



Study the Impact of Carbon and Nitrogen Sources on Some Biological Characteristics of Two Biocontrol Agents: *Trichoderma harzianum* and *Trichoderma viride*

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ABSTRACT

The results indicate that the use of nitrogen sources and carbon sources significantly affected the growth of *Trichoderma harzianum* and *Trichoderma viride*. Aspartic acid achieved the highest colony area for *T. harzianum* (60.79 cm²), while the highest colony area was for *T. viride* with alanine (51.50 cm²) and the lowest with cysteine (28.26 cm²). Arginine also showed a positive effect on the sprouting rate and biomass; the sprouting rate was 8.1 for *T. harzianum* and 10.2 for *T. viride*, while the biomass recorded the highest value of (0.230 g) for *T. harzianum* and (0.207 g) for *T. viride*. Glucose was the most effective source; *T. harzianum* colony area was (55.39 cm²), followed by sucrose and fructose, while cellulose was the least effective (13.85 cm²). Carbon sources also enhanced ant-pathogenic activity and reduced pH to 5.4 and 5.5 compared to the control groups (6.9 and 6.8).

Keywords: Carbon sources, nitrogen sources, *Trichoderma harzianum*, *Trichoderma viride*, sporulation.

INTRODUCTION

Non-pathogenic microorganisms (Guzmán-Guzmán *et al.*, 2023). *Trichoderma* is characterized by its high ability to symbiotically associate with plant roots, enhancing their growth and increasing their resistance to many pathogens (Tyśkiewicz *et al.*, 2022). *Trichoderma* plays an effective role in biological control against pathogens through mycoparasitism, antifungal metabolites-induced systemic resistance (ISR), and the secretion of cell wall-degrading enzymes, such as chitinase and glucanase. It also acts as a biostimulant by increasing nutrient availability and producing plant growth regulators, such as auxins and gibberellins (Alfiky and Weisskopf, 2021). Carbon nutrition is one of the primary factors affecting fungal growth, as carbon sources provide the energy needed for vital processes and the construction of living cells (Sun *et al.*, 2021; Arai *et al.*, 2023). Fungi, including *Trichoderma* utilize a variety of carbon compounds, classified as monosaccharides, disaccharides, and complex sugars. The different types of carbon sources play an important role in determining the growth rates, sporulation, and the production of enzymes and secondary metabolites (Borin and Oliveira, 2022). The type of sugar used by *Trichoderma* can significantly influence growth rate and the production of enzymes needed for biological control and the decomposition of organic matter (De La Cruz *et al.*, 1993; Steyaert *et al.*, 2003).

Glucose is the most efficient source, as it is easily absorbed without the need for enzymatic hydrolysis. This process accelerates metabolism, sporulation, and the production of cell wall-degrading enzymes (Sharon *et al.*, 2011; Gajera *et al.*, 2013). Sucrose requires hydrolysis by inverses into glucose and fructose before use; however, it is commonly utilized in low-cost media (Schulz and Rizvi, 2023). Cellulose is not used as a primary energy source in media due to its slow decomposition. However, it stimulates the production of cellulase and glucosidase enzymes, enhancing *Trichoderma*'s ability to decompose organic matter in the soil (Hamdan and Jasim, 2021). Nitrogen nutrition is a key factor that significantly influences fungal growth and reproduction including *Trichoderma* (Locatelli *et al.*, 2022). Nitrogen is an essential element for many cellular processes, including the synthesis of proteins, nucleic acids, and enzymes. *Trichoderma* relies on various nitrogen sources to support its growth and development, the most important of which are amino acids. Although amino acids provide easily absorbable nitrogen, fungi may prefer some amino acids over others depending on their metabolic needs and stress conditions (Ivana *et al.*, 2021; Mitrović *et al.*, 2021).

MATERIAL AND METHODS

Cultivation

T.harzianum and *T. viride* from the Plant Protection Department of the College of Agriculture and Frosty University of Mosul were used to study carbon and nitrogen nutrition. A minimum medium (g/L) was used, containing 5.0 g ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) and 15.0 g monopotassium phosphate (KH_2PO_4). The salts were sterilized separately, and 2% agar was added to prepare the solid medium. After sterilization, the following components were added:

Glucose 20.0%, hydrated magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) 0.6%, calcium chloride (CaCl_2) 0.6%, hydrated ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) 0.005%, hydrated manganese sulfate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$) 0.0016%, and hydrated zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) 0.0014% (Zhao *et al.*, 2021).

Effect of carbon sources on the biological properties of *T. harzianum* and *T. viride*

The effect of five carbon sources on the biological properties of the two fungi was tested: Cellulose, fructose, glucose, lactose maltose. the carbon sources were added to the minimal medium at a final concentration of 0.2% (w/v) as a substitute for the primary carbon source. The liquid and solid media were then sterilized.

Effect of nitrogen sources on the biological properties of *T. harzianum* and *T. viride*.

Five amino acids were selected for this study: Alanine, arginine, histidine, aspartic acid, and cysteine. amino acids (0.1% w/v) were added to a sterile, refrigerated (at 40°C) minimal medium as an alternative to the primary nitrogen source.

***T. harzianum* and *T. viride* colony growth measurement**

The minimal medium was enriched with the antibiotic Ampicillin at a concentration of 150 mg/L. It was then distributed into glass Petri dishes and inoculated with a 5 mm disc taken from the edge of a 5-day-old colony of both *T. harzianum* and *T. viride*. Three replicates were used for each fungus species. After inoculation, the dishes were incubated at 28°C, and the colony area was measured using ImageJ software after 7 days.

Sporulation

The number of spores was counted using a hemocytometer.

Antagonism activity

The antagonistic activity of *T. harzianum* and *T. viride* against *Fusarium solani* was evaluated using the dual culture technique. Media containing carbon or nitrogen sources were poured into petri dishes and allowed to solidify. The antagonistic capacity was calculated based on the area of *F. solani* colony using ImageJ software.

Inhibition percentage was determined according to the following equation:

$$\text{Percentage inhibition} = [(AF - AT)/AF] \times 100$$

Where:

AF = *F. solani* colony area in the control treatment

AT = *F. solani* colony area in the experimental treatments (Nofal *et al.*, 2021)

Biomass measurement

Flasks containing minimal medium with different carbon and nitrogen sources were inoculated with a 5 mm disc taken from a 5-day-old colony of *T. harzianum* and *T. viride*. Flasks were incubated for two weeks at 25°C. Cultivations were filtered using Whatman No. 1 filter paper of known weight. The filter papers were dried at 70°C until constant weight.

Biomass weight was measured using the equation:

$$\text{Biomass} = \text{weight of filter paper with biomass} - \text{weight of empty filter paper.}$$

Antibiosis activity

Fungal culture filtrates from *T. harzianum* and *T. viride* cultures grown on a minimal medium were tested for antibiosis activity. The filtrate was re-filtered using 0.45 µm and then 0.22 µm Millipore filters. Fifty percent of the filtrate was used with a sterile PDA medium, with the agar content adjusted before sterilization. The control treatment was a sterile PDA medium, free of culture filtrate. The media were poured into Petri dishes and inoculated with (0.5 cm) discs taken from a 4-day-old fresh colony of the pathogenic fungus *F. solani*. The dishes were incubated at 25°C for 7 days. The percentage of inhibition was measured according to the method described above in Antagonism Activity.

Determination of pH

The pH of the fungal culture filtrate produced by growing *Trichoderma spp.* on M9 medium was measured using a pH meter.

Statistical analysis

The experiments were carried out using a completely randomized design (CRD) with three replicates. Means were compared using the least significant difference (LSD) at a P value of 0.05 (Ruíz *et al.*, 2024).

RESULTS AND DISSECTION**Effect of different nitrogen sources on colony area (cm²), sporulation (LOG10), and biomass (Biomass g) of *T. harzianum* and *T. viride*.**

Colony area: (Table 1) shows that Aspartic acid was the most effective for *T. harzianum*, recording the highest colony area (60.79 cm²), followed by histidine and arginine (45.34 cm²). The highest value for colony growth was achieved with alanine for *T. viride* (51.50 cm²), while cysteine recorded the lowest value (28.26 cm²).

Sporulation: Aspartic acid had the highest effect on sporulation for *T. harzianum*, with a value of (8.1) followed by arginine (6.3) while sporulation was lower in the treatment with histidine and cysteine. Cysteine was the most effective in sporulation for *T. viride* with a value of 10.2, followed by alanine (9.8).

Biomass: *T. harzianum* recorded the highest biomass of Arginine (0.23 g), followed by cysteine (0.19 g), indicating that these acids stimulate the formation of Fungal biomass. *T. viride* had the highest biomass when using arginine (0.20 g), while values were lower with aspartic acid (0.14 g). Values in the control treatments were very low compared to the other treatments, with *T. harzianum* recording the lowest values for colony growth (6.28 cm²), reproduction (3.2), and biomass (0.05 g). *T. viride* also recorded very low values in all parameters (4.15 cm²) in growth, 2.6 in reproduction, and (0.15 g) in biomass.

Table 1: Effect of nitrogen sources on *T. harzianum* and *T. viride* colony area Sporulation Biomass

Fungi	Nitrogen sources	Colony area (cm ²)	Sporulation log ¹⁰	Biomass (g)
<i>T.harzianum</i>	Aspartic acid	60.79	8.1	0.16
	Histidine	45.34	5.4	0.18
	Arginine	45.34	6.3	0.23
	Cysteine	40.69	5.3	0.19
	Alanine	24.61	4.7	0.18
	Control	6.28	3.2	0.05
<i>T.viride</i>	Aspartic acid	45.34	6.3	0.14
	Histidine	31.15	6.6	0.16
	Arginine	31.15	6.5	0.20
	Cysteine	28.26	10.2	0.17
	Alanine	51.50	9.8	0.16
	Control	4.15	2.6	0.15
L.S.D		15.41	2.75	0.04

Effect of nitrogen sources on *T. harzianum* and *T. viride*, Antagonism, Antibiosis and PH

For *T. harzianum* result in (Table 2) shows that cysteine was the most effective, recording the highest antagonism activity value (87.52), followed by arginine (81.91) and histidine (77.14). Arginine for *T. viride* recorded the highest antagonism activity (84.41), followed by histidine (79.54) and cysteine (80.32). In both species, the control treatment had the least activity. Cysteine was also the most effective for *T. harzianum*, recording the highest value (98.7), followed by arginine (91.43) in *T. viride*. The effect was similar across the different amino acids, with arginine (85.79), cysteine (82.17), and histidine (84.73) recording the highest values. The control was the least active (57.20) for *T. harzianum* and 55.74 for *T. viride*. The pH did not change significantly with the addition of amino acids, ranging from 6.4 to 6.8. The control for *T. harzianum* had a significantly higher pH (9.9).

Table 2: The Effect of nitrogen sources on *T. harzianum* and *T. viride*, Antagonism, Antibiosis and pH

Fungi	Nitrogen sources	Antagonism Activity	Antibiosis Activity	pH
<i>T.harzianum</i>	Aspartic acid	75.47	59.18	6.7
	Histidine	77.14	58.87	6.4
	Arginine	81.91	91.43	6.7
	Cysteine	87.52	98.7	6.7
	Alanine	71.14	79.89	6.8
	Control	62.45	57.20	9.9
<i>T.viride</i>	Aspartic acid	71.17	78.48	6.5
	Histidine	79.54	84.73	6.5
	Arginine	84.41	85.79	6.6
	Cysteine	80.32	82.17	6.6
	Alanine	79.44	81.45	6.6
	Control	52.15	55.74	6.9
L.S.D		14.71	6.41	3.2

Effect of different carbon sources on colony area (cm²), sporulation (log¹⁰) and biomass (Biomass g) of *T. harzianum* and *T. viride*.

Colony area: Glucose was the most effective for *T. harzianum* recording the highest colonization area (55.39 cm²), followed by sucrose (52.78 cm²) and Fructose (41.83 cm²). Cellulose had the least effect on growth (13.85 cm²), while the control treatment showed the lowest value (4.15 cm²). Glucose also recorded the highest colonization area (55.39 cm²), followed by fructose (51.50 cm²). Cellulose had the least effect on *T. viride* (20.42 cm²), reflecting the poor ability of this fungus to use it as a carbon source. The control treatment showed the lowest growth (4.15 cm²).

Sporulation: For *T. harzianum*, glucose was the best sporulation-stimulating agent (6.4), followed by fructose (6.2), and then sucrose (6.1). Maltose for *T. viride* recorded the highest reproduction rate (7.2).

Biomass: For *T. harzianum*, fructose was the best source of biomass (0.21 g), followed by glucose (0.20 g) and then sucrose (0.19 g). For *T. viride*, Fructose was also the highest (0.21 g), while cellulose had the lowest value (0.18 g), which may indicate that it is consumed differently in this species compared to *T. harzianum*. The control treatment showed the lowest values in all parameters for both *T. harzianum* and *T. viride*. Reproduction was very low in the control treatment (3.1 and 2.6, respectively).

Table 3: The effect of different carbon sources on colony area (cm²), sporulation (log¹⁰) and biomass (Biomass g) of *T. harzianum* and *T. viride*.

Fungi	Carbon sources	Colony area cm ²	Sporulation log ¹⁰	Biomass (g)
<i>T.harzianum</i>	Fructose	41.83	6.2	0.21
	Glucose	55.39	6.4	0.20
	Sucrose	52.78	6.1	0.19
	Maltose	26.40	5.9	0.10
	Cellulose	13.85	4.7	0.11
	Control	4.15	3.1	0.04
<i>T.viride</i>	Fructose	51.50	6.7	0.21
	Glucose	55.39	6.8	0.22
	Sucrose	36.29	6.2	0.19
	Maltose	29.20	7.2	0.19
	Cellulose	20.42	4.8	0.18
	Control	4.15	2.6	0.09
L.S.D		15.41	2.75	0.045

Effect of carbon sources on *T. harzianum* and *T. viride*, Antagonism, Antibiosis and pH

Antagonism: For *T. harzianum* result in (Table 4) shows that *T. harzianum*, fructose, maltose, and glucose were the most effective in inhibiting *F. solani*, with values of (85.94) and (85.56), respectively, followed by Fructose (83.92). For *T. viride*, sucrose was the most efficient in antagonistic activity (82.88), followed by glucose (78.10), and then maltose (78.87). Cellulose showed the lowest values in both species (69.85) for *T. harzianum* and (78.00), For *T. viride*, indicating its weak effect on inhibiting *F. solani*. The control treatment was the least effective in both species.

Antibiosis: For *T. harzianum*, maltose had the best effect (96.92), followed by sucrose (89.78), and then fructose (80.12). For *T. viride*, glucose and sucrose had the highest effect 83.20 and 84.24, respectively, while cellulose was the least effective in this activity.

pH: All carbon sources lowered the pH of the environment compared to the control treatment (6.9) for *T. harzianum* and (6.8) for *T. viride*. Glucose had the greatest acidifying effect, reaching pH (5.4) for *T. harzianum* and (5.5) for *T. viride*. In contrast, cellulose and maltose recorded the highest pH values (6.5-6.7).

Table 4: The effect of carbon sources on *T. harzianum* and *T. viride*, antagonism, antibiosis and pH

Fungi	Carbon sources	Antagonism activity	Antibiosis activity	pH
<i>T.harzianum</i>	Fructose	83.92	80.12	6.3
	Glucose	85.56	57.80	5.4
	Sucrose	80.43	89.78	6.1
	Maltose	85.94	96.92	6.5
	Cellulose	69.85	78.45	6.5
	Control	61.32	56.16	6.9
<i>T.viride</i>	Fructose	69.88	77.06	6.2
	Glucose	78.10	83.20	5.5
	Sucrose	82.88	84.24	6.4
	Maltose	78.87	80.68	6.3
	Cellulose	78.00	79.58	6.7
	Control	51.21	49.21	6.8
L.S.D		18.11	8.31	2.9

T. harzianum and *T. viride* have demonstrated the ability to utilize a variety of sugars and amino acids as their sole carbon or nitrogen source. Different *Trichoderma* species have their own environmental preferences. However, *Trichoderma* species are distributed worldwide. Our study demonstrates that the widespread distribution of *T. harzianum* and *Trichoderma viride* is supported by their ability to utilize a variety of nutritional factors for their growth and development (Mohamed *et al.*, 2022; Guzmán-Guzmán *et al.*, 2023). Previous findings support studies indicating that amino acids play a critical role in stimulating fungal growth, as they are used as essential nitrogen sources in protein metabolism and biosynthesis (Hai-Ru *et al.*, 2011; Velmourougane and Prasanna, 2017). Aspartic acid in *T. harzianum* and alanine in *T. viride* were found to be the most conducive to colony growth, reflecting the role of these acids in promoting cell division and reproduction. Previous research has also indicated that arginine and cysteine contribute to the construction of cell walls and enhance fungal resistance to environmental stresses (Jayaswal *et al.*, 2003; Struck, 2015). Furthermore, the results showed that amino acids significantly enhanced fungal reproduction compared to the control group, with cysteine and arginine recording the highest reproduction rates. This is consistent with studies that have found that these acids stimulate spore production and biomass increase by stimulating metabolic pathways responsible for the production of proteins and enzymes necessary for fungal growth (Rajput *et al.*, 2014; Kamble and Kamble, 2021). Adding amino acids also contributed to lowering the pH of the fungal environment, which may help improve nutrient absorption and nutrient utilization efficiency.

Regarding the effect of carbon sources, the results confirmed that soluble sugars, such as glucose and sucrose, were the most stimulating for colony growth and fungal reproduction. This is consistent with studies indicating that these sugars are used as the primary energy source in biological processes (Rajput and Shahzad, 2015). Cellulose had the least effect, reflecting the need for specialized enzymes to digest it. This is consistent with previous studies showing that some fungi lack the ability to rapidly degrade cellulose compared to mono- and disaccharides (Sneniakuv, 2020; Gelain *et al.*, 2021).

The results demonstrate that cysteine and arginine, the amino acids, and glucose and maltose, the carbon sources, enhanced the antagonistic activity and bioproduction of antimicrobial compounds. These findings are supported by research showing that some amino acids play a role in the production of fungal antibiotics, while simple sugars are energy sources that support the secretion of these compounds (Danielson and Davey, 1973; Jun *et al.*, 2013; Sun *et al.*, 2021).

CONCLUSIONS

The results showed that amino acids and carbon sources had varying effects on the growth and reproduction of *T. harzianum* and *T. viride*. Aspartic acid was the most stimulating factor for *T. harzianum*, while cysteine had the highest growth rate in *T. viride*. Regarding carbon sources, glucose and fructose were the most effective in promoting fungal growth and reproduction, while

cellulose had the least effect. The study also demonstrated that some amino acids and carbon sources played a role in antifungal activity, with glucose and maltose showing the highest inhibitory capacity against *F. solani*, with minor effects on pH.

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دراسة تأثير مصادر النيتروجين والكربون على بعض الصفات الحيوية لنوعي المقاومة الحيوي:

Trichoderma harzianum and *Trichoderma viride*

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الملخص

تشير النتائج إلى أن استخدام مصادر النيتروجين ومصادر الكربون أثر بشكل ملحوظ على نمو *Trichoderma harzianum* و *Trichoderma viride*. حقق حامض الأسبارتيك أعلى مساحة مستعمرة *Trichoderma harzianum* (60.79 سم²)، بينما كانت أعلى مساحة مستعمرة *Trichoderma viride* مع الأليين (51.50 سم²)، وأقلها مع السيستين (28.26 سم²). كما أظهر الأرجينين تأثيراً إيجابياً على معدل الإنبات والكتلة الحيوية؛ حيث بلغ معدل الإنبات 8.1 *Trichoderma harzianum* و 10.2 *Trichoderma viride*، بينما سجلت الكتلة الحيوية أعلى قيمة، وهي 0.230 غ *Trichoderma harzianum* و 0.207 غ *Trichoderma viride*. كان الجلوكوز المصدر الأكثر فعالية؛ إذ بلغت مساحة المستعمرة 55.39 سم² T. *harzianum*، يليه السكر والفركتوز، بينما كان السليلوز الأقل فعالية (13.85 سم²). كما عززت مصادر الكربون النشاط الممرض، وخفضت الرقم الهيدروجيني إلى 5.4 و 5.5 مقارنةً بمجموعتي السيطرة (6.8 و 6.9).

الكلمات الدالة: مصادر الكربون، مصادر النيتروجين، *Trichoderma harzianum*، *Trichoderma viride*، التبويض.