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PROPHYLACTIC EFFECT OF NIGELLA SATIVA L. POWDER AGAINST OXIDATIVE STRESS INDUCED BY CYPERMETHRIN IN BROILERS

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ABSTRACT

Unsexed 60 Lohmann broiler chicken of 1-day-old were weighed and reared up to 2 weeks and allotted to 4 dietary treatment groups each of 15 birds. The first group received normal broiler fattening feed free of any additive and served as control. The 2nd group was fed on 10 g/kg diet nigella sativa powder (NS) whereas, the 3rd group was fed on 600 mg/kg diet cypermethrin and 4th group was fed on 10 g/kg diet NS and 600 mg/kg diet cypermethrin (CYP), for a month up to 44 - days old. NS induced a significant improvement in live body weight (LBW), body weight gain (BWG), feed intake (FI) and performance index (PI) and feed conversion ratio (FCR) in CYP intoxicated broilers. CYP residue levels in peritoneal fat, breast, thigh muscles, liver and kidney were below the maximum residue level (MRL).

Dietary NS induced a significant reduction in CYP residues in peritoneal fat, and thigh muscles, liver and accumulation of CYP residues were the highest in liver followed by peritoneal fat, thigh, breast and kidney. CYP induced a significant increase in serum ALT, AST, ALP, urea, creatinine, hepatic lipid peroxide, malondialdehyde (MDA) levels as well as hepatocellular DNA damage (% DNA in tail, tail length and the olive tail moment (OTM)), while evoked a significant decrease in the activities of hepatic catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GHS px) and superoxide dismutase (SOD).

However, NS powder significantly ameliorated oxidative stress, biochemical disorders and geno-toxicity produced by cypermethrin.

Keywords:

Cypermethrin - Nigella sativa - Growth Performance - oxidative stress-DNA-broilers.

INTRODUCTION

Cypermethrin (CYP), type II synthetic pyrethroid, as it contains an alpha-cyano group, and owing to its lipophilic properties, it tends to accumulate in tissues and organs, primarily in the central and peripheral nervous system. It has become a major class of insecticides used to control of ectoparasites those infecting caged layer hens. Emulsifiable concentrate or CYP dust applied as a spray and dust, respectively affecting some biochemical parameters when applied on 3-fold higher doses than recommended by the manufacturer in laying hen **(Chernaki-Leffer *et al.,* 2013).** CYP is commonly used in agriculture, forestry as well as in public and animal health programs. Although considered nontoxic, it has adverse effect on the hepatic and renal system **(Sushma and Devasena, 2010).** Given its rapid metabolism, the mean residence time of pyrethroids in the body is not long. Metabolism occurs by means of cytochrome enzymes (and metabolic products are eliminated from the body mostly in urine. In foods and household pest control products are important exposure sources for pyrethroids **(Palmquist *et al.,* 2012).**

Nigella sativa Linn. Belongs to family Ranunculaceae is an herbaceous annual plant that commonly known as black cumin and black seed. Its health enhancing potentials are due to active ingredients (thymoquinone, carvacrol, thymol) that are mainly concentrated in fixed or essential oil. Its active ingredients protect the body from nephrotoxicity and hepatotoxicity **(Darakhshan *et al.,* 2015).** It is suggested that seed, its oil and extracts act as antioxidant. The supplementations of diets with appropriate antioxidant or formulation containing bioactive compounds are useful in reversing the sequential distortion due to free radicals **(Bourgou *et al.,* 2012).** The seeds hold fixed and essential oils, sterols, flavonoid triglycerides, fatty acids, proteins, alkaloids, tocopherol and spooning **(Matthaus and Ozcan,2011).**

Nigella sativa (NS) seeds have immunostimulant effects, thus maintaining broiler chicken in good health and appear to be a multipurpose feed growth promoter and may be promising in improving broiler performance **(Al-Mufarrej, 2014).** NS has an excellent potential as alternative to vaccines and antibiotics in improving poultry immunity and reducing mortality due to therapeutic properties. The majority of therapeutic properties NS are due to the presence of thymoquinone (TQ) which is a major bioactive component of the essential oil **(Vafaee *et al.,* 2016).**

Therefore, the current study was conducted to investigate the possible protective effect of a natural growth promoting substance (Nigella sativa) against hepatorenal injury, oxidative stress and genotoxicity induced by cypermethrin intoxication in broilers.

MATERIAL AND METHODS

Broiler Ration:

Broiler ration obtained from Fat Hens Co. Kafr El-Zayat, Gharbia Governorate. Diet formulation based on nutrient requirements by **NRCS (2003).**

Table (1): Composition of the experimental basal diets.

Starter- diet			Finisher grower diet		
Ingredients	**Chemical composition**	**%**	**Ingredients**	**Chemical composition**	**%**
Yellow corn 54.00 **Soybean meal (46%cp)** **Yellow corn glutine (60%)** **Soybean oil** **Dicalcium Phosphate (762)** **Limestone 0.92** **NaCI 0.25** **Vit + Min mix (1/447)** **Sodium bicarbonate** **DL-methionine** **L-Lysine hydrochloride**	**Protein** **Fat** **Fibers** **Energy**	**23%** **5.33%** **3.26%** **3000 K.K.**	**Yellow corn 54.00** **Soybean meal (46%cp)** **Yellow corn glutine (60%)** **Soybean oil** **Dicalcium Phosphate (762)** **Limestone 0.92** **NaCI 0.25** **Vit + Min mix (1/447)** **Sodium bicarbonate** **DL-methionine** **L-Lysine hydrochloride**	**Protein** **Fat** **Fibers** **Energy**	**21%** **6.22%** **3.06%** **3100 K.K.**

Cypermethrin 10% E.C:

It is manufactured by cenavisa Laboratories' Cemi Pendra Estela s/n 43205 Reus (Spain). Chemical formula $C_{22}H_{19}Cl_2NO_3$. Molar mass 416.30 g/mol. Chemical class: synthetic pyrethroid. α- cyano -3- phenoxybenzyl (1RS) - cis, Trans - 3- (2, 2-diclorovinyl) - 2, 2- dimethyl-cyclopropane carboxylate of 10% purity. Common name: cypermethrin, alphamethrin, beta-cypermethrin, zeta-cypermethrin.

Nigella sativa L. Seed powder: (Family Ranunculaceae):

Dried seeds of NS were purchased from the Egyptian Ministry of Agriculture and Land Reclamation markets, Giza, Egypt. The seeds washed, air-dried in an oven at 40 °C overnight and freshly grinded to a coarse powder using an electric grinder and mixed with ration.

Experimental Design:

Sixty-One-Day old unsexed Lohmann apparently healthy broiler chicken obtained from a local hatchery were used. Initially the chicken was reared at brooding house up to 2 weeks. Feed and water provided ad libitum. The birds were weighed and allotted to 4 dietary treatment groups each of 15 chicken. The first group received normal broiler fattening feed free of any additive and served as control, the second group were fed on 10 g/kg diet freshly prepared NS powder according to **Miraghaee *et al.,* (2011).** Whereas, the third group were fed on 600 mg/kg diet cypermethrin (CYP) according to **NešKović *et al.,* (2013).**

The 4th group fed on 10 g/kg diet NS plus 600 mg/kg diet cypermethrin (CYP) for a month (up to 44-days old). Blood samples were collected from brachial wing vein, left to clot in clean dry tubes, and then centrifuged at 3000 rpm for 10 minutes. The sera were kept frozen at - 20°C for biochemical analysis. At the end of the experiment all birds were sacrificed by cervical dislocation and specimens of muscle, liver, kidney and peritoneal fats from each group were taken and prepared for further investigation.

Growth Rate Measurements:

Terminal live body weight (LBW), daily body weight gain (BWG) and daily feed intake (FI) were recorded. Feed conversion ratio (FCR) (kg feed consumed / kg of live weight gain (LBWG) and the performance index (PI) = LBW (kg) / FCR × 100 were calculated.

Residue Analysis:

Ration was analyzed before treatments. Serum, peritoneal fat, muscles (breast and thigh), liver and kidney samples collected from the sacrificed birds (control and treated). Ten grams of

each sample was extracted with 3g NaHCO3, 10-gram NaSO4 and 20 ml ethyl acetate in falcon tube in ultrasonic bath for 3 minutes. Then it was centrifuged for 3 minutes at 3200 g and the crude extract was filtered by 0.20µm PIFE filter. Finally it was injected in Hewlett Packard GC Model 6890 equipped with Ni63- electron Capture detector and so residues of cypermethrin were determined using gas chromatography method described by **Niewiadowska *et al.* (2010)** in Department of Pesticides Residues and Pollution, Center Agriculture Pesticide Laboratory (CAPL).

Liver and Kidneys Function Tests:

Activities of serum aspartate aminotransferase (AST) and alanine amino transferase (ALT). Serum alkaline phosphates (ALP) were estimated according to the method described by **Sahoo *et al.* (2014).** Urea and creatinine were determined according to **Newman and Price (1999)** using kits for Biodignostic Cairo, Egypt.

Measurement of Hepatic Lipid Peroxidation and Antioxidants Activities:

Liver specimens have been cleared of surrounding fat, cut into small pieces, washed with saline solution, and weighed out. Liver tissues were homogenized in ice-cold isotonic physiological saline solution to form homogenates at a concentration of 0.1g/ml. Samples were centrifuged at 3500 rpm for 10 min at - 4ºC, then the supernatant was obtained and used for antioxidant enzyme measurements by spectrophotometric methods. Superoxide dismutase (SOD) activity was estimated as described by **Spitz and Oberley (1989).** Catalase (CAT) activity was determined according to the method of **Aebi (1984).** Hepatic glutathione peroxidase (GPx) activity was assessed according to **Flohe and Gunzler (1984).**

Non-enzymatic reduced glutathione (GSH) was determined according to **Lin Hu *et al.* (1988).** Lipid peroxides formation was determined as Malondialdehyde (MDA) **according to Jentzsch, *et al.* (1996)** by kits of Biodignostic, Cairo, Egypt.

Comet Assay:

The liver tissue samples (200 - 250 mg) were placed in 0.5 mL of cold phosphate buffered saline (PBS) and finely minced to obtain a cell suspension. The slides mounted with cells were covered with coverslips and kept in the refrigerator for 3-5 min to solidify the low-melting agarose. DNA damage is commonly measured at the level of individual cells using the so-called comet assay (single-cell gel electrophoresis) by the method described by

Tice *et al.*,(2000) in Pathology Department,Animal Health Research institute (AHRI),Dokki, Cairo.

Statistical analyses:

Data were statistically analyzed using analyses of variance (F-test) followed by Duncan's multiple range test. A probability at level of 0.05 or less was considered significant. Standard errors were also estimated using IBM SPSS statistics program version 20.

RESULTS

Table (2) revealed that CYP induced a significant decrease in life body weight (LBW), body weight gain (BWG), feed intake (FI) and performance index (PI) ($p<0.05$) and pronounced increase in feed conversion ratio (FCR) in comparison to control groups. However, Nigella sativa (NS) induced a significant improvement in all these parameters in CYP-intoxicated broilers. CYP residues were not detected (ND) in broiler ration before pesticide treatment.

Table (2): Effect of supplementation of cypermethrin (600mg/kg diet) and/or nigella sativa powder (NS) (10 g/kg diet) on growth performance and feed efficiency of chicken broilers at age of 44 days.

Treatments / Parameters	Group 1 (Control)	Group 2 NS	Group 3 CYP	Group 4 CYP+ NS	F-Prob
Final Live body weight (gm/bird)	2213.33± 2.03a	2459.00± 4.93 b	1711.33± 5.94 c	1981.67 ± 2.19 d	0.000*
Daily live body weight gain (gm/ bird)	61.60 ± 0.53 a	68.62± 0.31b	47.51± 0.29 c	55.47 ± 0.29 d	0.000*
Daily feed intake (gm/bird)	107.49± 0.28 a	116.15± 0.08 b	86.56 ± 0.29 c	98.45 ± 0.29 d	0.000*
Feed conversion ratio (FCR)	1.74 ± 0.01 a	1.69± 0.01b	1.82± 0.01 c	1.77 ± 0.01 d	0.000*
Performance Index (P.I)	126.81± 0.51 a	145.22± 0.48 b	94.04 ± 0.62 c	111.75 ± 0.09d	0.000*

Data were represented as means±SE. *significantly difference using ANOVA test at P <0.05 Mean in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

Table (3) revealed that CYP residues were not detected (ND) in organs and tissues of control groups (-ve & +ve), as well as in serum of all treated groups and were below the maximum residue level (MRL) in peritoneal fat, breast and thigh muscles, liver and kidney in all treated groups. However, dietary NS powder induce a significant reduction in CYP residues in peritoneal fat, and thigh muscles, liver ($P < 0.05$) (F1). CYP residues were significantly different in different organs and tissues ($P < 0.05$) (F2) and accumulation of CYP residues in the liver were the highest in liver followed by peritoneal fat, thigh, breast and kidney. In addition, recovery percentage of CYP were (80%-98%) for all tested samples determined by GC method and this range was accepted.

Table (3): Cypermethrin residue in serum (ppm) and tissues (mg/kg) of broilers received dietary cypermethrin (CYP) (600mg/kg) and/or Nigella sativa powder (NS) (10g/kg) at age of 44 days.

Samples \ Groups		Group 1 (Control)	Group 2 NS	Group 3 CYP	Group 4 CYP+ NS	F_1-Prob	MRLs Codex, 2009) (mg/kg)
Residues (mg/kg)	Serum	ND	ND	ND	ND	-	-
	Peritoneal fat	ND a	ND a	0.042± 0.0009 b	0.034± 0.0020 c	0.000*	2 mg/kg
	Breast muscle	ND a	ND a	0.013± 0.0009 b	0.012± 0.0006 b	0.000*	2 mg/kg
	Thigh muscle	ND a	ND a	0.017± 0.0030 b	0.012± 0.0005 c	0.000*	2 mg/kg
	Liver	ND a	ND a	0.062± 0.0018b	0.054± 0.0009 c	0.000*	2 mg/kg
	Kidney	ND a	ND a	0.001± 0.0002 a	0.001± 0.0006 c	0.059	2 mg/kg
	F_2-Prob	-	-	0.000*	0.000*		

Data were represented as means±SE. * significantly difference using ANOVA test at $P < 0.05$
Means in the same row with different letters are significantly different (Duncan multiple range test $P < 0.05$). F1: between different treatments F2: between different tissue samples.

Table (4) demonstrated that CYP produced significant increase in serum aminotransferase enzymes (ALT and AST) as well as serum ALP and urea and creatinine (P<0.05) in comparison to control group. Furthermore, dietary NS induce a significant improvement in hepatorenal function in CYP- intoxicated broilers.

Table (4): Effect of dietary cypermethrin (CYP) (600mg/kg) and/or Nigella sativa seeds powder (NS) (10g/kg) on liver and kidney function in broilers at age of 44 days.

Groups / Parameters	Group 1 (Control)	Group 2 NS	Group 3 CYP	Group 4 CYP+ NS	F-Prob
ALT(IU/L)	25.33±0.88a	21.00±0.58 b	39.50±0.29c	28.67±0.88 d	0.000*
AST (IU/L)	35.42±0.36a	21.33±0.37 b	44.00±0.58 c	41.17±0.72 d	0.000*
ALP (IU/L)	118.63±0.69a	104.27±0.88b	142.17±0.60c	115.83±0.93d	0.000*
Creatinine (mg/dl)	0.42±0.01a	0.40±0.01a	0.71±0.01b	0.46±0.01c	0.001*

Data are represented as means of 15 samples ± S.E. * significantly difference using ANOVA test at P <0.05. Means in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

An oxidative damage in liver was revealed by pronounced decline in the activities of non-enzymatic antioxidant GSH and hepatic enzymes CAT, GSH-Px and SOD (P<0.05) and a significant increase in the lipid peroxide malondialdehyde (MDA) content (P<0.05) in CYP intoxicated broilers compared to control groups (P<0.05).In addition, treatment of CYP-intoxicated broilers with NS prevented depletion of hepatic GSH content and antioxidant enzymes. While, induced a significant decrease in hepatic lipid peroxidation (Table 5).

Table (5): Effect of dietary cypermethrin (CYP) (600mg/kg) and/or Nigella sativa seeds powder (NS) (10g/kg) on hepatic enzymatic and non-enzymatic antioxidants activity and lipid peroxidation in broilers at age of 44 days.

Parameter / Groups	Group 1 (Control)	Group 2 NS	Group 3 CYP	Group 4 CYP+ NS	F-Prob
SOD (U/g)	56.41±0.30a	70.72±0.36 b	33.62±0.31 c	51.10±0.21 d	0.000*
CAT (U/g)	2.56±0.24 a	3.01±0.02 b	1.06±0.06 c	1.92 ±0.08 d	0.000*
GSH (U/g)	4.04±0.27 a	5.17±0.16 b	1.36±0.27 c	5.08±0.14 b	0.000*
GSH- Px (U/g)	22.60±0.30 a	24.57±0.29 b	16.41±0.25 c	19.38±0.23 d	0.000*
MDA (nmol/g)	6.23±0.19 a	5.48±0.27 b	9.17±0.12 c	7.46±0.25 d	0.001*

Data are represented as means of 15 samples ± S.E. * significantly difference using ANOVA test at P <0.05. Means in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

Comet assay revealed that cypermethrin-induced genotoxicity that confirmed by hepatocellular DNA damage. The DNA damage was evidenced by an increased percentage of DNA in tail, comet length, tail length and the olive tail moment (OTM) (P<0.05) (Table 6), as well as micronucleus appearance in the cytoplasm Fig. (1 C). In comparison to intact DNA in control groups Fig. (1 A,B). However, NS significantly reduced DNA damage [% DNA in tail, comet length, tail length and the olive tail moment (OTM)] in liver cells of CYP- intoxicated broilers on dietary NS (P<0.05) Fig. (1 D).

Table (6): Effect of dietary cypermethrin (CYP) (600mg/kg) and/or Nigella sativa seeds powder (NS) (10g/kg) on hepatocellular DNA in broilers at age of 44 days.

Groups / Parameters	Group 1 (Control)	Group 2 NS	Group 3 CYP	Group 4 CYP+ NS	F-Prob
Comet Length (px)	22.66±0.28a	22.40±0.33 a	31.30±0.35 b	25.00±0.58 c	0.000*
%DNA in Head	98.48±0.29a	98.34±0.37 a	93.93±0.17 b	97.19±0.13 c	0.000*
%DNA in Tail	2.19±0.22 a	1.93±0. 39 a	5.39±0.47 b	3.51±0.23 c	0.000*
Tail Length (px)	1.06±0.07 a	1.03±0.03 a	6.33 ±0.20 b	2.46±0.25 c	0.000*
Olive Tail Moment	0.18±0.02a	0.17±0.01a	0.32±0.01b	0.22±0.01c	0.000*

Data are represented as means of 15 samples ± S.E. * significantly difference using ANOVA test at P <0.05. Means in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

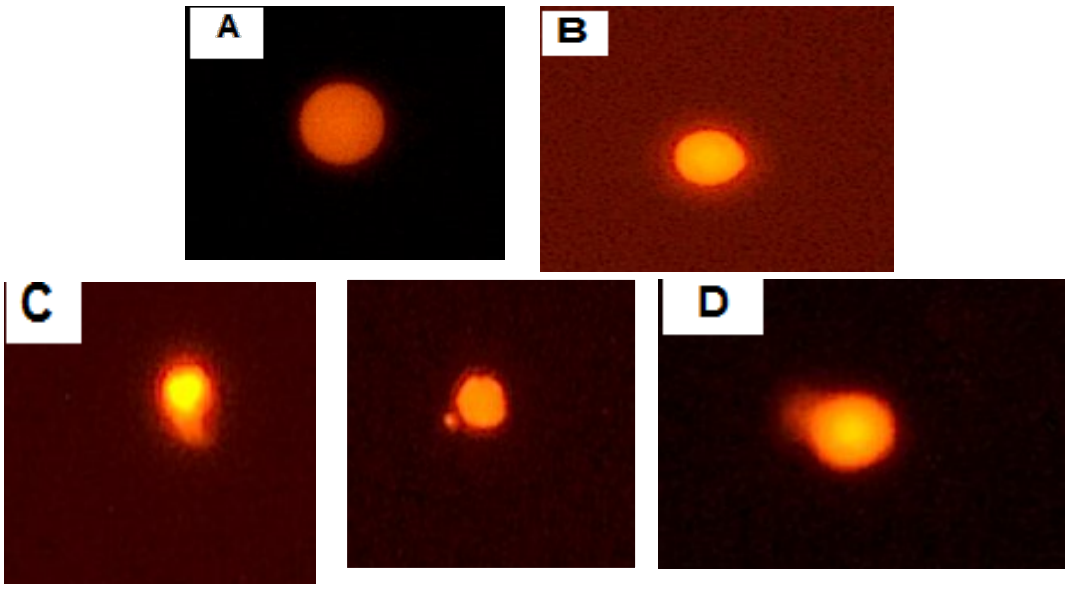

Fig. (1): Hepatocellular comet assay in broilers showing: **(A & B):** Intact DNA **(C):** Left: Cell have extensive DNA damage. Right: Micronucleus appearance in the cytoplasm **(D):** Cell have moderate DNA damage.

DISCUSSION

CYP-intoxicated broilers showed more prominent signs of toxicity that emphasized by a significant reduction in body weight as reported by **Sharaf *et al.* (2010).** Interaction with sodium channels is not the only mechanism of alpha-cyan pyrethroids (type II pyrethroids) but also cause a long-lasting protraction of sodium permeability of the nerve membrane during excitation **(Schleier and Peterson, 2011).**

Black cumin seeds were composed of 8.6% of moisture, 21.69% of CP; 6.05% of CF; 29.46% of EE; 4.50% of total ash, and 29.70% of nitrogenfree extract. They also contained macro -minerals (mg/100gm), i.e., Ca (572), P (540), Mg (264), Na (17.8) and K (810), and microminerals (mg/100gm), i.e., Cu (2.65), Zn (6.21), Fe (9.68) and Mn (8.50). Various scientists across the globe provided evidence about the composition of black cumin seeds. Overall, moisture, EE, CP, total ash and total carbohydrates contents were 3.8 to 9.0%, 22.0% to 40.35%, 20.85% to 31.2%, 3.7% to 4.7% and 24.9% to 40.0%, respectively **(Ayaşan, 2011).** It was found that compounds isolated from NS (including thymoquinone, carvacrol, t-anethole and 4-terpineol) have appreciable free radical scavenging properties **(Burtis and Bucar, 2000)** and the possible mode of action may be due to the activity of these ingredient. The seeds of NS (black

cumin) with thymoquinone as its main active constituent are mainly used for medicinal purposes. NS seeds and its extracts have important activities such as antioxidant and hepatoprotective. The beneficial impacts of NS in enhancing feed conversion ratio and growth performances could be due to its pharmacologically active substances and high nutritive value and may be the most suitable alternative to antibiotics and growth promoter especially in poultry nutrition **(Abd El-Hack *et al.,* 2016)**.

Many active components of NS have been identified, including thymoquinone, dithymoquinone, nigella, p-cymene and pinene. NS is also a source of minerals such as magnesium, calcium, phosphorus, potassium,iron, cobalt,zinc and manganese that are considered essential cofactors in various enzyme functions and vitamins. The active ingredients and pharmacologically active substances maintain health and improve the performance of poultry. NS significantly increased body weight gain (BWG) in broilers **(Khan *et al.* 2012).**NS have been reported to stimulate secretion of digestive enzymes (lipase and amylase) and intestinal mucous in broilers, to stimulate feed digestion, to impair adhesion of pathogens and to stabilize microbial balance in the gut, leading to better feed utilization and assimilation **(Sogut *et al.* 2012).**

Feeding birds with diets containing plant-derived phytonutrients such as carvacrol and thymol significantly improved the immune response in chickens and lowered poultry infectious diseases. Supplementing the diet of broilers with carvacrol caused an improvement in body weight gain **(Lillehoj *et al.,* 2011).** NS might have a good effect on protein metabolism.

Both NS seeds and their extracts supplemented diet increased feed intake when added in the broiler diet **(Ismail, 2011).**

In this study, CYP residue levels in the liver, peritoneal fat, breast muscle and leg muscle of broiler chickens was low in all treated groups and were below the maximum residue level (MRL) (2 mg/kg) established in **(Codex, 2009).** Similar to our study, accumulation of CYP residues in the liver and tissues was low. CYP residues in liver were higher than other investigated tissues **(NešKović *et al.,* 2013).** As CYP is fat-soluble, the highest residues could be expected in the fat of broilers. The MRL was 0.007, 0.007 0.048, mg/kg for poultry muscle, liver and fat respectively. Parent CYP was a significant part of the residue in fats. Metabolites at low levels were produced by ester cleavage and hydroxylation of the phenoxy ring. DCVA (3-(2, 2-dichlorovinyl) 2, 2-dimethylcyclopropane carboxylic acid) was a major part of the

residue in muscle and liver. In birds, pyrethroids are eliminated two to three times faster via ester hydrolysis and oxidation and this occur due to higher metabolic rates of birds **(JMPR, 2009).** CYP is very low in toxicity to birds. The cypermethrin residue levels were usually below detection limits in the organs and tissues of chickens.CYP residues in kidneys were much lower as compared to fat in broiler chicken. The liver contained the highest residue while, residues in breast and leg muscle were too low. The low residues in final poultry products (meat), is explained by the fact that birds have very effective mechanisms for the detoxification of CYP so it is rapidly metabolized and eliminated from the organism.

CYP with smaller quantities of metabolites often present only at trace levels. **(USA, FAO, 2010).** Despite of reduced toxicity, CYP is hazardous for chickens. Toxicity in liver and kidney were observed in broilers administered CYP **(Dharmendra *et al.,* 2012).** The increase or decrease of enzyme activity depends on the intensity of cellular damage. Rarely serum AST and ALT levels decrease in toxicology studies. The increase in the level of ALT and/or AST is a good indicator of hepatic toxicity **(Hayes, 2007).** In broilers, **NešKović *et al.* (2013)** found that AST activity was significantly increased in the CYP-treated chickens. ALP activity was significantly decreased. CYP did not accumulate in organs and tissues of chickens. These findings point to the possible adverse effects of cypermethrin on liver functions. CYP induces biochemical alterations in broiler chicken. CYP, have been shown to increase liver enzymes in the liver of broiler chicken. Possibly dehydration with CYP treatment led to increased serum proteins first **(Sharaf *et al.,* 2010).** Creatinine was significantly increased, as it is a waste product of protein metabolism and are held in blood in cellular damage **(Aslam *et al.,* 2010).** Powdered NS seeds from 1- 2 % had a protective effect against nephrotoxicity and hepatoxicity induced by chemicals **(Durrani *et al.,* 2007).** Carvacrol has been reported to exhibit numerous bioactivities in cells in addition to hepatoprotective properties of carvacrol and metabolic, synergistic, and mechanistic aspects **(Friedman, 2014).** Similar to our results, **Hermes *et al*. (2010)** observed that NS seeds decreased serum aspartate transaminase (AST) and alanine transaminase (ALT) in broilers.

The potential to induce oxidative stress is one of the toxic mechanisms of action of pesticides. By causing several negative changes within the cell and in the cell membrane, oxidative stress reduces the ability of cells to maintain their normal functions, and thereby, causes adverse effects through its direct or indirect impact on the tissues, organs and systems of the body

(Mustafa and Abdollahi, 2013). CYP is one of the most common contaminants in the ecosystem that induce hepatic oxidative stress **(Jin *et al.,* 2011).** Increased lipid peroxidation could be a consequence of depleted GSH stores induced by CYP -induced oxidative stress. Treatment with thymoquinone (TQ) restores normal levels of GSH,which might have resulted from the TQ-mediated reduction of peroxidative activity among cells. TQ increased and maintained the activities of the antioxidant defence mechanism of SOD and CAT enzymes significantly. In addition, TQ protected cell proliferation leading to enhanced regeneration after tissue damage **(Ince *et al.,* 2012).**

The antioxidant impacts of NS seeds could be due to the active components such as carvacrol, thymoquinone, 4-terepinol and anethole **(Toma e*t al.,* 2015).** NS may decrease the production of hydroxyl (OH), hydrogen peroxide (H2O2) and superoxide (O2) radicals, which are produced by aerobic respiration in the organisms, leading to an increase in lipid peroxidation. NS protect against oxidative stress by inhibiting free radical production and by regulating glutathione. Inclusion of NS seeds in the diet resulted in a significant decrease in erythrocyte MDA concentration and increased GSH concentration in chickens. NS treatment decreased lipid peroxidation and increased GSH levels in various tissues such as liver, kidney and serum **(Tuluce *et al.,* 2009).** NS powder supplementation resulted in a higher level of GSH-Px and SOD, while lipid peroxidation (MDA) level, and were significantly lower in muscle.

It was suggested that NS supplementation was effective at improving broiler performance and meat quality by enhancing antioxidant activities and suppressing lipid peroxidation in broilers meat **(Rahman and Kim, 2016).** The antioxidative status of broilers was improved due to the antioxidant property of thymol and carvacrol by elevating the activity of antioxidant enzymes SOD and GSH-Px and decreased MDA level in liver **(Hashemipour *et al.,* 2013).**

Two thousand and twelve Cypermethrin (CYP) could induces toxic effects and DNA damage in broilers chicken. Because of genetic damage, i.e., damage to the chromosomes, fragments lagging in the course of anaphase or lagging acentric chromosomes or cytoplasmic chromatin-containing bodies are failed to be incorporated into daughter nuclei (clast genesis), results in the development of significantly higher numbers micronuclei in red blood cells. Micronucleus appearance in the cytoplasm is considered as biomarker of DNA damage.

The DNA damage cause different consequences in different body systems **(Sharaf *et al.,***

2010). Exposure of chick embryos to and cypermethrin induced DNA damage as emphasized by an increased tail moment in the comet assay. Further, the presence of micronucleate erythrocytes and various abnormal cells including dacrocytes, microcytes, arthroplasties, and squashed/notched nuclei in the blood smear indicate the presence of insecticide-induced genotoxicity **(Uggini and Suresh, 2013).**

NS has also been shown to have anti-genotoxicity properties in a variety of biological species **(Babazadeh *et al.*, 2012).** NS oil (NSO) not only protected hepatocytes from the severe toxicity through enhancement of antioxidant enzymes activities, (SOD) and (CAT) in serum, but also provided a DNA protection as revealed by the comet assay **(Tuorkey, 2017).** Black seed extract significantly reduced DNA damage in leukocyte cells **(Cetin *et al.*, 2008).** Treatment with carvacrol controlled the damage of DNA and reduced mitochondrial enzymes. The existence of hydroxyl group (OH) which linked to aromatic ring is suggested to be the reason for the highly antioxidant activity of carvacrol and restoring the concentrations of lipid peroxidation products, as well as enzymatic and non-enzymatic antioxidants to normal either in vitro or in vivo as explained by **(Guimaraes *et al.*, 2010).**

Conclusions and Recommendations:

It was concluded that cypermethrin residue levels were below detection limits in the organs and tissues of broiler chickens, but was continue to be a threat for poultry. On the other hand, NS supplementation to the broilers diet act as detoxifying agent and might boosts up growth rate and improve feed conversion ratio by increasing feed intake. Furthermore, NS could be considered as a promising therapeutic agent against hepatoxicity, nephrotoxicity and genotoxicity induced by cypermethrin.

It was recommended to control the overuse of pesticides in agriculture and poultry farms, as well as to incorporate NS seeds powder in broilers diet in order to increase mass- production and to protect them against pesticides toxicity.

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التأثير الوقائى لمسحوق حبة البركة المضاد للأكسدة الناتجة عن السيبرميثرين فى دجاج التسمين

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الملخص العربى

لقد قمنا بتقسيم عدد 60 كتكوت إلى 4 مجموعات كل مجموعة تتكون من 15 كتكوت. المجموعة الأولى الضابطة وتتغذى على العلف فقط والمجموعة الثانية تتغذى على العلف مضاف له 10جم/كجم مسحوق حبة البركة والمجموعة الثالثة تتغذى على العلف المضاف له 600 مجم/كجم مبيد السيبرميثرين و المجموعة الرابعة تتغذى على العلف المضاف له 600 مجم/كجم مبيد السيبرميثرين بالإضافة إلى 10جم/كجم مسحوق حبة البركة لمدة شهر.

و لقد لاحظنا نقص فى إستهلاك العلف و وزن الطائر و معدل تمثيل الغذاء وكذلك فى أنزيمات السوبر اكسيد ديسميوتيز SOD و الكاتاليز CAT والجلوتاثيون بيروكسيديز GPx مع زيادة ملحوظة فى إنزيمات الكبد (ALP, AST, ALT) و(اليوريا والكرياتينين) و احد نواتج أكسدة الدهون المالون دايالدهايد , MDA بالإضافة إلى التأثير السلبى على الحامض النووى DNA فى خلايا الكبد كنتيجة لتعرض الطائر لمبيد السيبرميثرين فى العلف ولقد قمنا بالتأكد من خلو العلف من السيبرميثرن فى بداية التجربة وقبل إضافة المبيد ولقد كانت متبقيات السيبرميثرين فى الحدود المسموح بها فى جميع الأنسجة وكان أعلى تركيز للمبيد فى الكبد يليه الدهون ثم العضلات والكلى بينما حدثت زيادة فى إستهلاك العلف وزيادة فى وزن الطائر و تحسن واضح فى معدل تمثيل الغذاء ووظائف الكبد و الكلي ومضادات الأكسدة و تحسن ملحوظ فى حالة الحامض النووى DNA و نقص فى تركيز المبيد فى الأنسجة نتيجة لإضافة مسحوق حبة البركة للعلف.

لذلك نوصى بتشجيع مربى الدواجن على إضافة مسحوق حبة البركة للأعلاف نظرا لتأثيرها الفعال في زياة الكتلة الإنتاجية ولأنها تعتبر بديل أمن للمكملات الغذائية الصناعية المستوردة باهظة التكلفة وكذلك حماية الطائر من التأثير السلبى للمبيد.