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EFFECT OF *CESTRUM AURANTIACUM* LEAF EXTRACT AND KNO_3 ON SEED HEALTH OF BLACK GRAM

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ABSTRACT

An investigation was carried out to analysed seed health of black gram (Vigna radiata L.) by using Cestrum aurantiacum Lindl. leaf extracts and KNO_3 by some reliable physiological and biochemical parameters. It was found from the experimental results that the Cestrum leaf extracts (1:1 and 1:2) exert inhibitory effect whereas KNO_3 (100 and 500 µg ml^{-1}) exert promotive effect on black gram seeds. The extracts of Cestrum reduced the percentage germination and increased T_{50} values. KNO_3 treated black gram seeds shows promotive effect on dehydrogenase activity and it was concentration dependent. Cestrum leaf extracts shows profuse leaching of amino acids and enhanced soluble carbohydrate contents. It was also found that leaf extract treated black gram seeds contain reduced amount of insoluble carbohydrates and nucleic acids in comparison to control.

KEY WORDS

Cestrum aurantiacum, leaf extract, KNO_3, black gram, seed health.

INTRODUCTION

Seeds are the basic input in agriculture. But, storing of seeds is a serious problem in tropical and subtropical countries where high temperature and high relative humidity greatly accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigour in many states of India is much more acute because of its semiarid climate where high relative humidity prevailing during the major part of a year which is very favourable for the growth of microorganisms, particularly fungi (1, 2, 3).

Keeping in mind the problem of seed storing in our country, an attempt was made in this investigation to get rid of such problems by employing *Cestrum* leaf extracts and KNO_3 at various concentrations on black gram seeds.

The prime objective of this investigation is to probe the efficacy of the seed pretreating agents on seed health of black gram by analysing germination behaviour and metabolic status of seeds.

MATERIALS AND METHODS

Experiments of the present investigation were carried out by using healthy, mature leaves of *Cestrum aurantiacum* Lindl., collected from Darjeeling Government College campus, Darjeeling, West Bengal. Fully viable seeds of black gram (*Vigna radiata* L.) are used as the bioassay material. After proper washing the leaves of *Cestrum* (500g) were thoroughly homogenized by using mortar and pestle by adding distilled water. The homogenate was strained using fine cloth and then centrifuged at 4000*g* for 15 minutes. The supernatant was then made up to 500 ml using distilled water and this were considered as 1:1 (w/v) proportion stock solution of leaf extract. From this stock solution another concentration grade in the proportion of 1:2 (w/v) was prepared using distilled water and thus two

concentration grades of solution were prepared. These two concentration grades of leaf extracts were used for experimental purposes (4, 5).

After surface sterilization (0.1% $HgCl_2$ for 90 seconds) the seed samples of black gram are separately presoaked in *Cestrum* leaf extracts (1:1 and 1:2 w/v) and KNO_3 (100 and 500 µg ml^{-1}), or distilled water for 6 hours (h) and then dried back to the original dry weight of the seeds.

From the treated seed samples germination behaviour (percentage and T_{50} values (h) of seed germination), leaching of free amino acids, soluble carbohydrates, insoluble carbohydrates, nucleic acid contents and dehydrogenase activity were recorded.

To analyse the percentage germination, four groups of 100 seeds i.e. 400 seeds of each treatment were transferred to separate Petri dishes containing filter paper moistened with distilled water. Germination data were recorded after 48 hours of seed soaking following the International Rules for Seed Testing (6). The time required for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al.* (7). For recording TTC (2, 3, 5-triphenyl tetrazolium chloride) stainability, dehusked seeds of each treatment were allowed to imbibe 0.5% (w/v) TTC solution in Petri dishes and kept overnight in dark. Percentage of TTC stained seeds was calculated from the total number of seeds of each treatment (8). Extraction and estimation of the enzyme dehydrogenase was done as per the method of Rudrapal and Basu (9).

Leaching of free amino acids and soluble sugars from seeds was analysed after immersing seeds in distilled water for 24 hours. Insoluble carbohydrates as well as DNA and RNA contents were analysed from the treated seed samples. Quantification of soluble and insoluble carbohydrates was done following the method of McCready *et al.* (10) and amino acid according to Moore and Stein (11). Extraction and estimation of nucleic acids (DNA and RNA) contents was done following the methods of Cherry (12) modified by Choudhuri and Chatterjee (13). Statistical analysis of the data was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits and as per the method of Panse and Sukhatme (14).

RESULTS AND DISCUSSION

The effect of different concentrations of aqueous extracts from leaves of *Cestrum* plant was found inhibitory to various parameter *viz.* seed germination behaviour and metabolism of black gram seeds. Data clearly revealed that seed pretreated with KNO_3 (500 µg ml^{-1}) significantly alleviated the seed germination and metabolism. It is found that *Cestrum* leaf extracts 1:2 shows more inhibitory over control where as KNO_3 treated (500 µg ml^{-1}) seeds show more promoting effect (Table 1). Sarihan *et al*, 2005 (15), Bian *et al*, 2013(16) reported that KNO_3 treatments promote germination percentage of *Plantago lanceolata* and *Sorbus pohuashanensis* respectively. D'Abrosca *et al,* reported that *Cestrum parqui* contained some phytotoxic terpenoids that can inhibit seed germination (17).

Soluble carbohydrate levels increase in leaf extracts (1:2) than the other treatments. It is also found that insoluble carbohydrate contents remain high in case of KNO_3 (500 µg ml^{-1}) treated seeds (Table 2).

Seeds treated with KNO_3 (500 µg ml-1) shows higher amount of DNA and RNA contents than other treatments (Table 2). The leaching of free amino acids content is higher in *Cestrum* leaf extracts than KNO_3 (100 and 500 µg ml^{-1}) treated seeds. The activity of enzyme dehydrogenase shows the reverse trend of result than leaching of free amino acids (Table1 and Table 2).

The results therefore point out that although deterioration is a common phenomenon in treated and control samples of the seed species; the catabolic processes within the treated seed samples remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environment. Available reports show that during seed ageing a loss of some vital cellular components including protein, carbohydrates, nucleic acids etc. occurred. Thus, our result corroborates the findings of previous reports (18,19).

From our experiment it is evident that *Cestrum* leaf extracts showed inhibitory effects whereas KNO_3 exerted promotive effect on black gram seeds. Thus, a conclusion can be drawn from this investigation that *Cestrum* leaf extracts treatment is detrimental for black gram seed health but KNO_3 treatment is good for the black gram seed health.

TABLE 1: Effect of seed pre-treatments with *Cestrum* leaf extracts (1:1 and 1:2) and KNO_3 (100 and 500 µg ml^{-1}) on percentage (%) germination, T_{50} values (h) and dehydrogenase enzyme contents (unit $min^{-1}g^{-1}$fr. wt.) of black gram seeds.

Treatments	Percentage (%) of germination	T_{50} (h)	Dehydrogenase activity
Control	91.50	26.22	75.43
Cestrum leaf extract (1:1)	76.32	31.44	66.20
Cestrum leaf extract (1:2)	68.13	35.22	63.50
KNO_3 (100 µg ml^{-1})	93.50	25.66	78.37
KNO3 (500 µg ml^{-1})	95.00	25.26	81.43
LSD (P=0.05)	**1.85**	**0.17**	**3.22**

TABLE 2: Effect of seed pretreatments with *Cestrum* leaf extracts (1:1 and 1:2) and KNO_3 (100 and 500 µg ml^{-1}) on soluble carbohydrates (SC), insoluble carbohydrates (IC), nucleic acid (DNA and RNA) contents and leaching amino acids (LAA) of black gram seeds.

Treatments	SC (µg ml^{-1})	IC (µg ml^{-1})	DNA (µg g^{-1} fr. wt.)	RNA (µg g^{-1} fr. wt.)	LAA (µg ml^{-1})
Control	81.10	89.24	55.12	94.12	45.45
Cestrum leaf extract (1:1)	85.21	80.25	48.60	88.50	48.23
Cestrum leaf extract (1:2)	88.11	83.34	52.47	92.92	52.56
KNO_3 (100 µg ml^{-1})	75.23	91.23	67.55	96.36	38.32
KNO_3 (500 µg ml^{-1})	72.10	95.24	72.32	98.89	36.34
LSD (P=0.05)	**1.03**	**1.45**	**1.25**	**1.54**	**1.28**

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