

Acute Oral Toxicity of two Products from a Microbial Pest Control Agent (*Beauveria bassiana*) on Physiological Status Aspects of Male Albino Rats

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ABSTRACT

Background: Synthetic pesticides have accumulated in environment causing harm to humans and ecosystems. As a result, the use of biopesticides in agriculture and public health has expanded as a substitute for traditional pesticides. **Purpose:** To investigate the acute oral toxicity of *Beauveria bassiana*, on physiological status aspects of male rats. **Material and Methods:** Metabolic crude (MC), and wettable powder formulation (2.5% WP) from the local isolate of *B. bassiana* (AUMC 9896) were tested on adult Sprague Dawley (SD) male rats by single oral dose. **Results:** There was no evidence of death or toxic symptoms in any of the treated groups. In contrast, each product caused a significant increase in the body weight gain and relative liver weights of *B. bassiana*-exposed male and reduced the brain somatic index with WP only as compared to the control. The studied bioinsecticide also caused a substantial rise in total erythrocyte and absolute differential leucocyte counts, while red blood cell distribution width (RDW) and platelet count (Plt) were decreased significantly. Furthermore, male rats exposed to both types of *B. bassiana*, aspartate aminotransferases (AST), total protein (TP), albumin (Alb), AST/ALT, triglyceride, and very low-density lipoprotein cholesterol (VLDL) were elevated compared to the untreated group, whereas alkaline phosphatase (ALP) activity, globulin (Glb), Alb/Glb, urea content, and low-density lipoprotein cholesterol (LDL) count fluctuated between increased and decreased. Also, *B. bassiana*-treated rats had lower serum cholesterol and high-density lipoprotein cholesterol levels (HDL) values.

Conclusion: These results suggest that both treatments have slight effects on complete blood count (CBC) of treated male rats and marked effect on liver function, lipid profile, body weight gain and somatic index of the liver and brain.

Keywords: Acute oral toxicity, Bioinsecticide, *Beauveria bassiana*, Hematotoxicity, Hepato- and renal toxicity, Lipid profile, Male rats.

INTRODUCTION

Alternative pest management strategies that are less damaging and safe to non-targeted pests, plants, animals, and humans are being used to protect agricultural products due to the environmental and human dangers connected with the widespread use of synthetic pesticides [1].

Biological management with entomopathogenic organisms (EPO) such as bacteria, viruses, nematodes, fungi, and protistes agents are most of these techniques [2]. For more than 150 years, many species (EPO) have been used as biological control agents of insect pests [3] in row and glasshouse crops, orchards, ornamentals, range, turf and lawn, stored products, and forestry, as well as for the control of pest and vector insects of veterinary and medical importance [4].

More than 1000 species of entomopathogenic fungi (EPF) from 85 genera have been found to infect pests in agricultural, veterinary, and medical pests [5]. In the Hyphomycetes class, about 100 mycoinsecticides are commercially registered worldwide and among them, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, a cosmopolitan anamorphic genus of soilborne necrotrophic entomopathogenic fungi, is one of the most useful fungi, based-mycopesticides [6]. *Beauveria* is the most well-known entomopathogenic

fungus due to its global distribution and wide range of target pests. It was discovered more than 60 years ago that it may induce diseases in insects. *Beauveria* has become the most studied alternative control agent since then, and its conidia are now the basis for several commercial insecticides on the market [7].

Although several mycoinsecticides, as EPF, have previously been developed for commercial use in microbial control of insect pests and are marketed in a few countries, not all their bio-safety processes and risk assessments for mammals and humans have been evaluated [8]. As a result, registration of bioproducts based on microorganisms, particularly *B. bassiana*, pays special attention to the following aspects: 1- Allergic characteristics, 2- Toxic metabolite risks, 3- Genetic recombination and natural strain displacement, and 4- Impact on biodiversity [4].

Furthermore, acute toxicity studies in an animal model (oral, dermal, and inhalation) are one of a set of biosafety protocols that must be designed to provide information on health hazards while considering the periods in which workers are in direct or indirect contact with the microbial agent when used for pest control. As a result, the goal of this investigation is to determine the oral LD₅₀ of *Beauveria bassiana* culture (AUMC 9896)



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local strain as metabolic crude (MC) and formulation (2.5% WP, 1×10^8 Conidia/ml) on male rats during a single acute oral dosage.

MATERIALS AND METHODS

The microbial agent:

The microbial pest control agent, *Beauveria bassiana* (AUMC 9896), was used in current experiment. It was isolated from soil in Bioinsecticide Production Unit (BIPU), Plant Protection Research Institute, Agricultural Research Center and identified in Mycological Center, Faculty of Science, Assiut University by [9].

Preparation and Mass Production of the Bioinsecticide:

Crude extract of *B. bassiana* (AUMC 9896) was prepared according to Valencia, *et al.* [10] and adjusted it to a concentration of 1×10^7 conidia/ml. Before the experiment, the viability of the fungus was assessed. Method of Posada-Flórez, [11] was used for mass production of *B. bassiana* on boiled rice using one ml of conidial suspension (10^7 conidia/ml) to produce the mass culture of fungal conidia. The conidia were added with the powdered additives (2.5%) therefore one gram of wetttable powder formula (WP) contained to 1×10^8 conidia as descriptive by [12].

Ethical Statement:

The Ethics Committee of Zagazig University's Institutional Animal Care (ZU-IACUC), Zagazig, Sharkia, Egypt, approved all experiment protocols, which followed the "Guide for the Care and Use of Laboratory Animals" for the use and welfare of experimental animals. Zagazig University's Committee published the ethical procedures and policies under No. (ZU-IACUC/2/F/156/2020).

Animals:

Adults in good health in the trials, male Sprague Dawley (SD) albino rats, *Rattus norvegicus* albinus, were purchased from the Egyptian Company for Biological Products and Vaccines (Helwan Farm). Thirty male rats were kept in a typical day/night cycle (12 Light: 12 Dark) at room temperature (22 ± 3 °C) with a relative humidity (RH) of less than 70%. To acclimate the animals, they were given commercial diet and water *ad libitum* for one week prior to the start of the experiment. The animals were divided into two groups: the first group was used to calculate the oral LD₅₀ of *B. bassiana* (AUMC 9896), and the second group was used to assess the acute oral toxicity of two products on physiological status features of the animals.

Estimation of Oral LD₅₀:

The acute oral toxicity investigation followed the Organization for Economic Co-operation and Development's (OECD) [13] Guideline No. 425. The animals were selected at random, marked to enable for

individual identification, and housed in their cages for at least 5 days prior to dosing to allow them to acclimate to the laboratory environment. All animals were fasted overnight before being weighed, and the dose of the supplied test *Beauveria bassiana* (*Bb*) products (MC and WP) was estimated based on the fasting body weight of each animal body weight. The *Bb* preparations were given as a single dosage through a stomach tube. One animal was dosed at 0.5 ml/100 g body weight (bw) by *Bb* MC and 1 ml/100 g bw containing 1.1×10^{10} conidia/100 g bw by *Bb* WP. Each animal was observed carefully for up to 48 hours before deciding on whether and how much to dose the next animal. Conduct the main test to determine the LD₅₀ if the animal dies. Two more animals were tested if the first one lives. If both animals live, the LD₅₀ exceeds the limit dose, then the experiment is over (i.e., carried out to full 21-day observation without dosing of further animals).

Acute Oral Toxicity Study:

After calculating the acute oral LD₅₀ value, the acute oral toxicity study was conducted according to EPA's [14] "OPPTS 885.3050 Acute oral toxicity/pathogenicity". Five males in each group (3 groups) were used. The 1st group was control, 2nd, and 3rd groups gavaged orally with a single dosage (= LD₅₀ values) MC and WP of *Bb* respectively. Onset and duration of toxic symptom effects, as well as the body weights of the treated animals, were monitored and recorded daily for 21 days. The % of changes in body weight (bw) were determined according to equation Mansour *et al.* [15]

$$\text{Weekly body weight gain (\%)} = \frac{(\text{Final bw} - \text{Initial bw})}{(\text{Initial bw} \times \text{No. Weeks})} \times 100$$

The animals were weighed, sacrificed, and dissected at the end of the observation period, and some vital organs (liver, kidneys, brains, spleen, heart, lung, and testes) from both control and treated animals were carefully dissected out, washed with physiological saline (0.9% NaCl), dried, weighed individually (absolute organ weight), and tissue somatic index (relative organ weigh, %) were calculated.

Blood Sample Collection:

On the 21st day of the observation period, two parts of blood sample were taken from the retro-orbital plexus. The 1st part was collected into a specially prepared EDTA-treated tube for hematological analysis (Complete blood picture, CBC). The 2nd part was used to the clinico-biochemical analysis using serum obtained by putting blood samples into non-heparinized clean dry centrifuge tubes and allowing them to clot at room temperature for roughly 20 minutes before centrifuging at 3600 rpm for 15 minutes in the second stage. Serum samples were separated into dry, clean-capped tubes and stored at -40°C until the analysis was finished.

Hematological analysis:

Determining the hematological parameters (CBC) followed the method published by **Theml et al.** [16] using Auto Hematology Analyzer (Countender 20+, SFRI SAS, France). The hematological aspects were carried out by measurement of total cell counts, [red blood corpuscles (RBCs), white blood corpuscles (WBCs), and platelets (Plt)], hemoglobin (Hb), hematocrit (Hct), red cell distribution width (RDW) and absolute differential WBC counts (DLC). Also, the erythrocyte indices values such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were estimated.

Clinico-Biochemical analysis:

Commercial diagnostic kits were used to determine clinico-biomarkers of liver and kidney functions, as well as lipid profiles in serum by suitable methods. Aminotransferases, [alanine (ALT) and aspartate (AST)], and alkaline phosphatase (ALP) activity and total protein and albumin levels were measured as liver aspects. For kidney functions were assayed by the level of creatinine and urea. Lipid profiles *vis* total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol levels (HDL) were estimated. Additionally, calculate of the AST/ALT ratio, globulin concentrations, and the Alb/Glb ratio (A/G ratio) were done, and by using Friedewald equation, the serum concentration of low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) were estimated from triglycerides.

Statistical Analysis

The AOT425StatPgm "Acute Oral Toxicity (Guideline 425) Statistical Program" used the maximum likelihood approach to compute the LD₅₀. For the

analysis of physiological aspects, any data expressed as a percentage was first translated into angular transformation values ($\arcsin \sqrt{\text{percent}}$). All data were analyzed with IBM's Statistical Package for the Social Sciences (SPSS) for Windows (version 25, Chicago, USA) and represented as means \pm S.E (n = 5 animals). To examine the differences between means, one-way analysis of variance (ANOVA) was used, followed by the Least Significant Difference (LSD) test. At (p \leq 0.05, 0.01, and 0.001) difference from the control, all values of the results data were regarded significant (*), high significant (**), and very high significant (***).

RESULTS

Oral LD₅₀ Study:

Throughout the trial with the tested entomopathogenic fungi, no symptoms of toxicity, mortality, or pathogenicity were seen in any group. The estimated of acute oral LD₅₀ for *B. bassiana* (AUMC 9896) two products recorded more than 5000 mg/ kg bw and $> 1.1 \times 10^{10}$ conidia/kg bw with metabolic crude (MC) and formulation (WP) products respectively.

Acute Oral Toxicity Study:

The changes in body weight and tissue somatic index (relative organ weights) are index to the physiological status of animals. The finding in figure (1) revealed that the body weight gain (BWG), BWG/week and % BWG/week of treated animals with both products increased significantly when compared to untreated group. Also, treatment with *B. bassiana* induced highly significant alter of somatic liver and brain indexes by two products (MC and WP) and WP only respectively. The bioinsecticide, on the other hand, had no effect on the tissue somatic index (Table 1) of the remained organs when compared to the control group.

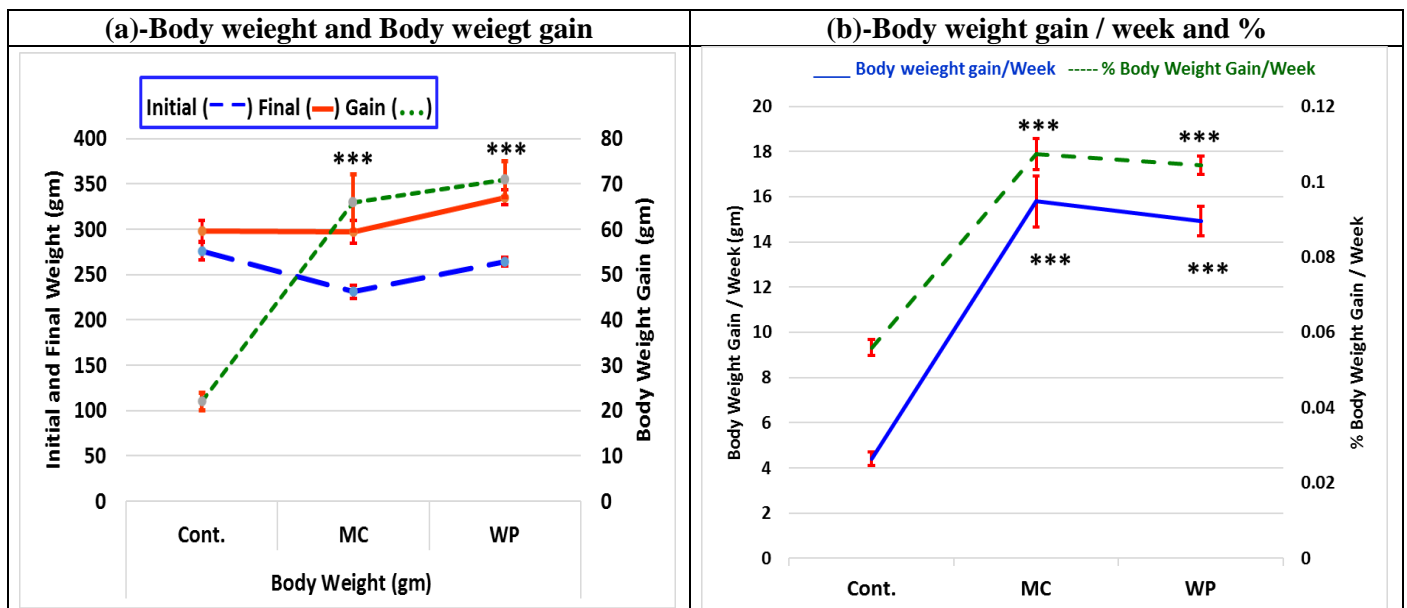


Fig. (1): Influence on body weight of male rats after acute exposure to *B. bassiana* products.

n = 5, Data are presented as (M ± SE), MC = Metabolic Crude, WP = Wettable Powder (2.5%), *** significant at P ≤ 0.001. The percentages were transformed into angular transformation values (arcsin √√; percent).

Table (1): Influence on tissue somatic index (%) of male rats after acute exposure to *Beauveria bassiana* products.

Treatments	Control	<i>Beauveria bassiana</i>	
Organs		MC	WP
Brain	4.248 ± 0.0345	4.236 ± 0.1108	3.836 ± 0.0224**
Heart	3.210 ± 0.0537	3.390 ± 0.1534	3.191 ± 0.0190
Lung	3.859 ± 0.0358	4.258 ± 0.1052	3.607 ± 0.1939
Liver	9.058 ± 0.3695	10.77 ± 0.2477**	10.36 ± 0.0665**
Kidney	4.459 ± 0.1839	4.508 ± 0.0280	4.43 ± 0.0502
Spleen	2.894 ± 0.0895	3.598 ± 0.3920	3.208 ± 0.0578
Testes	5.584 ± 0.2487	5.703 ± 0.0657	5.426 ± 0.1467

n = 5, Data are presented as (M ± SE), MC = Metabolic Crude, WP = Wettable Powder (2.5%), ** significant at P ≤ 0.01, The percentages were transformed into angular transformation values (arcsin √√; percent).

Complete blood count (CBC) results presented in table (2) showed significant elevation in white blood corpuscles (WBCs) of treated males with both product (MC and WP) as well as, in the absolute differential WBC counts (DLC) such as lymphocytes, monocytes, eosinophils, and neutrophils except eosin level was decreased in male of *Bb* crude treatment (MC). Meanwhile, WP of *B. bassiana* caused drastically reduction in red blood cell distribution width (RDW) as well as, reduce of platelet count (Plt) was showed in all male rats exposed. In comparison to untreated rats, no significant changes in erythrocytes, hemoglobin (Hgb), hematocrit (Hct), or erythrocyte indices (MCV, MCH, and MCHC) were observed in all treated animals.

Table (2): Influence on hematological parameters of male rats after acute exposure to *Beauveria bassiana* products.

Treatments	Control	<i>Beauveria bassiana</i>	
CBC		MC	WP
RBCs (× 10 ⁶ /μl)	6.492 ± 0.0807	6.452 ± 0.1067	6.382 ± 0.0174
WBCs (× 10 ³ /μl)	6.278 ± 0.2298	8.984 ± 0.3339***	9.52 ± 0.2417***
Hgb (g/dl)	14.62 ± 0.1020	14.38 ± 0.2728	14.18 ± 0.1744
Hct (%)	38.29 ± 0.1453	38.35 ± 0.3970	38.28 ± 0.1888
Platelet (10 ³ /mm ³)	790.0 ± 18.41	738.2 ± 21.26*	694.8 ± 2.332**
RDW (%)	22.88 ± 0.1393	22.43 ± 0.18165	21.84 ± 0.1870***
MCV (fl/cell)	59.22 ± 1.084	59.68 ± 0.5748	60.1 ± 0.3728
MCH (Pg/cell)	22.52 ± 0.1562	22.28 ± 0.1020	22.2 ± 0.2074
MCHC (g/dl)	38.12 ± 0.4609	37.32 ± 0.2332	36.92 ± 0.2396*
Differential WBC Counts (k/ μl)			
Eosinophils	0.14 ± 0.00603	0.0878 ± 0.00318***	0.188 ± 0.0049***
Neutrophil	0.702 ± 0.0566	1.08 ± 0.0635***	1.222 ± 0.0594***
Monocytes	0.28 ± 0.0127	0.4132 ± 0.0073***	0.4324 ± 0.0055***
Lymphocytes	5.112 ± 0.1177	7.452 ± 0.2987***	7.678 ± 0.3115***

n = 5, Data are presented as (M ± SE), MC = Metabolic Crude, WP = Wettable Powder (2.5%), *, ** and *** significant at P ≤ 0.05, 0.01 and 0.001, The percentages were transformed into angular transformation values (arcsin √√; percent).

In the exposed male rats with both types of *B. bassiana*, clinico-biochemical measures of liver function such as aspartate aminotransferase (AST) activity, AST/ALT ratio, total protein, and albumin (Alb) were higher than in the control group, as shown in table (3). On the other hand, no significant alternations were recorded in ALT activity of treated males. ALP activity and A/G ratio globulin (Glb) levels reduced, whereas these measures rose with formulation

product (WP). Furthermore, when compared to untreated male rats, globulin levels were higher with crude (MC) and decreased with formulation (WP).

Table (3): Influence on liver functions of male rats after acute exposure to *Beauveria bassiana* products.

Treatments Functions	Control	<i>Beauveria bassiana</i>	
		MC	WP
ALT (U / l)	22.31 ± 0.5201	21.95 ± 0.2535	21.98 ± 0.6359
AST (U / l)	27.74 ± 1.284	41.06 ± 1.748***	52.49 ± 2.779***
ALP (U / l)	187.3 ± 8.150	75.10 ± 4.787***	241.8 ± 10.17***
TP (g / dl)	7.501 ± 0.0831	9.645 ± 0.1092***	7.840 ± 0.0602*
Alb (g / dl)	5.450 ± 0.0156	5.934 ± 0.0835***	6.201 ± 0.0434***
Glb (g / dl)	2.051 ± 0.0911	3.710 ± 0.0365***	1.639 ± 0.1033**
AST / ALT	1.241 ± 0.0327	1.870 ± 0.0726***	2.387 ± 0.0882***
Alb / Glb	2.677 ± 0.112	1.600 ± 0.0184***	3.845 ± 0.2486***

n = 5, Data are presented as (M ± SE), MC = Metabolic Crude, WP = Wettable Powder (2.5%), *, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.

In terms of renal function, (Figure 2), urea concentration increased and decreased significantly in WP and MC-treated rats compared to the control group. Moreover, changes in creatinine levels of the treated male rats were not significant.

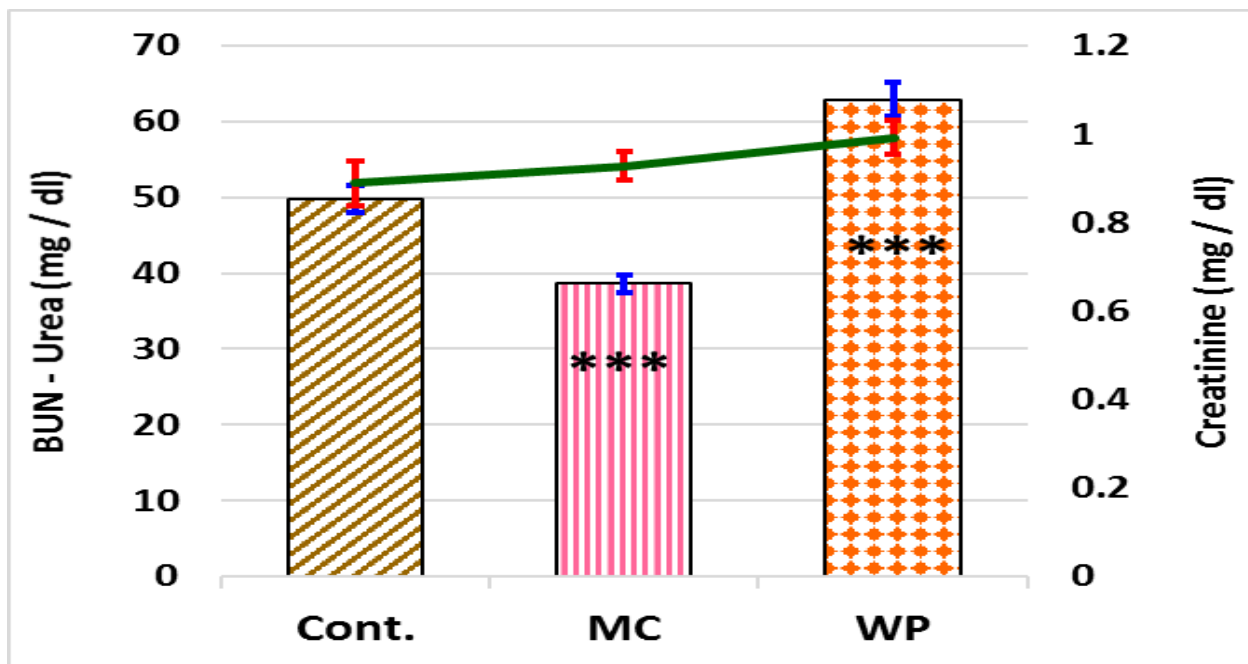


Fig. (2): Influence on kidney functions of male rats after acute exposure to *Beauveria bassiana* products.

n = 5, Data are presented as (M ± SE), MC = Metabolic Crude, WP = Wettable Powder (2.5%), *** significant at P ≤ 0.001.

The lipid profile analysis of *Bb*- treated male rats was illustrated in Fig. (3). The data revealed a significant drop in serum total cholesterol and HDL levels in both exposed rats compared to unexposed group whereas, triglyceride and VLDL contents were diminished in treated animals. Meanwhile in males treated with *Bb* formulation and metabolic crude products, LDL levels were raised and declined respectively.

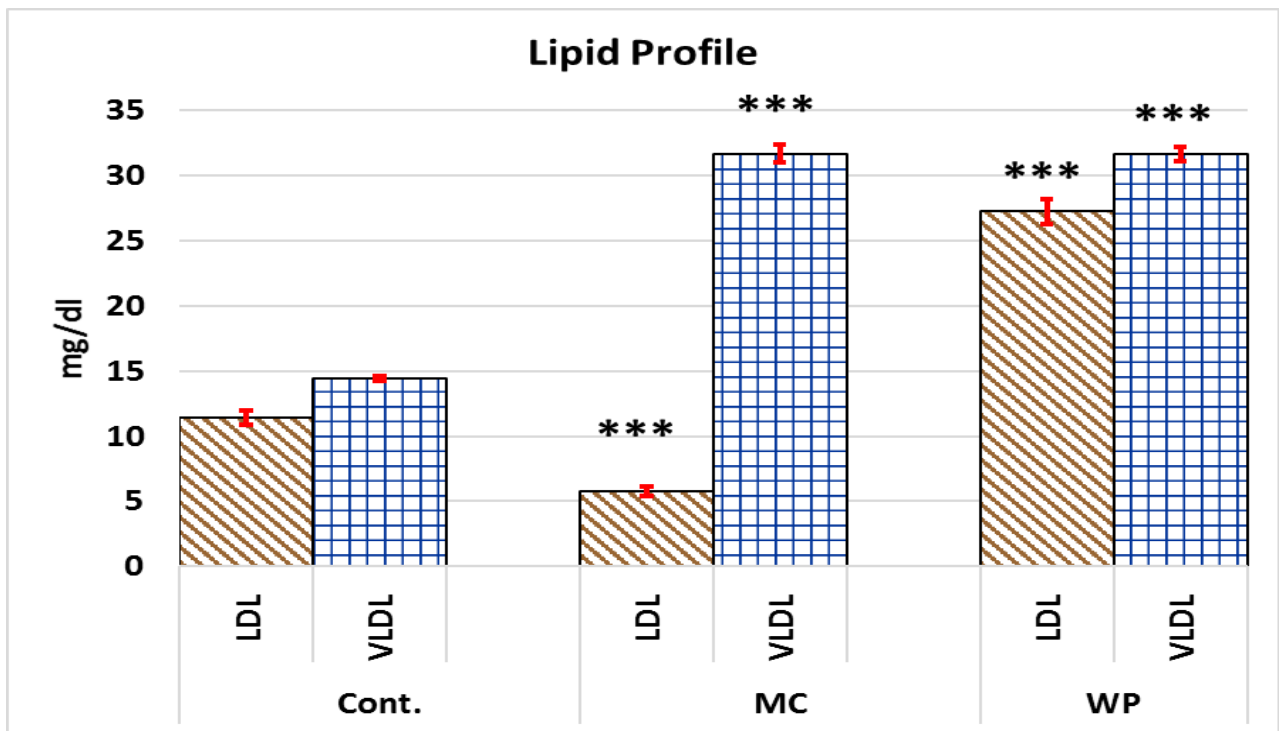
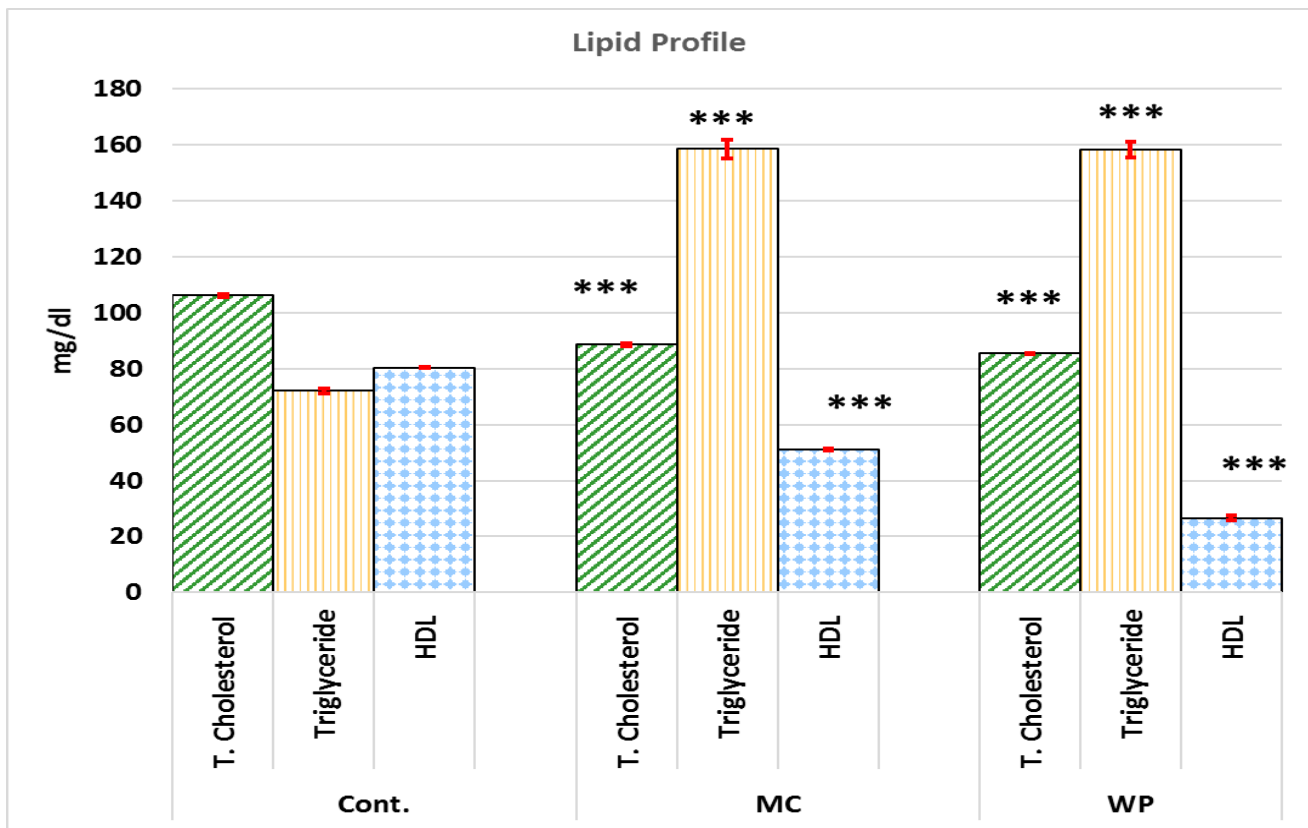


Fig. (3): Influence on lipid profile of male rats after acute exposure to *B. bassiana* products.

n = 5, Data are presented as (M ± SE), MC = Metabolic Crude, WP = Wettable Powder (2.5%), *** significant at P ≤ 0.001.

DISCUSSION

Similar results with the acute oral LD₅₀ estimated for two products of *B. bassiana* (AUMC 9896) were reported by EPA [17,18] showed that at a dose of 4.05 x 10⁹ CFU/animal (using the hemacytometer method) or 3.20 x 10⁸ CFU/animal (using the dilution plate count method), *B. bassiana* (HF23 and GHA128924), was not hazardous, infective, or pathogenic to rats. For acute oral toxicity/pathogenicity effects in mammals, the technical grade active ingredient (TGAI) of strain *B. bassiana* (HF23 and GHA128924) was classified as toxicity category IV in the two experiments. Also, the results agree with Mancebo *et al.* [19] who recoded no infectivity or pathogenicity was observed by *B. bassiana* (strain ATCC 74040) in rats after 21 days exposure to 1.8 x 10⁹ colony forming units kg⁻¹, and the acute oral LD₅₀ for rats was > 18 x 10⁸ CFU kg⁻¹.

Also, there was no mortalities and no signs of pathogenicity or treatment-related toxicity in rats orally dosed with *B. bassiana*, which was in accordance with EFSA [20] by 1.9 x 10⁸ CFU/animal of strain ATCC 74040, EFSA [21] by strain 147. Additionally, M-MA Document [22] reported that LD₅₀ of *B. bassiana* (strain PPRI 5539) > 5000 mg/kg for Sprague-Dawley female rats and > 2000 mg/kg for female CRL(WI)BR Wistar rat and, EFSA [21] showed that oral LD₅₀ of *Beauveria bassiana* 147 greater than 1.8 x 10⁹ CFU/kg bw.

Finally, we can classify *B. bassiana* (AUMC 9896) two products as category 5 according to GHS classification (The Globally Harmonized System of Classification and Labelling of Chemicals) or Category IV according to EPA's Office of Pesticide Programs (OPP) classification based on the results of acute oral LD₅₀ estimates (> 5000 mg/kg).

Body and organ weight reflects, in toxicological studies, the overall state of metabolism, and the capability of the organism to maintain its normal growth and development. It is also an indicator of detrimental consequences of any chemicals such as organ dysfunction, detoxification process, and organ toxicity [23].

Exposure of male rats to both *B. bassiana* products (MC and WP) resulted in a significant substantial rise in the liver somatic index. This impact could be attributed to *B. bassiana*'s toxic potential, which results in increased liver weight in acute dose exposed animals, as well as increased serum AST activity. This increase is in accordance with research from Eweis *et al.* [24] with *Beauveria bassiana* Vuillemin in rats.

Under normal settings, humoral factors regulate quantitative and qualitative equilibrium between all blood cells, ensuring a balance between cell synthesis (primarily in the bone marrow) and cell breakdown (mostly in the spleen, liver, bone marrow, and the diffuse reticular tissue) [16].

The increase in WBC count may reflect activation of the animal's defensive mechanisms and immune system, as well as mobilization of the immune system and/or a transfer in the leukocytic pool from the spleen to the peripheral blood [25]. In addition, Abd El-Ghany [4] revealed that two recent occurrences of disseminated mycoses represent the most severe human cases of *Beauveria* infections. Both infections occurred in patients with acute leukemia who were highly immunocompromised.

This study found a significant rise in AST activity, AST/ALT ratio, total protein and albumin levels recorded by this study indicate that the two products of a biopesticide are hepatotoxic. This is agreement with the earlier observations of the effect of abamectin by Eweis *et al.* [24] they found slight effects in total proteins, and alkaline phosphate in rats after treatment with *B. bassiana* Vuillemin.

The elevated AST levels in *B. bassiana*-treated male rats could be attributable to hepatotoxicity, which causes permeability changes and lysosomal enzyme leakage, which increases enzyme release [26]. Muscle inflammation related to dermatomyositis can also result in AST > ALT [27].

These findings revealed an increase in serum ALP in male rats exposed to the *B. bassiana* form (WP). ALP is usually released because of increased synthesis caused by a variety of liver diseases. As well as the loss of epithelial cells in the gastrointestinal tracts may be the cause of elevated serum ALP [28].

The elevations of total protein and albumin concentrations of *Bb* products-treated male were observed in the present study after treatment. Abnormally high levels of total protein can be related to conditions that cause chronic inflammation and with some types of infections that affect the immune system. The A/G ratio can be high or low because of abnormal changes to levels of albumin, globulins, or both [29] as showed in our results in treated male rats.

The current study found an increase in urea concentrations in male rats given a single acute dosage of *B. bassiana* products. Increases in BUN and/or serum creatinine aren't always due to kidney disease; they could be due to dehydration, hypovolemia, or protein catabolism. On the other hand, urea decreasing in male treated with *Bb* MC may be dysfunction of hepatic parenchymal cells (HPCs) or hepatocytes to detoxification ammonia that produced during metabolism, by forming urea which is eliminated by the kidneys [30].

Cholesterol, one of the body's main lipid fractions, is primarily generated in the liver and serves as a precursor to steroid hormones in cell physiology. Almost all the cholesterol in plasma is either attached to lipoproteins like VLDL, intermediate-density lipoprotein (IDL), or LDL or effluxes to HDL. LDL normally carries about 70% of total plasma cholesterol

but relatively small amounts of triglyceride. HDL contains about 20% to 30% of plasma cholesterol and only a small number of triglycerides, and most plasma triglycerides are present in VLDL [31].

Kozłowska et al. [32] demonstrated that *Beauveria bassiana* is a well-known biocatalyst for the transformation of dehydroepiandrosterone (DHEA, a steroid compound synthesized from cholesterol) by hydroxylation and oxidation process. On the other hand, elevation of triglyceride and VLDL as well as depression of HDL and LDL in presented data may be due to hypertriglyceridemia. Also, **Remaley et al.** [31] reported in pathologic states characterized by hypertriglyceridemia, both LDL and HDL, however, acquire increased core triglycerides, which promotes their lipolysis and the generation of smaller, denser forms of LDL and HDL.

The adverse effects of *Beauveria bassiana* on physiological status aspects (CBC, liver and kidney functions, and lipid profile) of male albino rats in the current study could be attributable to many biologically active secondary metabolites (SMs) that *Beauveria* spp. strains produce. These SMs are volatile organic compounds (VOCs), alkaloids (tenellin, bassianin, pyridovericin, pyridomacrolidin) [33], non-peptide pigments (oosporein), non-ribosomally synthesized cyclodepsipeptides (beauvericins, allobeauvericins, and bassianolides) and cyclopeptides (beauveriolides), [19,34].

Several researchers discovered that these SMs have biological activity in the following ways:

- 1- Inhibit hemocyte (erythrocyte) membrane ATPase activity with tenellin and bassianin [34].
- 2- Apoptosis (programmed cell death) is induced by Beauvericins (BEAs) via the mitochondrial pathway, which includes a reduction in reactive oxygen species generation, loss of mitochondrial membrane potential, release of cytochrome c, activation of caspase-9 and -3, and cleavage of poly (ADP-ribose) polymerase (PARP) [33].
- 3- The most well-known SM generated by *B. bassiana* and other *Beauveria* spp. is beauvericin (BEA). It has ionophoric effects (increase cytoplasmic Ca²⁺ concentration) on mammalian cell walls in general, causing ATP depletion and activating calcium sensitive cell apoptosis pathways [33] and cytolysis [19]. It's also a particular cholesterol acyltransferase inhibitor that's harmful to both murine and human cell lines [19].

These metabolites (SMs) have a wide range of chemical and biological properties, as well as various potential consequences on humans and the environment depending on the strain [34]. Some of them have been identified as having dangerous qualities (as mentioned above), while others have no information available [35]. These metabolites and their unique actions are of

toxicological importance in terms of registration and risk assessment [34].

CONCLUSION

The effects of each product type (MC and WP) of the microbial pest control agent, *Beauveria bassiana* (AUMC 9896), on physiological status aspects (CBC, liver and kidney functions, and lipid profile) of male albino rats were seen in the acquired data. Other effects were also body weight increase as well as the somatic index of the liver and brain. The negative impact of *B. bassiana* formulation (WP) on complete blood count (CBC) and tissue somatic index was greater than metabolic crude (MC). As a result, regulatory organizations (OCED, EPA) advocate conducting 6-pack testing (acute studies) employing microbial pathogenic control agents (MPCAs) or microbial pathogenic control products (MPCPs) to address human health and safety concerns.

In the future, more prolonged vertebrate pathogenicity/toxicity studies of *B. bassiana* local strains-based biopesticides should be conducted through various administration routes, periods, and formulation types to ensure ecosystem integrity and avoid potential risks to workers, operators, consumers, residents, and bystanders during the production process and application.

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