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# The Possible Induction of Resistance in *Lupinus termis* L. Against *Fusarium oxysporum* by *Streptomyces chibaensis* and its Mode of Action. II: Alleviating Oxidative Stress Associated with Infection

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## ABSTRACT

In understanding of how a pathogen as *Fusarium oxysporum* cause dramatic changes at the biochemical levels of a host and how a curing actinomycetes as *Streptomyces chibaensis* is able to successfully induce defenses against the pathogen is the basis of the present work. Soil infestation with *Fusarium oxysporum* induced the prevalence of oxidative stress. This was generally underlain with an obvious decrease in activity levels of peroxidase, catalase and polyphenol oxidase in both roots and shoots. During the 1st and 2nd stages of growth *F. oxysporum* evoked the accumulation of high level of phenols in roots of *Lupinus termis* with a concomitant decrease in shoots. The trend was reversed during the 3rd stage in root and shoot tissues. During 1st stage of growth as a result of soil inoculation with *S. chibaensis* culture, detoxifying enzymes (peroxidase, polyphenol oxidase) act synergistically. Considerable increase in enzymatic activity in shoots and roots compared to the control was recorded, while catalase enzyme behaved antagonistically. On the other hand, during 2nd and 3rd stages under the same treatment, peroxidase, catalase and polyphenol oxidase were all enhanced in plants root, shoot (except shoot polyphenol oxidase activity level). Shoots of *L. termis* harvested from soil invested *with S. chibaensis* culture applied with *F. oxysporum* culture showed low levels in polyphenol oxidase activity, while the other two enzymes scored high levels.Generally, *S. chibaensis* tended to decrease the phenol content of both roots and shoots of *L. termis* plants when added alone or with *F. oxysporum* either before or after it. Changes, in N, K and Zn contents refer to their obvious decrease and increase in response to *F. oxysporum* and *S. chibaensis*, respectively, with a concomitant increase in Fe content in both cases. The protein banding pattern of leaves (55 DAS) refer to the over expression of new proteins having a role in both susceptibility of *L. termis* to *F. oxysporum* (70, 50, 27 and 4.5 KD) and in resistance induced by *S. chibaensis* as those belong to the pathogen related proteins and the chitinases.

**Key Words**: *Lupins*, Streptomycetes*; Fusarium*; Oxidative stress; Defense mechanisms

## INTRODUCTION

The resurgence of interest in the use of introduced microorganisms for biological control of plant pathogens during the last decade has been driven in part by trends in agriculture toward greater sustainability and increased public concern for hazards associated with the use of synthetic pesticides. Rapidly evolving technologies from molecular biology and genetics have provided new insights into the underlying mechanisms by which biocontrol agent's function, and have allowed evolution of the behavior of microbial inoculants in natural environments to a degree was not previously possible (Thomashow & Weller, 1996).

Control of plant diseases has major economical significance as well as a crucial role in food safety. The latest discoveries in disease genetics provide an opportunity to engineer disease resistance in plants, application of plant's own defense response, might be an options to traditional crop protection (Osbourn, 2001). Potential use of bacteria for plant disease management reviewed by Backman *et al.* (1997).

Induced resistance is the phenomenon that a plant, once appropriately stimulated, exhibits an enhanced resistance upon "challenge" inoculation with a pathogen. On the contrary to pesticides which depend on its mode of action on direct pathogen suppression, induced resistance via biotic factors, imply the stimulation of natural defense mechanisms in plants (Hammerscmidt & Kuc, 1995).

The general effects of rhizobacteria on host plants range from deleterious to neutral to beneficial (Glick, 1995; Lazarovits & Nowak, 1997). Rhizobacteria that exert beneficial effects on plant development are termed "plant growth-promoting rhizobacteria" (PGPR) (Kloepper & Schroth, 1978) because their application is often associated with increased rates of plant growth. PGPR also provide benefits to plants by suppression of soil borne pathogens (Schippers *et al.,* 1987) and through induction of systemic resistance (Hoffland *et al.,* 1995).

The term systemic acquired resistance (SAR) has been commonly used in cases where induced resistance results from prior inoculation with necrotizing pathogens or application of chemical agents (Van Loon, 1998). While the

term induced systemic resistance (ISR) is an alternative term sometimes used to denote induced resistance by non-pathogenic biotic agents, e.g. PGPR (Van Wees *et al.*, 1997).

Of the hazards left off pathogenic organisms is the prevalence of oxidative burst (oxidative stress). Peroxidases play an important role in one of the earliest observable aspects of plants defense strategy (Wojtaszek, 1997), direct production of antimicrobial radicals (Baker & Orlandi, 1999). Salicylic acid and hydrogen peroxide are the only two molecules known so far, to have a proven role in the onset and maintenance of SAR (Sharma *et al.*, 1996; Parker, 2000). In cucurbits several peroxidase isoforms are associated with induced resistance (Hammerschmidt *et al.*, 1982). Acidic peroxidases increased systemically after cucumber, muskmelon and watermelon infection (Smith & Hammerschmidt, 1988).

PGPR induced higher levels of some defense enzymes 2-5 days after bacterization and before challenge with the pathogen (Chen *et al.*, 2000). Moreover a positive correlation between poly-phenol oxidase activity and disease resistance has been suggested in many reports (Retig, 1974; Bashan *et al.*, 1987). Rapid changes in its activity after infection of resistant wheat plants points to its possible participation in the defense mechanism (Tyagi *et al.*, 2000).

Accumulation of secondary metabolites as phyto-alexins, phenolic compounds, pathogen related proteins and other defense related proteins were induced by PGPR (Colling *et al.*, 1993). De Ascensao and Dubery, (2000) found difference in phenolic compounds and the enzymes involved in cell wall strengthening, namely phenyl alanine ammonia lyase, cinnamyl alcohol dehydrogenase, peroxidase and polyphenol oxidase during exploring the biochemical basis of tolerance in banana to *Fusarium* wilt caused by *F. oxysporum*.

The objective of the present work was carried out to search for the possibility of inducing resistance in *Lupins termis* L. against *Fusarium oxysporum* by inoculating soil with one of the PGPRs *Streptomyces chibaensis*. This will be achieved via following its potential in alleviating the oxidative stress associated with the pathogenic fungi. The present work search also for the efficiency of *Streptomyces chibaensis* in induction of synthesis of novel proteins having a role in defense against *F. oxysporum*.

## MATERIALS AND METHODS

**Biological materials.** White Lupin (*Lupinus termis* L. cv. Giza 1) seeds were supplied by the Ministry of Agriculture, Giza, Egypt. Surface sterilization was done with 70% (v/v) ethanol for 60 min and 30% (v/v) hydrogen peroxide for 20 min and then soaked for 4 h in sterile distilled water (Kudryavtseva *et al.*, 1998).

The present work also employed the use of water culture suspension of both *Streptomyces chibaensis* and *Fusarium oxysporum* which were kindly provided by Microbiology Section, Department of Botany, Faculty of Girls, Ain Shams University, Cairo.

**Experimental design.** Earthen pots (35 cm diameter) were equally filled with garden soil, and divided into three main groups (32 pots in each group). Pots were arranged inside the botanical garden of Botany Department, Faculty of Girls, Ain Shams University. Previously sterilized seeds were sown at the rate 10 per pot at the first of December. After emergence, seedlings were thinned per pot as possible for comparable ones. Plants were irrigated regularly with tap water. The experiment was conducted under natural conditions of temperature and irradiance.

**First treatment.** Twenty days after sowing (DAS), the pots were divided into three groups: the 2nd group of pots was inoculated with *Streptomyces* culture suspension at the rate of 30 ml per pot ($T_1$), while the 3rd group of pots was infested with 30 ml of *Fusarium* culture suspension per pot ($T_2$). Same supporting dose was given again to the 2nd and 3rd groups of pots after 10 days later. The first group of pots was left untreated serving as control.

**Second treatment.** The 2nd group of pots previously inoculated with *Streptomyces chibaensis* ($T_1$), was subdivided into two batches. One batch of them was further inoculated with 30 ml of *Fusarium* culture suspension after ten days of *Streptomyces* treatment ($T_3$). The 3rd group of pots (*Fusarium* treated ones) was also subdivided into two batches; one of them was further inoculated with 30 mL of *Streptomyces* culture suspension after ten days of *Fusarium* treatment ($T_4$).

**Harvesting time.** The plants were harvested in the 33, 55 and 75 days old to determine phenols and certain nutrients content of shoot and root of plants harvested from various treated and untreated soils and tissues were oven dried at 80°C for two days. Fresh tissues were cooled in liquid nitrogen and stored at -4°C to assay the activity of some enzymes (peroxidase, catalase and polyphenol oxidase), estimation of soluble protein and detection of protein pattern.

**Biochemical Methods**

**Electrophoresis.** Denaturating polyacrylamide gel (SDS-PAGE) electrophoresis was used for protein profile according to (Laemmli, 1970) method.

For soluble proteins, peroxidase and catalase extraction, 2 g of frozen tissues were homogenized with 10 mL of 50 mM phosphate buffer solution (pH 7.0), grounded with a mortar and pestle, and centrifuged at 15000 g for 25 min in a refrigerated centrifuge (Fu *et al.*, 2000).

Soluble protein content was estimated according to Lowry *et al.* (1951) method and Copeland (1994) modifications, using soluble bovine serum albumin as a standard.

**Peroxidase [EC 1.11.1.7] activity assay.** Crude extract was used for assaying the enzyme activity spectrophotometrically according to Kar and Mishra (1976) method**.** Five mL of reaction mixture "containing 125

µmole of phosphate buffer pH (6.8); 50 µmole catechol; 50 µmole hydrogen peroxide and 1 mL of the crude enzyme extract" was incubated for 5 min, after which the reaction was terminated by heating for 15 min (Garge *et al.,* 1999). Enzyme activity was expressed as change in optical density (O.D.) $g^{-1}$ $h^{-1}$.

**Catalase [EC 1.11.1.6] activity assay.** To 0.1 mL crude enzyme extract, 20 mM hydrogen peroxide and 50 mM phosphate buffer pH (7.0) were added in a total reaction mixture of 3 ml following Aebi (1974) method. Reaction mixture was left for 5 min, then the reaction was stopped. Absorbance was taken at 240 nm using UV lamp Spectrophotometer 21.

**Polyphenol oxidase [EC 1.10.3.2] extraction and activity assay.** Tyagi *et al*. (2000) method was followed, in which 500 mg frozen tissues (shoot, root) were grounded to extract the enzyme using 0.2 M sodium phosphate buffer (pH 6.0) in a pre-chilled mortar and pestle. Homogenate was filtered through four layered pre-washed cheese cloth and then centrifuged at 13000 g for 15 min at 5°C. For routine assay of polyphenol oxidase, method of Retig (1974) was followed with suitable modifications. Absorbance was read at 426 nm using Spectrophotometer 21.

**Total phenol.** From completely dried plant tissues (shoot, root), certain weight was used to extract total phenol by 0.3% HCl in MeOH, left over night. The extract was centrifuged and supernatant was retained. For complete extraction, the previous steps were repeated again. Supernatants were collected and evaporated to dryness. Residue was dissolved in 5 mL distilled water. Certain aliquot was taken from the extract and increased up to final volume 7 mL using $H_2O$, shaken well. Then 0.5 mL of Folin-phenol reagent was added and the mixture was vigorously shaken using vortex. One mL of 35% sodium carbonate solution was added, shaken and left to stand for one hour. Absorbance was recorded at 630 nm. Standard sample solution using tannic acid was used for calculating the concentrations of the unknown samples. Zero absorbance was set using all the reagents minus Folin (Malik & Singh, 1980).

For the analysis of macro and micronutrients (K, N, Fe and Zn), digested oven dried tissues were prepared. The potassium content was determined following Mengel and Kirkby (1980) method using flame photometer (Jenway PFP7, Gransmore Green, Dunmow, UK). Total nitrogen was estimated by conventional micro-Kjeldahl according to Jacobs (1978) method. While iron and zinc were determined by the atomic absorption following Allan (1961), Christian and Feldman (1970) method, respectively.

## RESULTS AND DISCUSSION

Oxidative stress induced by pathogenic infection or other stress factors generate highly reactive oxygen species (ROS) (Stajner *et at.,* 1995; Heiser *et al.,* 1998). Several enzyme systems exist within the cell for the neutralization of these ROS and thus for control of free radical formation. In addition, non-enzymatic antioxidants could be involved in scavenging singlet oxygen and so diminish oxidative stress (Elstner *et al.,* 1994).

*Fusarium oxysporum* is a common soil borne pathogenic fungus that elaborates a large number of phytotoxic products. The latter, in addition to other effects, induce the accumulation of reactive oxygen species.

Peroxidases are considered among the scavengers of hydrogen peroxide. They are involved in several plant defense responses, including lignifications (Walter, 1992), cross-linking of cell wall proteins, wound-healing and production of antimicrobial radicals (Lamb & Dixon, 1997). Thus, there are physiological events which insure the potential importance of peroxidases in plant-pathogen interactions (Dalisary & Kuc, 1995; Repka *et al.,* 2000).

The changes observed in peroxidase activity in the present work in response to *Fusarium* and *Streptomyces* treatments indicate its implication in defense mechanism against *Fusarium* and also in resistance strategy induced by *Streptomyces*. Thus, *Fusarium* via decreasing the activity level of peroxidase in both roots and shoots of *L. termis* (Table I) refer to its severe sensitivity to *Fusarium* and to its crucial role in *Fusarium* pathogenesis.

Increased activity of peroxidase, on the other hand, induced by *S. chibaensis* (Table I) when applied alone or in combination with *F. oxysporum* either after or before *F. oxysporum* investment, can cause a massive lignification of infected tissues and provide a physical and/or chemical barrier to the invading pathogenic *F. oxysporum*. In this connection, Albert and Anderson (1987) found that plant root colonization by one of plant growth promoting rhizobacteria was associated with increased peroxidase activity and enhanced lignification of stems or leaves in bean (Anderson & Guerra, 1985) and wheat (Frommel *et*

**Table I. Changes of peroxidase enzyme activity of *Lupinus termis* L. shoots, roots at different stages of growth and development as affected by soil inoculation with *Streptomyces chibaensis, Fusarium oxysporum* or both of them. Enzyme activity is expressed as change in optical density at 420 nm $g^{-1}$ fresh weight $h^{-1}$**

| | Stages | Days after sowing | | |
|---|---|---|---|---|
| | Treatments | 35 | 55 | 75 |
| Shoots | Control | 50 | 144 | 128 |
| | T1 | 176 | 196 | 194 |
| | T2 | 40 | 119 | 100 |
| | T3 | | 179 | 342 |
| | T4 | | 284 | 302 |
| Roots | Control | 122 | 260 | 309 |
| | T1 | 192 | 288 | 365 |
| | T2 | 100 | 200 | 102 |
| | T3 | | 280 | 370 |
| | T4 | | 272 | 360 |

T1: *S.chibaensis* treatment; T2: *F.oxysporum* treatment; T3: *S.chibaensis* followed by *F.oxysporum* treatment; T4: *F.oxysporum* followed by *S.chibaensis* treatment

**Table II. Changes of catalase enzyme activity of *Lupinus termis* L. shoots, roots at different stages of growth and development as affected by soil inoculation with *Streptomyces chibaensis, Fusarium oxysporum* or both of them. Enzyme activity is expressed as change in optical density at 240 nm $g^{-1}$ fresh weight $h^{-1}$**

| | Stages | Days after sowing | | |
|---|---|---|---|---|
| | Treatments | 35 | 55 | 75 |
| Shoots | Control | 388 | 846 | 418 |
| | T1 | 308 | 954 | 1252 |
| | T2 | 296 | 588 | 300 |
| | T3 | | 1238 | 1879 |
| | T4 | | 1015 | 1501 |
| Roots | Control | 104 | 254 | 297 |
| | T1 | 99 | 416 | 398 |
| | T2 | 103 | 245 | 237 |
| | T3 | | 269 | 512 |
| | T4 | | 350 | 300 |

**Table III. Changes of polyphenol oxidase enzyme activity of *Lupinus termis* L. shoots, roots at different stages of growth and development as affected by soil inoculation with *Streptomyces chibaensis, Fusarium oxysporum* or both of them. Enzyme activity is expressed as change in optical density at 420 nm $g^{-1}$ fresh weight $h^{-1}$**

| | Stages | Days after sowing | | |
|---|---|---|---|---|
| | Treatments | 35 | 55 | 75 |
| Shoots | Control | 24 | 80 | 152 |
| | T1 | 30 | 44 | 62 |
| | T2 | 28 | 100 | 216 |
| | T3 | | 36 | 52 |
| | T4 | | 68 | 100 |
| Roots | Control | 26 | 60 | 64 |
| | T1 | 28 | 92 | 68 |
| | T2 | 18 | 32 | 50 |
| | T3 | | 62 | 70 |
| | T4 | | 97 | 76 |

**Table IV. Changes of phenol content of *Lupinus termis* L. shoots, roots at different stages of growth and development as affected by soil inoculation with *Streptomyces chibaensis, Fusarium oxysporum* or both of them. Values are expressed as mg phenol $g^{-1}$ fresh weight**

| | Stages | Days after sowing | | |
|---|---|---|---|---|
| | Treatments | 35 | 55 | 75 |
| Shoots | Control | 354 | 255 | 356 |
| | T1 | 329 | 240 | 175 |
| | T2 | 205 | 237 | 421 |
| | T3 | | 195 | 286 |
| | T4 | | 304 | 84 |
| Roots | Control | 76 | 125 | 261 |
| | T1 | 65 | 72 | 206 |
| | T2 | 313 | 209 | 82 |
| | T3 | | 78 | 145 |
| | T4 | | 54 | 163 |

T1: *S.chibaensis* treatment; T2: *F.oxysporum* treatment; T3: *S.chibaensis* followed by *F.oxysporum* treatment; T4: *F.oxysporum* followed by *S.chibaensis* treatment

*al.,* 1991). Moreover, Lagrimini *et al.* (1997) reported that peroxidase seems to be involved in host defense and stress induced lignification. Thus peroxidase, besides its role in growth and morphogenesis, physiological events insure its potential importance in plant-pathogen interactions.

The results obtained here refer to marked decrease in activity level of catalase of both roots and shoots of *L. termis* in response to *F. oxysporum* (Table II). So, it was concluded that the phytotoxins produced by *F. oxysporum* may induced the suppression of catalase activity, which beside other mechanisms alleviated the level of ROS and other peroxides (Jarabac & Harvey, 1993; Balakumar *et al.*, 1996). In this regard, the accumulation of peroxides and other active oxygen species has been described as an early event in the host pathogen recognition, playing an important role in plant defense (Mehday, 1994; Baker & Orlandi, 1999). *Streptomyces chibaensis* when applied alone and during the early stage of growth cause reduction in catalase activity in both shoots and roots. On the other hand, and during 2nd and 3rd stages when added alone or in combination with *F. oxysporum* it enhanced the activity level of catalase in roots and shoots. Through this way *S. chibaensis* may exert a regulatory role on the level of $H_2O_2$ (Hirt, 2000; Kovtun *et al.*, 2000).

The results (Table III) showed that soil inoculation with *F. oxysporum* reduced the activity level of polyphenol oxidase (PPO) in roots at stages of growth. Such decrease in PPO refers to the susceptibility of *L. termis* roots to *F. oxysporum*. A positive correlation between PPO activity and disease resistance has been suggested (Mathur & Vyas, 1995; Paulitz & Chen, 1998). However, marked increase in activity level of the enzyme detected in shoots (Table III) of the same plant may refer to the less sensitivity of shoot system for *F. oxysporum* or to the predominance of defense system in its tissues.

It is clearly shown that icrease in PPO activity in roots of *L. termis* as a result of soil inoculation with *S. chibaensis* either alone or with *F. oxysporum* added to the soil after or before it throughout the plant development (Table III). Such increase may provide the plants with enhanced resistance to pathogen invasion by providing increased contents of oxidized quinone derivatives which impede pathogen growth. Nevertheless an increase in enzyme activity level was recorded particularly at 1st stage in shoot as a result of soil infestment with *S. chibaensis* culture suspension, a reverse pattern was scored during 2nd and 3rd stages, and also when *F. oxysporum* suspension was added to the soil either after or before *Streptomyces* inoculum, suggesting that *S. chibaensis* via increasing activity of PPO at the first stage blocked the invasion process at its beginning and hence will decide the fate of host-parasite relationship since the early stages of infection process. The decline in its activity in latter stages may be because PPO enzyme is age specific. Our results are consistent with those of Balakumar and Selvakumar (1998), Tyagi *et al*. (2000) and Orcutt and Nilsen (2000).

**Table V. Effect of soil inoculation with *Streptomyces chibaensis, Fusarium oxysporum* or both of them on certain elements content of shoots, roots of *Lupinus termis* L. during growth and development. Values are expressed as mg $g^{-100}$ dry weight**

| Parameters | | Iron | | | Nitrogen | | | Zinc | | | Potassium | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stages | | Days after sowing | | | | | | | | | | | |
| | Treatments | 35 | 55 | 75 | 35 | 55 | 75 | 35 | 55 | 75 | 35 | 55 | 75 |
| Shoots | Control | 3.36 | 7.38 | 5.90 | 24000 | 20000 | 15000 | 7.50 | 6.00 | 7.50 | 1267 | 1130 | 1000 |
| | T1 | 9.94 | 12.76 | 15.26 | 27000 | 21000 | 17000 | 14.00 | 6.90 | 7.60 | 1322 | 1182 | 1200 |
| | T2 | 6.90 | 9.00 | 10.22 | 25000 | 15000 | 8000 | 7.00 | 5.00 | 6.40 | 1337 | 1011 | 303 |
| | T3 | | 11.90 | 12.90 | | 22000 | 20000 | | 5.50 | 9.00 | | 1223 | 1000 |
| | T4 | | 10.80 | 12.00 | | 20400 | 15900 | | 6.50 | 7.50 | | 131 | 1050 |
| Roots | Control | 6.50 | 10.90 | 16.80 | 10000 | 13000 | 12010 | 9.5 | 9.5 | 9.5 | 979 | 826 | 767 |
| | T1 | 18.00 | 20.00 | 21.20 | 24000 | 17000 | 19000 | 10.5 | 10.5 | 10.5 | 1258 | 904 | 1192 |
| | T2 | 7.30 | 11.50 | 17.30 | 12800 | 10000 | 7900 | 8.0 | 8.5 | 7.0 | 858 | 700 | 290 |
| | T3 | | 16.20 | 19.50 | | 19000 | 18200 | | 13.5 | 6.0 | | 840 | 870 |
| | T4 | | 14.30 | 14.00 | | 14000 | 12800 | | 11.0 | 9.0 | | 860 | 883 |

T1: *S.chibaensis* treatment; T2: *F.oxysporum* treatment; T3: *S.chibaensis* followed by *F.oxysporum* treatment; T4: *F.oxysporum* followed by *S.chibaensis* treatment

Generally, different trends of PPO activity in roots and shoots in response to *F. oxysporum* and *S. chibaensis* either alone or together as in ($T_3$ and $T_4$) treatments may be due to a difference in isozyme pattern of PPO as was reported by (Tyagi *et al.*, 2000).

The data obtained in the present work (Table IV) indicated accumulation of high level of total phenols in root tissues at the first and second stages of growth in response to soil infestation with *F. oxysporum*. These results highlighted the intervention of soluble phenolic compounds in the susceptibility of *L. termis* roots to *F. oxysporum*. However, the decline observed in phenol content of the shoots of the same plants during the same period may refer to the less sensitivity of shoots to *F. oxysporum* (Table IV). The previous trend was reversed during the third stage of growth in both roots, shoot tissues. Moreover, it was reported that plants may respond to infection by non-pathogenic and pathogenic rhizosphere microorganisms by activating a set of inducible defense substances that may afford only local protection. Thus, it seems that root invasion by *F. oxysporum* led to the accumulation of one of the defensive substances (phenols) in the more susceptible tissues (roots). In this connection, Khalifa and Gameel (1983) found large differences in phenolic compounds between lines of cotton differing in leaf curl virus resistance.

Following the changes in phenol content of roots and shoots induced by *S. chibaensis* when applied alone or in combination with *F. oxysporum* ($T_3$ and $T_4$) it is obvious that it tends to decrease, in most cases, the phenol content (Table IV). Regulation of phenolic compounds by *S. chibaensis* and *F. oxysporum* observed in the present research seems to be via PPO at certain stages and via peroxidative enzyme at other stages (Tables I & III).

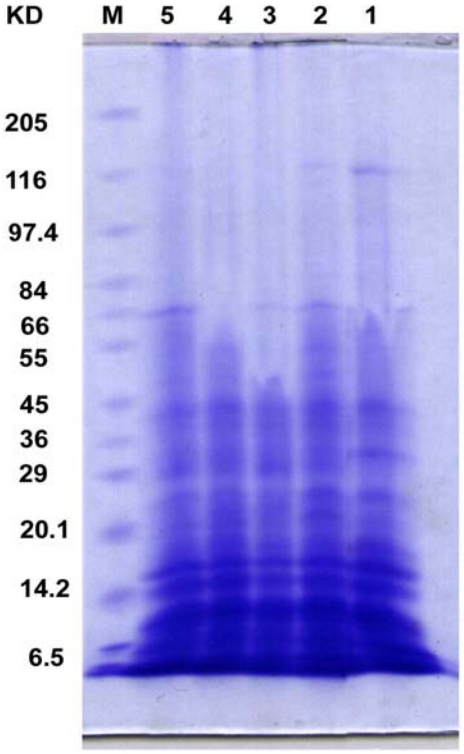
Several inorganic nutrients have been associated with changes in disease development in plants. Nitrogen is one of the most severely limiting nutrient elements in pathogenesis. From data in (Table V), it appears that soil infestation with *F. oxysporum* led to marked increase in nitrogen content of both roots and shoots at the early stage of growth followed by a sharp decrease at the second and third stages. High nitrogen level at the early stage may be a plant response or defense to ward off infection and to compensate damage results from invasion and destruction of the host tissues (Orcutt & Nilsen, 2000). The decline in nitrogen content observed at latter stages, could be correlated with pathogenesis when root tissues were really attacked affecting its ability to take up water and nutrients from soil or they may be leached out from macerated tissues also the infection site may acts as a sink center. In response to *S. chibaensis* alone or *Streptomyces and Fusarium* together ($T_3$ and $T_4$ treatments) marked increases in nitrogen content of shoots and roots were observed. Increased level of nitrogen throughout the three stages may represent one of the strategies employed by *S. chibaensis* in inducing resistance against *F. oxysporum*.

Potassium is very important in osmotic adjustment in plants and thus may have a critical role relative to the interaction of a developing pathogen and the host at the site of infection. Thus, decreased level of $K^+$ in roots of *L. termis*, in response to growth in *F. oxysporum* pathogenized soil might decrease osmotic pressure of the host tissues which tend to increase the susceptibility of root tissues to infection by *Fusarium*. Moreover, decreased level of $K^+$ in shoots, of the same plants, particularly at 2nd and 3rd stages (Table V) will increase the spread of pathogen or its toxins inside the host tissues (Albrecht, 1996) via altering the stomata function.

On the contrary, the existence of *Streptomyces* in growth medium either alone or with *Fusarium* ($T_3$ and $T_4$) led to marked increase in $K^+$ content in both shoots and roots of *L. termis*. *S. chibaensis* via increasing the level of $K^+$ impede the flow of nutrients from host to pathogen and retard the spread of *F. oxysporum* and/or its toxins.

Having a glimpse on the iron content of shoots and roots of *L. termis* harvested from soil inoculated with *S. chibaensis*, *F. oxysporum* either separately or together ($T_3$ and $T_4$), it is evident that there were obvious increase (Table V) compared with the control.

**Fig. 1. SDS-PAGE electrophoresis analysis (55 DAS) plant leaves. Lanes 1- Plants taken from untreated soil. 2- Plants taken from *S. chibaensis* inoculated soil. 3- Plants taken from *S. chibaensis* followed by *F. oxysporum* inoculated soil. 4- Plants taken from *F. oxysporum* inoculated soil. 5- Plants taken from *F. oxysporum* followed by *S. chibaensis* inoculated soil. M- Indicates molecular mass marker**




The essential micronutrient zinc is reported among other micronutrients which can increase resistance in pants to pathogens (Hardham, 1992; Shirasu *et al.,* 1999). In the present work a good correlation exist between the susceptibility or resistance of *L. termis* to *F. oxysproum* and its content of Zn. *Streptomyces* tended to increase it, in general, in both roots and shoots whether inoculated alone or with *Fusarium* ($T_3$ and $T_{4)}$. *Fusarium* investment, led to obvious decrease in Zn content of both roots and shoots throughout the plant developmental stages (Table V).

In this regard, Zn was shown to reduce the incidence of root disease in rice and wilt disease in cotton when applied as a root dip prior to transplant or as a soil amendment, respectively (Agrios, 1988). Zn has also been reported to be essential in some fungi for growth, sporulation and virulence as well as the formation of pathogen-produced toxins (Hardham, 1992).

Consistent with results of Hussain (1995), who found that the total mineral content of diseased leaves of sensitive cultivar of cotton was lower compared with the resistant.

The electrophoretic protein patterns of leaves (at the age of 55 days) have been carried out and the results obtained are illustrated in Fig. 1. These results indicate that the protein bands having molecular masses 24, 15, 12, 6.6, 4.5 KD occurred in leaves of the untreated and the variously treated plants. Evident quantitative variations were observed particularly with regard to the protein having molecular mass 4.5 KD where the highest concentration was recorded in case of the treatment involved the *Streptomyces* and *Streptomyces* followed by *Fusarium* (Fig. 1). Plant defensins represent a family of 5 KD proteins, occurring in many, if not all plant species. Several members of the plant defensins are potent inhibitors of fungal growth, suggesting a role in plant defense (Penninckx *et al.,* 1998).

On the other hand, the protein having molecular mass (70 KD) was detected in the plants leaves harvested from untreated and treated soils with the exception of its complete absence in the case of *Fusarium* treatment. It is worthy to mention that this protein represents one of the $Ca^{2+}$-dependent kinases (CDPK) which acts as a signal protein in resistance mechanisms (Romeis *et al.,* 2000). Thus, its absence in *L. termis* plants in response to *Fusarium* may retard the triggering of resistance gene transcription which leads to pathogen related proteins. This condition is expected to dramatically increase the severity of *Fusarium* and susceptibility of *L. termis* to it.

Fig. 1 indicates also that among the different treatments, unique protein (27 KD) was recorded only in leaves of the plants grown in soil inoculated with *Streptomyces* followed by *Fusarium* ($T_3$). This protein was reported to show chitinase activity (Viswanathan & Smaiyappi, 1999). Chitinases belong to the pathogenesis related (PR) proteins which hydrolyze chitin, a major cell wall component of the higher fungi (Boller, 1988). Thus, *Streptomyces* via inducing the synthesis of this protein may inhibit the growth of *Fusarium*. In this regard Viswanathan and Samiyappi (1999) found that the activity level of chitinase was increased to nearly 150% in PGPR-treated sugarcane tissues. Moreover, as a result of chitin hydrolysis by the action of chitinase an elicitor may be released which was reported to enhance the speed and magnitude of plant defense mechanism (Hadwiger & Beckman, 1980; Keen & Yoskikawa, 1983).

Novel protein band was recorded only with *Fusarium* investment with apparent molecular mass 50 KD. Such occurrence may have important role in pathogenesis (Erickson *et al.,* 1999; Shirasu *et al.,* 1999). It may target and interfere with the spread of certain signal proteins, leading to delaying in plant defense responses which were needed to be expressed fast and in critical time.

As regard to the mode of action of *S. chibaensis* in alleviating the *F. oxysporum* passive effects in *L. termis* plants, the following mechanisms (which were extracted from the results obtained in the present work and from those obtained by others) may be suggested, enhancing plant cell wall mechanical defense via accelerating polymerization of lignin monomers; detoxification for the pathogen toxins

effects through the oxidative enzymes as they are age specific. Additive strategy may be via altering gene expression (enhancing or repressing) inventing primitive conditions to future subsequent attack.

Via these mechanisms, *S. chibaensis* could be recommended as an efficient inducer of systemic resistance in *L. termis*.

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