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Trichoderma harzianum as fungicide and symbiont: is it safe for human and animals?

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Abstract

Trichoderma species are considered as biological control agents against numerous phytopathogenic fungi. They are also helpful for plants as plant symbiont. This study aimed to identify harmful effects of *Trichoderma* in laboratory animals. In the first step, inhalation toxicity was studied. Six rats as control received a spray of bio-formulation without spores. Ten rats as treatment A received 1.00×10^6 colony-forming unit (CFU) of *Trichoderma* spores and ten rats as treatment B received 1.00×10^7 CFU per test of *Trichoderma* spores. The harmful effects of *Trichoderma* were obvious especially in the lungs, liver and kidney, and some blood parameters were abnormal. In the second step, we studied acute oral toxicity by gavage. Four rats as control received bio-formulation without spores. Six rats as treatment A received 1.00×10^6 CFU per test of *Trichoderma* spores. Six rats as treatment B received 1.00×10^7 CFU per test of *Trichoderma* spores. The harmful effects of *Trichoderma* were noticeable more in the liver and kidney tissues. For dermal toxicity study, two rabbits as control received bio-formulation without spores by rubbing on the surface of the skin. Treatment groups A and B received 1.00×10^6 and 1.00×10^7 CFU per test of *Trichoderma* spores, respectively (four rabbits for each group). The liver and kidney and some blood parameters were abnormal. *Trichoderma* has some harmful effects on tissues and organs and although it is a natural product, it should be used under cautions.

Introduction

Trichoderma species are considered as biological control agents against numerous phytopathogenic fungi, including *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina.*[1-4] There are some reports of the usage of *Trichoderma* as an anti-parasite (*Toxocara canis*, *Leishmania amazonensis* and malaria) agent.[5-7] *Trichoderma* has been used for controlling human breast and cervical cancer.[8] Its species have been reported as pathogens of reptiles[9] and the list of infections that could be caused by *Trichoderma* spp. in humans is extensive.[10] *Trichoderma longibrachiatum* is the main human pathogen species within the genus and has been isolated with increasing frequency in recent years.[11] It produces toxic peptides called trilongins. Exposure to *T. longibrachiatum* cannot be treated with antimicrobial agents and dramatically weakens the immune system.[12] However, *Trichoderma* species also appear to belong to the growing list of emergent pathogens, with an increasing number of reports of invasive infections.[13,14] The problem is not limited to immunocompromised patients.[15] The antagonistic activity of *Trichoderma* isolates (NAS110) against plant pathogenic fungi: *R. solani*, *F. oxysporum*[16,17] *was* investigated in previous studies.[18-20]

To use *Trichoderma* as a fungicide and symbiotic, it is necessary to determine its potential side effects on the body of laboratory animals. This study aimed to determine the acute oral, dermal and pulmonary toxicity/pathogenicity of this agent according to the known scientific protocols.

Materials and Methods

***Trichoderma* preparation.** *Trichoderma harzianum* NAS110 was obtained from the microbial collection of Plant Pathology Laboratory (Nuclear Science and

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Technology Research Institute, Karaj, Iran). It was transferred to sterile potato dextrose broth under aseptic conditions and incubated at 28.00 ˚C for 72 hr. Then, the mycelial masses were transferred on potato dextrose agar medium and incubated in the same temperature conditions for 7 days. Spores were washed using saline solution from the surface of petri dishes and spore's suspension population was adjusted using a Neubauer slide (Paul Marienfeld, Lauda Konigshofen, Germany) at a concentration of 1.00×10^6 spore mL^{-1}. The spores were pelleted by centrifugation at 4,500 *g* for 10 min and washed twice in sterile saline solution. The washed spore suspension in distilled water was used as biomaterial of bio-formulation with components described as follow. Component of effective material (biomaterial) *T. harzianum* NAS110 included 2.00% *Trichoderma* spore, 98.00% of sucrose (Sigma-Aldrich, St. Louis, USA) as treatment A, and component of bio-formulation Trichofarm™ included 0.20% *Trichoderma* spore, 4.80% sucrose, 4.80% starch (Alphachemika Co., Mumbai, India), 0.20% Arabic gum (Sigma-Aldrich), and 90.00% kaolin (Sigma-Aldrich) as treatment B. All animals (rats and rabbits) were purchased from the animal house of Iran Pasteur Institute, Tehran, Iran. The study was carried out in accordance with the guidelines and regulations approved by the Animal Experimentation Committee of the Tabriz University (Approval Code: FVM.REC.1396.937; Approval Date: 02 October 2022), Tabriz, Iran.

Acute pulmonary pathogenicity (inhalation test). Twenty-six healthy young Wistar albino rats (13 of each sex) weighting 180 - 200 g (10 weeks old) were obtained and kept in metal and plastic cages. Food and water were *ad libitum*. The light and dark cycle was 12/12 hr and the temperature was set at 23.00 ˚C. After 1 week acclimatization period, they were divided as follows: Six animals (three of each sex) as control received a spray of bio-formulation without spores (Sucrose, Starch, Arabic gum, Kaolin with distilled water in the nose). Ten animals (five of each sex) as treatment A received 1.00×10^6 colony-forming unit (CFU) of *Trichoderma* spores. Ten animals as treatment B received 1.00×10^7 CFU of *Trichoderma* spores with intranasal route. The animals were then monitored for 21 days and were anesthetized 21 days after dosing by intraperitoneal injection of 70.00 mg kg^{-1} ketamine (Alfasan, Woerden, Netherlands) and 7.00 mg kg^{-1} xylazine (Alfasan) and after losing the pedal reflex, they were decapitated. Blood samples were collected of them. After necropsy, tissue specimens from the liver, kidney, spleen, heart and left lung were taken and fixed in buffered 10.00% formalin. After 1 week of fixation, the tissues were processed using a tissue processor and microscopic slides were made of them. The slides were stained by Hematoxylin and Eosin (H&E) and studied under a light microscope (ML2100; Krüss, Hamburg, Germany) and photomicrographs were created of them. The pathologic effects were classified as follow:

Liver) 0: No pathologic findings, 1: Slight hyperemia in sinusoids, 2: Hyperemia in sinusoids and central veins and/or hemorrhage, 3: Infiltrated inflammatory cells in tissue and/or cell degeneration, and 4: Scattered necrosis.

Heart) 0: No pathologic findings, 1: Hyperemia, 2: Hemorrhage and/or edema, 3: Infiltrated inflammatory cells in the tissue and/or cell degeneration, and 4: Scattered Necrosis.

Spleen) 0: No pathologic findings, 1: Hyperemia, 2: Hemorrhage, 3: Cell degeneration, and 4: Depletion of lymphoid tissue.

Kidney) 0: No pathologic findings, 1: Hyperemia, 2: Hemorrhage and /or protein secretion in renal tubules, 3: Cell swelling and/or degeneration of renal tubule epithelial cells and/or inflammation, and 4: Necrosis in the tubules.

Lung) 0: No pathologic findings, 1: hyperemia, 2: Hyperemia and slight hemorrhage and/or edema and emphysema, 3: epithelialization of type II pneumocytes, and 4: infiltrated inflammatory cells in tissue and/or scattered cell necrosis. The United States environmental protection agency preventing pesticides and toxic substances (7101) EPA712-c-96-318 February 1996. Microbial pesticide test guidelines OPPTS885-3150-Acute pulmonary toxicity/pathogenicity was followed.

Acute oral pathogenicity. Eighteen healthy young Wistar albino rats (nine of each sex) weighting 180 - 200 g (10 weeks old) were obtained and kept in metal and plastic cages. They were divided as follow: Four animals (two of each sex) as control received bio-formulation without spores, (sucrose, starch, Arabic gum and Kaolin) with distilled water with a stomach tube. Six animals (three of each sex) as treatment A received 0.20% (1.00×10^6 CFU) per test of washed spores of *Trichoderma* plus sucrose, starch, Arabic gum and kaolin with a stomach tube, six animals (three of each sex) as treatment B received 1.00×10^7 CFU per test of washed spores of *Trichoderma* plus sucrose with a stomach tube. Volume of suspension was 2.00 mL per 100 g body weight. The animals were fasted overnight before the test and after the substance had been administered, food was withheld for a further 3 - 4 hr. The animals were controlled for 21 days and were anesthetized using the previously mentioned protocol and after losing the pedal reflex were decapitated. Blood samples were collected from them. The rest of the procedure is like acute pulmonary toxicity. The United States environmental protection agency preventing pesticide and toxic substances (7101) EPA712-c-96-315 February1996. Microbial pesticide test guidelines OPPTS885-3050-Acute Oral Toxicity/pathogenicity was followed.

Acute dermal pathogenicity. Ten healthy young New Zealand white rabbits (five of each sex) weighting 2.50 - 3.00 kg were obtained and kept in metal cages. They received food and water *ad libitum*. The light and dark cycle was 12/12 hr and the temperature was set at

22.00 ˚C. After 1 week acclimatization period, they were divided as follow: Two animals (one of each sex) as control received bio-formulation without spores of *Trichoderma* by rubbing on the surface of the skin. Four animals as treatment A and four animals as treatment B. According to the protocol, acute dermal toxicity is the adverse effect occurring during or following 24 hr dermal exposure to a single dose of a test substance. The animals were monitored for 14 days and euthanized by an overdose of 60.00 mg kg^{-1} sodium thiopental (Hospira, Illinois, USA) 14 days after dermal exposure and blood samples were collected. The rest of the procedure was like the acute pulmonary toxicity. The United States environmental protection agency preventing pesticide and toxic substances (7101) EPA712-c-96-316 February 1996. Microbial pesticide test guidelines OPPTS885-3100-Acute Dermal Toxicity/pathogenicity was followed.

Blood analysis. The blood parameters and liver enzymes were measured in all dermal, oral and respiratory test groups. These parameters were white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count, carcinoembryonic antigen (CEA), glycosylated Hb, alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Statistical analysis. Data of our grading system were processed with SPSS Software (version 22.0; IBM Corp., Armonk, USA). For processing the pathologic data, the Kruskal-Wallis and Mann-Whitney U tests were used. For data of blood parameters, one-way ANOVA with the Tukey test as post hoc were used. The confidence interval was less than 0.05 in all the tests.

Results

Acute pulmonary pathogenicity (inhalation test). After 21 days of trial and microscopic study, information was gathered and shown in Table 1. In the group with *T. harzianum* spores, some pathologic effects were observed in liver (hemorrhage, hyperemia, infiltration of inflammatory cells), kidney (hyperemia,) and lung (edema, infiltration of inflammatory cells, type II pneumocyte epithelialization) but not in spleen and heart. There were no statistical differences between two treatment groups (Fig. 1).

Acute oral pathogenicity. After 21 days of trial, microscopic study information was gathered and is shown in Table 1. In the group with *T. harzianum* spore's treatment, some pathologic effects were observed in the liver (hyperemia, infiltration of inflammatory cells) and kidney (Hyperemia, hyaline cast formation), but not in the spleen and heart. Although some slight pathologic changes were observed in the lung (hemorrhage, infiltration of inflammatory cells), changes in the lungs were not statistically different between the control and treatment groups. There were no statistical differences between the two treatment groups (Fig. 2). With the comparison of oral and respiratory route of administration, just lungs were more affected in respiratory route and pathologic effects (edema, infiltration of inflammatory cells, epithelialization in the lungs) were more severe with respiratory administration.

Acute dermal pathogenicity. After 14 days of trial, microscopic study information was gathered and is shown in Table 1. In the group with *Trichoderma* spore's treatment, some pathologic effects were observed in the liver (hyperemia, infiltration of inflammatory cells), kidney (hyperemia, cell swelling in renal tubules) and lung (hyperemia, emphysema, epithelialization of type II pneumocytes) but not in the heart. Although some slight pathologic changes were observed in the spleen, the changes were not statistically significant between control and treatment groups. There were no statistical differences between two treatment groups (Fig. 3).

Blood sample tests. Blood samples were taken at the time of euthanasia and different parameters (Liver enzymes: ALP, ALT, AST and Blood parameters: CBC, CEA and glycosylated Hb) were measured in them. Liver enzymes (AST, ALT, and ALP) showed enhancement in all the test groups (oral, dermal, and inhalation), but there were no statistical differences between the two *Trichoderma* groups. This meant the substance was harmful to the liver which was shown in pathology, as well. The WBC count, RBC count and Hb have been changed with the use of *Trichoderma*. The WBC count has been enhanced with the usage of *Trichoderma*. The RBC count and Hb have been increased after the use in all three forms (oral, dermal, and respiratory). Information was shown in Tables 2 and 3.

Table 1. Pathogenicity of *Trichoderma* spores in different organs after inhalation, oral and dermal exposure are shown as mean ± standard error of mean.

Organ	Route	Control	Treatment A	Treatment B
Liver	Inhalation	0.00	2.67 ± 0.42*	2.00 ± 0.44*
	Oral	0.00	2.45 ± 0.31*	2.13 ± 0.28*
	Dermal	0.00	2.25 ± 0.48*	2.55 ± 0.29*
Kidney	Inhalation	0.00	2.67 ± 0.21*	2.67 ± 0.21*
	Oral	0.00	1.80 ± 0.29*	1.74 ± 0.34*
	Dermal	0.00	1.75 ± 0.48*	1.75 ± 0.25*
Spleen	Inhalation	0.25 ± 0.25	0.33 ± 0.21	0.33 ± 0.21
	Oral	0.17 ± 0.17	0.27 ± 0.13	0.45 ± 0.16
	Dermal	0.33 ± 0.33	0.33 ± 0.33	0.75 ± 0.25
Heart	Inhalation	0.00	0.55 ± 0.22	0.83 ± 0.48
	Oral	0.00	0.61 ± 0.27	0.69 ± 0.27
	Dermal	0.00	0.00	0.00
Lung	Inhalation	0.00	0.83 ± 0.48*	2.10 ± 0.36*
	Oral	0.00	1.24 ± 0.39	0.66 ± 0.34
	Dermal	0.00	1.51 ± 0.29*	2.01 ± 0.73*

* indicates statistical difference with the group control at $p \leq 0.05$.

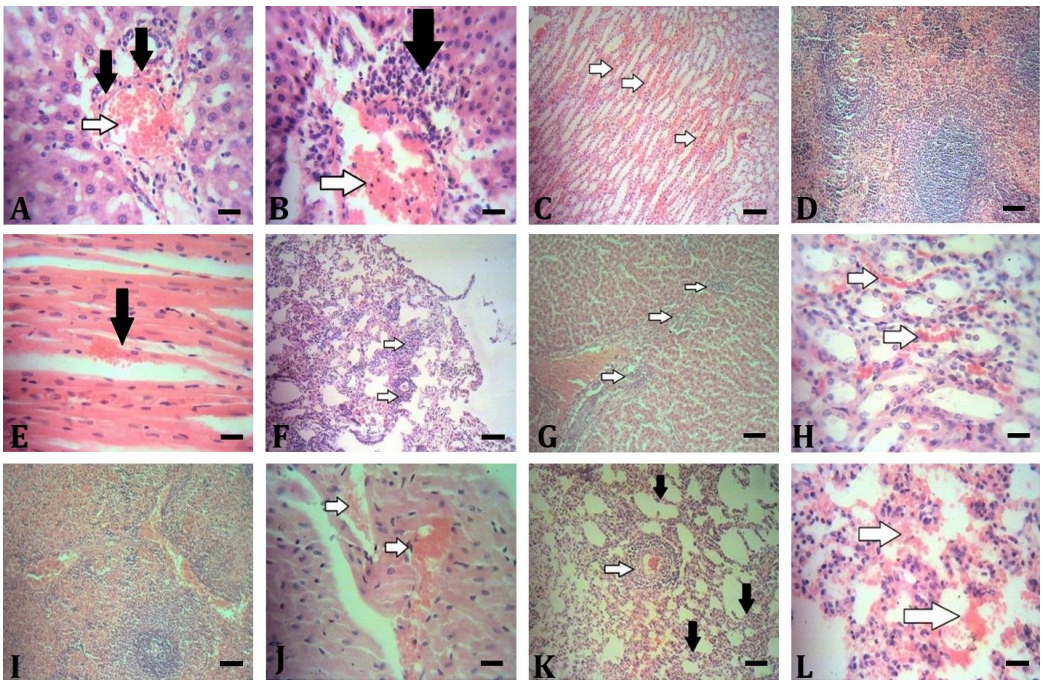

Fig. 1. Histopathogenicity of *Trichoderma* spore after inhalation exposure in different organs of treatments A (A – F) and B (G - L). **A)** Hemorrhage (black arrows) and hyperemia (white arrow) in the liver, **B)** Infiltration of inflammatory cells (black arrow) and hyperemia (white arrow) in the liver, **C)** Hyperemia in kidney (arrows), **D)** Normal spleen, **E)** Hyperemic heart muscle (arrows), and **F)** Infiltration of inflammatory cells in lung (arrows). **G)** Infiltration of inflammatory cells in liver (arrows), **H)** Hyperemia in kidney (arrows), **I)** Hyperemia in spleen (panoramic view), **J)** Hyperemia in heart muscle (arrows), **K)** Epithelialization of type II pneumocyte (black arrows) and Infiltration of inflammatory cells around a vessel (white arrow) in lung, and **L)** Edema in lung (arrows), (H&E staining, bars = 50.00 µm).

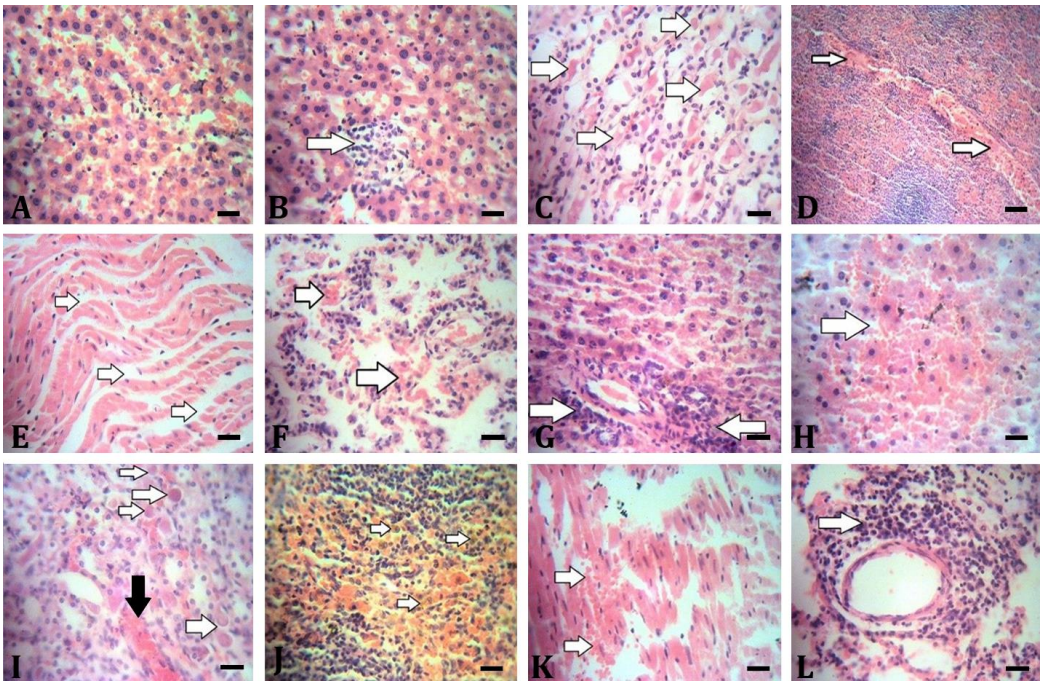

Fig. 2. Histopathogenicity of *Trichoderma* spore after oral exposure in different organs of treatments A (A – F), and B (G - L). **A)** Hyperemia in liver (panoramic view), **B)** Infiltration of inflammatory cells in liver (arrow), **C)** Protein secretion (hyaline cast and nephrotic syndrome) in renal tubules (arrows), **D)** Hyperemia in spleen (arrows), **E)** Edema in heart muscle (arrows), and **F)** Hemorrhage in lung (arrows). **G)** Infiltration of inflammatory cells in portal area of liver (arrows), **H)** Hemorrhage in liver (arrow), **I)** Protein secretion (hyaline cast and nephrotic syndrome) in renal tubules (horizontal white arrows) and hyperemia (transparent vertical arrow) in kidney, **J)** Hemosiderophages and hemosidrin pigments that is a sign of hemorrhage (arrows) in spleen, **K)** Hemorrhage in heart muscle (arrows), and **L)** Infiltration of inflammatory cells around a vessel in lung (arrow), (H&E staining, bar = 50.00 µm).

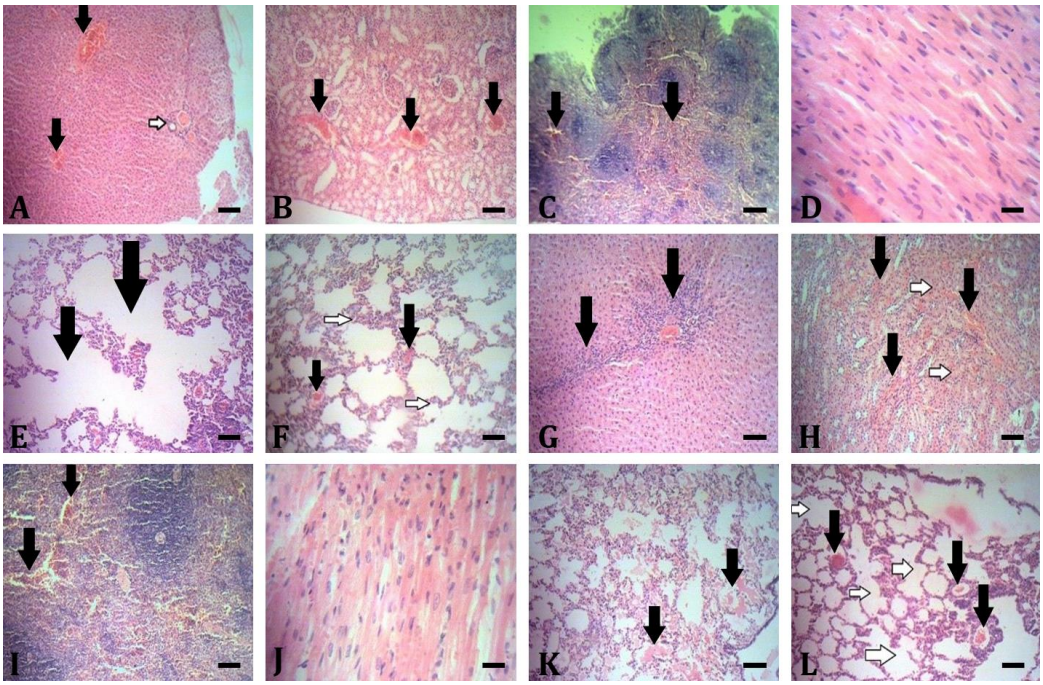

Fig. 3. Histopathogenicity of *Trichoderma* spore after dermal exposure in different organs of treatments A (A – F), and B (G - L). **A)** Hyperemia (black arrows) and slight infiltration of inflammatory cells in portal area of liver (white arrow), **B)** Hyperemia in kidney (arrows), **C)** Hyperemia in spleen (arrows), **D)** Normal heart muscle, **E)** Emphysema in lung (arrows), and **F)** Hyperemia (black arrows) and epithelialization of type II pneumocytes (white arrows) in lung. **G)** Infiltration of inflammatory cells in liver (arrows), **H)** Hyperemia (black arrows) and cell swelling in renal tubules (white arrows), **I)** Hyperemia (arrows) in spleen, **J)** Normal heart muscle, **K)** Pulmonary edema (arrows), and **L)** Hyperemia (black arrows) and emphysema (white arrows) in lung, (H&E staining, bar = 50.00 µm).

Table 2. Data of some blood parameters in experimental groups.

Groups	WBC ($\times10^3$ µL^{-1})	RBC ($\times10^6$ µL^{-1})	Hb (g dL^{-1})	HCT (%)	MCV (fL)	MCH (pg)	MCHC (%)	Platelet ($\times10^3$ µL^{-1})	Hb A1-C (%)
1-K	6.1 9 ± 0.97	7.62 ± 0.50	13.40 ± 0.11	36.50 ± 2.31	47.90 ± 1.70	16.00 ± 0.55	33.40 ± 2.60	432 ± 17.33	4.44 ± 0.17
2-K	4.72 ± 0.26	5.52 ± 0.17	15.50 ± 0.23	38.90 ± 2.30	45.70 ± 2.30	16.00 ± 0.46	35.00 ± 2.30	341 ± 23.00	4.03 ± 0.34
3-K	5.24 ± 0.24	7.14 ± 0.09	13.50 ± 0.26	36.80 ± 2.30	51.50 ± 1.70	17.20 ± 0.20	33.40 ± 2.00	234 ± 11.62	4.38 ± 0.10
4-T	3.59 ± 0.14[a]	9.72 ± 0.15[a]	15.40 ± 0.17[a]	41.40 ± 2.61	42.60 ± 1.30	15.80 ± 0.80	37.20 ± 1.70	440 ± 31.80	5.70 ± 0.24
5-T	2.80 ± 0.17[b]	9.58 ± 0.20	15.30 ± 0.09[b]	42.50 ± 2.45	44.40 ± 0.20	16.00 ± 0.88	36.00 ± 2.30	384 ± 25.40	5.75 ± 0.21
6-T	4.53 ± 0.17	8.67 ± 0.32[c]	14.00 ± 0.40[c]	42.10 ± 1.30	48.60 ± 2.70	16.10 ± 0.14	33.30 ± 2.10	207 ± 49.00	5.30 ± 0.09
7-F	3.30 ± 0.19[d]	9.07 ± 0.17[a]	15.40 ± 0.12[a]	43.20 ± 0.68	47.60 ± 2.20	17.00 ± 0.52	35.60 ± 3.20	389 ± 5.78	4.46 ± 0.11
8-F	3.07 ± 0.37[be]	9.68 ± 0.37[b]	14.90 ± 0.05b	43.10 ± 1.47	44.50 ± 2.80	15.40 ± 0.58	34.60 ± 2.88	332 ± 29.48	5.35 ± 0.23
9-F	4.38 ± 0.15[cf]	7.56 ± 0.50	14.90 ± 1.29[c]	43.90 ± 2.05	58.10 ± 3.03	19.70 ± 0.29	33.90 ± 2.21	441 ± 17.32	5.91 ± 0.21
Normal range	3.40 - 14.00	6.90 - 12.20	12.00 - 18.90	35.50 - 70.20	44.00 - 62.50	13.00 - 19.00	26.50 - 58.00	325 - 888	< 6.00

WBC: White blood cells, RBC: Red blood cells, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, and MCHC: Mean corpuscular hemoglobin concentration.
K: Formulation material without *Trichoderma* (1: Oral, 2: Dermal, 3: Respiratory).
T: *Trichoderma harzianum* NAS110 (bio-material)-1.00 × 10^7 colony-forming unit (4: Oral, 5: Dermal, 6: Respiratory).
F: Bio-formulation of *Trichoderma harzianum* NAS110 - 1.00 × 10^6 colony-forming unit (7: Oral, 8: Dermal, 9: Respiratory).
[a] indicates statistical significance with 1-k, [b] indicates statistical significance with 2-k, [c] indicates statistical significance with 3-k, [d] indicates statistical significance with 4-T, [e] indicates statistical significance with 5-T, and [f] indicates statistical significance with 6-T ($p < 0.05$).

Table 3. Data of liver enzymes and carcinoembryonic antigen test in experimental groups.

Groups	AST ($U\ L^{-1}$)	ALT ($U\ L^{-1}$)	ALP ($U\ L^{-1}$)	CEA ($ng\ mL^{-1}$)
1-K	148 ± 18.70	138 ± 15.20	330 ± 21.50	0.33 ± 0.07
2-K	440 ± 23.60	218 ± 10.68	142 ± 9.33	0.21 ± 0.02
3-K	320 ± 20.27	220 ± 16.70	165 ± 10.89	0.29 ± 0.02
4-T	153 ± 18.00	171 ± 13.10	296 ± 10.11	0.30 ± 0.03
5-T	943 ± 72.35	240 ± 10.31[b]	288 ± 0.17[b]	0.25 ± 0.02
6-T	332 ± 14.46[c]	401 ± 17.70	485 ± 6.00[c]	0.39 ± 0.04
7-F	361 ± 14.43[a]	233 ± 16.25[ad]	250 ± 11.54[a]	0.10 ± 0.02
8-F	597 ± 20.22[be]	108 ± 17.30[be]	290 ± 17.30[b]	0.33 ± 0.03
9-F	187 ± 11.78[f]	203 ± 18.40[f]	198 ± 17.90[f]	0.42 ± 0.12
Normal range	55.00 - 362	40.00 - 170	230 - 355	< 2.50

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, and CEA: Carcinoembryonic antigen.
K: Formulation material without *Trichoderma* (1: Oral, 2: Dermal, 3: Respiratory).
T: *Trichoderma harzianum* NAS110 (bio-material)-1.00×10^7 colony-forming unit (4: Oral, 5: Dermal, 6: Respiratory).
F: Bio-formulation of *Trichoderma harzianum* NAS110 - 1.00×10^6 colony-forming unit (7: Oral, 8: Dermal, 9: Respiratory).
[a] indicates statistical significance with 1-k, [b] indicates statistical significance with 2-k, [c] indicates statistical significance with 3-k, [d] indicates statistical significance with 4-T, [e] indicates statistical significance with 5-T, and [f] indicates statistical significance with 6-T ($p < 0.05$).

Discussion

With the usage of *Trichoderma harzianum*, different organs showed pathologic features but, in the liver, (hemorrhage, hyperemia, and infiltration of inflammatory cells), kidney (hyperemia, hyaline cast formation, cell swelling in renal tubules), and lung (edema, emphysema, infiltration of inflammatory cells, type II pneumocyte epithelialization) changes were statistically significant. Changes in the lungs were statistically significant just in the inhalation test group but pathologic findings existed in all the organs in all tests. Dose of 1.00×10^7 CFU showed more pathologic effects than dose of 1.00×10^6 CFU and the hazards were dose dependent. Liver enzymes (AST, ALT, and ALP) showed enhancement in all test groups (oral, dermal, and inhalation), however, there were no statistical differences in the two *Trichoderma* groups. This meant the substance was harmful to the liver which was shown in pathology, as well. The WBC count, RBC count and Hb have been changed with the use of *Trichoderma*. The WBC count has been enhanced with the usage of *Trichoderma*. The RBC count and Hb have been increased after the use in all three forms (oral, dermal, and respiratory).

Trichoderma species can cause localized infections such as pulmonary mycetoma, peritonitis, sinusitis, otitis, brain abscess and fatal disseminated disease.[11] The conidia of *Trichoderma* molds that are inhaled can interact with the airway epithelium causing symptoms

Trichoderma species can cause localized infections such as pulmonary mycetoma, peritonitis, sinusitis, otitis, brain abscess and fatal disseminated disease.[11] The conidia of *Trichoderma* molds that are inhaled can interact with the airway epithelium causing symptoms similar to those of Stachybotrys (black mold). The symptoms that follow the inhalation include, however, are not limited to sneezing, asthmatic attacks, prolonged coughing and infections of the lungs (e.g., pneumonia). *Trichoderma* species are a serious threat to immunocompromised patients. One species in this genus, *Trichoderma longibrachiatum*, is extremely harmful and toxic to human.[21]

In one case, thoracic tomodensitometry revealed bilateral pneumothorax, pneumopericardium and a dense lesion of the right apex.[14] Also, in one case *Trichoderma harzianum* was isolated from a pediatric patient with hematological malignancy (acute lymphoblastic leukemia). The child had pulmonary involvement and the mold was resistant to treatment.[22]

In a comprehensive search, 16 well-documented published cases of invasive *Trichoderma* infection have been found.[14] Skin lesions (necrotizing ulcerative) were observed in three patients.[14] *Trichoderma* species have also been isolated from food and contaminated food may explain the digestive involvement.[14,23] Direct microscopic examination of sputum, bronchoaspiration, and bronchoalveolar lavage fluid samples revealed the presence of fungal septate hyphae.[14] A recent case of otitis externa in ahealthy 12-year-old boy was resolved following treatment. In one research, *T. longibrachiatum* was administered to mice. The mortality of the infected mice was correlated with inoculum size.

The mortality rate of mice challenged with 1.00×10^4 and 1.00×10^5 CFU per animal was 25.00%; the rate was 62.50% in those challenged with 1.00×10^6 CFU per animal. All mice infected with 1.00×10^7 CFU per animal were died.[23] In another survey, inhaled spores could reach the alveoli and interacted with the airway epithelium. The fungal spores were found to trigger histamine release from the bronchiole associated lymphoid tissue Cells however, relatively high concentrations (0.10 - 2.00 $mg\ mL^{-1}$) were needed. A similar dose response was obtained in basophil histamine release. Mucosal mast cells from the airways were susceptible to the potentiating effect of *Trichoderma*.[24]

Some of these fungi that can act as human pathogens also produce mycotoxin. For example, trichodermin, a mycotoxin in the trichothecins group, is secreted by *Trichoderma brevicompactum*. Commonly, it contaminates the food source and consumption of these mycotoxin leads to vomiting and gastroenteritis.[25,26]

Trichoderma longibrachiatum is a human pathogen to immunosuppressed individuals, still other species can also cause infection in this type of individuals, such as

T. citrinoviride and *T. harzianum*. The list of illnesses that could be caused by *Trichoderma* spp. is extensive, including peritonitis and intra-abdominal abscess in patients undergoing continuous ambulatory peritoneal dialysis, liver infection, acute invasive sinusitis and disseminated infections of transplant recipients, brain abscess, skin infection, necrotizing stomatitis and pulmonary infections of patients with hematological malignancies, fungemia by contaminated saline, rhinosinusitis, pulmonary myeloma and fibrosis, hypersensitivity pneumonitis, endocarditis, otitis externa, cerebrospinal fluid infection and allergic reactions.[10,18] *Trichoderma* spp. bears a toxin called gliotoxin. Some of the effects of *Trichoderma* is referred to gliotoxin. Gliotoxin has high persistence in the water, soil and air, therefore, it is a risk to human health. This toxin is toxic for both animals and humans being lethal in relatively low concentrations.[27]

Gliotoxin can affect innumerable cell mechanisms leading to the death of the cells. The effects of gliotoxin on cells seem to be related not only to the concentration, but also to the cell type. In human and some animals, it is known that gliotoxin inhibits the function of the NADPH (reduced nicotinamide adenine dinucleotide phosphate) enzyme complex.[25] It can also induce apoptotic cell death of thymocytes, peripheral lymphocytes, macrophages and others.[15,28] According to references the prevalent species in the majority of *Trichoderma*-based products is *T. harzianum* (83.00%).[29]

This research showed that *T. harzianum* had pathologic effects on different organs and the blood with oral, dermal and respiratory usage and it must be used with care in agriculture.

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Conflict of interest

The authors declare no competing financial interest.

References

1. Sahampoor L, Zaker Tavallaie F, Fani SR, et al. *In vitro* efficiency of *Trichoderma harzianum* mutants in biocontrol of *Fusarium oxysporum f. sp. radicis-cucumerinum*. J Crop Prot 2020; 9(2): 285-300.
2. Harman GE. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology 2006; 96(2): 190-194.
3. Lava A, Babaeizad V, Tajick Ghanbary MA, et al. Effects of Trichoderma and endophytic fungus *Piriformospora indica* on cucumber physiology and Fusarium wilt disease of Cucumber. Annals of RSCB 2021; 25(4): 20742-20755.
4. Orojnia S, Habibi D, Shahbazi S, et al. Investigation the effect of different formulations of mutated trichoderma with gamma ray on soybean morphological indices [Persian]. Crop Physiol IZU Ahvaz 2021; 12(48): 97-115.
5. Abdel-Latif AMH, Haggag WM. Biocontrol potential of salinity tolerant mutants of *Trichoderma harzianum* against *Fusarium oxysporum*. Braz J microbiol 2006; 37(2): 181-191.
6. Lopes DS, Dos Santos UR, Dos Anjos DO, et al. Ethanolic extract of the fungus *Trichoderma asperelloides* induces ultrastructural effects and death on *Leishmania amazonensis*. Front Cell Infect Microbiol 2020; 10: 306. doi: 10.3389/fcimb.2020.00306.
7. de Souza Maia Filho F, da Silva Fonseca AO, Persici BM, et al. *Trichoderma virens* as a biocontrol of *Toxocara canis*: *in vivo* evaluation. Rev Iberoam Micol 2017; 34(1): 32-35.
8. Abd El-Rahman AA, El-Shafei SM, Ivanova EV, et al. Cytotoxicity of *Trichoderma* spp. cultural filtrate against human cervical and breast cancer cell lines. Asian Pac J Cancer Prev 2014; 15(17): 7229-7234.
9. Munoz FM, Demmler GJ, Travis WR, et al. *Trichoderma longibrachiatum* infection in a pediatric patient with aplastic anemia. J Clin Microbiol 1997; 35(2): 499-503.
10. Mukherjee PK, Horwitz BA, Singh US, et al. Trichoderma: biology and applications. London, UK: CAB International 2013; 1-10.
11. Trabelsi S, Hariga D, Khaled S. First case of *Trichoderma longibrachiatum* infection in a renal transplant recipient in Tunisia and review of the literature. Tunis Med 2010; 88(1): 52-57.
12. Chouaki T, Lavarde V, Lachaud L, et al. Invasive infections due to *Trichoderma* species: report of 2 cases, findings of *in vitro* susceptibility testing, and review of the literature. Clin Infect Dis 2002; 35(11): 1360-1367.
13. Tang P, Mohan S, Sigler L, et al. Allergic fungal sinusitis associated with *Trichoderma longibrachiatum*. J Clin Microbiol 2003; 41(11): 5333-5336.
14. Akagi T, Kawamura C, Terasawa N, et al. suspected pulmonary infection with *Trichoderma longibrachiatum* after allogeneic stem cell transplantation. Intern Med 2017; 56(2): 215-219.
15. Scharf DH, Remme N, Heinekamp T, et al. Transannular disulfide formation in gliotoxin biosynthesis and its role in self-resistance of the human pathogen *Aspergillus fumigatus*. J Am Chem Soc 2010; 132(29): 10136-10141.
16. Ghasemi S, Safaie N, Shahbazi S, et al. The role of cell wall degrading enzymes in antagonistic traits of *Trichoderma virens* against *Rhizoctonia solani*. Iran J Biotechnol 2020; 18(4): e2333. doi: 10.30498/IJB.2020.2333.
17. Soufi E, Safaie N, Shahbazi S, et al. Gamma irradiation

induces genetic variation and boosting antagonism in *Trichoderma aureoviride*. Arch Phytopathol Plant Prot 2021; 54: 1649-1674.
18. Schuster A, Schmoll M. Biology and biotechnology of Trichoderma. Appl Microbiol Biotechnol 2010; 87(3): 787-799.
19. Shahbazi S, Zaker Tavallaie F, Daroodi Z. Morphological and molecular identification of *Fusarium* spp. associated with carnation *Dianthus caryophyllus* in Mahallat, Iran. J Crop Prot 2021; 10(3): 461-471.
20. Zin NA, Badaluddin NA. Biological functions of *Trichoderma* spp. for agriculture applications. Ann Agri Sci 2020; 65: 168-178.
21. Harman GE. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T22. Plant Dis 2000; 84(4): 377-393.
22. Kantarcioğlu AS, Celkan T, Yücel A, et al. Fatal *Trichoderma harzianum* infection in a leukemic pediatric patient. Med Mycol 2009; 47(2):207-215.
23. Prelle A, Spadaro D, Garibaldi A, et al. A new method for detection of five alternaria toxins in food matrices based on LP-APCI-MS. Food Chem 2013; 140(1-2): 161-167.
24. Larsen FO, Clementsen P, Hansen M, et al. The indoor microfungus *Trichoderma viride* potentiates histamine release from human bronchoalveolar cells. APMIS1996; 104(9): 673-679.
25. Zeilinger S, Gruber S, Bansal R, et al. Secondary metabolites in *Trichoderma* – Chemistry meets genomics. Fungal Biol Rev 2016; 30(2): 74-90.
26. Woo SL, Ruocco M, Vinale F, et al. *Trichoderma*-based products and their widespread Use in agriculture. Open Mycol J 2014; 8: 71-126.
27. Vidal-García M, Redrado S, Domingo MP, Marquina P, et al. Production of the invasive Aspergillosis biomarker Bis (methylthio) gliotoxin within the genus *Aspergillus*: *In Vitro* and *in Vivo* metabolite quantification and genomic analysis. Front Microbiol 2018; 9: 1246. doi: 10.3389/fmicb.2018.01246
28. Vargas WA, Mukherjee PK, Laughlin D, et al. Role of gliotoxin in the symbiotic and pathogenic interactions of *Trichoderma virens*. Microbiology (Reading) 2014; 160(Pt 10): 2319-2330.
29. Topolovec-Pintarić S. Trichoderma: Invisible partner for visible impact on agriculture. IntechOpen 2019; doi:10.5772/intechopen.83363.