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RESEARCH ARTICLE

INFLUENCES OF *NIGELLA SATIVA* ON ANTIOXIDANT ACTIVITY IN BROILERS TOXIFIED WITH SYNTHETIZED ORGANOPHOSPHORUS COMPOUNDS.

Al Shimaa M Ibrahim¹, El-Said E Esmail¹ and Essam S Soliman^{2*}.

1. Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia, Egypt, 41522.
2. Department of Animal Hygiene, Zoonosis & Animal Behavior, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt, 41522.

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Abstract

Nigella has historical and religious uses for preventing and treating many different kinds of diseases. Influences of *Nigella* on bird performance, biochemical, antioxidant and immunological status in birds toxified with synthesized diazinon were studied. 150 broiler chick were received and divided into three groups; G1 and G2 were supplemented with *Nigella* (1.4 gm / 100 gm; 2.8 gm / 100 gm ration; respectively) and G3 is control. G1 and G2 were toxified with synthesized diazinon (100 ppm / 1 L drinking water). A total of 180 samples (60 serum, 60 plasma and 60 intestinal swab) were collected during the study period. The results revealed the ability of *Nigella* to maintain biochemical parameters as TP; Alb; Glob; ALT; AST; Urea and Creatinine after prolonged deviation. Glutathione peroxidase was sharply decreased, Glutathione Reductase was sharply increased indicating the stimulation action. Superoxide Dimutase was significantly increased until the 4th week of age followed by significant decrease. Immunoglobulins (IgG, IgM) were increased at the 2nd week of age followed by significant decrease by the protective effects of *Nigella*. On the other hand, IgE was increased and maintained at high levels. A sharp decline in both Total Bacterial Count and Total Enterobacteriaceae Count until the 4th week of age followed by sharp increase in both types in all treated groups suggesting the failure of the protective action. A follow up with the supplementation in higher doses may protect from the further changes in biochemical, antioxidant, immunological and bacteriological aspects.

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Introduction:-

Nigella sativa was known in traditional and religious medicine for almost centuries in India, China, Far East and Middle East as a natural treatment for many diseases and as a flavoring agent that claimed a medicinal usage traced back to the ancient Egyptians, Greeks, and Romans; **Elkhayat et al., (2016)**. *Nigella* seeds have many medicinal actions such as bronchodilator, hypotensive, antibacterial, antifungal, anti-diabetic, anticancer; **Periasamy et al. (2016)**, analgesic, anti-inflammatory, antidepressant; **Sahak et al., (2016)** and immune-potentiating actions.

Corresponding Author:- Essam S Soliman.

Address:- Department of Animal Hygiene, Zoonosis & Animal Behavior, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt, 41522.

Nigella was able chemically to reduce the levels of triglycerides and glucose once they were elevated in the body, as well as can stabilize cholesterol metabolism. Despite of its large benefits in the medicinal field, *Nigella* seeds were neglected and left behind although, *Nigella sativa* may possibly induce P-glycoprotein activity, **Hammad Shafiq et al., (2014)**.

Diazinon is one of the most commonly used organophosphorus compounds; and have many applications in the veterinary and agricultural fields; **Ghoneim et al., (2001)**. Diazinon is an organic phosphate and cholinesterase enzyme inhibitor that commonly used for the control of common chicken lice and other variety of insect in poultry farms. Diazinon was not recommended to be used in the presence of a live bird as chickens will consume the diazinon crystals, results in lacrimation, diarrhea, dyspnea, and death. Postmortem finding from toxicity may include lung edema, fatty livers, severe enteritis and the inclusion of diazinon crystals in the crop and gizzard contents.

The present study aimed for evaluating the influences of supplementary different concentration of *Nigella sativa* on antioxidant activity [superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase], blood chemistry, bacterial load and immunological status before and after toxifying birds with synthesized diazinon.

Material and methods:-

Experimental birds and study design:-

150 broilers (one day old chicks) were purchased and placed under the same environmental conditions for acclimatization. The birds were divided into 3 groups (G1, G2, and control G3) according to the supplemented concentration of *Nigella sativa*. G1 supplemented with 1.4% *Nigella sativa* (1.4 gm / 100 gm ration); G2 supplemented with 2.8% *Nigella sativa* (2.8 gm / 100 gm ration) and G3 was kept as control. G1 and G2 were toxified with synthesized diazinon (100 ppm / 1 L drinking water; LD50= 6.31 mg / kg) after it have been compared to the commercial form for its chemical and physical properties as well as the compound stability.

Diazinon:-

Diazinon was synthesized in the laboratory and the reaction mixture was then washed first with 400 mL of 0.1 N hydrochloric acid and then with 400 mL of 0.1 N sodium hydroxide solution. After Separating off the washing liquids, the xylene was distilled off in vacuum for the complete removal of the volatile constituents, the diazinon was finally distilled in a high vacuum (approximately 1 mbar) at 60 °C. The yield of 0,0 diethyl -0- (2- isopropyl -4-methyl -6 – pyrimidinyl) the ionophosphate was 280 g = 92.1% of the theoretical yield, **Budavari, (1996); Hansch et al., (1995); Geller et al., (2003)**.

Sampling and samples preparation:-

Blood samples: A total of 180 samples were collected during the study period, these samples were collected on a weekly basis starting from 14th day old (zero sample), and three post challenge sample collections (P1, P2, and P3). **Serum blood samples** (60 samples) were left in a cool place for 2-3 hrs, then centrifuged at 3000 rpm / 5 min. The serum samples were separated in Eppendorf tube of 3 mL capacity and kept frozen until examination for Blood biochemical parameters as Total Protein (TP); Albumin (ALB); Globulin (Glob); liver enzymes as alanine aminotransferase (ALT); aspartate aminotransferase (AST); Cholesterol (CHOL); Triglycerides (TG); Urea (Ure); Creatinine and Glucose (Gluc), the parameters were measured calorimetrically using UV1100 spectrophotometer; **Young, (1995-2001)**, immunological assay was also performed on serum samples (IgG, IgM, IgE) using DIFFU plate method; **Berne, (1974), Heremans and Masson, (1973)**. **The whole blood samples** (60 samples) were centrifuged at 4000 rpm / 10 min; then aspirate off the plasma and stored at -80 °C. The erythrocytes were washed 3-4 times with 5 mL sodium chloride 0.9% solution and centrifuged after each wash at 4000 rpm / 10 min. Finally the washed erythrocytes were re-suspended in cold distilled water and mixed; the lysate were stored at -80 °C for anti-oxidant assay as superoxide dismutase; SOD (**Nishikimi et al., 1972**), glutathione peroxidase; GSH-Px (**Paglia and Valentine, 1967**).and glutathione reductase (**Goldberg and Spooner, 1983**).

Intestinal swab (60 samples); from the original dilution of all swab sample, 1 mL was transferred aseptically to a test tube containing 9 mL sterile 0.1% buffered peptone water (w/v) to prepare a dilution of 10⁻², then from which tenfold decimal serial dilution up to 10⁻⁶ were prepared to cover a wide range of samples contamination which could be easily counted. The dilutions were subjected to two types of bacterial counts; Total Bacterial Count (TBC) and Total Enterobacteriaceae Count (TEC).

Bacterial Counts:-

Total Bacterial Count (TBC); The number of Aerobic spore forming micro-organisms in samples was carried out using drop Plate Method by **Zelver et al., (1999)** and **Herigstad et al., (2001)** using standard plat count agar. The inoculated and un-inoculated control plates were incubated at 37 °C for 24-48 hours. **Total Enterobacteriaceae Count (TEC);** was performed using drop Plate Method; **Zelver et al., (1999)** and **Herigstad et al., (2001)** with Eosine Methylene Blue Agar (EMB). Plates were inverted and incubated at 37 °C for 24-48 hours. Counting the colonies on the plates showed 30-300 colonies per plates; **Cruickshank et al., (1975, 1980)**. Five typical colonies were selected and cultured onto MacConkey agar plates, and incubated at 37 °C for 24 hr, the pure colonies on MacConkey agar plates were inoculated onto nutrient slant and incubated at 37 °C for 24 hr and kept for further identification.

Statistical analysis:-

Statistical analysis was run through SPSS (version, 16) for windows Levesque, (2007). The obtained data were analyzed statistically using factorial experiments of Analysis of Variance (ANOVA) with general linear model procedures (GLM) for all tested groups, times and their interactions.

Results:-

Proteinogram in Table 1 revealed that total protein, Albumin and Globulin revealed non-significant ($P \leq 0.05$) differences between G1, G2 and control at different sampling times. As well as, ALT, AST (Table 2) showed non-significant differences ($P \leq 0.05$) in all groups at all sampling times. Urea (Table 2) revealed a non-significant increase ($P < 0.05$) in G1 and G2 compare to control at P1, P2 and a non-significant decrease ($P < 0.05$) in G1 and G2 compare to control at P3. Creatinine revealed significant decline ($P \leq 0.05$) in G2 and control compared to G1 at zero time and a highly significant decrease ($P < 0.01$) in control compared to G1 and G2 at P1 sampling time. Meanwhile, at P2 and P3 there was a non-significant difference ($P > 0.05$) between groups.

Triglycerides and cholesterol (Table 3) showed a highly significant decrease ($P < 0.01$) in G1 and G2 compared to control at zero sampling time and a non-significant difference ($P > 0.05$) between groups at P1, P2 and P3 sampling times. Glucose showed a highly significant decrease ($P < 0.01$) in G1 and G2 compared to control group at P1 and significant decrease ($P \leq 0.05$) in G1 and control groups compared to G1 at P2 sampling time.

Glutathione peroxidase (Table 4) showed a highly significant ($P < 0.01$) decrease in G2 and control groups compared to G1 at zero and P1 sampling times, and a non-significant difference ($P > 0.05$) between all groups at P2 and P3 sampling times. Glutathione reductase and Superoxide dimutase revealed significant difference ($P \leq 0.05$) between groups at zero and P2 sampling times.

Immunoglobines (IgG) revealed a highly significant difference ($P < 0.01$) in Table (5) between groups during all sampling times and non-significant ($P > 0.05$) decrease between G2 and control compared to G1 at P1 sampling time. IgM profile showed a highly significant difference ($P < 0.01$) between groups at all sampling times; a non-significant decrease ($P > 0.05$) between G2 and control compared to G1 at zero sampling time; a non-significant decrease ($P > 0.05$) between G1 and G2 compared to control at P2 sampling time and a non-significant difference ($P > 0.05$) between G1 and control at P3 sampling time; Table (5). IgE revealed a highly significant difference ($P < 0.01$) between groups at zero and P1 sampling times, and a non-significant decrease ($P > 0.05$) in G1 and G2 compared to control at the same sampling time. A significant difference ($P \leq 0.05$) at P2 and non-significant difference ($P > 0.05$) at P3 sampling time, Table (5).

TBC revealed in Table 5 a highly significant ($P < 0.01$) difference between all groups at all sampling times, and a non-significant decrease ($P > 0.05$) between G2 and control compared to G1 at P2 sampling time. Meanwhile, TEC showed a highly significant difference ($P > 0.01$) between all groups at all sampling times and non-significant ($P > 0.05$) difference between G1 and control; G1 and G2; G2 and control and G2 and control at zero, P1, P2, and P3 sampling times; respectively.

IgM and IgE (Table 6) showed a highly significant strong positive correlation ($r = 0.646$; $P < 0.01$) with TBC. Table (7) revealed a significant weak negative correlation ($r = -0.394$; $P \leq 0.05$) between TBC and Superoxide Dimutase. TEC in Table (7) showed a significant weak negative correlation with SOD ($r = -0.336$; $P \leq 0.05$).

Discussion:-

A variety of medicinal plants as well as their extracted and purified constituents have shown many therapeutic effects. On a wide spectrum, medicinal plants was able to serve as therapeutic, medicinal alternatives, safer choices and effective treatment. Animals, broilers and human-being in a variety of cultures can be treated or prophylactic with medicinal plants. *Nigella sativa* from family *Ranunculaceae*, have been used for centuries as food preservative and medicinal plant. The oil constituents; thymoquinine (TQ), have shown a potential medicinal properties, as it possess anti-oxidant effects by enhancing the oxidant scavenger system, which as a consequence causes antitoxic effects induced by several problems; **Salem, (2005)**. The present study showed that Glutathione peroxidase possess a highly significant decrease in G2 and control groups compared to G1 at zero and P1 sampling times, and a non-significant difference between all groups at P2 and P3 sampling times. Glutathione reductase and Superoxide dimutase revealed significant difference between groups at zero and P2 sampling times.

Several diseases serve to contribute oxidative damage to many biological structures during their pathophysiological course especially in cardiovascular diseases and cancer. The oxidative damage was caused by imbalance between the pro-oxidant (free radicals) and the anti-oxidant (scavenging) mediators, as pro-oxidant predominated either by the increased generation of the free radicals contributed by excessive oxidative stress conditions, or by the poor scavenging capability in the body. Free oxygen radicals; O_2 , OH^\cdot , and NO^\cdot that are developed during a stress condition; are electrically charged and attack cells causing tearing of the cellular membranes and created a havoc with the nucleic acids, proteins, and enzymes existed in animal or human body, causing the cell to lose its structure and function completely. ROS are produced mainly by certain cells of immune system as macro- phages and neutrophils;

Maxwell, (1999); Schulz et al., (2000); Hogg, (1998).

The present study showed a sharp increase in the lipid profile and glucose, and this was attributed to diazinon intoxication. The elevated levels was declined latter as the bird proceed in age and this was attributed to the ability *Nigella sativa* to decrease the lipid peroxidation, increase the anti-oxidant mechanism and prevent the lipid-peroxidation-induced liver damage. *N. sativa* also was able to decrease the elevated glucose and MDA concentrations, increased the lowered GSH and ceruloplasmin concentrations. These results were supported with the finding from experimentally infected rabbits in **Meral et al., (2001); Mariod et al., (2009)**, and **Umar et al., (2012)**.

Nigella sativa showed beneficial immunomodulatory properties, as it was able to stimulate T cell- and natural killer cell-mediated immune responses. The oil and its active ingredients proved an excellent anti-microbial and anti-tumor properties. Gathering these beneficial and medicinal influences, *N. sativa* seed is a promising source for active ingredients that would be with potential therapeutic modalities in different clinical settings; **Salem, (2005)**. Immunoglobines (IgG, IgM) in the present study revealed a highly significant difference between groups during all sampling times with potential increase followed by decrease from the 3rd week of age proving the presence of immune barrier, that did not last for a period of time.

Table 1. Biochemical changes in proteinogram by groups supplemented with different *Nigella sativa* concentrations against Diazinon (OPC) toxicity.

Time	Group	Total Protein g/dl	Albumin g/dl	Globulin g/dl
Zero	G1	7.373±2.445 ^a	1.828±0.242 ^a	5.546±2.653 ^a
	G2	9.181±5.032 ^a	1.054±0.385 ^a	8.127±4.650 ^a
	G3	18.048±4.347 ^a	0.887±0.489 ^a	17.161±3.927 ^a
P1	G1	39.181±10.161 ^a	2.161±0.088 ^a	37.020±10.077 ^a
	G2	35.108±10.282 ^a	2.164±0.702 ^a	32.945 ±9.585 ^a
	G3	25.687±11.704 ^a	0.754±0.154 ^a	24.933±11.858 ^a
P2	G1	32.048 ±9.338 ^a	1.805±0.199 ^a	30.243±9.537 ^a
	G2	29.157±1.193 ^a	1.867±0.500 ^a	27.289±0.997 ^a
	G3	32.024±3.842 ^a	1.573±0.225 ^a	30.451±3.681 ^a
P3	G1	26.675±8.058 ^a	1.763±0.225 ^a	24.912±8.216 ^a
	G2	24.988±3.463 ^a	1.218±0.057 ^a	23.770±3.517 ^a
	G3	26.434±6.637 ^a	1.418±0.270 ^a	25.016 ±6.368 ^a

G1 Broilers supplemented with 1.4% *Nigella sativa*

G2 Broilers supplemented with 2.8% *Nigella sativa*

G3 control

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$).

Table 2. Biochemical changes in liver & kidney function by groups supplemented with different *Nigella sativa* concentrations against Diazinon (OPC) toxicity.

Time	Group	ALT U / L	AST U / L	Urea mg / dl	Creatinine mg / dl
Zero	G1	3.200±1.124 ^a	3.733±0.309 ^a	18.052±0.567 ^a	2.057±0.313 ^a
	G2	1.653±0.107 ^a	1.750±0.926 ^a	23.095±0.677 ^a	0.766±0.154 ^b
	G3	1.493±0.232 ^a	1.867±0.617 ^a	25.982±5.404 ^a	1.117±0.360 ^{ab}
P1	G1	0.853±0.053 ^a	9.450±4.554 ^a	23.364±0.828 ^a	0.951±0.037 ^a
	G2	0.800±0.562 ^a	5.133±3.739 ^a	19.361±1.544 ^a	0.412±0.102 ^b
	G3	0.800±0.640 ^a	3.967±3.106 ^a	8.545±5.079 ^b	0.341±0.156 ^b
P2	G1	1.547±0.282 ^a	9.217±2.510 ^a	19.900±0.491 ^a	0.484±0.039 ^a
	G2	5.013±2.399 ^a	5.017±3.365 ^a	12.933±5.543 ^a	0.362 ±0.237 ^a
	G3	4.587±2.356 ^a	5.367±3.285 ^a	13.818±6.034 ^a	0.823±0.365 ^a
P3	G1	1.600±0.277 ^a	5.950±2.865 ^a	18.938±1.447 ^a	0.973±0.504 ^a
	G2	1.547±0.533 ^a	4.200±3.676 ^a	20.131±1.485 ^a	1.048 ±0.435 ^a
	G3	2.507±1.102 ^a	2.683±1.184 ^a	28.907±5.530 ^a	0.908 ±0.417 ^a

G1 Broilers supplemented with 1.4% *Nigella sativa*

G2 Broilers supplemented with 2.8% *Nigella sativa*

G3 control

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$).

Table 3. Biochemical changes in lipid profile & glucose function by groups supplemented with different *Nigella sativa* concentrations against Diazinon (OPC) toxicity.

Time	Group	Triglycerides mg / dl	Cholesterol mg / dl	Glucose mg / dl
Zero	G1	87.255±22.162 ^b	26.667±1.633 ^b	882.143±43.055 ^a
	G2	146.078±21.502 ^b	81.569±37.650 ^b	990.476±55.380 ^a
	G3	378.431±43.965 ^a	236.340±18.573 ^a	915.476±124.750 ^a
P1	G1	533.333±163.866 ^a	184.052±55.219 ^a	1060.714±49.616 ^b
	G2	209.804± 4.273 ^a	130.980±32.236 ^a	1141.667±111.161 ^b
	G3	187.255± 47.191 ^a	191.373±59.268 ^a	1505.952 ±45.612 ^a
P2	G1	730.392±535.101 ^a	327.843±17.544 ^a	182.143±98.263 ^b
	G2	296.078±45.953 ^a	260.131±66.850 ^a	1192.857±154.812 ^a
	G3	408.824±109.313 ^a	212.549±104.265 ^a	783.333±281.172 ^{ab}
P3	G1	767.647±518.602 ^a	321.830±11.680 ^a	291.667±101.526 ^a
	G2	343.137±57.242 ^a	149.804±62.796 ^a	780.952±275.535 ^a
	G3	381.373±118.688 ^a	303.007±82.010 ^a	1016.667±503.872 ^a

G1 Broilers supplemented with 1.4% *Nigella sativa*

G2 Broilers supplemented with 2.8% *Nigella sativa*

G3 control

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$).

Table 4. Antioxidant enzymatic changes by groups supplemented with different *Nigella sativa* concentrations against Diazinon (OPC) toxicity.

Time	Group	GSH-Px mU / mL	GSH-Rx U / L	SOD U / mL
Zero	G1	7943.462±1685.959 ^a	475.582±415.297 ^b	2817.118±1154.865 ^b
	G2	2042.604±38.907 ^b	1714.773±328.218 ^{ab}	6357.759±563.306 ^a
	G3	1465.488±85.043 ^b	3215.200±541.572 ^a	4418.103±731.341 ^{ab}
P1	G1	5362.647±590.086 ^a	2411.400±1004.750 ^a	7635.468±2017.330 ^a
	G2	3423.794±291.801 ^b	1976.008±1935.818 ^a	4602.833±173.482 ^a
	G3	1990.729±244.611 ^c	864.085±90.613 ^a	6834.975±3214.343 ^a
P2	G1	635.477±103.751 ^a	569.358±448.788 ^b	6434.729±1534.552 ^{ab}
	G2	1841.586±745.713 ^a	7890.637±113.872 ^a	8097.290±203.645 ^a
	G3	1660.021±1195.042 ^a	3436.245±2346.301 ^{ab}	2478.449±1522.926 ^b
P3	G1	1614.630±622.508 ^a	3262.088±529.168 ^a	1631.773±226.769 ^a
	G2	1018.060±640.322 ^a	4903.180±535.825 ^a	1862.685±333.381 ^a
	G3	486.334±198.704 ^a	3262.088±487.462 ^a	2924.877±512.417 ^a

G1 Broilers supplemented with 1.4% *Nigella sativa*
G2 Broilers supplemented with 2.8% *Nigella sativa*
G3 control
Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).
Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$).

Table 5. Immunological and bacteriological changes by groups supplemented with different *Nigella sativa* concentrations against Diazinon (OPC) toxicity.

Time	Group	IgG mg / dl	IgM mg / dl	IgE IU / mL	TBC CFU / mL	TEC CFU / mL
Zero	G1	8.433±0.384 ^a	9.900±0.058 ^a	6.667±0.219 ^b	13.8×10 ³ ±600.925 ^b	1.4×10 ¹ ±2.404 ^b
	G2	6.100±0.058 ^b	4.067±0.067 ^b	6.467±0.176	16.2×10 ³ ±635.959 ^a	1.1×10 ² ±7.638 ^a
	G3	3.867±0.949 ^c	4.500±0.500 ^b	10.667±0.667 ^a	4.5×10 ³ ±404.145 ^c	1.4×10 ¹ ±2.333 ^b
P1	G1	21.100±2.098 ^a	12.133±0.353 ^b	8.800±0.208 ^b	3.4×10 ³ ±338.296 ^a	5.8×10 ¹ ±6.009 ^a
	G2	8.567±0.384 ^b	5.800±0.404 ^c	7.467±0.06 ^b	3.1×10 ² ±12.019 ^c	4×10 ¹ ±8.083 ^a
	G3	4.833±0.833 ^b	19.667±1.856 ^a	28.400±6.032 ^a	1.3×10 ³ ±28.868 ^b	0.0±0.000 ^b
P2	G1	5.367±0.470 ^b	3.233±0.186 ^b	13.600±2.346 ^{ab}	2.4×10 ² ±38.442 ^a	7.5×10 ² ±31.798 ^a
	G2	4.033±0.033 ^c	2.233±0.186 ^b	8.033±0.786 ^b	3.5×10 ¹ ±3.180 ^b	0.0±0.000 ^b
	G3	8.933±0.470 ^a	24.667±1.453 ^a	17.700±1.762 ^a	4.3×10 ³ ±1.856 ^b	0.0±0.000 ^b
P3	G1	8.767±0.384 ^a	5.467±0.418 ^a	9.400±0.643 ^a	22×10 ³ ±1527.525 ^b	12.3×10 ³ ±1452.966 ^a
	G2	7.233±0.722 ^a	3.733±0.504 ^b	10.833±1.837 ^a	41×10 ³ ±577.350 ^a	1.7×10 ³ ±378.594 ^b
	G3	4.900±0.802 ^b	6.167±0.088 ^a	13.567±1.065 ^a	9.3×10 ² ±8.819 ^c	1.7×10 ³ ±44.096 ^b

G1 Broilers supplemented with 1.4% *Nigella sativa*
G2 Broilers supplemented with 2.8% *Nigella sativa*
G3 control
Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).
Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$).

Table 6. Correlation co-efficient of immunoglobulins with Total Bacterial Count (TBC) Above Diagonal and Total Enterobacteraceae Count (TEC) Below Diagonal.

r	TBC	IgG	IgM	IgE
TEC	1	0.24	-0.304	-0.248
IgG	0.043	1	0.278	-0.158
IgM	-0.185	0.278	1	0.646
IgE	-0.096	-0.158	0.646	1

** Correlation is highly significant ($P < 0.01$). * Correlation is significant ($P < 0.05$).
NS. Correlation is non-significant ($P > 0.05$).

Table 7. Correlation co-efficient of antioxidants with Total Bacterial Count (TBC) Above Diagonal and Total Enterobacteraceae Count (TEC) Below Diagonal.

r	TBC	GSH-Px	GSH-Rx	SOD
TEC	1	-0.015	0.114	-0.394
GSH-Px	-0.191	1	-0.327	0.136
GSH-Rx	0.074	-0.327	1	0.021
SOD	-0.366	0.136	0.021	1
**. Correlation is highly significant (P < 0.01). *. Correlation is significant (P < 0.05). NS. Correlation is non-significant (P > 0.05).				

Conclusion:-

Nigella sativa supplementations (1.4%, 2.8%) were able to protect the birds from intoxication at once up to the 4th week of age. Although it maintained the biochemical parameters in a normal levels after little deviation resulted from intoxication. Antioxidants were severely stimulated by the *Nigella sativa* supplementations (1.4%, 2.8%) to initiate its protective effect. A follow up with the supplementation in higher doses may protect from the further changes in biochemical, antioxidant, immunological and bacteriological aspects.

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