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Evaluation of Some Treatments in Combating the Mung Plant Against Chronic Pneumonia Caused by The Fungus Macrophomina Phaselina

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Abstract: The study was conducted in Diyala Province, specifically in Al-Miqdadiyah district. The pathogenic fungus Macrophomina phaseolina was isolated from mung bean plants showing symptoms of wilting and yellowing of leaves. The treatment of the pathogenic fungus significantly outperformed the control in terms of disease management, achieving the highest infection rate and severity, which reached 73.8% and 0.76, respectively.

The study revealed, through testing the local variety of mung beans, its sensitivity and resistance to the disease, as well as its impact on growth characteristics.

Additionally, the study demonstrated the evaluation of the effectiveness of three types of plants (fenugreek, mung beans, and soybeans) in a laboratory disease test to inhibit the growth of plant seeds. Fenugreek proved to be the most sensitive, with a 30% damage rate, while mung beans and soybeans showed 20% damage.

Some field treatments and agricultural applications have contributed to reducing the infection rate and severity. One of these treatments is the biocontrol agent, T. harzianum, which played a significant role in this regard.

The study also showed the significant impact of several factors and agricultural applications in reducing the infection rate and severity. These factors include salicylic acid, potassium phosphate, the fungicide Amistar (active ingredients: difenoconazole and azoxystrobin), both individually and in combination, as well as their application methods on seed treatment and seedling irrigation.

The use of these treatments demonstrated significant superiority, except for the combination of the biocontrol agent T. harzianum with salicylic acid and potassium phosphate, and when using the seedling irrigation method. In this case, it achieved a notable reduction in infection rate and severity, with T1.P1 reaching 31.4% and 0.33%, and T1.S1 reaching 27.8% and 0.31%, compared to the control treatment contaminated with the pathogenic fungus. The influence of these treatments inversely reflected on the reduction in loss of growth characteristics, with the total wet and dry weight of both roots and shoots in the treatment of the combination of the biocontrol agent T. harzianum, salicylic acid, and potassium phosphate being 3.66 g and 34.61 g, respectively, and 3.08 g and 34.91 g, respectively, compared to the contaminated control, which had 0.24 g and 12.9 g, respectively.

Keywords: Mung plant, chronic pneumonia, treatment

Introduction

Mung bean (Vigna radiata L.) is considered a strategic leguminous crop cultivated in numerous agricultural lands around the world. Due to its high protein content in its seeds, ranging from 19% to 29%, and elevated carbohydrate levels of 62% to 65%, along with other essential nutrients, it plays a crucial and prominent role in ensuring some of the human food security needs, especially as the world's population density continues to increase (Ali et al., 1990).

Mung bean is characterized by a short growing season, typically ranging from 90 to 130 days. It has a wide ecological range, making it relatively drought-tolerant. It is often introduced in crop rotation systems due to its ability to significantly enhance soil fertility and its suitability for nitrogen fixation through root nodules containing nitrogen-fixing bacteria that develop on its root surfaces (Ali et al., 1990; Allahmoradi et al., 2011).

Mung bean is susceptible to various pathogens during its growing season, including viral, fungal, and bacterial infections. The fungus Macrophomina phaseolina is responsible for charcoal rot disease in mung beans and is considered one of the most significant factors that limit its cultivation, reducing both yield quantity and quality, and consequently leading to substantial economic losses (Su et al., 2001).

The pathogenic fungus Macro phomina phaseolina belongs to the Ascomycetes fungal division and the family Botryosphaeriaceae. It falls under the order Botryosphaeriales. This fungus is known to inhabit the soil (Lucking et al., 2009), and its microscopic fungal particles can persist in the soil for extended periods, sometimes exceeding 15 years, depending on environmental conditions (Baird et al., 2003; Shaner et al., 1999).

Macrophomina phaseolina is recognized for its ability to infect the roots and seeds of agricultural crops like mung beans (Jyotsha et al., 2008).

In recent years, plant diseases have become a significant factor limiting the growth and productivity of many agricultural crops, especially those caused by pathogens that reside within the soil, such as the fungus Macrophomina phaseolina. Although infection occurs in the early stages of plant growth, the symptoms often do not appear until the plant has reached more advanced stages of its life, such as flowering and the development of root nodules (Malvick, 2004; Pilar et al., 2012).

Due to the difficulty in early disease diagnosis and the widespread use of chemical pesticides to combat plant diseases, there is growing concern about the impact of excessive chemical pesticide usage. Many researchers and organizations, including the United States Environmental Protection Agency (USEPA), the Food and Agriculture Organization (FAO), and the World Health Organization (WHO), have highlighted the negative consequences of chemical pesticide use on soilborne pathogens. The excessive use of chemical pesticides not only contributes to genetic changes within tissues and causes diseases such as cancer and various other health issues but also adversely affects non-target organisms, disrupting stable ecosystems and reducing microbial activity in the rhizosphere (Al-Zubaidi, 1992; Gold et al., 2001; Sande et al., 2011; Adeola, 2012; Thuy, 2015).

While chemical methods for controlling pathogenic fungi persist and continue to be used due to their quick results and applicability over large agricultural areas, the current global trend in most countries is to reduce or eliminate the use of chemicals in disease control. Considerable efforts and resources have been dedicated to finding cost-effective and environmentally friendly biocontrol agents that can be applied individually or in combination with other factors without causing negative effects on humans, animals, or other organisms (Abood et al., 2003; Koch, 2005).

Fungi from the Trichoderma genus have received significant attention in the field of biological control. They have been used to treat various plant diseases on a wide range of crops (Diana et al., 2008; Al-Nasseri, 2001; Al-Chabi and Matroad, 2009).

The study aimed to utilize multiple methods with their respective interventions to demonstrate the extent of stimulating plant resistance to the pathogen M. phaseolina and combating this pathogen effectively. Additionally, the study sought to achieve high productivity and lower costs by reducing the use of chemical pesticides, thus promoting a safer environment.

Mung bean crops are susceptible to a wide range of pathogens, but one of the most significant diseases that reduces both the yield and quality of the crop is charcoal rot, which is caused by the fungus M. phaseolina (Su et al., 2001).

2-2 Spread of Charcoal Rot Disease and Its Economic Impact

Charcoal rot disease, caused by the fungus Macrophomina phaseolina, is considered one of the most serious diseases affecting a wide range of agricultural crops worldwide, under varying climatic conditions, whether wet or dry (Abawi and Pastor-Corrales, 1990).

According to Villeneuve and Maigning (2008), Purkayastha et al. (2006), and Al-Jubouri (2011), charcoal rot disease can affect over 500 plant species, significantly impacting economically important agricultural crops such as mung beans, watermelons, cotton, soybeans, beans, peas, field peas, white and yellow maize.

This disease is prevalent in many cultivated regions around the world, resulting in severe economic losses. In recent decades, Egypt's mung bean production has remained stagnant due to the crop's exposure to wilt diseases caused by Fusarium oxysporum f.sp. sesami (Zaprometoff) and charcoal rot (M. phaseolina), leading to substantial yield losses ranging from 50% to 100% (El-Bramawy and Abdul Wahid, 2006; Gaber et al., 1998; El-Shakhess and Khalifa, 2007).

Many studies have highlighted the severity of this disease on various crops. For instance, Bruton et al. (1985) emphasized that charcoal rot disease in mung beans is prevalent in tropical and subtropical regions, previously describing it as one of the most dangerous diseases affecting mung bean crops in Texas. In recent years, charcoal rot has become a significant issue for mung bean farmers in Arizona, as its prevalence has increased significantly, especially in fields using drip irrigation. The cause of this increase is attributed to consecutive mung bean cultivation in the same field for two or three seasons without proper crop rotation practices. Additionally, there is a possibility of salt accumulation due to irrigation near the boundaries of the humid area and on the soil surface (Hanks and Ashcroft, 1980).

According to Wrather et al. (1997), the total crop loss due to charcoal rot in soybeans in Brazil, Argentina, the United States, Paraguay, and Canada reached approximately 1.23 million tons in 1994, with an estimated value of around 272.26 million dollars.

Charcoal rot disease in India can lead to crop losses ranging from 10% to 70% in mung bean crops (Thirumalachar, 1955). As for peanut (Arachis hypogaea L.) crops, losses have varied from 4% to 72%, depending on the variety and type grown (Mathur and Jeshi, 1967).

In their study on controlling charcoal rot disease in mung beans, Karim and Alwan (2016) highlighted the negative impact the disease has on all parts of the mung bean plant. This includes reducing the roots' ability to absorb vital nutrients for growth and the transfer of toxins and metabolic substances into the tissues of the infected plant, disrupting the plant's biological processes. The disease also causes the swelling and death of plant roots.

2-3 Macrophomina phaseolina Fungus, the Causative Agent of Charcoal Rot Disease

The first recorded infection of the M. phaseolina fungus in Iraq was in 1949 by the researcher Hansford (Mihail, 1992; Su et al., 2001).

This fungus has been given various names by several scientists over the years. In 1913, the scientist Taubanhus named it Sclerotium bataticola. In 1924, the scientist Small named it Rhizoctonia lamellifera due to the rot observed on the roots of some field crops, describing the fungus as having black, stony particles. In 1925, it was renamed Rhizoctonia bataticola by the scientist Jones-Briton, and finally, in 1927, the scientist Ashby gave it the name Macrophomina phaseolina for the conidial stage (Al-Ani, 1988).

Macrophomina phaseolina is one of the plant pathogens that are consistently present in the soil (soil-borne). This fungus belongs to the class of incomplete fungi (Class: Deuteromycetes) and the order Coelomycetes. Its isolates vary in terms of the size of sclerotia and the presence or absence of conidial structures. The conidial stage is not commonly found in soybeans but is prevalent in peanuts. Initially, the conidial structures are deposited on the plant's surface, and upon maturation, these structures burst. They have a diameter ranging from 100 to 200 microns, and their color is initially black, becoming darker with age. Their shape can be spherical or flattened, and they have a small or indistinct opening. The conidia are simple, aseptate, cylindrical, measuring 14-33 by 6-12 microns, and they are single-celled. The cell shape can be oval or ellipsoidal (Dhingra and Sinclair, 1977).

The sclerotia of the Macrophomina phaseolina fungus are black, smooth-surfaced, and can be round, rectangular, or irregular in shape. These sclerotia vary in size, shape, and thickness from one isolate to another. They are composed of a mass of fungal hyphae connected due to the presence of melanin. Each sclerotium is made up of approximately 50-200 individual cells (Dhingra and Sinclair, 1977).

2-4 Family Range and Fungal Pathogenicity

The fungus M. Phaseolina is one of the important fungi that inhabit the soil and cause diseases in plant families. It does not specifically target a particular type of plant but infects a wide range of plant species, potentially affecting more than 500 types. It thrives in various environmental conditions (Purkayastha et al., 2006).

This fungus primarily affects economically significant agricultural crops, including white and yellow maize, beans, peas, watermelon, mung bean, and groundnut (Bouhot, 1968; Dhingra & Sinclair, 1975; Adam, 1986; Gray et al., 1990; Hall, 1991; Diourte et al., 1995; Villeneuve & Maigning, 2008). It is also a pathogen of softwood forest trees (McCain & Scharpf, 1989), and it infects bushes and fruit trees (Songa & Hillocks, 1996). The fungus is known for its high capacity to infect both crops and vegetables, whether they are in plastic greenhouses or open fields. It can cause various diseases during the growing season, such as root rot, damping-off, and stem rot (Sinclair et al., 1984; Wrather et al., 2001; Das et al., 2008; Al-Hilaly, 2008; Al-Jubouri, 2011).

Numerous attempts have been made to describe the fungal community of M. Phaseolina in different parts of the world, relying on variations in pathogenicity (Karunanithi et al., 1999), morphological characteristics (Fernandez et al., 2006), and physiological properties (Purkayastha et al., 2006).

In his study conducted in the United States, Wylie (1988) discovered that, in addition to sesame plants, some of the most important plant families affected by the fungus M. Phaseolina include peppers, citrus fruits, peanuts, soybeans, chickpeas, sweet and regular potatoes, sunflowers, beans, cowpeas, white maize, and yellow maize.

Several researchers in their studies conducted in Iraq and Pakistan found that the fungus has infected a wide range of crops and vegetables, such as cotton, beans, sugar beets, okra, and cowpeas (El-Behadli & Al-Azawi, 1979; Shanida et al., 1991).

According to Farr et al. (1989), some of the key plant families affected by the fungus M. Phaseolina include the ones mentioned earlier.

2-5 Morphological and Physiological Characteristics of Fungi

The fungus Macrophomina phaseolina is an opportunistic pathogen, not limited to infecting a specific plant family but with a broad host range. It can persist in the soil for a long time, possibly up to 15 years, depending on environmental conditions. It produces microsclerotia on infected tissues, which serve as a source for initial root infections in subsequent seasons due to its association with plant debris. This makes Macrophomina phaseolina a challenging fungus to control. Sclerotia are active in dry soil but lose their pathogenicity in wet soil after a specific period, typically 7-8 weeks

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after the loss of the plant host (Shaner et al., 1999; Baird et al., 2003; Abawi & Paster-Corrales, 1990).

Several studies have shown that Macrophomina phaseolina exists in different forms and appearances. Isolates have been found that form both conidial and microsclerotial phases, while others only form microsclerotia (Su et al., 2001; Ndiaye, 2007). These isolates differ in their growth rate, pathogenicity, and susceptibility to infection. Faster-growing isolates with abundant production of microsclerotia tend to be more pathogenic compared to slower-growing isolates with limited microsclerotium production (Purkayastha et al., 2006).

The pathogenicity of isolates can also vary between different plant species or even within the same plant species. For instance, isolates taken from one cotton plant variety may have different pathogenic capabilities on other cotton varieties. The fungal colonies can display different colors, ranging from white to dark brown or gray, which may intensify with time. Hyphae typically branch at acute angles, and growth in certain isolates may occur in concentric rings (Aboshosha et al., 2007; Rayatpanah et al., 2012).

4-2-4 effect of experimental parameters on the rate and severity of infection.

The results of the statistical analysis in Table (4-2) show significant differences between the treatments. The Fungicide treatment resulted in a decrease in the infection rate, which was 23.5%, compared to the control disease treatment, which had the highest average of 73.8%. As for the severity of infection, the results indicate significant differences between the treatments. The Fungicide treatment demonstrated its effectiveness in reducing the severity of infection, with a severity score of 0.29, compared to the control disease treatment, which had a score of 0.76.

The reduction in the infection rate and severity may be attributed to the biocontrol ability of the fungus Trichoderma harzianum on the direct parasitism of the pathogenic fungus. Trichoderma harzianum works by breaking down the cells of the pathogenic fungus, as well as secreting enzymes that degrade external and internal chitin, as well as 4-B-N-acetylglucosaminidases enzymes within the environments containing chitin, serving as carbon sources. However, two of these enzymes, Chitinase and Glucanase, are particularly important due to their ability to break down the cell walls of the pathogenic fungus. Trichoderma harzianum affects the pathogenic fungus through various mechanisms and produces competitive substances that inhibit the pathogen.

Trichoderma harzianum also induces resistance in plants by secreting several enzymes such as Peroxidase, β-1,3-glucanase, Chitinase, Polyphenol oxidase, Phenylalanine ammonialyase, and total phenols. These results are in line with previous findings by Hall et al. (2001) and Larkin (2004). Trichoderma harzianum's high capability to enter the fungal hyphae of the pathogen, feed on its materials, and ultimately lead to the death of the pathogen may explain these effects (Al-murad, 2011).

Table (4-2) illustrates the measurement of the infection rate and severity in the treatments.

Treatment	Infection Rate (%)	Infection Severity	Treatment	Infection Rate (%)	Infection Severity	Treatment	Infection Rate (%)	Infection Severity
T1	**31.9**	**0.38**	**T2 with S1**	**29.9**	**0.32**	**S1 with P3**	**43. 6**	**0.45**
T2	**36.2**	**0.42**	**T2 with S2**	**31.3**	**0.35**	**S2 with P1**	**51.7**	**0.52**
S1	**38.7**	**0.44**	**T2 with S3**	**38.7**	**0.41**	**S2 with**	**61.6**	**0.67**

						P2		
S2	41.7	0.47	T1 with P1	31.4	0.33	S2 with P3	28.8	0.31
S3	29.7	0.38	T1 with P2	29.6	0.35	S3 with P1	21.5	0.24
P1	41. 1	0.47	T1 with P3	38.9	0.37	S3 with P2	24.5	0.23
P2	38.8	0.42	T2 with P1	26.6	0.27	S3 with P3	32.7	0.44
P3	34.4	0.45	T2 with P2	33.4	0.35	Fungicide	23.5	0.29
T1 with S1	27.8	0.31	T2 with P3	39.3	0.45	control	0	0
T1 with S2	27.9	0.28	S1 with P1	21.7	0.26	control disease	73.8	0.76
T1 with S3	25.4	0.21	S1 with P2	32.8	0.39			
L.S.D.	11.369	12.585						

4-3 The Effect of Experimental Parameters on Vegetative Characteristics

4-3-1 The Effect of Experimental Parameters on Root and Shoot Dry Weight

The results of the statistical analysis in Table (4-3) for vegetative characteristics indicate significant differences in both root dry weight and shoot dry weight.

Regarding root dry weight, the results show significant differences among the treatments. The highest average root dry weight was recorded in the Control treatment, with 5.577 grams, which did not differ significantly from the Control Amistar and Trich1+potass3 treatments, with averages of 5.507 and 4.65 grams, respectively. The lowest value was observed in the Control W disease treatment, with 0.24 grams.

As for shoot dry weight, the results indicate highly significant differences between the treatments. The highest values were found in the Trich1+potass3 and Trich2 with potass1 treatments, with 47.57 grams, which did not differ significantly from several other treatments. The lowest value was in the Salicy1 with potass1 treatment, with 7.83 grams.

The superior performance of the Amistar treatment, as in the Control, in root dry weight can be attributed to its stimulation of plant growth, increased root dry weight, and enhancement of nutrient elements. This is in line with the findings of Singh (1991) and Al-Woka'a and Al-Alawi (2011), which demonstrated significant improvement in growth and productivity.

The biofungicide T.harzianum works by increasing the availability or breakdown of nutrients such as phosphorus and sulfur due to the enzymes it produces, which enhances nutrient absorption by plants, thereby increasing the activity of the root system. Additionally, it plays an effective role in improving soil properties and nitrogen fixation (Al-Samarrai et al., 2009).

Whereas the treatment Trich2 with potass1 demonstrated its effectiveness in shoot dry weight, and in the Trich1+potass3 treatment, the explanation for this increase can be attributed to potassium and its role in enhancing the efficiency of the photosynthetic process. Potassium promotes the deposition of dry matter in the plant. Spraying with potassium sulfate increases shoot dry weight by facilitating its absorption by the leaves, which, in turn, leads to increased water and nutrient use efficiency. This positively affects the photosynthetic process and, consequently, enhances the yield of this process and increases shoot dry weight (Shirin et al., 2010).

Additionally, potassium plays a physiological role as it contributes to regulating moisture balance in plant tissues, maintains cell turgor, regulates leaf movement, and various bending processes (Chaves et al., 2005). This aligns with the findings of Al-Taher (2014).

Table (4-3) illustrates the measurement of root dry weight and shoot dry weight in the treatments:

The parameters	The dry root weight	The dry root weight	The parameters	The dry root weight	The dry root weight
Control	5.577	35.36	Salicylic acid 3	1.5	19.76
control Amistar	5.507	36.07	Tricho 1high	3.82	38.51
control W disease	0.24	12.9	Tricho2Low	3.093	37.45
Potassium phosphate1	0.973	16.83	Trich1 with potass1	3.663	34.61
Potassium phosphate2	1.81	20.59	Trich1 with potass2	4.053	36.29
Potassium phosphate3	1.585	20.53	Trich1 with potass3	4.49	38.06
Salicy1 with potass1	1.01	7.83	Trich1 with Salicy1	3.085	34.91
Salicy1 with potass2	0.993	9.98	Trich1 with Salicy2	3.34	31.71
Salicy1 with potass3	0.81	10.55	Trich1 with Salicy3	4.133	37.66
Salicy2 with potass1	0.914	11.8	Trich1+potass3	4.65	47.57
Salicy2 with potass2	0.38	10.37	Trich2 with potass1	3.097	47.57
Salicy2 with potass3	0.75	11.93	Trich2 with Salicy1	2.733	29.86
Salicy3 with potass1	1.127	16.84	Trich2 with Salicy2	2.972	30.55
Salicy3 with potass2	0.655	13.95	Trich2 with Salicy3	3.078	34.43
Salicy3 with potass3	0.913	8.94	Trich2 with Salicy3	3.56	27.76
Salicylic acid 1	2.003	19.5	Trich2+Salicy2	3.44	37.75
Salicylic acid 2	2.027	17.94			
L.S.D.	1.9737	17.649			

5-1 Conclusions

1- Lab testing showed that the concentrations of potassium phosphate, salicylic acid, and T. harzianum inhibited the growth of the diseased fungus colony, affecting the infection rate and intensity in field applications. This also reduced losses in growth characteristics and yield.

2- Treating seeds with a solution of salicylic acid in seedling production contributed to rapid growth in seedlings.

3- Macrophomina phaseolina, the pathogenic fungus, inhibited the germination of mung bean, fenugreek, and soybean seeds. The germination rates for these plants were 20%, 30%, and 20%, respectively, under the influence of the pathogenic fungus.

4- The fungicide effectiveness of Amistar, containing the active ingredients difenoconazole and azoxystrobin, was demonstrated in plant protection. It reduced the infection rate and intensity when applied through soil drenching with the chemical fungicide Amistar, supplied by Syngenta, a Swiss company, at a concentration of 2 ml per liter and added at a rate of 50 ml per plant.

5-2 Recommendations

1- Encouraging the use of biological products, including a combination of different biological agents, such as the biocontrol agent T. harzianum, for leguminous plants as a safe alternative in combating soil-borne diseases like charcoal rot in mung bean plants.

2- Promoting the use of seedling production methods with seed soaking in a salicylic acid solution to enhance rapid seedling growth. This method is effective for vigorous growth, disease resistance, and minimizing yield losses.

3- We recommend the use of the fungicide Amistar, containing active ingredients difenoconazole and azoxystrobin, for controlling root rot fungi, including the causative agent of charcoal rot (Macrophomina phaseolina).

4- It is advisable to use combinations of potassium phosphate with salicylic acid or with T. harzianum through soil drenching.

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