

Assessment of the haematological parameters and biochemical indices of albino rats fed with spores of *Trichoderma viride*

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Abstract. The haematological as well as some enzymes biomarkers of albino rats orogastically dosed with different concentrations of *Trichoderma viride* as well as *T. viride* subjected to various treatments were evaluated. Generally, red blood cell count, haemoglobin content and packed cell volume of the control group were significantly higher than that of animals dosed with different concentrations of *T. viride*. The same trend was observed when *T. viride* was amended with 1% lactose, sodium nitrate and sodium chloride. There were no significant differences in the alanine aspartate, alkaline phosphatase and alanine transaminase values of animals dosed with lower concentrations of *T. viride*. This study suggests that lower concentration of *T. viride* could be used in controlling fungal phytopathogens.

Keywords: *Trichoderma viride*; Enzyme biomarkers; Albino rats; Heamatology; Orogastically dosed.

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Introduction

Phytopathogenic fungi are known cause a lot of diseases to economically important crops worldwide (Gohel et al., 2006; Heydari and Pessarakli, 2010; Todorova and Kozhuharova, 2010). These diseases have been controlled by the application of large quantities of chemical fungicides. However, their extensive use causes serious pollution problem in the environment (Raghunathan and Divakar, 1996). Therefore, there is the need for an alternative (Zegeye et al., 2011).

According to Daayf et al. (2003)

Biological control using microorganisms to control these diseases provides protection essentially through production of antibiotics or other molecules that are deleterious to the pathogen's development, competition with the

pathogen for nutrients and space or induced plant resistance (Daayf et al., 2003).

It employs natural enemies of pests or pathogens to eradicate or control their population. The induction of plant resistance using non-pathogenic or incompatible microorganisms is also a form of biological control (Cook, 1993; Schouten et al., 2004).

A host of plant pathogenic fungi such as *Rhizoctonia solani*, *Pythium aphanidermatum*, *Fusarium oxysporum*, *Fusarium culmorum*, *Gaeumannomyces graminis* var. *tritici*, *Sclerotium rolfsii*, *Phytophthora cactorum*, *Botrytis cinerea* and *Alternaria* spp. have been effectively controlled by *Trichoderma* species (Jones and Stewart, 1997; Kucuk and Kivanc, 2003; Dolatabadi et al., 2011).

Trichoderma spp. are free-living fungi that are common in as well as on plant surfaces (Islam et al., 2011). *Trichoderma* species lytic enzymes such as chitinases, glucanases and proteases which are essential in biocontrol of plant diseases (Gajera and Vakharia, 2012). Previous study by Ekundayo et al. (2015) has shown the antagonistic activities of *T. viride* against *Sclerotium rolfsii*, soil borne pathogen of many agricultural and horticultural crops. However, there is dearth of information on the toxicological effects of this particular fungus. Therefore, this present investigation was conducted to determine the effects of this fungus on the haematology and some enzymes biomarkers of albino rats.

Materials and methods

Fungal isolates and treatment conditions

Trichoderma viride, an isolate of maize cob was obtained from the Department of Microbiology, The Federal University of Technology, Akure (FUTA). The fungus was cultivated using LAB M potato dextrose broth in conical flask while other flasks containing *T. viride* was amended separately with 1% NaNO₃, lactose and NaCl.

Experimental animals

A total number of 30 albino rats were obtained from the Department of Animal Production, The Federal University of Technology, Akure, and were caged in a well ventilated conditions. Prior to the experiments, the animals were weighed and stabilized for a period of 8 days by giving them grower mash prepared by Guinea Feed Nigeria Limited and water *ad libitum*.

Animal grouping and administration of *T. viride*

The animals were grouped into ten with three rats in a group for a period of 7 days. The treated albino rats were orogastically dosed with the different treatments: 1, 0.5, 0.1 mL *T. viride*, 1 mL *T. viride* supplemented with 1% NaNO₃, 1 mL *T. viride* supplemented with 1% NaCl, and 1 mL *T. viride* supplemented with 1% lactose. There were 4 sets of control, (1) animals fed with 1 mL NaNO₃, (2) those fed with 1 mL lactose, (3) those fed with 1 mL NaCl, and (4) those fed with clean water only. After the expiration of 7 days of treatment, the animals were sacrificed and their haematological parameters and enzyme biomarkers were determined (Adebolu et al., 2011).

Haematological analysis of blood samples

The blood samples were collected into EDTA anticoagulant bottles. Haematological parameters; packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), mean cell haemoglobin concentration (MCHC) and differential leucocyte count for lymphocytes, neutrophil, eosinophil, basophil, and monocytes were determined according to standard methods (D'Armour et al., 1956; Wintrobe et al., 1961; Aning et al., 1998).

Determination of liver functioning tests

Some liver functioning tests, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined according to the protocols of Babson et al. (1966) and Momoh et al. (2013).

Data analysis of results

The data collected were then subjected to Analysis of variance (ANOVA) and the means were separated by Duncan's New Range Multiple Test at $P = 0.05$, using SPSS version 15.0 Microsoft Windows XP.

Results

Table 1 shows the haematological parameters of albino rats subjected to various treatments. The results show a significant difference in the erythrocyte sedimentation rate of all the treatments. The highest erythrocyte value was obtained from $\text{NaNO}_3 + T. viride$. There was no significant difference in the packed cell volume of albino rats fed with basal diet and lactose. The least PCV was obtained from rats fed with $\text{NaNO}_3 + T. viride$. Also, the highest haemoglobin content was obtained from control group although there was no significant difference in the haemoglobin content of lactose and NaCl fed albino rats. The white blood cell count of albino rats fed with 1.0 mL of *T. viride* was significantly higher than that of control group.

Table 1. Haematological parameters of albino rats under various treatments.

Treatment	ESR (%)	PCV (%)	HB (%)	WBC ($\times 10^3$)/mm ³	RBC (%)
<i>T. viride</i> 1 mL	8.00 \pm 1.32a	27.00 \pm 1.00d	9.00 \pm 0.33e	17.57 \pm 1.60a	6.47 \pm 0.45de
<i>T. viride</i> 0.5 mL	4.67 \pm 0.58b	29.67 \pm 0.58c	9.89 \pm 0.19d	14.27 \pm 0.25b	8.00 \pm 0.87bc
<i>T. viride</i> 0.1 mL	2.50 \pm 0.50de	34.00 \pm 1.00b	11.38 \pm 0.40b	10.13 \pm 0.68d	8.90 \pm 0.36b
NaNO_3 (1 mL)	4.33 \pm 0.76bc	30.00 \pm 1.00c	9.99 \pm 0.36d	11.23 \pm 0.45cd	8.80 \pm 0.44b
$\text{NaNO}_3 + T. viride$ (1 mL)	8.33 \pm 0.29a	25.33 \pm 0.58d	8.39 \pm 0.25e	15.90 \pm 1.40b	5.87 \pm 0.65e
lactose (1 mL)	1.00 \pm 0.00f	38.00 \pm 1.00a	12.81 \pm 0.20a	11.67 \pm 0.35cd	9.10 \pm 1.05b
Lactose + <i>T. viride</i>	3.33 \pm 1.04cd	31.67 \pm 1.53c	10.57 \pm 0.55cd	14.80 \pm 1.47b	7.17 \pm 0.29cd
NaCl (1 mL)	1.33 \pm 0.58ef	37.00 \pm 1.73a	12.22 \pm 0.56a	12.23 \pm 0.60c	8.53 \pm 0.35b
NaCl + <i>T. viride</i> (1 mL)	2.33 \pm 0.58de	31.00 \pm 1.00c	10.76 \pm 0.52bc	18.13 \pm 0.81a	7.40 \pm 0.46cd
Control	1.00 \pm 0.00f	38.33 \pm 1.53a	12.75 \pm 0.49a	10.33 \pm 0.42d	10.30 \pm 0.82a

Note: Values are means of 3 replicates with standard deviation. Values followed by similar alphabets along each column are not significantly different from each other at $P \leq 0.05$. Keys - ESR: Erythrocyte sedimentation rate; PCV: Packed cell volume; Hb: Haemoglobin; WBC: White blood cell; RBC: Red blood cell.

The white blood cell differential count of albino rats treated with different concentrations of *T. viride* and *T. viride* supplemented diets presented in Table 2. Lymphocytes of control and albino rats fed with 0.1 mL of *T. viride* were significantly higher than other treatments. Also, there were significant differences in the monocytes, neutrophils, eosinophis and basophils of the different treatments.

Table 2. White blood cells differential counts of albino rats under various treatments.

Treatment	LYM (%)	MON (%)	NEU (%)	EOS (%)	BAS (%)
<i>T. viride</i> 1 mL	61.33±1.53c	7.67±1.16b	28.00±1.00bc	2.00±0.00a	1.00±0.00c
<i>T. viride</i> 0.5 mL	65.33±.53b	4.33±2.08c	27.67±1.53bc	1.67±0.58b	1.00±0.00c
<i>T. viride</i> 0.1 mL	69.67±0.58a	7.67±0.58b	21.00±1.00e	1.33±0.58bc	0.33±0.58c
NaNO ₃ (1 mL)	65.00±3.60b	4.33±2.08c	25.67±2.08cd	1.33±0.58bc	3.33±0.58a
NaNO ₃ + <i>T. viride</i> (1 mL)	62.67±1.16bc	7.00±1.00b	26.33±1.53cd	2.00±0.00a	2.00±0.00b
lactose (1 mL)	61.67±1.53c	9.00±1.00a	28.00±1.00bc	1.00±0.00c	0.33±0.00c
Lactose + <i>T. viride</i>	65.67±1.16b	7.00±1.00b	24.33±2.08d	2.00±0.00a	1.00±0.00c
NaCl (1 mL)	60.33±0.58c	7.33±0.58b	29.33±0.58b	2.00±0.00a	1.00±0.00c
NaCl + <i>T. viride</i> (1 mL)	56.67±1.16d	7.00±1.00b	33.33±1.53a	2.00±0.00a	1.00±0.00c
Control	70.33±1.53a	7.00±1.00b	20.33±0.58e	2.00±0.00a	0.33±0.58c

Note: Values are means of 3 replicates with standard deviation. Values followed by similar alphabets along each column are not significantly different from each other at $P \leq 0.05$. Keys - Lym: Lymphocyte; Mon: Monocyte; Neu: Neutrophil; Eos: Eosinophil; Bas: Basophil.

Biochemical indices of albino rats treated with *T. viride*

The AST values of animals fed with different concentrations of *T. viride* and *T. viride* supplemented with sodium chloride were significantly lower than the control. However, there was no significant difference in *T. viride* supplemented with lactose and sodium nitrate as well as the control (Figure 1). The ALP and ALT of *T. viride* supplemented with lactose, sodium chloride and sodium nitrate were higher than the control (Figures 2 and 3).

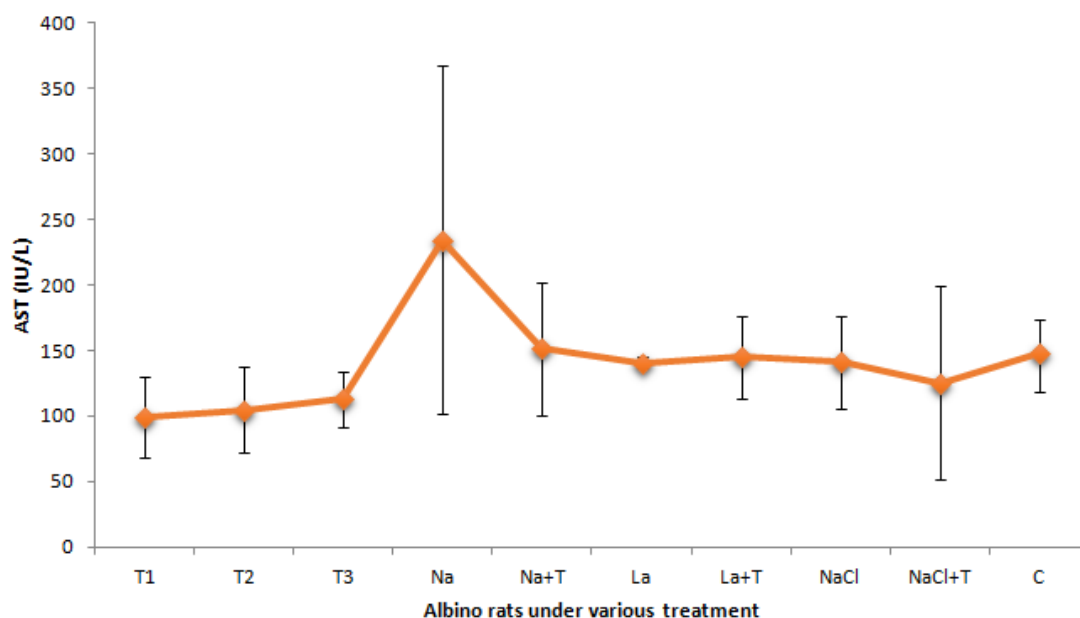


Figure 1. Alanine aspartate (AST) of albino rats under various treatments of *Trichoderma viride*. Keys - T1: 1.00 mL of *T. viride*; T2: 0.50 mL of *T. viride*; T3: 0.10 mL of *T. viride*; Na: Sodium nitrate (NaNO₃); Na+T: Sodium nitrate (NaNO₃) + *T. viride*; La: Lactose; La+T: Lactose + *T. viride*; NaCl: Sodium chloride; NaCl+T: Sodium chloride + *T. viride*; C: Control.

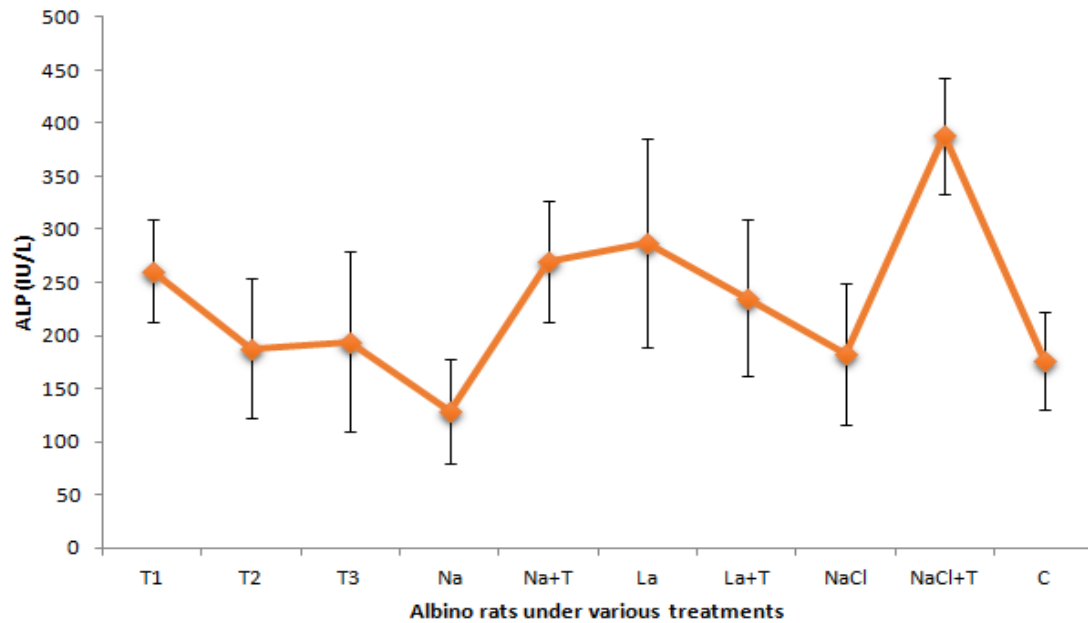


Figure 2. Alkaline phosphatase of albino rats under various treatments. Keys - T1: 1.00 mL of *T. viride*; T2: 0.50 mL of *T. viride*; T3: 0.10 mL of *T. viride*; Na: Sodium nitrate (NaNO_3); Na+T: Sodium nitrate (NaNO_3) + *T. viride*; La: Lactose; La+T: Lactose + *T. viride*; NaCl: Sodium chloride; NaCl+T: Sodium chloride + *T. viride*; C: Control.

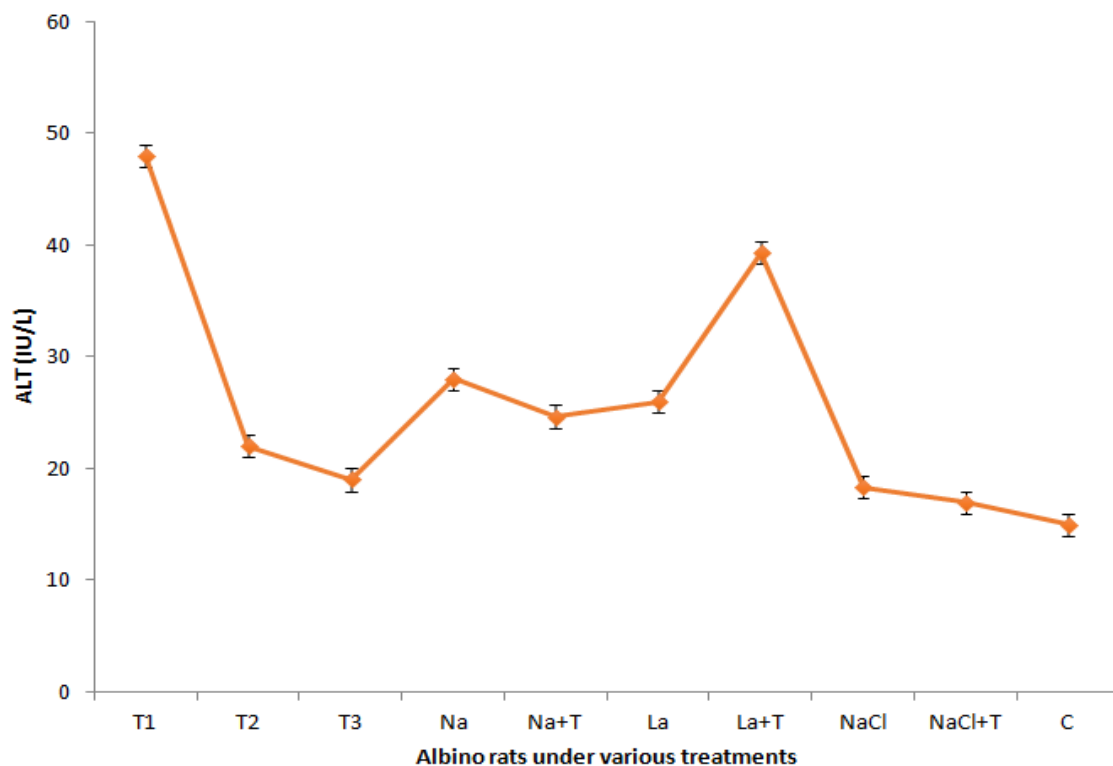


Figure 3. Alanine transaminase of albino rats under various treatments. Keys - T1: 1.00 mL of *T. viride*; T2: 0.50 mL of *T. viride*; T3: 0.10 mL of *T. viride*; Na: Sodium nitrate (NaNO_3); Na+T: Sodium nitrate (NaNO_3) + *T. viride*; La: Lactose; La+T: Lactose + *T. viride*; NaCl: Sodium chloride; NaCl+T: Sodium chloride + *T. viride*; C: Control.

Discussion

Haematological parameters are often used to determine the negative effects of a foreign substances on the blood. There was significant increase in the red blood cell (RBC), haemoglobin concentration (Hb) and packed cell volume values of the untreated rats (control group) similar to the findings of Fasanu and Oyedapo (2008) and Ogunmefun et al. (2013). The decrease in RBC of treated group suggests that *T. viride* does not relatively possess haematinic property but causes anaemia. It can also be an indication that *T. viride* impaired the production of blood cells or the rate of destruction of matured red cells was greater than the rate of its production (Odeyemi et al., 2008).

Neutrophils which are matured cells usually attack and destroy bacteria in the blood (Dacie and Lewis, 1995). The significant increase in the neutrophils implied enhancement in the ability of the blood component to phagocytose. "Lymphocytes are the main effector cells of the immune system" (McKnight et al., 1999). Lymphocyte values were lowered in the treated groups showing that the immunological status of the body was not enhanced.

The biochemical indices are useful parameters in assessing the functionality of the liver and kidney (Odeyemi et al., 2008). The ALP is a marker enzyme of damage for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan et al., 2004). Enzymes from diseased or damaged tissues may become recognizable in the serum presumably by leakage through altered cell membrane of the rat organs (Akanji and Ngaha, 1989). Supplementation of *T. viride* with sodium chloride, lactose and sodium nitrate caused a significant increase in the ALT and ALP indicating liver damage. The increase in ALT and ALP of rats fed with 1.00 mL of *T. viride* was an indication of the damage inflicted on the plasma membrane. "This increase may be due to disruption of the ordered lipid-bilayer of the membrane structure of the affected organ" (Yakubu et al., 2003). Decrease in alanine aspartate (AST) values of the different concentrations of *T. viride* proved there was no damage to the organs.

Conclusion

This research has shown that *T. viride* had dose and parameter specific effects on the haematological and biochemical parameters investigated. I equally acknowledge all authors whose works have been cited in the course of preparation of this manuscript.

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Conflict of interest

Author declares that there are no conflict of interest.

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