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Biochemical changes in rice plant due to application of carbofuran

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Abstract
Carbofuran is a widely used insecticide for the control of a number of major rice-pests and caused a resurgence of rice leaf folder. An investigation was undertaken to study the effect of carbofuran application on different biochemical activities of the rice plant. Results indicated that gross weight of all the plant tissues (root, stem, and leaf) observed were more 21 days after treatment of carbofuran and highly pronounced in stem followed by leaf at 28 days after treatment. The average gross weight of the whole plant on 28 days after treatment was 55.7 gm in the untreated control compared with 122.8 gm in the treated plants. As regards soluble amino acids and chlorophyll, an increase of both the parameters in treated plants compared to the control plants was observed from 14 days after application of carbofuran. The difference in chlorophyll content was more on 14 days after treatment 3.55 and 5.40 mg/gm of fresh tissue in control and treated plants, respectively; whereas the difference in total soluble amino acid content was more on 28 days after treatment. The activity of acid phosphatase was more only in the root and leaf in treated plants than in control.

Keywords: Carbofuran, rice plant, biochemical analysis, acid phosphatase

1. Introduction
Pests are major biotic constraints to maximize rice yield. Sustainable management of the crop is needed to control the main pests that limit rice growth and development. It is necessary to use pesticides, including herbicides, fungicides, and insecticides [1]. Carbofuran is a widely used insecticide for the control of a number of major rice-pests. Carbofuran is observed to cause leaf folder resurgence in the field [2, 3]. Hydroponic studies have indicated striking differences in greater root development in rice seedlings, enhanced plant height, more total and productive tiller, promotion of rapid maturation and increased yield between carbofuran treated and untreated plants [4], whereas Mahrub and Pollet [5] were unable to observe any positive action on rice growth in farmers' field. Furthermore, information on the effect of carbofuran application on the biochemical factors of rice plant is scanty. The results of a study on the effects of carbofuran on acid phosphatase activity in tomato plants showed that enzymatic activity increased significantly and was not toxic compared to control. The results also indicated that the growth of tomato plants was significantly higher in all the cases of carbofuran treatment [6]. In another study, the total free amino acid content and the total chlorophyll content decreased in brinjal leaf as an effect of the application of carbofuran [7]. In view of the above, an investigation was undertaken to study the effect of carbofuran application on different biochemical activities of the rice plant.

2. Materials and methods
Effect of carbofuran on different biochemical activities of rice var. TN 1 was studied in a pot experiment in the greenhouse. Furadan 3G @ 1.5 kg a.i./ha was applied 7 days after transplanting (DAT) in standing water in 22 cm-diameter pots containing 9.0 kg of pulverized soil. The experiment was carried out in completely randomized design and replicated 10 times. Plant samples were taken on the 14th, 21st and 28th day after application of carbofuran. Different physical characters were measured and biochemical parameters of the plant tissue were estimated.

2.1 Estimation of acid phosphatase
The method described by Agoreyo [8] was followed for the estimation of acid phosphatase. Plant tissues were sterilized with 1% (v/v) sodium hypochloride solution for 10 min, washed

with water three times and soaked in water for 24 h. The tissues (15 g) were ground in a pre-chilled mortar in 30 ml of 20 mM sodium acetate buffer (pH 5.0) containing NaCl 0.9% (w/v). The homogenate was centrifuged at 6,000 rpm for 30 min. The supernatant filtered through cotton was used as the crude extract and conserved at 4 °C. Acid phosphatase activity was based on the conversion of *p*-NPP into *p*nitrophenol (*p*NP) [9]. The standard acid phosphatase assay was performed in a total volume of 250 ml, containing 100 mM sodium acetate buffer (pH 5.0), substrate *p*NPP (5 mM) and enzyme preparation. The reaction mixture was incubated at 37 °C for 10 min, then 2 ml of Na_2CO_3 (2%, w/v) were added to stop the reaction and absorbance were measured at 410 nm using a spectrophotometer. *p*NP was used as a standard. Under defined as 1 _mol of *p*NP released per min. Specific activity was defined as the units of enzyme activity per mg of protein.

2.2 Estimation of chlorophyll

Samples were collected on the day of analysis and refrigerated until just before analysis. The methodology described by Arnon [10] was followed for the estimation of chlorophyll. The sample was ground in pre chilled mortar and 4-5 ml of 90% alkaline acetone (acetone kept on ice) was added. The sample filter was ground vigorously for approximately 30 seconds while keeping the tube on ice. Contents of grinding tube were dumped into a 15ml graduated centrifuge tube. Pestle and grinding tube were rinsed with 90% acetone into the graduated centrifuge tube and centrifuged for 15 minutes at medium speed. The volume of extract in the centrifuge tube was recorded. The cuvette was filled with extract and absorbance read at 750 (turbidity blank), 665, 663, 645, and 630 nm on a spectrophotometer. Acetone was used as a reference. 25 µl of 2N HCl was added to the extract and mixed thoroughly and reared at the previous wavelengths after 1 minute.

2.3 Estimation of total soluble amino acid

One gram of the leaf was weighed and made into small pieces and plunged immediately in boiling alcohol. Then, it was cooled and ground thoroughly in a mortar with pestle with hot alcohol and filtered through Whatman No. 41 filter paper and made up to ten ml volume with alcohol. Amino acid was estimated by following Moore and Steins modified ninhydrin method [11]. One ml of the alcoholic extract was pipetted in a test tube and a drop of methyl red indicator was added, and neutralized with 0.1 N sodium hydroxide. To each test tube, one ml of ninhydrin reagent was added and mixed thoroughly. The tubes were heated by placing in hot water bath for 20 min. Five ml of the diluent solution was added and mixed thoroughly. The tubes were heated by placing in hot water bath for 20 min. Five ml of the diluents solution was added to the mixture, while it was still in a water bath; one blank was maintained with one ml of distilled water instead of a sample. The tubes were removed and cooled under a running tap water and the contents were mixed thoroughly. After getting stable purple color, the absorbance of the solution was read at 570 nm. The amount of amino acid was calculated using a standard curve prepared from glycine.

3. Result and discussion

Results indicated that all the tissues examined (root, stem, and leaf) recorded higher gross weight on 21 days after application of carbofuran compared to control. The difference in gross weight between the treated and the control plants was highly pronounced in the stem followed by the leaf at 28 days after treatment (Fig. 1). In respect of length of leaves and plant height, the phytotonic effect due to carbofuran treatments was recorded by Skaria [12]. The average gross weight of the whole plant on 28 days after treatment was 55.7 gm in the untreated control compared with 122.8 gm in the treated plants.

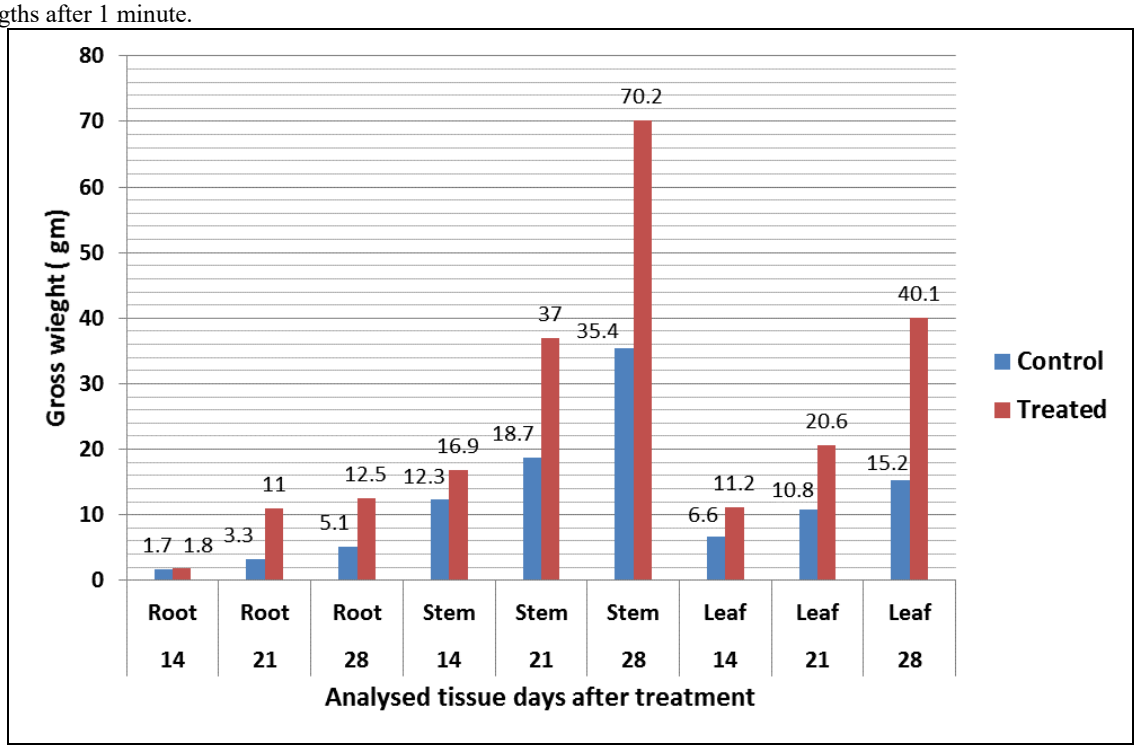

Fig 1: Effect of application of carbofuran on the gross weight of different tissues of rice plant

As regards soluble amino acids and chlorophyll increase of both the parameters in treated plants compared to the control plants was observed from 14 days after application of carbofuran. The difference in chlorophyll content was more on 14 days after treatment 3.55 and 5.40 mg/gm of fresh tissue in control and treated plants, respectively; whereas the difference in total soluble amino acid content was more on 28 days after treatment (Fig. 2 & 3).

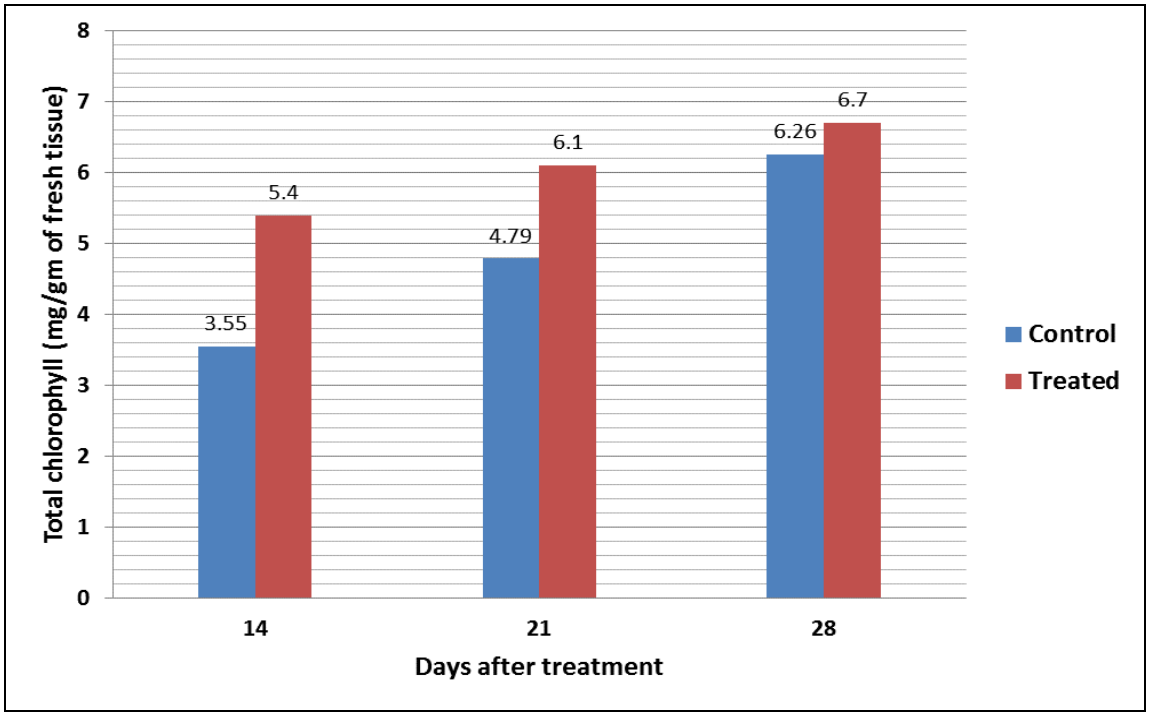

Fig 2: Effect of application of carbofuran on total chlorophyll content of growing rice leaf

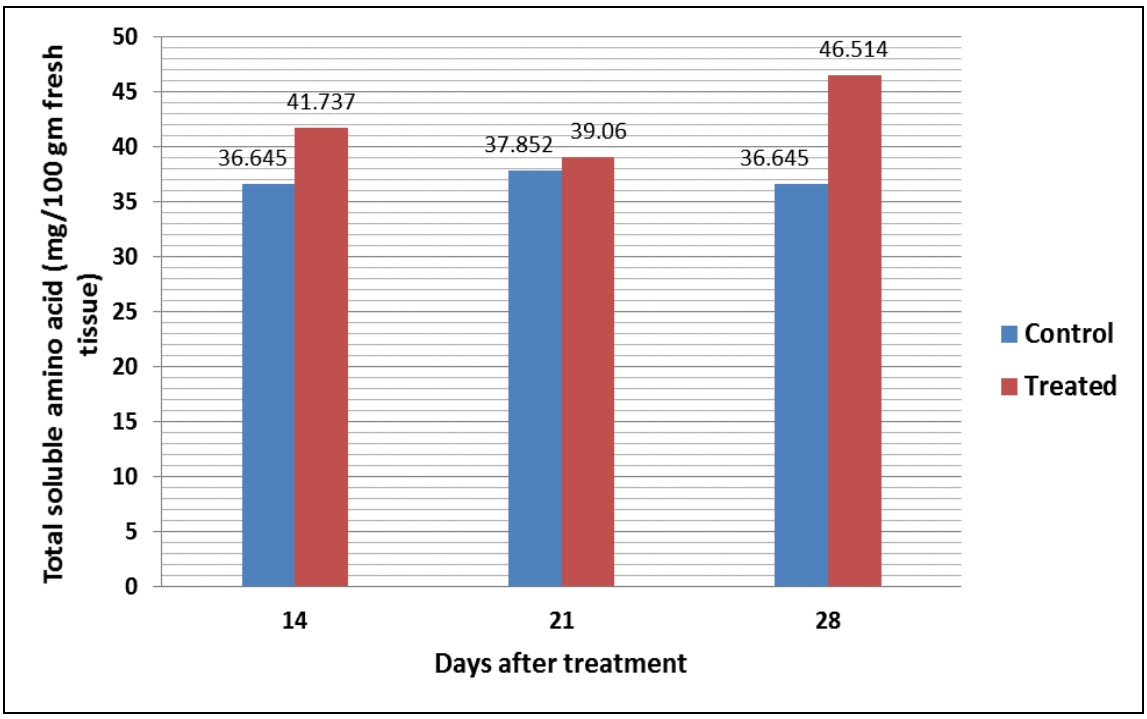

Fig 3: Effect of application of carbofuran on total soluble amino acid content of growing rice leaf

Hydrolysis of phosphate esters is a critical process in the energy metabolism and metabolic regulation of plant cells [13]. It increases P uptake by the plant and crop yield. Acid phosphatases play a major role in the mineralization of organic phosphorous in soil [14]. The beneficial effect of carbofuran treatment on the acid phosphatase helping in the regulation of growth process in treated developing rice plants was observed. The activity of acid phosphatase was more only in the root and leaf in treated plants than in control (Fig. 4). The maximum activity was observed on the 14th day after treatment in the root (0.0535 gm protein/minute) and on the 28th day after treatment in leaf (0.1199 gm protein/minute).

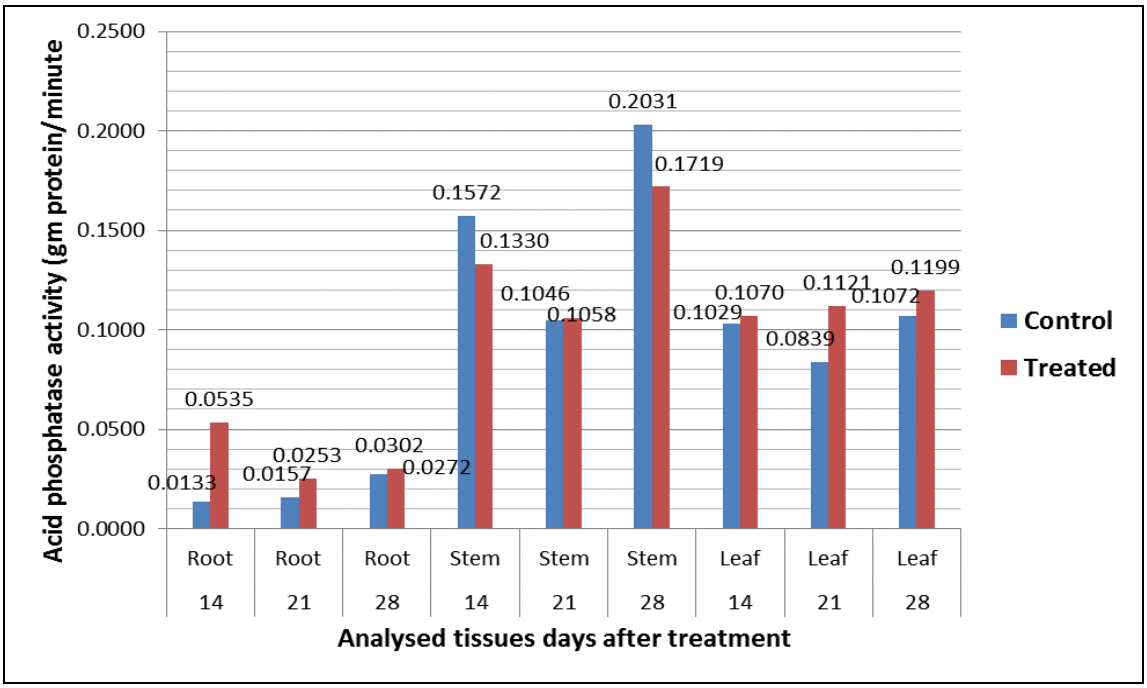

Fig 4: Effect of application of carbofuran on acid phosphatase activity of different tissues

In the present investigation increase in chlorophyll content, soluble amino acids, acid phosphatase activity and overall growth of the plant was observed in carbofuran treated plants which could possibly make them favorable for leaf folder infestation.

4. Conclusion

This study evaluated the effect of application of carbofran on gross weight, total chlorophyll, total soluble amino acids and acid phosphatase content in different tissues (root, stem, and leaf) of the rice plant on different weeks after application of carbofuran. In general, the activity of all the parameters studied increased with the application of carbofuran compared with the control except the level of acid phosphatase which was less only in the stem with respect to control. The level of acid phosphatase was four times more in the roots on 14 day after application (DAA) of carbofuran. The gross weight of root increased more than three times on 21 DAA of carbofuran, whereas, that of stem and leaf increased by about two times compared to the control. The total chlorophyll content increased more than one and half times on 14 DAA of carbofuran which decreased gradually over the weeks. There was an insignificant increase in the total soluble amino acid content in the rice leaves of treated plants compared to the control plants.

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