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Effect of vitamin B12 on the oxidative stress of *Sitophilus oryzae* under different time course

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Abstract

The use of vitamins is widely distributed nowadays to overcome the deleterious impacts of oxidative stress especially along ecosystem structure and function. *Sitophilus oryzae* is considered as a serious pest to all stored products. The deleterious insect infestation may result from the presence of oxidative stress. To decline the impacts of oxidative stress and restore the homeostasis between antioxidants and oxidants, vitamin B12 was used. Here, the effect of vitamin B12 on the insects oxidative stress status was examined in adult insect *S. oryzae*. Around 250 insects were treated with 2 µg/mL commercial vitamin B-12 under different time course (0, 6, 12, 18, and 24 h). The results showed that the time course has a direct positive elevation effect on the total antioxidant capacity, DPPH, and reducing power of *S. oryzae*. While, the oxidative stress parameters showed a fluctuation pattern of insect protein carbonyls and lipid peroxides. The highest application time of 2 µg/mL vitamin B12 (24 h) resulted in a drastic depletion in the levels of protein carbonyls amount with the factor of –2.1-x with respect to control value ($p < 0.001$). These findings suggest the ability of insects to protect against oxidant production.

Keywords: *Vitamin B, Antioxidants, Oxidative stress, Sitophilus oryzae, Reactive oxygen species, Bioremediation*

1. Introduction

Environmental stress can increase the production of Reactive Oxygen Species (ROS) in living organisms. In aerobic cells, ROS are produced from molecular oxygen as result of normal cellular metabolism (Hermes-Lima, 2004; and Sena and Chandel, 2012). Yet, exogenous sources, such as un-proper waste management especially drugs, can directly or indirectly influence the level of ROS in cells of different organisms (Amado *et al*., 2006; Sureda *et al*., 2006; Dos-Anjos *et al*., 2011; Abdelfattah *et al*. 2017; Yousef *et al*., 2019; Abdelfattah 2021; and Abdelfattah and Renault 2021).

As ROS exceed normal level, it leads to oxidative stress and cause a serious damage to macromolecules inside living organisms, such as DNA damage, protein carbonylation, lipid peroxidation, and enzyme inactivation (Halliwell and Gutteridge, 1984). Proteins, especially enzymes can also be damaged by ROS (Davies, 1987; and Costa *et al*., 2007). This damage may occur as a direct amino acid oxidation such as lysine,

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tyrosine, tryptophan, proline, arginine, histidine, methionine, and cystiene (Levine *et al*., 2000). The oxidative amino acids may result in formation of repairable oxides such as protein-methionine sulphoxide, cysteine sulphonic acid derivatives, and unrepairable oxides and carbonyls (Costa *et al*., 2007).

The other protein damage may result from interactions between lipid peroxidation products, and proteins to form cross-linked proteins (Pardini, 1995). While, the lipid peroxidation is a chain reaction that may be initiated by primary free radical to yield lipid-free radical. Then, lipid peroxidation propagation occurs by interaction of lipid radical (L•) with molecular oxygen to form lipid peroxyl radical. This results in a chain reaction of lipid peroxidation which propagates and yield lipid hydroperoxides. Finally, the reaction can be stopped by a termination reaction such as recombination of lipid peroxyl radicals. Previous accepted knowledge revealed that protein carbonyls amount, and lipid peroxide concentration considered as biomarker of oxidative stress (Kaviraj *et al*., 2014).

Protection against xenobiotics, including ROS-mediated environmental pollutants, can be realized by two main mechanisms: (i) the avoidance of stress, which cannot be achieved by organisms living in polluted areas or (ii) the intensification of the antioxidative defense of the organism (Migula *et al*., 2004). Antioxidants response - which includes total antioxidant capacity, reducing power ability, 2,2 diphenyl-1-picylhydrazyl (DPPH) inhibition percentage, reduced glutathione (GSH), α-tocopherol, β-carotene, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbate peroxidase (APOX), polyphenoloxidase (PPO), glutathione reductase (GR), acetylcholine esterase (AChE), and glutathione-s-transferase (GST)—are supposed to be important indicators of oxidative stress (Lushchak, 2011).

Vitamin B12 (B12), or cobalamin, is considered as an essential water-soluble vitamin. It has a vital way in maintaining neuronal health. Besides that, B12 may have antioxidant properties, and its deficiency or overuses may thus contribute to oxidative stress and the onset of age-related diseases (Van De Lagemaat *et al*., 2019). In addition to, B12 may play a key role in modulating immune responses as its low B12 status lead to increase the basal interleukin-6 production in some previous studies. *Sitophilus oryzae* L. is considered as a primary insect pest of stored rice in warm climate areas and it is a very effective insect pest that damaging stored grains worldwide (Oryzae, 2006).

The present study was performed to investigate the potential effect of vitamin B12 against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). So, the potential use of oxidative stress parameters as a bioremediation agent will be discussed in this paper. Therefore, the levels of total antioxidant capacity, reducing power ability, DPPH, protein carbonyls amount, and lipid peroxides concentration were measured after 6, 12, 18, and 24 post injection of 2 μg / mL vitamin B12 to adult *S. oryzae.*

2. Materials and methods

2.1. Insect treatment and sample preparation

From the Entomology Department, Faculty of Science, Cairo University, Egypt, the *S. oryzae* adult was supplied from a colony reared under the rearing conditions (12:12 L:D; 34° ± 2; 75% RH). Each 10 mL of B12 (2 μg / mL) were injected to 50 adult and leave for 6, 12, 18, and 24 h post injection. The control specimens with 0 h values, was injected with distilled water. After time spent, the specimens were stored at –20°C until use. Each experiment was done in three replicates.

2.2. Determination the antioxidants levels

The total antioxidant capacity was measured according to the procedure of Prieto *et al*. (1999). The method include homogenated sample with the following 0.25 mL 0.6 M sulfuric acid, 0.5 mL 28 mM sodium phosphate, and 0.25 mL 4 mM ammonium molybdate, then incubate at 95ÚC for 90 min. The absorbance was measured at 695 nm.

The reducing power concentration of the samples was done according to methodology of Oyaizu (1986). In this protocol, samples are mixed with 1 mL 0.2 M pH = 6.6 phosphate buffer and 0.5 mL 1% potassium ferricyanide and incubated in water bath at 50°C for 20 min after that 1 mL 10% of TCA was added to mixture and centrifuged at 2000 g for 10 min at 4°C. The absorbance was measured at 480 nm after adding 1.5 mL of 0.1% ferric chloride to the reaction.

The other sensitive, accurate, low sample concentration and low-cost biochemical analysis, (DPPH), was determined by Blois (1958). The reaction mixtures include 1 mL of 0.5 M DPPH to different concentration of sample and incubated for different time before measuring absorbance at 525 nm. DPPH assay is based on scavenging capability measurement. The nitrogen atom contains old electron which is reduced by delivery a hydrogen atom from antioxidants to hydrazine.

2.3. Oxidative damage concentration

From Levine *et al*. (1990), we used the procedure for the protein carbonyls assay, with the below-described modifications. In 5 mL ice-cold phosphate buffer (60 mL of 50 mM phosphate buffer, 10 mL of 0.1% Triton X-100, 5 mL of 0.05 mM $CaCl_2$; then completed to 100 mL with distilled water after adjusting pH to 7.0 with 2M HCl or NaOH) samples were homogenized. The samples were centrifuged at 2000 ×*g* for 10 min at 4°C after homogenization (mortar, 10 strokes / 30 sec), a 800 μL aliquot of the supernatant mixed with 200 μL of 10 mM 2, 4-dinitrophenyl hydrazine (DNPH) prepared in 2 M HCl. The samples were incubated for 30 min at room temperature, precipitated with 10% Tricholoroacetic Acid (TCA), and left for 10 min at 4°C. At 5000 ×*g* the samples were centrifuged for 7 min at 4°C. The pellet was washed four times with an ethanol / ethyl acetate (1:1) mixture, and redissolved in 1 mL of sodium phosphate buffer (60 mL of 150 mM phosphate buffer, 30 mL of 3% sodium dodecyl sulphate, adjusted to a final volume of 100 mL with distilled water after adjusting the pH to 6.8 with 2M HCl or NaOH). Finally, the absorbance was measured at 366 nm, and the rate of protein carbonyls concentration was expressed as OD / mg protein.

As Hermes-Lima *et al*. (1995) method, the lipid peroxides concentration was measured. The samples were homogenized in ice-cold methanol (1:5, w / v). At 4°C, the samples were centrifuged at 2000 *g* for 10 min after homogenization (mortar, 10 strokes / 30 sec). For the assay, 5 mL aliquot of the supernatant was used. The following components were consecutively added to the samples (200 μL of supernatant): 400 μL of 1 mM $FeSO_4$, 200 μL of 0.25 M H_2SO_4, and 200 μL of 1 mM xylenol orange. The absorbance was measured at 580 nm. Lipid peroxides concentration was expressed as mM cumene hydroperoxides / μg protein.

Spectrophotometrically, the total protein concentration of samples was determined by Bradford (1976) method. Briefly, 0.9 mL of the dye reagent (10 mg COBB + 5 ml methanol + 10 ml 85% O-phosphoric acid, completed to 100 ml with distilled water) were added to 0.1 mL of each sample in a separate test tube. The contents of the tube were mixed by gentle shaking and left to stand for 2 min. The OD of the protein sample was measured at 595 nm. 2% albumin concentration was used as a standard.

2.4. Statistical analysis

The effect of both control treatment and vitamin B-12 treated samples on the oxidative stress parameters levels of adult *S. oryzae* were assessed by performing *Kruskal-Wallis H ($p < 0.05$)*. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.).

3. Results and discussion

The present work discussed the ability of using oxidative stress parameters of the *S. oryzae* adult as an indicator of vitamin B12 effects. The treated insects were injected with 2 μg / mL B12 a long different time of application and the oxidative stress parameters were evaluated in different insects adult (Figures 1-2). Also, the relation among time post injection of B12 and insect oxidative stress parameters were evaluated as a time series predictions with estimation of residual autocorrelation factors (R-ACF), ACF, residual partial autocorrelation factors (R-PACF), and PACF (Figues 3-5).

As, the problem of oxidative stress factors estimation, or more specifically, the mechanisms of using the oxidative stress, are considered as a key research point dealing with phenomena of bioremediation, toxicology, and adaptation. In recent studies, these researches topics have gained a significant value, mainly due to the increase stressing levels as results of the human and environments-related activities pressure (Zhang *et al*. 2019; and Abdelfattah, 2021). These abnormal variations need organisms' adaptation. The living organism's responses can be vital as it can allow the studying of specific defense mechanisms against various and different stress factors (Abdelfattah and Dorrah, 2015; Abdelfattah, 2016; Renault *et al*., 2016; Abdelfattah *et al*., 2017; Yousef *et al*., 2017 and 2019; Abdelfattah, 2020 and 2021; Nassar *et al*., 2020; Abdelfattah and Lim, 2021; Abdelfattah and Renault, 2021; Abdelfattah *et al*., 2021 a, b and c).

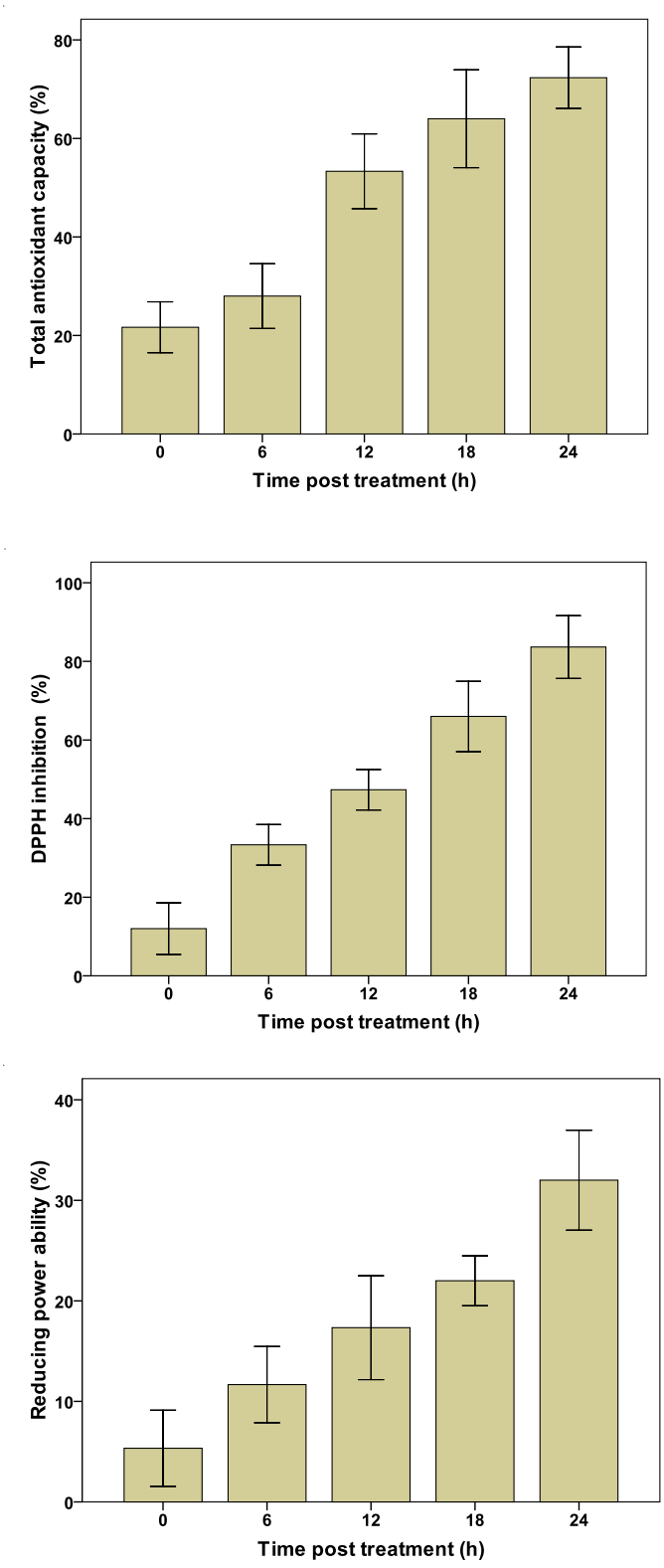
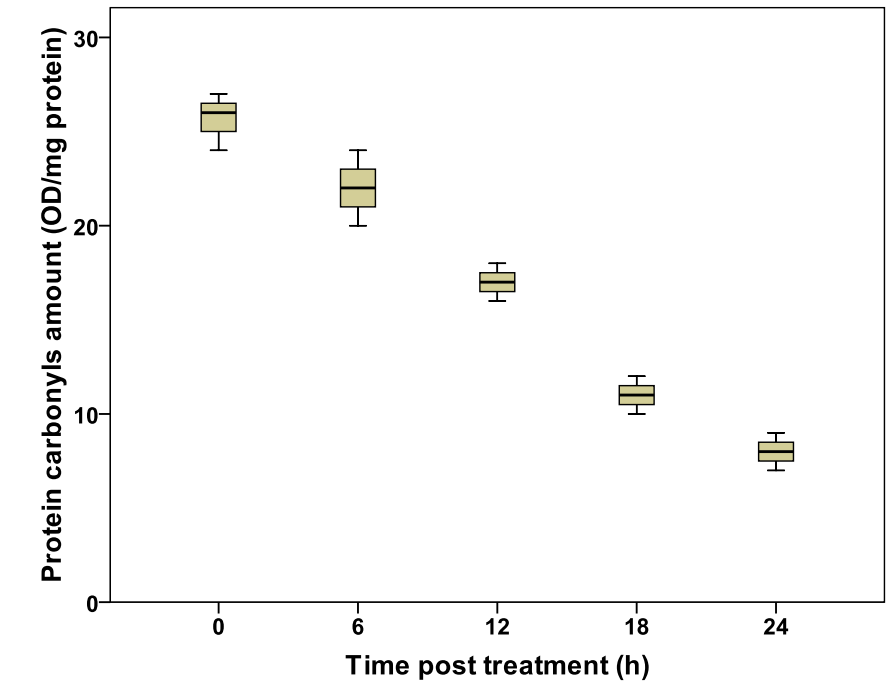


Figure 1: Effects of different concentration of commercial vitamin B12 on the (mean, P25, P75) of total antioxidant capacity, reducing power, and 2,2 diphenyl-1-picylhydrazyl (DPPH) measured in adult of *Sitophilus oryzae*. Adult insects were injected with different time course post injection of vitamin B12: 0, 6, 12, 18 and 24 h. 'small letter' at the top of the fig. report the statistical comparisons among control and vitamin B12-treated larvae at each case separately (ANOVA, *Tukey2 s-b* test, $p < 0.05$).



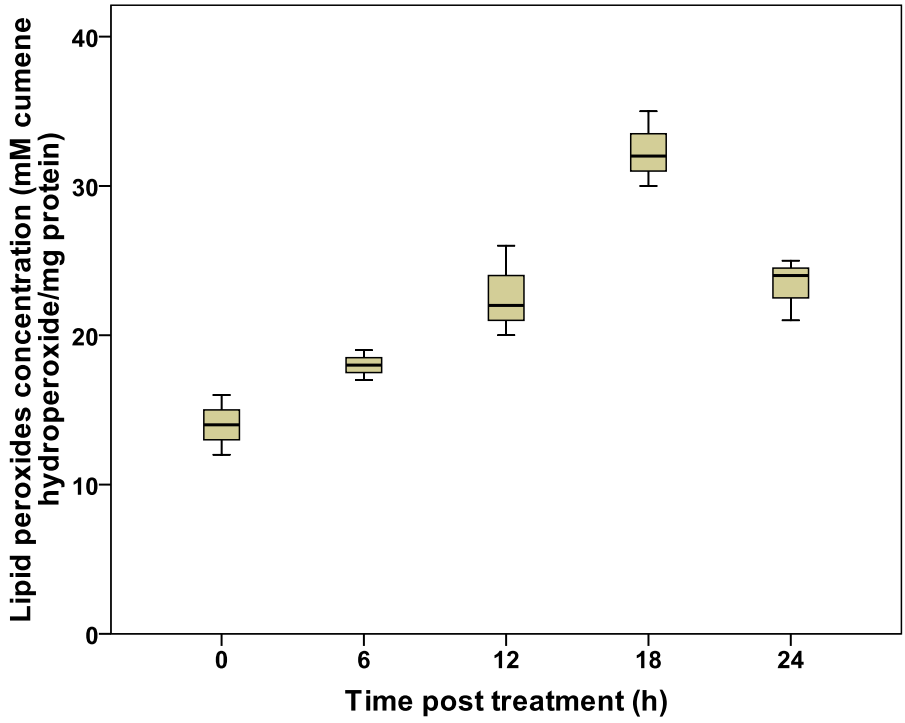
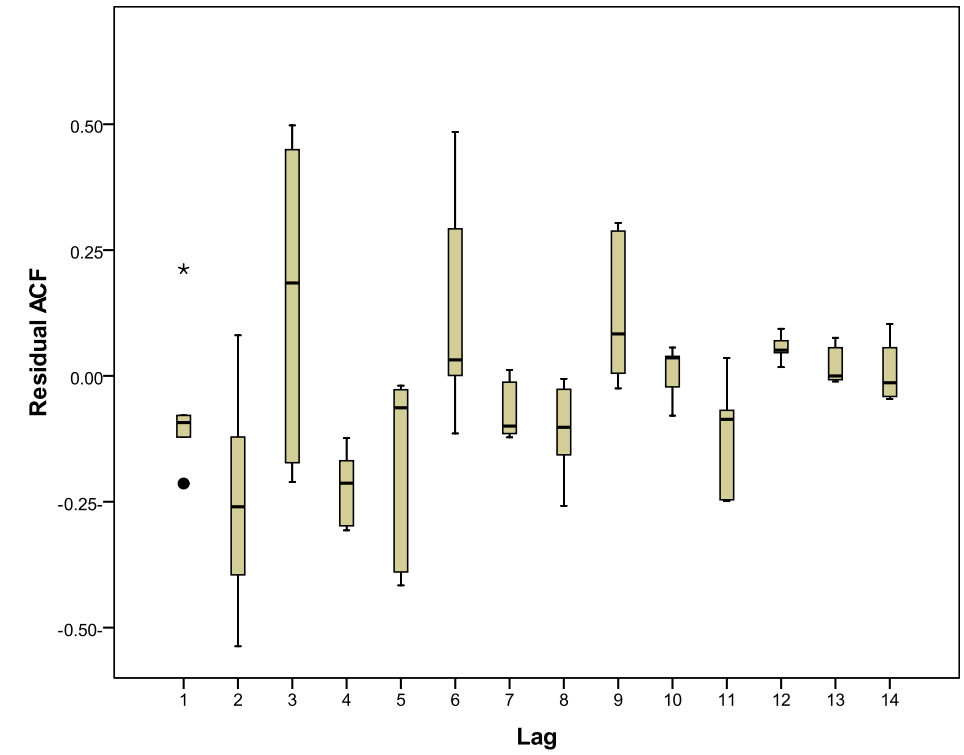


Figure 2: Effects of different concentration of commercial vitamin B12 on the oxidative stress amount (mean, P25, P75) of protein carbonyls and lipid peroxides measured in adult of *Sitophilus oryzae*. Adult insects were injected with different time course post injection of vitamin B12: 0, 6, 12, 18 and 24 h. 'small letter' at the top of the fig. report the statistical comparisons among control and vitamin B12-treated larvae at each case separately (ANOVA, *Tukey2 s-b* test, $p < 0.05$).



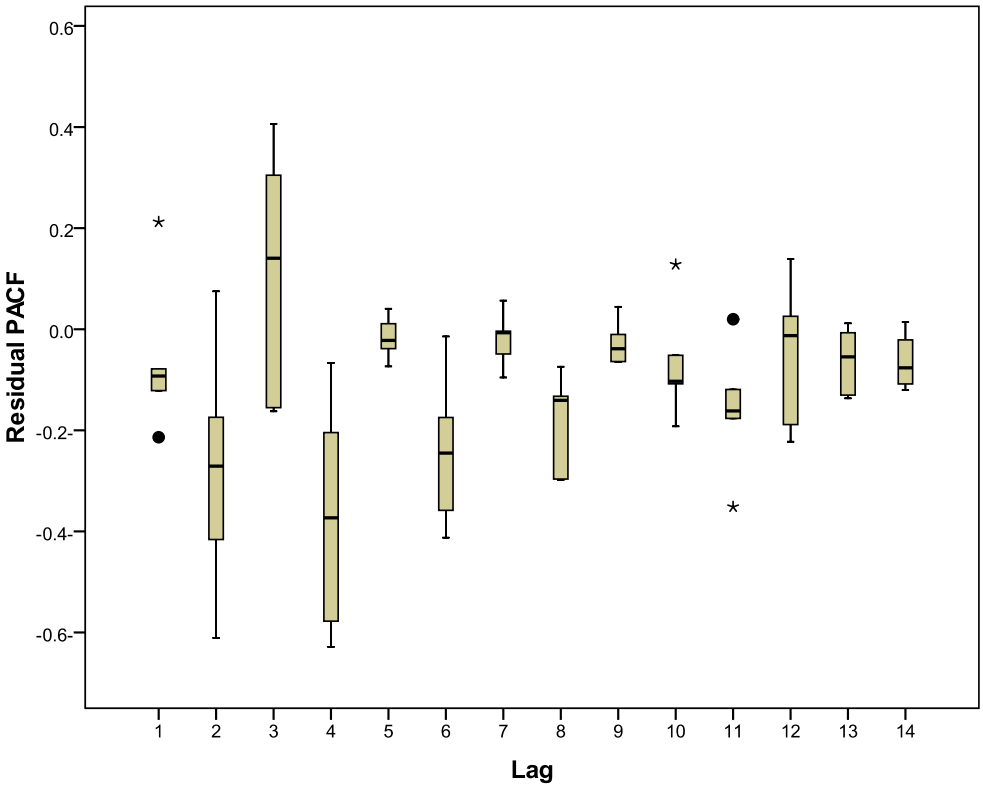
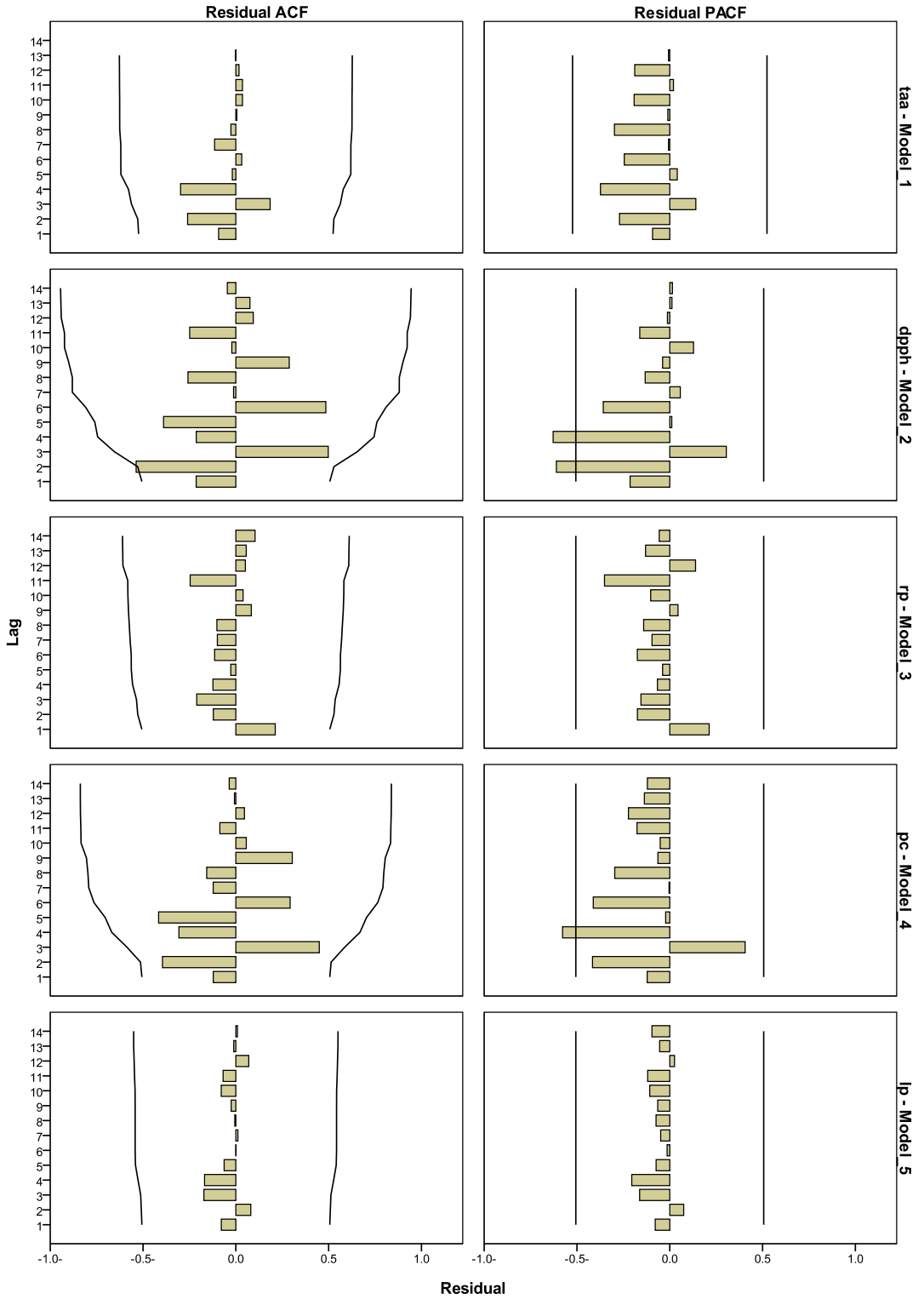


Figure 3: The residual autocorrelation function (ACF), and residual partial autocorrelation function plot (PACF) of the residual series between the forecasted series and the real (differential) series of time course post injection of vitamin B12 (0, 6, 12, 18 and 24 h) into adult *Sitophilus oryzae*



Note: Solid lines represent the upper and lower confidence limit coefficient.

Figure 4: The autocorrelation function (ACF), and partial autocorrelation function plot (PACF) of the residual series between the forecasted series and the real (differential) series of time course post injection of vitamin B12 (0, 6, 12, 18, and 24 h) on the tested oxidative stress parameters total antioxidant capacity (tta), reducing power ability (rp), 2,2 diphenyl-1-picylhydrazyl (dpph), protein carbonyls amount (pc), and lipid peroxides levels (lp) of adult of *Sitophilus oryzae*.

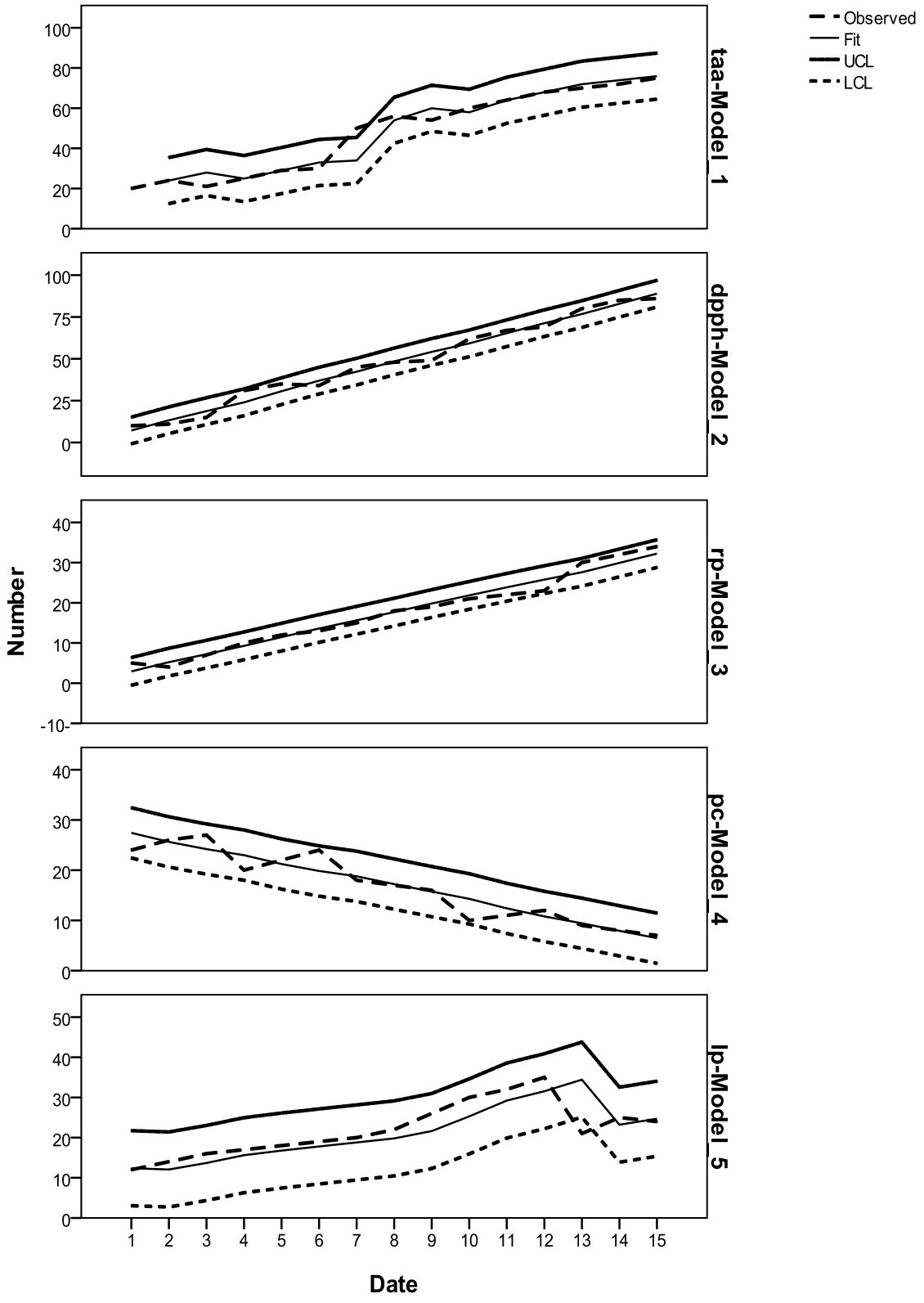


Figure 5: Time series of observed, fit, upper limit, lower limit, and forecast of time course post injection of vitamin B12 (0, 6, 12, 18, and 24 h) on the tested oxidative stress parameters total antioxidant capacity (tta), reducing power ability (rp), 2,2 diphenyl-1-picylhydrazyl (dpph), protein carbonyls amount (pc), and lipid peroxides levels (lp) of adult of *Sitophilus oryzae*

Oxidative stress can be occurred as a result of overproduction of ROS and depletion the antioxidants system. For example, Van De Lagemaat *et al*. (2019) study revealed the antioxidant properties of vitamin B12 includes the depletion in the concentration of O_2, amounts of protein carbonyls and lipid peroxides. In the same line, the present results showed a significant decrease in the protein carbonyls amount of adult *S. oryzae* from 6 to 24 h post injection time, comparing to control values, yet, the lipid peroxides levels showed a significant decrease, with respect to control at 24 h post injection with B12 (Figure 2). Importantly, the highest application time of 2 μg / mL vitamin B12 (24 h) resulted in a drastic depletion in the levels of protein carbonyls amount with the factor of –2.1-x with respect to control value ($p < 0.001$) (Figure 2). This result recommends the reality of a threshold time of vitamin B12 administration to avoid production of oxidants. Also, this research results demonstrated a strong negative person correlation between application time of vitamin B12 and protein carbonyls amount with a polynomial type of equation and the accuracy was checked by the value of chi square. However, there was a positive correlation among vitamin B12 and levels of lipid peroxides, total antioxidant ability, reducing power ability and DPPH inhibition percentage.

As little information on the effects of vitamin B12 on the oxidative stress parameters especially total antioxidant ability and reducing power, the knowledge collected from other treatment studies was considered for interpreting the results of this study. Slowiñska *et al.* (2016) measured the effect of age and pesticide exposure on the total antioxidant capacity in honeybee haemolymph and seminal plasma. The results showed the elevation of TAA levels with increased with age of bees ($p \leq 0.05$). However, the exposure of pesticides, imidacloprid (IMD) doesn't affect TAA of haemolymph of 30-day-old honeybees. Similarly to the depletion levels of TAA in the *S. oryzae* adult insects injected with vitamin B12 with respect to control values especially at 6 h more than 24 h post injection (Fig. 1). Also, Slowiñska *et al.* (2016) study showed the depletion of TAA in haemolymph of 1-day-old bees was lower in treatments with the addition of 5 and 200 ppb IMD compared with controls ($p \leq 0.05$). This result emphasized that honeybees antioxidant protection can be disturbed by exposure to pesticides especially IMD. The same observation of decrease the capacity of total antioxidant was occurred with the increasing of relative humidity. However, the TAA, reducing ability and DPPH analysis in the present study showed a significant elevation especially at 24 h post injection of B12 (Figure 2). Similarly, the exposure to UV light for 30 min resulted in increased total antioxidant capacity in *Helicoverpa armigera* adults (Meng *et al.*, 2009). However, the results of Howden and Kilby (1960) reported that the normal patterns of different reducing ability were occurred as a result of age variation in the haemolymph of *Schistocerca gregaria*.

Meng *et al*. (2009) study focused on using ultraviolet (UV) light (backlight), with the range 320-400 nm, as an effectors' on the insect oxidative stress elevation of *Helicoverpa armigera* adults. The results showed an increase in the total antioxidant capacity, protein carbonyl content and activities of SOD, CAT, POX or GST. However, the antioxidant capacity and SOD activity returned to control levels. These results confirm the hypothesis that *S. scaptersici* increases the level of oxidative stress in *L. migratoria* larva. Also, the study of van Sambeek and Wiesne (1999) showed that the *Heterorhabditis megidis* and *Steinernema feltiae nematodes* can used against the orthopteran insects. The death rate of *Locusta migratoria* and *Schistocerca gregaria* was positively correlated with the nematode-inoculated sand percentage.

4. Conclusion

In the present study, the effects of time course of vitamin B12 were examined in the adult *S. oryzae*. The results emphasized that vitamin B12, may be used as exo-non enzymatic antioxidants to scavenging the ROS, also, B12 is considered as a vital micronutrient for living organisms metabolism processes. Here, the results revealed that the adult of the *S. oryzae* which applied with vitamin B12 were characterized by high levels of antioxidants, as total antioxidant capacity, reducing power capacity, and DPPH, however, the levels of protein carbonyls were decline especially after 24 h post injection. These findings suggest the ability of insect to protect against oxidant production.

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Ethical approval and consent to participate

This paper does not contain any studies with human participants or animals that require ethical approval.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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