Al-Furat Journal of Innovations in Agricultural Sciences (FJIAS)
https://doi.org/10.46649/fjias.v2i2.002

Integrated Management of Okra Root Rot Disease Caused by the Fungus *Rhizoctonia solani*

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Received (15/01/2026), Received in revised form (20/03/2026)
Accepted (24/05/2026), Available online (29/06/2026)

FJIAS 2026, 2(2): 12-25

Abstract. *The aim of this study was to evaluate the effectiveness of the biological pesticide NOVO TREAT, the biological control agent Trichoderma harzianum, and the chemical fungicide Metalaxyl, in addition to the two control treatments, infected and non-infected, in controlling the okra root rot disease caused by the fungus Rhizoctonia solani. The study showed that the biological pesticide NOVO TREAT was the best in inhibiting the growth of the fungus Rhizoctonia solani. This is due to the active ingredients in the biological pesticide, which are responsible for the induction of plant resistance against the pathogenic fungus. This led to the improvement of plant growth in all the studied plant vegetative and root parts by stimulating plant growth, improving the quality of plant yield, and productivity. In addition, the treatment led to the improvement of the quality of the plant's environment and the development of plant immunity against pathogenic agents by activating plant defense mechanisms. The biological pesticide NOVO TREAT was highly efficient in improving plant growth in terms of plant height, leaf length, and leaf width. Plant height increased to 34.344 cm, leaf length increased to 12.855 cm, and leaf width increased to 11.222 cm. There was a significant increase in the amount of chlorophyll in the plant in comparison to the non-infected control treatment, in which the plant's height was 6.833 cm, the plant's leaf length was 5.966 cm, and the amount of chlorophyll in the plant was 2.524 SPAD. The biological pesticide NOVO TREAT played an effective role in the induction of plant resistance against the fungus Rhizoctonia solani, resulting in the improvement of plant growth in several ways. The biological control agent Trichoderma harzianum was the second treatment in inhibiting the growth of the fungus Rhizoctonia solani in the plant, resulting in the improvement of plant growth in terms of plant height, which reached 28.633 cm, and plant leaf length, which reached 11.266 cm. The chemical fungicide Metalaxyl was the third treatment in improving plant growth in terms of plant vegetative and root parts, while the infected control treatment showed the least significant values among the treatments*.

Keywords: *Rhizoctonia solani, NOVO TREAT, Trichoderma harzianum.*

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1. INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is an important vegetable crop belonging to the Malvaceae family, believed to have originated in Ethiopia and Sudan, and is widely cultivated in tropical and subtropical regions worldwide. It is mainly consumed as tender green pods rich in vitamins and minerals, while its seeds contain considerable amounts of oil and protein, with oil characterized by high levels of unsaturated fatty acids such as linoleic acid. Despite its nutritional importance, its utilization remains relatively limited, although its fiber can be used in industries such as paper production [1].

This plant is commonly used in culinary and medicinal practices for treating various conditions, such as helminthic infections, dysentery, inflammation, and irritation of the stomach, intestines, and kidneys. Apart from its nutritional value, okra mucilage has been widely investigated for its functional properties, especially regarding antidiabetic activity. Hence, it is considered a potentially valuable adjunct therapy and nutritional supplement for diabetes control. In addition, okra mucilage is considered a potentially valuable resource for pharmaceutical and food industries. This is because it can be extracted by employing various methods without significantly affecting its physicochemical properties and biological activity. Therefore, researchers have increasingly demonstrated interest in okra mucilage. The findings of research on its applications underscore the significance of exploring this potentially valuable source of bioactive compounds with intriguing technological properties [2, 3].

The fungus Rhizoctonia solani is regarded as one of the most important species within the Rhizoctonia genus. This is a soil-borne plant pathogenic fungus characterized by considerable diversity in morphology, host plants, and pathogenic aggressiveness. Despite its extensive history as a devastating pathogen, which has caused damage to economically important plants across the world, and limited information on host-pathogen interactions and molecular mechanisms, effective control measures have been largely based on integrated control strategies employing biological and chemical control methods. This pathogen is commonly known to infect seedlings and has been reported as an important disease in North America [4; 5; 6]. This species has been reported to demonstrate considerable variability in colony morphology, biochemical, and molecular characteristics, as well as pathogenicity and aggressiveness [4; 7].

The color of the mycelial mat and the color, size and abundance of sclerotia are particularly used in distinguishing Rhizoctonia into different species. For instance R. solani forms buff to brown mycelial mats on Potato Dextrose Agar (PDA) At the microscopic level, all Rhizoctonia species look more or less similar They produce non-sporulating hyphae with 90 degree branches. Each hypha is divided into cells by cross walls called septa, and each septum has a tiny pore in the center named dolipore. Hyphal branching usually takes place close to septa Hyphae have a distinct constriction at branch points. Although Rhizoctonia species cannot be separated using micro-morphology, their sexual spores (basidiospores teleomorphic stage) look quite different [22]..

Biological control agents are an important tool in the field of agriculture to limit the loss of agricultural crops caused by plant pathogens, thereby contributing to an increase in food production in the world. Biological control agents are an alternative to the use of chemical pesticides in agricultural fields. Fungi that belong to the Trichoderma genus, such as Trichoderma harzianum, are the most used biological control agents in the field of agriculture.

https://doi.org/10.46649/fjias.v2i2.002

Trichoderma harzianum has been used in combination with other beneficial microorganisms such as plant growth-promoting bacteria in the control of plant diseases. Trichoderma harzianum uses multiple approaches to control plant pathogens, including mycoparasitism, antibiotic production, and induction of plant defense mechanisms. Trichoderma harzianum is known to antagonize other organisms such as nematodes and plant pathogens, thus acting as biological control agents in the field of agriculture [6; 7; 9].

The biological pesticide NOVO TREAT is an environmentally friendly pesticide that helps in the development of plant resistance against pathogenic agents. The biological pesticide NOVO TREAT inhibits the ability of the pathogen to infect the plant by the use of the active ingredients Bacillus amyloliquefaciens and fusaricidin, which are plant resistance inducers against pathogens, acting as novel antimicrobial agents [2].

Metalaxyl fungicide was first registered as a pesticide in the United States in 1979. Metalaxyl is a systemic fungicide that is used to control various plant diseases caused by pathogenic agents such as oomycete pathogens in the soil [10].

The aim of the study was to limit the severity of the root rot disease in okra plants, thus increasing the yield of the plant while limiting the use of chemical pesticides that may have a negative impact on the plant, the soil, and the environment. The study aims to encourage the use of biological pesticides that are environmentally safe, thus improving the quality of the soil while inhibiting the development of the pathogenic fungus in the plant.

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2. MATERIALS AND METHODS

Isolation and Identification of the Pathogen

The inoculum of *Rhizoctonia solani* was prepared by culturing the fungus on Petri dishes containing Potato Dextrose Agar (PDA) medium. The medium was allowed to solidify before inoculation. The fungal isolate was obtained from infected okra (*Abelmoschus esculentus* L.) roots collected and isolated in the laboratories of the College of Medicinal and Industrial Plants. After the fungal growth appeared on the plates, a small portion from the margin of the actively growing pure colony was transferred using a sterile loop previously sterilized by flame. The inoculated plates were incubated at 27°C for five days. After incubation, the cultures were stored in a refrigerator at 4°C until further use, and the isolate was periodically subcultured when necessary to maintain its viability.

For artificial infection, each pot planted with okra seeds was inoculated with one Petri dish containing the fungal colony in order to induce infection, and the plants were monitored until disease symptoms appeared.

Effect of the Biological Pesticide NOVO TREAT, the Biocontrol Agent *Trichoderma harzianum*, and the Chemical Fungicide Metalaxyl on Infection Caused by *Rhizoctonia solani*

The effects of the biological pesticide NOVO TREAT, the biological control agent *Trichoderma harzianum* (T-22), and the chemical fungicide Metalaxyl were evaluated under greenhouse conditions. Plastic pots with a diameter of 24 cm filled with sandy soil were used. The soil was sterilized using 37% formalin prior to planting. The experiment was conducted according to a Randomized Complete Block Design (RCBD) with three replicates, and treatments were randomly distributed.

Okra seeds were planted in the pots after inoculating the soil with the pathogenic fungus as described previously, while some pots were kept without inoculation to serve as the non-infected control. Both biological and chemical treatments were applied according to the following treatments:

1. Infected control (soil inoculated with the pathogen).
2. Non-infected control (soil not inoculated with the pathogen).
3. Biological control agent *Trichoderma harzianum* (T-22) alone. The fungal suspension was prepared at a rate of 5 g/L obtained from Bioglobar Tr Company, where each gram contained 4×10^6 spores. The suspension was applied as a soil drench at 20 ml per pot.
4. Biological pesticide NOVO TREAT, obtained from Atomes Company (Canada). The active ingredients of the pesticide are *Bacillus amyloliquefaciens* and fusaricidin. The solution was prepared according to the manufacturer's recommendations at 1.25 ml/L, and applied at 5 ml per pot.
5. Chemical fungicide Metalaxyl (5%), obtained from Hangzhou Tionlong Biotechnology Co., Ltd. The fungicide solution was prepared at a concentration of 2 g/L and applied at 10 ml per pot.
6. The process of planting useful pots on sterile soil was carried out as in the control pots, with one plant per pot, in addition to removing pots contaminated with pathogenic fungi, with one plant per pot, as the number of pots in each repetition was 3 pots, and the total number of pots used in the experiment was 45 pots.

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7. The okra crop was planted on 17/5 when the environmental conditions were suitable for cultivation, with temperatures ranging between 25 and 35 degrees Celsius and humidity being ideal for the plant. The harvesting process was carried out after 60 days, and measurements were taken at intervals, with the average of those measurements being taken through statistical analysis of that data.
8. The chlorophyll content in the leaf was estimated using a SPAD spectrophotometer. The chlorophyll content of okra plants was estimated, and the experimental results showed that this method outperformed many previous methods, as the chlorophyll value was obtained using a spectrophotometer. Furthermore, the chlorophyll content in the leaf could be estimated, and this method contributed to increased accuracy by using an optical system that provides reflectance and transmittance information. It is worth noting that the required equipment is inexpensive, and this measurement method is consistent with what was reported by [23].

Studied Traits

The following growth and yield parameters were recorded: Plant height (cm), Number of leaves, Leaf width (cm), Chlorophyll content (SPAD value), Number of fruits, Fresh root weight (g), Fresh shoot weight (g), Leaf length (cm) and Root length (cm).

3. RESULTS AND DISCUSSION

Effect of Different Treatments on Some Growth Traits of Okra Plants Infected with *Rhizoctonia solani* (Plant Height, Leaf Length, and Leaf Width)

The results of the statistical analysis presented in Table (1) indicated significant differences between the applied treatments and their effects on the vegetative and root characteristics of the infected okra plants with the fungus *Rhizoctonia solani*. The results indicated that the biological pesticide NOVO TREAT was the best treatment in increasing the height of the infected plants, reaching 34.344 cm. This is due to the presence of the active ingredient Bacillus amyloliquefaciens and fusaricidin, which induce resistance in the infected plants against the causative fungus. There was no significant difference between the biological pesticide NOVO TREAT and the biological control agent, since the height of the infected plants treated with the biological control agent T. harzianum reached only 28.633 cm, while significant differences were observed between the results of the NOVO TREAT treatment and the results of the other treatments used in the experiment.

As for the length of the infected plants' leaves, the results indicated significant differences between the biological pesticide NOVO TREAT and the biological control agent T. harzianum compared with the results of the other treatments used in the experiment. The length of the infected plants' leaves reached 12.855 cm when the NOVO TREAT treatment was used, while the length of the infected plants' leaves reached only 11.266 cm when the biological control agent T. harzianum treatment was used, while the length of the infected plants' leaves reached only 8.933 cm when the infected plants were treated with the chemical fungicide Metalaxyl treatment, while the infected control treatment recorded the least value (5.966 cm), indicating significant superiority over the results of the other treatments used in the experiment.

As for the width of the infected plants' leaves, the results of the statistical analysis presented in Table (1) indicated significant superiority over the results of the other treatments used in the experiment, where the width of the infected plants' leaves reached 11.222 cm when the NOVO TREAT treatment was used, while the infected plants' leaves treated with the biological control agent T. harzianum reached only 9.177 cm, while the infected control treatment recorded the least significant value (4.866 cm) [11; 12; 13].

Table 1. Effect of Different Treatments on Some Growth Traits of Okra Plants Infected with *Rhizoctonia solani*

Treatments	**Plant height (cm)**	**Leaf length (cm)**	**Leaf width (cm)**
Infected control	6.822 c	5.966 d	4.866 d
Non-infected control	14.700 b	9.444 bc	6.688 c
NOVO TREAT	34.344 a	12.855 a	11.222 a
Trichoderma harzianum	28.633 a	11.266 ab	9.177 b
Metalaxyl	20.967 b	8.933 c	7.344 c

Means with similar letters within each column are not significantly different according to Duncan's Multiple Range Test at the 0.05 probability level.

Effect of Different Treatments on Some Growth Traits of Okra Plants (Number of Leaves, Number of Fruits, and Root Length) Infected with Rhizoctonia solani

The results of the statistical analysis, as shown in Table (2), indicated that the differences between the treatments used, including their effects on the vegetative growth of the okra plants infected with the Rhizoctonia solani fungus, were significant. The biological pesticide NOVO TREAT had the highest effectiveness. There was a significant difference in the number of leaves, as compared to all the treatments used in the experiment. It reached 20.333 leaves/plant. This value was significantly different from the other treatments. The biological control agent Trichoderma harzianum came in second, reaching 16.333 leaves/plant. There was no significant difference between the chemical fungicide Metalaxyl and the non-infected control, reaching 10.111 and 10.000 leaves/plant, respectively. As for the infected control, the value reached 5.111 leaves/plant.

As for the number of fruits, the statistical analysis indicated that the NOVO TREAT treatment significantly surpassed all other treatments, reaching 10.777 fruits/plant. There was no significant difference between the biological control agent Trichoderma harzianum, which recorded 8.222 fruits/plant, and the chemical fungicide Metalaxyl, which recorded 7.111 fruits/plant. On the other hand, the infected control treatment recorded the least value, which was 4.111 fruits/plant.

As for the root length, the NOVO TREAT treatment significantly surpassed all other treatments, reaching 18.733 cm. This may be due to the capacity of the biological pesticide to induce resistance in the plant against the disease-causing agent, R. solani, which consequently inhibits the growth of the root system. Trichoderma harzianum, the biological control agent, ranked second, reaching 13.033 cm. On the other hand, the infected control treatment recorded the least value, which was 5.944 cm. The high efficacy of the biological pesticide may be due to the presence of the active ingredients Bacillus amyloliquefaciens and fusaricidin, which induce resistance in the plant, consequently improving the growth of the root system. These results agree with the results obtained by [14; 15; 16].

Table 2. Effect of Different Treatments on Some Growth Traits of Okra Plants Infected with *Rhizoctonia solani*

Treatments	**Number of leaves**	**Number of fruits**	**Root length (cm)**
Infected control	5.111 d	4.111 d	5.944 d
Non-infected control	10.000 c	5.888 c	10.244 bc
NOVO TREAT	20.333 a	10.777 a	18.733 a
Trichoderma harzianum	16.333 b	8.222 b	13.033 b
Metalaxyl	10.111 c	7.111 bc	9.089 c

Effect of Different Treatments on Fresh Shoot Weight of Okra Plants Infected with Rhizoctonia solani

The results of the statistical analysis showed that significant differences existed among the used treatments in the experiment concerning the fresh weight of the shoot system (Figure 1). The biological pesticide NOVO TREAT showed the highest significant increase in fresh weight of the shoot system compared to the other used treatments, reaching 13.111 g, showing a significant difference compared to the infected and non-infected control treatments. This could be explained by the high efficiency of NOVO TREAT in controlling the infection caused by the pathogen Rhizoctonia solani, which led to a significant increase in the fresh weight of the shoot system.

The second-best treatment was Trichoderma harzianum, a biological control agent, which showed a significant ability to inhibit the infection caused by the pathogen Rhizoctonia solani, resulting in a significant increase in the fresh weight of the shoot system, reaching 11.255 g. The chemical fungicide Metalaxyl and the non-infected control treatment showed no significant difference between them concerning the effect on the growth of the plant. These results are in agreement with the results obtained by [11; 17]

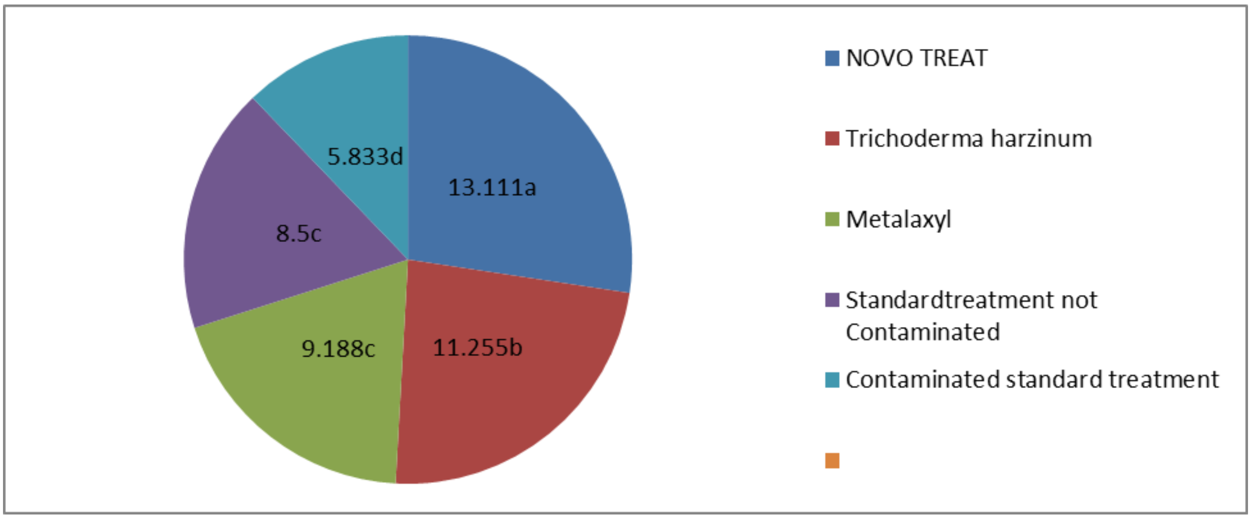

Figure 1. Effect of different treatments on the fresh shoot weight (g) of okra plants.

Effect of Different Treatments on Fresh Root Weight of Okra Plants Infected with Rhizoctonia solani

The results of the statistical analysis on the effect of the biological agents, chemical fungicide, and infected and non-infected control treatments on the fresh weight of the root system are presented in Figure 2, indicating a significant variation in the effect of different treatments on the fresh weight of the root system. Among the different treatments, the biological pesticide NOVO TREAT showed the highest fresh weight of the root system, which was 13.155 g, as presented in the figure below. It is clear that the biological pesticide was the most effective in increasing the fresh weight of the root system compared to the other treatments. This is due to the high capacity of the biological pesticide to stimulate the plant's physiological activities, including photosynthesis and transpiration, which enhanced the qualitative and quantitative vegetative characteristics of the plant.

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https://doi.org/10.46649/fjias.v2i2.002

The second most effective treatment in controlling the infection was the biological control agent Trichoderma harzianum, which showed an increase in the fresh weight of the root system to 10.988 g. The chemical fungicide, Metalaxyl, showed a moderate effect on the fresh weight of the root system, which was 9.333 g. No significant variation was observed between the chemical fungicide and the non-infected control treatment, which showed a fresh weight of 8.888 g. However, the infected control treatment showed the least effect on the fresh weight of the root system, with a value of 5.877 g.

These findings are in accordance with the findings obtained by [18; 19] which showed that the biological control agents, such as the fungus antagonist Trichoderma harzianum T-22 and the bacterial biocontrol agent Bm Mega Flu, when used either individually or in combination, significantly reduced the incidence and severity of the diseases caused by the pathogens that affect the vegetative parts as well as the root system of the plants when compared to the infected control treatments.

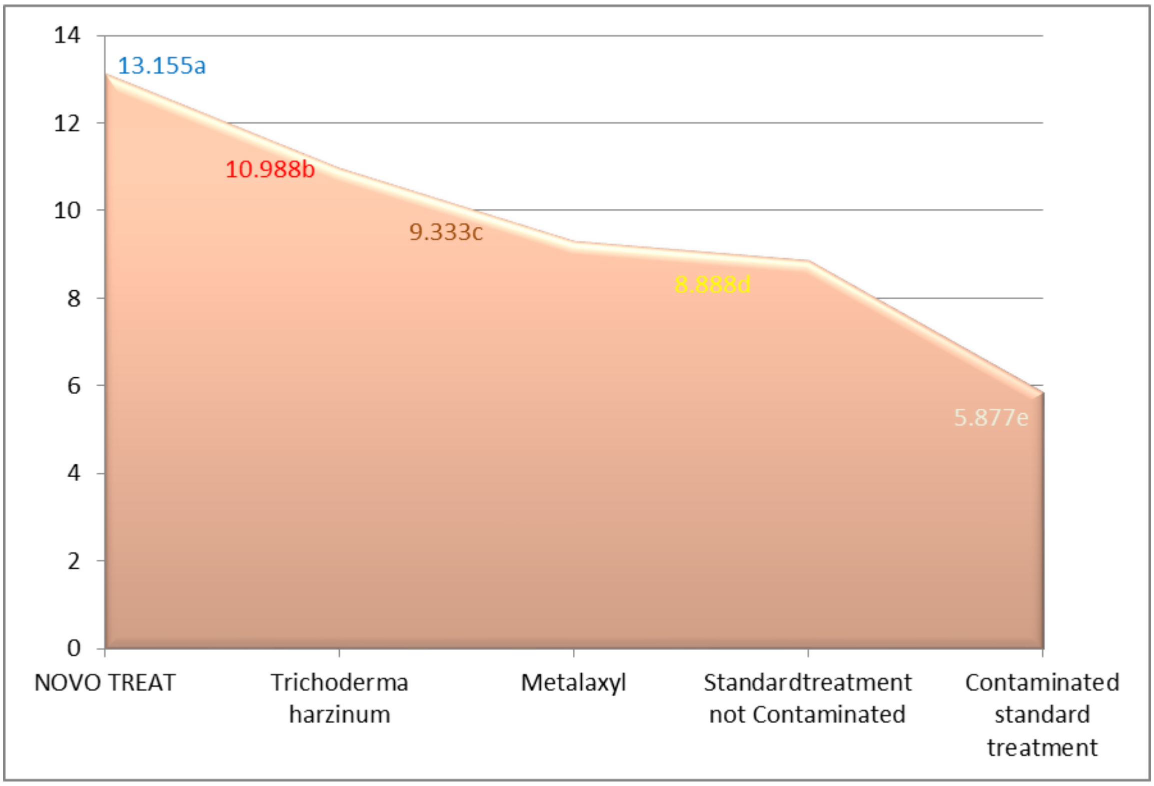

Figure 2. Effect of different treatments on the fresh root weight (g) of okra plants.

Effect of Different Treatments on Chlorophyll Content (SPAD) in Okra Plants

The findings obtained by analyzing the effects of the different treatments on the chlorophyll content in the okra plant leaves are shown in Figure 3. According to the findings, the biological treatments, such as the biological pesticide NOVO TREAT, showed a significant and positive effect on the chlorophyll content in the okra plant leaves when

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https://doi.org/10.46649/fjias.v2i2.002

compared to the other treatments. The NOVO TREAT treatment showed the highest value in terms of chlorophyll content, which was 11.701 SPAD units, with a significant difference when compared to the other treatments used in this study.

The biological control treatment Trichoderma harzianum showed the second highest value in terms of chlorophyll content in the okra plant leaves, with 9.004 SPAD units. The chemical fungicide Metalaxyl showed the third highest value in terms of chlorophyll content in the okra plant leaves when compared to the other treatments used in this study, with a significant difference when compared to the infected control treatments as well as the non-infected control treatments. The infected control treatment showed the lowest value in terms of chlorophyll content in the okra plant leaves, with 2.524 SPAD units. These findings are in accordance with the findings obtained by [20; 21], which showed that biological control is one of the most important environmentally friendly methods used in the control of plant diseases.

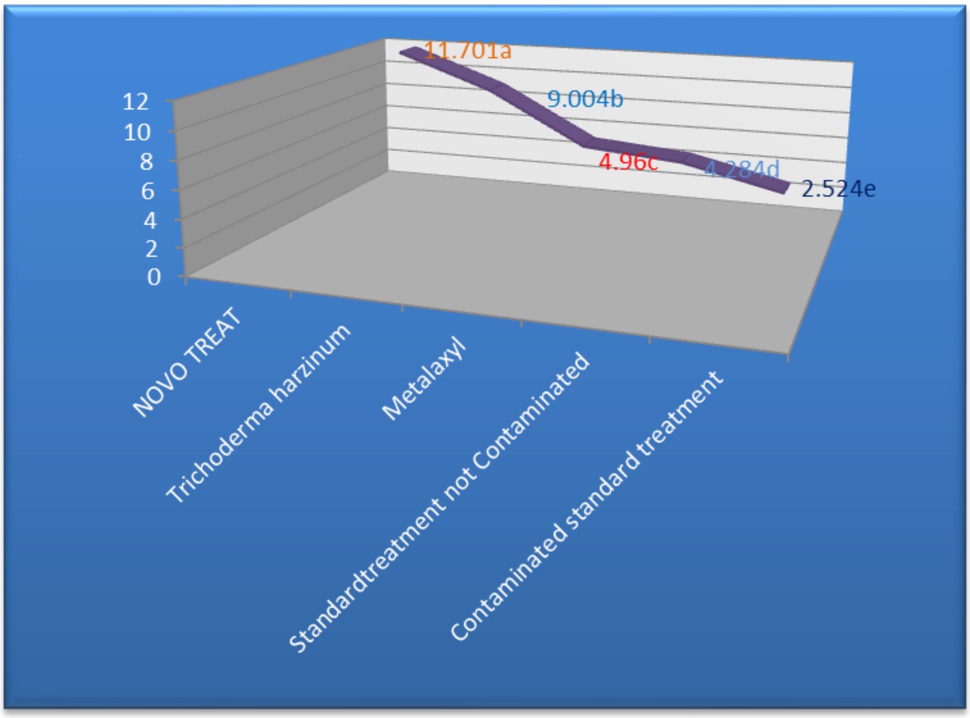

Figure 3. Effect of different treatments on chlorophyll content (SPAD) in okra plants.

https://doi.org/10.46649/fjias.v2i2.002

4. CONCLUSIONS

The results of the present study revealed that the application of the biological pesticide NOVO TREAT to okra plants significantly increased the resistance of the plants to the causal agent of okra root rot disease, *Rhizoctonia solani*. The treatment effectively activated the plant's defensive mechanisms, leading to significant improvements in the growth traits of the plants compared to the other treatments used in the experiment. The biological control agent *Trichoderma harzianum* also showed a positive effect in reducing disease infection and improving vegetative growth characteristics of okra plants. The results of the study show the potential of biological control agents as effective alternatives to chemical fungicides in the control of plant diseases. Therefore, the use of biological pesticides such as NOVO TREAT and biological control agents is a promising and environmentally safe approach for reducing the use of chemical pesticides, minimizing their adverse effects on the environment, and promoting the development of sustainable agriculture.

https://doi.org/10.46649/fjias.v2i2.002

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