



ORIGINAL ARTICLE

Effect of Fungicide Antracol on Blood Enzymes in Snake Headed Fish, *Channa punctatus* (Bloch.)**Rashmi Sharma and Surendra Singh**

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Email: dr.rashmisharma1976@gmail.comReceived: 14th Jan. 2017, Revised: 13th March 2017, Accepted: 16th March 2017**ABSTRACT**

Chemicals are used extensively in form of different categories of pesticides in agriculture to improve crop yield. Such chemicals even fungicides effect on individual species, change in species abundance and production are predictable in communities exposed to chemical stress. Fishes are found to be extremely sensitive to any environmental alterations. Fungicides have been found as underground and aquatic pollutants and their bioaccumulation may cause damage to aquatic animals. Antracol, Zn containing dithiocarbamate has tendency of bioaccumulation due to the presence of heavy metal as zinc. The effect of organometallic compounds on aquatic organisms is currently attracting wide spread attention, particularly in studies related to biochemical observations and envisage the toxic effects.

Key words: Fungicide Antracol, Blood Enzymes, *Channa punctatus*

INTRODUCTION

In this modern era, there is a competition among the nations for development in every field. For a nation to progress socially, economically as well as politically, development processes in different field are very necessary. Development leads into industrialization. The growth of industry is an important feature of civilization. Agriculture is also an important sector of the economy of any nation. It is true that the economic development in modern times has come to be associated with industrialization can follow only on the sound heels of agriculture. Indian agriculture is the backbone of Indian economy. In comparison with the other countries as regards the share of agriculture in national income we find that India contributes much more, as a vast populated country. The current strategy for agricultural development is mainly bent towards intensifying cultivation in selected areas through the spread of high yielding varieties and multi-cropping programmes which aim at producing two or more crops of short duration as against one crop under the traditional varieties. For this purpose, so many devices are used to increase in per capita income and production; such devices are in the form of fertilizers, pesticides and different mechanical tools also.

For improving productivity, several types of xenobiotics (chemical compounds) are used by the peasants. The extensive use of the chemical compounds in crop production has not only contributed to the increase in yield per unit area and quality of product but sometimes their use may be harmful. These chemical compounds escape into the biosphere from the zones of usage, both in particulate and in vapour form, and contaminate the air, water and soil masses. Under the name chemical compounds are grouped a large number of pesticides that may be an insecticide, a herbicide or a fungicide. Fungicides offered new standards of control for diseases such as grey mould, powdery mildew and rusts. There are several groups of fungicides which have selective toxic activity effective against certain life processes in the fungi. Fungicides spread over the crops to protect them fungal attacks. The extremely use of fungicides has not only protect the crops but also has undesired effects on human beings including other living beings. These compounds spread over crops also move into water bodies due to surface runoff. In excess, they cause immediate and mass scale deaths, of aquatic communities. The surface water provides a good potential for culture and capture fisheries. In other country almost about 59% of surface water has been polluted in both urban as well as rural areas due to industrial waste waters and agricultural runoff. So water

pollution is a serious environmental problem which has considerable economic repercussion on rural economic activities like agriculture.

Illiteracy and not proper knowledge about the use of these chemical compounds results a disturbed ecosystem. Fungicides and other undesirable chemicals constitute a sufficiently powerful cause of ecological disturbance. The whole ecosystem will be affected when any of the components of the ecosystem is affected by aquatic pollution such as fishes. Fish is a significant source of food production, and can definitely boost our production of protein rich food compaign. India, presently is the world's seventh largest nation, with fish catches, both from coastal waters and from various inland water i.e. rivers, lakes reservoirs and irrigation ponds. Fish farming also adds substantially to our national income. Communities and ecosystems are hierarchical systems with emergent properties, stability, diversity, and the like that in the part reflect the integral effect of interactions among system components. A key factor in aquatic pollution is the adequate supply of such chemicals.

The Antracol, an organometallic fungicide is introduced by Bayer (India) limited as a contact fungicide. Today the area under potato crops is steadily increasing around the world. Also on the increase are the demands made of the farmer in a fiercely competitive market. Fungal diseases constitute the main threat to the harvest. Late blight of potato (*Phytophthora infestans*), the most important fungal disease, spreads very quickly and can cause an untreated canopy to collapse within a few days if the weather is warm. It is widely used by peasants in treatment of potato late blight fungus in rural areas around Agra (U.P.). Antracol has remained popular due to its special mode of action, because it affects several sites in the metabolism of fungal cells. Active constituent in Antracol is propineb that belongs to the dithiocarbamates class of compounds. It is found in wettable powdered form. Like organophosphates, the carbamates also interfere in cholinergic transmission. The carbamate enters the synapse and inhibits the acetylcholine-esterase as a result the actylcholine contains to depolarize the post synaptic membrane, causing prolonged stimulation resulting into the failure of the nerves or effector tissue. This experiment shows distinctive band pattern of changes in enzyme's biochemical values.

MATERIALS AND METHODS

Maintenance of Fish in Laboratory:

Channa punctatus, an air breathing snake headed murrel, ranging from 12cm to 15 cm in length and 35 gm to 45 gm in weight were collected from local fresh water ponds in Aligarh and transported to laboratory during the month of September, when room temperature ranged from 30°C to 35°C and water temperature from 25°C to 28°C. The experiments were generally carried out in aquarium tanks containing 25 litre tap water and each aquaria measuring 75cmX37.5cmX37.5cm. These fishes were acclimatized to laboratory conditions and fed on small pieces of liver and boiled egg yolk according to feeding schedule. Tap water free from chlorine, underground water was used as the diluting water. It was stored in a large aquarium for dechlorination and changed every two days or week if it give foul smell.

Properties of Experimental Compound:

Propineb 70WP, Zinc containing dithiocarbamate has been selected for present study.

Trade name: Antracol, Bayer 46131

Other synonyms: Methylzineb, Mezineb, Airone, Taifen and Zipromat

Chemical name: Zinc, (N,N' - Propylene-1, 2-bis (dithiocarbamate)

Action: Fungicide

Form: Wettable powder

Colour: White

Odour: Weak characteristic odour

Melting point: 150-160°C

pH value: 6.0 - 8.0

Molecular weight: 279.774 amu

Molecular formula: (C₅ - H₈N₂ - S₄ - Zn)_x

Field Application and Formulation:

Propineb 70WP (Antracol), a contact fungicide is used as a foliar spray for the control of fungal diseases in different crops. It shows solid formulation i.e. a wettable powder and based on propineb technical containing 70% w/w. Propineb as active ingredient and balance auxiliaries as inert materials.

Determination of LC₅₀ and Preparation for Biochemical Estimations:

Evaluation of a chemical's hazard to aquatic life is generally done using LC₅₀ test in which the value reported is the concentration in water lethal to 50% of the test population in a given time, usually 96hrs (Johnson and Finlry, 1980). To determine the mean lethal concentration, well acclimatized active *Channa punctatus* were chosen and divided into four groups (A, B, C & D). Each group consisting of four individuals and kept into the standard solution of compound Antracol 70WP that was prepared by different doses, 2000mg, 4000mg, 6000mg and 8000mg, in different aquarium tanks. The mortality of survival number of test fishes of each group was recorded after 96 hrs. The LC₅₀ was assessed by plotting the experimental results on graph paper, taking the logarithms of the concentration on abscissa and the mortality percentage on the ordinate. Actually the probit values are plotted in place of the mortality percentages. The data were analysed statistically by log dose/probit regression line method (Finney, 1971). Regression line was drawn on the basis of two variables log dose and empirical probit on a simple graph paper and used to determine the expected probit necessary for LC₅₀ determination.

The blood was collected after severing the caudal Peduncle of the living fish by a scissors. Further, serum was separated out *vide infra*. Centrifugal tubes filled with blood, were allowed to stand vertically at slight angle to clot for about three minutes. It was then centrifuged at 3000 rpm for 15 minutes. The supernatant was serum and separated by a fine rubber bulb pipette in separate test tube. The serum samples were used for the estimation of serum biochemical parameters.

Biochemical Estimations and Their Statistical Analysis:

Biochemical alterations in blood enzymes of *Channa punctatus* after intoxication to sub-lethal concentration (1/10th value of LC₅₀ for 96 hrs) of Antracol within 90 days at different intervals 15th, 30th, 60th and 90th days of experiment. The serum lactate dehydrogenase was estimated by UV kinetic (IFCC and SFBC kit method). Succinate dehydrogenase enzyme was estimated by the method of Beatty, *et al.*, (1966). In the present investigation, computer software assisted analysis is used for different statistical calculations based on Fischer and Yates, 1950.

RESULTS AND DISCUSSION

Antracol is an organo metallic compound which is widely used for the control of fungal diseases in plants as a fungicide. It has been selected as studying material. The results clearly reveal that exposure to propineb influenced the biochemical parameters of blood enzymes in *Channa punctatus* (Bloch.).

Level of blood enzyme lactate dehydrogenase of fishes in present study, is increased and from 835.00U/L to 2267.50U/L with respect to increase exposure time (15days, 30days, 60days and 90days) to propineb. Lactate dehydrogenase level is also increased after exposure to carbamate pesticide sevin in *Channa punctatus* has been reported by Sastry and Siddiqui (1996). Sastry and Siddiqui (2004) have also reported decreased level of lactate dehydrogenase in liver tissue of *Channa punctatus* after intoxication to endosulfan. Tissue breakdown elevates levels of lactate dehydrogenase and therefore a measure of it indicates of hemolysis. In medicine, lactate dehydrogenase is often used as a marker of tissue breakdown as lactate dehydrogenase is red blood cells and can function as a marker for hemolysis. It can also be used as a marker of myocardial infarction as elevated level. So lactate dehydrogenase levels uses are assessment of tissue breakdown in general, this is possible when there are no other indicators of hemolysis. It is used to follow-up cancer (especially lymphoma) as cancer cells have a high rate of turn over with destroyed cells leading to an elevated lactate dehydrogenase activity (Simin, *et al.*, 1983). It begins to diffuse out of these tissues and blood lactate rises. So this lactic acidosis is associated with myocardial infarction, severe congestive cardiac failure, pulmonary edema and blood loss which lead to shock.

Table 1: LDH and SDH activity (u/l) in control and treated *Channa punctatus* after treatment with Antracol

S.No.	Treatment Days	No. of fishes	LDH activity		SDH activity	
			Control	Treated	Control	Treated
			Mean ± S. Em.	Mean ± S. Em.	Mean ± S. Em.	Mean ± S. Em.
1.	15	4	835.00±7.07	937.75±20.77***	415.50±3.32	365.75±4.04****
2.	30	4	835.00±7.07	1249.25±4.81****	415.50±3.32	221.25±4.26****
3.	60	4	835.00±7.07	1435.25±9.10****	415.50±3.32	191.75±2.68****
4.	90	4	835.00±7.07	2267.50±150.50****	415.50±3.32	98.25±9.26****

* Non-significant (P>0.05) ; ** Significant (P<0.05) ; *** Highly significant (P<0.01) ; **** Very highly significant (P<0.001)

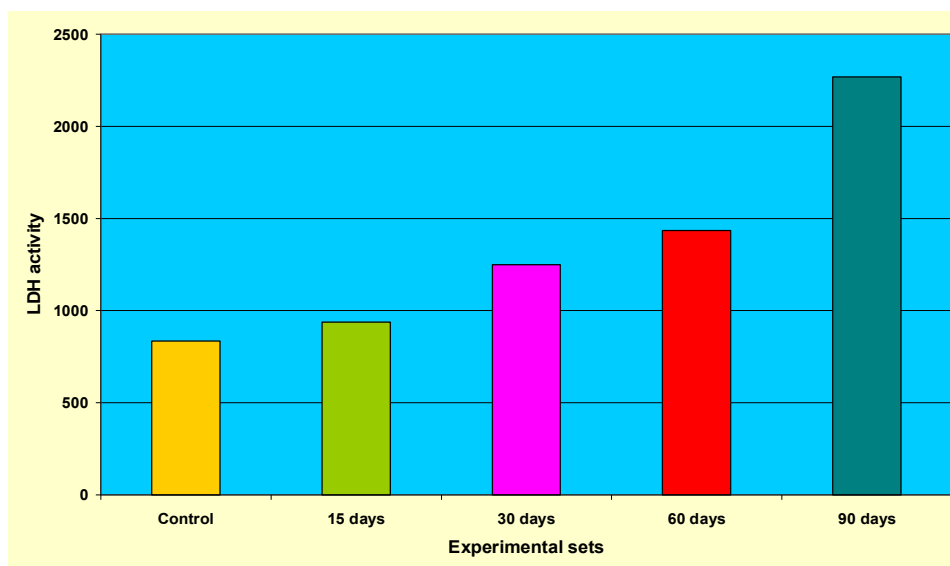


Fig.1: LDH activity (u/l) in control and treated *Channa punctatus* after treatment with Antracol

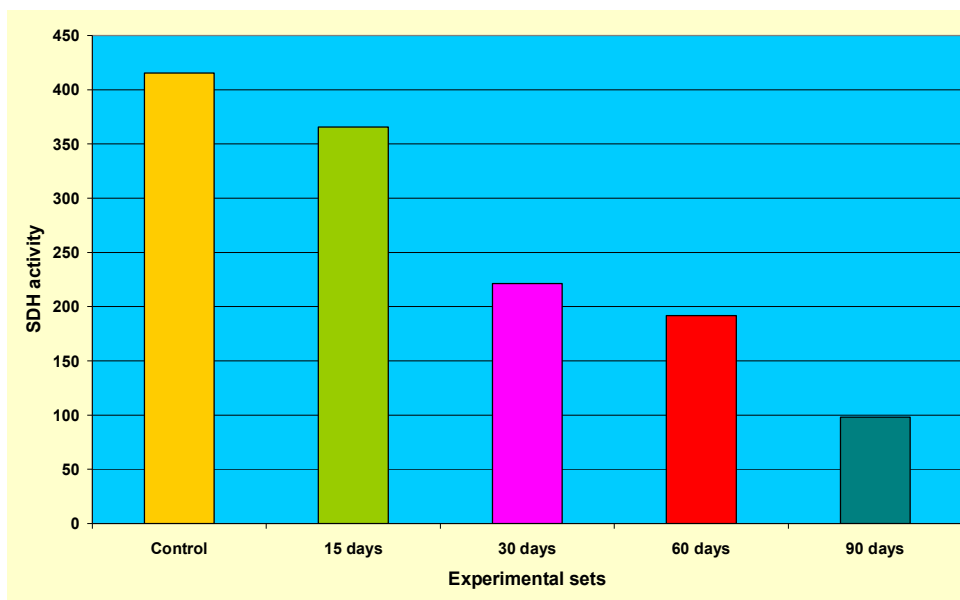


Fig.2: SDH activity (u/l) in control and treated *Channa punctatus* after treatment with Antracol

Level of succinate dehydrogenase is decreased from 365-98U/L with respect to increase exposure time (15day-90day) to propineb. Amemarie, *et al.*, (1978) also showed inhibition in succinate dehydrogenase due to ubiquinone type inhibitors include carboxin and thenoyltrifluoroacetone. Similarly, same conditions in succinate dehydrogenase level due to high rates of superoxide production in skeletal muscle (Dalal and Battacharya 1994). Succinate dehydrogenase level is also decreased in *Channa punctatus* after exposure to carbamate pesticide sevin by Sastry and Siddiqui (1996). Same conditions also been seen in *Channa punctatus* after intoxication to endosulfan by Sastry and Siddiqui (2004). Succinate dehydrogenase activity of muscle and liver of detergent treated fish *Channa striatus* decreased up to 24hr exposure has been reported by Soranraj and Ranjith Singh (2002).

Carbonyl group is known to inhibitor of succinate dehydrogenase enzyme as carbamate (Rajaa Hussain and George, 2010). The decreased succinate dehydrogenase activity levels in the present investigation indicate the decreased operation of Krebs cycle probably by limiting the flow of substrates into the cycle or impairment of mitochondrial organisation. Succinate dehydrogenase functions not only in mitochondrial energy generation but also has a role in oxygen sensing and tumor suppression and therefore is the object of ongoing research. Several deeds have been given in favour of present study. Lastly it may be said that results of the present study clearly document that intoxication to antracol with varying time exposure 15 days, 30 days, 60 days and 90 days disturbs metabolic pathway of blood enzymes in the fish *Channa punctatus*.

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