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Protection faba bean crop against root rot disease using chitosan nanoparticles and mycorrhizal fungi (A Review).

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ABSTRACT **: Root rot is one of the most deadly diseases that affect faba bean plants. This study proved the effectiveness of chitosan, chitosan nanoparticles (chitosan NPs), and arbuscular mycorrhizal fungi (AMF) as a secure substitute and a method of control against root rot disease caused by Fusarium oxysporum. It also enhanced the physiological characteristics of faba bean plants and their capacity to directly inhibit pathogens or induce them to fight diseases. Arbuscular mycorrhiza, chitosan NPs, and chitosan all have the power to promote plant growth. They have an impact on a variety of physiological functions in plants, such as nutrient intake, cell division, enzyme activation, growth parameters, mineral content, photosynthetic pigments, and the production of phenol and total soluble sugar, which leads to increased yield. Also, this study demonstrated that chitosan NPs have higher antifungal effects than chitosan and are more effective when combined with mycorrhizal fungi than without them. In addition, a low concentration of chitosan NPs has a higher effect than a high concentration.**

Key words: Arbuscular Mycorrhiza, Fusarium oxysporum, Chitosan nanoparticles, root rot, faba bean.

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I. INTRODUCTION

The faba bean is one of the most important food and feed crops for human and animal consumption in Egypt. The vital importance of faba bean seeds is due to their high nutritional value in vitamins, carbohydrates, and protein (Sahile *et al*., 2011). Also, a good source of zinc, calcium, iron, antioxidants, and fiber gives humans the most essential elements and proteins required for life (Etemadi *et al*., 2019 and Merga *et al*., 2019). In addition, it improved soil fertility by fixing atmospheric nitrogen (Boubekeur *et al*., 2012 and Mohsen *et al*., 2013). Numerous dangerous diseases afflict this strategic crop, harming the plant and lowering the production yield. One of the main ailments that affect faba bean production is root rot disease, which is commonly caused by many pathogens, i.e.: *Rhizoctonia solani, Fusarium solani, Fusarium oxysporum, Sclerotinia spp., and Pythium sp*., causing seed rot, seedling fall-over, damping off, and death (Abd El-Hai and Elsaidy, 2016). According to McKenzie and Morrall (1975), root rot disease in the faba bean was caused by *Fusarium sp.* and *Rhizoctonia sp*. to varying degrees (20% and 60%, respectively). In addition, Lamari and Bernier (1985) noted that *Rhizoctonia solani* caused faba bean root rot in Manitoba. In Alberta, faba bean root rot was associated with Fusarium infection. Sumar *et al*. (1981) observed that approximately 40% of faba bean fields were affected in 1978 and 70% in 1979. According to surveys conducted in central Alberta between 2004 and 2006, root rot was the most prevalent faba bean disease (Chang *et al*. 2014). According to FAO (2018), the production of faba beans in Egypt has plummeted from 246801 metric tons in 2008 to 119104 metric tons in 2016, and the cultivated area has shrunk from 71.445 hectares in 2008 to 34.314 ha in 2016. Fusarium sp. is frequently the cause of root rot disease. Elongate, red lesions in the upper tap root and lower hypocotyl are signs of root rot. The lesions then grew bigger and more numerous. Root rot disease is commonly caused by *Fusarium sp*. The symptoms of Fusarium root rot are elongate, red lesions in the lower hypocotyl and upper tap root. Then the lesions increased in size and number. The most diseased plant may die (Abd El-Hai and El-Saidy, 2016). Faba bean infected roots exhibit block rot symptoms, which may spread to the stem base. According to Al-Kahal *et al*. (2009), *Fusarium oxysporum* infection can occur early, causing seed rot, seedling death before emergence, or seedling damping-off after emergence. The fungus Fusarium can infect tissue as it grows. Fusarium fungus symptoms include secondary root rot and darkening of the plant's vascular tissues (orange or red to black hue) (Lv *et al*., 2021).

Fungicides are occasionally successful in controlling certain infections chemically. However, it has a number of drawbacks, including being one of the greatest environmental pollutants, being carcinogenic, and blocking the root nodule (Rhizobium inoculants). As a result, we must combat faba bean disease using alternative, safe, and effective approaches in order to manage these challenges. Numerous studies show that mycorrhizal fungi are crucial in the prevention and treatment of plant diseases (Akthar and Siddiqui 2008). In order to protect bean plants against Fusarium root rot infection, mycorrhizal fungi were employed as biocontrol agents (Rashad and Askar, 2010). According to Berger *et al.* (2016), Chandra *et al*. (2018) showed that chitosan nanoparticles have higher effects against pearl millet downy mildew by generating nitric oxide. And against potato and tomato bacteria wilt (Khairy *et al*., 2022). In order to combat Fusarium root rot disease and improve physiological qualities in faba bean, this work used a new, safe management strategy that took advantage of the synergistic interaction between arbuscular mycorrhiza fungi (AMF), chitosan, and chitosan NPs.

Fusarium oxysporum

Fusarium oxysporum f.sp. cubense is one of the causal organisms of root rot disease. The first plant pathologist to isolate it from bananas in Cuba was Smith (1910), named due to its Cuban source. *Fusarium oxysporum* f. sp. cubense, recorded under vegetative compatibility group (VCG) 01213/16, has been known by surname Tropical Race 4 (TR4), then renamed *F. odoratissimum*. Recently, the pathogen has spread all over the world. Snyder and Hansen (1940) created special forms to classify *Fusarium oxysporum*, more recently known as *Fusarium oxysporum* f. sp. cubense, as a seed-born fungus that can cause several diseases such as root rot and damping-off in a wide range of plants worldwide.

Controls of root rot disease

For the previous reasons, it is necessary to control this disease, improve yield and seed quality, and induce plant resistance against it. Chemical fungicides such as Rizolex T.50 wp, Cyproconazole, Prodione, and Hexaconazole sometimes give good results against these diseases. However, they are one of the most well-known environmental contaminants and have a number of negative effects, including carcinogenicity (Epstein *et al*., 1967; Ismail *et al*., 2004) and the inhibition of root nodule bacteria by Rhizobium inoculants. Fungicides generally endanger the delicate balance of the environment and public health (Elad, 1992). Additionally, a lot of chemical fungicides too are expensive.

Therefore, to overcome these difficulties, it is urgent to apply alternative, safe, and efficient methods against this disease.

Bio-control agents and natural safe compounds are alternative safe agents, and mycorrhizal fungi and nanoparticles (NPs) are examples of these alternative agents.

1-Arbuscular Mycorrhizal (AM) fungi as antifungal agents

Mycorrhiza is one of the most important fungi used as a natural source of nutrients in agriculture (Begum *et al*., 2019). Mycorrhiza is a term derived from the Greek words "mukés", meaning fungus, and "rhiza," meaning root. Selosse *et al*. (2015) observed that arbuscular mycorrhiza fungi's (AMF) symbiosis with plants had been reported about 400 million years ago. Symbiotic associations between fungi and a plant's root can act as biocontrol for plants by destroying pathogens or inducing the ability of plants to attack pathogens (Salam *et al*., 2017; Begum *et al*., 2019) and increasing crop yield (Kaur *et al*., 2014). Russell and Bulman, (2005) observed that the AM fungi obtain their activity from symbiosis with vascular and non-vascular plants.

According to Schüßler *et al*. (2001), arbuscular mycorrhizal fungi (AMF) are classified in the fungal phylum Glomeromycota, which is organized into 4 orders, 8 families, and 10 genera. These genera include Scutellospora, Glomus, Acaulospora, and Gigaspora. According to research by Spatafora *et al*. (2016), the phylum Mucoromycota, which has been divided into four orders (Archaeosporales, Glomerales, Diversisporales, and Paraglomerales) and 25 genera, contains the sub-phylum Glomeromycotina, which includes the AMF species.

Mycorrhizal morphology

AMF spores are generated in the soil and have greater spore diameters (50–500 m) than those of other soil fungi. The vegetative mycelium is multinucleate and aseptate, and AMf is asexual reproduction. Their hyphae are created by rich dichotomous branching that results in haustoria-like structures (Peterson *et al*., 2004; Rashad, 2010).

Mycorrhiza is divided into three types according to morphological and anatomical structure (Roth-Bejerano *et al*., 2014). Fig. (1).

1-Ectomycorrhiza;

The ectomycorrhiza appear as an extracellular fungal growth on the root cell but do not penetrate the cortex cells of plant roots (Gutie´rrez *et al*. 2003).

2-Endomycorrhizas

Endomycorrhiza appear as inter- and intracellular fungal structures called vesicles and arbuscles (Slama *et al*., 2010 and Bowles *et al*., 2016).

Vesicles are lipid-filled sack-like structures within roots that act as storage organs and sometimes as propagative structures (Peterson *et al*., 2004).

Arbuscular; appear as a hyphae network with highly branched structures.

There are two forms.

The pair type is characterized by the growth of hyphae from one cell to the next.

Arum type is characterized by the growth of hyphae in the space between plant cells.

3-Ectendomycorrhiza: endo and ectomycorrhiza characterize the growth of hyphae from inside and between plant cells.

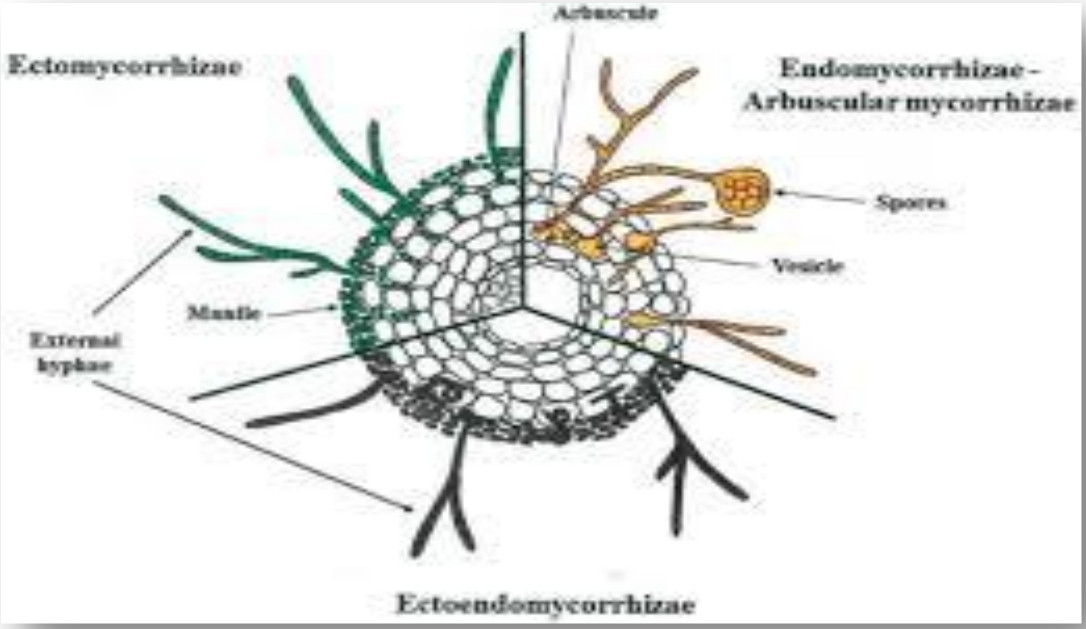

Fig. (1). Root cross section: illustrating different types of mycorrhizal relationships (Monika *et al*., 2022)

Arbuscular mycorrhizal fungi act as biocontrol agents through various mechanisms.

Indirect effect on the pathogen

AMF has tolerance impacts and nutritional effects on plants by increasing photosynthesis capacity, accelerating nutrient absorption, and activating antioxidant enzyme activities. Beltrano *et al*. (2013), Sun *et al*. (2018), and Begum *et al*. (2019) reported that AMF could induce plant resistance against different environmental stresses through fungus hyphae, increasing the absorption area of the plant root and hence increasing water and nutrient absorption from the soil (Rouphael *et al*., 2015; Bowles *et al*., 2016); Tahat *et al.* (2008); and Pringle *et al*. (2009) reported that AMF changes the architecture of the host plant (root morphology), increasing growth and branching. This might affect the interaction between the population of rhizospheric microorganisms and pathogen development. For example, the effect of *Glomus mosseae* on tomato roots leads to an increase in root size, root branching, length, and surface area.

The transpiration process was improved as a result of the growth of stomata conductivity (Yin *et al*., 2010; Raminder *et al*., 2014; Chandrasekaran *et al*., 2019). AMF releases enzymes (chitinase, peroxidase, protease, and cellulase) that allow the plants to digest substrates. Some AMF produce phytohormones (indole-acetic acid, gibberelline, zeatin, and abscisic acid) that have an effect on plant growth and morphology and stimulate nodulation and nitrogen fixation, which improve soil quality and plant resistance (Dar and Reshi 2017). In addition, many researchers have reported that AMF has a physiological effect on plants by increasing the phenolic levels in the plant, such as isoflavonoids or flavonoids, syringic acid, lignin, coumaric acid, etc.;

Rashad and AL-Askar (2010) used mycorrhizal fungi as biocontrol agents and protection of bean plants against Fusarium root rot infection, and their resistance was associated with an increase in the phenolic content, defense enzymes, and phytoalexins (Rashad and AL-Askar, 2010; Baslam *et al*., 2011). Like Yin *et al*. (2010), AM fungi can increase the activity of protective enzymes (e.g., catalase, ascorbate, and peroxidase) in strawberries under drought stress. Furthermore, induction of hydrolytic enzymes such as chitinase, b-glucanase, and superoxide dismutase induces the host defense mechanism against pathogens such as Fusarium wilt (Pozo *et al*., 2002; El-Khallal, 2007). Jahromi *et al*. (2008) showed a reduction in proline levels in AM plants under salt stress. On the contrary, Kaya et al. (2009) found an increase in proline accumulation in AMF plants. In addition, Nell *et al.* (2010) have proven that AMF increases the essential mineral nutrients (phosphate and nitrogen) and some microelements (copper and zinc) that increase the potential tolerance against pathogen infection. An increase in lignin deposition in plant cell walls restricts the spread of pathogens.

Direct effect on pathogen:

The direct effect of AMF on the pathogen is shown as competition for nutrients or space that occurs between microorganisms having the same physiological requirements. Competition could be directed to the root space (root tissues) by AM fungi. A mature AMF colonization, which is distinguished by the presence of arbuscular, seems to be a prerequisite for biocontrol.

There are several studies indicating that AMF plays an important role in plant disease management (Akthar and Siddiqui 2008). Mycorrhizal fungi were used as biocontrol agents against Fusarium root rot infection in bean plants (Rashad and Askar, 2010). Matsubara *et al*. (2001) observed that several species of mycorrhizal fungi, such as *Glomus fasciculatum* and *Gigaspora margarita*, decrease rot root disease caused by *Fusarium oxysporum f. sp.* Alike, Harriar, and Watson (2004) stated the importance of mycorrhizal fungi in the biocontrol of *Fusarium*, *Cylindrocladium*, *Pythium*, *Rhizoctonia*, *phytophthora*, *Aphanomyces*, *Macrophomina*, *Sclerotinium*, and *Verticillium*. Moreover, John and David (2004) reported that arbuscular mycorrhizal fungi could potentially affect powdery mildew in cucumbers.

2- Chitosan (CHT) and Chitosan NPs as antifungal agents

Chitosan and chitosan NPs are an eco-friendly alternative to the management of plant diseases (Hoang *et al*., 2022). Offering preventive and stimulating effects against plant pathogens.

i- Chitosan

Chitosan, a natural, cheap, and safe product of chitin deacetylation, was tested in agriculture in the late 1980s by Malerba and Cerana (2016). Chitosan was reported as an anti-pathogen (Allan and Hadwiger, 1979). Meng *et al*. (2010) indicated that chitosan prevented the growth of *Alternaria kikuchiana* Tanka and *Physalospora piricola* Nose in pear fruits (*Pyrus pyrifolia* L). In addition, several studies investigating that chitosan inhibited and prevented the growth of *Botrytis cinerea* in gray mold disease (Reglinski *et al*., 2010), *Rhizoctonia solani* in rice sheath blight (Liu *et al*., 2012), and *Fusarium oxysporum* in cowpea (*Vigna unguiculata* L.) (Berger *et al*., 2016),

ii - Chitosan NPs and Nanotechnology (NT):

Nanoscience, or nanoparticles (NPs), and natural products (chitosan) are the latest and most important biocontrol agents for plant diseases.

The term nanotechnology was first used by Prof. Norio Tanguchi at Tokyo Science University in 1974. The prefix (nano) means the tiny particles of 10^{-9} size, or one billionth of a meter (one nanometer nm) = one millionth of a millimeter (mm); human hair is 80 nm wide, a red blood cell is 7000 nm, and a strand of DNA is 2.5 nm wide (Mousa *et al*., 2019). Nanotechnology appeared at the end of the 1980s and has been developed and applied in China since the middle of the 1990s.

Properties

Nanomaterials can be classified according to their shape, properties, and size. NPs have unique chemical and physical properties due to their nano-scale size and high surface area *(*Ibrahim *et al*., 2017*).* Their nano-scale size leads to differences in their optical properties, which appear in different colors due to absorption in special visible regions. Due to their unique characteristics, properties, structures, shape, and size (Saad *et al*., 2021; El-Saadony *et al*., 2021).

Importance and uses:

Nanotechnology can be used in many fields such as chemistry, materials, medical science, biology, and physics, and in other important fields, it can be used to operate genes freely, to produce excellent properties in animal and plant species, and as vectors for treating various diseases by absorbing some medicine that can inhibit cancer cells by producing nano-biological cells. Several nanotechnology-based products are finding applications in industries such as

medical devices, imaging, sports, biosensing, electronics, drugs, environmental cleanup, cosmetics and sunscreens (NCPI 2011; USEPA 2007), agriculture, textiles, food, etc. According to Sharon *et al*. (2010) and Sastry **et al**. (2011), as more items using nanotechnologies migrate from research and development into production and commerce, they will have a growing impact on the global economy. Conventional methods for creating nanoparticles typically involve the processing of atoms, molecules, and particles in a liquid or vacuum environment. These methods require a lot of capital (Mandal et al., 2006; Anandan et al., 2008) as well as resources (Pugazhenthiran *et al*., 2009). According to Kashyap *et al*. (2013), fungi were employed to create a number of nanoparticles that are now widely used in all domestic and international industries and professions. This type of nano-product is called green nanotechnology, which is the biosynthesis of nanoparticles (NPS) by microorganisms such as bacteria, actinomycetes, fungi, yeasts, and viruses.

Nanotechnology in agriculture:

Nanotechnologies for instance have the ability to improve agricultural production by improving seed germination, growth, yield percentage, plant quality, and the storage period of vegetables and fruits, (Yifen *et al*., 2019). Urea nanoparticles increased the agronomic efficiency of nitrogen fertilization by 44.5% and the yield of grain by 10.2% versus normal urea (Shiwen *et al*., 2015). It also enhances the productivity of crops by increasing the efficiency of agricultural inputs to facilitate site-targeted controlled delivery of nutrients (Shang *et al.,* 2019) and accelerated adaptation of plants to progressive climate change factors such as extreme temperatures, water deficiency, salinity, alkalinity, and environmental pollution with toxic metals without threatening existing sensitive.

Nanotechnology is an eco-friendly alternative for the management of plant diseases and is used in many agricultural fields. Nano-fertilizers can be used to increase crop yields by increasing absorbable nutrients (Hoang et al., 2022). Furthermore, nano-pesticides, i.e., nano-insecticides, nano-fungicides, nano-bactericides, and nano-herbicides, have been used to protect plants from plant diseases (Duhan *et al*., 2017; Hassani Saad *et al*., 2022). Christian *et al*. (2013) proved that the Zn NPs were significantly more inhibitory to *Fusarium graminearum* growth than Zn in micro-sized form. Nano-copper biosynthesized by *Streptomyces griseus* may be used as an efficient fungicide that has the ability to manage red root-rot disease in tea plantations (Ponmurugan *et al*., 2016). According to Sharma *et al*. (2017), certain nanoparticles, such as nickel ferrite nanoparticles ($NiFe_2O_4$) and cobalt ferrite ($CoFe_2O_4$) nanoparticles, can be used as fungicides in plants. These particles were successfully tested for antifungal activity against *Dematophora necatrix, Colletotrichum gloeosporioides*, and *Fusarium oxysporum*. The reduction of Fusarium wilt in Capsicum spp. caused by ferrite nanoparticles proved that both of them can be utilized as fungicides to manage plant diseases. Alumina silica nanoparticles' excellent antifungal activity against tomato root rot caused by *Fusarium solani* was studied by Shenashen *et al*. (2017) in both laboratory and greenhouse settings.

Chitosan NPs as antifungal agents, Further to the previous investigation, many studies concluded that chitosan nanoparticles are more effective against various plant fungal diseases due to their being one of the best material choices for the preparation of nanoparticles in different applications due to their nontoxic properties, biodegradability, being soluble in acidic conditions, and the free amino groups (positive charge) on their polymeric chains (protonates) (Phaechamud, 2008). As well, Khairy *et al.* (2022) stated that chitosan nanoparticles are considered one of the alternative methods of disease management. Chandra et al. (2018) proved that chitosan nanoparticles induce resistance against pearl millet downy mildew by generating nitric oxide. Furthermore, Suryadi *et al*. (2019) found that nano-chitosan demonstrated an inhibitory effect of up to 85.7% against *Colletotrichum gloeosporioides*. Additionally, it might prevent 61.2% of the germination of *C. gloeosporioides* spores. Moreover, Saharan et al. (2015) stated that nanoparticles caused inhibition of mycelia growth and spore germination in *Alternaria solani* and *Fusarium oxysporum*. Cu-chitosan nanoparticles were found to be most effective in disease control in tomato plants, with values of 87.7% in early blight and 61.1% in Fusarium wilt and Alternaria solani (10, 70%) at 0.03, 0.04% of nano-chitosan (Popova et al., 2020). Moreover, Saharan *et al*. (2015) stated that nanoparticles caused inhibition of mycelia growth and spore germination in *Alternaria solani* and *Fusarium oxysporum*. Cu-chitosan nanoparticles were found to be most effective in disease control in tomato plants, with values of 87.7% in early blight and 61.1% in Fusarium wilt and *Alternaria solani* (10, 70%) at 0.03, 0.04% of chitosan NPs (Popova *et al*., 2020). Moreover, chitosan NPs were found to reduce the sheath blight disease by 75.01% compared with chitosan. In addition, the effect of chitosan NPs on plants is different depending on the characteristics of the NPs and the type of active ingredient loaded,. Chitosan NPs-loaded copper could inhibit the growth of *F. oxysporum* by 60.1% and *A. solani* by 84.2 at 0.1% (Saharan *et al*., 2015); chitosan NPs-loaded zinc inhibited spore germination and the growth of *Curvularia lunata* (Choudhary *et al*., 2019).

Chitosan and chitosan NPS as antifungal agents mechanisms

Chitosan and chitosan NPs exhibit antimicrobial properties against a broad spectrum of plant pathogens. It's important to note that chitosan's and nano-chitosan's efficacy can change based on the type of pathogen, plant species, application technique, and environmental circumstances. To find the best application techniques for certain crops and diseases, field tests and extensive research are required. Chitosan and chitosan NPs inhibit the growth and development of pathogens by destroying their cell walls, messing with their enzyme systems, and inhibiting their replication. Chitosan NPs, with their increased surface area, can have even greater antimicrobial effects. Plant diseases caused by pathogens can be efficiently suppressed by chitosan and chitosan

NPs. They can be applied to plant surfaces as coatings, films, or sprays to prevent pathogen assault. Through the formation of a physical barrier, they stop pathogens from infecting plant tissues. In addition, the plant's defensive systems may be stimulated by chitosan and chitosan NPs, leading to an improved immune response. This entails the induction of genes involved in defense, the creation of antimicrobial substances, and the strengthening of the cell walls of the plant. As a result, pathogen resistance in plants increases, lessening the severity of disease. Chitosan and chitosan nanoparticles can serve as carriers for beneficial extracts or compounds with antimicrobial properties. The nanoparticles are improving their efficacy in combating plant pathogens.

Synergistic of Chitosan and Mycorrhiza against plant disease

Although chitosan has an antagonistic effect on pathogenic fungi, it does not have an impact on mycorrhizal fungi (Hassan *et al*., 2017and El-Gazzar *et al*., 2018), which mentioned that chitasanase and chitinase enzymes produced by plants have an adverse effect on the cell wall of pathogenic fungi but have no harmful impact on mycorrhiza. Chitosan is composed of polysaccharides that stimulate beneficial microorganism activity (mycorrhiza and Rhizobium bacteria) within the soil. Chitosan NPs treatment improved AMF colonization (52%), increasing the levels of auxins and strigolactones in roots (Saleh *et al*., 2023). Amerany et al. (2020) concluded that chitosan, mycorrhiza, and compost are among the best treatments for tomato growth. Furthermore, Ingle *et al*. (2017) and Tian *et al*. (2019) have shown that interaction between various nanoparticles (NPs) and mycorrhizae affects its growth positively or negatively according to the NPs type, speciation, size, concentration, and soil physicochemical properties. As well as Shams (2019), reported that using nano-chitosan-urea as spray on the plant´s foliar in the presence of mycorrhiza (AMF) led to a high kohlrabi knob yield.

Conclusions

Pure chitosan, chitosan nanoparticles, and mycorrhizal fungi enhanced the growth parameters of the faba bean plant, including mineral content material and photosynthetic pigments, and had an induction impact on defense-related enzyme activity and general phenol contents when infected with *Fusarium oxysporum.* Furthermore, they have a resistance effect against the infection of a root rot pathogen (*F. oxysporum*). Chitosan NPs have a higher antifungal effect than chitosan and are more effective when combined with mycorrhizal fungi than without them. So it is recommended to use mycorrhizae and chitosan nanoparticles to overcome the injurious effects of Fusarium root rot and enhance the growth and physiological activities of the plant.

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