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SHORT COMMUNICATION

AMELIORATIVE ROLE OF CERTAIN NATURALLY OCCURING PROTECTIVE SUBSTANCES IN ANTAGONISING THE PHYTOTOXIC EFFECTS OF COPPER SULPHATE ON NITRATE REDUCTASE ACTIVITY IN *PISUM SATIVUM* L.

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The present investigation was carried out in the winter season of 2002-2003 to study the nitrate reductase (NR) activity in terms of soluble protein in the leaves of *Pisum sativum* as influenced by different concentrations of $CuSO_4$. Nitrate reductase activity was inhibited with increasing concentration of $CuSO_4$ (0.01, 0.05, 0.1 and 1 mM). Use of plant growth regulators (PGRs), *viz*. IAA, GA_3 and kinetin and certain protective substances, such as EDTA (0.1M), methionine (1.0 mM), sucrose (5 mM), phosphorus (KH_2PO_4 5 mM) and KNO_3 (5 mM) in combination with least inhibitory concentration of copper sulphate (0.01 mM) were found to increase nitrate reductase activity in the leaves of *Pisum sativum* L.

***Key words*: Copper sulphate, nitrate reductase, PGRs, protective substances**

Phytotoxic effects of heavy metal ions have been widely reported (Woolhouse, 1983). The physio-biochemical aspects of metal toxicity, however, have been explored only in a few processes. The relatively strong affinity of heavy metal ions for side chain ligands of protein (Vallee and Ulmer 1972, Hampp *et al*. 1976) indicated that enzyme activities and other functional proteins are one of the primary targets of metal toxicity. Nitrogen metabolism is very important for providing the organic nitrogen essential for plant growth and nitrate reductase (NR) is the key enzyme in this process. Present study was undertaken to evaluate the effect of $CuSO_4$ on NR activity in the leaves of *Pisum sativum* L. with or without PGRs and protective substances.

A field experiment was conducted during winter season of 2002-2003 in the Departmental field of Life Sciences, Tripura University. Uniformly bold and healthy seeds were surface sterilized with 95% ethanol for 2 min, thoroughly washed with distilled water and sown in the field containing alluvial soil. The size of the plot for each treatment was 1m x 1m. Three replicates were taken for each treatment. The plants were raised under natural condition. The plants were watered as per requirement. A total of two Experiments were conducted. In the Experiment-I, solutions of $CuSO_4$ (0.01, 0.05, 0.1 and 1 mM, prepared in distilled water) were sprayed on the foliage of 15-day-old plants followed by assay of *in vivo* NR activity and soluble protein in the leaves on 7^{th} and 14^{th} day after spray by the methods of Hageman and Hucklesby (1971) and Lowry *et al*. (1951) respectively.

In the Experiment-II, leaf *in vivo* NR activity were assayed to evaluate the effects of $CuSO_4$ in combination with PGRs and certain protective substances on 4^{th} day after spray. This experiment was conducted into two sets (viz. set- I & set-II). Set-I consisted of experiments with PGRs, whereas, set-II included experiments with protective substances. Distilled water and the least inhibitory concentration of $CuSO_4$ were taken as T_0 and T_1 respectively in the both sets. Other treatments for set I and set II were as under:

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For set I various treatments were: T_2 (10 µg/ml IAA), T_3 (10 µg/ml IAA + 0.01 mM $CuSO_4$), T_4 (10 µg/ml GA_3), T_5 (10 µg/ml GA_3 + 0.01 mM $CuSO_4$), T_6 (10 µg/ml kinetin), and T_7 (10 µg/ml kinetin + 0.01 mM $CuSO_4$).

For set II various treatments were: T_2 (5 mM sucrose), T_3 (5 mM sucrose + 0.01 mM $CuSO_4$), T_4 (5 mM KH_2PO_4). T_5 (5 mM KH_2PO_4 + 0.01 mM $CuSO_4$), T_6 (5 mM KNO_3), T_7 (5 mM KNO_3 + 0.01 mM $CuSO_4$) T_8 (0.1 M EDTA), T_9 (0.1 M EDTA + 0.01 mM $CuSO_4$), T_{10} (1.0 mM methionine), and T_{11} (1.0 mM methionine + 0.01 mM $CuSO_4$). Three replications were used for each treatment.

The results of experiment I showed that the *in vivo* NR activity in the leaves of sprayed plants decreased with increase in the concentration of copper sulphate (0.01 to 1.0 mM) (Fig. 1) up to 14 days after spray. The 0.01 mM concentration was least inhibitory, whereas, 1 mM was maximum inhibitory. The copper ions showed a concentration depended inhibitory effect on NR activity. The inhibitory effect of Cu^{2+} ions was recouped after 14 days of spray, which indicated that due to its dilution in the plant (*Pisum sativum*) with age, the inhibitory effect is overcome. The inhibitory effect of Cu^{2+} and Hg^{2+} ions on *in vivo* and *in vitro* NR activity in the leaves of *Vigna mungo* were also observed by Siddiqui *et al*. (1982). The inhibitory effect with Hg^{2+} and Cu^{2+} was probably due to

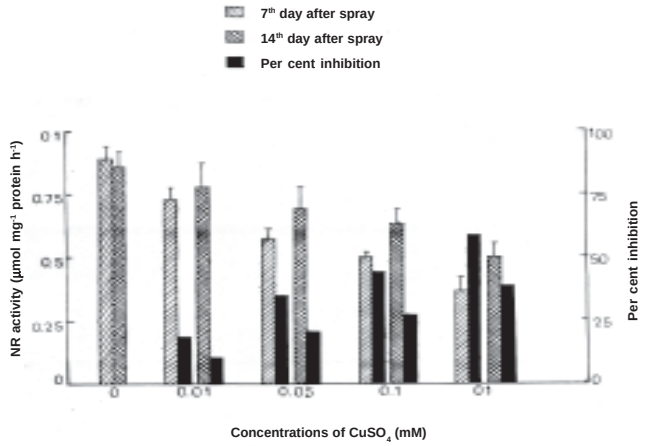

Fig. 1. *In vivo* nitrate reductase (NR) activity in the leaves of *Pisum sativum* sprayed with different concentrations of copper sulphate. CD at 5% level is 0.18 and 0.194 on 7th and 14th days after spray application respectively.

the attachment of these ions to the thiol group (-SH group) of the enzyme because Hg and Cu are known to inactivate –SH group of enzyme (Kanamorie and Matsumoto 1972, Takimoto and Tanaka 1973). In experiment II the NR activity was inhibited by the spray of copper sulphate (Fig. 2). In the treatment set where PGRs were added to copper sulphate solution the NR activity increased with respect to control. Maximum NR activity was noticed with $CuSO_4$ + GA_3 combination. This may be due to action of PGRs on protein synthesis via enhanced synthesis of enzymes of N-metabolism in general and of nitrate reductase in particular. The enhancement in the synthesis of NR protein with GA_3 has been reported in *Vigna mungo* (Srivastava *et al*. 1981). Similar explanation may hold true in the present study also where GA_3 enhanced the NR activity in *Pisum sativum*. The results showed that among protective

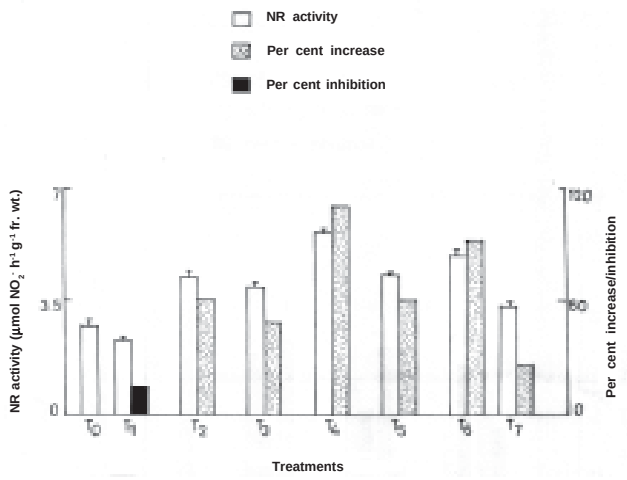

Fig. 2. Effect of foliar spray of copper sulphate in combination with PGR on *in vivo* NR activity on 4th day after spray. T_0- control (water spray), T_1- 0.01 mM $CuSO_4$, T_2 -10 µg/ml IAA, T_3 –10 µg/ml IAA + 0.01 mM $CuSO_4$, T_4 -10 µg/ml GA_3, T_5 -10 µg/ml GA_3 + 0.01 mM $CuSO_4$, T_6 -10µg/ml kinetin, T_7 -10 µg/ml kinetin + 0.01 mM $CuSO_4$. CD at 5% level is 0.336.

substances $CuSO_4$ + methionine combination was most effective followed by $CuSO_4$ + EDTA > $CuSO_4$ + sucrose > $CuSO_4$ + KNO_3 > $CuSO_4$ + KH_2PO_4 (Fig. 3). Copper may decrease NR activity by suppressing the synthesis of enzyme molecules or by inhibiting the activity of existing molecules. Supply of sucrose protected the inhibitory effect of copper sulphate. Sucrose is known to enhance the stability of NR and mobilization of

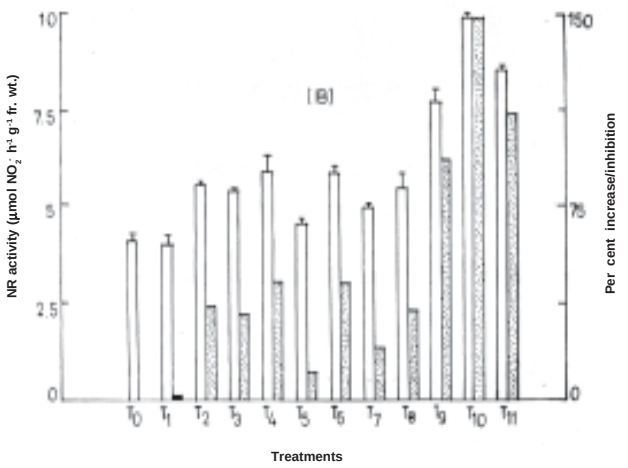

Fig. 3. Effect of foliar spray of copper sulphate in combination with certain protective substances on *in vivo* NR activity on 4th day after spray. T_0- control (water spray), T_1- 0.01 mM $CuSO_4$, T_2 - 5 mM sucrose, T_3 -5 mM sucrose + 0.01 mM $CuSO_4$, T_4 -5 mM KH_2PO_4, T_5 -5 mM KH_2PO_4 + 0.01 mM $CuSO_4$, T_6 - 5 mM KNO_3, T_7 -5 mM KNO_3 + 0.01 mM $CuSO_4$, T_8 - 0.1 M EDTA, T_9 -0.1 M EDTA + 0.01 mM $CuSO_4$, T_{10} - 1.0 mM methionine, T_{11}- 1.0 mM methionine + 0.01 mM $CuSO_4$. CD at 5% P is 0.68.

endogenous nitrate pool (Puranik and Srivastava 1993). Methionine, a sulphur containing amino acid, increased enzyme activity and significantly reversed the inhibitory effect of copper sulphate. Application of EDTA showed favourable effects in reducing the heavy metal toxicity and increasing NR activity. These results are in agreement with the observations of Madhavi and Charyulu (1998), who reported that chelating agents (EDTA, gypsum and serpentine soil) provide protection against lead, cadmium and mercury for the growth and metabolism of *Trigonella foenumgracum*. Verma and Singh (1996) observed that the growth and yield of black-gram grain and straw increased significantly over control due to phosphate and copper interaction.

It is thus suggested that compounds such as EDTA (0.1M), methionine (1.0 mM), sucrose (5 mM) and phosphorus in the form of KH_2PO_4 (5 mM) and KNO_3 (5 mM) along with GA_3 may counteract the toxic effect of copper sulphate in the leaves of *Pisum sativum* L.

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