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Ameliorative effect of *Spirulina platensis* and *Erythrina variegata* on oxidative stress in imidacloprid intoxicated white leghorn cockerels

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Abstract

The present experiment was conducted to evaluate the ameliorative efficacy of *Erythrina variegata* leaf powder (EVLP) and *Spirulina platensis* (SP, Blue green algae) as feed additives on antioxidant parameters following simultaneous administration of imidacloprid for 60 days in white leghorn cockerels. Forty two white leghorn cockerels weighing 250-275 g of 4-6 months age were selected for this study. Cockerels were divided equally and randomly into six groups T1 to T6 and fed on as control (T1), *Spirulina platensis* @ 0.2% (T2); imidacloprid @ 50 ppm (T3); *Spirulina platensis* @ 0.2% plus imidacloprid @ 50 ppm (T4); *Erythrina variegata* leaf powder (1%) (T5); and *Erythrina variegata* leaf powder (1%) plus imidacloprid @ 50 ppm (T6) for 60 days. Blood samples were collected at 0, 30 and 60 days interval, tissue samples were collected at the end of the study. A significant ($P<0.05$) decline in RBC catalase and SOD, and an increase in LPO in RBC, liver, kidney and testes was observed in imidacloprid treated group which returned to normalcy following simultaneous medication with SP and EVLP in T4 and T6 groups after 60 days. Findings of this study indicated that administration of imidacloprid @ 50 ppm in feed for 60 days produced oxidative stress which was ameliorated following simultaneous dietary supplementation of *Spirulina platensis* and *Erythrina variegate* @ 0.2% and 1%, respectively. Further, *Spirulina platensis* was more potent in reversing the oxidative stress induced toxic effects in cockerels.

Keywords: Imidacloprid, *Erythrina variegata, Spirulina platensis*, oxidative, cockerels.

Abbreviations: EVLP: *Erythrina variegata* leaf powder, SP: *Spirulina platensis*, LPO: Lipid peroxidase, SOD: Superoxide dismutase, IMI: Imidacloprid, MDA: Malondialdehyde.

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Introduction

Increasing feed price and consumer concerns about chemical feed additives forced poultry industry to look for newer and safer feed additives, which could improve feed utilization/and product quality. Herbal preparations are widely used as feed additives for enhancing growth, reducing feed cost by improving feed conversion ratio and for building better immunity in broiler production (1, 2). Furthermore, these herbal feed additives have no side effects on the health of birds and increase the performance of broiler by increasing live weight gain, feed conversion ratio (3) and immunity (4, 5).

Imidacloprid, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, a chloronicotyl compound, is an globally used insecticide for crop protection during the last decade due to its low soil persistence and high insecticidal activity (6). It is fastest growing in sales as insecticide globally because of its low selectivity for insects and apparent safety for humans (7, 8).

Spirulina platensis (blue green algae) is widely distributed and found mainly in Africa, Asia and South America. Beneficial effects of supplementation of poultry diet with *Spirulina platensis* on immunity, productivity and reproductive performance are reported by various authors (9,10,11). The genus *Erythrina* comprises

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of about 110 species of trees and shrubs. Studies on phytochemical constituents of *Erythrina variegata* (Coral tree) species have demonstrated alkaloids and flavonoids as major constituents (12). Different parts of *E. variegata* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. It is potentially effective for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds.

Keeping in view, the indiscriminate use of insecticide imidacloprid, birds are frequently exposed to Imidacloprid through various sources and in search of herbal growth promoters and antioxidative medicament for poultry industry, the experiment was conducted to evaluate the effects of dietary addition of *Erythrina variegata* leaf powder (EVLP) and *Spirulina platensis* (SP) on amelioration of antioxidative damage caused by imidacloprid in white leghorn cockerels.

Materials and Methods

This study was conducted at the Instructional Poultry Farm, G.B. Pant University of Agriculture & Technology, Pantnagar. Commercial formulation of Imidacloprid, Midas 17.8 % SL purchased from local market of Pantnagar was used in the study.

Plant materials: *Erythrina variegata* leaves used in the study were collected from university campus and taxonomically identified vide letter no. *CHN/64/2012/ Tech-II dated 2.10.2012*, Office of Scientist 'F', Central National Herbarium, Botanical Survey of India, Botanical garden, Howrah 711-103 as *Erythrina variegata* (L.). *Spirulina platensis* powder (Spirulina DXN) manufactured by twenty first century pharmaceuticals, Chennai were used in the study.

Experimental animals: Forty two male white leghorn cockerels weighing 250-275g of 4-6 months age were procured from Instructional Poultry Farm, Pantnagar. The animals were kept in deep litter system under standard managemental conditions. Chicks were acclimatized to experimental conditions for two weeks before the commencement of experiment. The experimental diets were prepared by supplementation of commercial poultry feed with imdacloprid (50 ppm), EVLP (1 %) and SP (0.2%), respectively as per the experimental design (Table 1). Experimental chicks were maintained as per ethical guidelines for the management of experimental birds. The experimental protocol was approved by IAEC vide approval number is IAEC/CVASc./VPT/170.

Experimental design: The birds were divided into six groups with seven chickens in each group. Imidacloprid-based commercial formulation, *E. variegata* leaf powder (EVLP) and *Spirulina platensis* were given orally in the feed for 60 days (Table 1). Blood samples for anti-oxidative parameters were collected from wing vein after proper restraining of birds at 0, 30 and 60 days intervals. Organs (liver, kidney and testes) were collected after sacrificing experimental chicks humanely on day 60 after completion of the study.

Table 1. Experimental design for evaluation of protective effect of *Erythrina variegata* leaf powder and *Spirulina platensis* in male white leghorn cockerels

Groups	Treatment	No. of birds
I	Control	7
II	*Spirulina platensis 0.2%* in feed	7
III	Imidacloprid @ 50ppm in feed	7
IV	*Spirulina platensis 0.2%* + Imidacloprid @ 50ppm in feed	7
V	*Erythrina variegata 1 %* in feed	7
VI	*Erythrina variegata 1 %* + Imidacloprid @ 50ppm in feed	7

Estimation of antioxidative parameters: Estimation of oxidative stress related parameters were determined at 0, 30th and 60th day in RBCs and in tissues of liver, kidney and testes after 60 days. Absorbance was read using double beam UV-VIS spectrophotometer after(BIORAD Smart Spec™ Plus).

Sample collection

Separation of erythrocytes: Freshly collected heparinised blood samples (2 ml) were centrifuged at 2500 rpm for 15 min. Plasma and buffy coat were removed and erythrocyte pellet was washed thrice with 0.15 M NaCl. The 33% dilution of the packed RBC was made in PBS (pH 7.4). The washed erythrocyte pellets were suspended in PBS of pH 7.4 and kept at 4°C till further analysis.

Preparation of tissue homogenate: Frozen liver, kidney and testes samples were partially thawed and 200 mg of each sample was weighed and taken in 2 ml of ice-cold saline for LPO and SOD estimation. Fresh samples of tissue homogenates were prepared using Polytron homogenizer, Kinematica under cold conditions. The homogenates were centrifuged for 10 min at 3000 rpm and the supernatant was collected and used for estimation of LPO and SOD level.

Measurement of Lipid peroxidation: Membrane peroxidative damage in erythrocytes and tissue homogenates was determined in terms of malondialdehyde (MDA, *nM MDA/ml*) production by the method of Rehman (13).

Measurement of Superoxide dismutase activity: The activity of superoxide dismutase (SOD, U/gm of protein) in erythrocyte lysate and tissue homogenates was determined by method of Marklund and Marklund (14).

Measurement of Catalase activity: The activity of catalase in erythrocyte lysate was determined by method described by Aebi (15).

Statistical analysis: Data collected were subjected to the two way analysis of variance (ANOVA) using the SPSS 16.0 software. Data are presented as Mean±SE, n=7. Statistically significant difference was considered at 5% level (16).

Results and Discussion

Antioxidant markers in RBC

The results of LPO assessed by as malondialdehyde level (nM MDA/ml) are shown in Figure 1. A significantly ($P<0.05$) higher MDA levels was observed in imidacloprid treated group as compared to control after 30 and 60 days whereas chicks fed on both SP and EVLP with imidacloprid revealed significantly lower LPO levels than group III at 30 and 60 days. The high MDA is indicative of higher lipid peroxidation that was ameliorated by the treatment with SP and EVLP during the study. A significant ($P<0.05$) decrease in SOD activity was seen in imidacloprid treated group III as compared to control after 60 days (Figure 3). Mean SOD level in group IV (SP + IMI) and VI (EVLP + IMI) showed a significant increase as compared to imidacloprid treated group III after 30 and 60 days. A significant ($P<0.05$) decrease in SOD activity in imidacloprid treated cockerels resulted in enhanced oxidative stress. Whereas, treatments with *Spirulina platensis* and EVLP in combination with imidacloprid revealed reversal of oxidative damage. As depicted in Figure 5, a significant ($P<0.05$) decrease in catalase activity was seen in imidacloprid treated group as compared to control after 30 and 60 days. Mean catalase level in group IV (SP + IMI) and VI (EVLP + IMI) showed a significant increase as compared to imidacloprid treated group III after 30 and 60 days. A significant increase in catalase activity was seen in group II (SP) after 30 and 60

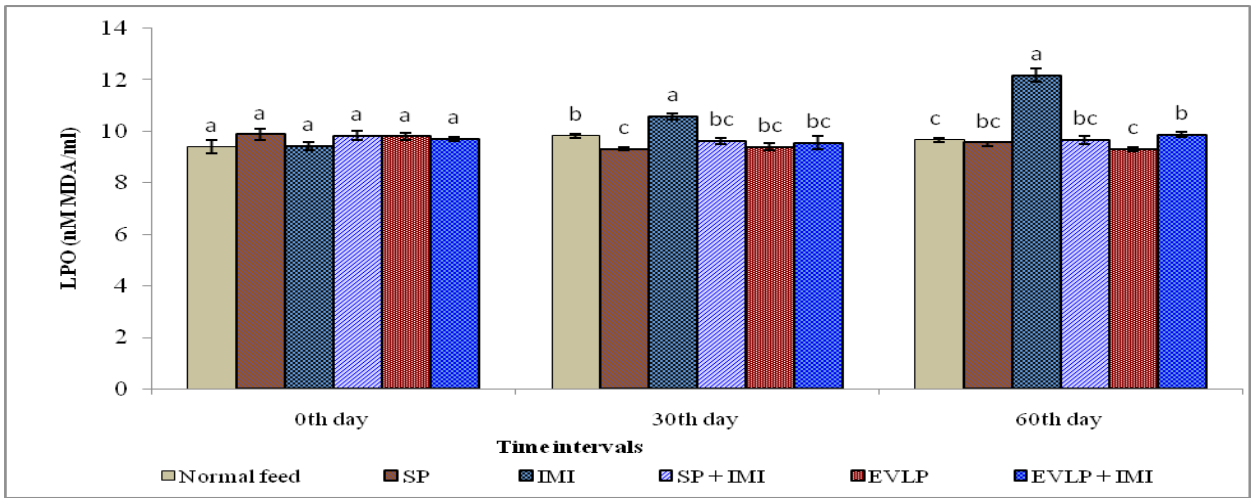


Figure 1. Effect of feeding of 0.2% *Spirulina plantesis* (SP) and 1% *Erythrina variegata* leaf powder (EVLP) daily as feed supplement for 60 days on LPO (nM MDA/ml) (Mean±SE, n=7) in RBC of imidacloprid intoxicated WLH cockerels. Data at the same sampling time with different letters are significantly different ($p < 0.05$).

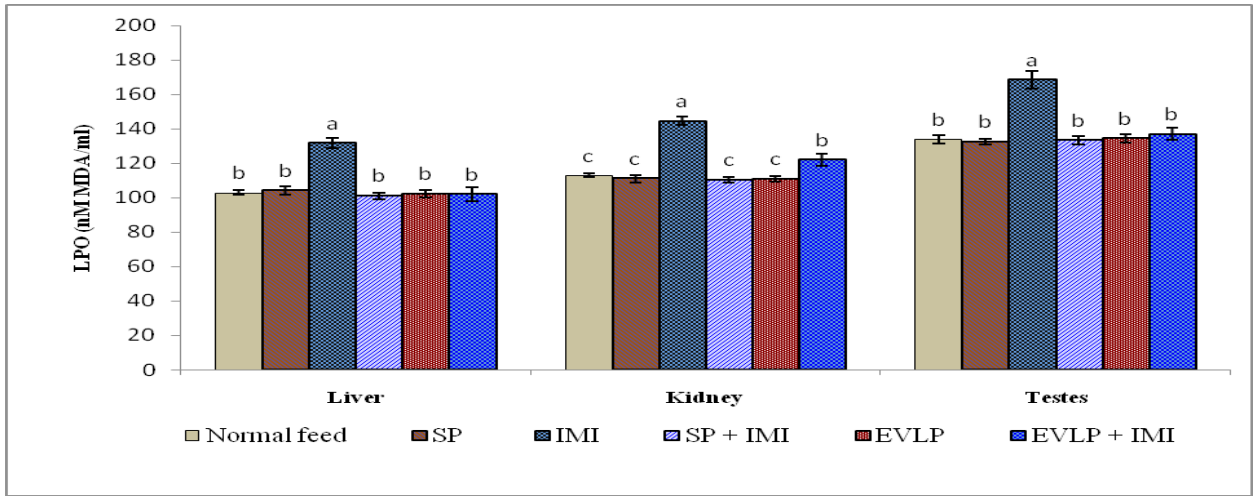


Figure 2. Effect of feeding of 0.2% *Spirulina plantesis* (SP) and 1% *Erythrina variegata* leaf powder (EVLP) daily as feed supplement for 60 days on LPO (nM MDA/ml) (Mean±SE, n=7) in liver, kidney and testes of imidacloprid intoxicated WLH cockerels. Data for the same organ with different letters are significantly different ($p < 0.05$).

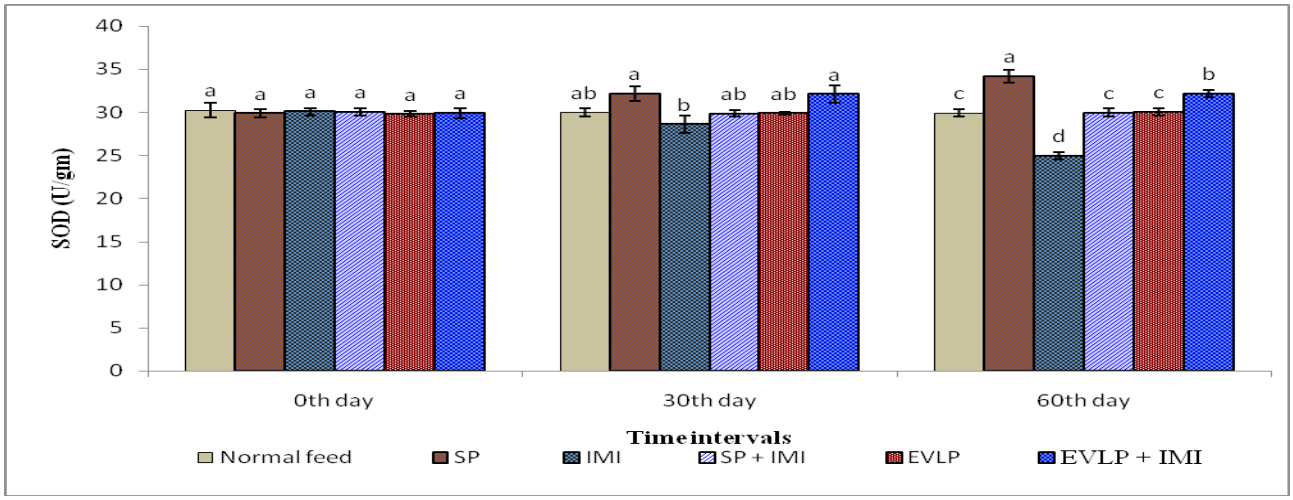


Figure 3. Effect of feeding of 0.2% *Spirulina plantesis* (SP) and 1% *Erythrina variegata* leaf powder (EVLP) daily as feed supplement for 60 days on SOD (U/gm) (Mean±SE, n=7) in RBC of imidacloprid intoxicated WLH cockerels. Data at the same sampling time with different letters are significantly different ($p < 0.05$).

days and in group V (EVLP) after 60 days as compared to control. There was a significant (P<0.05) decrease in catalase activity in imidacloprid treated cockerels. Chicks fed on *Spirulina platensis* and EVLP with imidacloprid revealed an ameliorative effect on catalase activity. *Spirulina platensis* and EVLP treated chicks of group II and V revealed a significantly high catalase activity than control.

Antioxidant markers in tissues

Malondialdehyde levels (LPO) in liver, kidney and testes (nM MDA/ml) are presented in Figure 2. A significantly higher MDA levels were observed in liver, kidney and testes of imidacloprid treated chicks (group III) as compared to other groups. Malondialdehyde levels (LPO) of kidney tissue in group VI (EVLP + IMI) was significantly lower in comparison to group III and other groups. The results of SOD activity in liver, kidney and testes (U/g) are shown in Figure 4. A significant (P<0.05) decrease in SOD activity was seen in imidacloprid treated group III as compared to control after 60 days. Mean SOD level in group IV (SP + IMI) and VI (EVLP + IMI) showed a significant increase as compared to imidacloprid treated group III after 30 and 60 days. A significant (P<0.05) decrease in SOD activity

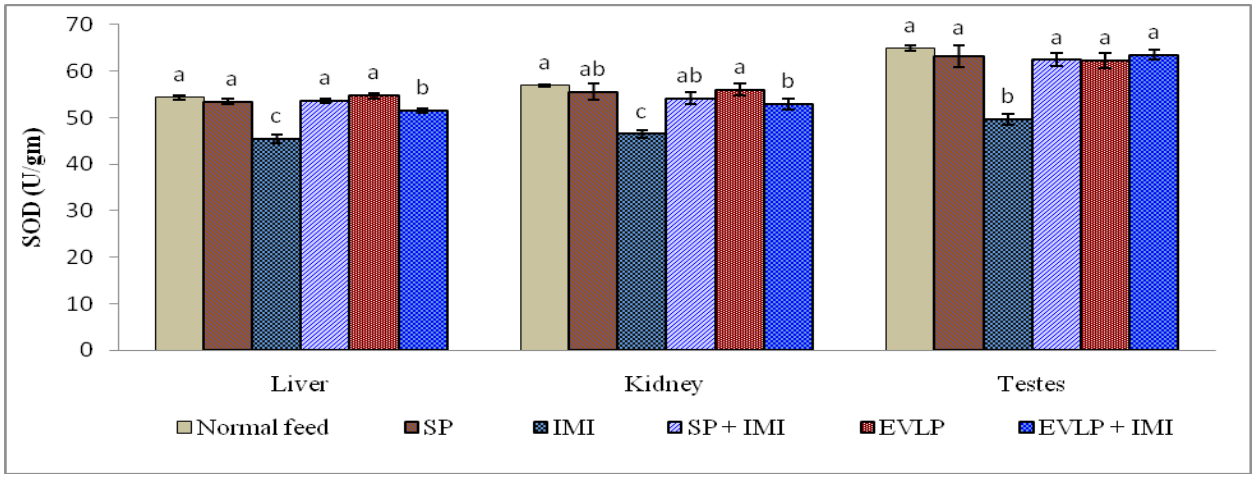


Figure 4. Effect of feeding of 0.2% *Spirulina plantesis* (SP) and 1% *Erythrina variegata* leaf powder (EVLP) daily as feed supplement for 60 days on SOD (U/gm) (Mean±SE, n=7) in liver, kidney and testes of imidacloprid intoxicated WLH cockerels. Data for the same organ with different letters are significantly different ($p < 0.05$).

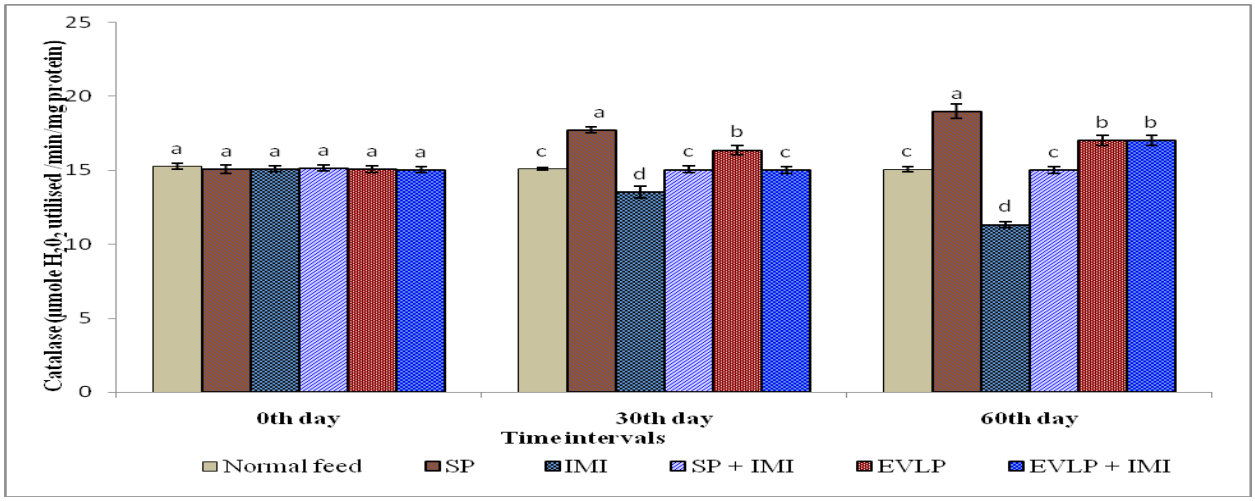


Figure 5. Effect of feeding of 0.2% *Spirulina plantesis* (SP) and 1% *Erythrina variegata* leaf powder (EVLP) daily as feed supplement for 60 days on Catalase (µmole H_2O_2 utilised/min/mg protein) (Mean±SE, n=7) in RBC of imidacloprid intoxicated WLH cockerels. Data at the same sampling time with different letters are significantly different ($p < 0.05$).

in imidacloprid treated cockerels resulted in enhanced oxidative stress. Whereas, treatments with *Spirulina platensis* and EVLP in combination with imidacloprid revealed its reversal.

Oxidative stress occurs when the production of the active oxidants and free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) supersedes the antioxidative defense mechanism. LPO in membranes is regulated by the availability of substrate in the form of polyunsaturated fatty acids (PUFA), the availability of inducers such as free radicals and the excited state molecules to initiate propagation, the antioxidant defense status of environment and physical status of membrane lipids (17). An increase in the levels of malondialdehyde indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms (18). In the present study, an increase in lipid peroxidation in imidacloprid treated cockerels in red blood cells, liver, kidney and testes might be due to the excessive production of ROS leading to oxidative stress. In agreement to our findings, an increase in MDA levels in liver, kidney and heart tissues was also reported by Ranjan *et al.* (19) following oral administration of imidacloprid @ 22.50 and 45 mg/kg

b wt orally in rats for 90 days. Protective effect of *Spirulina platensis* in imidacloprid intoxicated cockerels might be due to their content of antioxidant active constituents such as C-phycocyanins, β- carotene, vitamins, minerals, proteins, lipids and carbohydrates (20). Decline in MDA levels following administration of *Spirulina platensis* @ 1000 mg/kg b wt against deltamethrin toxicity in rats was also reported by Abdel-Daim *et al.* (21). The protective effect by EVLP might have been be due to the presence of flavonoids and phenolic contents (22).

It is well known that, superoxide dismutase is the major antioxidant enzyme containing copper, zinc, and manganese. The protective effects of cellular Cu/Zn-SOD against oxidative tissue damage can mainly be attributed to their breakdown of the superoxide radicals into hydrogen peroxide and oxygen, and reduction in hydroxyl radical formation which is the prime initiators of lipid peroxidation. Thus a reduction in SOD activity may increase superoxide anions which predict to oxidative damage in cell membranes and other cellular structures. Superoxide dismutase diminishes the level of superoxide anion by converting it to hydrogen peroxide while catalase and glutathione peroxidise decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. In present study, a significant decrease in SOD activity in imidacloprid treated chicks might have increased ROS production in red blood cell, liver, kidney and testes of cockerels. The protective effect shown by *Spirulina platensis* and EVLP might be due to the presence of antioxidants phycocyanins and flavonoids, respectively. An increase in SOD activity indicating protective effect of *Spirulina platensis* @ 1000 mg/kg b wt against deltamethrin toxicity in rats was also reported by Abdel-Daim *et al.* (21) and by Mahima *et al.* (23) following bromobenzene induced toxicity in rats. A reduction in SOD activity in liver, kidney and testes is an indication of an increase in accumulation of superoxide anion in the tissues causing detrimental effect on these tissues. The oxidative damage produced in liver, kidney and testes are also supported by histopathological findings (24).

Catalase is an antioxidant enzyme produced naturally within body tissues, and catalyses the conversion of hydrogen peroxide into water and oxygen, thus, preventing the formation of carbon dioxide in the blood. Catalase works closely with superoxide dismutase to prevent free radical damage to the body. SOD converts the dangerous superoxide radical to hydrogen peroxide whereas catalase subsequently converts H_2O_2 to harmless water and oxygen. Catalase is one of the most efficient enzymes of the cells; each catalase molecule can convert millions of hydrogen peroxide molecules every second. Catalase is located in the cell's peroxisome and prevents natural formation of hydrogen peroxide induced detrimental effect on the cell by antioxidant action. The significant ($P<0.05$) reduction in catalase activity in imidacloprid treated cockerels might have caused oxidative stress following imidacloprid exposure. The protective effect shown by *Spirulina platensis* and EVLP may be attributed to the presence of antioxidants phycocyanins and flavonoids, respectively. Similar increase in catalase activity were also reported by Abdel-Daim *et al.* (20) indicating protective effect of *Spirulina platensis* @ 1000 mg/kg b wt against deltamethrin toxicity in rats and by Mahima *et al.* (23) against bromobenzene induced toxicity, following administration of *Spirulina fusiformis* @ 400 mg/kg b wt in rats. Gupta *et al.* (25) reported that the supplementation of feed with 1% EVLP and 0.2% SP, simultaneously given with imidacloprid for 60 days produced a protective effect on body weight gain and haematobiochemical parameters.

Thus, it is concluded from the present study that the supplementation of feed with *Spirulina platensis* and *Erythrina variegata* have protective effect against imidacloprid induced oxidative stress in cockerels.

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Conflict of Interest: None Declared

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