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Short Communication

Effects of Dimethoate and Bacilar fertilizer on biochemical and immunological parameters in common carp, *Cyprinus carpio*

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Abstract: Fish exposure to agrochemicals can suppress their immune system and survival. Therefore, this study investigates adverse effects of sublethal concentrations of Dimethoate alone or combined with Bacilar (an organophosphorus pesticide and bio-fertilizer) on the innate immune parameters of common carp, *Cyprinus carpio*, within 14 days. No significant changes were found in levels of total protein, immunoglobulin, the activity of lysozyme and complement C3 in fish exposed to Bacilar alone; however, globulin and complement C4 level indicated a significant reduction. Fish exposure to Dimethoate alone or combined with Bacilar resulted in a decrease in the activity of ACH50, lysozyme, complement C3, C4 and levels of total protein, globulin, and immunoglobulin in compared with the control group. In conclusion, the results of this study showed that innate immune parameters decreased in fish exposed to dimethoate and/or Bacilar. As consequences: Dimethoate or/and Bacilar have the immunosuppressive effect on fish.

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Introduction

The presence of different pollutants, such as pesticides and chemical or organic fertilizers in aquatic ecosystems near agricultural farms can be a serious threat to aquatic organisms (Fazilat et al., 2017). Dimethoate, with the chemical formula of O, O-dimethyl S-methyl carbamoyl methyl phosphoro-dithioate and the organophosphorus pesticide – Bacilar – which is the biological product of *Bacillus subtilis*, along with some micronutrients are used in agriculture. Therefore, the introduction of these compounds into surface waters can bring about alterations in biochemical parameters of blood (Fazilat et al., 2017), cause oxidative stress (Dogan et al., 2011) and behavioral disorders in fish.

Changes in the activity of innate immune parameters (Ahmadi et al., 2014), alterations in the gene expression of cytokines IL-6, IL-8, IL-10 and TNF-α (Chen et al., 2014), and an increase in expression of gene atrophies LC3-II, dynein (Chen et al., 2015) and Hsp60, Hsp70, and Hsp90 genes (Xing et al., 2015) in the head kidney and spleen, alterations in expression of immunoglobulin M (IgM), complement C3, and lysozyme (LYZ) (Ma and Li, 2015) are a number of damages incurred to the immune system of fish exposed to different pesticides and agrochemical compounds. These changes can increase fish sensitivity to biological and environmental infections. Thus, this study evaluates changes in the immune parameters of common carp treated with varied concentrations of either Dimethoate or Dimethoate and Bacilar.

Materials and Methods

This study is conducted according to the Ethics of Animal Experimentation in Iran. Common carp, *Cyprinus carpio*, were divided into 9 groups and tested for 14 days under different conditions: Fish under standard environmental conditions, or the control group (Group I); fish treated with 16 and 32 μg L^{-1} of Dimethoate (respectively Groups II and III); Fish treated with 0.1 and 0.2 ml L^{-1} of Bacilar bio-fertilizer (respectively Groups IV and V); fish treated with 16 μg L^{-1} of Dimethoate + 0.1 ml L^{-1} of Bacilar (Group

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Table 1. Biochemical parameters of *Cyprinus carpio* exposed to Dimethoate alone or combined with Bacilar for a period of 14 days.

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulins (g/dl)	Immunoglobulins (mg/ml)	Lysozyme (U/ml)	ACH50 (U/ml)	Complement C3 (mg/dl)	Complement C4 (mg/dl)
Control	4.8±0.2^{c}	2.1±0.6^{a}	2.7±0.5^{b}	15.6±3.3^{c}	23.3±2.4^{b}	92.0±8.5^{e}	15.5±2.9^{c}	16.3±0.4^{c}
16 µg/l Dimethoate	4.0±0.1^{ab}	3.0±0.5^{bc}	0.9±0.5^{a}	2.7±1.1^{a}	13.5±3.0^{a}	81.2±3.6^{cd}	10.9±1.8^{a}	10.2±0.6^{a}
32 µg/l Dimethoate	3.9±0.4^{ab}	2.7±0.3^{b}	1.13±0.4^{a}	8.0±4.0^{b}	14.0±3.6^{a}	70.6±2.0^{b}	8.4±1.2^{a}	8.9±1.9^{a}
0.1 ml/l Bacilar	4.6±0.7^{c}	3.0±0.3^{bc}	1.6±0.8^{a}	15.8±5.6^{c}	22.3±4.5^{b}	83.0±3.7^{cd}	15.7±4.7^{c}	14.1±0.9^{b}
0.2 ml/l Bacilar	4.4±0.3^{bc}	3.5±0.4^{c}	0.9±0.5^{a}	17.8±4.7^{c}	19.9±0.5^{b}	90.6±5.9^{e}	14.1±2.8^{bc}	13.6±0.6^{b}
16 µg/l Dimethoate & 0.1 ml/l Bacilar	4.1±0.7^{ab}	3.1±0.7^{bc}	1.0±0.8^{a}	6.3±3.6^{ab}	12.9±2.2^{a}	84.8±5.0^{d}	10.8±1.9^{a}	13.8±0.4^{b}
16 µg/l Dimethoate & 0.2 ml/l Bacilar	4.0±0.3^{ab}	3.1±0.6^{bc}	0.9±0.6^{a}	7.3±3.7^{ab}	15.7±3.2^{a}	78.0±1.8^{c}	11.4±1.1^{ab}	14.0±1.5^{b}
32 µg/l Dimethoate & 0.1 ml/l Bacilar	4.1±0.4^{ab}	3.2±0.4^{bc}	0.9±0.4^{a}	6.5±3.7^{ab}	12.2±0.9^{a}	61.4±2.0^{a}	10.4±1.4^{a}	10.5±2.0^{a}
32 µg/l Dimethoate & 0.2 ml/l Bacilar	3.7±0.2^{a}	2.8±0.3^{b}	0.9±0.4^{a}	4.0±1.9^{ab}	12.2±0.8^{a}	62.6±2.1^{a}	9.9±1.8^{a}	10.4±2.3^{a}

Data are presented as mean±S.D. Significant differences between values when compared with control group were characterized by alphabetical symbol (P<0.01).

VI); fish exposed to 32 µg L^{-1} of Dimethoate + 0.1 ml L^{-1} of Bacilar (Group VII); and fish exposed to 32 µg L^{-1} of Dimethoate + 0.2 ml L^{-1} of Bacilar (Group IX). Blood samples were collected from all groups. The following methods measured immune parameters of plasma: total immunoglobulin by Amar and colleagues' method (Amar et al., 2000), alternative complement pathway activity (ACH50) by Yano's method (Yano, 1992) and based on hemolysis of sheep red blood cells (ShRBC) (Baharafshan Research Company), lysozyme activity in blood plasma via *Micrococcus luteus* (Actinobacteria: Micrococcaceae) (Lange et al., 2001), the activity of complements C3 and C4 via immunoturbidimetry (Abdollahi et aal., 2016), and total protein, albumin and globulin (Johnson et al., 1999) by Pars Azmun Kits. After checking the normality of data via Shapiro-Wilk, data analysis was done by one-way ANOVA, using SPSS 22. The means were compared by Duncan Test at a 99% confidence level ($\alpha = 0.01\%$).

Results

No mortality was observed in fish during the experiment; however, an increase in mucus secretions, changes in color and behavior, such as unbalanced swimming, swimming on the surface, and neurological reactions are among the significant behavioral changes in fish exposed to Dimethoate alone or combined with Bacilar on the final days of the experiment. Biochemical parameters of Common carp exposed to Dimethoate alone or combined with Bacilar for 14 days are presented in Table 1. The results of this study indicated that Bacilar alone had no significant effects on levels of total protein, immunoglobulin, lysozyme activity, and complement C3 in fish. However, levels of these parameters in fish treated with either Dimethoate or Dimethoate and Bacilar were significantly reduced. Fish exposure to Bacilar and Dimethoate decreased globulin and complement C4 levels. There was a significant increase in albumin levels in fish treated with Bacilar and Dimethoate compared to that of the control. No significant changes were found in ACH50 activity in fish treated with 0.2 ml L^{-1} of Bacilar; however, ACH50 activity was significantly reduced in other experimental groups.

Discussion

The immunotoxicity of agrochemicals on fish has been widely investigated (Marchand et al., 2017; Chen et al., 2015; Ma and Li, 2015). In the present study, immunological parameters in plasma of fish exposed to Dimethoate and/or Bacilar fertilizer are evaluated. A significant decrease in levels of total protein may indicate liver necrosis or disturbance in the physiological performance of fish treated with Dimethoate or Dimethoate and Bacilar. Dimethoate can reduce levels of total protein in plasma, especially globulins, by reducing appetite, causing a disturbance in the absorption of amino acids in intestines, increased activity of proteolytic enzymes, the increased rate of proteins break down in the liver and preventing protein synthesis in the liver (Narra, 2017). Liver failure, malnutrition as well as a biochemical reaction between agrochemicals and the amino acid sequences of proteins found in blood may account for lower plasma total protein. Similar results are reported in fish exposed to different pesticides (Ahmadi et al., 2014). An increase in albumin can be attributed to its role in distributing the pesticide in the blood (Tarhoni et al., 2008).

Total immunoglobulin is a main element of blood adaptive immunity in bony fish and a well-known biomarker in evaluating the immunotoxicity of fish exposed to environmental pollutants (Li et al., 2013). In this study, a decrease in total immunoglobulin levels is due to disturbance in the adaptive immune system in fish exposed to Dimethoate alone or both Dimethoate and Bacilar. A reduction of total immunoglobulin levels in the blood may be due to an insufficient synthesis of immunoglobulins, or changes in the gene expression involved in the biosynthesis of Ig's subunits (Ghazy et al., 2017). Similar effects were observed in other species exposed to different agrochemical compounds (Narra, 2017).

Lysozyme, a vital element of the innate immune system of bony fish, is very sensitive to pollution-induced stress (Ahmadi et al., 2014). Lysozyme is expressed to a great extent in hematopoietic cells, granulocytes, monocytes, and macrophages (Merlini and Bellotti, 2005). Therefore, a reduction in the activity of lysozyme in fish exposed to Dimethoate alone or Dimethoate and Bacilar indicates the influence of this pollutant on lysozyme biosynthesis and thus disturbance in the innate immune system of common carp. Furthermore, the significant decrease in lysozyme activity in plasma of fish exposed to Dimethoate and Bacilar may indicate debility of defense mechanisms against bacterial agents. The activity of lysozyme is reduced in fish treated with different pesticides (Li et al., 2013).

The complement system, as the first element of the innate immune system, plays a vital role in the immune system and affects the adaptive immune system by stimulating the reproduction of B cells (Li et al., 2013; Pushpa et al., 2014). Both C3 and C4 are glycoproteins that are produced by liver cells, macrophages, and monocytes. New molecular and cellular findings indicate that complement proteins are synthesized in different parts (Løvoll et al., 2007). Therefore, the reduced activity of ACH50, C3, and C4 in fish exposed to Dimethoate or Dimethoate and Bacilar may disturb the biosynthesis of these elements in hepatocytes, macrophages, and monocytes. A reduction in total complement and its elements in fish exposed to different pollutants demonstrate a deficiency in the innate immune system (Ahmadi et al., 2014).

In general, our results indicate that exposure to agrochemicals, such as Dimethoate and Bacilar can suppress the innate immune system in common carp. A decrease in ACH50, total protein, globulin, immunoglobulin, lysozyme, and complement C3 and C4 activity may increase fish sensitivity to infectious pathogens.

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چکیده فارسی

اثرات دایمیتوات و کود باسیلار بر فراسنجه‌های بیوشیمیایی و ایمنی در ماهی کپور معمولی (*Cyprinus carpio*)

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چکیده:
قرار گرفتن ماهیان در معرض مواد شیمیایی مورد استفاده در کشاورزی می‌تواند موجب سرکوب سیستم ایمنی و کاهش بقای آنها گردد. بنابراین در این مطالعه به بررسی اثرات نامطلوب غلظت‌های زیرکشنده دایمیتوات به تنهایی و توام با کود باسیلار (یک آفت‌کش فسفره آلی و یک کود زیستی) بر فراسنجه‌های ایمنی ذاتی ماهی کپور معمولی در طی ۱۴ روز، پرداخته شده است. تغییر معنی‌داری در سطح پروتئین تام، ایمنوگلبولین و فعالیت لیزوزیم و کمپلمان C3 در ماهیان در معرض کود باسیلار به تنهایی یافت نشد. با این حال، سطح گلبولین و کمپلمان C4 به‌طور معنی‌داری کاهش نشان داد. قرار گرفتن ماهیان در معرض دایمیتوات به تنهایی و یا توام با کود باسیلار موجب کاهش فعالیت کمپلمان تام ACH50، لیزوزیم، کمپلمان C3، C4 و سطح پروتئین تام، گلبولین و ایمنوگلبولین تام در مقایسه با ماهیان گروه کنترل گردید. نتایج این مطالعه نشان داد که پارامترهای ایمنی ذاتی در ماهیان در معرض دایمیتوات و یا باسیلار کاهش یافت. در نتیجه، دایمیتوات و باسیلار دارای اثر مهار کنندگی بر سیستم ایمنی ماهیان هستند.

کلمات کلیدی: مواد شیمیایی کشاورزی، ماهی کپور، مسمومیت ایمنی.