The Efficacy of vermicompost, effective microorganisms (EM1), and humic acid in managing cucumber root rot caused by *Rhizoctonia solani* in Iraq

Duha Ayed Abd Hazza Al Masoudi[1]*, Abdal Zahra Jabbar Al Muhammadawi[1]

[1]Plant Protection department, Agriculture College, University of Kerbala, Kerbala, Iraq
***Corresponding author e-mail: duha.a@s.uokerbala.edu.iq**
https://doi.org/10.59658/jkas.v12i1.3250

Received:
Oct. 12, 2024

Accepted:
Nov. 15, 2024

Published:
Mar. 15, 2025

Abstract

This research aims to manage cucumber root rot disease caused by the fungus *Rhizoctonia solani* using Vermicompost, the microbial product Effective Microorganisms (EM1), and humic acid under field conditions. The field survey results confirmed the spread of cucumber root rot disease in the surveyed areas, with infection rates ranging between 48.0% and 46.23% while disease severity between 28% and 38%. Several types of fungi associated with root rot disease were isolated and identified, with *Rhizoctonia solani* being the most frequently occurring pathogenic fungi, followed by *Fusarium spp*. and *Macrophomina phaseolina*. The results also showed that all tested isolates of *Rhizoctonia solani* were pathogenic and caused a significant reduction in the germination rate of cucumber seeds. Additionally, the results demonstrated the ability of the microbial product EM1 to inhibit the growth of *Rhizoctonia solani* on P.D.A with the highest inhibitory effect was observed at concentrations of 5%, 10%, and 15%, where the percentage of inhibition reached 100%. The field experiment results showed that all tested treatments reduced disease severity to varying degrees. The treatment combining all control factors (EM1 + Vermicompost + Humic Acid) recorded the highest inhibition rate of disease severity of 6.66%, compared to 77.77% in the pathogen-only treatment. This combination effectively controlled the pathogen and significantly limited the spread of the infection. The combination of the bio-product and Vermicompost ranked second, reducing disease severity to 13.33%, followed by the combinations of the Bio-preparation with humic acid and Vermicompost with humic acid, with disease severities of 22.22% and 26.66%, respectively.

Keywords: *Cucumis sativus* L., Root rot diseases, *Rhizoctonia solani*, Effective Microorganisms (EM1), Vermicompost.

Introduction

cucumber (*Cucumis sativus* L.), belong to the family Cucurbitaceae, is one of the most important vegetable crops due to its nutritional value. Every 100 g of fresh cucumber contains approximately 96 g of water, 3 g of carbohydrates, 1 g of protein, 12 calories, 1 mg of phosphorus, 0.03 mg of iron, 0.03 mg of vitamin B, 0.04 mg of

vitamin B2, 0.20 mg of niacin, and 8 mg of ascorbic acid [1]. Cucumber also has medicinal uses, such as maintaining the radiance of human skin, alleviating nervous disorders, detoxifying the body, relieving headaches, and quenching thirst [2]. Nutrition experts recommend consuming cucumber with its peel, as it contains numerous nutrients, including vitamins K, A and C, minerals like iron, potassium, phosphorus, copper, and magnesium [3].

Fungi such as *Fusarium spp*., *Rhizoctonia solani* and *Macrophomina phaseolina* exhibit distinctive symptoms of root rot disease, depending on environmental conditions and the age of the plant at the time of infection. Symptoms include rot at the base of the stems and root cortex in mature plants, followed by yellowing of the foliage after the destruction of the root system. Signs of wilting appear during the hottest parts of the day. These fungi infect plants at various stages of growth, ultimately causing plant death [4].

The use of biological control methods as alternatives to pesticides has become necessary as they are effective methods and preserve the safety of the environment as these living organisms are used, including Vermicompost from earthworms, are used to recycle household organic waste and urban waste that causes environmental pollution, transforming it into high-quality organic fertilizer containing essential nutrients for soil and plants [5]. This fertilizer Vermicompost, reduces the need for harmful and expensive chemical fertilizers. It increases the production of high-quality crops that are safe for health and the environment, free of pollutants, and restore the natural flavor of produce. Vermicompost also improves soil porosity and aeration through the tunnels dug by earthworms, enhances soil fertility, and increases the availability of essential nutrients. It balances soil pH levels. Biocontrol agents and fertilizers play a significant role in promoting sustainable crop production and protection. Typically, these biological agents are strains isolated from soil [6,7] used to control diseases by employing environmentally friendly, antifungal microorganisms.

While not considered an essential fertilizer, the use of Humic acid (HAH.A.) is a deeply organic material that enhances soil fertility and improves soil structure. It has a unique ability to modify the physical properties of soil and can promote plant growth by activating microbial performance altering soil fixation properties from a chemical perspective. Humic acid contains several organic compounds that help boost plant growth, yield, and root system development [8]. It activates certain enzymes and inhibits others, increases plant resistance to harsh environmental conditions such as high temperatures and salinity, enhances cell membrane permeability, and stimulates various biochemical reactions in the plant [9].

Additionally, beneficial microorganisms play an effective role in protecting plants from diseases. Biofertilizers are environmentally friendly, cost-effective, non-toxic, and easy to apply; they help maintain soil structure and biodiversity in agricultural lands. Thus, they are an excellent alternative to chemical fertilizers [10,11]. Biofertilizers, or microbial inoculants, are organic products containing specific microorganisms derived from plant roots and rhizosphere regions. They have been proven to improve plant growth and productivity by 10-40% [12]. These microbial inoculants

colonize the root zone and plant's interior, promoting plant growth when applied to seeds, plant surfaces, or soil. Not only do they enhance soil fertility and crop productivity by adding nutrients to the soil, but they also protect the plant from pests and diseases. They have been shown to promote root system growth, extend lifespan, and reduce damage [13]. This study was conducted to identify the fungal pathogen causing cucumber root rot in the Karbala Governorate, Iraq. Based on the above, the study aimed to isolate the fungal pathogens associated with cucumber root rot disease and provide environmentally friendly solutions to limit its spread.

Materials and Methods

Field survey

The field survey was conducted during the 2023-2024 season in Kerbala Governorate, covering the following areas: Al-Wand, Al-Salamiyah, Mustafa Khan and Al-Ibrahimiyah, from 10th September 2023 to 5th November 2023. Samples were collected from plants showing symptoms of root rot disease, such as wilting, yellowing of leaves, and general stunted growth, with noticeable brown rot on the main and secondary roots. Plants were randomly uprooted from each site by intersecting diagonals. Both healthy and infected plants were examined, then placed in labeled polyethene bags and stored in a refrigerator at four °C until the fungi associated with the roots were isolated from each sample. The percentage of infection was also calculated.

Isolation and identification of fungi accompanying cucumber roots

The isolation process from cucumber plant samples showing symptoms of infection was carried out the day after the field survey. The plants were brought to the laboratory, and the infected roots were washed under running water for five minutes to remove soil. The roots were then cut into small pieces, each 0.5 cm long, and surface-sterilized by immersing them in a sodium hypochlorite solution (1% free chlorine) for three minutes. Afterwards, the pieces were rinsed with sterile distilled water for two minutes and dried with filter paper. The pieces were transferred using sterile forceps to Petri dishes, with four pieces in each dish (dish in each dish (diameter 9 cm) containing Potato Dextrose Agar (P.D.A) sterilized in an autoclave at 121°C and 1 atm pressure for 15-20 minutes, with the addition of the antibiotic tetracycline after sterilization. The plates were incubated in an incubator at 25±2°C for three days, after which the accompanying fungi were examined and purified by transferring small pieces from the edges of the fungal hyphae to the centre of a new Petri dish containing P.D.A. The plates were incubated for four days, and the fungi were examined under a compound microscope. The fungi were identified at the genus and species levels based on the appearance of fungal colonies and the characteristics of the hyphae, spores, and structures formed using established taxonomic keys [14,15,16,17]. The percentage of fungal occurrence and frequency was also calculated.

Preservation of fungal isolates

The fungal isolates of *Rhizoctonia solani* . were preserved in test tubes containing a growth medium composed of 20 g potato extract, 20 g carrot and 16 g agar, sterilized in an autoclave. The tubes were placed in a slanted position until the medium solidified.

Afterwards, the tubes were inoculated by adding a 0.5 cm disc from the edges of five-day-old fungal colonies. The tubes were incubated at 25±2°C for seven days. Once the fungal growth was complete, the tubes were stored in a refrigerator at 4°C.

Pathogenicity testing of *Rhizoctonia solani* . using cucumber seeds

Ten isolates of *Rhizoctonia solani* . were tested for their pathogenicity according to the method of [18]. Petri dishes with a diameter of 9 cm containing the P.D.A medium, sterilized in an autoclave for 20 minutes, were prepared, and the antibiotic tetracycline was added to the medium. After the medium solidified, the dishes were inoculated at the center with a 0.5 cm disc taken from the edge of a five-day-old *Rhizoctonia solani* colony. The dishes were then incubated at 25±2°C for three days. Following this, radish seeds, which had been surface-sterilized using a sodium hypochlorite solution (1% free chlorine) and whose germination ability had been previously tested, were placed in a circular arrangement near the edge of the Petri dish, with 10 seeds per dish and three replicates for each isolate. A control treatment (without the pathogenic fungi) was also included. The dishes were incubated at 25±2°C, and after seven days, the results were recorded by calculating the germination percentage.

Pathogenicity test of *Rhizoctonia solani*. isolates on cucumber seedlings grown in plastic pots under greenhouse conditions

The pathogenicity of three fungal isolates of *Rhizoctonia solani*., namely (DH1, DH4 and DH7), which exhibited high inhibitory capacity in the pathogenicity test on cucumber seeds in the laboratory, was tested. The test was conducted on cucumber seedlings grown in plastic pots under greenhouse conditions.

Preparation of *Rhizoctonia solani*. fungal vaccine

Local millet seeds (*Panicum miliaceum* L.) were used to prepare fungal inoculum following the method of [19]. The millet seeds were thoroughly washed with water to remove impurities and dirt. They were soaked in water for six hours and then left on a piece of gauze for half an hour to remove excess moisture. Subsequently, 50 grams of the seeds were placed in 250 ml glass flasks, sterilized for one hour in an autoclave. After 24 hours, the sterilization process was repeated, and the flasks were allowed to cool. The flasks were inoculated by placing five 0.5 cm diameter discs of P.D.A (Potato Dextrose Agar) medium, each containing fungal growth of *Rhizoctonia solani*. isolates, separately in each flask. The flasks were then incubated at a temperature of 25±2°C for 15 days, with shaking every three days to ensure proper aeration and distribution of the fungal inoculum across all millet seeds.

Pathogenicity test of *Rhizoctonia solani*. isolates on cucumber seedlings grown in plastic pots

The pathogenicity of the fungal isolates was tested on 10-day-old cucumber seedlings. The seeds were surface-sterilized and dried on sterile filter paper, then sown in 15 cm diameter plastic pots, each containing 1 kg of sterilized loamy soil. After ten days of germination, the pots were inoculated with the fungal isolates grown on millet seeds. The inoculum (10 g per pot) was mixed thoroughly with the soil around the seedlings near the roots. The control treatment was left without fungal inoculation. The experiment was conducted with four replications Fourteen days after fungal

inoculation, the percentage of root rot disease was calculated. The disease severity on the root system was assessed based on a 5-point disease index, as follows:
(0=Healthy plant, 1 =Infection of root hairs, 2 = Infection of root hairs and roots, 3 =Infection of root hairs and stem and 4 = Plant death), the percentage of disease severity was calculated using [20].

Molecular diagnosis of selected fungal isolates

The pathogenic fungal isolate *Rhizoctonia solani* . (DH4), isolated from cucumber root rot cases due to its high virulence and disease-causing ability, was targeted for genetic and molecular analysis. The study aimed to investigate and compare the genetic composition with the genomes of globally identified isolates. The isolate was identified through ITS-rDNA region sequence analysis, where the PCR products were sent to Macrogen in South Korea for nucleotide sequencing of the Internal Transcribed Spacer (ITS) region. After receiving the nucleotide sequences of the fungal isolates, the sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) to compare them with available data in the National Center for Biotechnology Information (NCBI) database, specifically those about globally identified fungal isolates.

The fungal isolate that did not show a 100% match with any nucleotide sequences in the NCBI database was further analyzed using MEGA X software (version 11). This software was used to analyze the sequences and construct a phylogenetic tree to determine the relationships between this isolate and similar ones registered in the NCBI database. The phylogenetic tree was constructed using the Neighbor-Joining method based on the nucleotide sequence of the ITS region of the fungal isolates.

Evaluation of the antagonistic potential of effective microorganisms (EM1) in inhibiting the growth of *Rhizoctonia solani*. isolates

The EM1 solution was activated by mixing the inactive basic EM1 solution, as detailed in the Table, with molasses and sterilized warm water free of chlorine. The mixture consisted of 5 ml of the inactive EM1 solution, 5 ml of molasses, and 90 ml of sterilized chlorine-free water, then placed in a tightly sealed plastic container. The container was kept in a warm location away from sunlight for 20 days at a temperature between 30-40°C. During this period, the container was opened multiple times to release gas and allow a layer of sediment to form at the bottom, as suggested by [21].

Subsequently, 1%, 3%, 5%, 10% and 15% of the activated EM1 solution were added to 100 ml glass flasks, which were then filled to 100 ml with P.D.A growth medium, with each concentration prepared separately. The mixtures were poured into Petri dishes, and after solidification, a 0.5 cm diameter disc of pathogenic *Rhizoctonia solani* isolates, aged five days, ere inoculated in each dish's centre. This was done in triplicate for each isolate, with a control treatment that consisted of inoculating the center of the dish solely with fungal isolates. The Petri dishes were incubated at a temperature of 25±2°C for seven days, after which the colony diameter and percentage of inhibition were measured based on the control treatment to evaluate the efficacy of the microbial preparation.

Assessment of the effect of integrating the bio-preparation (EM1), Vermicompost, and humic acid against root rot disease of cucumber caused by the fungi *Rhizoctonia solani* under field conditions

Based on the results of laboratory and greenhouse experiments, several treatments demonstrating significant efficacy against the pathogenic fungi *Rhizoctonia solani* were selected for field application in a greenhouse, where the soil was sterilized using solar pasteurization. The experiment was conducted according to a Randomized Complete Block Design (RCBD) with three replications. The experiment included nine treatments as follows:

Sterilized soil only (Control), Contamination with the pathogenic fungi *Rhizoctonia solani* only (R.s. only), addition of the bio-preparation (EM1) at a rate of 5 mL plant-1+ combined with contamination by the pathogen (R.s + EM1), Addition of Vermicompost at a rate of 300 mL plant^{-1} + combined with contamination by the pathogen (R.s + Vermi), addition of humic acid at a rate of 5 mL plant^{-1} + combined with contamination by the pathogen (R.s + Humi), Addition of (EM1) at a rate of 5 mL plant^{-1} + Vermicompost at a rate of 200 mL plant^{-1} + combined with contamination by the pathogen (R.s + EM1 + Vermi), Addition of (EM1) at a rate of 5 mL plant^{-1} + humic acid at a rate of 5 mL plant^{-1} + combined with contamination by the pathogen (R.s. + EM1 + Humi), addition of humic acid at a rate of 5 mL plant^{-1} + Vermicompost at a rate of 200 mL plant^{-1} + combined with contamination by the pathogen (R.s. + Vermi + Humi) and Integrated treatment: Addition of (EM1) at a rate of 5 mL plant^{-1} + Vermicompost at a rate of 100 mL plant^{-1} + humic acid at a rate of 5 mL plant^{-1} + combined with contamination by the pathogen (R.s + EM1 + Vermi + Humi).

The treatments (M1 + Vermi + Humi) were added to the field, mixed thoroughly with the soil, and irrigated. The mixtures were left for two days before planting cucumber seeds at a density of 15 seeds per treatment, with five seedlings per replicate. The inoculum of the pathogenic fungi was added ten days after germination, and weeds were manually removed during the growth stage. After 30 days of fungal inoculation, three plants from each replicate were taken to calculate the disease's percentage of infection and severity.

Statistical analysis

Complete randomized design (C.R.D.) was used in laboratory and greenhouse experiments, while field experiments were analyzed according to the complete randomized block design (RCBD). Data were analyzed using the Statistical Analysis System GenStat12 program, and significant differences between means were compared using the least significant difference (L.S.D.) test at a significance level of 0.05 [22].

Results and Discussion

Field survey of cucumber root rot disease

The field survey results, as presented in Table (1), in cucumber fields located, showed the prevalence of cucumber root rot disease across all surveyed regions. The infection rate ranged from 28% to 48%, while the severity of infection varied between 20% and 37.7%. The highest infection rate was 48.07%. The increased infection rates in these

fields could be attributed to the repeated cultivation of cucumber or other crops from the same Solanaceae family in these fields, which leads to the accumulation of fungal inoculum of the pathogens, particularly (sclerotia) bodies that persist in the soil for extended periods. Additionally, host residues and favorable environmental conditions, especially optimal temperatures for the growth of pathogenic fungi, contribute to disease spread. Agricultural practices such as tilling and weeding, which can cause injuries to the roots, further facilitate fungal invasion. The severity of the disease also varied depending on crop and soil management practices, regular irrigation, control measures, and environmental factors such as temperature and humidity, which significantly impact the increase of fungal inoculum.

The highest severity of infection was recorded in Al-Salamiyah, reaching 37.7%, followed by 24% in the Al-Ibrahimiyah area. The increased infection rates could be attributed to a lack of attention to crop cultivation and inadequate agricultural practices, such as repeated planting, irregular irrigation, imbalanced fertilization, poor fertilizer quality, poor fertilizer quality, and soil type. Additionally, the repeated crop cultivation without crop rotation leads to the accumulation of fungal inoculum. Cucumber root rot diseases threaten cucumber cultivation in many countries worldwide, which is why integrated pest management (I.P.M.) strategies are increasingly being adopted.

Table (1): Field survey of cucumber root rot disease in some areas of Kerbala Governorate.

Area	Injury percentage (%)	Injury severity (%)
Kerbala - Al-Salamiyah	48.07	37.7
Kerbala - Al-Ibrahimiya	38.46	24.00
Kerbala - Al-Wand	29.00	23.46
Kerbala - Mustafa Khan	28.00	20.00

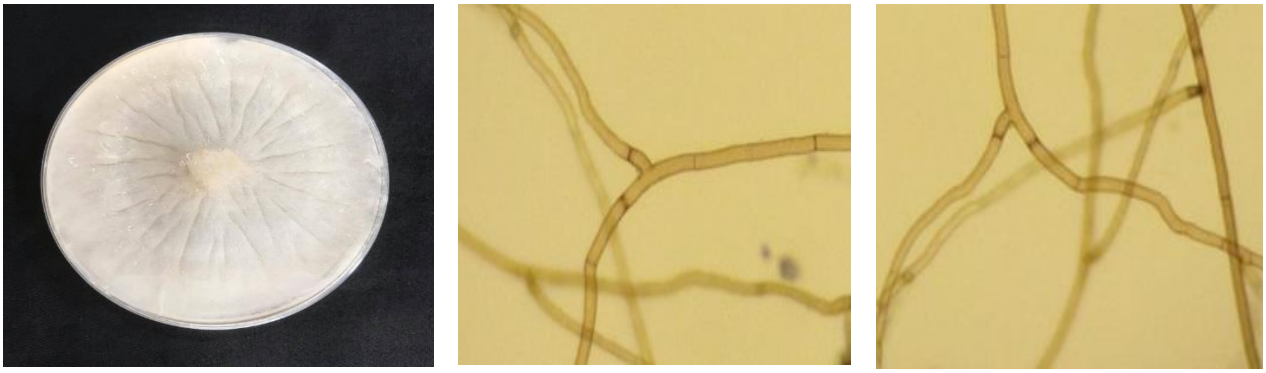
Isolation and identification of fungi associated with the infected roots of cucumber plants

The fungal isolation and identification results, as shown in Table (2), revealed the presence of several fungal species isolated and identified from the roots of infected cucumber plants. These fungi belong to six genera: *Rhizoctonia sp., Alternaria sp., Aspergillus spp., Fusarium spp., Macrophomina sp., and Pythium sp.*, with *Rhizoctonia solani* being the most dominant fungi. Its occurrence rate in the samples reached 61.4%, with a frequency rate of 32.10%. This dominance could be attributed to the accumulation of fungal growth, especially the sclerotia that remain viable in the soil for several years, even without a host. Other factors include inadequate soil management practices, such as the lack of proper fertilization, crop rotation, deep ploughing, the use of contaminated seeds, and the absence of resistant cultivars. These findings are consistent with the results reported by [23].

Table (2): Isolation and diagnosis of fungi associated with cucumber roots.

Fungi	Percentage of appearance (%)	Frequency percentage (%)
Rhizoctonia solani	61.4	32.10
Fusarium spp	45.3	27.00
Fusarium solani	21.6	15.2
Fusarium oxyspoum	12.0	5.11
Macrophomina sp	45.3	8.22
Pythium sp	21.6	4.16
Alternaria spp	13.4	4.12
Aspergillus spp	12.0	1.22

The fungi *Rhizoctonia solani* is a soil-borne plant pathogen with significant variation in morphology, host range, and aggressiveness. Despite its history as a destructive pathogen of economically important crops, traditional identification is primarily based on vegetative characteristics. The fungal hyphae are multinucleate and appear transparent when young but turn brown as they age; hyphal branches typically originate from a distal septum and exhibit a characteristic constriction at the branching point. Most members of this species, though not all, produce uninucleate cells and sclerotia [17].



Rhizoctonia solani *Rhizoctonia solani* *Rhizoctonia solani*

Figure (1): Examples of some traits of pathogenic fungal isolation

Pathogenicity Test

The pathogenicity of the fungal isolates was tested using cucumber seeds in a potato dextrose agar (P.D.A) medium. The results of the study, presented in Table (3), showed that all tested *Rhizoctonia solani* isolates were pathogenic, causing a significant reduction in the germination rate of cucumber seeds compared to the control treatment. The isolates varied in their effect on seed germination. The germination rates in treatments DH4, DH2 and DH7 were 4%, 0% and 5%, respectively, while the germination rate in the other isolates ranged between 12% and 32%, compared to 86.04% in the control. The differences in the impact of fungal isolates on seed germination may be attributed

to the amount of toxic substances secreted by the isolates or their ability to produce cell wall-degrading enzymes. Based on this experiment, the focus was placed on isolates DH7, DH4 and DH2 in the subsequent plastic pot experiment.

Table (3): Detection of pathogenic fungal isolates using cucumber seeds.

Fungi	Isolate code	Germination percentage (%)	Inhibition percentage (%)
Rhizoctonia solani	DH1	32	62.79
Rhizoctonia solani	DH2	5	94.18
Rhizoctonia solani	DH3	24	72.09
Rhizoctonia solani	DH4	0	%100
Rhizoctonia solani	DH5	18	79.06
Rhizoctonia solani	DH6	30	65.11
Rhizoctonia solani	DH7	4	95.34
Rhizoctonia solani	DH8	12	86.04
Control		86	0.00
L.S.D $_{0.05}$		3.2	

Pathogenicity Test of Rhizoctonia solani Isolates on Cucumber Seedlings Grown in Plastic Pots

The results of the pathogenicity test on cucumber seedlings (Table 4) indicated that all the fungal isolates tested exhibited a high pathogenic ability, infecting all the plants with a 100% infection rate for all fungal isolates compared to the control treatment, which had a 0.00% infection rate. When calculating the percentage of disease severity, the fungal isolate (DH4) was distinguished by having significantly higher pathogenic virulence than the other two isolates, recording the highest disease severity percentage of 82.5%. In contrast, the other two isolates recorded 43.4% and 35.6%, respectively. Therefore, the *Rhizoctonia solani* isolate (DH4) was selected for subsequent field experiments.

Table (4): Pathogenicity of *Rhizoctonia solani* isolates on cucumber seedlings grown in plastic pots.

Fungi	Isolate code	Disease percentage (%)	Injury severity percentage (%)
Rhizoctonia solani	DH2	100	35.6
Rhizoctonia solani	DH4	100	82.5
Rhizoctonia solani	DH7	100	43.5
Control		0.00	0.00
L.S.D $_{0.05}$			9.65

Molecular Identification

The results of the nucleotide sequence analysis of the *Rhizoctonia solani* (DH4) isolate, which was isolated from cucumber root rot cases and characterized by its high virulence in causing root rot disease in cucumber, confirmed that the isolate belongs to *Rhizoctonia solani*. This represents the first recorded instance of this species causing root rot disease in cucumber plants in Iraq. The fungal isolate was registered with the National Center for Biotechnology Information (NCBI) under the accession number PP467926.1. The molecular nucleotide sequences showed a high match percentage, ranging from 99.82% to 99.46%, with the ITS gene region (Fig. 2) compared to equivalent nucleotide sequences retrieved from the GenBank database at NCBI using the BLAST program. Additionally, nucleotide sequence analyses were performed using the MEGA software to analyze the isolates and construct a phylogenetic tree (Fig. 3) showing the relationship between this isolate and similar ones recorded in NCBI. The tree was built based on the molecular nucleotide sequence of each isolates' ITS region.

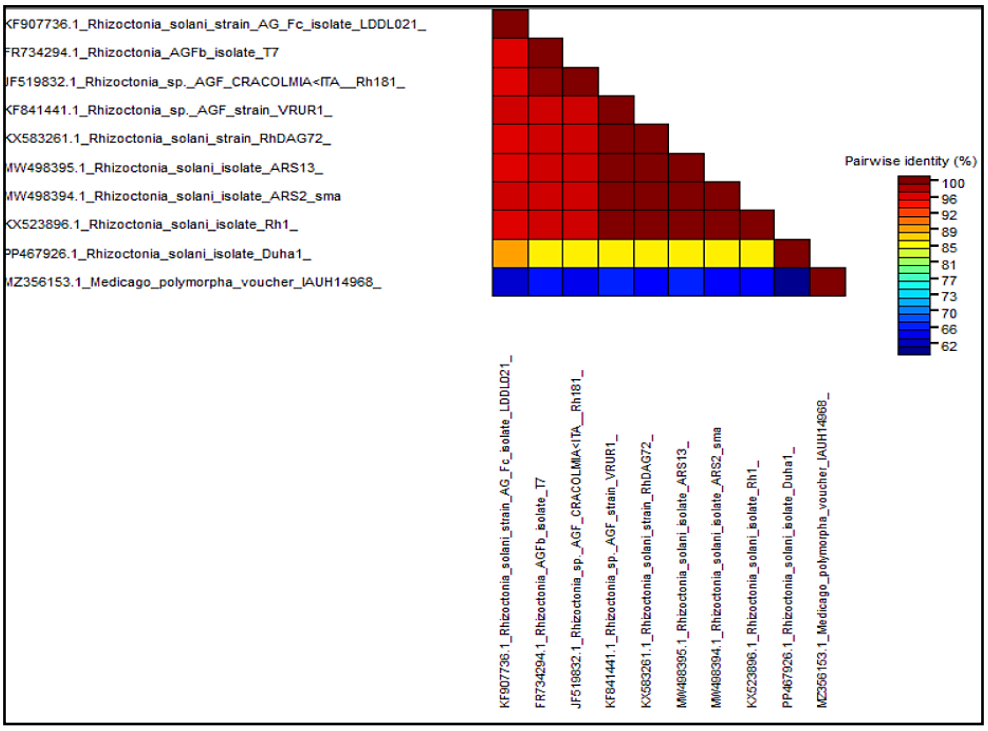


Figure (2): The similarity percentage between the *Rhizoctonia solani* isolate Duha-1 (marked with a black dot) and the corresponding global isolates of the same fungi based on the sequences of their nitrogenous bases of the ITS-rDNA region. This figure was created using the Sequence Demarcation Tool version 1.2 program.

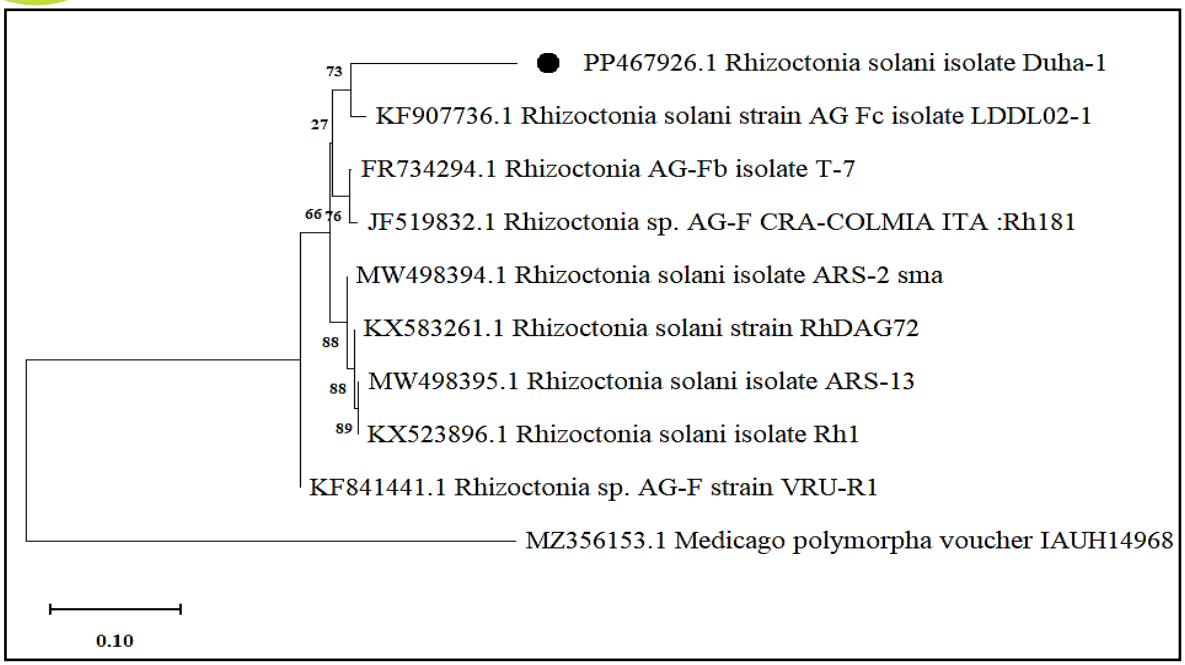


Figure (3): The genetic tree of the fungi *Rhizoctonia solani* isolate Duha-1 (marked with a black dot) which was constructed based on the sequences of its nitrogenous bases of the ITS-rDNA region in addition to the sequences of global strains of the same pathogenic fungi obtained from the GenBank data repository. The genetic distances were calculated using the neighbor-joining method.

Due to the importance of accurate fungal classification, molecular identification aims to detect and diagnose fungal isolates using PCR molecular identification precisely aims to accurately detect and diagnose fungal isolates through the use of polymerase chain reaction (PCR) and determining the sequence of nitrogenous bases in the amplified .N.A.D.N.A. products from isolated fungi. This approach allows for identifying genetic similarities and differences between fungal isolates at the species level. The technique relies on .N.A.D.N.A. analysis for precise diagnosis, revealing the extent of genetic variation among the studied species by using genetic fingerprinting methods such as Random Amplified Polymorphic .N.A.D.N.A. (RAPD) via PCR and Restriction Fragment Length Polymorphism (RFLP) [24].

Many researchers have employed this technique to diagnose various fungi, including Rhizoctonia solani [25,26], because morphological characteristics on culture media, along with microscopic examination challenges, may not provide sufficient or definitive evidence to differentiate between fungal groups, including *Rhizoctonia solani*. Given the importance of accurate fungal classification, several methods have been adopted, but the most precise and modern approach is the molecular diagnosis, which is highly sensitive and specific [27].

Evaluation of the antifungal activity of various effective microorganisms (EM1) concentrations on the growth inhibition of *Rhizoctonia solani* on P.D.A medium.

The results of the test demonstrated the ability of the Effective Microorganisms (EM1) preparation to inhibit the growth of *Rhizoctonia solani* on P.D.A medium at

varying concentrations. The EM1 preparation showed the highest efficacy at concentrations of 5%, 10% and 15%, achieving a 100% inhibition rate compared to the control treatment with *Rhizoctonia solani* alone, which recorded a 0.00% inhibition rate. In contrast, 1% and 3% concentrations resulted in inhibition rates of 33.33% and 43.33%, respectively. Therefore, the concentration of 5% was selected for use in subsequent field experiments.

Effect of integration between the biological preparation (EM1), earthworm waste (Vermicompost) and humic acid against cucumber root rot disease caused by the fungi *Rhizoctonia solani* in the field.

The field experiment results indicated that all tested treatments significantly reduced the severity of infection, albeit to varying degrees. The integration treatment combining all control factors (EM1 + Vermicompost + Humic Acid) recorded the highest inhibition of root rot severity, with an infection rate of 6.66%, compared to the pathogen-only treatment, which exhibited a severity rate of 77.77%. This integration effectively controlled the pathogenic agent and substantially limited the spread of the disease (Table 5). The treatment combining the EM1 preparation with Vermicompost ranked second in reducing infection severity, achieving an infection rate of 13.33%. This was followed by the combinations of the EM1 preparation with humic acid and the Vermicompost with humic acid, which resulted in infection rates of 22.22% and 26.66%, respectively. Notably, the EM1 preparation outperformed the other individual treatments, reducing infection severity to 46.66% compared to the pathogen-only treatment at 77.77%.

Table (5): Effect of integration between the biological preparation (EM1) and earthworm waste (Vermicompost) and humic acid against cucumber root rot disease caused by the fungi *Rhizoctonia solani* in the field.

Treatments	Infection percentage (%)	Injury severity percentage (%)
Control	0.00	0.00
R.s. only	100	77.77
R.s.+EM1	77.77	46.66
R.s.+Vermi	88.88	53.33
R.s.+ Humi	100	60
R.s.+EM1+Vermi	44.44	13.33
R.s.+ EM1+ Humi	55.55	22.22
R.s .+ Vermi + Humi	55.55	26.66
R.s.+EM1+Vermi + Humi	22.22	6.66
L.S.D $_{0.05}$	12.18	10.08

From the above, we can conclude that the most common and recurring fungi are *Rhizoctonia solani*, which are characterized by their toxicity because they can produce a wide spectrum of mycotoxins. The results of molecular diagnosis confirmed their

toxicity by matching them with toxic fungi. However, using some inhibitory substances such as the biological preparation (EM1), earthworm waste (Vermicompost), and humic acid had an effective effect, and these substances can be used to limit the growth and reproduction of these fungi.

References

1) Chakraborty S., and Rayalu S. (2021). Health beneficial effects of cucumber. pp. 21.

2) Trak, N.H.D.T.H., and Chauhan M.F.H.D.D (2022). Skin Care Secrets from Herbal World. Notion Press. Eisbn. pp. 979.

3) Al-Maliky, A.A.T (2023). Study of cucumber damping- off and root rot caused by *Pythium aphanidermatum* (Edson) Fitz. and their integrated control. Master Thesis, College of Agriculture, University of Basrah. pp. 95.

4) Moses, R.T (2006). Biological and chemical control of fungal seedling diseases of cowpea. University of Pretoria, pp. 67.

5) Joshi, R., Singh J., and Vig A.P (2015). Vermicompost as an effective organic fertilizer and biocontrol agent: effect on growth, yield and quality of plants. Reviews in Environmental Science and Bio/Technology, 14: 137-159.

6) Fasusi, O.A., Cruz C., and Babalola O.O (2021). Agricultural sustainability: microbial biofertilizers in rhizosphere management . Agriculture, 11(2): 163

7) Elnahal, A.S., El-Saadony M.T., Saad A.M., Desoky E.S.M., El-Tahan A.M., Rady M.M., and El-Tarabily K.A (2022). The use of microbial inoculants for biological control, plant growth promotion, and sustainable agriculture: A review. European Journal of Plant Pathology, 162(4): 759-792.

8) Canellas, L.P., Olivares F.L., Aguiar N.O., Jones D.L., Nebbioso A., Mazzei P., and Piccolo A (2015). Humic and fulvic acids as biostimulants in horticulture. Scientia horticulturae, 196: 15-27.

9) Shahrajabian, M.H., and Sun W (2024). The importance of salicylic acid, humic acid and fulvic acid on crop production. Letters in Drug Design & Discovery, 21(9): 1465-1480.

10) Riaz, U., Mehdi S.M., Iqbal S., Khalid H.I., Qadir A.A., Anum W., and Murtaza G (2020). Bio-fertilizers: eco-friendly approach for plant and soil environment. Bioremediation and biotechnology: sustainable approaches to pollution degradation, 189-213.

11) Thomas, L., and Singh I (2019). Microbial biofertilizers: types and applications. Biofertilizers for sustainable agriculture and environment, 1-19.

12) Kawalekar, J.S (2013). Role of biofertilizers and biopesticides for sustainable agriculture. J. Bio. Innov., 2: 73–78.

13) Raghuwanshi, R (2012). Opportunities and challenges to sustainable agriculture in India. Nebio, 3: 78–86.

14) Parmeter, J.R., and Whitney H.S (1970). Taxonomy and nomenclature of the perfect state. In J. R. Parmeter (Ed.), Rhizoctonia solani: Review. Biology and

systematics of the form genus rhizoctonia biology and pathology. University of California Press. pp. 7–19.

15) Ellis, M.B (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute Kew, survey England.1- 608.

16) Booth, C (1977). *Fusarium*. Laboratory guide to the identification of the major species. pp. 58.

17) Summerell, B.A. and Leslie J.F (2006) The *Fusarium* Laborator manual. pp. 388.

18) Bolkan, H. A., and Butler, E.E (1974). Studies on heterokaryosis and virulence of Rhizoctonia solani. Phytopathology, 64(5): 13-522.

19) Dewan, M.M (1988). Identify and frequency of occurrence of fungi in roots of wheat and rye grass and their effect on take – all and host growth. Ph. D. Thesis Univ. Wes. Australia. pp. 201.

20) McKinney, H.H (1923). Investigations of the rosette disease of wheat and its control. Journal of Agricultural Research, 23(7): 2-11.

21) Higa, T. and Wididana G (1991). The concept and theories effective microorganisms. in: parr, j.f., s.b. hornick an whitman (eds.), proc. first international conference on kyusei nature farming, us department of agriculture, washington, pp: 118.

22) Al-Rawi K.M., and Khalafallah A (2000). Design and download of agricultural experiments. Dar Al-Kutub for Printing and Publishing, University of Mosul, Republic of Iraq. pp. 488.

23) Al Mousawi, M.A. and Juber K.S (2012). Isolation and identification of the Ecthogen causing root and stem rot disease on cowpea and evaluation of the Azotobacter vinelandii efficacy for controlling the disease under labrotary condetions. The Iraqi Journal of Agricultural Sciences, 43(2): 67-75

24) Edwardes, I.P., and Turco R.F (2005). Inter-and intraspecific resolution of nrDNA TRFLP assessed by computer - simulated restriction analysis of a diverse collection of ectomycorrhizal fungi. Mycol. Res. 109(2): 212-226.

25) Alaei, H., Amir H.M., and Ali D (2012). Molecular characterization of the rDNA-ITS sequence and a PCR diagnostic technique for *Pileolaria terebinthi*, the cause of pistachio rust. Phytopathologia Mediterranea, 51: 488-495.

26) Alhussaini, M.S., Moslem M.A., Alghonaim M.I., Al-Ghanayem A.A., Al-Yahya A.A., Hefny H.M., and Saadabi A.M (2016). Characterization of Cladosporium species by Internal Transcribed Spacer-PCR and microsatellites-PCR. Pakistan Journal of Biological Sciences, 19(4): 143-157.

27) Wickes, B.L., and Wiederhold N.P (2018). Molecular diagnostics in medical mycology. Nature communications, 9(1): 1-8.