Int. j. adv. multidisc. res. stud. 2022; 2(3):144-150

Received: 02-04-2022
Accepted: 12-05-2022

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Biological control mechanisms of *Trichoderma* species to induce resistance in Leguminous plants

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Abstract

when plant is functionally not normal, it is diseased, A plant's aberrant functioning usually results in a decline in yield quality and quantity, the result of interaction of a plant environment is disease, and it is frequently influenced by abiotic and biotic factors (temperature, humidity, microorganisms, etc.) this is detected as signals for the mechanisms activation of response of plant. Leguminous plants, for example, are affected by a varied variety of fungal pathogen such as Fusarium spp., *Sclerotinia* spp., *Pythium* spp., *Rhizoctonia* spp., *Botrytis* spp.) resulting in significant economic losses for certain crops.

The use of chemical synthesis fungicides is one method of disease control. Its application to the soil directly or on the seed can be beneficial against fungi that harm crops during or soon after germination by reducing their incidence and improving plant emergence. Fungicide applications intended at preventing yellowing, wilting and root rot are harm caused by fungi, on the other hand, are frequently inefficient and impractical due to the huge amount of soil to which they need be directed, the use of biocontrol agents as a technique to control infectious diseases of legume, primarily fungi caused those, can lessen the negative impacts of plant pathogens while also promoting beneficial responses in the plant. Biocontrol agents are thought to have distinct advantages over synthetic fungicides, such as fewer non-target and environmental effects, efficacy against fungicide-resistant infections, lower development of resistance risk, and use in organic farming circumstances where synthetic fungicides are prohibited, theses done by many mechanisms called biological control mechanisms which consist of two ways Direct biological control and Indirect biological control.

Keywords: Biological Control, Sustainability, Species, Leguminous Plants

1. Introduction

Biological control in its direct sense means organizing the pest community by using its biological enemies, and the phenomenon of natural hostility between living things that Roberts described in 1874 for the first time in antagonism did not benefit man from it in combating plant diseases until 1921 when Hartley contamination the soil of forest nurseries with thirteen species of fungi as an attempt To combat damping of pine seedling (Chen and Duan, 2015) [13], Weinding was the first to use the fungi *Trichoderma* as a biological resistance agent in 1932, as he was able to prove the ability of *T. lignorum* to control the activity of many types of soil pathogenic fungi, then moved from the field of laboratory experiments to application in the field. In 1934, the incidence of damping-off disease in lemons caused by *Rhizoctonia solani* was reduced by adding *T. ligonorum* to sterilized soil. It was also found that the growth of the bio-resistant fungi *Trichoderma* on sterilized oats and its addition to contaminated soil caused a decrease in the disease of seedlings and seed rot. Cucumbers and peas (Agrios, 2005) [2].

1.1 Induce resistance in Leguminous Plants by *Trichoderma* species

Pulses are members of the Fabaceae (Leguminous), family, which is the world's third biggest plant family. They are considered to have invented before 90 million years, with a period of diversification starting in the early Tertiary epoch. Only a few of the Fabaceae family's 20,000 species and 700 genera are classified leguminous plants, for example genus *Vicia, Phaseolus, Lens, Cicer* and others (FAO, 2016) [15].

when plants are functionally not normal, there were diseased, A plant's aberrant functioning usually results in a decline in quality and quantity yield, the result of interaction of a plant environment is Disease, and it is frequently influenced by abiotic and biotic factors (temperature, humidity and microorganisms etc.) this is detected as signals for the mechanisms activation of response of plant.

Leguminous plants, for example, are affected by a varied variety of fungal pathogen such as Fusarium spp., *Sclerotinia* spp., *Pythium* spp., *Rhizoctonia* spp., *Botrytis* spp.) resulting in significant economic losses for certain crops (Anon., 2005 and Malmierca *et al*., 2014) [4, 29].

The use of chemical synthesis fungicides is one method of disease control. Its application to the soil directly or on the seed can be beneficial against fungi that harm crops during or soon after germination by reducing their incidence and improving plant emergence. Fungicide applications intended at preventing (Beebe *et al*., 1991 and Valenciano *et al*., 2004 [43]). applications of Fungicide intended at preventing fungal damage such as yellowing, wilting and root rot, on the other hand, are frequently inefficient and impractical due to the huge amount of soil to which they need be directed (Shoresh *et al*., 2010) [41].

In order to assure food safety and long-term sustainability, the allowed goods number by plant protection has been decreased. As a result, integrated production by organic farming, non-chemical methods and other fields should be prioritized (FAO, 2016) [15].

The use of biocontrol agents to control diseases of legume fungal infections can lessen the negative impacts of plant pathogens while also promoting beneficial in the plant responses (Shoresh *et al*., 2010) [41]. Biocontrol agents are thought to have distinct advantages over synthetic fungicides, for example environmental effects and non-target, efficacy against resistant of fungicide infections, synthetic fungicides are lower development of resistance risk, and use in organic farming circumstances prohibited (FAO, 2016) [15]. Strains from bacterial genera like *Pseudomonas*, *Agrobacterium, Bacillus, Streptomyces* as well as fungal genera like *Trichoderma, Gliocladium, Candida, Ampelomyces, Coniothyrium*, have demonstrated to be effective biocontrol agents against pathogenic microorganisms, *Trichoderma* is known for its high efficiency against pathogenic fungi, thus it may be used in a variety of ways in resistance. It can be added as a suspension or as a powder laden with seeds. (Vinale *et al.*, 2008) [47].

Trichoderma spp. (Teleomorph: Hypocrea) is a fast growing opportunistic invasive and produce of glucanases, chitinases, and proteases, as well as compounds having antibacterial activity as secondary produce, found in soil. Many species of *Trichoderma* are also well-known as biocontrol agents of pathogenic fungal (Lorito *et al*., 2010) [28], antibiosis, competition for nutrients with pathogenic fungi and Mycoparasitism are the principal biocontrol methods in direct confrontation used by *Trichoderma* with fungal pathogens (Afzal *et al.*, 2008 and Shoresh *et. al*., 2010) [1, 41]. *Trichoderma* species have evolved opportunistic systems for nutrient absorption and solute transport, as well as adaptability to abiotic stressors, *Trichoderma* has the ability to help legumes develop. *Trichoderma* also creates organic acids like citric or fumaric acid, which lowers pH in soil and allows phosphates and other micronutrients like manganese, magnesium and iron to be soluble in crops of legume. *Trichoderma* spp. colonize the root surface of legumes and cause significant changes in plant metabolism, such as the accumulation of phytoalexins and the down- or up-regulation of defense-related gene expression. species of *Trichoderma* cause substantial changes in plant metabolism after colonize the surface of the root (Shoresh *et al*., 2010) [41].

Trichoderma interaction physically is limited to the epidermis' initial cell layer and the bark of root with plants. biocontrol of strains of Trichoderma can also as defense response induce the genes involved expression, as well as plant growth promotion, nutrient uptake and root development. *Trichoderma* spp. are known for their important agricultural benefits, for example their ability to increase crop yield in field conditions and protect crops from disease. species of *Trichoderma* have been related to biocontrol and biotechnological applications in almost every case, strains' versatility of *Trichoderma* in suppressing pathogen which caused diseases, *Trichoderma* strains have evolved many methods of actionو both proliferate and fight other fungi and to improve root and plant growth since they grow best when there are abundant healthy roots (Monte, 2001, Howell, 2003 and Benítez, *et al*., 2004) [33, 24, 6].

2. Biological control mechanisms of *Trichoderma* spp.

2.1 Direct biological control: It includes:

2.1.1 direct parasitism

Direct parasitism by *Trichoderma* spp. involves direct attack against other fungi. This process is complex and includes a series of events, namely, identification of the host fungi, attack, penetration, and killing of the host fungi. The enzymes that degrade the walls of the mycelium of pathogens, namely Protease, Lipase and B, are involved in this mechanism. Glucosidase, Phosphamidase, and Esterase, and *Trichoderma* spp. can be recognized by the fungi. On the presence of its host fungi in its biosphere by sensing the products of cell wall degrading as a result of the action of a group of cell wall degrading enzymes CWDEs, including Glucanases and Chitinases and proteases, as a small amount of the exochitnases enzyme is secreted to its biosphere, and when this enzyme works to break down the walls of other fungi, it produces several substances, including oligomers, which work to continuously stimulate the production of exochitinases enzymes, and then the fungi *Trichoderma* spp. by direct intrusion into his family (Harman, 2000) [21].

2.1.2 Antagonism

The mechanism of antibiosis depends on the chemical compounds produced and released in the external environment by the fungi *Trichoderma* spp. Which affect the pathogens negatively, and the antibiotics produced by the fungi *Trichoderma* are Harzianic acid and Alamethicins. and Richolin and Peptaibols and many others (Brion, *et al*., 2003) [9].

2.1.3 Increasing plant tolerance to different environmental stress conditions

The different mechanisms that stimulate plant growth by the fungi *T. harzianum* lead to the assumption, that the fungi stimulate the formation of a dense root system deep in the plant and then achieves physiological benefits for the plant, especially in the dry season. The roots endemic to some of its isolates can withstand drought and help heal wounds by oxidation Lipids and cellular proteins (Harman, 2000) [21].

2.1.4 Competition for food sources

Because microorganisms' numerical density is determined by nutrients, competition for limited resources is one of the most significant biological controls against plant infections, and the relationship of pathogenic fungi to plant roots depends on the numerical density in causing disease and reducing it is among the priorities in determining that relationship. *Trichoderma* spp. High competitive ability for nutrients due to its high growth, development and reproduction speed, as well as having a high pollen energy that enables it to control the interstitial space around the roots of the plant and its positive reflection on the growth of those plants (Vey, *et al*., 2001) [45].

Competition between pathogens and *Trichoderma* would be formed in order to gain oxygen, more nutrients, light, and other resources (Paulitz,1990) [36]. *Trichoderma* is a fierce competitor for both nutrients and space. It can be found in practically all soils and habitats with large levels of organic matter. It would be a good decomposer of fungal material and plant in those areas. Furthermore, some *Trichoderma* species have a high level of metabolomic plasticity, allowing them to develop on a variety of carbon sources and nitrogen, *Trichoderma* also has the ability to rhizosphere colonization, which could be crucial for its usage as an effective agent of biological control (Howell, 2003) [24].

Trichoderma Genes related ability to compete in rhizosphere colonization and soil were also described (Chacón *et al*., 2007) [12], including the first functionally analyzed transport of oligopeptide in mold fungi and two heat shock proteins overexpressed in *Trichoderma* strain biocontrol and in plants, improving their resistance to abiotic stresses (Vizcaíno *et al*., 2006 and Montero *et al*., 2007) [48, 34].

2.1.5 Inhibition of enzymes pathogen

The biocontrol fungi inhibit the action of the pathogen's enzymes responsible for its ability pathogenicity of plants represented by enzymes degrading the cell walls of the plant host and causing infection (Vey e*t al.*, 2001) [45]. The two isolates NaM 1185 and T-39 of *T. harzianum* produced the enzyme Srineprotase that inhibits the enzymes degrading pectin, cutinolytic and cellulose produced from the pathogenic fungi *Bipolaris cinerea*, which plays a major role in the analysis of host cell walls (Brion *et a*l., 2003) [9].

2.1.6 Production of hydrolytic enzymes

The fungi *Trichoderma* spp. A group of production of Lytic Enzymes that break down the cell walls of other fungi are known as Cell-Wall-Degrading CWDEs (Roy *et al*., 2006) [40].

2.1.7 Increase the availability of nutrients

Cause microorganisms, including mushrooms spp. *Trichoderma* increasing the availability of mineral nutrients as well as the production of metabolic compounds in the soil that promote plant growth Promoting Substances and this falls within the concept of biofertilizer (Roy *et al.*, 2006) [40].

2.2 Indirect biological control

It includes:

2.2.1 Promote plant growth

Bjorkman (2004) [7] showed that this mechanism was proposed to explain the phenomenon of stimulating the growth of plants inoculated with *T. harzianum* and that the rates of increase in the growth rates of plants inoculated with *T. harzianum* could not be completely attributed to the hypotheses of the activity of auxin-like compounds that may be produced by this isolate from the fungi and suggested that This mechanism works in conjunction with other mechanisms to stimulate plant growth, including the production of plant growth regulators by the fungi *T. harzianum* to explain the stimulation of the growth of corn plant roots, as the fungi increases the secretion of these substances at the ends of the roots. the positive effect of some isolates of *T.harzianum* in stimulating plant growth, which instructed to produce plant growth regulators that work in harmony with other mechanisms, including increasing the readiness and absorption of plant nutrients, as well as some isolates of the fungi *T. harzianum* increase seed germination and significantly reduce the time required for germination (Hoyos-Carvajal *et al*., 2009) [25].

species *of Trichoderma* have evolved abiotic stress adaption opportunistic mechanisms as well as solute transport as rhizosphere invaders and nutrition intake. The induction of cell wall extension and expansion, secondary root development, lateral hair of root creation, and a faster photosynthetic rate all aid these activities in the plant (Hermosa *et al*., 2013) [22].

The effect of concentration of *Trichoderma inoculum* on emergence of plant was tested in a peanut culture, *Arachis hypogaea*, without and with a fungicide that might be employed in conjunction with the antagonist species to increase seed emergence and protection. Given that fungicides are only effective for a short period of time and do not spread through the soil via the root system, combining chemical and biological agents could improve seed and root protection (Rojo *et al*., 2007) [39].

In another study, When the peanuts were treated with four species of *Trichoderma* (*T.viride*, *T.atroviride*, *T.harzianum*, *T.virens*) shoot length was decreased between 10.48% in *T. atroviride* and 51.78% in *T. harzianum*. *T. harzianum* increased the length of the shoot by 51.78 % and *T.atroviride* increased the shoot weight by 14.04 % (*T.atroviride*) and 80.38 % (*T. harzianum*) compared to the control plant; the roots weight was increased by 64.42 %, and nodules number per plant was increased by 30.97% when they inoculated with *Trichoderma* spp., *T. harzianum* can be an effective strategy for enhancing plant growth and development by improving the microbial community in the rhizosphere, *Trichoderma* boosted early development in peanut plants in this study, increasing yield (Kamaruzzaman *et al*., 2016) [26].

When a bean plant (*P. vulgaris*), was in contact with *Trichoderma*, it grew larger than when the pathogen was not present. *T. harzianum* was also found to boost the bean plants growth when compared to plants cultivated without it, according to other researchers, In the presence of R. *solani*, however, treatment *T. harzianum* with plants did not shrink in size (Pereira *et al*., 2014 and Mayo *et al*., 2015) [37, 31].

Bean plants exposed to *T. velutinum* isolation increased their hypocotyl diameter by 4.75 %, their root system length by 10.75 %, and their dry weight of root system and aerial parts by 4.27 % and 5.51 %, respectively, as compared to control plants. When *R. solani* infected plants, *T. velutinum* T028 caused an increase of 8.76 % in hypocotyl diameter, 11.05 % in dry weight of aerial parts and 3.43 % in dry weight root system, 21.15 % in root system length, when compared to the pathogen-free of plant control (Mayo, *et al*., 2016 A) [30].

These findings are consistent with those of Yedidia, *et al*. (2001) [49] found that *T. harzianum* had a much stronger effect on cucumber plants, increasing root length by 75.0 %, aerial parts by 95.0 %, dry weight by 80.0 %, and blade size by 80.0 % when compared to untreated controls.

In comparison to plants grown without *T. harzianum*, *T.*

harzianum was able to promotion of bean plants growth (Pereira *et al*., 2014) [37]. The effect of farnesol on bean plants development xwas investigated in another investigation. This chemical, which is apparently generated by fungi from farnesyl pyrophosphate, is a self-regulatory signaling molecule that elicits a reaction across the local fungal population by accumulating in the extracellular space. The plants were cultivated 14 days in hydroponic culture under controlled conditions with varied farnesol concentrations (10,100,1000, 2000 and 5000 μM) in the nutrition solution, and their wet and dry weights of root systems and aerial parts were measured. At doses of 10 μM and 100 μM farnesol, the results demonstrated a negative influence on growth, which could be related to synthesis of abscisic acid. Bean plants, on the other hand, showed increased root systems and aerial parts development when given 2 mM of farnesol (Mayo *et al*., 2016 B) [32].

In chickpea plants inoculated with *F. oxysporum* f. sp. *Ciceris*, *Trichoderma* spp. significantly reduced the incidence of wilt. In comparison to control plants, the incidence was reduced by 65.85%. In addition, the *Trichoderma* presence improved the culture germination by 13.36% to 8.95% compared to control plants, increased the shoot weight 31.31%, and increased the root weight 41.30% compared to control (Dubey *et al*.,2007) [14].

Trichoderma creates organic acids such as fumaric and citric acids, which lower pH of soil and allow phosphates solubilization and other micronutrients including iron, manganese, and magnesium (Bentez, *et al*., 2004) [6]. The quantity of manganese, iron, copper and zinc in beans leaf biomass is reduced by *T. harzianum*, whereas the amount of copper in the seeds is increased (Öğüt and Er, 2006) [35]. Investigations have shown that *Trichoderma species* especially *T. harzianum* may solubilize manganese (IV) oxide, iron (III) oxide, phosphates and zinc, all of which are solubility highly or limitly, due to chelation processes and activity of oxidation-reduction (Altomare *et al*., 1999) [3]. The increase in nutrients, particularly phosphorus, may encourage the plants growth that come into touch with soils containing this fungi species, *T. atroviride* has been demonstrated to create and breakdown indole acetic acid, which, when combined with ethylene produced by rhizosphere bacteria, induces an increase in plant growth (Gravel *et al*., 2007) [18].

Cowpea (*Vigna unguiculata)* was inoculated with *Trichoderma* isolates, in compared to the control treatment, there was dry matter increased and phosphorus levels in aerial portions, The biological control agents' ability to solubilize phosphate could explain this result. The nitrogen and carbon availability, as well as type of grown plants, may all influence *Trichoderma* phosphate solubilization ability (Grayston *et al*.,1997) [19], as well as the type of phosphate solubilized (Barroso and Nahas, 2005) [5]. Kapri and Tewari (2010) [27] *in vitro* found similar results after inoculating *Trichoderma* into cultures of chickpea and reporting an increase in solubilization ability of phosphate, as well as a significant effect on production of biomass, these studies demonstrated *Trichoderma* sp. potential as promoters of plant growth due to their indole acetic acid production capacities and phosphate solubilizing.

2.2.2 Stimulating resistance in plants

Resistance is induced in plants grown in media containing Trichoderma spp. as follows:

2.3 Induced resistance

Induced resistance has been known in plants for more than fifty years, and it must be induced by an inducing factor, a Latin word meaning elector. The induced factor can be defined as a factor or substance if the living cell system is exposed to it or to them, even if it is in a low concentration. This system begins to build or by increasing the concentration of certain substances. The stimuli are usually biotic or abiotic (chemical) agents that result in plant resistance to a wide range of pathogens and extreme growth conditions. The chemical stimuli are organic, such as tannins and salicylic acid, some are mineral, such as phosphate and cobalt salts, and some are natural, such as plant hormones, and some are synthetic. The following types of induced resistance can be identified:

2.3.1 Induced local resistance

Localized resistance Induced occurs in an area close to the induction zone and is represented by toxic changes including accumulation of phytoalexin, oxidation of phenols, and hypersensitivity (Riches and Holmes, 2005) [38].

2.3.2 Induced systemic resistance

It includes the induction of plant defense responses at the level of tissue systems and organs

Stimulating the structural chemical defense mechanisms in plant parts far from the site of infection (Vidhyasekaran, 2004) [46], including:

1. Defense proteins

These Defense-Related Proteins, especially those related to pathogenesis-related proteins (PR Proteins), whose synthesis is induced by the elicitor factor as a result of infection with proteins originally in the plant, are among the main defenses in the plant and are classified into fourteen groups (Vidhyasekaran, 2004) [46].

Trichoderma produces many biologically active secondary metabolites, this comprises certain natural chemical substances associated with its survival, ability to compete with organisms, symbiosis, transport, and differentiation, Antibiotics, which have the potential to limit microbial development, are also included in this category (Ghisalberti and Sivasithamparam, 1991) [17].

Mayo *et al.* (2015) [31] used an antifungal assay on membranes to determine the capacity of isolates of *Trichoderma* to create compounds with activity inhibitory against *R. solani*, The percentages of range of inhibition from 86 to 58 %. In other experiments, the proportion of inhibition in assays membrane utilizing *T.atroviride* T11 and *T.virens* T59 ranges from 100% to 84.7 % (Campelo *et al*., 2010) [11].

2. Plant Defense Proteins

They are small molecular weight alkaline peptides, and more than 80 of the genes that code for the construction of these proteins were identified in the plant, and they were called γ-thionin, then the name was changed to defense proteins (Thomma *et al*., 2002) [42].

3. synthetic of L-lignin and the compounds from L-lignin

Phenylalanine Ammonia lyase, after induction by a living or abiotic agent, works by building lignin primers and other cyclic compounds and is itself toxic for pathogenic organisms such as Phytoalxin compounds, these precursors condense in the Shikimic Acid Pathway to form Lacticin, which is the most complex organic compound on the face of nature (Agrios, 2005) [2].

4. Defensive response to changes in plant metabolism

Various changes in the phenylpropanoid pathway include stimulating and activating the enzymes Phenylalanine Ammonia Lyase PAL, 4-Cinnamate Hydroxylase, and 4-Coumarate CoA Ligase, leading to an increase in the activity of these enzymes and the accumulation of many compounds, many of which were known to be active against microorganisms, including acid, Benzoic Acid, Salicylic Acid, Derivatives of Jasmonic Acid, and Ethylene (Gailite, *et al*., 2005 and Boller and Felix, 2009) [16, 8].

Auxins are phytohormones that are produced by plants, as they encourage cell division and expansion through their effect in activating basic vital activities, the work of membranes and the growth of plant cell walls (Grotewold, 2005 and Veitch, 2009) [20, 44]. Auxin Indol Acidic Acid (IAA) is considered one of the essential factors for stimulating the growth and development of strawberry fruits, especially when used in the early stages, as it works on stimulating growth, delaying flower aging and reducing the activities of fruit ripening (Hopkins and Huner, 2004) [23], stated that auxins are organic substances that move from the areas of processing in the plant to the areas of influence, as they have a catalytic role in the growth and development of the plant. Callis (2005) [10] noted that the use of auxins works to stimulate the vegetative, fruitful and root growth of plants.

3. Conclusions

leguminous plants, one of many plants, are affected by many pathogenic fungi *Fusarium* spp., *Sclerotinia* spp., *Pythium* spp., *Rhizoctonia* spp., *Botrytis* spp.) resulting in significant economic losses for certain crops. Chemical synthesis fungicides is one method of disease control. Its application directly to the soil or on the seed may be beneficial against that harm fungal crops during or soon after germination by reducing their incidence and improving plant emergence.

In order to assure safety of food and long-term sustainability, the number of allowed goods by protection plant has been decreased. As a result, organic farming, integrated production by non-chemical methods, and other fields should be prioritized.

The use of biocontrol agents as a technique to control legume infectious diseases, primarily those caused by fungi, can lessen the negative impacts of plant pathogens while also promoting beneficial responses in the plant, Strains from bacterial genera like *Pseudomonas*, *Agrobacterium*, *Bacillus*, *Streptomyces* as well as fungal genera like *Trichoderma*, *Gliocladium*, *Candida*, *Ampelomyces*, *Coniothyrium*, have demonstrated to be effective biocontrol agents against pathogenic microorganisms, *Trichoderma* is known for its high efficiency against pathogenic fungi, thus it may be used in a variety of ways in resistance. It can be added as a suspension or as a powder laden with seeds.

Many species *of Trichoderma* are well-known as fungal biocontrol agents phytopathogenic, antibiosis, competition for nutrients with pathogenic fungi and Mycoparasitism, are the principal biocontrol methods used by *Trichoderma* in direct hostility with pathogenic fungi. *Trichoderma* species have evolved opportunistic systems for nutrient absorption and solute transport, as well as adaptability to abiotic stressors, *Trichoderma* has the ability to help legumes develop. *Trichoderma* also creates organic acids like citric or fumaric acid, which allows phosphates and other micronutrients like manganese, iron, lowers soil pH and magnesium to be soluble in crops of legume. *Trichoderma* spp. colonize the legumes root surface and cause significant changes in plant metabolism, such as the phytoalexins accumulation and the up- or down-regulation of expression defense-related gene. species of Trichoderma colonize the surface of root and cause in plant metabolism a substantial change.

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