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EFFECT OF COPPER NITRATE TRIHIDRATE ON GROWTH PARAMETERS OF *VIGNA ACONITIFOLIA* (JACQ.) MARECHAL.

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ABSTRACT Seed germination is important aspect for survival and continuation of generation of any plant species. The present study was carried out to investigate effect of three test concentration of Copper nitrate on seed germination, root length, weight of seeds, total RNA and total Protein of Turkish gram *Vigna aconitifolia* (Jack.) Marechal. The study revealed that seed germination was inhibited 100% when seeds of *Vigna aconitifolia* were exposed to 10mM, 100mM and 1000mM test concentrations.The average highest seed weight 0.34±04 mg was found in seeds exposed to the concentration 10mM followed in control i.e. 0.246±04m, the average highest total RNA content was found 0.18±0.02 mg in seeds exposed to 10mM concentration followed by distilled water i.e. 0.11+0.05 mg. The total protein content of seeds exposed at 100mM test concentration was found highest i.e. 0.107±0.03 followed by control and test concentration 1.0 i.e.0.101+0.07 and 0.08+0.03. The present study revealed that all tested copper nitrate concentrations possess germination inhibiting effect. Presence of Copper nitrate in industrial effluents with these millimolar concentrations may affect agriculture especially Turkish gram farming.

KEYWORDS : Copper nitrate, *Vigna aconitifolia,* Germination, Pollution.

Introduction:

The seed germination is important for the survival and perpetuation of many plant species. Heavy metals, such as Hg, Cd, Pb, Cu, Zn and Ni, are essential micronutrients and plays vital role in plants growth, but found to be toxic to organisms at high concentrations [1]. Seeds are more tolerant to various stresses, but after imbibition and subsequent vegetative developmental processes, they become stress-sensitive in general. Heavy metals like cadmium, copper, lead and mercury had been reported for its adverse effect on pollen germination and tube growth of apricot, *Armanica vulgaris* and cherry *Cerasus avium* [2].

Use of copper nitrate in various industrial processes has increased several folds. As per the National Health Haz Map Copper nitrate is used in "light sensitive reproductive papers, nickel plating baths, pesticides (wood preservatives, fungicides, herbicides, and insecticides), pyrotechnic compositions, aluminium brighteners, solid rocket fuel catalysts, paints/varnishes, drugs, flotation of cinnabar, cancer treatment, electrolysis and electroplating, and electronics; Also used as a ceramic colour, a mordant and oxidant in textile dyeing and printing, a metal colouring reagent (burnishing iron, giving a black "antique" finish to copper, and colouring zinc brown), a nitrating agent for aromatic organosilicon compounds, an organic catalyst, a drilling mud dispersant, and a corrosion inhibitor" [3].

matki or moth bean *Vigna aconitifolia* (Jacq.) Maréchal is one of the minor pulses of India. R. N. Adsule reported "It is widely grown in India and the Far East and the National Academy of Sciences has identified moth bean as a possibly more significant food /protein source for the future (NAS, 1978)" [4]. The plant *Vigna aconitifolia* is considered to be native of India and Pakistan. It is consumed as whole or sprouts by large population in Maharashtra. It has very low agricultural inputs and gives high yield. Because of this its production in Rajasthan has been reported increased from 325.1 thousand tonnes in 2008-09 to 774.7 thousand tonnes in 2010-11 [5].

The objective of study was to discover the effect of three concentration of Copper nitrate aqueous solution on germination and parameters like seed weight, total RNA and Protein contents of *Vigna aconitifolia* germinating seeds.

Materials and Methods

Seed collection: Seeds of *Vigna aconitifolia* (Jacq.) Marechal belong to family *Fabaceae* were collected from local market of Kalyan. Healthy seed of same size, shape and colour were segregated by visual examination and used for experiment.

Preparation of Test concentrations: Test solutions of **Cu** $(NO_3)_2.3H_2O$ of 10mM, 100mM, and 1000mM concentrations **were** prepared in 1 litter sterilized distilled water. Control was prepared by using sterilized distilled water without adding Cu $(NO_3)_2.3H_2O$.

Seed Treatment: Seeds were surface sterilised with 0.1% $HgCl_2$ for 1 min and washed with sterilized distilled water thrice in aseptic condition to avoid fungal infection to seeds. 50 seeds were soaked separately in each test concentration for 6 hrs.

Seed Germination Test

Petri dishes of size 9 cm diameter were lined with filter paper and sterilized by autoclaving at 15 lbs for 20 min. For experiment set petri dishes were aseptically irrigated with 10 ml of copper nitrate and control set was prepared by using sterilized distilled water**.** Ten seeds were placed aseptically keeping distance 1 cm between seeds in each petri dish. The experiment was run with three replicates. Petri dishes ware then incubated in dark for seven days at room temperature. Seeds were analysed every day for germination rate. Seed weight, root length, shoots length, estimation of RNA and total proteins were recorded after 7 days for seven seeds.

Estimation of RNA was determined by Orcinol method A standard graph was drawn by plotting the values as a function of RNA concentration [6].

Estimation of Proteins: were estimated by the method of Lowry et al. (1951) using Bovine serum albumin (BSA) as standard [7].

Results and Discussion:

Result of effect of various test concentrations of copper nitrate (Cu $(NO_3)_2 3H_2O$) against seed germination rate, root length and shoot length of *V. aconitifolia* was shown in table 1. All test concentrations showed toxic effect against seeds of *V. aconitifolia*. Theses concentrations showed 100% seed germination inhibition. The highest seed germination was observed only in control set. All experimental test concentrations showed 100% root and shoot formation while control set showed average root length and shoot length 7.23+46 and 7.8+46 respectively. Results of effects of various test concentrations of copper nitrate on seed weight, of *V. aconitifolia* was shown in graph1. The highest seed weight recorded was 0.34+04 mg in seeds exposed 10mM to test concentration followed in control i.e. 0.246+04mg. Total high RNA content 0.18+0.02 mg was found in seeds exposed to test concentration 10mM followed by distilled water i.e. 0.11+0.05 mg. The protein content of seeds in test concentration 100mM was highest i.e. 0.107+0.03 followed by control and test concentration 1.0 i.e.0.101+0.07 and 0.08+0.03.

At higher concentrations like 100mM and 1000m M of $Cu(NO_3)_2 3H_2O$ 100% inhibition in cell this is may be because of initiation of Programmed Cell Death (PCD) with disruption of protein structure. Hung et.al. 2007 reported copper induced Programmed Cell Death in rice roots [8]. The protein inhibition and disruption is due to copper becomes reactive species and attack on sulfhydryl groups in proteins [9]. The gain in average weight and average total RNA of seeds of *V. aconitifolia* exposed to the lower concentration i.e. 10m M because initially the lower intake of copper in seed act as micronutrients which plays a vital role in several important metabolic pathways in plants and also act as cofactor for several proteins including structural proteins. The gain in weight of seeds may be because of higher uptake of water along with copper. But on longer exposure it becomes toxic to seeds and inhibits the germination of seeds.

Copper (Cu^{+2}) is an essential trace element needed for the normal growth and development of cereal crops. Therefore, optimum copper concentration ensures a normal growth and development of plants [10]. Higher concentration, copper was shown to inhibit plant growth by hampering important cellular processes such as photosynthesis and respiration [11]. Excess amount of copper concentrations produce the oxidative stress by increase in the levels of reactive oxygen species (ROS) within subcellular compartments. Copper induced reactive species are superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (.OH), all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids [12].

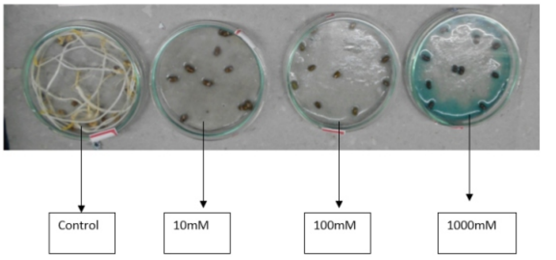
Redox cycling between $Cu\ 2^+$ and Cu^+ can catalyse the production of highly toxic hydroxyl radicals, with subsequent damage to DNA, lipids, proteins and other biomolecules[13]. Thus, at high concentrations, Cu can become extremely toxic causing symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth [14, 15]. At the cellular level, toxicity may result from i) binding to sulfhydryl groups in proteins, thereby inhibiting enzyme activity or protein function; ii) induction of a deficiency of other essential ions; iii) impaired cell transport processes; iv) oxidative [14,16].

Soil pollution by heavy metals is serious environmental problem. Rapid industrialization has accelerated the environmental pollution by many folds. Disposal of industrial solid waste and effluent without proper treatment will increase degree of accumulation of heavy metals in top soil and leeching of metals into the ground water [16,17,18,19]. Heavy metals at high concentrations cause serious threat not only to plant community but associated fauna in that area. The present study concluded that if the Vigna aconitifolia exposed to the 10mM, 100mM or 1000 mM concentrations of Copper nitrate effluent may 100% inhibit seed germination and subsequently survival and perpetuation of *Vigna aconitifolia* species.

Table 1 showing results of effect of various test concentrations of Cu (NO3)2 $3H_2O$ on Seed germination rate, root length and shoot length.

Test Parameter	Control.	10mM	100 mM	1000 mM
Mean of seed germination	8.3+1.5	0	0	0
Mean Shoot length in cm	7.23+4.6	0	0	0
Mean Root Length in cm	7.8+4.6	0	0	0
Mean Weight of seeds in g	0.246+0.04	0.341+0.40	0.077+0.01	0.098+0.01
Mean Concentration of RNA in mg	0.114+0.05	0.185+0.02	0.114+0.08	0.075+0.04
Mean Concentration of protein in mg	0.101+0.07	0.037+0.00	0.107+0.03	0.087+0.03

Plate 1 : Showing results of effect of different concetration of Copper nitrate germination of *Vigna aconitifolia* after seven days .



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