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Effects of dietary Kemin multi-enzyme on survival rate of common carp (*Cyprinus carpio*) exposed to abamectin

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Abstract

The aim of this study was utilizing Kemin multi-enzyme in common carp (*Cyprinus carpio*) diet and investigating the impact on fish survival rate exposed to the lethal concentration of abamectin. For this purpose, 350 fish were divided into two experimental diets, containing 0 and 1000 mg Kemin kg^{-1} of diets for 6 weeks and weighed end of this stage. Then, LC_{50} of abamectin for fish dietary consumed Kemin multi-enzyme (treatment group) was determined with different concentrations of abamectin (0, 0.25, 0.5, 1, 2, 3, 6 mL L^{-1}) for 96 hours; also, this testes were repeated for the other group (didn't fed with Kemin multi-enzyme-control group). Analysis of the data showed significant difference between treatment and control groups in terms of fatalities (p<0.05). Higher mortality was related to the Kemin multi-enzyme group and lower mortality related to control group. The 96h LC_{50} of abamectin for *Cyprinus carpio* that were fed with Kemin multi-enzyme and control group were 0.369 and 1.205 mg L^{-1}, respectly. However, there was significant different between weights of treatment (10.43±0.58 gr) in compared with control group (7.56±1.32 gr) at the end of 6 weeks. Results of this study showed that use of Kemin multi-enzyme although increase the fish growth, but due to its effects, such as high intestinal absorption levels, it can increase the toxicity of abamectin (as an example of popular pesticides) and mortality rate.

Keywords: Kemin multi-enzyme, Growth performance, Pesticides, Abamectin, Toxicity, Common carp.

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Introduction

Enzymes are organic catalyst and trigger or expedite chemical reactions which otherwise, the reactions were carried out with less speed (Purich, 2010). Kemin multi-enzyme contains phytase, lipase, xylanase, protease, beta-glucanase, alpha-amylase, pentosanaze, hemicellulase, cellulase and pectinase (Ghomi *et al.*, 2012; Adelian *et al.*, 2014). Several studies have been done in the field of multi-enzyme and enzyme and its effects on fish (Farhoudi *et al.*, 2013). Ghomi *et al.* (2012) by using Kemin multi-enzyme in Beluga (*Huso huso*) fingerlings diets showed that this enzyme could contribute to increase weight gain and specific growth rate of the fish. Adding enzymes to the diet of fish resulted in improved feed efficiency.

Abamectin (i.e. Doramectin) is a bunch of powerful insecticide that comes from a fungus named *Streptomyces avermitilis* (Hedayati *et al.*, 2014). Abamectin is popular among relatively vast range of farmers and ranchers; this can create a risk to food safety and animal health (Al-Kahtani, 2011). Presently, Abamectin is the active components of some insecticides and nematocide products used in agriculture and the most used agents in veterinary medicine for several years in prevention of parasitic diseases (Hedayati *et al.*, 2014). Abamectin is highly insoluble in water, due to their lipophilicity, so distribution in soil is limited. Some of these pesticides cause environmental pollution and interfere in life cycle of organisms especially in vertebrates and fishes (Nazifi *et al.*, 2000, Yalsuyi *et al.*, 2017, Vajargah *et al*., 2018). Numerous studies have been done on the toxicity of Abamectin in aquatic organisms; for example, Hedayati *et al.* (2014), studied the toxicity of Abamectin on common carp fingerlings; also, Novelli *et al.* (2011), studied the effect of Abamectin concentration in survival of Daphnia.

Common carp (*Cyprinus carpio*) is one of the oldest cultured species. So that, common carp is main production of fish farms in worldwide; also, Asia and Europe are the main centers for rearing carp (FAO, 2016). Best growth is obtained when water temperature ranges between 23 and 30 °C. The fish can survive during the cold winter periods. Moreover, salinity up to 5% is tolerated and optimal pH range is 6.5-9.0. The species can survive in low oxygen concentration (0.3-0.5 mg L^{-1}) as well as super saturation.

Pesticides and other type pollutions (i.e. heavy metal) through irrigation and rainfall flowed into the soil and eventually led to pollution of surface and underground water sources (Lin *et al.*, 2007; Ziyaadini *et al.*, 2017). This led to the spread contaminants in the food chain to humans and other consumers (Norouzi *et al*., 2017; Yalsuyi and Vajargah., 2017a). Therefore, water pollution is one of the factors threatening the aquatic health and productions.

The use of digestive multi-enzymes enhances the digestive system and increases the efficiency (Hidalgo *et al*., 1999; Lin *et al.*, 2007). On the other hand, exposure to some pesticides can

affect food ingestion efficacy (Hedayati *et al.*, 2014; Vajargah *et al*., 2014). For example, Vajargah *et al.* (2013) studied toxicity of Diazinon and Deltamethrin on tench (*Tinca tinca*) larvae and fingerling. As well, Chorehi *et al.* (2013) studied the toxicity of Diazinon on Caspian vimba, *Vimba vimba persa* (Cypriniformes: Cyprinidae) and Vajargah and Hedayati (2014) studied toxicity of tricholorofon on four viviparous fish: *Poecilia latipinna, P. reticulate, Gambusia holbrooki* and *helleri* (cyprinodontiformas: poecilidae). The results of all researches showed pesticides can be absorbed through the digestive tract and thereby weakening the performance of fish and poisoning. On the other hand, enzyme can reduce food viscosity and enhance food digestibility (Shirzadi *et al.*, 2010).

In this study, the effects of Kemin multi-enzyme were studied on the digestive tract and growth factors, as well as survival after exposure to lethal amounts of abamectin as a toxin agent.

Materials and methods

At the first 350 common carps with average weight of 6±1.3 g were taken from fish farm in Golestan province and transferred into the aquaculture center of fisheries department, GAU University. After two weeks of adaptation, 42 fish were randomly selected and divided into 2 groups (control and treatment) with 3 replications. Fish weight was measured at the starting of the experiment. Physicochemical condition of the water before and during the experiment was almost constant (water temperature 25±2 ˚C, dissolved oxygen 7-9 mL L^{-1}, pH 7.4 to 8 and Ammonia 0.025±0.003 ml L^{-1}).

The Kemin multi-enzyme level in the treatment group diets was 1000 mg kg^{-1} of food and its value was zero for the control group. Fish were fed with experimental diet 3 times daily at 3% of body weight for 6 weeks. At the end of 6 weeks, feeding was stopped and the individual weight of the fish from each group was measured. The aim of evaluate toxicity of abamectin, according to Hedayati *et al.* (2017) and laboratory facility, 147 Fish were placed for 96 hours exposed to Abamectin (active emulsion 1.8% Mahan Manufacturing Co.).

Dead fish were removed from the tank and mortality rate was recorded at the time of 0, 24, 48, 72 and 96 h. Finally, according to the Hedayati *et al.* (2014), LC_{50} of Abamectin for fish that had consumed Kemin multi-Enzyme was determined. By using digital scale 1,000 milligrams of Kemin multi-enzyme per kilogram of food, dissolved in the gelatin solution, and sprayed on the diet and air dried for five hours. Abamectin acute toxicity test (50% mortality) was estimated in the nominal concentration of Abamectin at intervals 24 hours (24 hours LC_{50}), 48 hours, 72 hours and 96 hours using the software SPSS (IBM SPSS Statistic 20), through the probit test with confidence level 95% (Vajargah *et al.*, 2017).

Results

During the 96 h LC_{50}, there was no death in the control group (zero concentration Abamectin). Daily mortality was counted and recorded (Table 1).

Table 1: Mortality rate of common carp (control and treatment groups) exposed to acute toxicity of Abamectin.

Concentration (mg L^{-1})	Number of fish	Number of mortality							
		24 h		48 h		72 h		96 h	
		Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
0	21	0	0	0	0	0	0	0	0
0.25	21	0	0	0	0	1	1	6	2
0.5	21	0	0	0	0	5	4	17	5
1	21	0	0	0	0	4	8	12	12
2	21	4	2	18	9	19	15	21	17
3	21	6	5	19	11	21	17	21	20
6	21	21	9	21	13	21	19	21	21

The results of this study indicated 96 h LC_{50} of Abamectin for control group was 1.205 mg L^{-1} (Table 2); also, 96h LC_{50} of pesticide Abamectin for treatment group was 0.369 mg L^{-1} (Table 3). In case of 96 h LC_{50} of Abamectin, there was significant difference (p<0.05) in the control group (1.205 ml L^{-1}) compared to the treatment group (0.369 ml L^{-1}).

Table 2: Lethal concentration of Abamectin for common carp (not fed with Kemin multi-enzyme) exposed to acute toxicity of Abamectin.

Point	Concentration (mg L^{-1})			
	24 h	48 h	72 h	96 h
LC_{10}	2.671	1.110	0.202	0.115
LC_{20}	3.812	2.160	0.573	0.489
LC_{30}	4.635	2.917	1.131	0.759
LC_{40}	5.338	3.564	1.609	0.990
LC_{50}	**5.996**	**4.169**	**2.055**	**1.205**
LC_{60}	6.653	4.774	2.501	1.421
LC_{70}	7.356	5.421	2.979	1.652
LC_{80}	8.179	6.179	3.537	1.922
LC_{90}	9.320	7.229	4.312	2.296
LC_{95}	9.474	8.096	4.952	2.605

Table 3: Lethal concentration of Abamectin for common carp (fed with Kemin multi-enzyme) exposed to acute toxicity of Abamectin.

Point	Concentration (mg L^{-1})			
	24 h	48 h	72 h	96 h
LC_{10}	1.991	0.875	0.289	0.101
LC_{20}	2.458	1.157	0.547	0.193
LC_{30}	2.795	1.361	0.732	0.259
LC_{40}	3.082	1.535	0.891	0.316
LC_{50}	**3.351**	**1.698**	**1.040**	**0.369**
LC_{60}	3.620	1/861	1.188	0.422
LC_{70}	3.907	2.035	1.347	0.479
LC_{80}	4.244	2.239	1.532	0.545
LC_{90}	4.710	2.521	1.790	0.637
LC_{95}	5.096	2.755	2.003	0.713

There was a significant difference (p<0.05) in mortality rate between treated and control group in 1.205 mg L^{-1} of Abamectin (Fig. 1); the highest mortality rate related to the treated group and the lowest was observed in control group.

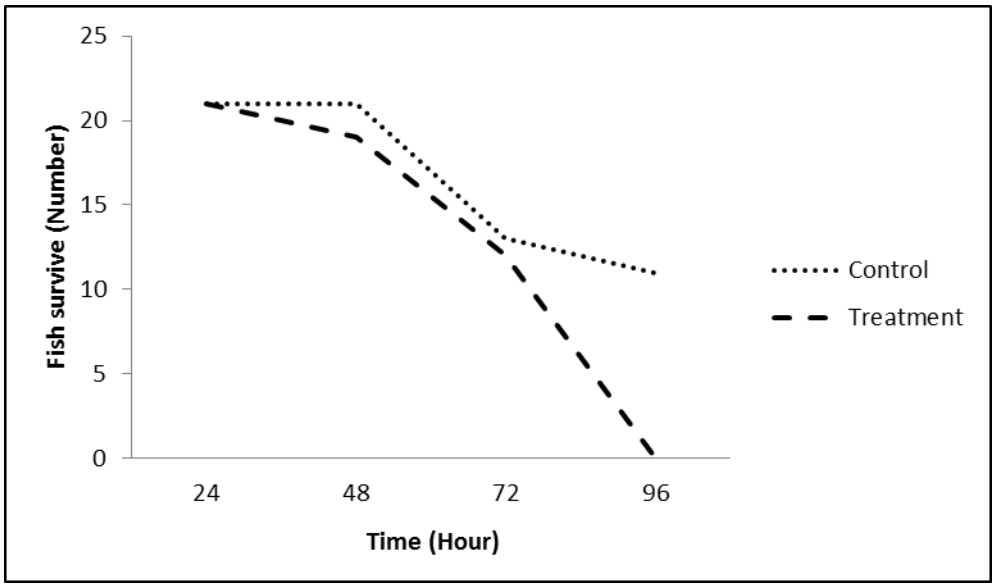

Figure 1: Changes in the fish population's exposure to the concentration of 1.205 mg L^{-1} Abamectin during 96 hours. At the time of 24 hours in both groups was no mortality.

Fish exposed to higher concentrations of Abamectin showed acute symptoms, such as increasing shake operculum, fast swimming, dark color and restlessness and death with open mouth. There was a significant difference (p<0.05) between control group (7.56±1.32 g) and treated group (10.43±0.58 g) in weight gain.

Discussion

With the growing population and industrial development, pollution of water resources has become a global problem (Bela and Prasad, 2008; Yalsuyi and Vajargah, 2017b). Variety of pesticides (i.e. Abamectin, Ivermectin and Doramectin) commonly used on farms for pigs, cattle and sheep (Suarez, 2002); and previous studies had shown 98% of the pesticide consumed is excreted unchanged from the body of this animals (Tišler and Eržen, 2006).

In relation to the effect of multi-enzymes on the toxicity of agricultural pesticides, there were no similar studies. The results of this study illustrate the addition of Kemin multi-enzyme to the diets would improve feeding and growth of fish, along with increasing their susceptibility to the pesticides. The mortality rate in fish that had received Kemin multi-enzyme was higher than the control group (had not Kemin multi-enzyme). Abamectin is absorbed through the digestive system; since the enzyme can reduce

the viscosity and enhance food digestibility (Findrik and Vasic-Racki, 2009). Farhangi *et al.* (2007) reported that the use of enzymes can improve gastrointestinal function in fish.

Hedayati *et al.* (2014) stated 96h LC_{50} of Abamectin for common carp was 12 mL L^{-1}; while, 96h LC_{50} of Abamectin for common carp (treatment group) in percent study was 0.369 mL L^{-1}. The test conditions for both studies was similar; so, the major difference between 96h LC_{50} of Abamectin was related to Kemin multi-enzyme.

Mireles-Arriaga *et al.* (2015), studied the use of enzymes (exogenous enzyme) in animal feed and they stated that use of digestive enzymes in animal feed could lead to improved digestive function and increased the growth of animal. The result of their study was similar with the results of percent study. Hidalgo *et al.* (1999) studied the effect of digestive enzymes on various fish species with different habits. For this purpose, they were fed rainbow trout (*Oncorhynchus mykiss*), gilthead Seabream (*Sparus aurata*), European eel (*Anguilla anguilla*), common carp (*C. carpio*), goldfish (*Carassius auratus*), and tench with equal amounts of the digestive enzymes. The results of their study showed that adding enzymes to fish diets enhance their growth. The results of their study were similar with the results of our study.

Carvalho *et al.* (1997), found that the common carp larvae fed on diets with high levels of protein hydrolysates. A group of fish received only one type of protein and a type protein hydrolysates; another group of fish received several proteins and enzymes. The result of their study showed growth of larva fed by a diet with protein hydrolysates as the only source of nitrogen, significantly lower than the larva fed on mixture of intact proteins and protein hydrolysates. They also stated that digestive enzymes can improve the growth performance. The result of their study was similar with the results of percent study.

Previous studies also found the effect of multi-enzyme on growth, blood biochemical factors and maturation of fish (Farhangi and Carter, 2007; Yildirim and Turan, 2010; Adelian *et al*, 2014). The result of present study clearly illustrate improved growth and feeding parameters using multi-enzymes in the diet of common carp (along with increased susceptibility to Abamectin toxicity; because multi-enzyme can clearly increase intestinal absorption and some poisons (i.e. Abamectin) can be absorbed through the intestinal wall (Hidalgo *et al.*, 1999; Hedayati *et al.*, 2014). Nowadays, various types of enzymes can be used in fish diets. Thus, the increased use of multi-enzymes in the diet of fish alongside the increasing use of pesticides can reduce fish resistance against toxins and increased cost of production. Therefore, the study of synergism impact of them will be the interesting subject of future studies.

Reference

Adelian, M., Imanpoor, M. R., Taghizade, V. and Mazandarani, M., 2014. Utilizing Kemin multi-enzyme in the diet of common carp

(*Cyprinus carpio*). 2nd National Conference on Fisheries and Aquatic animals in Iran. Islamic Azad University, Bandar Abbas, Iran.

Bela, Z. and Prasad, R., 2008. Impact of pollution on fresh and marine water resources. *Journal of Pollution Research*, 273, 461-466.

Chorehi, M.M., Ghaffari, H., Hossaini, S.A., Niazie, E.H. N., Vajargah, M.F. and Hedayati, A., 2013. Acute toxicity of Diazinon to the Caspian vimba, *Vimba vimba persa* (Cypriniformes: Cyprinidae). *International Journal of Aquatic Biology*, 1(**6**), 254-257.

Farhangi, M. and Carter, C.G., 2007. Effect of enzyme supplementation to dehulledlupin-based diets on growth, feed efficiency, nutrient digestibility and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, 38, 1274-1282.

Farhoudi, A., Abedian, A.M., Nazari, R.M. and Makhdoomi, C.H., 2013. Changes of digestive enzymes activity in common carp (Cyprinus carpio) during larval ontogeny. *Iranian Journal of Fisheries Sciences*, 12(2), 320-334.

FAO, 2016. The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. Food and agriculture organization of the United Nations, Rome. 200 pp.

Findrik, Z. and Vasić-Rački, D., 2009. Overview on reactions with multi-enzyme systems, *Chemical Biochemical Engineering*, 4, 545–553.

Ghomi, M.R., Shahriari, R., Faghani H., Nikoo, M. and Elert, E.V., 2012. Effects of exogenous dietary enzyme on growth, body composition, and fatty acid profiles of cultured great sturgeon *Huso huso* fingerlings. *Aquaculture International*, 20(**2**), 249–254.

Hedayati, A., Vajargah, M.F., Yalsuyi, A.M., Abarghoei, S. and Hajiahmadyan, M., 2014. Acute toxicity test of pesticide Abamectin on common carp (*Cyprinus carpio*). *Journal of Coastal Life Medicine*, 2(**11**), 841-844.

Hidalgo, M.C., Urea, E. and Sanz, A., 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, 170(**3**), 267–283.

Lin, S., Mai, K. and Tan, B., 2007. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture Research*, 38(**15**), 1645–1653.

Nazifi, S., Firoozbakhsh, F. and Bolouki, M., 2000. Evaluation of serum biochemical parameters in experimental intoxication with trichlorofon in silver carp (*Hypophthalmichthys molitrix* Valencrennes). *Journal of the Faculty of Veterinary Medicine*, 55, 55-60.

Mireles-Arriaga, A.I., Espinosa-Ayala, E., Hemández-Garcia, P.A. and Márquez-Molina, O., 2015. Use of exogenous enzyme in animal

feed. *Life Science Journal*, 12(**2**), 23-32.

Novelli, A., Vieira, B.H., Cordeiro, D., Cappelini, L.T.D., Vieira, E.M. and Espíndola, E.L.G., 2011. Lethal effects of Abamectin on the aquatic organisms *Daphnia similis*, *Chironomus xanthus* and *Daniorerio*. *Chemosphere*, 86, 36-40.

Norouzi, M., Bagheri, T., Ghodrati, S.H. and Amirjanati, A., 2017. Toxic heavy metal concentration in soft tissues of gray mullet Liza aurata (Mugilidae: Perciformes) during the sexual maturity and sexual rest. *Iranian Journal of Fisheries Sciences*, 16(3), 920-934.

Hedayati, A., Yalsuyi, A.M. and Vajargah, M.F., 2017. Acute toxicity test as a method to assessment toxicity of pollutants. *Annals of Aquaculture and Research*, 4(**1**), 1036.

Purich, D.L., 2010. Enzyme kinetics: catalysis & control: a reference of theory and best-practice methods. Elsevier . 920 P.

Shirzadi, H., Moravej, H. and Shivazad, M., 2010. Influence of non starch polysaccharide-degrading enzymes on the meat yield and viscosity of jejunal digesta in broilers fed wheat/barley-based diet. *African Journal of Biotechnology*, 9 (10), 1517-1522.

Suarez, V.H., 2002. Helminthic control on grazing ruminants and environmental risks in South America. *Veterinary Research*, 33, 563–573.

Tišler, T. and Eržen, N. K., 2006. Abamectin in the aquatic environment. *Journal of Ecotoxicology*, 15, 495-502.

Vajargah, M.F., Hossaini, S.A., Niazie, E.H.N., Hedayati, A. and Vesaghi, M.J., 2013. Acute toxicity of two pesticides Diazinon and Deltamethrin on Tench (*Tinca tinca*) larvae and fingerling. *International Journal of Aquatic Biology*, 1(**3**), 138-142.

Vajargah, M.F., Hedayati, A., Yalsuyi, A.M., Abarghoei, S., Gerami, M.H. and Farsani, H.G., 2014. Acute toxicity of Butachlor to Caspian Kutum (*Rutilus frisii Kutum* Kamensky, 1991). *Journal of Environmental Treatment Techniques*, 2(**4**), 155-157.

Vajargah, M.F. and Hedayati, A., 2014. Toxicity of tricholorofon on four viviparous fish: *Poecilia latipinna, Poecilia reticulate, Gambusia holbrooki* and *helleri* (cyprinodontiformas: poecilidae). *Journal of Coastal Life Medicine*, 2(**7**), 511-514.

Vajargah, M.F., Yalsuyi, A.M. and Hedayati, A., 2017. Acute toxicity of povidone-iodine (Betadine) in common carp (Cyprinus carpio L. 1758). *Pollution*, 3(**4**), 589-593.

Vajargah, M.F., Yalsuyi, A.M., Hedayati, A. and Faggio, C. 2018. Histopathological lesions and toxicity in common carp (*Cyprinus carpio* L. 1758) induced by copper nanoparticles. *Microscopy research and technique*, 2018, 1-6.

Woynarovich, A., Bueno, P.B., Altan, O., Jeney, Z., Reantaso, M.,

Xinhua, Y. and Van Anrooy, R., 2011. Better management practices for carp production in central and Eastern Europe, the Caucasus and central Asia. FAO Fisheries and Aquaculture Technical, 566 P.

Yalsuyi, A.M and Vajargah, M.F., 2017a. Acute toxicity of Silver nanoparticles in Roach (*Rutilus rutilus*) and Goldfish (*Carassius auratus*). *Journal of Environmental Treatment Techniques*, 5 (**1**), 1-4.

Yalsuyi, A.M. and Vajargah, M.F. 2017b. Recent advance on aspect of fisheries: A review. *Gournal of Coastal Life Mediciene, 5(**4**), 141-148.*

Yalsuyi, A.M., Hedayati, A., Vajargah, M.F. and Mousavi-Sabet, H., 2017. Examining the toxicity of Cadmium chloride in common Carp (Cyprinus carpio) and Goldfish (Carassius auratus). *Journal of Environmental Treatment Techniques*, 5(**2**), 83-86.

Yildirim, Y.B. and Turan, F., 2010. Effect of exogenous enzyme supplementation in diets on growth and feed utilization in African catfish, *Clarias gariepinus*. *Journal of Animal and Veterinary Advances*, 9(**2**), 327-331.

Ziyaadini, M.., Yousefiyanpour, Z., Ghasemzadeh, J. and Zahedi, M.M., 2017. Biota-sediment accumulation factor and concentration of heavy metals (Hg, Cd, As, Ni, Pb and Cu) in sediments and tissues of Chiton lamyi (Mollusca: Polyplacophora: Chitonidae) in Chabahar Bay, Iran. *Iranian Journal of Fisheries Sciences*, 16(**4**), 1123- 1134.