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Effect of Pesticide on Nitrate Reductase Activity in *Trigonella Foenum* i.e., Fenugreek

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Abstract

Nitrate reductase (NR) is the key enzyme of nitrate assimilation subjected to inhibition by excessive use of pesticides. Now a day's pesticides are used commonly at a large scale on field. In the present study, the effect of pesticides on nitrate reductase activity has been studied by in vivo assay in *Trigonella foenum-graecum*. The four pesticides Confidor, Omite, Karathane, Chlorpyrifos+Cypermethrinwere used for study at various concentrations such as 25, 50,100 and 200 µl/100 ml. The application of pesticides showed decrease in activity of nitrate reductase enzyme. Out of these pesticides confidor affected the nitrate reductase activity maximum. The nitrate reductase activity in control plant was found to be 0.460U ± 0.005 which was reduced up to 0.192 ± 0.008U in presence of confidor. Even in the tissue specific assay it was seen that the leaves and stem of the plant, the NR activity was affected maximum than other parts. The use of pesticides lowers the NR activity of the *Trigonella* plants thereby affecting its nitrogen metabolism.

Keywords: Nitrate reductase; Confidor; Omite; Karathane; Chlorpyrifos+cypermethrin; *In vivo* NR-Assay

Introduction

Nitrate reductase (NR; EC 1.6.6.1.3) is one of the most important enzymes in the assimilation of exogenous nitrate—the predominant form of nitrogen available to green plants growing in soil. It is monomer composed of 100 KD polypeptide and one each of FAD, Heme ion and molybdenum

$$NO_3 + NADPH_2 \xrightarrow{\text{Nitrate reductase}} NO_2 + H_2O + NADP$$

Nitrate reduction, the most important stage of nitrogen turnover in nature, has several functions:

1. Utilization of NO^{3-} as a source of nitrogen (nitrate assimilation);
2. Production of metabolic energy during NO^{3-} utilization as terminal acceptor of electrons (nitrate respiration), and
3. Dissipation of excess of reducing energy to maintain oxidation–reduction balance (nitrate dissimilation).

It is an enzyme that is being used to help clean up the environment, by conversion of nitrate to nitrite and has great potential to be part of the solution to the global problem of excess nitrate and related nitrogen nutrients in water sources [1]. Nitrate is currently one of the most hazardous pollutants [2-4]. Activity of this enzyme in plants gives a good estimate of the nitrogen status of the plant and is very often correlated with growth and yield. Nitrate reduction processes are perhaps most significant in maintaining water quality. NR enzyme is responsible not only for nitrate reduction, but also for nitrate uptake in *Chlamydomonas*, *Chlorella sorokiniana* [5,6]. Assimilatory Nitrate reduction, performed by a variety of bacterial and eukaryotic organisms [7,8] includes the reduction of nitrate and nitrite compounds, often mediated by nitrate or nitrite reductase enzymes, for synthesis of amino acids. The ability of nitrate to induce nitrate reductase for nitrate metabolism in higher plants has been widely accepted [9]. The nitrate reductase activity is inhibited by heavy metals like Cr^{3+} and Cu^{2+} irreversibly [10]. It has also been reported that insecticides (kelthane and fenvalerate) caused some inhibition to enzyme activities [6]. In some experiments 15 different pesticides were evaluated to study their effect on growth and nitrogen assimilation of the *Azolla Mexicana* [11]. It was seen that phenolic herbicides were detrimental causing 75% reduction in nitrogen fixation and nitrate reduction. Choramber (3 amino, 2-5 dichlorobenzoic acid) and benomyl (methyl 1-butyl carbamoyl 2-bezimidazolyl carbamate) caused 84-99% reduction in nitrogen fixation [12]. The toxic insecticide Trichlorfon induced inhibition of nitrogen uptake in Cyanobacteria [13]. In the present study the effect of pesticide on nitrate reductase enzyme activity is studied invivo in Fenugreek i.e., Trigonella foenum. The plant material was selected since it is easy to grow and have short life cycle.

Materials and Methods

Plant material

The present study involves the use of *Trigonella Foenum-graecum* i.e., Fenugreek. The seeds of the fenugreek were purchased from the market and grown in different pots in the laboratory. The plantlets were identified as the *Trigonella foenum-graecum* with the help of Department of Botany, Yashvantrao Chavan Institute of Science, Satara.

Pesticides

From a common survey of around 100 farmers using pesticides around Satara City, Confidor, Omite, Karathane, Chlorpyrifos

+Cypermethrinwere selected as the most commonly used pesticides on fields for the present study.

In vivo Nr Assay

Nitrate reductase (NR) activity can be directly assayed using cut plant tissues. In a solution containing nitrate, phosphate buffer and propanol, nitrate can readily enter plant material. In this case, the plant cell supplies NR and the reductant NADH. The propanol in the assay medium increases cell permeability and makes the leaf segments essentially anaerobic. Under anaerobic conditions, the reduction of nitrite to ammonia is inhibited, and the conversion of nitrate to nitrite is enhanced Because of the sensitivity of the reaction to molecular oxygen all operations should be carried out in dim room light to reduce photosynthetic activity. With this approach the NR activity associated with all four inducing treatments can be assayed by one group [14].

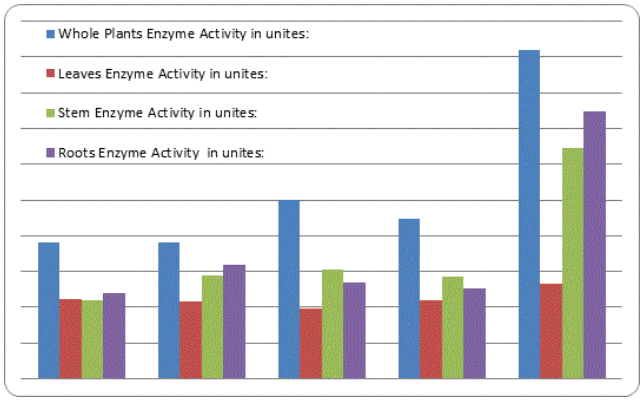
Weigh out four 0.5 g leaf samples for each of the inducing treatments, cut the tissue into 1.0-1.5 cm squares segments. Prepare duplicate screw cap test tubes for each inducing condition. You will need one set for time=0 (t0) and another set for time=30 min. (t30).

Place the leaf samples in the appropriate tubes. Add 10 ml of In vivo assay solution to each tube and cap the tube.(100 mM phosphate buffer, pH 7.5 ,30 mM KNO_3, 5% (v/v) propanol) Place the two tubes in a boiling water bath for 5 min. and then allow to cool to room temperature. Place all tubes, t_0 and t_{30}, in a shaking water bath at 30°C and incubate for 30 min. At the end of the 30-min. incubation period, place the t_{30} tubes in a boiling water bath for 5 min. and then cool to room temperature. To detect nitrite in the assay tubes, add 1 ml 1% sulfanilamide in 3 N HCl, and 1ml of 0.02% N-(1-naphthyl)-ethylenediaminehydrochloride. Mix thoroughly and place tubes in the dark at room temperature for 15 min. Determine OD of each standard tube at 540 nm and calculate the enzyme activity.

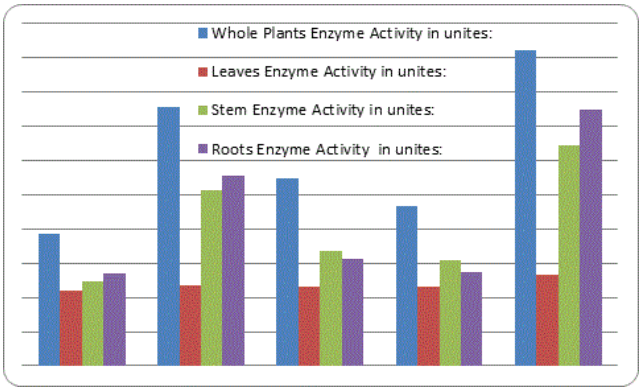
(Note: All the experiments were repeated three times and the data presented is the mean of the readings.)

Results

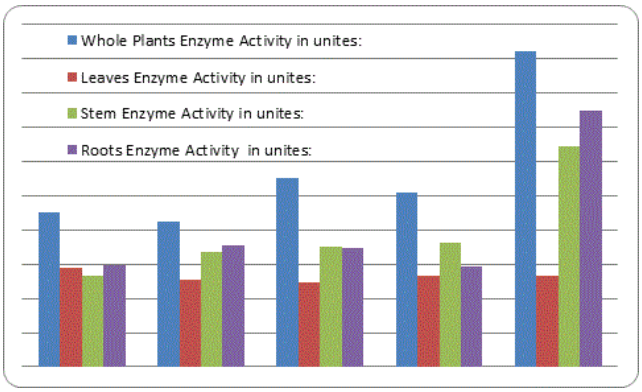
The results are shown in graphical representation (Graphs 1-4).



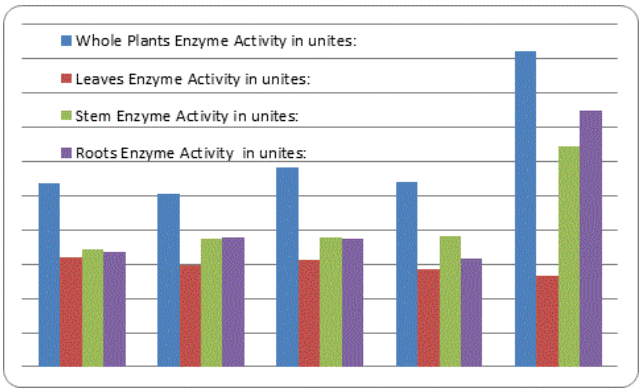
Graph 1: Enzyme Activity at concentration 200 µl/100 ml.



Graph 2: Enzyme Activity at concentration 100 µl/100 ml.



Graph 3: Enzyme Activity at concentration 50 µl/100 ml.



Graph 4: Enzyme Activity at Concentration 25 µl/100 ml.

Discussion

In the present investigation from the result clearly indicates the inhibition of nitrate reductase activity after using the pesticides Confidor, Omite, Karathane, Chlorpyrifos+Cypermethrin, on *Trigonella Foenum-graecum*. Nitrate reductase activity of control pot of whole plant was found to be 0.460 units however it is severely

affected after using confidore and is reduced to 0.192 units. After studying different parts of the plant it is observed that leaves are the most affected by the use of pesticides than root and stem.

Different pesticides used in the current study showed the inhibitory effect on the nitrate reductase in the following order Confidor>Chlorpyrifos 50%+Cypermethrin 5% >Karathane>Omite.

All the pesticide under investigation showed the inhibitory effect on the nitrate reductase, thereby affecting the ability of the plant to utilize the nitrate.

Similler studies on insecticides such as kelthane and fenvalrate caused inhibition of nitrate reductase in fungi from root nodules and faba bean (Vicia faba L).

Chromium and copper metals irreversibly inhibited nitrogen reductase in *Aspergillus niger*.

Several pesticides have been proved to affect growth and nitrogen assimilation of *Azolla anbaena* symbiosis (especially *Azolla mexicana*). Toxic insecticide trichlorfon inhibits the nitrogen and Ammonium uptake in cyanobacteria. Cadmium, acetochlor and bensulfuron methyl have toxic effect on nitrogen metabolism and plant growth in rice, seedlings resulting in marked decrease in the fresh weight and the activities of nitrate reductase (NR).

Number of researchers proved in different plants use of pesticides leading to inhibition of nitrate reductase. In the present investigation the commonly used pesticides showed similar inhibitory effect on *Trigonella Foenum-graecum* and compelling us to use nitrate reductase friendly methodology in place of above pesticides.

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