Research Article

Separate and combined effects of Dimethoate pesticide and bio-fertilizer on the activity of enzymes involved in anaerobic pathway, neurotransmission and protein metabolism in common carp, *Cyprinus carpio* (Teleostei: Cyprinidae)

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Abstract: The objective of this study was to investigate the effects of combination of Dimethoate pesticide and Bio-fertilizer, two frequently used chemical in agriculture, on blood biochemical and physiological parameters of common carp, *Cyprinus carpio* to understand their potential hazard to freshwater organisms. A total of 270 fish specimens were selected and allocated into nine treatments including: control group, A (0.016 mg/L pesticide), B (0.032 mg/L pesticide), C (0.1 ml/L fertilizer), D (0.2 ml/L fertilizer) and combined treatments: AC, AD, BC, BD, in triplicate. After 14 days of experiment, bloods were sampled from caudal vein. The blood biochemical enzymes including Acetylcholinesterase (AChE), Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT) and Creatine phosphokinase (CPK), were measured. AChE showed a decreasing trend in all treatments except in control fish. ALP significantly decreased in groups received higher doses of fertilizer and combined treatment of lower doses of pesticide with both doses of fertilizer (D, AC, AD treatments). AST and LDH did not show any significant differences in all treatments compared to the control group. ALT in combined AD treatment showed a significant increase compared to the control group and other treatments. CPK in B and combined treatments significantly increased in comparison with the control group. The results of this study showed that the effects of combined treatments of dimethoate pesticide and bio-fertilizer were significantly different from those in separate treatments of given chemicals.

Keywords: Dimethoate pesticide, Bio-fertilizer, Common carp, Biochemical parameters.


Introduction

Pesticides usually used in agriculture to protect corps and animals from insects, weeds and diseases. These pesticides transfer by run off into the aquatic ecosystems and pass into aquatic animals such as fish through food chain which cause health problem to aquatic organisms. Entrance of pollutants into the aquatic environment would damage the physiological and biochemical process in the body of aquatic organisms, specially fishes (Agrahari et al. 2007).
Evidences show that frequent and continuous use of pesticides in high concentrations could have adverse physiological effects on aquatic organisms (Monteiro et al. 2006). Organophosphates (Ops) are believed to have inhibitory effect on cholinesterase activity in the nervous system (Agrahari et al. 2007). Acetylcholine is a neurotransmitter that is able to have stimulatory and inhibitory effects on the muscles. Cholinesterase is widely used in fresh water system as an indicator of exposure to OPs (Fulton & Key 2001).

Dimethoate \[\text{C5H12NO3PS}_2\], IUPAC NAME: O,O Dimethyl S-(N-methylcarbamoylmethyl) phosphor dithioate\] is a systemic OP pesticides used for a wide range of pests (Singh, 2013). It is commercially available as Rogor and similar to other OPs pesticides has AChE inhibitory effect. Dimethoate is highly toxic and reported as carcinogens by USEPA due to induction of tumors in rats (Singh 2009).

Previous research reported that the 96h LC\(_{50}\) concentration of Dimethoate is 21.42 mg/L for Colisa fasciatus, 17.9 mg/L for Channa punctatus, 2.98 mg/L for Heteropneus fossilis and 65 mg/L for Clarias batrachus. However, it has been reported to be more toxic in cyprinids; 0.007 mg/L 96h LC\(_{50}\) value for Catla catla and 1.60 mg/L for Cyprinus carpio (Dey & Saha 2014).

Nowadays, chemical-fertilizers are replaced by bio-fertilizers that are propounded to enhance the fertility of soil in sustainable agriculture (Wue et al. 2005). Bio-fertilizers contain microorganisms that are able to convert macronutrients from unavailable to available form, during biological processes and affect the development of root system and better seed germination (Rajendran & Devaraj 2004).

Lactate dehydrogenase (LDH) is an enzyme in all tissues, including the heart, kidney, liver, skeletal muscle, brain, red blood cells and gills and are widely used as an indicator of abnormalities and tissues damages. Also Creatine Phosphokinase (CPK) enzyme, functions in heart muscle, gills and brain. Measurement of the level of this enzyme in plasma indicates the damage of muscle fibers and other tissues. Moreover Alkaline Phosphatase (ALP) is an enzyme found in in different body tissues with level changes corresponded to damage and dysfunction of target tissues. Finally, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are two enzymes found in different tissues and its blood levels are commonly measured to ensure the normal function of liver as a main detoxifying organ in vertebrates. Because these enzymes may enter to plasma because of cell destruction by stressors (Banaee & Sureda 2011).

Here we have tested the singular and combined toxicity effect of Dimethoate pesticide and bio-fertilizer on the blood parameters of common carp, Cyprinus carpio, one of the most important cultured fish species.

**Materials and Methods**

**Fish husbandry condition:** A total of 270 common carp (mean body weight 35±5 g) were purchased from a fish farm in Ahvaz, Khuzestan province, Iran. The fish were randomly divided into 27 tanks with 70L capacity equipped with aerators and 50% daily water exchange. For acclimation purpose, fish were kept in laboratory conditions (25±2 oC, PH 7±0.2, 16L: 8D photoperiod and 6±1 mg/L O\(_2\)) for two weeks and fed with commercial diet twice a day at 2% body weight. Dimethoate pesticide (40%) (Arya Chemistry Company, Iran) and bio-fertilizer (F.S.P Market Company) were used during the experiment after sublethal toxicity determination (LC\(_{50}=1.6\text{mg/l}\)). Doses 0.01 (0.016 mg/l) and 0.02 (0.032 mg/l) of dimethoate were used as sublethal concentration. Also, fertilizer was used at 0.1 and 0.2 ml per liter of water.

**Treatments:** Fish individuals were selected and allocated into 9 groups in triplicates and exposed to following treatments for 14 days: Control group (without any chemical), Group A (0.016 mg/L pesticide), Group B (0.032 mg/L pesticide), Group C (0.1 ml/L fertilizer), Group D (0.2 ml/L fertilizer) and combined treatments AC, AD, BC and BD.

Every day morning 50% of test solution of each
treatment was replaced with new water containing equal doses of chemicals during 14 days of experiment. At the end of the experiment, fish subsampled from each treatment were anesthetized by 150 mg/l clove powder and blood sample was collected from caudal vein by heparinized syringe and plasma separated by centrifugation at 6000rpm for 15 min.

**Biochemical assay:** The activity of Acetylcholinesterase (AChE) was determined by using commercial kit (ParsAzmoon, Iran) following the manufacturer’s instruction. Lactate dehydrogenase (LDH) activity was measured by conversion of pyruvate to L-lactate by monitoring the NADH oxidation using commercial kit (ParsAzmoon, Iran). Aspartate aminotransferase (AST) was measured in a coupled reaction with malate dehydrogenase in the presence of NADH. For alanine aminotransferase (ALT) measurement, the enzyme reaction with alanine and α-ketoglutarate and formation of glutamate and pyruvate was applied.

Creatine phosphokinase (CPK) and alkaline phosphatase (ALP) were determined using commercial kit (ParsAzmoon, Iran) following the manufacturer’s instruction.

**Statistical analysis:** Data has been expressed as mean ±SD. The normality of data was tested by Kolmogorov-Smirnov test. Data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Duncan test at P≤0.05 significant level. All statistical analysis were performed in SPSS (Release 21 IBM) software.

**Results**

The blood level of AChE enzyme in B, C and BC treatments significantly decreased compared to the control group. Also, in the combined treatments of pesticides and fertilizer, a greater reduction was observed in enzyme level by increasing the doses of pesticides (Fig. 1). There was no significant difference in the circulating level of ALP enzyme in A, B, C and BC, BD treatments in comparison with control group while D, AC and AD treatments showed significant reduction in enzyme level in comparison with the control group (Fig. 2). The results of present study showed no significant differences in LDH enzyme in treated fish in comparison to control group. Only a significant decrease was observed in AC group in comparison to A and AD treatments (Fig. 3).

The results of this study revealed that the levels of CPK enzyme in all combined groups were significantly higher than those in control group and separate treatments. Treatment of fish with combination of lower concentration of pesticide with both levels of fertilizer (treatments AD and BC) led to highest level of CPK (Fig. 4). There was no significant difference in AST enzyme level among all treatments and compared to control group (Fig. 5). Based on the obtained results, there were also no significant differences in ALT enzyme levels among all treatments, except in group treated with...
combination of pesticide (at lower doses) and fertilizer (in higher doses) which was significantly higher than all treatments and control group (Fig. 6).

**Discussion**

AChE has been considered as a common indicator for altering brain neural function, which is mostly used...
as an indirect measurement of acetylcholinesterase activity (Scott & Sloman 2004). AChE is a neurotransmitter that has excitability and inhibitory effects. Reducing the enzyme level in freshwater fish was observed after exposure to diazinon and Fenpropathrin (Banaee et al. 2014a, b) and Chlorpyrifos (Scott & Sloman 2004). The AChE reduction in all treatments combined with fertilizer may conclude the effect of fertilizer in intensifying pesticides toxicity.

Alkalin phosphatase plays an integral role in metabolism within the liver and development within the skeleton. Due to its widespread functions in body hemostasis of organisms, its concentration in the bloodstream is used as a biomarker in determining malfunction of liver, kidney and other tissues involved in body highness. The level of ALP significantly increases subsequent to contamination of body with any chemical led to damage of liver and renal tissues. Therefore increasing the ALP levels subsequent to separate use of dimetoate pesticide may related to damage and malfunction in the liver and kidney of treated fish. In the present study the circulation level of ALP decreased following treated of fish with higher doses of bio-fertilizer or in treatments used combined treatment of fertilizer and lower level of pesticides. Taking these together, it is concludable that bio-fertilizer reduced the toxictody in lower doses pesticide, but not in higher doses. It seems that fertilizer reduces the bioavailibility of pesticides, but to work well, the doses of fertizer should be increased in higher doses of pesticide. The activity of ALP increased in blood plasma of Rhamdia quelen, Oreochromis mosambicus and cyprinus carpio during exposure to different pesticides and pollutant chemicals (Saha & Kaviraj 2009). Alkaline phosphatase level change has been shown as an indicator for damages of liver and liver dysfunction (Banaee & Sureda 2011). The ALP enzyme apperad to have also play an important role
in glycogen metabolism, so it can stimulate glucose synthesis in the liver to overcome energy required during stress conditions. Therefore, another explanation to decrease of ALP level in some combined treatments of pesticides and fertilizer is that perhaps with increase in pollutant concentration, the level of the enzyme increases in liver to provide energy required in detoxification process and this minimize the enzyme level in plasma (Banaee et al. 2016).

Lactate dehydrogenase is one of the most important liver enzymes and glycolytic enzymes mainly found in the heart and other tissues. LDH converts pyrovic acid to lactic acid (Bernet et al. 2001). LDH is in the cytoplasm of tissue cells of fish specially skeletal muscle, heart, liver, kidney. Following stress exposure in fish, glycogen and glucose catabolism process produce lactate in tissues and subsequently the level of LDH enzyme increases. In contrast, decreasing the amount of LDH enzyme could be related to intensive liver tissue damage under conditions with exposing to the contaminants (Agrahari et al. 2007). Reduction of the amount of LDH enzyme in fish can be related to changes in the metabolic process. Besides, it can be caused by catabolism of lactate dehydrogenase in fish muscle (Velisek et al. 2009). LDH decreased in *Labeo rohita* and *Catla catla* fish after exposed to dimethoate (Hussain et al., 2016), and in contrast increased in blood plasma of common carp exposed to phosalon (Kaya et al. 2015).

In accordance with our study, the CPK enzyme levels were increased after exposure of common carp to organophosphate pesticides, bifenthrin (Banaee & Sureda 2011) and phosalone (Kaya et al. 2015). In contrast, the amount of this enzyme decreased in *Alburnus mossulensis*, after exposure to Fenpropathrin (Banaee et al. 2014b). CPK provides energy from cetaine in tissues with higher activty. In the present study increase in level of CPK enzyme in the combined treatments was observed by increasing the dose of pollutant wich probably show the higher energy requirment to help the fish to come out of pesticide and fertilizer stress.

AST and ALT enzymes are important to metabolism of cellular nitrogen, liver glucose and oxidation of amino acid. AST and ALT enzyme are typically found in hepatocyts, and also are present in heart, kidney, skeletal muscle and pancerase. Increasing the activity of these enzymes play an important role in amino acid oxidation or transformation during gluconeogenesis (Rao 2006). In this study, there were no significant changes in the levels of AST in different treatments but the ALT enzyme increased significantly in group treated with combination of pesticide (at lower doses) and fertilizer (in higher doses). AST and ALT increased in blood plasma of common carp exposed to deltamethrin (Vani et al. 2011), and chlorpyrifos (Banaee et al. 2014a), however the level of AST enzyme decreased in carp exposured to deltamethrin (Vani et al., 2011). Vani et al. (2011) and Shakoori et al. (1996) suggested inhibition of AST activity shows that required energy to overcome stress is not mediated trough it route in Krebs cycle but throuth ALT which accounts for its increased activity.

**Conclusion**
The results of this study showed that the effects of combined treatments of dimethoate pesticide and bio-fertilizer were significanitly different from those in separate treatments of given chemicals.

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**References**


مقاله پژوهشی

اثرات انفرادی و ترکیبی آفت کش دیمیوتات و کود زیستی بر فعالیت آنزیم‌های دخیل در مسیرهای بی‌هوازی، انتقال عصبی و متابولیسم پروتئین در ماهی کپور معمولی (Cyprinus carpio) مسئول فیزیولوژی و بیوشیمیایی

نسترن فضیلت، آریا وزیرزاده، مهدی بنایی، حمید فرهاذی

چکیده: هدف این تحقیق بررسی اثرات آفت کش دیمیوتات و کود زیستی، دو ماده شیمیایی پراستفاده در کشاورزی، بر فراسنجه‌ها біохімічна та фізіологічна активність крові копор мідної для درک اثرات منفی این مواد بر موجودات آبزی بود. به این منظور ۲۷۰ قطعه ماهی کپور معمولی تهیه و در ۹ گروه با سه تکرار تقسیم شدند. تیمارها شامل گروه شاهد، گروه های A و B به ترتیب شامل ۱/۰ و ۱/۵ میلی گرم دیمیوتات، گروه های C و D به ترتیب شامل ۱/۰ و ۱/۵ کود زیستی باسیلار و تیمارهای ترکیبی شامل AC، AD، BC و BD بود. بعد از ۱۴ روز از سیاهرگ دمی ماهیان خونگیری شد و استیل کولین استراز، آلکالین فسفاتاز، آسپارتات امینو ترانسفراز، لاکتات دهیدروژناز، آلانین امینو ترانسفراز و کراتین فسفوکیناز اندازه‌گیری گردید. استیل کولین استراز در همه تیمارها بجهت تیمار A به‌طور معنی‌داری کاهش یافت. آلکالین فسفاتاز به‌طور معنی‌داری در تیمارهای دریافت کننده دوز بالای دیمیوتات کاهش یافت. آسپارتات امینو ترانسفراز نیز در تیمارهای دریافت کننده دوز بالای کود کاهش یافت. لاکتات دهیدروژناز نیز در تیمارهای دریافت کننده دوز بالای کود کاهش یافت. آلانین امینو ترانسفراز در تیمارهای دریافت کننده دوز بالای کود کاهش یافت. کراتین فسفوکیناز نیز در تیمارهای دریافت کننده دوز بالای کود کاهش یافت. نتایج این تحقیق نشان داد که اثرات انفرادی سم و کود مورد مطالعه منتفی است، اما اثرات ترکیبی این مواد بر موجودات آبزی معنی‌دار است.

کلمات کلیدی: آفت کش دیمیوتات، کود زیستی، کپور معمولی، فراسنجه‌های بیوشیمیایی.