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Theme

Characterization of mechanisms involved in the effects of PGPR and Biocontrol of some *Streptomyces* spp. strains isolated from Steppic soils on tomato seedlings.

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My Deep Gratitude goes to the God First And Foremost and I am Truly Grateful for My Parents Who Have Devoted Their Lives for Me.

I would also like to Thank My Astoundingly Supportive True Friend and Sister **Nadia**, My Two Brothers **Ahmed** and **Abdelkader** do Thanks Also Goes to All of My Friends.

I Would Like To Thank All of The Professors Who I Have Encountered During Three Years In Major Biology, End Especially The Professors of Plant Biology And Physiology, plant Biotechnology Major Whom Information's And Teachings Where Very Valuable And I Must Give A Special Thanks To Doctor **TOUATI Mostefa** For His Valuable Advices And His Generous Help, I Must Also Give a Thank you To The Co-Supervisor **Toumattia O** . Also a Special Thanks To The Jury Members : **Mostefa D, Adli B, Bezini E.**

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FORL: *Fusarium oxysporum f. sp. radicis-lycopersici*

ufc : colony forming units

PGPR: Plant Growth-Promoting Rhizobacteria

ISR: Induced Systemic Resistance

SAR: Systemic Acquired Resistance

BCA: Biological Control Agent

GP: Germination percentage

T: Control T

TR: Thirame (Control TR)

FW: Fresh weight

DW: Dry weight

WC: Water content

Chl: Chlorophyll

CMP: Cell Membrane permeability

TSS: Total soluble sugars

PRO: Proline

POD: Peroxidase

CAT: Catalase

Introduction

Tomatoes (*Solanum lycopersicum)* are economically important crops in the world in general and in Algeria in particular. Because of its importance as food, studies were conducted to improve productivity, fruit quality, and resistance to biotic and abiotic stresses.

Tomato has been widely used not only as food, but also as a research material. The tomato plant has many interesting features such as fleshy fruit, a sympodial shoot, and compound leaves, which other model plants (e.g., rice and Arabidopsis) do not have. Most of these traits are agronomically important and cannot be studied using other model plant systems. In addition to its agronomical importance, the tomato belongs to the extremely large family of the Solanaceae plants and is closely related to many commercially important plants such as potato, eggplant, peppers, tobacco, and petunias. Knowledge obtained from studies conducted on tomato can be easily applied to these plants, which makes tomato important as a research material. Because of these facts, tomato serves as a model organism for the family Solanaceae and, specifically, for fleshy-fruited plants (Kimura and Sinha. 2008).

Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens, whereas others trigger beneficial effects. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect providing growth promotion. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR (Plant growth-promoting rhizobacteria) (Kloepper et al, 1980).

Due to the intensive cultivation systems, tomato plants are particularly exposed to the risk of phytopathological problems especially those caused by soil borne pathogens. The case of the genus Fusarium represent a serious danger for tomato plants growth and often cause important economic and yield losses. For many years, the management of soil borne epidemics mainly relied on application of soil fumigants (Burhan et al. 2005).

The terms “biological control” and its abbreviated synonym “Biocontrol” have been used in different fields of biology, most notably in entomology and plant pathology. In plant pathology, biological control is defined as the reduction in the amount of the inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man. The organism that suppresses the pathogen is called Biological Control Agent (BCA) (Cook and Baker, 1983).

The use of BCAs to manage soil borne epidemics is gaining more and more interest in modern agriculture as confirmed by the numerous researches. However, they often show variable performance and the crops protection is not always as effective as it should be expected. Identifying new antagonist microorganisms is of fundamental importance in horticulture in the world of today (Pal and Gardener, 2006).

Bacteria of the genus Streptomyces represent a significant fraction of the soil microflora. They can establish beneficial relationships with plants by colonizing the rhizosphere and entering the root tissues. These features together with the wide number of antifungal compounds they produce, make streptomyces a promising antagonist of soil borne pathogens as well as a promising PGPR agent (Burhan et al. 2005).

This research project aimed to study four Streptomyces strains as BCAs (against Fusarium) and as PGPR on tomato seedlings (marmande cultivar).

The project is organized as follows:

First, the literature review section contains information about the different aspects studied in this work. The second section is devoted to the description of the materials and methods used to evaluate the PGPR and the biocontrol effects of four Streptomyces strains codified as (TM52, IA1, D54, and D15). The third section reports detailed results of our project and their discussion. At the end of this work, a conclusion is provided.

Literature Review

I.1. The agronomical status of tomatoes in the world and in Algeria in particular:

Tomato (*Solanum lycopersicum*, formerly, *Lycopersicon esculentum* Mill.) is the second most important vegetable crop after potato in the world. In addition to being consumed as a fresh vegetable, it is also used as a salad, in ketchup, as a puree, a pickle and in many other forms, depending up on the growing area. It is estimated that 4.6 million ha of tomatoes are grown annually worldwide producing more than 126 mt. In addition to being an important vegetable crop worldwide, tomato is also used as a model plant species for genetic studies related to fruit quality, stress tolerance (biotic and abiotic) and other physiological traits. It is widely adapted to a variety of climates spanning the tropics to temperate regions. In order to meet the demand for tomatoes, and because of its economic contribution to the agriculture industry it is also grown in greenhouses. Furthermore, new approaches are adopted for the improvement of tomato production (Panthee and Chen. 2010).

Table 01: The world's leading tomato producers in 2011 (FAO, 2011).

The ranking	The country	Production: (Million tons)	The ranking	The country	Production: (Million tons)
1	China	48.57	8	Brazil	4.41
2	India	16.82	9	Spain	3.82
3	United States	12.62	10	Uzbekistan	2.58
4	Turkey	11.00	11	Mexico	2.43
5	Egypt	8.10	12	Russia	2.20
6	Iran	6.82	13	Ukraine	2.11
7	Italy	5.95	14	Tunisia	1.28

In Algeria, the Spaniards introduced tomatoes in the 17th century. Its cultivation began in the West and more precisely in Oran around 1905 (Benabadji, 1977), then it gradually expanded to reach the entire coastal region, particularly the Algiers coast. Today, tomato cultivation occupies a transcendent place in the agricultural economy. Almost 23,500 ha are devoted to it annually, generating an average production of 7,900,000 quintals with average yields of around 336Qx / ha in 2011(MADR, 2011).This production remains low and quite distant from those recorded in other countries of the Mediterranean basin such as Tunisia, Morocco, Spain, France and Italy where the yields vary between 400 Qx / ha and 1040 Qx / ha for the growing season (FAO, 2011).

This status is related in part to different biotic and abiotic factors. Greenhouse tomato production requires a humid environment and optimum temperatures of 20 ° C to 25 ° C (Chaux and Foury, 1994), which corresponds to climatic requirements for the propagation of phytopathogenic agents (Baptista, 2012).Tomatoes are attacked by more than 20 kinds of fungi, 19 virus species and 7 bacterial species as well as several pests (Blancard, 2009).

The cultivation of tomatoes in Algeria ranks second after potato. The climatic conditions of the tomato growing regions are very favorable for obtaining good yields (Zidani, 2009).

Table 02: Variation of Algerian tomato production throughout the years from 2001 till 2011 (FAO, 2011).

Years	Production (tons)	Yield (Hg / Ha)	Cultivated area (Ha)
2001	830,531.00	208,518.96	39,830.00
2002	814,941.00	191,705.72	42,510.00
2003	887,097.00	193,985.63	45,730.00
2004	1, 092,270.00	233.695.63	46,729.00
2005	1, 023,450.00	241,641.88	42,354.00
2006	796 ,160.00	256,784.39	31,005.00
2007	567,313.00	282,540.47	20,079.00
2008	559,249.00	284,532.69	19,655.00
2009	641,034.00	308,352.49	20,789.00
2010	718,240.00	336,412.18	21,350.00
2011	790,000.00	336,170.21	23,500.00

I.2. Tomato plants:

I.2.1. General description and taxonomy:

Tomato is an herbaceous, dicotyledonous plant of the Solanaceae family, grown for its fruit. The term refers to both the plant and the fleshy fruit. The tomato is a perennial, usually grown as an annual. It is a plant with indeterminate growth (monopodial stem), but there are certain varieties with a limited growth (monopodial then sympodial stem after 4 or 5 leaves). Leaves are alternate, compound, imparipinnate (odd number of leaflets) and include 5 to 7 leaflets with lobes. The fleshy fruits are berries with two or more boxes. They can weigh from a few grams to nearly two kilograms. Their shape is generally spherical but can be more or less flattened, more or less ribbed, heart-shaped or pear. The reproductive system is formed by inflorescences of a certain type. The tomato is usually self-pollinated, but over-fertilization is possible. The fruits are green then usually turn red when ripe. However, they can be yellow, pink, orange, white, black or even two-tone at maturity (Ranc, 2010).

Tomato belongs to the Solanaceae family of flowering plants. This family of plants includes more than 3000 species including many that are economically important. Even though there are more than 7000 varieties of tomatoes, they all represent only one species of tomato that is cultivated, *Solanum lycopersicum* (Olmstead, 1997).

Table 03: Taxonomy of tomato plants (Olmstead, 1997).

kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	Solanum
Species	*Solanum lycopersicum*

I.2.2. Anatomy:

I.2.2.1. Vegetative system:

I.2.2.1.1. Stem and leaves:

Leaves are lateral organs that are produced from the flanks of the shoot apical meristem. Leaf development can be divided into three continuous and overlapping phases: initiation, primary morphogenesis, and secondary morphogenesis or histogenesis. *Solanum lycopersicum* has small compound leaves with thick, rounded leaflets. Leaflets are initiated from the marginal blastozone at the primary morphogenesis stage and go through similar developmental stages as leaves the tomato is a short-lived perennial plant, grown as an annual plant, typically growing to1-3 m tall, with a weakly woody stem that usually scrambles over other plants (Shukla et al, 2013).

I.2.2.1.2. Roots:

Tomatoes can have either a fibrous root system or a taproot system; plants grown from seed usually have a tap root system, where plants grown from cuttings have fibrous root systems (Shukla et al, 2013).

I.2.2.2. Reproductive system:

I.2.2.2.1. The flower:

The flowers of the tomato are hermaphrodite and actinomorphic. The chalice has five or more sepals, green in color. The corolla has as many petals as sepals, welded at the base. The androcea has five stamens or more, with lateral dehiscence, introrses. The elongated anthers form a cone constricted around the pistil. The latter consists of several fused carpels, forming an ovary superior bilocular or multilocular and centrally located. Depending on the cultivar and the environmental conditions, the style may be in the internal position in the stamen cone (Brevistyle flower), flush, or slightly protruding (longistyle flower) (Ranc, 2010).

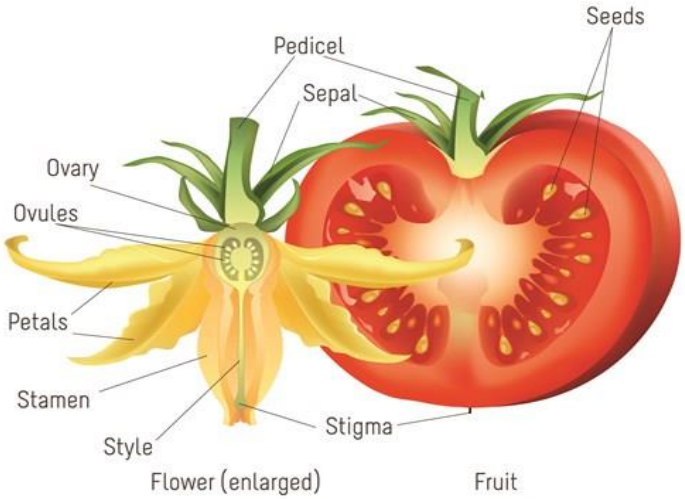


Fig 01: Longitudinal section of a tomato flower and fruit (Ranc, 2010).

I.2.2.2.2.The fruit:

Tomato is a deshiscent fruit belonging to the family of fruits, which is derived from the increased development of the ovary after fusion of the carpel walls. Thus, the tomato fruit is characterized by a complex structure associating different tissus with specific characteristics, especially at maturity (Fig 01). Locular cavities (Lodges) are delimited by the outer pericarp, the septum or radial pericarp and the internal columella or pericarp. The seeds carried by the placenta are wrapped in the locular tissue (gel) which occupies, in varying proportions. At the level of the outer pericarp,

three parts are distinguished : the skin or exocarp protected by a fine cuticle, the mesocarp and the endocarp which constitute the flesh (Zidani. 2009).

The tomato has a good nutritional density with 95% of water and 5% of dry matter composed of 50% of sugars (fructose and glucose), 25% organic acids (citric and malic acids), 8% minerals, 2% amino acids, carotenoids and other secondary metabolites, it is also a source of fiber (2g / 100g), ie a quarter of the recommended dietary intakes. The tomato also contains many minerals and trace elements and like most fruits and vegetables, it brings a lot of potassium (245.0 mg / 100g)which makes of it an appreciable source of this important mineral. It can also provide 50 to 160 mg of vitamin C and 22.5 to 90 mg of vitamin E. Among the phyto constituants, it contains polyphenols (ferulic acid, chlorogenic acid, caffeic acid), flavonoids (quercitin, kaempferol, rutin and naringenin), and carotenoids, especially lycopene (zidani. 2009).

I.2.4.Centre of origin:

The tomato originated in the Andean region of South America and in Central America. The tomatoes are believed to have originated in the coastal trip of western South America, from the equator to about 30° latitude south (Posada. 2016).Tomato is now a cosmopolitan crop with major production in temperate regions, even though its origins lay in tropical regions (Atherton and Rudich, 1986).

I.2.5.Climate requirements and phenology:

Tomatoes require a warm climate for growth and do not tolerate frost. The usual life cycle in cultivation spans one spring and summer. Its optimum temperature is around 26ºC (day) and 12ºC (night). Plants require minimum temperatures above 18ºC for vegetative growth, but can survive at lower temperatures (12ºC). Tomato requires frequent irrigation to delay maturity and prolong plant productivity, Tomatoes grow well on most mineral soils, but they prefer deep, well-drained sandy loams (Atherton and Rudich, 1986).

I.3.Fusarium:

I.3.1.Taxonomy and description:

The genus Fusarium is well known for its important role in phytopathology, this group of pathogen has a large number of species with parasite specificity for a

wide range of host plants, it's responsible for diseases known as fusariosis such as crown and root rot, The telluric fungi belonging to the genus Fusarium are the most damaging of the crops of economic interest (Agrios, 2005).

I.3.1.1. Fusarium oxysporum f. sp. radicis-lycopersici:

Fusarium oxysporum f. sp. radicis-lycopersici leading to fusarium crown and root rot is one of the most destructive soilborne diseases of tomatoes occurring in greenhouse and field crops it is a necrotrophic pathogens affecting tomato crops on a worldwide scale Despite such major economic impact, little is known about the molecular mechanisms regulating *Fusarium oxysporumf.sp. radicis-lycopersici* resistance in tomato (Hedbas et al 2013).

Table 04: Taxonomy of *Fusarium oxysporum* (Agrios, 2005).

kingdom	fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Nectriaceae
Genus	Fusarium
Species	*Fusarium oxysporum*

I.3.1.2. Fusarium solani:

Fusarium solani is a cosmopolitan species and is classified into the section Martiella. *Fusarium solani* can be distinguished into 50 subspecific lineages and most of them have not been further described formally. The species is among a well known plant pathogen, causing various types of diseases on a wide range of plants and there are at least 111 plant species from 87 genera that are commonly infected by *Fusarium solani* (Hafizi et al, 2013).

Table 05: Taxonomy of *Fusarium solani* (Porter et al, 2015).

Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Nectriaceae
Genus	Fusarium
Species	*Fusarium solani*

I.3.2. Development of disease:

Infection occurs through the wounds and natural holes created by the newly formed root. The disease caused by Fusarium genus is characterized by a long period of incubation. When infection occurs immediately after planting, external symptoms appear immediately before harvest. If however infection occurs during the production of seedlings, the disease may manifest itself at the time of flowering (Hedbas et al 2013).

I.3.3. Conditions for disease development:

Fusarium genus seems to prefer rather low temperatures. Its thermal optimum would be between 18ºC and 20ºC and its most serious attacks will occur at temperatures between 10ºC and 20ºC (Agrios, 2005).

I.3.4. Symptoms:

The fungus, after initially infecting secondary roots, moves into larger roots and eventually invades the plants vascular system. Crown rot-infected plants then start to show a unilateral vein-clearing. Slow wilting follows this and the plants become stunted and yellow, beginning with the older leaves and then spreading to the whole plant. Eventually the entire plant turns brown and dies. Although a parasite of the root and the collar, the fungus causes browning of the vessels up to 30 cm above the collar and brown longitudinal necrotic lesions form on the stem (Agrios, 2005).

I.4. The PGPRs:

I.4.1. Agro-Biotechnological interest of PGPR:

Plants provide raw material for industries producing food, pharmaceuticals, cosmetics, and fragrance flavor imparting biochemical. Therefore, there is an urgent need for conservation, sustainable utilization and management of plant genetic resources to meet the growing requirements of food, fodder, fiber, health, water and other needs.

This led to the idea that bacteria present in the roots are beneficial for the growth of plants. The major roles of these bacteria are: (a) to supply nutrients to crops; (b) to stimulate plant growth, e.g., through the production of phytohormones; (c) to control or inhibit the activity of plant pathogens, (d) to improve soil structure; and (e) bioaccumulation or microbial leaching of inorganic compounds (Sowinski et al. 2007).

Rhizoshpere mainly consists of bacteria termed rhizobacteria, which by direct or indirect means exert a positive effect on plants. These bacteria that benefit the plants by

stimulating its growth are termed as PGPR (plant growth promoting rhizobacteria) (kundan et al. 2015). PGPR also offer ecofriendly alternatives to chemical control of plant diseases and pests as well as chemical fertilizers

Mechanisms used by PGPR can be direct or indirect; the former entails the secretion of growth regulators and the latter occurs through the production of antimicrobial compounds that reduce the deleterious effects of phytopathogens (Sowinski et al. 2007).

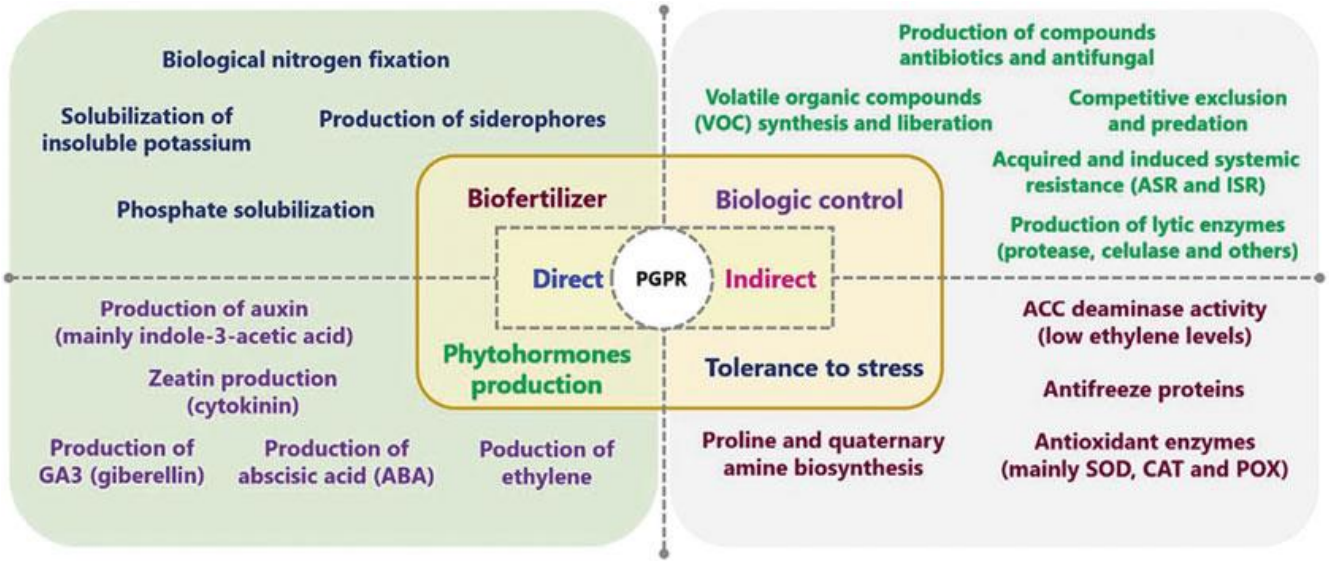


Fig 02: Direct and indirect mechanisms mediated by plant growth-promoting rhizobacteria (PGPR) with beneficial effects on host plants (Ahmed and kibrat. 2014).

I.4.2. Direct mechanisms of PGPR:

I.4.2.1. Biological nitrogen fixation:

Nitrogen fixation is considered as an important trait of PGPRs as it directly provides nitrogen to the plant. Nitrogen-fixing strains are marketed as biofertilizers for 20 years and they are considered important for agriculture.

Bacterial strains possessing the trait of nitrogen fixation are classified in to two categories. First category includes root/legume-associated symbiotic bacteria which possess the specificity and infect the roots to produce nodule. Other group of bacteria is the so-called free-living nitrogen fixers which do not possess specificity to plant. Although free-living nitrogen fixers do not penetrate the plant's tissues, yet these bacteria live sufficiently close to the root such that the atmospheric nitrogen fixed by the bacteria that is not used for their own benefit, but is taken up by the plant but is taken up by the plant. The genes for nitrogen fixation, called nif genes are found in both symbiotic and free living systems. The process of

N2 fixation is carried out by a complex system known as nitrogenase which changes nitrogen to Ammonia. Structure of nitrogenase was elucidated as a two-component metalloenzyme consisting of dinitrogenase reductase which is the iron protein and dinitrogenase which has a metal cofactor. N2-fixing system varies among different bacterial genera (Ahmed and kibrat. 2014).

I.4.2.2. Phosphate solubilization:

Phosphorus (P), the second important plant growth-limiting nutrient after nitrogen, is abundantly available in soils in both organic and inorganic forms. Despite of large reservoir of P, the amount of available forms to plants is generally low. To overcome the P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. Plants absorb fewer amounts of applied phosphatic fertilizers and the rest is rapidly converted into insoluble complexes in the soil this operation is not only costly but also environmentally undesirable. In this context, organisms coupled with phosphate solubilizing activity may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilizers, the solubilization of inorganic phosphorus occurs as a consequence of the action of low molecular weight organic acids which are synthesized by various soil bacteria. Conversely, the mineralization of organic phosphorus occurs through the synthesis of a variety of different phosphatases, catalyzing the hydrolysis of phosphoric esters. Importantly, phosphate solubilization and mineralization can coexist in the same bacterial strain. Besides providing P to the plants, the phosphate solubilizing bacteria also augment the growth of plants, enhancing the availability of other trace elements by synthesizing important plant growth promoting substances (Ahmed and kibrat. 2014).

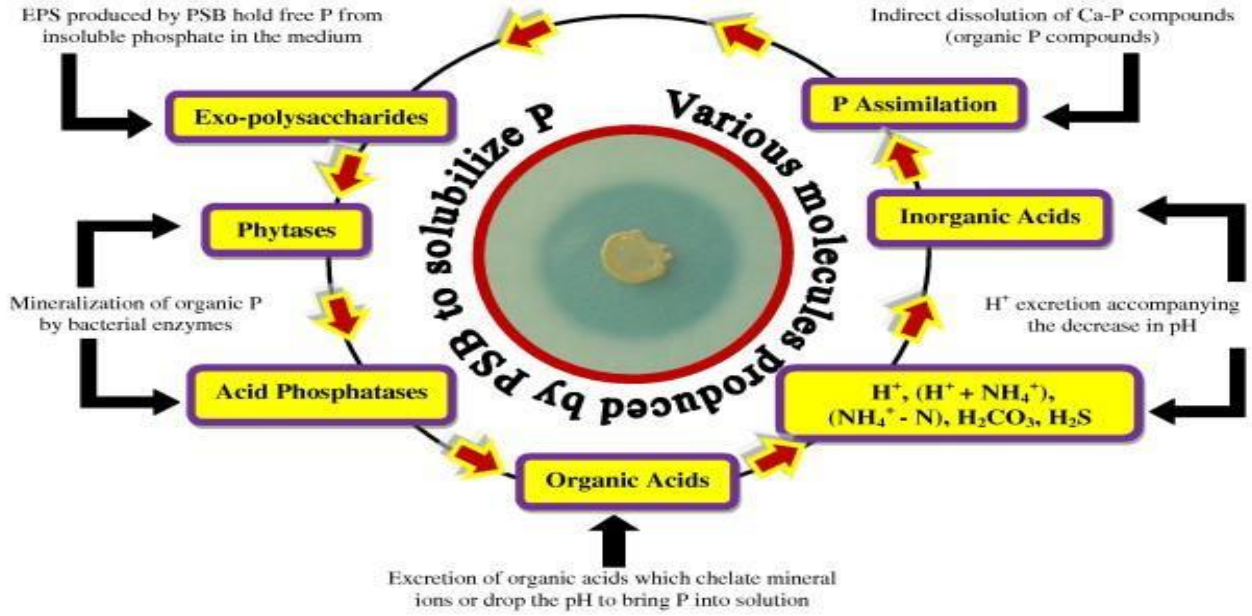


Fig 03: Various organic/inorganic substances produced by phosphate solubilizing bacteria responsible for phosphate solubilization in soils (Ahmed and kibrat. 2014).

I.4.2.3. Potassium solubilization:

Potassium is the third major essential macronutrient for plant growth. As more than 90% of potassium exists in the form of insoluble rock and silicate minerals, the concentration of soluble potassium is usually very low in soil. Potassium deficiency has become a major constraint in crop production. The ability of PGPR to solubilize potassium rock by producing and secreting organic acids has being widely investigated. Thus, applying potassium-solubilizing PGPR as biofertilizer to improve agriculture can reduce the use of agrochemicals and support eco-friendly crop production (Goudaa et al, 2018).

I.4.2.4. Iron solubilization:

Iron is one of the essential elements for all living cells; it is abundant in the soil, often in an insoluble form, ferric iron (Fe^{3+}). To acquire it, the bacteria resort to the synthesis of siderophores. This synthesis takes place only in a situation of iron deficiency. Siderophores bind ferric iron and transform it into its soluble form which is ferrous iron (Fe2 +), they are also used in the biological fight against phytopathogenic fungi (Goudaa et al, 2018).

I.4.2.5. Phytohormone production:

Soil micro-organisms, particularly the rhizosphere bacteria, possess the potential toproduce classes of well-known phytohormones including auxins, gibberellins, cytokinins, ethylene, and abcisic acid. Plant responds to any phytohormone in the rhizosphere that is supplemented externally. These phytohormones can mediate processes including plant cell

enlargement, division, and extension in symbiotic as well as non-symbiotic roots (Goswami et al. 2016).

I.4.2.5.1. Indole acetic acid:

Indole-3-acetic acid (indole acetic acid, IAA) is one of the most common as well as the most studied auxins, and much of the scientific literature considers auxin and IAA to be interchangeable terms. Its main function is cell division, cell elongation, differentiation, and extension. But it has been known that plant responses to IAA vary from plant to plant in terms of sensitivity. Generally, IAA released by rhizobacteria interferes with many plant developmental processes. There are several ways by which IAA supports the plant. IAA helps in the apical dominance, and also stimulates lateral and adventitious root development and growth. Besides development, IAA plays crucial role in leaf and flower abscission. Thus IAA can be considered as major auxin involved because it plays overall role in growth stimulation (Goudaa et al, 2018).

I.4.2.5.2. Ethylene:

Ethylene hormone in plants is the simplest molecule with a wide range of biological activities. It is produced by plant endogenously and induces different physiological changes in plants at molecular level. The production of ethylene varies within the plant species and types of tissues. This gaseous hormone is formed by breakdown of methionine that is present in all the cells, It effects plant growth by root initiation, fruit ripening, seed germination, and inhibiting root elongation. The major effect seen is fruit ripening and thus called aging. 1-aminocyclopropane- 1-carboxylate (ACC) deaminase is a vital enzyme present in plant growth promoting rhizobacteria (PGPR), which regulates ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into alpha-ketobutyrate and ammonia hormone in plants. The PGPR ACC activity is thought to decrease root ethylene production, which in turn can alleviate the repressing effect of ethylene on root growth (Goudaa et al, 2018).

I.4.2.8. Cytokinins:

Cytokinins are phytohormones that promote cell division in plant roots and shoots. Their main function is cell growth and differentiation. As they also affect apical dominance so the farmers use them to increase the overall yield. Cytokinins can be produced in soil and pure culture by PGPR and this is an emerging alternate to enhance plant growth to improve yield and quality of crops, playing crucial role in sustainable development (Goudaa et al, 2018).

I.4.2.9. Gibberellins:

Gibberellins are chemicals produced naturally by plants and are involved in several aspects of germination. They stimulate the enzyme (alpha amylase) and help in hydrolysis of starch present in many seeds into glucose to be used in cellular respiration. Gibberellins are plant hormones that influence and control plant developmental processes like stem elongation, germination, dormancy, flowering, sex expression and leaf and fruit senescence. Lastly gibberellins act as a chemical messenger and help by breaking dormancy. Several studies revealed that many soil bacteria in general, and PGPB in particular, can produce either cytokinins or gibberellins or both (Goudaa et al, 2018).

I.4.3. Indirect plant growth promotion:

Several PGPR are known to reduce the effects of plant stresses by limiting phytopathogen caused damage. This can occur via local antagonism of soilborne pathogens, or by induction of systemic resistance against pathogens throughout the entire plant (Pathak et al. 2017).

I.4.4. Biocontrol of soilborn deseases:

A PGPR can have plant growth enhancing activity as its primary effect and as its secondary effect, it reduces the disease by enabling the plant to outgrow and thereby “escape” the disease. However, there are many specific examples of PGPR with direct biocontrol activity (Doornbos et al. 2012).

I.4.5. Modes of Action of PGPR as Biocontrol Agents:

For successful and sustainable biocontrol under field conditions, it is imperative that the mode of action of the BCA strains being used is known. The mode of action involved will be a determining factor in the type of disease control strategy to beimplemented (Doornbos et al. 2012).

I.4.5.1. Production of Antifungal Metabolites:

PGPR including those associated with cereal crops produce various types of antifungal metabolites capable of reducing or suppressing infection by pathogenic fungi in several crops (Barness et al, 1991).

I.4.5.1.1. Antibiotics and Siderophores:

Antibiosis is an attractive and a highly effective mode of action of rhizobacteria in the suppression of soilborne infections in a number of crops. Most biocontrol strains of PGPR produce one or several groups of antibiotics, which inhibit fungal pathogens. The potential uses of antibiotic producing PGPR as biocontrol agents have been reported in many crops. Biocontrol PGPRs also exert their antagonistic activity against plant pathogens by means of

secretion of siderophores. The siderophores bind most of the Fe+3 in the rhizosphere and effectively prevent the proliferation of fungal pathogens by depriving them of available iron. Suppression of the pathogens arises because iron deficiency causes growth inhibition, decrease in nucleic acid synthesis, inhibition of sporulation, and causes changes in cell morphology (Barness et al, 1991).

I.4.5.1.2. Cell Wall Degrading Enzymes:

One of the major mechanisms used by biocontrol agents to control soilborne pathogens involves the production of cell wall degrading enzymes. Cell wall degrading enzymes such as b-1, 3-glucanase, chitinase, cellulase, and protease secreted by biocontrol strains of PGPR exert a direct inhibitory effect on the hyphal growth of fungal pathogens. Chitinase and b-1,3-glucanase degrade chitin, an insoluble linear polymer of b-1,4-N-acetylglucoseamine, which is the major component of the fungal cell wall (Farag et al. 2013).

I.4.6. Induction of Systemic Resistance:

Induced systemic resistance (ISR) is the state of defensive capacity developed by the plant when stimulated by diverse agents including rhizobacteria. Once resistance is induced in plants, it will result in nonspecific protection against pathogenic fungi, bacteria, and viruses. The mode of action of disease suppression by nonpathogenic rhizosphere bacteria should be distinguished from pathogen induced systemic acquired resistance (SAR). Colonization of the plant root system by rhizobacteria can indirectly lead to reduced pathogen attack through induction of systemic resistance PGPR elicit ISR in plants by increasing the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reactions of the host. This results in the synthesis of defense chemicals such as chitinase, peroxidase, and pathogenesis-related proteins (Glick, 2010).

I.4.7. Root Colonization and Rhizosphere Competence:

Root colonization is an important prerequisite for bacteria to be considered as true PGPRs, and it is commonly believed that a biocontrol agent should colonize the rhizosphere and the surface of the plant it protects. Therefore, any given PGPR is often ineffective as a biocontrol agent against root disease if it does not colonize the roots efficiently (Crowley et al. 1988).

I.5. *Streptomyces* taxonomy and properties:

Actinomycetes are Gram-positive bacteria characterized by a genome with high G + C ratio. They are mostly aerobic, but some of them can grow anaerobically. Several actinomycetes form branching filaments and possess mycelial growth while some species

produce external spores. Out of all rhizosphere microbes, actinomycetes are regarded to be special in plant growth promotion because they exhibit many useful traits. Their filaments and ability to sporulate help them cleave strongly to the rhizospheric soil particles forming a strong bond with the plants. Streptomyces are important groups of soil bacteria from the actinomycetes family. Alongside Micromonospora, they are the most commonly described actinomycetes making up 1– 20% of the culturable soil microbes (El-Tarabily and Alkhajeh. 2016).

Waksman and Henrici introduced the genus Streptomyces in 1943. They are Gram-positive aerobic bacteria, members of the order Actinomycetales within the class Actinobacteria and have a DNA guanine and cytosine content of 69-78 mol% (Korn-Wendisch et al., 1992). The taxonomy of the genus Streptomyces has been of interest for researchers from 1940s, the need for patenting led to an over classification of the genus (Anderson and Wellington, 2001). Species described within the genus Streptomyces increased from approximately 40 to over 3000, but many of these strains were considered synonyms. To prevent over speciation, standard identification criteria and type strains were needed. The criteria were based on morphological features such as mycelia, soluble pigments, spore chain, spore surface, production of melanin pigment, and the utilization of a range of carbon sources (Shirling and Gottlieb, 1968). In 1980s and in following years, the numerical taxonomy was introduced (Kampfer et al., 1991). Each cluster is regarded as single species or species-group. Using the numerical classification approach, the 1989 edition of Bergey's Manual describes 142 species (Locci, 1989), in contrast to 463 species described in the 1974 edition (Pridham and Tresner, 1974).

I.6. Plant growth-promoting Streptomyces (PGPS) and their role as a biocontrol agent:

Most Streptomyces are efficient rhizosphere and rhizoplane colonizers. They can also be endophytes colonizing inner tissues of host plants (Sousa and Olivares 2016). These attributes may be due to features such as quorum sensing controlled gene expression, multiplication rate, antibiotics, siderophore, cellulases, phytohormones, amino acid synthesis, chitinase, lipase, and β-1,3-glucanase production. Exudate attraction of Streptomyces to the rhizosphere is accomplished by the chemotaxis movement of these microbes. In the agricultural sector, the emergence of PGPS either as biofertilizer or biocontrol has led to new discoveries into other ways these microbes can be useful. Streptomyces are not left out in this discovery, although many studies have focused on the biocontrol activities of these genera

due to its high production of bioactive compounds which are used as defense mechanisms (Shrivastava and Kumar, 2018).

The global attempts to discovering natural products as biocontrol agents for plant protection have notably been on the rise and actinomycetes, Streptomyces being the most proactive, appear to be a readily available natural choice in finding new ways to combat plant pathogens. Their abilities to control plant pathogens stem from the following traits: Synthesis of plant growth regulators, Siderophore production, Antibiotics production, Volatile compound secretion, Competition for nutrients. (Priya et al. 2017).

Materials and methods

The aim of this project was to study the effect of four *Streptomyces spp* strains (TM52, IA1, D15 and D54) as BCAs against *Fusarium oxysporum f. sp. radicis-lycopersici* and *Fusarium solani* and as a PGPR agent on tomato seedlings (*Solanum lycopersicum*. L. cv Marmande).

The work was carried out in the PFE laboratories at the Faculty of Nature and Life Sciences in Ziane Achour University of Djelfa.

II.1.Plant materials and bacterial strains:

Tomato seeds (*Solanum lycopersicum*. L. cv Marmande) were prepared and inoculated in a previous study with four streptomyces strains codified as : (TM52, IA1, D54, D15) as well as two groups of seeds were used as controls, the first group (T) include non-treated tomato seeds while the other marked as (TR) and represents tomato seeds treated with a chemical fungicide (Thirame) (**Fig 04)**.

Fig 04. Non-treated (right) and treated (left) Tomato Seeds.

II.2.Growth conditions:

The soil used in this experiment is a mixture of (1:1:1) agricultural soil, sand and compost. The soil was autoclaved twice for 20 min with a 24 h incubation period. The seeds were directly sown in plastic pots (8.5 cm high, of 10 cm square section) with a density of 6 seeds per pot.

A randomized complete block design was adopted. It included two treatments (PGPR effect and Biocontrol effect). Each treatment was replicated five times and each pot represents

a replicate i.e., (T, TR, TM52, IA1, D54, D15) x 5 = 30 pots for treatment (see annexes **Tab 06 and 07**).

II.3.Cultivation of plants and Disease induction:

Six equally spaced holes of about 2 cm depth were realized across the soil for each pot using a flame-sterilized Pasteur pipette.

For the Biocontrol treatment, two pathogens were used in this study: *Fusarium oxysporum f. sp. radicis*-lycopersici as a soil borne and *Fusarium solani* was sprayed on tomato seedlings after 17 days of growth. The FORL cell suspension (10^6 ufc/ml) was introduced into the 30 pots. Five ml of the FORL cell suspensions were introduced in each hole using a sterile syringe three days before sowing seeds to allow the disease to develop. After 17 days of growth, a second pathogen was sprayed on tomato seedlings and on the soil (*Fusarium solani* (10^6 ufc/ml)).

For PGPR treatment, the seeds were sown directly into the holes of each pot using a flame-sterilized forceps.

The experiment was conducted for 40 days under a photoperiod of 16 hours light "Daylight" and 8 hours of obscurity. The seedlings of each pot were irrigated by150 ml sterile tap water once every two days for the first 30 days, and then 50 ml every day for the last 10 days until harvest (**Fig05**).

Fig 05: Seed sowing

II.4. Studied parameters:

After 40 days of growth, the following parameters were measured:

II.4.1. Germination percentage:

Germination percentage was calculated as:

(number of germinated seeds / total number of seeds) x 100.

II.4.2.Fresh and dry weights:

In order to determine the fresh and dry weights, a plant from each pot of the different treatments was immediately weighed to determine mean fresh weight, and then the samples were dried in an oven at 70°C for 48 hours to determine the dry weight.

II.4.3.Water content:

Water content was calculated as the difference between fresh weight and dry weight:

WC=FW-DW

II.4.4.Measurement of photosynthetic pigments contents:

Samples of 200 mg of fresh material were cut into 2 mm fragments. were homogenized in the dark three times with 2ml 80% acetone The extraction was carried out by grinding the samples 3 times in the presence of 2 ml 80% acetone. Absorbance was measured at three wavelengths 447nm for carotenoids, 663 nm for chlorophyll a and 647 nm for chlorophyll b using an "BECKMAN DU 520" spectrophotometer. Contents of chlorophyll a, b, total chlorophyll (a + b) and carotenoids in the whole-pigment extract were calculated as described by Linchthentaler et al. (1987):

$$C_a(\text{mg/g FW}) = (12{,}25\ OD_{663} - 2{,}79\ OD_{647})\ (V/FW)$$

$$C_b\ (\text{mg/g FW}) = (21{,}50\ OD_{647} - 5{,}10\ OD_{663})\ (V/FW)$$

$$C_{(a+b)}(\text{mg/g FW}) = (7.15\ OD_{663} + 18.71\ OD_{647})\ (V/FW)$$

$$C_{(x+c)}(\text{mg/g FW}) = (1000\ OD_{447} - 1.82\ C_a - 85.02\ C_b)\ (V/198FW)$$

Where :

V : is the initial volume (6mL)

FW : is the initial fresh weight (200mg)

OD : is the corresponding optical density.

II.4.5.Determination of total soluble sugars:

Samples of 100 mg fresh weight from leaf tissue were macerated in 4 ml of 40% methanol for one hour in a water bath at 85 ° C. The total soluble sugars content was determined according to the phenol-sulfuric acid method (Dubois et al. 1956). 0.5 ml 5% phenol was added to 0.5 ml of the extract. After vortexing, 2.5 mL of concentrated sulfuric acid (H_2SO_4) were added. After a second vortexing, the mixture thus produced was allowed to cool for 15 minutes. The solution turns gradually to pink color. The optical density was measured at a wavelength of 487 nm using a "BECKMAN DU 520" spectrophotometer. The values were plotted on the calibration curve constructed from samples containing known pure glucose concentrations (curve 01 in annexes).

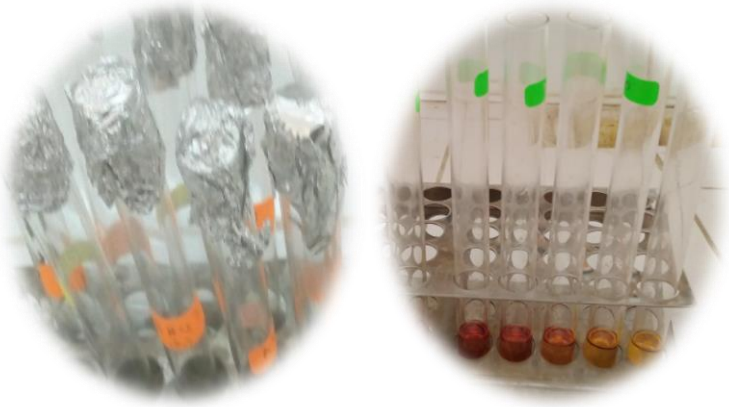


Fig 06: Determination of total soluble sugars

II.4.6.Determination of proline:

The proline content was determined according to the method described by Troll and Lindsley (1955) and repeated and simplified by Dreier and Goring (1974).

Samples of 100 mg fresh weight from leaf tissuewere macerated for one hour in a water bath at 85 ° C in test tubes to which 4 ml of 40% methanol were added. The samples whole were heated again at 85 ° C.

The test tubes were covered with aluminum foil to minimize losses of methanol by evaporation. After cooling, 1mL of the extract was removed to which was added 1mL of acetic acid (99-100%), 2 mL of Ninhydrin 3% and 1 ml of a mixture of 120 ml of distilled water plus 300 ml of acetic acid (99-100%) and 80 ml of 85% orthophosphoric acid (H3PO4, d = 1.7).The obtained mixture was boiled in a water bath for 30 minutes.

The solution turns gradually red. After cooling, 5 mL of benzene was added to separate two phases. After vortexing, the upper phase was recovered and dried by the addition of an anhydrous sodium sulphate spatula (N2SO4).The optical density was measuredat the wavelength of 528 nm using a UV-visible spectrophotometer of "BECKMAN DU 520 ".

The values were plotted on the calibration curve constructed from samples containing known concentrations of proline (curve 02 in annexes).

II.4.7.Measurement of cell membrane permeability:

Sampels of 0.5 cm long were cut from a leaf of each plant (a plant from each pot) and washed with distilled water. They were then placed in test tubes containing 10 ml of distilled

water. The conductivity of the solution was then measured (C1) for the first time with a calibrated conductivity meter. The samples were then put in a boiling water bath for 20 minutes and then cooled to room temperature. The conductivity was measured a second time (C2). The percentage of leakage of electrolytes was calculated according to the formula (Bin Yan et al. , 1996) :

$$\mathbf{MP\ (\%) = C_1 100\ / C_2}$$

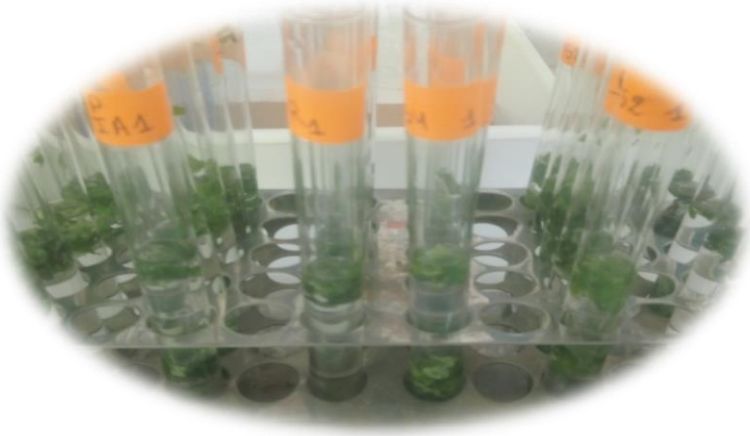


Fig 07: Measurement of cell membrane permeability

II.4.8.Determination of catalase (CAT) and peroxidase (POD) activities:

II.4.8.1.Extraction:

0.5 g of fresh weight from leaf tissue were ground in 7 mL of 50 mM SPB (Sodium Phosphate Buffer) (pH = 7) containing 1% (w / v) polyvinylpyrrolidone in an ice bath.The homogenate was centrifuged at 15000g / 20 min at 4 ° C. The supernatant was used for the determination of enzymatic activity.

Fig 08: Extraction of catalase and peroxidase

II.4.8.1.1.Determination of catalase activity (CAT):

CAT activity was measured using Chance and Maehly method with some modifications. The CAT reaction solution (3 ml) contained 1.9 ml of 50 mM SPB (pH 7.0), 1 ml of 15mM H2O2 and 0.1 ml of the enzyme extract. The reaction was initiated by adding the enzymatic extract. The changes in the absorbance of the reaction solution at 240 nm were read every 15 sec (1 min).

II.4.8.1.2.Determination of peroxidase activity (POD):

The reaction solution of POD (3ml) contained 2.8 ml of 5mM SPB (pH 7), 0.03ml H2O2 (40mM), 0.05ml guaiacol (20mM) and 0.1ml of the enzymatic extract. Absorbance changes at 436 nm were read every 15s (1 min).

II.5.Statistical analysis:

Statistical significance of differences between means was determined with ANOVA single factor followed by LSD's post hoc test at 1% significance level using the Statistica.

Results and Discussion

III.1.Results of the PGPR effect:

The analysis of variance of the different variables for the PGPR effect (**Table 08** in annexes) showed the following results :

High significant difference between the six groups (4 bacterial strains and 2 controls) for the PRO variable with $p < 0.01$.

Very high significant difference between the six groups for the CMP, TSS and GP variables with $p < 0.001$.

No significant difference between the six groups for the other variables.

The LSD test for Homogenous Groups are presented in annexes (see **Tables 09 – 12).**

III.1.1.Germination percentage:

Figure 09 shows the effect of the the four streptomyces strains on the tomato plant's percentage of germination. The seeds treated with the four strains have shown a higher percentage of germination in comparison to the two controls T and TR, in which the D54 showed the highest percentage of germination compared to the other strains, and the seeds treated with thirame have the lowest percentage of germination among the two controls.

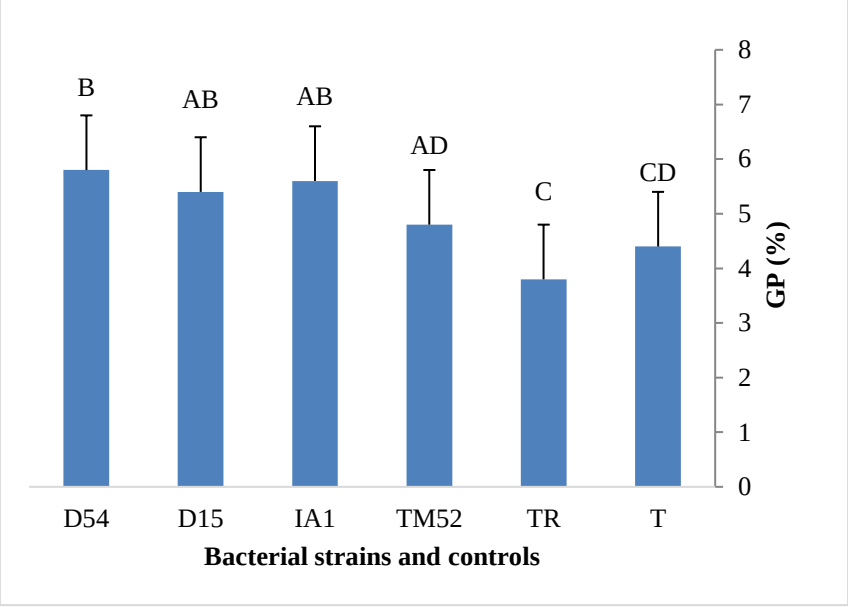


Figure 09: The effect of the four streptomyces strains on the tomato plant's percentage of germination.

III.1.2.Fresh weight:

Figure 10 shows the effect of the the four streptomyces strains on the tomato plant's production of fresh weight. No significant difference was observed for the production of fresh weight between the six groups.

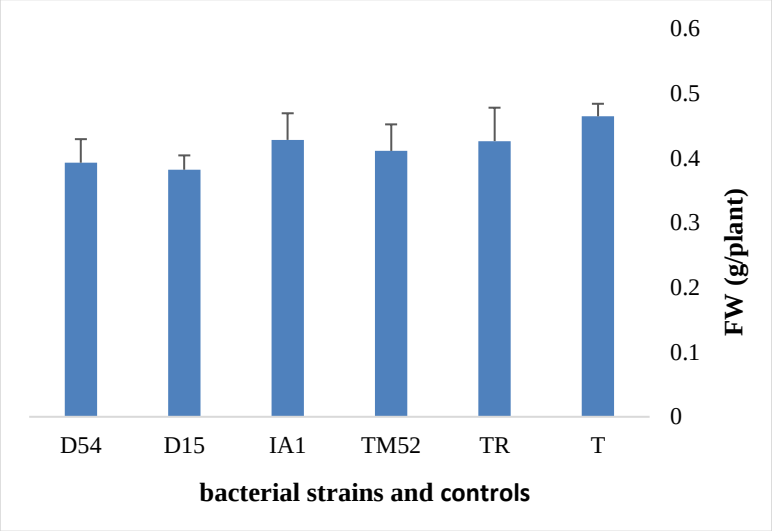


Figure 10: The effect of the four streptomyces strains on the production of fresh weight in tomato seedlings.

III.1.3.Dry weight:

Figure 11 shows the effect of the the four streptomyces strains on the tomato plant's dry weight. There is no significant difference in dry weight between the six groups

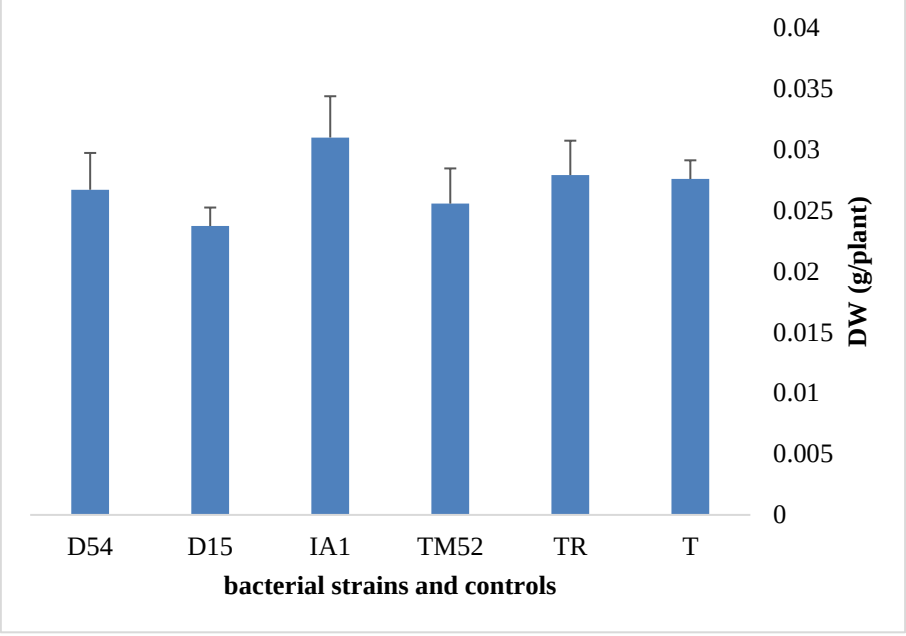


Figure 11 : The effect of the four streptomyces strains on the dry weight tomato seedlings.

III.1.4.Water content:

Figure 12 shows the effect of the four streptomyces strains on the water content of the tomato seedlings. There is no significant difference in the water content between the six groups.

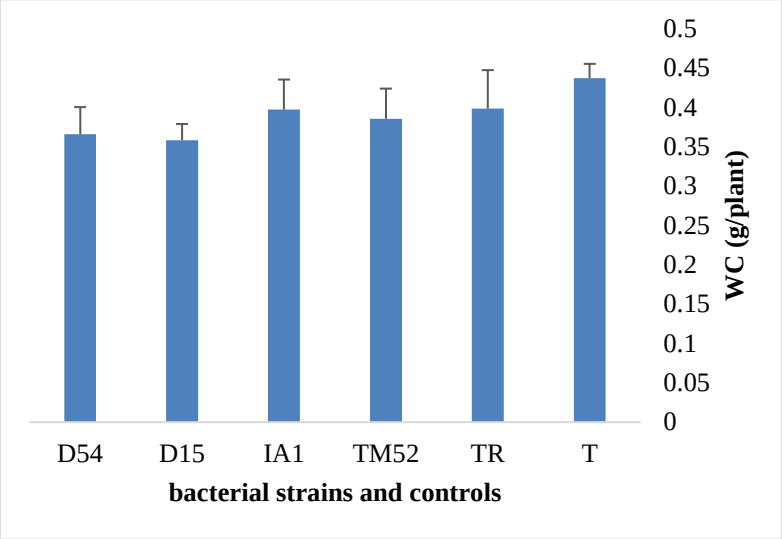


Figure 12 : The effect of the four streptomyces strains on the water content of the tomato seedlings.

III.1.5.Chlorophyll a:

Figure 13 shows the effect of the four streptomyces strains on the content of chlorophyll a in the leaves of tomato seedlings. There is no significant difference in the content of chlorophyll a between the six groups.

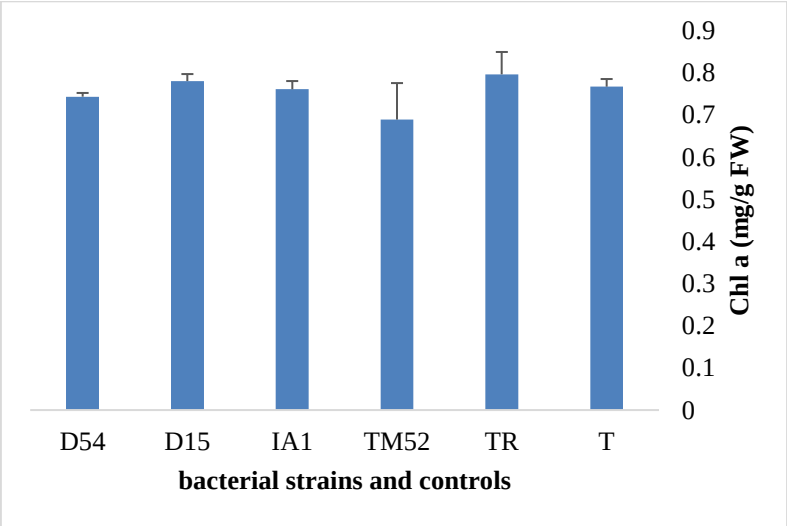


Figure 13 : The effect of the four streptomyces strains on the content of chlorophyll a in the leaves of tomato seedlings.

III.1.6.Chlorophyll b:

Figure 14 shows the effect of the four streptomyces strains on the content of chlorophyll b in the leaves of tomato seedlings. No significant difference was observed between the six groups for the content of chlorophyll b.

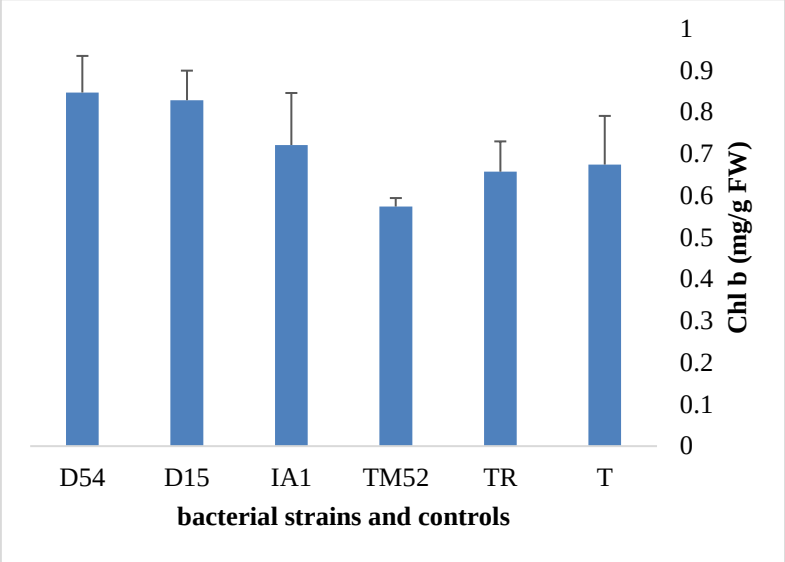


Figure 14: The effect of the four streptomyces strains on the content of chlorophyll b in the leaves of tomato seedlings.

III.1.7.Chlorophyll a+b:

Figure 15 shows the effect of the four streptomyces strains on the content of chlorophyll a+b in the leaves of tomato seedlings. There is no significant difference in the content of chlorophyll a+b between the six groups.

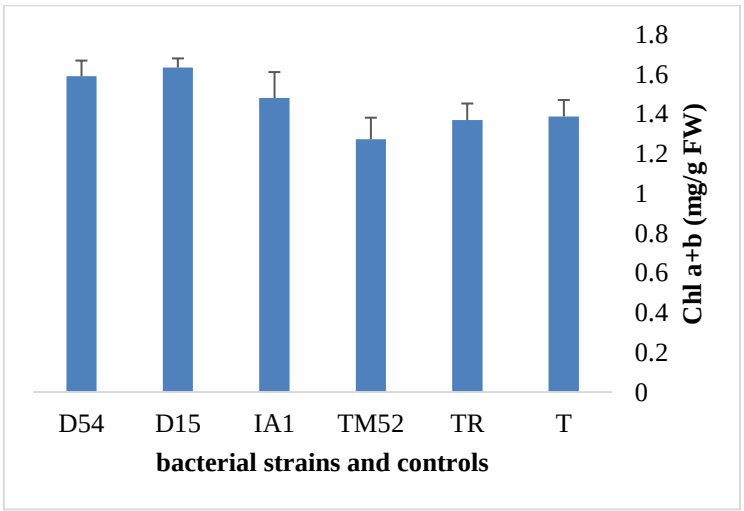


Figure 15 : The effect of the four streptomyces strains on the content of chlorophyll a+b in the leaves of tomato seedlings.

III.1.8. Carotenoids:

Figure 16 shows the effect of the four streptomyces strains on the content of carotenoids in the leaves of tomato seedlings. There is no significant difference in the content of carotenoids between the six groups.

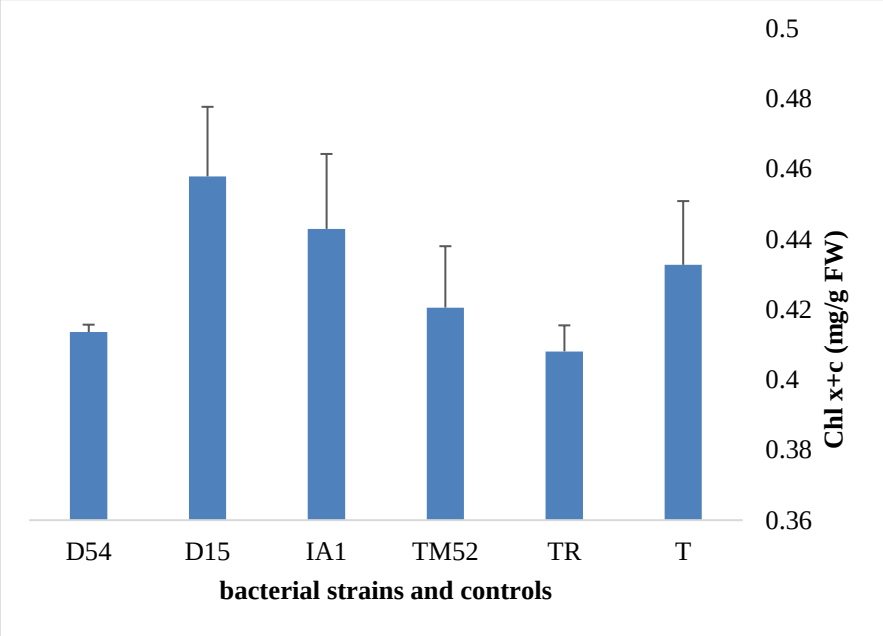


Figure 16 : The effect of the four streptomyces strains on the content of carotenoids in the leaves of tomato seedlings.

III.1.9. Cell membrane permeability:

Figure 17 shows the effect of the four streptomyces strains on the tomato plant's cell membrane permeability. The control seedlings T have the higher percentage of cell membrane permeability in comparison to the other groups. On the other hand seedlings treated with the D15 strain showed the lowest percentage of cell membrane permeability.

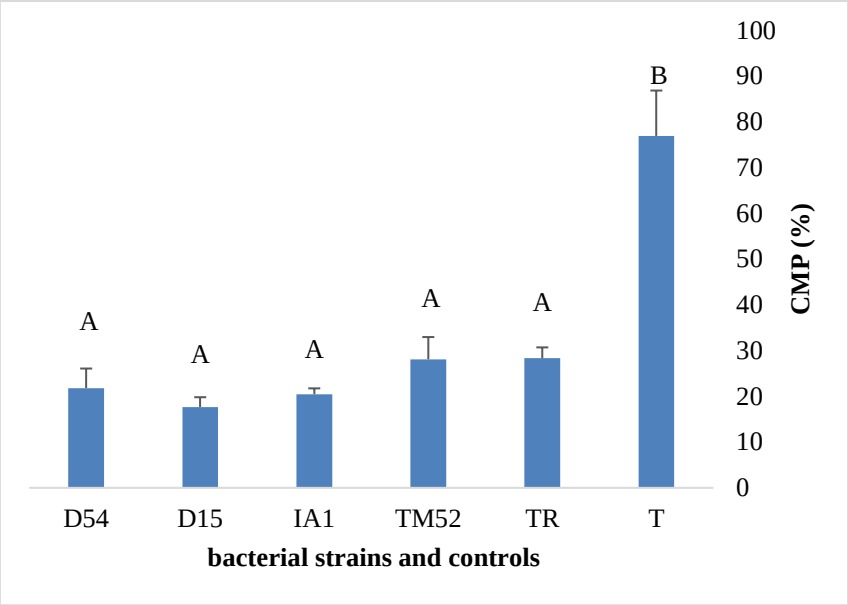


Figure 17 : The effect of the four streptomyces strains on the cell membrane permeability in the leaves of tomato seedlings.

III.1.10. Total soluble sugars:

Figure 18 shows the effect of the four streptomyces strains on the accumulation of total soluble sugars in the leaves of tomato seedlings. The seedlings treated with D15 have the lowest content of total soluble sugars,however, seedlings treated with IA1 accumulated high amounts of TSS .

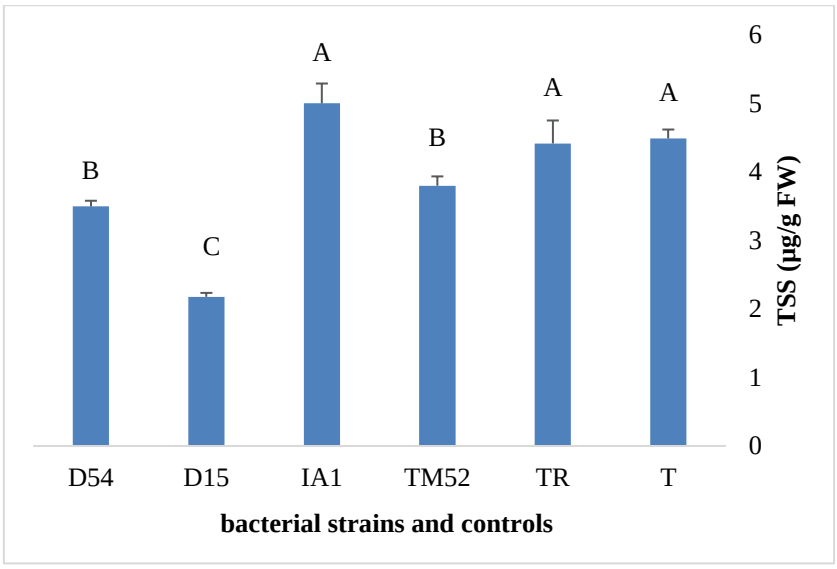


Figure 18 : The effect of the four streptomyces strains on the accumulation of total soluble sugars in the leaves of tomato seedlings.

III.1.11.Proline:

Figure 19 shows the effect of the four streptomyces strains on the accumulation of proline in the leaves of tomato seedlings. The seedlings treated with Thirame TR, have the highest content of proline, in the other hand, seedlings treated with D15 have the lowest amount of proline.

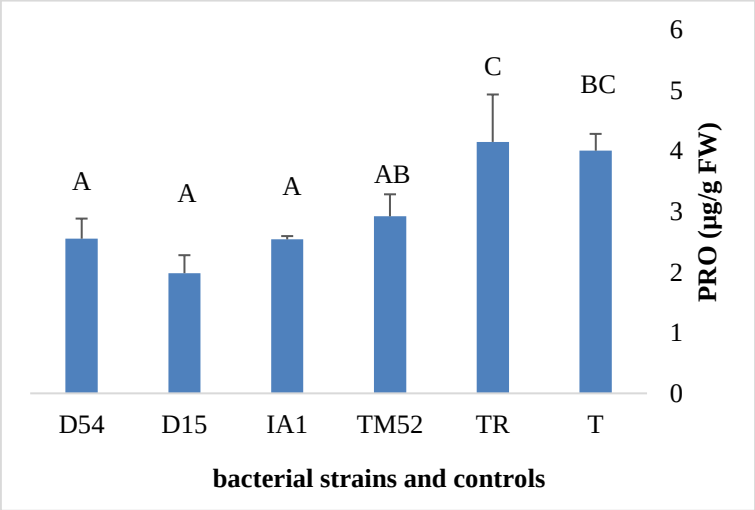


Figure 19 : The effect of the four streptomyces strains on the accumulation of proline in the leaves of tomato seedlings.

III.1.12.Peroxidase activity:

Figure 20 shows the effect of the four streptomyces strains on the activity of peroxidase in the leaves of tomato seedlings. No significant difference between the six groups for peroxidase activity.

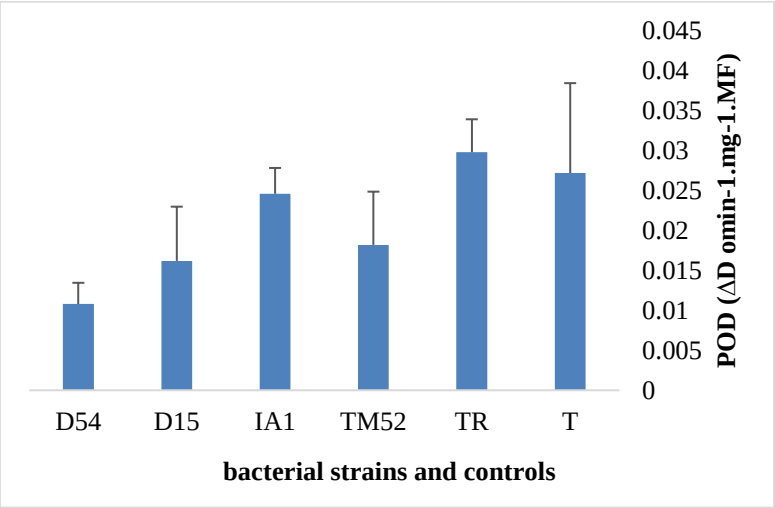


Figure 20 : The effect of the four streptomyces strains on the peroxidase activity in the leaves of tomato seedlings.

III.1.13.Catalase activity:

Figure 21 shows the effect of the four streptomyces strains on the activity of catalase in the leaves of tomato seedlings. There is no significant difference in the the activity of catalase between the six groups.

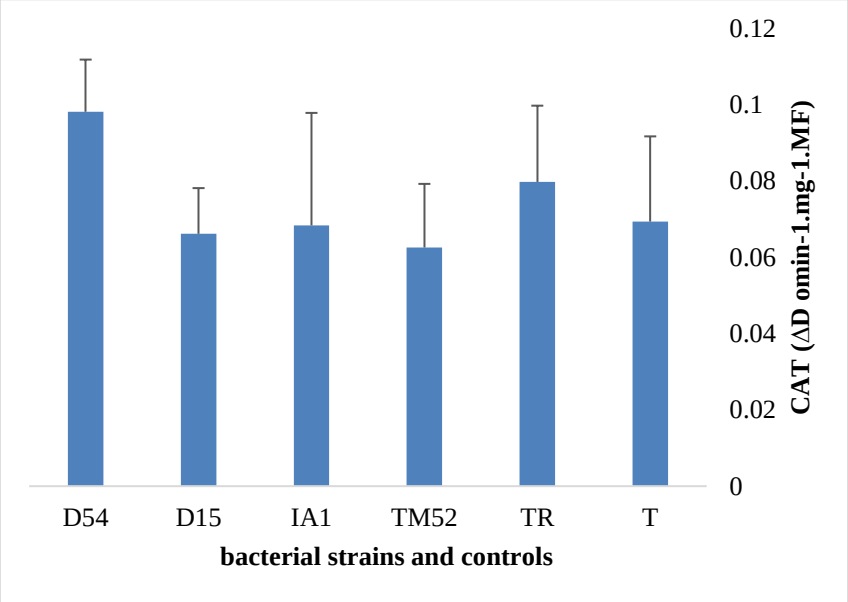


Figure 21: The effect of the four streptomyces strains on the activity of catalase in the leaves of tomato seedlings.

III.2.Results of the Biocontrol effect:

The analysis of variance of the different variables for the Biocontrol effect (**Table 13** in annexes) showed the following results :

Significant difference between the six groups (4 bacterial strains and 2 controls) for the POD variable with $p < 0.05$

Highly significant difference between the six groups for the PRO, DW variables with $p < 0.01$.

Very highly significant difference between the six groups for the CMP, TSS variables with $p < 0.001$.

No significant difference between the six groups for the other variables.

The LSD test for Homogenous Groups are presented in annexes (see **Tables 14 – 18).**

III.2.1.Germination percentage:

Figure 22 shows the effect of biotic stress (Fusarium) on the percentage of germination in tomato seedlings treated by different streptomyces strains. There is no significant difference in the percentage of germination between the six groups

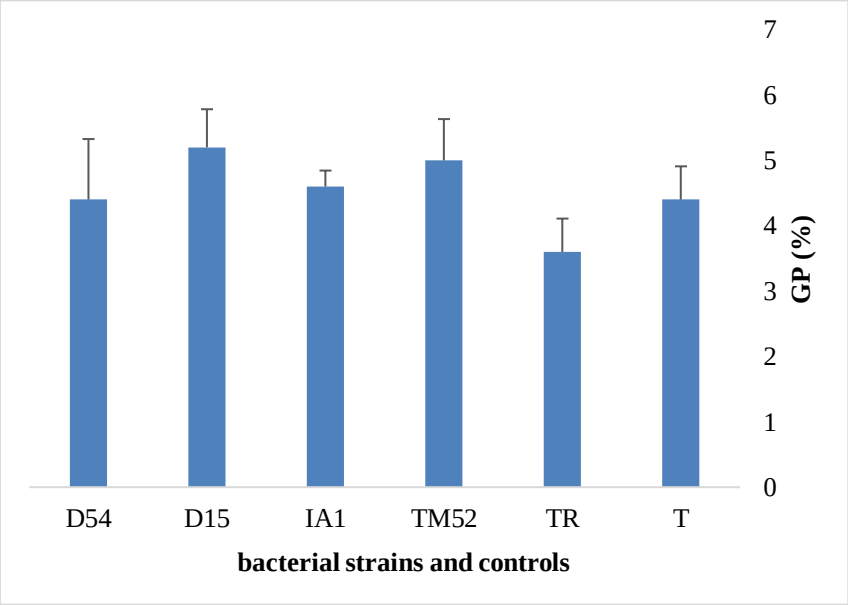


Figure 22: The effect of biotic stress (Fusarium) on the percentage of germination in tomato seedlings treated by different streptomyces strains.

III.2.2.Fresh weight:

Figure 23 shows the effect of biotic stress (Fusarium) on fresh weight production in tomato seedlings treated by different streptomyces strains. There is no significant difference in the production of fresh weight between the six groups.

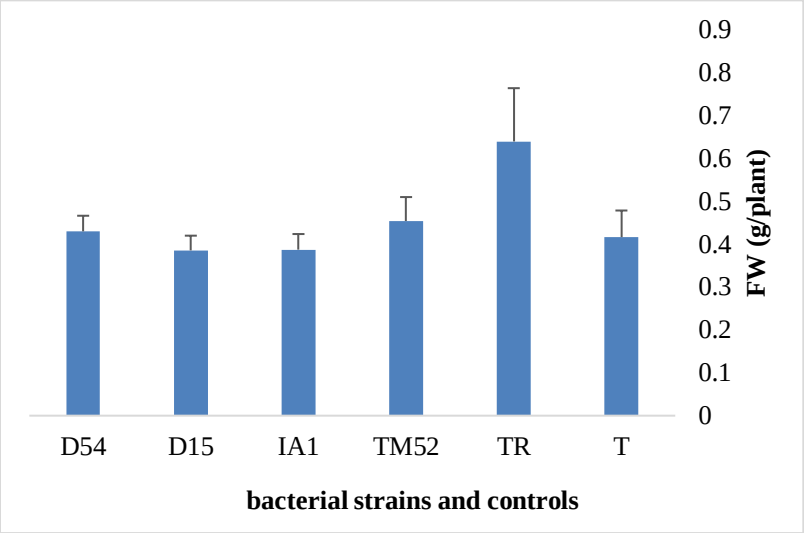


Figure 23: The effect of biotic stress (Fusarium) on fresh weight production in tomato seedlings treated by different streptomyces strains.

III.2.3.Dry weight:

Figure 24 shows the effect of the four streptomyces strains on the tomato plant's dry weight. Seedlings treated with Thirame showed the highest productivity of dry weight followed by the other groups.

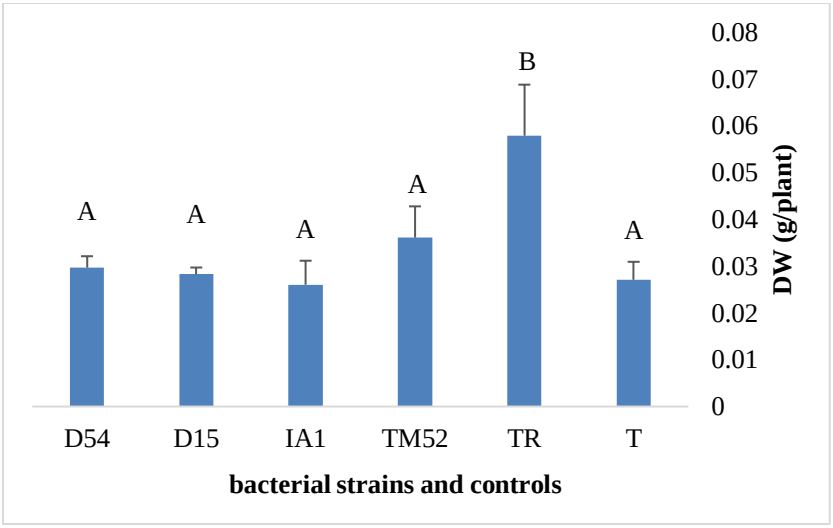


Figure 24: The effect of biotic stress (Fusarium) on dry weight production in tomato seedlings treated by different streptomyces strains.

III.2.4.Water content:

Figure 25 shows the effect of biotic stress (Fusarium) on water content in tomato seedlings treated by different streptomyces strains. There is no significant difference in the water content between the six groups.

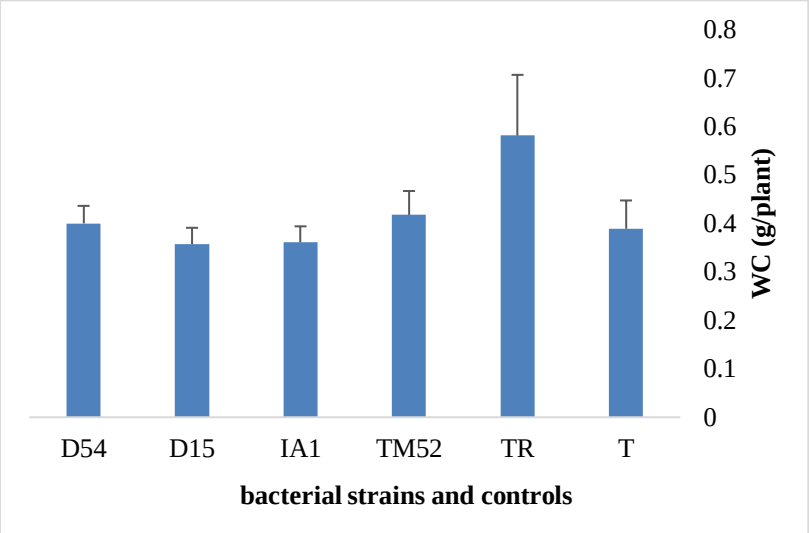


Figure 25: The effect of biotic stress (Fusarium) onwater content in tomato seedlings treated by different streptomyces strains.

III.2.5.Chlorophyll a:

Figure 26 shows the effect of biotic stress (Fusarium) on chlorophyll a content in tomato seedlings treated by different streptomyces strains. There is no significant difference in the content of chlorophyll a between the six groups.

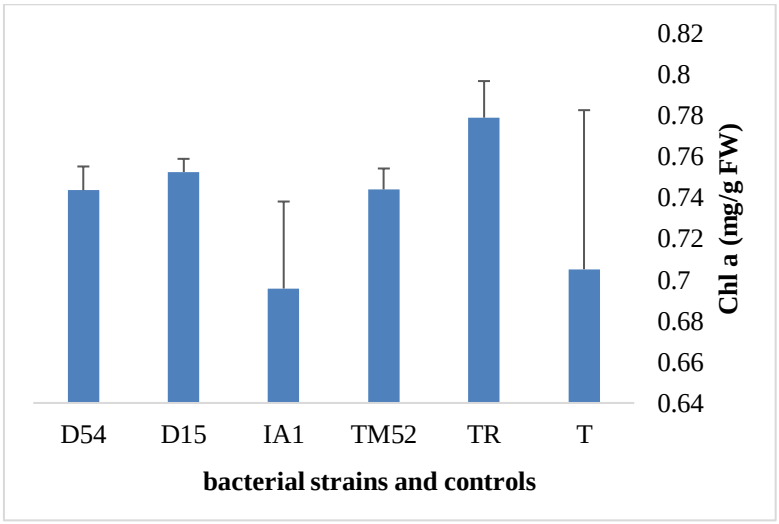


Figure 26: The effect of biotic stress (Fusarium) on chlorophyll a content in tomato seedlings treated by different streptomyces strains.

III.2.6.Chlorophyll b:

Figure 17 shows The effect of biotic stress (Fusarium) on chlorophyll b content in tomato seedlings treated by different streptomyces strains. There is no significant difference in the content of chlorophyll b between the six groups.

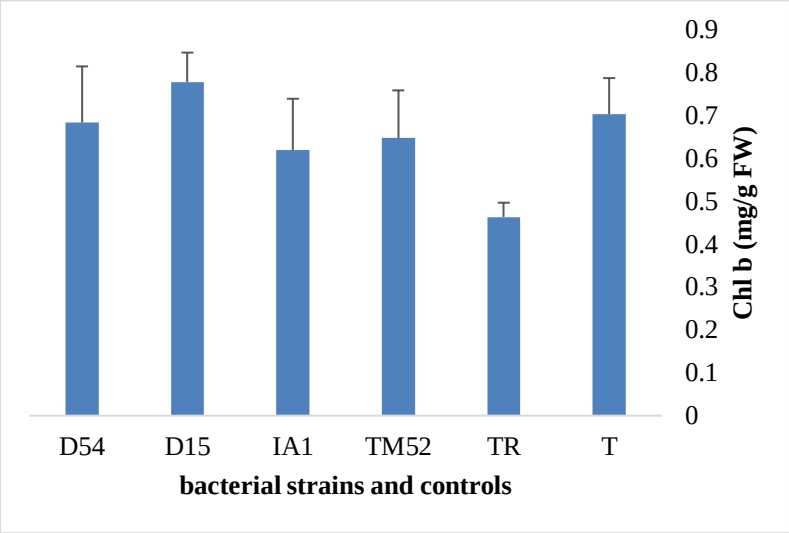


Figure 27: The effect of biotic stress (Fusarium) on chlorophyll b content in tomato seedlings treated by different streptomyces strains.

III.2.7.Chlorophyll a+b:

Figure 28 shows the effect of biotic stress (Fusarium) on total chlorophyll a+b content in tomato seedlings treated by different streptomyces strains. There is no significant difference in the content of chlorophyll a+b between the six groups.

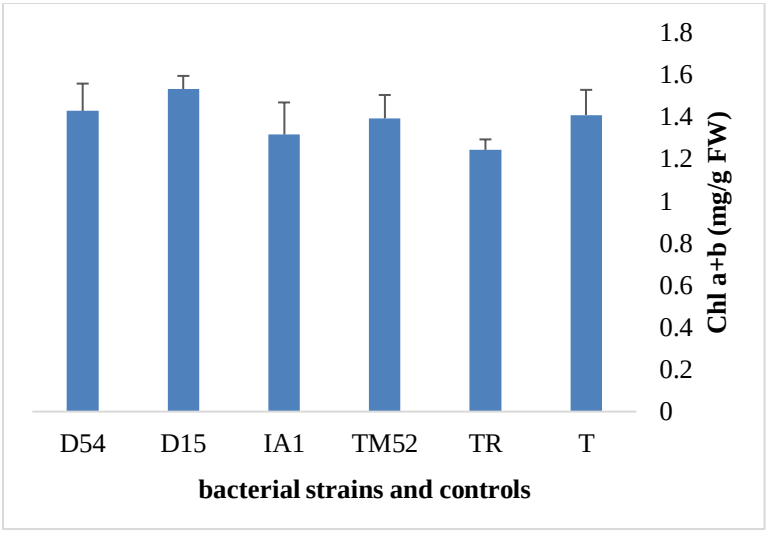


Figure 28: The effect of biotic stress (Fusarium) on chlorophyll a+b content in tomato seedlings treated by different streptomyces strain.

III.2.8.Carotenoids:

Figure 29 shows the effect of biotic stress (Fusarium) on carotenoids content in tomato seedlings treated by different streptomyces strains. There is no significant difference in the content of carotenoids between the six groups.

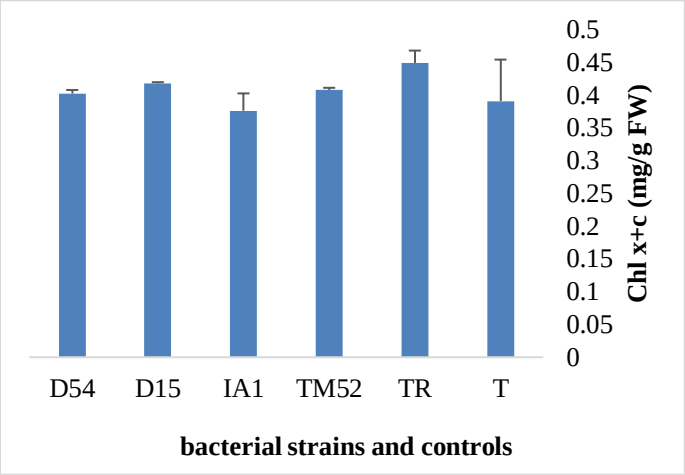


Figure 29: The effect of biotic stress (Fusarium) oncarotenoids content in tomato seedlings treated by different streptomyces strains.

III.2.9. Cell membrane permeability:

Figure 30 shows the effect of biotic stress (Fusarium) on cell membrane permeability in tomato seedlings treated by different streptomyces strains. The treatment by the different strains significantly decreased cell membrane permeability of tomato seedlings in comparison to control T. Seedlings treated with the IA1 strain showed the lowest percentage of cell membrane permeability.

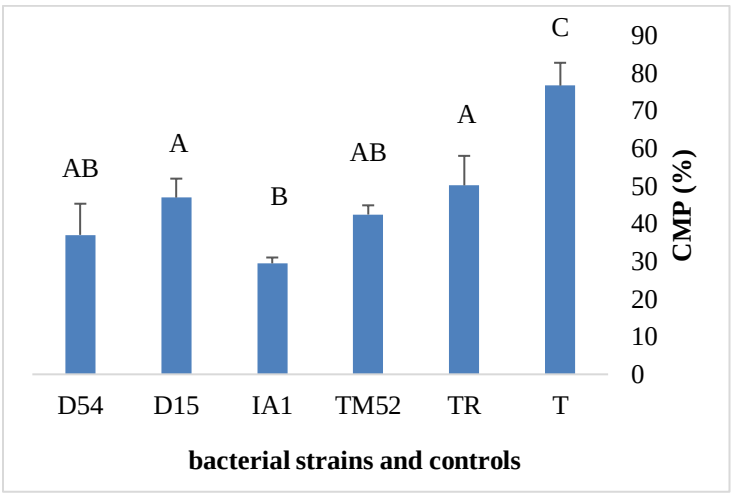


Figure 30: The effect of biotic stress (Fusarium) on cell membrane permeability in tomato seedlings treated by different streptomyces strains.

III.2.10. Total soluble sugars:

Figure 31 shows the effect of biotic stress (Fusarium) ontotal soluble sugars accumulation in tomato seedlings treated by different streptomyces. Control seedlings T significantly accumulated high amounts of total soluble sugars in comparaison to seedlings treated by streptomyces strains.

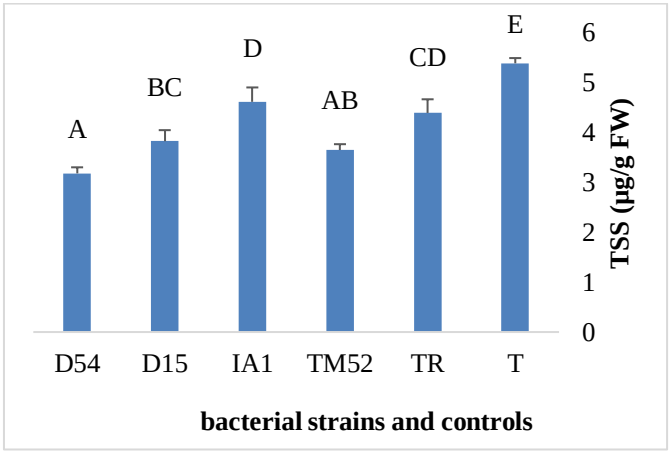


Figure 31: The effect of biotic stress (Fusarium) on total soluble sugars accumulation in tomato seedlings treated by different streptomyces strains.

III.2.11.Proline:

Figure 32 shows The effect of biotic stress (Fusarium) on proline accumulation in tomato seedlings treated by different streptomyces strains. Seedlings treated with the different streptomyces strains significantly accumulated less amounts of proline in comparaison to cotrols (TR, T).

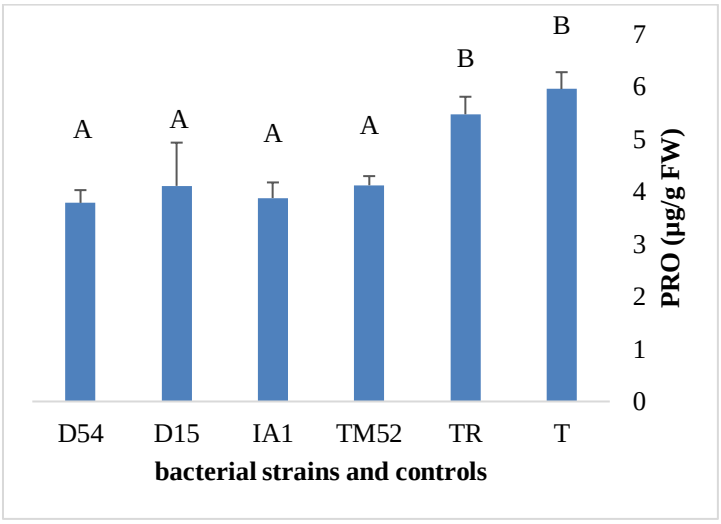


Figure 32: The effect of biotic stress (Fusarium) on proline accumulation in tomato seedlings treated by different streptomyces strains.

III.2.12.Catalase activity:

Figure 33 shows the effect of biotic stress (Fusarium) on catalase activity in tomato seedlings treated by different streptomyces strains. There is no significant difference in the activity of catalase between the six groups.

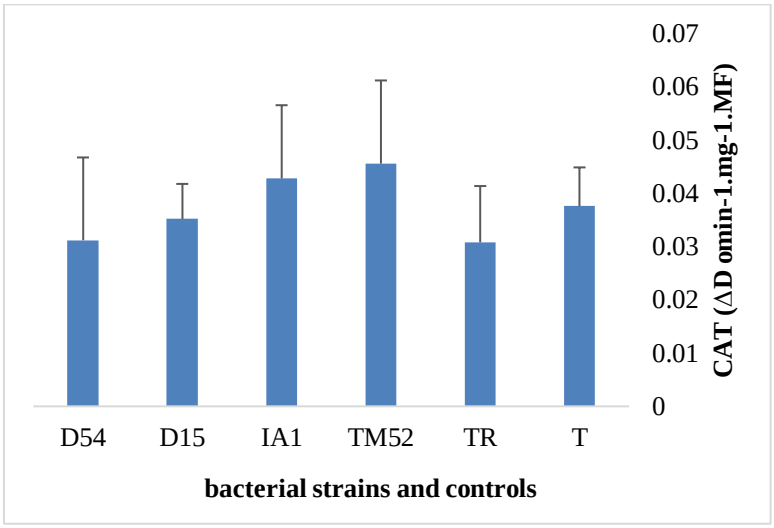


Figure 33: The effect of biotic stress (Fusarium) on catalase activity in tomato seedlings treated by different streptomyces strains.

III.2.13.Peroxidase activity:

Figure 34 shows the effect of biotic stress (Fusarium) on peroxidase activity in tomato seedlings treated by different streptomyces strains. Seedlings treated with TM52 showed low peroxydase activity in comparaison to other seedlings. However, seedlings treated with D15 strain was caracterized by the highest peroxidase activity.

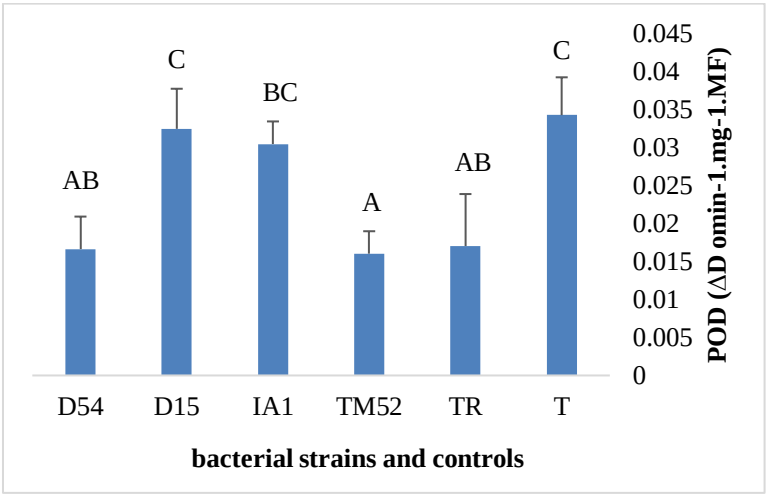


Figure 34: The effect of biotic stress (Fusarium) on peroxidase activity of in tomato seedlings treated by different streptomyces strains.

III.3.Discussion:

III.3.1.Germination percentage and Biomass:

It is important to calculate a biomass and the of germination because biomass is usually expressed as a net change in the weight because there can be significant changes to the biomass within the designated time (Weller and Cook, 1986).

For the PGPR effect, the results for the percentage of germination were particularly positive. The studied streptomyces strains (TM52, IA1, D15, D54) showed higher germination percentage than the two controls (T, TR). However, for the Biocontrol effect, no significant difference was noticed for this parameter.

Similarly, the results of fresh and dry weights showed no significant differences between groups were observed result for both PGPR and Biocontrol effects.

III.3.2.Photosynthetic pigments:

The measurement of photosynthetic pigments content is a very important parameter allowing to have valuable insights into plant growth and yield potential. (Peer and Schippers, 1988)

No significant effect was observed for the Biocontrol effect and For the PGPR effect in terms of photosynthetic pigments production.

III.3.3. Cell membrane permeability:

Cell membranes are important for allowing nutritive, respiratory and excretory processes in plants. To allow this to happen correctly the membranes are semi permeable. However, membrane permeability can be affected by several factors such as biotic or abiotic stress (Eeuwens and Schwabe, 1975)

The results of cell membrane permeability were significantly important for both PGPR and Biocontrol effects. The four studied bacterial strains have showed significant low percentage of cell membrane permeability in comparison to the two controls, which means that seedlings treated with the strains have preserved their membrane structure.

III.3.4. Total soluble sugars and proline:

Total soluble sugars content is not only the main photosynthate in higher plants, but also the main form of carbohydrate metabolism and temporary storage. The soluble sugars content plays a very important role in carbohydrate metabolism and has a close relationship with photosynthesis and production (Bing et al, 2011).

Over production of proline is a widespread response observed in plants experiencing various stresses. The determination of this amino acid is therefore very useful to assess the physiological status and more generally to understand stress tolerance in plants (Bates et al, 1973).

The results for the accumulation of total soluble sugars and proline showed significantly positive results in both effects. Because the rate of accumulation of total soluble sugars and of proline were remarkably lower with seedlings treated with the bacterial strains in comparison to the two controls.

Seedlings treated with (TM52, D15, D54) showed lower accumulation of total soluble sugars in comparison to the two controls in both effects.

Seedlings treated with all four bacterial strains showed lower accumulation of proline in comparison to the two controls in both effects.

Thus, these seedlings are more tolerant or more resistant to biotic stress (Fusarium).

III.3.5.Catalase and peroxidase activities:

Plants have the effective enzymatic antioxidant defense system including catalase (CAT) and peroxidase (POD). This system allow for scavenging of ROS leading to protection of plant cells from oxidative damage (Alici and Arabac, 2016)

For the PGPR effect, no significant effect was observed in both Catalase and peroxidase activity

As for the Biocontrol effect, the results showed a positive result only in Peroxidase activity.

Seedlings treated with all four bacterial strains showed lower Peroxidase activity in comparison to the control T (non treated seedlings).However only seedlings treated with TM52 showed lower peroxidase activity than the two controls (non treated control and control

treated with fungicide Thirame), seedlings treated with D54 showed equal peroxidase activity with control treated with chemical fungicide Thirame TR.

Thus, these seedlings are more tolerant or more resistant to biotic stress (Fusarium).

Conclusion

Conclusion

This work aimed to evaluate the PGPR and the Biocontrol effects of 4 streptomyces strains (TM52, IA1, D15, D54) on tomato seedlings (*Solanum lycopersicum*. L. cvMarmande). Two controls were used; the first was an ordinary control (non-treated seeds) and the second control represents tomato seeds treated with a chemical fungicide "Thirame" used to explore the effect of a chemical fungicide on seeds and in order to derive a comparison against with what is hopefully to be biopesticide "the bacterial strains".

The studied parameters in this work were: percentage of germination, biomass, chlorophyll content, cell membrane permeability, accumulation of total soluble sugars and proline and enzymatic activity.

According to the obtained statistical results, seeds treated with the bacterial strains showed an improvement under normal conditions as well as under biotic stress conditions.

For the PGPR effect, the strains "D15, D54, TM52" showed a high productivity than controls because they were characterized by an increase in the percentage of germinationand a decrease in the cell membrane permeability, accumulation of total soluble sugars and proline

For the Biocontrol effect, D15, D54, and TM52 strains revealed an important resistance towards the biotic stress because they showed an increase in the percentage of germination and a decrease in cell membrane permeability, accumulation of proline and total soluble sugars. In addition to that D54 and TM52 strains showed lower peroxidase activity.

In the light of the results of this modest work, we conclude that the D15, D54 and TM52 strains are the most efficient in terms of promoting tomato plants growth in normal conditions and in terms of promoting tomato plants resistance towards Fusarium. These results opens up prospects for exploitation of these four strains as excellent inocula for a field study to confirm their utility in improving tomato plants production and resistance to Fusarium.

References

Agrios G.N. 2005. Plant pathology. Fifth edition, Elsevier academis press, California 29101, 524-539.

Ahemad M, Kibret M. 2014 Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud UnivSci 26(1):1–20.

Anderson AS, Wellington E. 2001. The taxonomy of Streptomyces and related genera. International Journal of Systematic and Evolutionary Microbiology 51:797-814.

Anderson AS, Wellington E. 2001. The taxonomy of Streptomyces and related genera. International Journal of Systematic and Evolutionary Microbiology 51:797-814.

Atherton, J.G. and J. Rudich. 1986. the Tomato Crop: A Scientific Basis for Improvement, Chapman & Hall, London.

Baptista F J., Bailey B J.et Meneses JF. 2012. Effect of nocturnal ventilation on the occurrence of Botrytis cinerea in Mediterranean unheated tomato greenhouses. Crop Protection. 32 : 144-149.

Barness E, Chen YY, Hadar HMVR. 1991. Siderophores of Pseudomonas putida as an iron source for dicot and monocot plants. Plant and Soil 130(1), 231–241.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water stress studies. Plant Soil 39: 205-207.

Benbadji. 1977. Etude expérimentale de la croissance et de la production de la tomate sous l'action des concentrations différentes de Nacl et d'apport d'amendement. Thèse de Magistère. Institut National d'Agronomie. Alger.69p.

Bing LIU, Yan-sheng LI, Xiao-bingLIU, Cheng WANG, Jian JIN, S.J. Herbert. 2011. Lower total soluble sugars in vegetative parts of soybean plants are responsible for reduced pod number under shading conditions. Australian Journal of Crop Science. 5(13):1852-1857.

Blancard D. 2009. Les maladies de la tomate, identifier, connaitre, maitriser. Edition:Quæ. Paris. 691p.

Bohs L., Olmstead R. G. 1997. Phylogenetic relationships in Solanum (Solanaceae) based on ndhF sequences. Syst. Bot. 22: 5-17.

Burhan, A., A. Ghafoor and H. Badar. 2005. Forecasting and Growth Trends of Production and Export of Kinnow from Pakistan. Journal of Agriculture Social Sciences.

Chaux C. etFoury C. 1994. Productions légumières, Tome III : légumineuses potagères, légumes fruits. Edition : Lavoisier tec et doc. Paris. 563p.

Cook RJ, Baker KF. 1983. The nature and practice of biological control of plant pathogens. St. Paul, MN : American Phyto pathological Socirty.

Crowley DE, Reid CPP, Szaniszlo PJ, Florida DEC. 1988. Utilization of Microbial Siderophores in Iron Acquisition by Oat. Plant Physiology 87(3), 680–685.

Dilip R. Panthee, Feng Chen. 2010. Genomics of Fungal Disease Resistance in Tomato. Current Genomics, 11, 30-39.

Doornbos RF, Loon L, Van C. 2012. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agronomy for Sustainable Development 32(1), 227–243.

Dubois m. ; Gilles k a. ; Hamilton j k. ; Rebers p a. et Smith F., 1956 – Colorimetric method for determination of sugars and related substances. Anal. Chem., 28 : 350 – 356.

Dweipayan Goswami, Janki n. Thakker and Pinakin c. Dhandhukia. 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food &Agriculture, 2: 1127500.

Eeuwens cj, Schwabe ww. 1975. Seed and pod wall development in Pisum sativum L. in relation to extracted and applied hormones. J Exp Bot 26: 1-14.

El-Tarabily k, Alkhajeh A field performance of endophytic actinomycetes in relation to plant growth promotion and biological control of 1186 ApplMicrobiolBiotechnol (2019) 103:1179–1188 Fusariumoxysporum a pathogen of tomato. In, 2016 2016. vol 106. Amerphytopathologicalsoc 3340 pilot knob road, St Paul, MN 55121 USA, p 55–55.

EsmahandeAlici, Gulnur Arabaci. 2016. Determination of SOD, POD, PPO and CAT Enzyme Activities in Rumexobtusifolius L. Annual Research & Review in Biology 11(3): 1-7.

FAOSTAT. 2011. Food and Agriculture Organisation of the United Nations. Consultable à http://faostat.fao.org(vérifié le 25-05-2019).

Farag MA, Zhang H, Ryu C. 2013. Dynamic Chemical Communication between Plants and Bacteria through Airborne Signals: Induced Resistance by Bacterial Volatiles. Journal of Chemical Ecology 39(7), 1007–1018.

Glick BR. 2010. Using soil bacteria to facilitate phytoremediation. Biotechnology Advances 28(3), 367–374.

Hanna Habdas, Wojciech Szczechura and Mirosława Staniaszek. 2013. fusarium oxysporum f. sp. radicis-lycopersici – the cause of fusarium crown and root rot in tomato cultivation. journal of plant protection research. Vol. 53, No. 2.

Kampfer P, Kroppenstedt RM, Dott W. 1991. A numerical classification of the genera Streptomyces and Streptoverticillium using miniaturized physiological tests. Journal of General Microbiology 137:1831-91.

Kimura, S. and Sinha, N. 2008. Tomato (Solanumlycopersicum): A model fruit-bearing crop. Cold Spring Harb. Protoc. (This issue). doi: 10.1101/pdb.emo105.

Kloepper JW, Leong J, Teintze M, Schroth MN, 1980. Enhanced plant growth by siderophores produced by plant growthpromotingrhizobacteria. Nature, 286: 885–886.

Korn-Wendisch F, Kutzner H, Balows A, Truper H, Dworkin M, et al. 1992. The family Streptomycetaceae. The Prokaryotes.:921-95.

Lichthentaler h k. ; Buschmann. ; Rinderle u. et Schmuck c., 1986 – Application of chlorophyll fluorescence in ecophysiology. Radiation and environmental. Biophysics., 25 : 297 – 308.

Locci R. 1989. Streptomycetes and related genera. In Bergey's manual of systematic bacteriology, ed. ST Williams, ME Sharpe, JG Holt, pp. 2451-508. Baltimore (USA): Williams and Wilkins.

Luisa Posada. 2016. solanum lycopersicum. P5-7.

MADR. 2011. Ministère de l'Agriculture et du Développement Rural, Direction des statistiques. Alger.

Munees Ahemad and Mulugeta Kibret. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University – Science 26, 1–20.

Nicolas RANC. 2010. Analyse du polymorphisme moléculaire de gènes de composantes de la qualité des fruits dans les ressources génétiques sauvages et cultivées de tomate ; recherche d'associations gènes/QTL.

Pal KK, McSpadden Gardener B. 2006. Biological control of plant pathogens. The plant health instructor:1-25

Porter, L. D., Pasche, J. S., Chen, W., and Harveson, R. M. 2015. Isolation, identification, storage, pathogenicity tests, hosts, and geographicrange of Fusarium solani f. sp. pisi causing Fusarium root rot of pea. Plant Health Progress doi:10.1094/PHP-DG-15-0013.

PriyaShukla ,KatyaniBajpai, Shalini Tripathi, Sunil kumar, GirendraKumar Gautam. 2013. A Review on the Taxonomy, Ethnobotany, Chemistry and Pharmacology of SolanumLycopersicum Linn. International Journal of Chemistry and Pharmaceutical Sciences: Vol.1(8), 521-527.

Pridham TG, Tresner HD. 1974. Genus I. Streptomyces Waksman and Henrici 1943. In Bergey's Manual of determinative bacteriology, 8thedn, ed. RE Buchanan, NE Gibbons, pp. 748-829. Baltimore (USA): Williams and Wilkins.

Priya E, Thenmozhi R, Nagasathya A, Kumar DP, Thajuddin N, Muralitharan G (2017) Antagonistic potential of Streptomyces flavomacrosporus GACMPT-57 against plant pathogens. J MicrobiolBiotechnolRes 4(1):68–73.

Rajiv Pathak, AnupamaShrestha, JanardanLamichhane, Dhurva P. Gauchan. 2017. PGPR in biocontrol: mechanisms and roles in disease suppression. International Journal of Agronomy and Agricultural Research, Vol. 11, No. 1, p. 69-80.

R. Hafizi, B. Salleh, Z. Latiffah. 2013. Morphological and molecular characterization of Fusarium. solani and F. oxysporumassociated with crown disease of oil palm. Brazilian Journal of Microbiology 44, 3, 959-968

Rishi Kundan ,Garima Pant , Nitesh Jadon and Pavan Kumar Agrawal.2015. Plant Growth Promoting Rhizobacteria: Mechanism and Current Prospective. Journal of Fertilizers & Pesticides undan et al., J FertilPestic 2015, 6:2 DOI: 10.4172/2471-2728.

Shirling EB, Gottlieb D. 1968. Cooperative description of type cultures of Streptomyces III. Additional species descriptions from first and second studies. International journal of systematic bacteriology 18:279-392.

Shrivastava P, Kumar R. 2018. Actinobacteria: eco-friendly candidates for control of plant diseases in a sustainable manner new and future developments in microbial biotechnology and bioengineering. Elsevier, pp 79–91.

Sousa JAJ, Olivares FL. 2016. Plant growth promotion by Streptomycetes: ecophysiology, mechanisms and applications. ChemBiolTechnolAgric 3(1):24.

Susana Castro-Sowinski, Yoav Herschkovitz ,Yaacov Okon & Edouard Jurkevitch. 2007. Effects of inoculation with plant growth-promoting rhizobacteria on residentrhizosphere microorganisms, DOI:10.1111.

Sushanto Goudaa , Rout George Kerryb , Gitishree Dasc , Spiros Paramithiotisd , Han-Seung Shine , Jayanta Kumar Patrac, 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiological Research 206, 131–140.

Troll w. et Lindsley g., 1955 – Aphotometric method for the determination of proline. J. Biol. Chem., 215 : 655 – 660.

Van Peer R, Schippers B. 1988. Plant growth responses to bacterization with selected Pseudomonas Spp. strains and rhizosphere microbial development in hydroponic cultures. Canadian Journal of Microbiology.;35:456-463.

Weller DM, Cook RJ. 1986. Increased growth of wheat by seed treatments with fluorescens pseudomonads, and implications of phytium control. Canadian Journal of Plant Pathology.;8:328-334.

Zidani S. 2009. Valorisation des pelures de la tomate séchée en vue de leur incorporation dans la margarine. Mémoire de Magister. Université M'hamed Bougara Boumerdes, Faculté des sciences de l'ingénieur.114p.

Annexes

Randomized complete blocks:

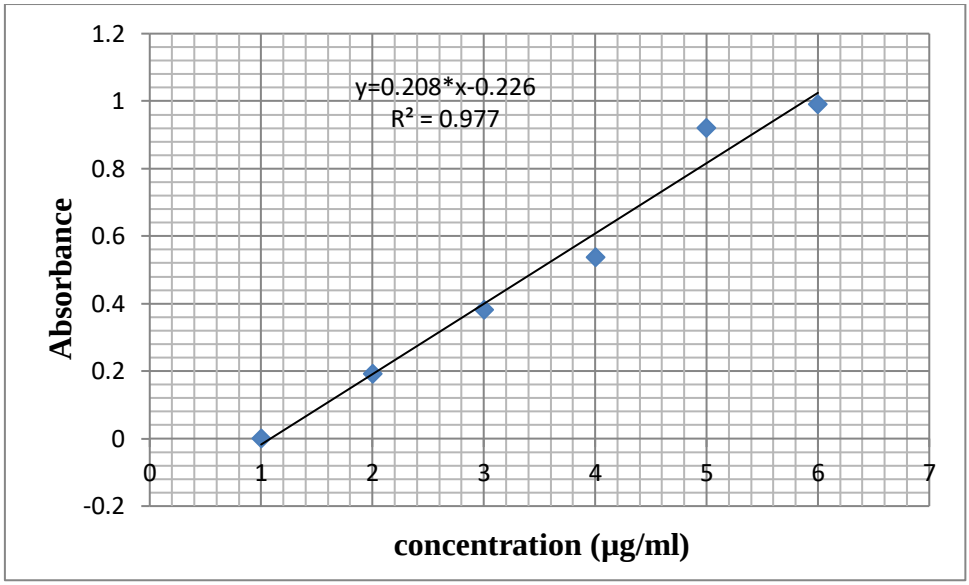
Tab 06: Randomized complete block of PGPR effect

T 5	TR 1	D15 1	D54 1	TM52 4	T 4
Ia1 3	T 3	TM52 1	D54 3	TM52 2	D15 4
TR 4	T 1	D545	D54 2	IA1 4	D15 3
TR 2	TM52 3	D15 5	TR 5	T 2	D15 2
TR 3	IA1 5	D54 4	TM52 5	IA1 1	IA1 2

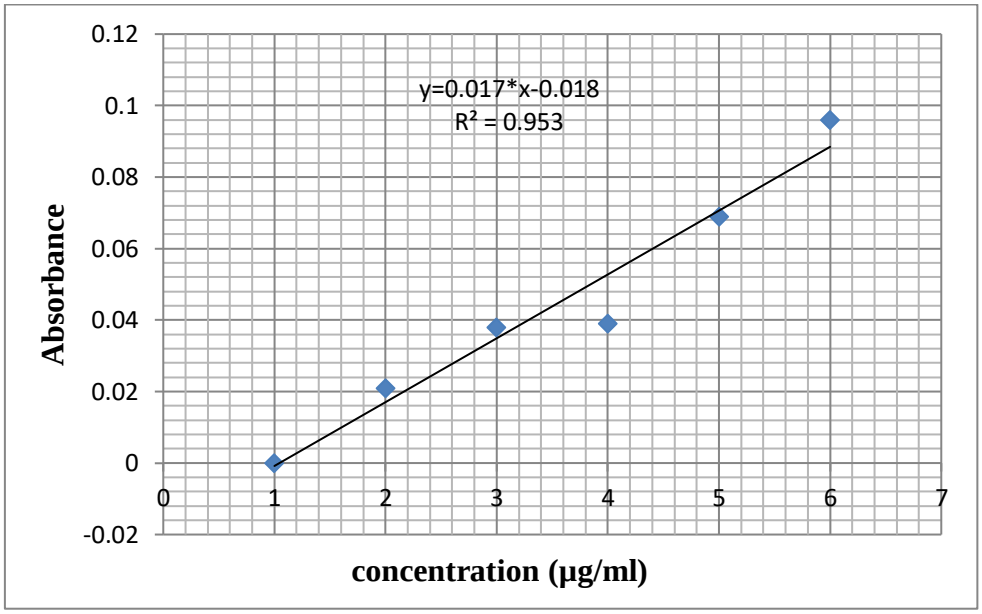
Tab 07: Randomized complete block of Biocontrol effect

TM52 3	TM52 1	IA1 4	TR 1	TR 5	D54 3
IA1 2	D54 5	D15 4	TR 2	IA1 1	TR 3
TM52 4	IA1 5	D54 4	T 3	T 2	TM52 5
D15 1	D15 5	TR 4	T 5	IA1 3	TM52 2
D15 3	D54 2	T 4	D15 2	D54 1	T 1

Calibration curves:



Curve 01: calibration curve of total soluble sugars



Curve 02: calibration curve of proline

Statistical results for the PGPR effect:

Tab 08: Analysis of Variance (PGPR effect)

Variables	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
GP	4156,45	5	831,290	3333,400	24	138,8917	5,98517	0,000992
FW	0,02	5	0,004	0,165	24	0,0069	0,62795	0,680105
DW	0,00	5	0,000	0,001	24	0,0000	0,86368	0,519587
WC	0,02	5	0,004	0,146	24	0,0061	0,64704	0,666371
Chl a	0,04	5	0,007	0,228	24	0,0095	0,73831	0,602183
Chl b	0,28	5	0,055	0,950	24	0,0396	1,39539	0,261376
Chla+b	0,48	5	0,096	1,023	24	0,0426	2,25046	0,081944
Chlx+c	0,01	5	0,002	0,031	24	0,0013	1,39414	0,261817
CMP	12444,46	5	2488,891	3040,105	24	126,6710	19,64846	0,000000
PRO	18,90	5	3,781	20,480	24	0,8533	4,43088	0,005325
TSS	24,78	5	4,955	4,829	24	0,2012	24,62627	0,000000
POD	0,00	5	0,000	0,005	24	0,0002	1,25719	0,314348
CAT	0,00	5	0,001	0,048	24	0,0020	0,43477	0,819811

Tab09 :LSD test; variable GP (PGPR effect) Homogenous Groups:alpha = 0,05000, Error: Between MS = 138,89 /df = 24,000

Treatment	GP - Mean	A	B	C	D
TR	63,33600			****	
T	73,33400			****	****
TM52	80,00000	****			****
D15	89,99800	****	****		
IA1	93,33200	****	****		
D54	96,66600		****		

Tab 10: LSD test; variable CMP (PGPR effect) Homogenous Groups: alpha = 0,05000 Error: Between MS = 126,67 /df = 24,000

Treatment	CMP - Mean	A	B
D15	17,67438	****	
IA1	20,46638	****	
D54	21,80426	****	
TM52	28,09640	****	
TR	28,32992	****	
T	76,92508		****

Tab 11:LSD test; variable PRO (PGPR effect) Homogenous Groups, alpha = 0,05000 Error: Between MS = 0,85333 /df = 24,000

Treatment	PRO - Mean	A	B	C
D15	1,983240	****		
IA1	2,541880	****		
D54	2,553080	****		
TM52	2,921800	****	****	
T	4,005560		****	****
TR	4,150820			****

Tab 12: LSD test; variable TSS (PGPR effect) Homogenous Groups:alpha = 0,05000 Error: Between MS =0,20122/df = 24,000

Treatment	TSS - Mean	A	B	C
D15	2,175460			****
D54	3,493280		****	
TM52	3,795580		****	
TR	4,411740	****		
T	4,484660	****		
IA1	4,996180	****		

Statistical results for the Biocontroleffect:

Tab 13: Analysis of Variance (Biocontrol effect)

Variables	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
GP	1851,770	5	370,354	11887,98	24	495,3324	0,74769	0,595758
FW	0,228	5	0,046	0,52	24	0,0219	2,08120	0,103003
DW	0,004	5	0,001	0,00	24	0,0002	4,15807	0,007314
WC	0,174	5	0,035	0,50	24	0,0209	1,66465	0,181524
Chl a	0,024	5	0,005	0,17	24	0,0070	0,68699	0,637941
Chl b	0,281	5	0,056	1,14	24	0,0474	1,18399	0,346206
Chla+b	0,244	5	0,049	1,46	24	0,0609	0,80307	0,558592
Chlx+c	0,016	5	0,003	0,10	24	0,0043	0,73762	0,602654
CMP	6578,966	5	1315,793	4006,65	24	166,9437	7,88166	0,000165
PRO	21,176	5	4,235	21,43	24	0,8929	4,74336	0,003733
TSS	15,505	5	3,101	4,95	24	0,2062	15,03978	0,000001
POD	0,002	5	0,000	0,00	24	0,0001	3,39677	0,018422
CAT	0,001	5	0,000	0,02	24	0,0007	0,24859	0,936415

Tab 14:LSD test; variable DW (Biocontrol effect) Homogenous Groups:alpha = 0,05000
Error: Between MS = 0,00018 / df = 24,000

Treatment	DW - Mean	A	B
IA1	0,026040	****	
T	0,027100	****	
D15	0,028280	****	
D54	0,029700	****	
TM52	0,036120	****	
TR	0,057900		****

Tab 15: LSD test; variable PRO (Biocontroleffect) Homogenous Groups: alpha = 0,05000 Error: Between MS =0,89289 / df = 24,000

Treatment	PRO - Mean	A	B
D54	3,782120	****	
IA1	3,873860	****	
D15	4,106140	****	
TM52	4,117320	****	
TR	5,469260		****
T	5,949720		****

Tab 16 :LSD test; variable TSS (Biocontrol effect) Homogenous Groups: alpha =0,05000 Error: Between MS =0,20619/df = 24,000

Treatment	TSS - Mean	A	B	C	D	E
D54	3,182320	****				
TM52	3,650680	****	****			
D15	3,827260		****	****		
TR	4,394440			****	****	
IA1	4,618040				****	
T	5,389640					****

Tab 17 :LSD test; variable CMP (Biocontroleffect) Homogenous Groups:alpha =0,05000 Error: Between MS = 166.94/df = 24,000

Treatment	CMP - Mean	A	B	C
IA1	29,43910		****	
D54	37,00256	****	****	
TM52	42,39666	****	****	
D15	46,89494	****		
TR	50,13516	****		
T	76,60516			****

Tab 18 :LSD test; variable POD (Biocontrol effect) Homogenous Groups: alpha =0, 05000 Error: Between MS =0, 00011 /df = 24,000

Treatment	POD - Mean	A	B	C
TM52	0,016000	****		
D54	0,016600	****	****	
TR	0,017000	****	****	
IA1	0,030400		****	****
D15	0,032400			****
T	0,034200			****

Abstract

The use of BCAs and PGPRs is considered to be an interesting solution for the production and protection of crops. The present work consist on studying the PGPR and Biocontrol effect of four streptomyces strains (TM52, IA1, D54, D15) on tomato seedlings in comparison with two controls. Tomato seeds (*Solanum lycopersicum*. L. cv Marmande) treated with the bacterial strains and controls were planted for 40 days, the following parameters were measured : Germination percentage, biomass, photosynthetic pigments, cell membrane permeability, accumulation of total soluble sugars and proline, catalase and peroxidase activities.

The statistical results obtained showed the efficiency of these strains"D15, D54, IA1 and TM52" in terms of promoting tomato plants growth in normal conditions and in terms of promoting tomato plants resistance under biotic stress conditions. These results open up the prospects for a field study in order to validate the usefulness of these strains in the improvement of tomato growth and it's resistance under biotic stress conditions.

Key words: Tomato, PGPR, BCA, biotic stress, controls,

Résumé

L'utilisation des agents de biocontrôle et les agents PGPR est considérée comme une solution intéressante pour la production et la protection des cultures. Le présent travail consiste à étudier l'effet PGPR et Biocontrol de quatre souches de streptomyces (TM52, IA1, D54, D15) sur des plants de tomate en comparaison avec deux témoins. Des semences de tomates (*Solanum lycopersicum.* L. cv Marmande) traitées avec les souches bactériennes et les témoins ont été plantées pendant 40 jours, les paramètres suivants ont été mesurés: pourcentage de germination, la biomasse, les pigments photosynthétiques, la perméabilité membranaire, l'accumulation des sucres totaux solubles et de la proline, les activités catalase et peroxydase.

Les résultats statistiques obtenus ont montré l'efficacité de ces souches "D15, D54, IA1 et TM52 " en termes de promotion de la croissance des plants de tomates dans des conditions normales et en termes de promotion de la résistance des plants de tomates dans des conditions de stress biotique. Ces résultats ouvrent les perspectives d'une étude de terrain visant à valider l'utilité de ces souches dans l'amélioration de la croissance de la tomate et sa résistance aux conditions de stress biotique.

Mots clés : Tomate, PGPR, ABC, stress biotique, témoins.

ملخص

يعتبر استخدام البكتيريا المساعدة في تحسين نمو النبات وعوامل المكافحة الحيوية حلاً مثيرًا للاهتمام لإنتاج المحاصيل وحمايتها . يتكون العمل الحالي من دراسة تأثير تحسين نمو النبات و تأثير المكافحة الحيوية لأربعة سلالات بكتيريا streptomyces المسمات كالتالي: (TM52, IA1, D54, D15) على شتلات الطماطم مقارنة بالشواهد. حيث تمت زراعة بذور الطماطم (*Solanum lycopersicum.* L. cvMarmande) التي تمت معالجتها بالسلالات البكتيرية بالإضافة الى الشواهد لمدة 40 يومًا. ثم تم قياس القيم التالية: النسبة المئوية للإنبات ، الكتلة الحيوية ، أصباغ التمثيل الضوئي ، نفاذية الغشاء الخلوي ، تراكم السكريات الكلية القابلة للذوبان والبرولين ، نشاط الكاتلاز و البيروكسيديز.

أظهرت النتائج الإحصائية التي تم الحصول عليه فعالية السلالات "D15 وD54 وIA1 وTM52 "من حيث تعزيز نمو نباتات الطماطم في الظروف العادية و من حيث تعزيز مقاومة نباتات الطماطم في ظل ظروف الإجهاد الحيوي. هذه النتائج تفتح الآفاق لدراسة ميدانية تهدف إلى التحقق من صحة فائدة هذه السلالات في تحسين نمو الطماطم و مقاومته الظروف الإجهاد الحيوي.

الكلمات المفتاحية: الطماطم، البكتيريا المساعدة في تحسين نمو النبات، عوامل المكافحة الحيوية، الاجهاد الحيوي، الشواهد.