1c **(O'Flynn *et al.*, 2004)** and phages YAB, Ib3 and BYM **(Quiberoni *et al.*, 2004)**. These results was confirmed by **Ackermann (2003)** who mentioned that the tailed phages were the predominate group of phages and *Siphoviridae* or phages with long, non-contractile tails are the predominate group of tailed phages (61 % of tailed phages). Furthermore, there was a characteristic trait in phage RSP7; a fork like base plate. This base plate of RSP7 was similar to base plate of eight *Siphoviridae* phages (designated as JS77.1) that had a characteristic trait; a fork like base plate **(Chibani-Chennoufi *et al.*, 2004)**

All four tested phages were tested for their ability to form plaques on a range of gram-negative and gram-positive bacteria. Each phage was shown polyvalent activity, where they lysed V strains of *R. solanacearum*, *Erwinia amylovora* and *Pseudomonas aeruginosa*. On the other hand, the other tested bacteria were not lysed. These phages were polyvalent and similar to P3, P5 and P6 phages **(Supriadi and Green, 1989)**, T, V, M phages **(El-Sawi, 1998)** and ΦS and ΦL phages **(El-Sayed *et al.*, 2001)**.The susceptibility of bacterial strains to phage attack differed and this may be due to variation of receptors molecules (adsorption blocking), restriction modification system in the host or other phage resistant system such as abortive infection **(Duckworth *et al.*, 1981 and O'Flynn *et al.*, 2004)**. Hence, the obtained results suggested that V strains of *R. solanacearum*, *E. amylovora* and *P. aeruginosa* may have the same receptor molecule for the studied phages.

In the present study all of four experimental phages have no lysogeny. So, no new strains were appeared against these phages. It is likely that these phages were obligate lytic phages and they did not converted into prophages. Therefore, these phages can be considered as promoting candidate to be used as biocontrol agents to control V strains of *R. solanacearum*.

Based on the aforementioned and results, four actinomycete strains (A11, A36, A39, and A84) are prone to be capable of inhibitory the growth of *R. solanacearum*, when double layer method was applied. Whereas, when agar-disc method was applied, only one strain (A11) had the ability to inhibit the growth of *R. solanacearum*. These results suggested that the inhibition of *R. solanacearum* was due to the ability of these actinomycete strains to produce quite potent antibacterial compounds.The four actinomycetes strains (A11, A36, A39, and A84) can inhibit the growth of *R. solanacearum* when the double layer

technique was applied to their ability to produce antibiotic(s) and/ or other metabolites on SPA medium. Meanwhile, only A11 strain can inhibit the growth of *R. solanacearum* when agar disc method was applied due to the ability of A11 strain to produce antibacterial compounds on starch-casein agar medium. While, the other three strains failed to do so on the later medium. Consequently, this means that the ability of actinomycetes to produce antimicrobial compounds is greatly affected by medium composition. These findings were similar to those of **Yuan and Crawford (1995)**, **Cundlif (1997), Oskay *et al.* (2004)** and **Badr (2006)**

The four selected actinomycete isolates were classified as the belong to genus *Streptomyces* due to the results that all of them have cell wall type I (**Lechevalier and Lechevalier, 1970 a**). These isolates were further differentiated on the basis of the colour of the colony as follows:

- White series includes the two isolates A11 and A84. These two isolates are characterized by rectiflexibles spore chain, smooth spore surface, non-distinctive colour of substrate mycelium, absence of soluble pigment, no ability to produce melanoid pigments. Moreover, these two isolates utilize D-glucose, D-fructose, mannose, sucrose, mannitol, L-rhaminose, L-insitol and starch. While, there were some differences between A11 and A84 in utilization of carbon source; where isolate A11 utilize D-xylose, arabinose, lactose and glycerol and does not utilize rafinose and D-galactose. Isolate A84 utilize rafinose and D-galactose and does not utilize D-xylose, arabinose, lactose and glycerol. Therefore, according to the diagnostic key of Bergey's manual (1989) **(Williams *et al.*, 1989)** and surveying the literature on the description of *Streptomyces* spp. In the articles of ISP (1966, 1968 a, &b, 1969 and 1972) **(Shirling**

and Gottelieb, 1966, 1968 a & b, 1969 and 1972) isolate A11 was classified as *Streptomyces mutabilis* **(Preobrazhenskaya and Ryabova, 1957)** and isolate A84 was classified as *S. pyridomyceticus* **(Okani and Unezawa, 1955).**

- Grey series includes isolate A36. This isolate was characterized by spiral spore chain, spiny spore surface, non distinctive colour of substrate mycelium, absence of soluble pigment, no ability to produce of melanoid pigments. This isolate utilize all the experimental carbon sources in this investigation. Therefore, isolate A36 was classified as *S. sparogenes* **(Owen *et al.* 1962)**.
- Red series includes isolate A39. This isolate was characterized by rectiflexibles spore chain, smooth spore surface, non distinctive colour of substrate mycelium, absence of soluble pigments, no ability of production of melanoid pigment. This isolate utilize D-glucose, mannose, sucrose, and starch while it does not utilize the other experimental carbon sources in this investigation. So, isolate A39 was classified as *S. luridus* **(Krasilinkov *et al.*, 1957**).

Perusal of the data obtained concerning the antibiotic production potential of these four *Streptomyces* species, they can be considered as promising candidates for biological control of brown rot of potato brown rot of potato incited by *R. solanacearum*.

From 16 tested essential oils, three tested essential oils (anise, thyme and lettuce essential oils) had the strongest antagonistic effect against *R. solanacearum*. They completely inhibited the growth of studied bacterium at plates of 9 cm diameter when filter paper disk method or fumigation method were applied for these oils. There were 9 tested essential oils had weak inhibitory effect against *R. solanacearum* and 4

essential oils had no antagonistic effect against the studied bacterium. These results indicated that the three strongest essential oils had both non-volatile and volatile antibacterial compounds that inhibited the growth of the tested bacterium. The non volatile antibacterial compound(s) had a high diffusion rate through SPA and high antibacterial properties. Also, these results suggested that the nine essential oils that had weak inhibitory effect against tested bacterium may be due to low inhibitory effect of non-volatile compounds of these oils and/ or low diffusion rate of these compounds through SPA medium. These results resembled those obtained by **Abd El-Aziz (2002)** who studied inhibitory effect of essential oil against *R. solanacearum* by fumigation method and contact method.

The antimicrobial action of the three selected essential oils as estimated against *R. solanacearum* by detecting of the MIC values appeared to depend on the test producer (i.e. agar disk method or diffusion method). The lowest MIC values were recorded with anise oil (≤ 0.25 % for agar disk method and 0.5 % for striking method), then theme oil (1 % for agar disk method and 2 % for striking method) and latter lettuce oil (> 2% for both used methods. These results indicated that anise oil was the most effective antagonized oil against *R. solanacearum*. These results were similar to those obtained by **Takarada *et al.* (2004)** who determined MIC values of manuka and tea tree essential oils against some oral bacteria and these MIC values were approximates to the MIC values of the three selected essential oils of the current study.

Selected essential oil (anise oil) seemed to exhibit a broad antimicrobial spectrum; showed inhibitory action towards eight microbes of the eleven test organisms. In general, anise oil inhibited the growth of gram-negative bacteria *(R. solanacearum* and *Escherchia coli*), gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilus*), fungi,

(*Rhizoctonia solani* and *Fusarium oxysporum*) and yeasts (*Lipomyces starkeyi, Candida lipolytica and Saccharomyces cerevisiae* El). These results indicated that anise oil possesses antimicrobial compounds that of broad spectrum against these different groups of microorganisms. These results were confirmed by those of **Hamido (2003)** who studied the wide antimicrobial activities of *Artemisia monosperma* and *Pulicaria incise* essential oils against gram-positive, gram-negative, fungi and yeasts.

The water extract of wheat straw (WE-WS) sterilized by autoclaving or filtration inhibited the growth of *R. solanacearum* and the tested WE of *Pleurotus* spp SWS also inhibited the same bacterial species except WE of *Pleurotus floridanus* SWS. These results may be due to that wheat straw contains antibacterial compound(s) which was soluble in water and so present in the water extracts (WEs). The difference in the inhibitory effect of WE of *Pleurotus* spp SWS may be due to the difference in their ability to produce extracellular enzymes. These results were confirmed by the results of **Tan and Wahab (1997)**. They reported that *Pleurotus sajor-caju* grown on cotton waste produces relatively low levels of three components namely cellobiohydrolase, CMCase and β-glucosidase. Also, **Velázquez-Cedeňo *et al.* (2002)** indicated that the two basidomycetes *Pleurotus ostreatus* and *P. pulmonarius* had the ability to produce some extracellular lignocellulytic enzymes. Furthermore, the inhibition zones of testes WEs sterilized by filtration had significantly increased than their corresponding ones sterilized by autoclaving. These results may be due to the antibacterial compound(s) present in WEs damaged partially or completely by heating.

On the other hand, WE-RS and WE of *Pleurotus* spp. SRS had stimulatory effect on the growth of *R. solanacearum*. This stimulatory effect may be due to RS contain water soulble stimulatory compound(s). The significant increase in stimulatory effect of WE of *Pleurotus* spp.

SRS than WE-RS may be due to the effect of extracellular enzymes secreted by *Pleurotus* spp. which can release more stimulatory compound(s). These results are in conformity with results of **Tan and Wahab (1997)** and **Velázquez-Cedeňo *et al.* (2002)**. The significant difference between the stimulation zones of *Pleurotus* spp. may be due to the enzymatic activity of each species; the type of secreted enzymes and their quantities. WE of *P. columbinus* SRS had the significant largest stimulation zone, so, this species had the best extracelluar activity comparing with the three tested species releasing stimulating compounds(s) of *R. solanacearum* growth which was/ were soluble in water. On the contradictory, *P. pulnonarius* had the less enzymatic activity releasing water soluble compound(s) that had stimulating effect on *R. solanacearum*.

All the eights tested fungal species inhibit the growth of R. solanacearum. Therefore, all of them may produce antibacterial exo-metabolite(s) active against *R. solanacearum*. The largest inhibition zone was reported with both *C. stercoreus* Egyptian strain and *A. campester* Egyptian strain, which may be due to the antibacterial metabolite(s) secreted by the previous strains were the most active against *R. solanacearum*. These results are in agreement with **Anke and Oberwinkler (1976)**, **Heim and Anke (1988)**, **Hwang *et al.* (2000)** and **Lui and Zhang, (2004)**. *Cyathus stercereus* was to produce polysaccharides which have antibacterial action against *R. solanacearum.* These results are in conformity with **El-Fallal and Moussa** (**2006)**.

In the greenhouse experiment, generally, phage cocktail had the best results followed by the treatments of coating by a thin layer of fine powder of PCSWS or WS. The worst results were recorded with the treatments of WE and 1g of fine powder of PCSWS or WS.

Moreover, the addition of another dose of tested bio-agents, after 70 days of planting, did not significantly affect the observed diseases incidence after 80 days and the percentage of total infected tubers for each pot at harvesting time and also in most cases of plant height. While these additional doses significantly increased the reported mean weight of tubers of each pot (in most cases). These results suggest that the application of the additional dose may be late or the short period between the application of this additional dose and reporting studied two factors after 80 days (only ten days) may affect on these reported data. Therefore, these results suggested that the time of application of the additional dose requires more study. Also, the doses of these bioagents at planting time and in the additional dose require more study. The time of planting in suspensions, filtrates or emulsions also require more investigation.

Phage cocktail had the highest recorded plant height after 80 days of planting and the mean weight of tubers after harvest. The disease incidence after 80 days and percentage of total infected tubers of phage cocktail presented the best category having the lowest values. These results were similar to **Tanaka, *et al.* (1990)** who used avirulent stain of *Pseudomonas solanacearum* (*R. solanacearum*) and its lytic phage to control bacterial wilt disease of tobacco. Also, these results are in agreement with **McKenna *et al.* (2001)** who used a virulent phage to control scab disease of potato. There are a number of potential advantages of using phages to control infections; in particular, phages which are highly specific for pathogenic bacterium and thus are harmless to the host. Low initial dose of the phage can be used since the virus multiplies in the bacterial cells, releasing new phage particles on lysis. This process of enhancement of the number of phages should continue until all the bacteria have been destroyed. The problem of development of resistance can be reduced by concurrent administration of a number of different

phages, each of which act on the same type of cells **(Hanlon *et al.*, 2001)**. Therefore, the use of phage cocktail surpassed than the use of each phage alone. Moreover, this may suggest that this phage cocktail might increase and/ or enhance the resistance of potato plants.

Moreover, the good results of plants coated by fine powder of PCSWS or WS came after those of phage cocktail in plant height and main weight of tubers for each pot. Also, no significant differences were recorded between disease incidence of plants of these bioagents and their percentage of total infected tubers of studied disease and those of phage cocktail. These results suggested that the coating of potato pieces at planting time may play an important role involving many mechanisms during plant life affecting on plant health and crop productivity; as improving soil physical properties, fertilizers and biocontrol agents as the following:

1. The direct inhibitory effects on studied pathogen by the water extracts of PCSWS or WS. The watering of plants cause leakage of these powders producing WE that inhibit *R. solanacearum* as revealed in this investigation.
2. Their ability to improve soil properties may be due to the role of PCSWS or WS in improvement of soil aeration, structure, drainage, moisture holding capacity, (**Abo El-Fadl *et al.*, 1995; He *et al.*, 1995 and Davey, 1996)**.
3. Their role as fertilizers may be due to increasing N mineralization significantly **(Gök *et al.*, 2002)**.
4. These powders of PCSWS or WS contain valuable amount of sugars, nitrogen, cations, hormones and some phenolic compounds **(Shukery *et al.*, 1999; Shen and Shen, 2001 and El-Fallal and Migahed, 2003)**.

5. The role of PCSWS or WS as a biocontrol agent may be due to that the coating of potato pieces plays a role as a physical barrier that reduces contact with the pathogen or the impacts of non-target chemicals **(Hau and Beutte, 1983** and **Ferguson and Shew, 2001)**.
6. The coating process change preferred niche of the pathogen diminishing the pathogen's ability to survive **(Bailey and Lazarovits, 2003)**.
7. These organic matters may directly or indirectly suppress disease by enhancing production of decomposition products by antagonistic microbial populations **(Huang and Huang, 1993; Shetty, *et al.,* 2000** and **Gamliel *et al.,* 2000)**.
8. The organic matter can stimulate the production of lytic enzymes involved in the degradation of plant pathogens. Microbial degradation of plant residues may produce secondary products with antimicrobial activities that inhibit the growth of plant pathogens **(Bailey and Lazarovits, 2003 and Bardin *et al*., 2004)**.

The results of the use of actinomycete *S. mutabilis* to manage bacterial wilt disease of potato showed that in green house trials *S. mutabilis* inhibited not only *R. solanacearum* but also other pathogens due to wide antimicrobial activity of *S. mutabilis* as mentioned before in the current study. Moreover, *S. mutabilis* may produce growth factors that stimulate the growth of potato plants in this experiment. The last suggestion had the agreement of **Koaze (1958), Turhan (1981), Tahvonen and Avikainen (1990), Mansour *et al.* (1994), El-Tarabily *et al.* (1997)** and **AlDesuqy *et al.* (1998).** In addition, the antimicrobial activities of *S. mutabilis* may be due to the production of lytic enzymes. This suggestion confirmed by **Lloyd *et al.* (1965), Beyer and Diekmann**

(1984) and Okazaki and Tagawa (1991), **Grigorova *et al.* (1997)** and **Yoshida *et al.*, 1997**).

The results of *Cyathus stercoreus* Egyptian strain are attributed to the mode of action of glucan extracted from *Cyathus stercoreus* that has antibacterial activity against the studied pathogen and has detected in this study. These results are in conformity with the results of **El-Fallal and Moussa (2006**). The antimicrobial activity of polysaccharides from basidiomycetes has been mentioned previously **(Wasser and Weis, 1999; Ershova *et al.*, 2003 and Guo *et al.* 2004)**. Moreover, **Halsall (1993)** showed that the nitrogenase activity of Beijerinckia indica B15 was stimulated by inoculation with *Cyathus stercoreus* both in axemic culture and in native soil. He found that *C. stercoreus* can degrade lignocelllase. So, it showed degree of ability to cross-feed diazotrophs. Its lignocellulotic ability has been also confirmed by **(El-Fallal and El-Diasty, 2006**).

There were no significant differences in the values of studied factors between the plants treated by two AO emulsions of concentrations (0.25 % and 0.5 %). The use of AO emulsion gave results that came only after phage cocktail and coated treatments and came before *S. mutabilis* and *Cyathus stercoreus* Egyptian strain. These results were similar to those of **Pradhanang *et al.*, (2003)** who used thymol, plamarosa oil and lemongrass oil to manage bacterial wilt (caused by *R. solanacearum*) of tomato under greenhouse conditions. These results ascribed to the wide antimicrobial activities of anise oil against a wide range of microorganisms as indicated in the current investigation. Also, anise oil inhibited the growth of some other pests as revealed by **Tunç and Sahinkaya (1998)** who reported that anise oil was toxic to two greenhouse pests; the carmine spider mite and cotton aphid.

The two combinations phage cocktail + *S. mutabilis* and phage cocktail + *Cyathus stercoreus* Egyptian strain did not have any significant effect on the disease incidence, after 80 days of planting, and percentage of total infected tubers, while there significant decrease the effect of phage cocktail on the recorded plant height, after 80 days of planting, and mean weight of tubers at harvesting time. These results suggested that there was antagonistic relationship between phage cocktail and *S. mutabilis*. This antagonism may be due to the extracellular compounds produced by *S. mutabilis* or *Cyathus stercoreus* Egyptian strain which inhibited the activity of one phage or more of the four phages in Phage cocktail. This suggestion was supported by results of **De Banchero *et al.* (1965), Waksman (1967)** and **Mansour *et al.* (1988)** who mentioned that actinomycetes produce antiviral compounds. Moreover, this suggestion was confirmed by **Amoros *et al.* (1997)** who found that extracts from fresh fruiting bodies of some basidiomycetes species inhibited some viruses.

Soaking potato pieces in filtrate WE-PCSWS or WE-W at planting time provided plant by water soluble fertilizers and antimicrobial compounds. However, the WEs filtrates may be exhausted or break down during the growth of potato plants and had no effect on studied bacterium in the last stages of potato growth.

Generally, the results of greenhouse experiment suggested that WS or PCWS powders can be used as a carrier for effective microbial biocontrol agents and using them in coating potato pieces at planting time. This suggestion can increase the antimicrobial activity and to take advantage of the other mentioned mode of action mechanisms of these organic matters.

Biological control of most plant diseases remains inconsistent and unpredictable in the field. The origins of this inconsistency are likely diverse and often not well understood. Environmental conditions, soil type, host plant cultivar, variation in pathogen response to inoculated antagonists and inoculation strategy have been all shown to significantly influence the efficacy of biological control **(Decan, 1991; Schisler *et al.*, 2000 and Rayan *et al.*, 2004)**.

The results of field experiment were different from those of greenhouse experiment. The actinomycete *S. mutabilis* had results in field experiment better than those of *S. mutabilis* in greenhouse experiment. These results may be due to the field soil differ from green house soil. Egyptian field soils are neutral to alkaline, which are favourable for actinomycetes **(Alexander, 1977 and Youssef *et al.*, 2001)**. Also, soil micro-flora of field was different from micro-flora of greenhouse soil. Furthermore, *R. solanacearum* strains naturally presented in field soil might be more sensitive to extracellular compounds produced by *S. mutabilis*.

The results of phage cocktail in field experiment were relatively lower than those of green house experiment. This difference in phage cocktail results might be due to the difference in soil properties and/ or due to the sensitivity difference of *R. solanacearum* strain(s) presented naturally in field soil from the experimental strain of *R. solanacearum* by which greenhouse soil was artificial infested.

Using phage cocktail treatment significantly increased plant height, number of tubers and weight of tubers more than the control treatment. Also, phage cocktail treatment significantly decreased the percentages of infected tubers by brown rot disease. These results were due to the efficacy of phage cocktail to destroy *R. solanacearum*. In addition, percentage of non marketable tubers of phage cocktail treatments

decreased significantly. These results confirm the suggestion that phage cocktail might enhance the induction of resistance of potato plant towards *R. solanacearum* infection. The addition of additional dose of phage cocktail did not have any significant influence on the tested parameters except percentage of non-marketable tubers. These results revealed that the field soil might hinder the phages in their search for a suitable host *R. solanacearum* and hinder its approval to rhizoplane of potato plant. These results might be due to the soil particles adsorbed phage particles and thereby limits the maximum rate at which they can encounter a suitable host cell **(Gill and Abedon, 2003)**.

Soaking potato pieces in *S. mutabilis* suspension significantly increased the plant height and weight of tubers. Therefore, these results confirm previous suggestion in greenhouse experiment that *S. mutabilis* produced growth factors (growth regulators). The amendment of (drenching) plants after 70 days by 100 ml of *S. mutabilis* suspension had no significant influence in the recorded parameters. These results might be due (as mentioned before with phage cocktail) to the adsorption of *S. mutabilis* cells on soil particles that attenuated their approval to rhizoplane zone and hinder their roles as antagonists and growth regulators.

The percentage of non-marketable tubers decreased significantly with *S. mutabilis* treatments from the percentage of control. These results might be due to the wide antimicrobial effect of *S. mutabilis*. Also, exracellular compounds of *S. mutabilis* might inhibit the growth of some harmful fauna.

The combination between phage cocktail and *S. mutabilis* in field experiment had results better than the results of green house experiment. These results might be due to the influence of *S. mutabilis* in field experiment was better than its influence under the other experiment.

Perusal of aforementioned the present investigation offers a contribution to integrated biocontrol of brown rot disease of potato. Meanwhile, it throws some light on the potentiality of bacterial viruses and/ or *Streptomycetes* in destroying the pathogen (*R. solanacearum*) or at least minimizing its decrease-producing activity.

VI. SUMMARY

The brown rot (bacterial wilt) disease of potato is worldwide disease that damage potato plantation all over the world. Therefore, this investigation was conducted to study the effectiveness of some biological agents to control the brown rot disease.

The causal agent of this disease was isolated from typical infected tubers collected from ten locations from Dakahlia and Damietta Governorates. This causal agent was identified as *Ralstonia solanacearum* biovar 2 which equivalent to race 3. Moreover, the pathogenicity test was carried out for the obtained isolates. All of the obtained isolates were virulent but their virulence varied from one isolate to another.

Ten soil samples, taken from root zone of healthy plants, were used for isolation of four lytic phages active against virulent strains *R. solanacearum*. These soil samples were collected from the root zone of healthy potato fields from different locations in Dakhlya and Damietta Governorates. Four phages were isolated that were designated as RSP4, RSP5, RSP6 and RSP7. These phages had clear plaques with different shapes and diameters. All the four isolated phages were tailed phages belonged to the order *Caudovirales*. The phage RSP4 had short tail, so it belonged to the *Podoviridae* family or Baradly's group C. The other three phages had long flexible non-contractile tails, so, they were belongs to the *Siphoviridae* family or Baradly's group B. The four isolated phages were polyvalent; they can infect more than one bacterial species. They can infect virulent isolates of *R. solanacearum*, *Pseudomonas aeruginosa* and *Erwinia amylovra*. No lysogenic bacteria appeared after incubation these phages for one week.

A total 40 different actinomycetes strains were isolated from ten soil samples taken from the root zone of healthy potato plants. These strains were tested as antagonists against *R. solanacearum*. By application double layer method, four actinomycetes stains (A11, A36, A39 and A84) inhibited the growth of *R. solanacearum*. Only one actinomycetes strain (A11) inhibited the growth of this bacterium. While, there was not any actinomycete strain inhibited the growth of this bacterium by application fumigation method. The antimicrobial activities of these four actinomycetes species were studied. A11 isolate had the higher antimicrobial activities and A84 had the lowest activities. The four selected actinomycetes strains were identified as the following; A11: *Streptomyces mutabilis,* A36: *S. sparogenes*, A39: *S. luridus* and A84: *S. pyridomyceticus*.

The antagonistic effects of 16 commercial essential oils against *R. solanacearum* were tested. By using filter paper disc method, 12 essential oils inhibited the growth of *R. solanacearum* and three of them (anise, thyme and lettuce oils) completely inhibited the growth of *R. solanacearum*. In addition, when the fumigation method was applied, these three oils cause completely inhibition for the growth of *R. solanacearum*. The minimal inhibition concentration (MIC) was determined for these three essential oils. The lowest values of MIC were recorded with anise oil (AO); ≤ 0.25 % in disc method and 0.5 % in striking method. The antimicrobial activity of anise oil is wide; it inhibited different species of bacteria, fungi and yeasts.

The antagonistic activities of filtrates of water extracts (WE) of three *Pleurotus* spp. spent wheat straw were tested against *R. solanacearum* by application filter disc paper method. Filtrates of WEs were sterilized by two methods; filtration and autoclaving. The three

filtrates of WEs of *Pleurotus* spp. spent wheat straw and WE-WS inhibited growth of *R. solanacearum* in case of sterilization by filtration. The largest diameter of inhibition zone was recorded with filtrate of *P. columbinus* spent WS (PCSWS). The reported diameters of inhibition zones of filtrates of WE-WS and WE-PCSWS in case of filtration method were larger from those in case of autoclaving method. Filtrates of WEs of rice straw and *Pleurotus* spp. spent rice straw had stimulatory effect for *R. solanacearum.* The largest diameter of stimulation zone was recorded with filtrate of WE of *P. columbinus* spent rice straw.

All tested eight basidiomycetes species had antagonistic effect on *R. solanacearum*. The largest recorded inhibition zone diameter was observed with *Cyathus stercoreus* Egyptian strain (CSE) strain and *Agaricus campester* Egyptian strain. Antimicrobial activities of CSE were studied against eleven different collected microbes by application agar disc method. CSE did not inhibit the growth of any tested microbe and no inhibition zones were formed.

Greenhouse experiment was conducted to reveal the effectiveness of the most active biocontrol agents against *R. solanacearum*. There were 31 treatments in this experiment; control, 15 treatments that treated only at planting time and 15 treatments treated at planting time and after 70 days of planting. Each treatment had 6 replicates. Lysate of phage cocktail of the four isolated phages, *S. mutabilis* suspension, *Cyathus. stercoreus* Egyptian strain suspension, two concentrations (0.25 % and 0.5 %) of anise oil emulsion and filtrates of water extract of WS and PCSWS were used for only soaking potato pieces for 10 minutes before planting or they used for soaking and drenching (100 ml for each corresponding treatment) after 70 days of planting. In addition, the suspensions of combinations between two or three of Phage cocktail, *S.*

mutabilis and *C. stercoreus* Egyptian strain were use in the same way. The fine powders of WS and PCSWS were used only for coating potato pieces at planting time or 1g of these fine powder was put in the planting hole at planting time or they used for one of the two previous treatment plus addition 1 g of fine powder on the soil surface near the plant. The recorded results indicated that the most effective agent was phage cocktail of the four isolated phages and the fine powders of PCSWS and WS when used for coating potato pieces at planting time. The lowest effective treatments were the treatments that treated by addition 1g of fine powder of wheat straw (WS) or *Pleurotus columbinus* spent wheat straw (PCSWS) in the planting hole at planting time. The addition of another dose after 70 days of planting lead to significant increase of only mean weight of tubers for each pot for most used bio-agents, while this addition did not cause significant difference for the other studied parameter.

Field experiment was conducted to show the effectiveness of phage cocktail, the most active actinomycete (*S. mutabilis*) and the combination of them for the control of brown rot disease under field conditions. Seven treatments were tested; control, three treatments of soaked potato pieces in suspensions of Phage cocktail, *S. mutabilis* and Phage cocktail + *S. mutabilis* and three treatments of soaked potato pieces at planting time in the previous suspension plus drenching by 100 ml of the respective suspension after 70 days. The results of Phage cocktail were more or less similar to the results of *S. mutabilis*, while their combination had similar recorded results.

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الملخص العربي

مرض العفن البني (الذبول البكتيري) في البطاطس عالمي الانتشار يصيب ويدمر زراعات البطاطس على مستوى العالم، لذلك فقد أجريت هذه الدراسة لمعرفة مدى كفاءة بعض وسائل المقاومة الحيوية المختلفة في مكافحة هذا المرض.

تم عزل المسبب المرضي من درنات مصابة بها أعراض نموذجية للإصابة، وهذه الدرنات تم تجميعها من مناطق مختلفة من محافظتي الدقهلية ودمياط. وتم تعريف المسبب المرضي *Ralstonia solanacearum* طرز ٢ (سلالة ٣). وتم إجراء اختبار المرضية للعزلات.

وتم عزل أربعة لاقمات بكتيرية تصيب السلالات الحادة مرضياً لبكتريا *R. solanacearum* من عينات تربة تم تجميعها من منطقة الجذر لنباتات بطاطس سليمة من حقول بطاطس في مناطق مختلفة من محافظتي الدقهلية ودمياط. وقد تم تسمية هذه اللاقمات (RSP٤, RSP٥, RSP٦ and RSP٧). وهذه اللاقمات البكتيرية لها روائق مختلفة الشكل ولها أقطار مختلفة الطول. وقد تم دراسة الشكل المورفولوجي لهذه اللاقمات باستخدام المجهر الإلكتروني، وقد وجد أن جميع هذه اللاقمات البكتيرية الأربعة لها ذيول وتنتمي لرتبة *Caudovirales*. وقد وجد أن اللاقمة البكتيرية RSP٤ تنتمي لعائلة *Podoviridae* "قصيرة الذيل" والثلاث اللاقمات الأخرى تنتمي لعائلة *Siphoviridae* "طويلة الذيل". وبدراسة المدى العوائلي لهذه اللاقمات الأربعة وجد أن هذه اللاقمات متعددة العوائل تستطيع أن تصيب أكثر من عائل، حيث وجد أنه تصيب السلالات الحادة مرضياً لبكتريا *R. solanacearum* بالإضافة إلى الأنواع البكتيرية التالية *Pseudomonas aeruginosa* and *Erwinia amylovra*. كما لم يتم ملاحظة ظهر أي سلالات مقاومة للاقمات من بكتريا *R. solanacearum* بعد التحضين لمدة أسبوع.

وقد تم عزل أربعين سلالة من الأكتينوميسيتات من عينات التربة المعزول منها اللاقمات البكتيرية. وقد تم اختبار قدرة هذه السلالات عل تثبيط نمو بكتريا *R. solanacearum* ، وباستخدام الطريقة ثنائية الطبقات وجد أن أربعة سلالات

(A١١, A٣٦, A٣٩ and A٨٤)لها القدرة على تثبيط هذه البكتريا، وباستعمال طريقة قرص الأجار وجد أن سلالة واحدة فقط (A١١) لها القدرة على تثبيط هذه البكتريا، بينما لم يوجد بكتريا لها هذه القرة بتطبيق طريقة التدخين. وقد تم اختبار القدرة التضادية للميكروبات للسلالات الأربعة السالفة الذكر، ووجد أن السلالة A١١ هي الأكثر قدرة تضادية بينما كانت السلالة A٨٤ الأقل في قدرة تضادية. وقد تم تصنيف هذه السلالات الأربعة بناءاً على الصفات المورفولوجية والفسيولوجية كما يلي:

A١١: *Streptomyces mutabilis*, A٣٦: *S. sparogenes*, A٣٩: *S. luridus* and A٨٤: *S. pyridomyceticus*.

وباختبار قدرة عدد ١٦ من الزيوت العطرية لإحداث تثبيط لبكتريا *R. solanacearum* باستخدام طريقة قرص ورق الترشيح وجد أن عدد ١٢ من هذه الزيوت له القدرة عل إحداث تثبيط لنمو هذه البكتريا، ومن هذه الزيوت يوجد ٣ زيوت لها القدرة على إحداث تثبيط كامل لنمو هذه البكتريا في طبق قطره ٩ سم، وهذه الزيوت هي: زيوت الينسون، والزعتر، والخس.

وباختبار قدرة هذه الزيوت الثلاث على إحداث تثبيط للبكتريا محل الدراسة بطريقة التدخين وجد أنها جميعها له القدرة على إحداث تثبيط كامل للبكتريا محل الدراسة كما في الطريقة السابقة، وبدراسة أقل تركيز مثبط (MIC) لهذه الزيوت، وجد أن أفضل نتائج كانت لزيت الينسون، حيث كانت القيمة المسجلة % ٠,٢٥ ≤ باستخدام طريقة قرص الأجار، بينما كانت القيمة المسجلة % ٠,٥ بطريقة التخطيط، وقد وجد أن زيت الينسون له قدرة عالية لتثبيط الأنواع المختلفة من الميكروبات.

وبدراسة قدرة راشح المستخلص المائي لمخلفات تبن القمح المستهلك لثلاث أنواع من فطريات *Pleurotus* spp. وأيضاً تبن القمح وجد أنها جميعاً تعطي تثبيطا للبكتريا محل الدراسة، وكان أفضل تثبيط لراشح المستخلص المائي للتبن المستهلك بفطر *P. columbinus* (WE-PCSWS)، وبدراسة القرة التثبيطية لـ WE-PCSWSولراشح المستخلص المائي لتبن القمح (WE-WS) وجد أنها قدرة

ضـعيفة، بنمـا كـان راشـح المـستخلص المـائي لقـش الأرز وقـش الأرز المـستهلك بالفطريات نفسها منشطاً لنمو البكتريا محل الدراسة.

وبدراسة قدرة ٨ فطريات بازيدية على تثبيط نمو البكتريا محل الدراسة، وجد أن جميعهـا لهـا القـدرة علـى هذا التثبـيط، وكانـت أفـضل النتـائج مـسجلة مـع فطـري *Cyathus stercoreus* Egyptian (CSE) strain & *Agaricus campester* Egyptian strain، وكانـت القـدرة التثبيطيـة لفطـر CSE ضـد ميكروبـات أخـرى ضـعيفة جـداً حيـث أنـه لـم يـستطع تثبـيط أي مـن ١١ نـوع مـن الميكروبات المستخدمة في هذه الدراسة.

وفـي تجربـة الـصوبة تـم دراسـة ٣١ معاملـة مختلفـة؛ ٣٠ معاملـة و معاملـة الكنترول، هذه الـ٣٠ معاملـة منهـا ١٥معاملـة تعامل فقط قبل الزراعـة و١٥ معاملـة أخـرى تعامـل مـرتين قبـل الزراعـة وبعـد ٧٠ يـوم مـن الزراعـة، ولكـل معاملـة ٦ مكـررات. وفـي هـذه التجربـة تـم نقـع تقـاوي البطـاطس فـي معلقـات خلـيط اللاقمـات البكتيرية (PhC)، والأكتينوميست (SM) *Streptomyces mutabilis* وفطر (CSE) *Cyathus stercoreus* Egyptian strain و راشـح WE-PCSWS و WE-WS و مـستحلب زيـت الينـسون (AO) لمـدة ١٠ دقـائق قبـل الزراعـة، وفـي معـاملات أخـرى يـتم النقـع قبـل الزراعـة بالإضـافة إلـى الـري بـ ١٠٠ مـل مـن المعاملات نفسها. كم تم التعامل مع تركيبة من اثنين أو ثلاثـة مـن المعلقـات التاليـة ، (PhC و SM و CSE) بنفس الطريقة. وبالنسبة للمسحوق الناعم من PCSWS و WS فقد تم استعمالها لتغطية التقاوي أو وضع ١ جرام منها في حفرة الزراعة قبل الزراعـة، أو تـتم التغطيـة أو إضـافة الـ ١ جـرام بالإضـافة إلـى أنـه بعـد ٧٠ يـوم تـتم إضافة ١ جرام من هذه المساحيق الناعمة بالقرب من النباتات فوق سطح التربة. وقد كانت أفضل النتـائج مـسجلة مـع PhC وأسوأها مسجلة مـع المـساحيق الناعمـة لـ PCSWS و WS التي وضعت في حفرة الزراعة، وقد كان لإضافة جرعـة إضـافية مـن العوامـل الحيويـة المـستخدمة تـأثير معنـوي فقـط مـع متوسـط وزن الـدرنات لكـل أصيص، بينما لم يكن لها تأثير معنوي على باقي العوامل التي تم دراستها.

وفي تجربة الحقل تم دراسة سبع معاملات؛ ستة معاملات والمعاملة الكنترول، لكل معاملة مكررة في ثلاث قطع. ثلاث معاملات يتم فيها نقع التقاوي في محلول أو معلق PHC أو SM أو PhC + SM قبل الزراعة لمدة ١٠ دقائق، وثلاث معاملات أخرى يتم فيها النقع بالإضافة إلى الري بـ١٠٠ مل من العوامل الثلاث السالفة الذكر. وقد وجد كانت نتائج خليط الاقمات البكتيرية والأكتينوميست *S. mutabilis* وتركيبة المزيج بينهما متقاربة و لايوجد بينها فروق معنوية، ولم يؤثر الري بـ١٠٠ مل من هذه العوامل الحيوية المستخدمة على أي من القياسات التي تم دراستها تأثيراً معنوياً إلا على متوسط وزن الدرنات لكل إصيص.

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كلية العلوم بدمياط

قسم النبات

دراسات على المقاومة البيولوجية لمرض العفن البني في البطاطس

رسالة مقدمة من

زياد موسى عبد المعطي أحمد

للحصول على درجة دكتوراه الفلسفة في العلوم –نبات (ميكروبيولوجي)

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الجيزة

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قسم النبات

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كلية العلوم بدمياط

قسم النبات

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عنوان الرسالة: # دراسات على المقاومة البيولوجية لمرض العفن البني في البطاطس

رسالة مقدمة للحصول على درجة دكتوراه الفلسفة في العلوم –نبات (ميكروبيولوجي)

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