Name: Khalid Habib

Department: Biosciences

Supervisor: Prof. Tasneem Fatma

Title: "Toxicological Impact of Carbamate Pesticide on Cyanobacterial Biofertilizer"

ABSTRACT

Pesticide contamination in aquatic ecosystem including paddy field is a serious global environmental concern. Cyanobacteria are also affected by pesticides as non-target organism. In present work *Calothrix brevissima* (cyanobacterial biofertilizer) was studied for their responses to a carbamate pesticide 'carbaryl' with special reference to fatty acid profile, electrolyte leakage, enzymatic and nonenzymatic antioxidant. The cyanobacterium was grown for 21 days with 0, 10, 20, 30 and 40 mg L^{-1} carbaryl pesticide in culture medium. Reduction in growth, photosynthetic pigments, total protein and polyunsaturated fatty acid (PUFA) content were accompanied with increased amount of malondialdehyde (MDA) and the enhanced cell membrane leakage indicated free radical mediated deleterious effect of carbaryl, while increased level of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione S-transferase **(**GST) glutathione reductase (GR), reduced glutathione (GSH) and oxidized glutathione (GSSG), sulfur containing amino acids (cysteine, cystine and methionine) and osmolytes (proline, glycine-betaine, sucrose, mannitol, trehalose and glycogen), indicated their probable role in free radical scavenging as well as stress enzyme protection against denaturation.

Findings:

1. Growth behavior of *C. brevissima* in presence of carbaryl (as biomass)- Visually yellowing and reduction in aggregate formation represented the adverse effect of carbaryl. Maximum yellowing and aggregation was observed at 40 mg L^{-1} carbaryl concentration. Carbaryl showed dramatic reduction from 10 mg L^{-1} on further increase of carbaryl upto 40 mg L^{-1} though there was gradual decrease but the rate of reduction was not so drastic.
2. Chlorophyll was adversely affected by carbaryl in *C. brevissima*. Chlorophyll content showed overall decreasing trend. The maximum amount of reduction was found in presence of 40 mgL^{-1} carbaryl. Carotenoid and phycobiliprotein contents also showed reduction with increasing concentration of carbaryl. Maximum reduction was detected in carotenoid and phycobilin at 40 mg L^{-1} of carbaryl concentration.
3. The total protein contents were decreased with increasing concentration of pesticide. The maximum protein reduction was observed in *C. brevissima* (40%) at 40 mg L^{-1} pesticide concentration. The decrease in protein content may also be due to presence of pesticide beyond their tolerance range, increased level of ROS or increased protease activity. It resulted retarded growth and decreased carbon and nitrogen assimilation.

4. The total carbohydrate content showed increasing trend with increasing concentration of carbaryl (0, 10, 20, 30 and 40 mg L^{-1}) in comparison to control. This may be the adaptive measure aimed at survival under carbaryl stress condition.
5. Malondialdehyde (MDA) content increased with increasing concentrations of pesticide suggesting formation of free radicals eliciting carbaryl toxicity. MDA increased gradually and significantly to 46% at 40 mg L^{-1} carbaryl exposure suggesting induction of oxidative stress to *C. brevissima*.
6. Carbaryl induced a dose-dependent membrane damage resulted in increased electrolyte leakage. The values were considerably increased (5% to 27%) respectively at 10 to 40 mg L^{-1} carbaryl. Membrane permeability may be due to pesticide induced cell membrane injury or oxidative damage.
7. The enzymatic antioxidant viz. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione S- transferase (GST) and glutathione reductase (GR) and non-enzymatic antioxidant (GSH & GSSG) were accelerated to enhance the detoxification of free radicals stimulated by the treatment of carbaryl.
8. Proline (a universal protectant of various stress condition), glycine-betaine, sucrose, mannitol, trehalose and glycogen content increased with increasing carbaryl concentration suggesting these osmoprotectants also play role in detoxification of pesticide.
9. The cysteine, cystine and methionine content significantly increased in *C. brevissima* with increasing concentrations of the pesticide. The order of enhancement in sulphur containing amino acids was cysteine (79%) > cystine (64%) > methionine (52%) at 40 mg L^{-1} which might be another adaptive defense strategy of the cyanobacterium against the pesticide.
10. Highest amount of fatty acid was in the form of α- linolenic acid (20.6%) while lowest amount of fatty acid was represented by myristic acid (2.68%). In terms of saturation its can be said that saturated fatty acid were present in highest amount (55.46%) and it was followed by polyunsaturated (45.77%) and monounsaturated (22.17%). This could be result of the high PUFA (α- linolenic, linoleic and oleic acid) content of *C. brevissima*. The high content of these PUFAs in *C.brevissima*, mainly α-linolenic acid, linoleic acid are essential PUFA and thus are necessary for good human health.
11. 57.54 and 24.10 kDa protein bands were observed in presence of only lower concentration of carbaryl while 21.99 kDa protein band appeared in presence of 30 and 40 mg L^{-1}. 14.12 kDa band was more expressed in 10 and 20 mg L^{-1} carbaryl, though it also existed in control and higher pesticide concentration. 29.85, 25.11, 23.71 and 10.59 kDa bands expressed only in 10 and 20 mg L^{-1} pesticide concentration along with control. 66.83, 42.16, 39.81, 16.78 and 14.96 kDa bands also appeared in control as well as carbaryl exposed cultures but their expression decreased with increasing pesticide concentration. Changes in protein profiling and newly formed protein might be helping cyanobacterium to tolerate adverse condition.