Evaluating the efficacy of sulfur and Trichoderma harzianum fungi against wheat (Territicum astivum L.) and their effect on production.

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ABSTRACT

Sulfur fertilizers were used in this experiment and the bio-fungus (Trichoderma harzianum) to studying the effect of each of them on the pathogen Alternaria alternata and on the total yield of wheat (Triticum aestivum L.). The results showed that the fertilization treatment with potassium sulfate added through the soil was significantly excelled compared to the control treatment with a percentage of decrease amounted to (66.31%) in the percentage of infection, the same treatment has excelled in reducing the severity of infection with a percentage of decrease amounted to (86.99%) compared to the control treatment in the growth stage (31). As for the growth stage (59), the superiority was for the bio-control factor for Trichoderma harzianum, with a decrease in the percentage of infection amounted to 84.22% compared to the control treatment. As for the severity of infection in the growth stage (59), the superiority was for the fertilization treatment with potassium sulfate with the bio-vaccine (T.harzianum) added through the soil at a percentage of decrease amounted to (67.47%) compared to the control treatment. While the fertilization treatment with potassium sulfate added through the soil was significantly excelled in reducing the severity and percentage of infection in the growth stage 77, with a percentage of decrease amounted to (97.78% and 98.81%), respectively, compared to the control treatment. The fertilization treatment with potassium sulfate had a significant excelling in the total yield, with an percentage of increase amounted to 30.17% compared to the control treatment.

Research paper from the MSc thesis for the first author.

تقييم كفاءة الكبريت والفطر الاحيائي *Trichoderma harzianum* ضد مرض التبقع الالترناري على محصول الحنطة *Triticum astivum* L. وتأثيرهما في الانتاج .

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الخلاصة

لقد استخدمت الاسمدة الكبريتية في هذه التجربة والفطر الاحيائي *Trichoderma harzianum* لغرض دراسة تأثير كل منهما في الممرض *Alternaria alternata* وفي الحاصل الكلي لمحصول الحنطة *Triticum astivum* L حيث اظهرت النتائج تفوق معاملة التسميد بكبريتات البوتاسيوم المضافة عن طريق التربة تفوق معنوي قياسا بمعاملة المقارنة وبنسبة انخفاض بلغت 66.31 % في نسبة الاصابة كما تفوقت نفس المعاملة في خفض شدة الاصابة بنسبة انخفاض بلغت 86.99 % قياسا بمعاملة المقارنة في مرحلة النمو31. اما في مرحلة النمو 59 فان التفوق كان لعامل المقاومة الحيوية الفطر *Trichoderma harzianum* وبنسبة انخفاض في نسبة الاصابة بلغت 84.22 % قياسا بمعاملة المقارنة , اما شدة الاصابة في مرحلة النمو 59 كان التفوق لمعاملة التسميد بكبريتات البوتاسيوم مع اللقاح الحيوي *T.harzianum* المضافة عن طريق التربة وبنسبة انخفاض بلغت 67.47 % قياسا بمعاملة المقارنة .في حين تفوقت معاملة التسميد بكبريتات البوتاسيوم المضاف عن طريق التربة تفوقا معنويا في خفض شدة ونسبة الاصابة في مرحلة النمو 77 وبنسبة انخفاض بلغت 97.78 % و98.81% على التوالي قياسا بمعاملة المقارنة. ولقد كان لمعاملة التسميد بكبريتات البوتاسيوم تفوق معنوي في الحاصل الكلي وبنسبة زيادة بلغت 30.17% قياسا بمعاملة المقارنة .

بحث مستل من رسالة ماجستير للباحث الاول

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered one of the important strategic cereal crops in the world. It covers a large part of the daily diet for a third or more of the world's population. In addition, it is an important source of energy (4). Iraq is considered one of the first original centers of emergence in the cultivation of wheat, and the factors for the success of cultivation in it are available and in a good way, while the productivity was not at the required level for several reasons, including those related to not resorting to modern technologies in the process of serving and managing the crop and One of the most important crops management processes is nutrient management, which has a prominent role in increasing productivity (17). The wheat plant is one of the crops whose vegetative growth is dense, which helps to increase the humidity, therefore it suffers from many diseases that lead to a loss in financial returns. It also reduces the quality of the crop and reduces the yield. Among these diseases are rust, Smut, and downy and Powdery mildew, The Septoria Leaf Blotch diseases that affect the wheat crop are considered one of the main diseases that affect wheat because of the damage they cause to the crop, and the percentage in the loss caused by Septoria diseases is estimated at (50%) (13). Among the diseases that affect the wheat crop is the disease caused by Alternaria leaf spot, which is from anamorphic fungi, and it is a widespread fungus that spreads in several places and it is a Saprobionts (15). The trends of modern scientific research are the use of modern methods and sustainable materials through which the risk of disease can be reduced. Therefore, plant nutrition by adding fertilizers may contribute to reducing the risk of infection, and many studies have shown that these nutrients may contribute to reducing the risk of Pathogenesis (12). Sulfur fertilizer is considered one of the fertilizers that work on the availability of some nutrients, and this, in turn, leads to raising the level of productivity and good growth in the wheat crop (10). Bio-control with Trichoderma harzianum fungus has also proven its effective role in reducing pathogen infection through some mechanisms that include competition, antagonism, and parasitism (20, 21). Since the Trichoderma spp. fungus is present in the soil, where it is a part of the rhizosphere that has the ability to grow and multiply in it and compete with microorganisms and other organisms where its role does not stop at biological control, but rather has a role in supporting plant nutrition and increasing the percentage of germination and production, where recent studies confirm the role of T.harzianum in the availability of nutrients and thus increase production (1, 10). Therefore, the study aimed to clarify the role of sulfur and Trichoderma harzianum in the controlling of Alternaria alternata fungus on the wheat crop (Triticum astivum L.) and the total yield of wheat.

2. MATERIALSAND METHODS

Preparation method of Alternaria alternate fungus:

The isolates of the fungus (Alternaria alternate) were brought from the University of Mosul, College of Science, Department of Life Sciences - Isolation Bank on 1/11/2019. The culture medium (PDA) was prepared by mixing it with water at a rate of (39 g / liter distilled water) and on a Magnet starrier to be mixed well and then put in the steam sterilizer until it was completely sterilized and homogeneous, and it was taken out of the device and cooled by leaving it in the laboratory until decrease the temperature of the medium and before it solidifies, the antibiotic is added to it and shaken well and the medium is poured into sterile dishes, it has then waited until the fungus hardens and activates the fungus, where a part of the fungal colony is transferred to the prepared medium in the dishes and left in the incubator for five to seven days until the growth is complete and covers all parts of the dish, After the growth of the fungus in the dishes was completed, the dishes were removed from the incubator and 10 ml of sterile distilled water was added to it, and through using a small brush, the spores in the dishes were collected and placed in sterile distilled water, and after all the 500 dishes used were completed, they were placed in plastic tubes and placed in the refrigerator at the freezing temperature of (-4 ° C).

location of the experiment and its treatments:

The experiment was conducted in the agricultural season (2019-2020) in agricultural land in Salah al-Din province, Sharqat district - Ashur sub-district, which is about 3 km from the Tigris River (east of the river), in order to evaluate the efficiency of sulfate, potassium chloride and the bio-control factor (Trichoderma harzianum) in controlling the pathogen (Alternaria alternata) that infects the wheat crop (Triticum aestivum L.) Sham 6 variety and on the total yield. In an experiment, its treatment was the addition of two types of fertilizers as sulfate and potassium chloride added through the soil and sprayed on the leaves, as well as mixing them with the bio-fungus Trichoderma harzianum as well as the bio-fungus Trichoderma harzianum added alone with one treatment. The experiment included nine treatments in addition to the control treatment, as shown in the table below, with three replicates (30 experimental units).

Table 1: Type of added fertilizers and date of their addition:

No.	Type of added substance	First batch	Second batch
1	K_2SO_4 fertilizer	2019/11/24	2020/3/10
2	K_2SO_4+Trichoderma fertilizer	2019/11/24	2020/3/10
3	KCL fertilizer	2019/11/24	2020/3/10
4	KCL + Trichoderma fertilizer	2019/11/24	2020/3/10
5	K_2SO_4 spry	2019/11/24	2020/3/10
6	K2SO4+Trichoderma spry	2019/11/24	2020/3/10
7	KCL spry	2019/11/24	2020/3/10
8	KCL + Trichoderma spry	2019/11/24	2020/3/10
9	Trichoderma	2019/11/24	2020/3/10
10	control	--------------	--------------

The design of the experiment:

The land was plowed after irrigating with a depth of (20-15 cm). An area of the field (180 m2) of the plowed area was used and this area was distributed to (30) experimental units. The dimensions of each experimental unit are 2x3 = 6 m2 and the distance between each two experimental units is 50 cm. The experiment was designed using The Randomized Complete Block Design (RCBD), with three replicates, and samples were distributed randomly.

Cultivation process:

The units were divided into straight lines with a rate of 10 lines, and the distance between one line and the other was 12 cm. Cultivarting was started on 11/24/2019 and It was planted at the internationally approved seed rate (350 grains.m^{-2}). The field was surrounded by a distance of (1 m) planted with barley to protect it from collateral damage.

Method of Calculation and adding Fertilizer

The fertilizer used in the experiment is a Potassium sulfate fertilizer, which is considered the main factor and its purpose are to obtain sulfur and potassium chloride fertilizer. The purpose of adding it to the balance of potassium with potassium present in potassium sulfate, which is considered a secondary factor of the experiment factors, the fertilizers were added through the soil in two batches to the wheat crop with the following proportions, the potassium sulfate at a rate of (333.3 kg.ha^{-1}). As for potassium chloride, it was added at a rate of (288.5 kg.ha^{-1}), potassium sulfate was added in the first batch with seed sowing on 11/24/2020 to six experimental units, three of which were added to potassium sulfate alone, while the remaining three were added to which a bio-vaccine (Trichoderma harzianum). Potassium chloride fertilizer has also been added to six experimental units, three of which only potassium chloride is added, while the remaining three are added with chloride a bio-vaccine (Trichoderma harzianum) in which it is added to the soil. Trichoderma harzianum was added separately to three experimental units. The bio-fungus (Trichoderma harzianum) has been added to its experimental units and according to the recommendation of the manufacturer, where it is mixed 2 g per square meter or liter of water. Fertilizers were added to the second batch of sulfates and chloride on 3/10/2020. This is for the treatments that are added to the experiment through soil. As for the treatments that are added by spraying the plants, they were also added in one batch, on the same date of 3/10/2020, and with the same quantities mentioned above, by a sprayer with a capacity of (5 liters) after dissolving the fertilizers in a quantity of water.

Calculating the concentration of Alternaria alternata:

The calculations were performed on the spores suspension that was kept in the refrigerator after it was taken out and examined. Samples were taken from it by Loop using the slide and placing the cover slide on the glass slide designated for counting. A very small drop of spores suspension is placed between the cover slid and the glass slide and placed under the microscope and the spores are counted in each of the squares inside the slide, which contains (16) squares. This process was performed on all samples in all plastic tubes and then entered into the equation for the calculation of concentration.

The calculation of concentration = $\frac{number\ of\ spores}{0.1}$ = the results $\times 10^3 \times 10^3$

The concentration varied between 1 x 10^6 and 1 x 10^7. More spores suspension were added to increase the concentration until it reached the desired concentration, which amounted to (1x10^7). A quantity of spore suspension was obtained about (7.5 liters) and the suspension was sprayed on 1/1/2020 at 5 Am by a sprayer with a capacity of (5 L) and for each experimental unit (250 ml) of spores suspension at a concentration of (1x10^7) and added to it (250 ml) of Distilled water with (1 ml) of Tween 20 and the field was completely covered with (7.5 L) spores suspension.

Growth stages:

Growth stage 31:

This stage represents the emergence of the first node on the stem, which their length amounted to 1 cm, and this stage represents the distance between the base of the plant and the upper part of the plant above the soil surface. At this stage, measurements of the disease were taken with rate of five plants from each experimental unit and the number of leaves in each plant was then calculated, examining the infected leaves and dividing the infected leaf in a manner similar to dividing the graphical sheets so that each of these squares represents a value of 3.12, according to the division of the paper from 3.12, 6.25, 12.5, 25, 50, 100 %.The severity of the infection was calculated in this way, where when the entire leaf (100) is infected, it is written with the number (1), and when half of the leaf (50) is infected, it is written with the number 0.5, as well as all the above numbers are calculated as follows: 0.25, 0.12, 0.6 and 0.3% by dividing it by 100 After the values of the affected leaves are collected, they are divided by the number of infected leaves, thus the severity of the infection in each plant obtain, and the severity of the infection for five plants that were taken from each experimental unit was then collected, and This will give the severity of infection for each experimental unit. As for the percentage of infection, it is calculated according to the equation:

The percentage of infection= number of infected leaves / total number of leaves x 100

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Growth Stage 59:

This stage of growth represents the stage of completion of the emergence of spikes, and at this stage the measurements were taken for Alternaria leaf spot, in the same way that the measurements were taken in the growth stage 31 and in the same manner.

Growth Stage 77:

This stage represents the milky stage, which means the stage of formation of milk in the grains. At this stage, measurements were taken in the same way that they were taken in the growth stage 31.

Study traits:

1- The severity and percentage of infection in stage 31.
2- The severity and percentage of infection in stage 59.
3- The severity and percentage of infection in stage 77.
4- Total yield ($tons.ha^{-1}$).

3. RESULTS AND DISCUSSION

The effect of adding sulfur and Trichoderma harzianum and the method of addition on Alternaria alternata disease in three stages of growth of the wheat crop and the total yield: -

Severity and percentage of infection in stage 31:

Table (2) shows that fertilization treatments with sulfur had an effect in the process of reducing the severity and percentage of infection with the pathogen Alternaria alternata, where the treatment with potassium sulfate added through the soil has excelled on all treatments and it had a significant superiority in the process of reducing the severity of infection and the percentage of infection compared to the control, with a percentage of decrease amounted to (87.07% and 66.31%), respectively. The reason for this may be attributed to the role of sulfur, which works to reduce the degree of soil reaction, which in turn leads to the availability of some of macro and micronutrients needed by the plant. The role of sulfur was the main element in the defense process inside the plant, and it works to reduce the percentage of pathogenic infection with fungi and in various stages of plant growth. This result agrees with (18) who showed that the fertilizers had significant effects on the disease caused by Z. tritici and the sulfur fertilizers significantly reduced the percentage of infected leaves, the severity of the disease and the concentration of spores in the Growth stages 31. Significant differences also emerged between the treatment of Trichoderma harzianum and the control treatment in reducing the severity and percentage of the pathogen Alternaria alternata, with a percentage of decrease amounted to (66.2% and 53%), respectively. This indicates that the bio-vaccine (Trichoderma harzianum) had a role in the process of reducing the severity of the infection and it works to fix some elements in the soil. Where Fayyad, (7) explained that the fungus Trichoderma harzianum has a great role in the process of disease resistance and limiting its spread and it has several mechanisms used in the process of controlling plant diseases, including competition and parasitism. It also produces some enzymes that have a role in cell wall degradation of pathogens that attack plants such as Chitinases, Glucanases and Cellulases. It also produces proteolytic enzymes and it works to secrete antibiotics that inhibit and kill pathogens, as well as be resistant to toxins produced by the pathogen. The fertilization treatment with potassium sulfate and Trichoderma harzianum that added through the soil was significantly excelled compared to the control treatment, with a percentage of increase amounted to (75.77 and 49.80%), respectively. This is due to the fact that the role was for both the sulfate and bio-vaccine, which work to reduce the degree of soil reaction, which increases the availability of the nutrients, which increases plant growth and its ability to resist diseases as shown in (7, 18). The fertilization treatment with potassium chloride that was also added through the soil was significantly excelled in reducing the severity and percentage of infection compared to the control treatment, with a percentage of increase amounted to (65.32 and 28.34%), respectively. This is attributed to the role of potassium in reducing the severity and percentage of infection as a result of activating many enzymes and the process of photosynthesis, as well as increasing plant growth and cell division, which gives plant resistance due to the increase in cell walls thickness and contributes to protein formation as a result of nitrogen fixation in the soil.

Table 2: Effect of fertilization with sulfur and Trichoderma harzianum and the method of adding fertilizers on the severity and percentage of infection on the pathogen Alternaria alternata at the growth stage 31: -

Treatment	The severity of infection	Percentage of infection
K_2SO_4	1.67	0.0347
K_2SO_4 & Trichoderma	3.13	0.0517
K_2SO_4 spry	11.46	0.0777
K_2SO_4 spry & Trichoderma	14.79	0.9073
KCL	4.48	0.0738
KCL & Trichoderma	4.38	0.0591
KCL spry	14.58	0.0915
KCL spry & Trichoderma	13.12	0.0893
Trichoderma	4.38	0.0484
Control	12.92	0.1030
L.S.D	8.016	0.03833

The severity and percentage of infection in stage 59:

Table (3) shows that the treatment of fertilization with harzianum Trichoderma, which was added alone, was significantly excelled in reducing the severity and percentage of the pathogen Alternaria alternata, with a percentage of decrease amounted to (75.11% and 84.22%), respectively, compared to the control treatment. This may be attributed to the role of Trichoderma harzianum in fixing some nutrients in the soil as a result of reducing the degree of soil interaction by the sulfur-oxidizing bacteria (Thiobacillus) that work in acidic conditions. It also works to reduce the infection with pathogens and controlling them with one of the mechanisms that it has in terms of parasitism and competition for food and others. Fayyad, (7) showed that the bio-controlling agent (Trichoderma harzianum) was used in controlling against several plant diseases, including root rot in tomatoes, Damping off, Rot disease palm pollen and many other diseases. It also explained that T.harzianum has several mechanisms that it uses in the control that enable it to kill and inhibit the pathogenic fungi of the plant, including parasitism on the fungi spinning of pathogenic fungi directly. This process is accompanied by the secretion of some enzymes that work to degrade cell walls, as well as proteolytic enzymes, as well as competition for place and nutrients, especially in the area around the root, in addition to the production of enzymes that degrade the cell walls of plant pathogenic fungi, including Chitinases, Glucanases, Cellulases, and proteolytic enzymes. It also secretes some antibiotics that kill the pathogenic fungi of the plant and stimulates the systemic control of the plant. In addition to the above, it inhibits the enzymes and toxins of the pathogen. The treatment of fertilization with potassium sulfate and bio-vaccine (T.harzianum) added through the soil was significantly excelled in reducing the severity and percentage of infection compared to the control treatment, with a percentage of reduction amounted to (67.96 and 42.47%), respectively. The treatment of fertilization with potassium sulfate and the bio-vaccine (T.harzianum) that added by spraying on the

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leaves was significantly excelled in reducing the severity and percentage of infection compared to the control treatment, with a percentage decrease amounted to (67.34 and 45.61%), respectively. This is due to both factors sulfur and T.harzianum, which work to increase the availability of nutrients, including microelements, as a result of reducing the degree of soil interaction and the sulfur-oxidizing bacteria of the genus Thiobacillus are active in acidic conditions, which work to reduce the degree of soil interaction and increase the availability of most nutrients in Soil, including sulfur and some other elements, where Valdebentio, (21) explained that the micro-organisms in the appropriate conditions can lead to bio-oxidation of sulfur in the soil and to bacteria with chemical nutrition of the genus Thiobacillus and some autotrophic microorganisms of bacteria and fungi an important role in that, where sulfur turns into sulfuric acid, which plays a role in Reducing the degree of soil interaction and increasing the availability of nutrients, which in this case acts as a fixer for the properties of the alkaline and lime soil. Singh, (19) indicated that when adding four levels of sulfur (45,30,15,0 kg of sulfur per hectare) to sandy clay soils planted with wheat crops, a significant increase in the concentration of sulfate in the soil and its availability in the soil solution increased. Whereas, the treatment of fertilization with potassium sulfate added through the soil was significantly excelled in reducing the severity and percentage of infection, with a percentage of decrease amounted to (60.80 and 57.52%), respectively. Fertilization treatment with added potassium sulfate, sprayed on leaves, was significantly excelled to reducing the severity and percentage of infection compared to the control treatment, with a percentage of decrease amounted to (49.14 and 45.11%), respectively. This is due to the role of sulfur, which increases the availability of the nutrients, including the trace elements, as a result of reducing the degree of soil interaction, because the sulfur-oxidizing bacteria, the genus Thiobacillus, are active in acidic conditions, which reduce the degree of soil reaction and increase the availability of most nutrients in the soil, including sulfur and some Other elements as shown in (21, 19).

Table 3: Effect of fertilization with sulfur and Trichoderma harzianum and the method of adding fertilizers on the severity and percentage of infection on the pathogen Alternaria alternata at the growth stage 59: -

Treatment	The severity of infection	Percentage of infection
K_2SO_4	0.252	0.0934
K_2SO_4 & Trichoderma	0.206	0.1265
K_2SO_4 spry	0.327	0.1207
K_2SO_4 spry & Trichoderma	0.210	0.1196
KCL	0.241	0.1825
KCL & Trichoderma	0.345	0.1936
KCL spry	0.364	0.1549
KCL spry & Trichoderma	0.299	0.1478
Trichoderma	0.160	0.0347
Control	0.643	0.2199
L.S.D	0.0959	0.08303

The severity and percentage of infection in stage 77:

Table (4) showed that the fertilization treatment with potassium sulfate added through the soil had a significant effect in the process of reducing the severity and percentage of infection excelling on all treatments compared to the control treatment, with a percentage of decrease amounted to (98.81% and 97.78%), respectively. Fertilization treatment with added potassium sulfate that added by spraying on leaves was significantly excelled to reducing the severity and percentage of infection, with a percentage of decrease amounted to (93.92 and 88.01%), respectively. This may explain that sulfur at this stage of growth has proven its role in the process of reducing the severity and percentage of infection after the second batch of fertilizers was added in the month of March of the year 2020, and after the addition, the amount of fertilizers has been enhanced and sulfur has an effective role in the process of reducing the degree of soil reaction and fixing some nutrients in the soil. The treatment of fertilization with potassium sulfate and T.harzianum added through the soil in reducing the severity and percentage of infection was significantly excelled compared to the control treatment, with a percentage of decrease amounted to (93.81 and 89.23%), respectively. The treatment of fertilization with potassium sulfate and T.harzianum added by spraying on leaves was significantly excelled in reducing the severity and percentage of infection compared to the control treatment, with a percentage of decrease amounted to (81.20 and 60.71%), respectively. This is due to both factors sulfur and T.harzianum, which work to increase the availability of nutrients, including microelements, as a result of reducing the degree of soil interaction and the sulfur-oxidizing bacteria of the genus Thiobacillus are active in acidic conditions, which work to reduce the degree of soil interaction and increase the availability of most nutrients in Soil, including sulfur and some other elements. where Valdebentio, (21) explained that the micro-organisms in the appropriate conditions can lead to bio-oxidation of sulfur in the soil and to bacteria with chemical nutrition of the genus Thiobacillus and some autotrophic microorganisms of bacteria and fungi an important role in that, where sulfur turns into sulfuric acid, which plays a role in Reducing the degree of soil interaction and increasing the availability of nutrients, which in this case acts as a fixer for the properties of the alkaline and lime soil. Singh, (19) indicated that when adding four levels of sulfur (45,30,15,0 kg of sulfur per hectare) to sandy clay soils planted with wheat crops, a significant increase in the concentration of sulfate in the soil and its availability in the soil solution increased. The bio-control factor works to give the plant the characteristic of resistance to the pathogens through the means it uses to resist the pathogens of parasitism and competition for food, and the enzymes, including Chitinases, Glucanases and Cellulases that work to inhibit the work of mycotoxins produced by the fungi attacking the plant, thus limit or Reduces the spread and growth of fungi within plant tissues. One researcher has shown that sulfur-containing compounds are directly or indirectly associated with defenses within the plant against microbial pathogens and there are many of these compounds, which include thionins, defensins, glucosinolates, phytoalexins, alliin, and glutathione (14). Some researchers have proven that sulfur is one of the necessary elements that plants need in strengthening defense lines against pathogens, and they have proven this in their research and found that there are many fungal diseases that are affected by the toxicity of sulfur, including most of the syndromes and Basidiomycota fungi and not Oomycota and bacteria (11). The treatment of fertilization with T.harzianum was significantly excelled in reducing the severity and percentage of infection, with a percentage of decrease amounted to (94.35 and 84.46%), respectively. This may be attributed to the role of Trichoderma harzianum in fixing some nutrients in the soil as a result of reducing the degree of soil interaction by the sulfur-oxidizing bacteria (Thiobacillus) that work in acidic conditions. It also works to reduce the infection with pathogens and controlling them with one of the mechanisms that it has in terms of parasitism and competition for food and others. Fayyad, (7) showed that the bio-controlling agent (Trichoderma harzianum) was used in controlling against several plant diseases, including root rot in tomatoes, Damping off, Rot disease palm pollen and many other diseases. It also explained that T.harzianum has several mechanisms that it uses in the control that enable it to kill and inhibit the pathogenic fungi of the plant, including parasitism on the fungi spinning of pathogenic fungi directly. This process is accompanied by the secretion of some enzymes that work to degrade cell walls, as well as proteolytic enzymes, as well as competition for place and nutrients, especially in the area around the root, in addition to the production of enzymes that degrade the cell walls of plant pathogenic fungi, including Chitinases, Glucanases, Cellulases, and proteolytic enzymes. It also secretes some antibiotics that kill the pathogenic fungi of the plant and stimulates the systemic control of the plant. In addition to the above, it inhibits the enzymes and toxins of the pathogen.

Table 4: Effect of fertilization with sulfur and Trichoderma harzianum and the method of adding fertilizers on the severity and percentage of infection on the pathogen Alternaria alternata at the growth stage 77.

Treatment	The severity of infection	Percentage of infection
K_2SO_4	0.0044	0.020
K_2SO_4 & Trichoderma	0.0230	0.097
K_2SO_4 spry	0.0226	0.108
K_2SO_4 spry & Trichoderma	0.0699	0.354
KCL	0.0433	0.183
KCL & Trichoderma	0.0815	0.225
KCL spry	0.0594	0.183
KCL spry & Trichoderma	0.1050	0.311
Trichoderma	0.0210	0.140
Control	0.3720	0.901
L.S.D	0.1002	0.01529

Total yield (tons.ha^{-1}):

Table (5) shows the superiority of fertilization treatment with potassium sulfate added through the soil on all treatments in increasing the total yield compared to the control treatment, with a percentage of increase amounted to (30.17%). The treatment of fertilization with potassium sulfate added by spraying on leaves was significantly excelled in increasing the total yield, with a percentage of increase amounted to (18.72%). This superiority is due to the role of sulfur in reducing the degree of soil reaction, which in turn leads to the availability of some nutrients in the soil, and it is done by oxidizing it inside the soil by the bacteria Thiobacillus that is present in the soil and some other microorganisms, and these organisms, in turn, represent sulfur in the form of amino acids as well as It leads to an increase of sulfur and nitrogen absorbed by the plant, which leads to an increase in plant growth, which is reflected in the increase in yield. Hussein (6) showed that sulfur has an important role in increasing the total yield of grains, and also mentioned that sulfur has excelled at the level of addition (4 mcg,ha-1) by giving it the highest average of the total weight of grains, which amounted to (10.83 tons.ha-1) compared to the control treatment that did not add to it sulfur, which gave the lowest value amounted to (9.11 tons.ha^{-1}), where this increase in yield was due to the role of sulfur in increasing the growth of the plant and its components, as well as increasing the yield. Al-Fahdawi and Khalil, (5) mentioned that sulfur had a significant effect in increasing the total yield of grains, and it was indicated that treatment S3 gave the highest value for the weight of total grain, which amounted to (8.32 tons.ha^{-1}) excelling on all treatments, while the total weight of the control treatment was (5.58 tons.ha^{-1}). Sulfur also

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affected nitrogen metabolism in plants through its contribution to nitrate reduction. These results agree with (2, 3) who stated that sulfur significantly affects the growth and yield trait of yellow corn and wheat when adding sulfur from its various sources in Iraqi soils. The treatment of fertilization with potassium sulfate and T.harzianum added through the soil was significantly excelled in increasing the total yield, with a percentage of n increase amounted to (24.91%). Whereas, the treatment of fertilization with potassium sulfate and T.harzianum inoculum added by spraying on leaves significantly excelled in increasing the total yield, with a percentage of increase amounted to (20.36%). This is due to both factors sulfur and T.harzianum, which work to increase the availability of nutrients, including microelements, as a result of reducing the degree of soil interaction and the sulfur-oxidizing bacteria of the genus Thiobacillus are active in acidic conditions, which work to reduce the degree of soil interaction and increase the availability of most nutrients in Soil, including sulfur and some other elements. where Valdebentio, (21) explained that the micro-organisms in the appropriate conditions can lead to bio-oxidation of sulfur in the soil and to bacteria with chemical nutrition of the genus Thiobacillus and some autotrophic microorganisms of bacteria and fungi an important role in that, where sulfur turns into sulfuric acid, which plays a role in Reducing the degree of soil interaction and increasing the availability of nutrients, which in this case acts as a fixer for the properties of the alkaline and lime soil. Singh, (19) indicated that when adding four levels of sulfur (45,30,15,0 kg of sulfur per hectare) to sandy clay soils planted with wheat crops, a significant increase in the concentration of sulfate in the soil and its availability in the soil solution increased. The bio-control factor works to give the plant the characteristic of resistance to the pathogens through the means it uses to resist the pathogens of parasitism and competition for food, and the enzymes, including Chitinases, Glucanases and Cellulases that work to inhibit the work of mycotoxins produced by the fungi attacking the plant, thus limit or Reduces the spread and growth of fungi within plant tissues. One researcher has shown that sulfur-containing compounds are directly or indirectly associated with defenses within the plant against microbial pathogens and there are many of these compounds, which include thionins, defensins, glucosinolates, phytoalexins, alliin, and glutathione (14). Some researchers have proven that sulfur is one of the necessary elements that plants need in strengthening defense lines against pathogens, and they have proven this in their research and found that there are many fungal diseases that are affected by the toxicity of sulfur, including most of the syndromes and Basidiomycota fungi and not Oomycota and bacteria (11). The treatment of fertilization with Trichoderma harzianum was significantly excelled in terms of the total yield compared to the control treatment, with a percentage of n increase amounted to (17.91%). This may be attributed to the role of the bio-fungus Trichoderma harzianum in the availability of some nutrients, including nitrogen in the soil, as a result of reducing the degree of soil interaction, as well as encouraging the growth of root hairs. These results agree with (9) that the fungus Trichoderma harzianum has the ability to available nitrogen and some other nutrients such as iron, zinc, and copper, and the production of biological materials that increase the availability of the nutrients in the plant.

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Table 4: Effect of fertilization with sulfur and Trichoderma harzianum, and the method of adding fertilizers on the total yield (ton/ha):

Treatments	K2SO4	K2SO4 & Trichoderma	K2SO4 spry
Their average	8155	7583	7006
Treatments	K2SO4 spry & Trichoderma	KCL	KCL & Trichoderma
Their average	7150	6854	6765
Treatments	KCL spry	KCL spry & Trichoderma	Trichoderma
Their average	6286	6266	6937
Treatment	Control		
average	5694		
LSD	189.9		

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