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Effect of some Pesticides Seed Dressers on Serum Biochemical Parameters of Japanese Quails (*Coturnix coturnix japonica*)

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ABSTRACT

Extensive use of pesticide products in agriculture can pose severe hazards to both humans and animals. Birds may be at risk of poisoning by ingesting seeds treated with these pesticides. The aim of the study was to evaluate the serum biochemical effects of two insecticide/fungicide combinations; Dress force® and Apron star®, commonly used for seed treatment in Japanese quails – Coturnix cortunix japonica. For this purpose, a total of 42 adult Japanese quails were randomly divided into seven equal groups, and then fed for eight weeks with seeds treated with Dress force at (2.0, 2.5 and 3.0g/kg) and Apron star at (2.0, 2.5 and 3.0g/kg), representing the low, recommended and highest doses respectively. Significant increase ($p<0.05$) in the transaminases (AST and ALT) and ALP values was recorded in the pesticides treated birds compared to the control. Total protein reduced significantly ($p<0.05$) at the highest dose. Dress force treated quails recorded significantly ($p<0.05$) higher blood urea nitrogen (BUN) values than Apron star at the low (2.0 g/kg) and recommended (2.5 g/kg) doses. In conclusion, both pesticide formulations influenced the biochemical parameters in adult quails with different degrees.

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INTRODUCTION

Pesticides are a group of chemicals used in agriculture to control diseases and insects and regulate plant growth, they can be derived from natural compounds or synthesized, different pesticide applications can be used as herbicides, insecticides, or fungicides (Sun *et al*., 2019). Deleterious effects of these herbicides and pesticides have extremely increased all over the world (Hussain *et al*., 2021). The persistent use of these compounds at crops and animal feed industries is harmful for target and non-target animals and the food web (Safdar *et al*., 2022).

These pesticides such as insecticides and fungicides are used as seed dressers for treating seeds to be planted to ensure adequate germination and emergence (Adejumo *et al*., 2015). This pesticide dressed seeds may be an important source of food for some birds in times of scarcity, sometimes they are not properly buried or often spilled from sowing machines, which makes them accessible to birds, representing a risk of pesticide intake (Lopez-Antia *et al*., 2013).

Avian species occupy a remarkable position in the ecosystem, are good indicator of a healthy ecosystem and are a sign of early warning to environmental issues (Sheikh *et al*., 2021; Luqman *et al*., 2021). These avian species die due to ingestion of pesticides and their remains may decompose rapidly or might be eaten by the scavengers (Arya *et al*., 2019).

Use of insecticides as seed treatments on some crops poses risks to small birds and ingestion of a few treated seeds could cause mortality or reproductive impairment to sensitive bird species (Gibbons *et al*., 2015). These insecticides known as neonicotinoids have been reported to decrease egg production, hatching interval, and eggshell thinning (Vohra and Khera, 2014). Acute effects of neonicotinoids could cause indirect mortality by making the birds more vulnerable to predators or to collision with automobiles (Millot *et al*., 2017).

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Imidacloprid, a neonicotinoid resulted in nervous disorders rock pigeon (*Columba livia domestica*) because of its nicotinic action (Abu Zeid *et al*., 2019). Imidacloprid treated seeds at the recommended application rate caused mortality of adult red-legged partridges Lopez-Antia *et al*. (2015).

Additionally, low concentrations of acetochlor affected the hematological, histopathological and serum biochemical parameters of Japanese quails (Arshad *et al*., 2024). Kalsom *et al.* (2023) also reported alterations in the hematological indices and histopathological changes in heart, spleen and testes of Japanese quails administered bifenthrin even at sub-lethal concentrations. Extensive degenerative alterations were observed in the liver, testes, kidneys and many cellular structures of birds (Suliman *et al*., 2020). Histopathological and biochemical parameters were also altered in different tissues of rats (Ahmed *et al.,* 2020).

Since *Coturnix japonica* is a well-suited model species for the study of insecticide risk assessment (Zhang *et al*., 2015) and plausible routes of exposure to these chemicals for quails include ingestion of coated seeds and environmental residues at greater concentrations than those predicted by normal use (Gobeli *et al*., 2017), this study aims to investigate the effect of some widely used seed treatment pesticides at different concentrations on some biochemical markers of the Japanese quail, *Coturnix coturnix japonica*.

MATERIALS AND METHODS

Experimental Design and treatment

The present study was carried out at the Department of Biology, Ahmadu Bello University, Zaria. A total of 42 adult Japanese quails of both sexes, weighing between 140 to 160g were bought from the Kundilla Veterinary Clinic Kano. The quails were fed commercial poultry feeds (Vital feed brand) and were allowed to acclimatize for two weeks.

Dress force® and Apron star® formulations were obtained from an agrochemical outlet in Zaria.

i) Dress force®: - An insecticide-fungicide mixture which comprises Imidacloprid 20%, metalaxyl-M 20% and Tebuconazole 2%
ii) Apron star®: - Insecticide-fungicide formulation with active constituent Thiamethoxam 20%, metalaxyl-M 20% and Difenoconazole 2%

Three application doses of both pesticide formulations were used, equal amount of millet seeds were treated with the pesticides at the following concentrations, 2.0 g/kg, 2.5 g/kg and 3.0 g/kg of seeds, to represent a dose below the recommended dose, the recommended dose for cereal coating and a dose higher than the recommended dose to assess the effects of potential abuse in the pesticides application respectively.

After acclimatization, the birds were divided into seven groups (A, B, C, D, E, F, and G) in wire cages, each containing 6 birds.

Group A served as control for both chemicals and were fed untreated seeds, Group B, C, D were fed seeds treated with 2.0g/kg, 2.5g/kg and 3.0g/kg of Dress force® representing the low, recommended and highest dose groups respectively, while group E, F and G were fed seeds treated with 2.0g/kg, 2.5g/kg and 3.0g/kg of Apron star® ® representing the low, recommended and highest dose groups respectively. The birds in all the treatment groups were fed *ad libitum* for 8 weeks.

Serum Biochemical Analysis

Blood was collected from the jugular vein of each bird into plain bottles for biochemical analyses, allowed to clot and then centrifuged at 3500 rpm for 10 minutes. The sera was harvested and used for the assay. The biochemical parameters evaluated include; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein and blood urea nitrogen (BUN) as liver and kidney function tests by colorimetric method using Randox assay kits and automated Biochemistry analyzer.

Statistical Analysis

The values were expressed as mean ± SEM, one-way analysis of variance (ANOVA) was used to analyze the different parameters using SPSS version 20.0. Duncan's multiple ranged test (DMRT) was used to separate the treatment means and differences were considered significant when $p < 0.05$. Student T-test was used to compare the mean effect of both toxicants at different dose levels.

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RESULTS

]The results on different serum biochemical parameters of quails fed Dress force® treated seeds are presented in Table 1. The quantity of the different liver biomarkers which include Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) varied across the groups. AST was significantly increased in quails of groups C and D, while ALT and ALP increased significantly in groups B-D in comparison with the untreated quails. The quantity of the kidney biomarkers which include the Total protein (TP) and Blood urea nitrogen (BUN) values also showed significant differences between the treated and control quails. TP reduced significantly in quails of the highest dose group (D), while the BUN values were significantly higher in the three dose groups (B, C, D,) in comparison with the control.

In quails fed Apron star® treated seeds AST, ALT and ALP were significantly higher in birds of the three dose groups (E, F, G), only quails fed seeds at the highest dose (G) had significantly lower value for the TP and BUN compared to the control (A), as shown in Table 2.

Table 1: Biochemical parameters of Japanese quails fed Dress force® treated seeds

GROUP	AST	ALT	ALP	TP	BUN
Control	15.25±1.38[c]	8.75±0.63[c]	79.00±2.08[c]	4.33±0.41[a]	17.25±1.25[b]
TDF1	18.33±2.03[bc]	12.50±0.65[b]	95.33±2.33[b]	3.73±0.37[ab]	23.25±1.11[a]
TDF2	22.67±1.76[ab]	14.00±0.91[ab]	105.67±2.73[a]	3.35±0.29[ab]	26.50±1.04[a]
TDF3	26.00±2.31[a]	16.25±0.85[a]	107.33±3.48[a]	2.67±0.18[b]	27.00±1.58[a]

Data = Mean ± SEM; Statistical tool: One way ANOVA followed by DMRT; mean with different superscripts along columns are significantly different ($p<0.05$).

TDF1= Treatment Dress force at 2.0g/kg, TDF2= Treatment Dress force at 2.5g/kg, TDF3= Treatment Dress force at 3.0g/kg; AST= aspartate aminotransferase, ALT= alanine aminotransferase, ALP= alkaline phosphatase, TP= total proteins, BUN= blood urea nitrogen

Table 2: Biochemical Parameters of Japanese quails fed Apron star® treated seeds

GROUP	AST	ALT	ALP	TP	BUN
Control	15.25±1.38[c]	8.75±0.63[b]	79.00±2.08[c]	4.33±0.41[a]	17.25±1.25[b]
TAS1	20.0±1.08[b]	13.00±0.58[a]	102.00±3.46[b]	3.93±0.18[a]	19.50±1.04[b]
TAS2	22.00±1.58[ab]	14.33±1.33[a]	105.00±4.42[b]	3.57±0.23[ab]	20.25±1.32[ab]
TAS3	25.75±1.65[a]	16.00±1.00[a]	119..33±4.63[a]	3.00±0.20[b]	24.00±1.41[a]

Data = Mean ± SEM; Statistical tool: One way ANOVA followed by DMRT; means with different superscripts along columns are significantly different ($p<0.05$).

TDF1= Treatment Dress force at 2.0g/kg, TDF2= Treatment Dress force at 2.5g/kg, TDF3= Treatment Dress force at 3.0g/kg; AST= aspartate aminotransferase, ALT= alanine aminotransferase, ALP= alkaline phosphatase, TP= total proteins, BUN= blood urea nitrogen

Comparison of the biochemical parameters of *C. coturnix japonica* fed Dress force® and Apron star® treated seeds

Comparison of biochemical parameters of both toxicants is shown in Figure 1. At the low dose (2.0g/kg), there was no significant difference in AST, ALT, ALP and TP values of the pesticide formulations; while BUN value was significantly higher ($p<0.05$) in birds of the dress force group recording 23.25±2.22 compared to 19.50±1.04 in birds of the apron star group (Fig 1a). At the recommended dose (2.5g/kg), AST, ALT, ALP and TP values of dress force and apron star treated quails were statistically ($p>0.05$) equal. But there was significant difference ($p<0.05$) in their BUN values with dress force recording significantly higher BUN value (26.50±1.04) compared to apron star (20.25±1.32) as shown in Fig 1b. The highest dose (3.0g/kg) did not produce significant differences in all the biochemical parameters of the pesticides formulations (Fig 1c).

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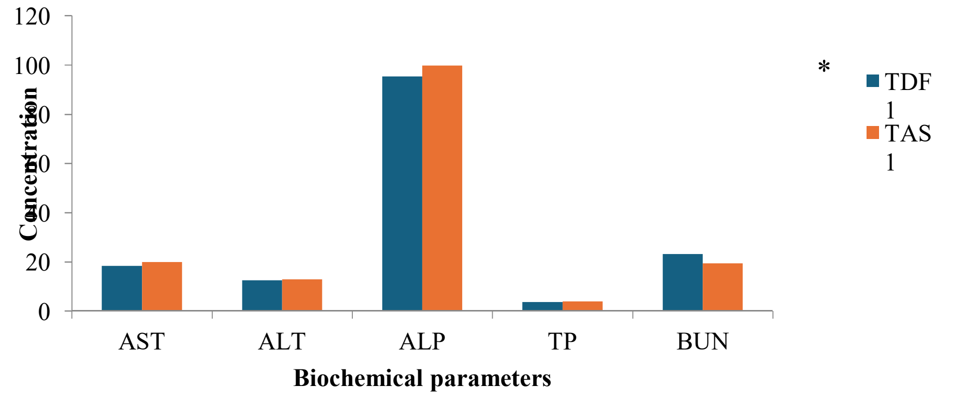
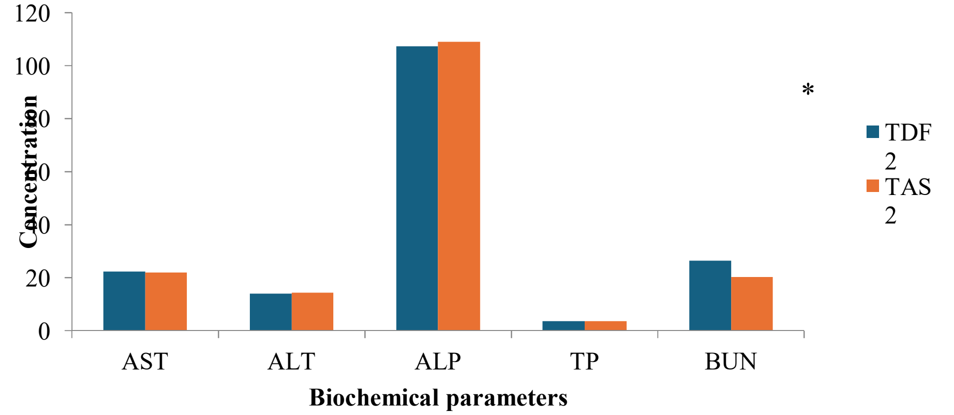
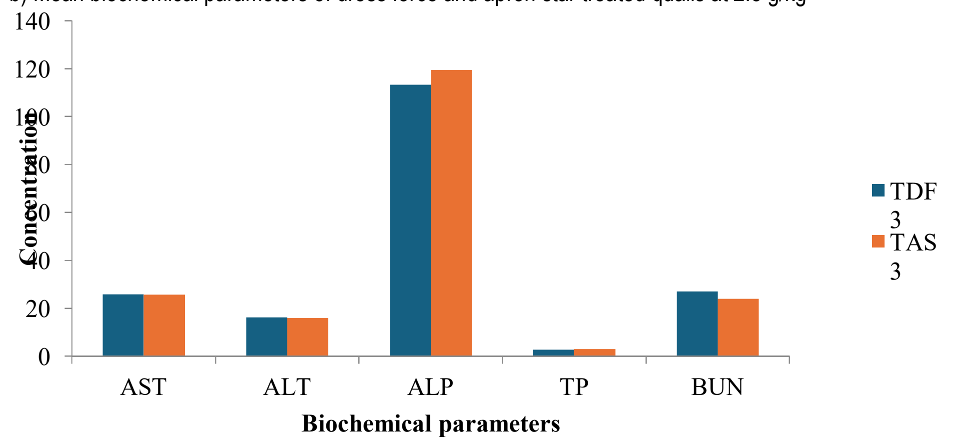


a) Mean biochemical parameters of dress force and apron star treated quails at 2.0 g/kg



b) Mean biochemical parameters of dress force and apron star treated quails at 2.5 g/kg



c) Mean biochemical parameters of dress force and apron star treated quails at 3.0 g/kg

Fig 1 (a-c): Comparison of the mean biochemical parameters of Dress force and Apron star treated Japanese quails at the different dose levels

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DISCUSSION

The pesticides had varying effects on the serum transaminases of the quails. Aspartate amino transferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are found in cells of different organs such as heart, kidneys, liver, brain, erythrocyte, intestine and lungs. The release of these intercellular enzymes into the blood and their increase are the most important clinical signs in the diagnosis of damage to cell membranes (Banaee, 2012). In this study, increase in AST, ALT and ALP quantities observed in the treated quails suggests that administration of these pesticides caused destructive changes in the hepatic cells leading to the release of the enzymes from the cytoplasm into the blood and since the liver is the main target organ of pesticide toxicity and detoxification mechanism, the change in the enzyme activities might be due to accumulation of the pesticides metabolites in the liver. The significant increase in AST and ALT indicates that the pesticide formulation is hepatoxic, while the significant increase in urea concentration indicates that the pesticide is possibly nephrotoxic this is confirmed with the histopathological changes observed in the liver and kidney of the birds. Similar findings were reported on neonicotinoids toxicity by Emam *et al*. (2018), Gul *et al*. (2017) and Khaldoun-Oularbi *et al.* (2018).

Reduction in total protein in the birds treated with the pesticide formulations might be because of decreased protein synthesis and reduction in dietary protein utilization, it might also be as due to the nephrotoxic effects of the toxicants. Elevation in serum BUN at the doses tested is an indication of functional damage of the kidney and may be due to conversion of toxic ammonia into non-toxic urea. Increased urea levels have been attributed to increased nitrogen retention and/or corrupted renal function by Hasheesh *et al.* (2002) and impaired glomerular function to remove the non-protein nitrogenous and metabolic substance of protein from the body (Hussain *et al.,* 2014).

CONCLUSION

In conclusion, this study observed varying biochemical effects of the pesticides following the continuous feeding of the treated seeds to the birds, even when used at the recommended doses, thus, careful attention should be given to the application of the pesticide seed dressers.

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