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Effect of calixin on growth and some enzyme activities of pea, mungbeam and chickpea

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The systemic fungicide calixin, a formulated product of the fungicide tridemorph (4-tridecyl 2, 4-dimethyl morpholine) was found to reduce overall growth of three leguminous plants, viz. *Pisum sativum* L., *Cicer arietinum* L. and *Vigna mungo* (L.) Heper at relatively high concentrations (50 and 100 mg kg^{-1} soil) in pot culture. Both dry weight and plant height were similarly affected. The root morphology was also changed at the concentrations of 50 and 100 mg. kg^{-1} soil. The fungicide also partially inhibited the dehydrogenase and protease activities of mungbean seedlings raised from seed presoaked in aqueous calixin solutions, though amylase activity remained unaffected. Fungicide did not have any effect on the germinability of mungbean seeds.

Key words : Calixin, *Pisum sativum.*, *Vigna mungo.*, *Cicer arietinum.*, protease, dehydrogenase, amylase

INTRODUCTION

Morpholine fungicides, particularly tridemorph have been reported to show toxic effects in some plants, like membrane-damage (Buchenauer, 1975), chlorosis (Siddiqui and Haahr, 1971) growth (Schmitt *et al.*, 1982; Hosokawa *et al.*, 1984; Bergmann *et al.*, 1982), inhibition of mitosis (Cortes *et al.*, 1982), and fresh weight (Fisher and Hayes, 1981), etc.

The present communication reports the results of an investigation on the effect of calixin on the growth of three legume plants viz. pea, mungbean, chickpea grown in pots and on germination and some enzyme activities of mungbean seedlings raised from seeds pretreated with calixin.

MATERIALS AND METHODS

Soil Sample

Soil was collected in unused polythene bags from the experimental garden of the Botany Department, Burdwan University. It was air dried in the shade, ground and sieved through a 100 mesh net.

Cultivation and harvesting of legumes

Healthy seeds of locally available varieties were chosen, cleaned by washing and soaked in distilled water for 4 h prior to surface sterilization (0.1% $HgCl_2$ solution, 5 min). The seeds were then thoroughly washed with sterilized water and sown in 15 cm × 10 cm clay pots (10 seeds/pot) containing 700.0 g of soil (pH 7.7, moisture content 12%). The potting soil consisted of 2 parts of clay-loam, 1 part of farm-yard compost and 1 part of sand. It was throughly mixed with calixin (0 to 100 mg. kg^{-1} on dry weight basis). After growth of the seedlings for 2 weeks, they were thinned to 6 plant per pot. There were 3 replicate pots for each treatment and control (without calixin).

Plants were harvested after 45 days by inverting the pots and carefully separating the roots. They were gently washed in tap water to remove adhering soil particles and the dry weight of plants (105°C, 24 h) and total length of fresh plants were measured.

Enzyme assays

Seeds were allowed to imbibe in aqueous calixin solution (0 to 100 mg. l^{-1} for 4 h and 10 seeds were placed on moist cotton-wool pads in 10 cm sterile petridishes for germination at 30°C. After 2, 4 and 6 days, 5 healthy seedlings from each treatment were removed for enzyme assay. For determining the dehydrogenase activity by the triphenyl tetrazolium chloride (TTC) method (Assare-Bomah and Fletcher, 1983), the seedlings were dipped in 0.1% aq. TTC solution for 3 h in dark, the root and hypocotyl portions were cut into pieces and an aliquot of 100 mg was extracted with two change of methanol. The extracts were pooled and optical density was measured at 486 nm in Spectronic 20 (Bausch & Lomb).

Protease activity was measured in the cell-free extracts (0.1 M phosphate buffer, pH 6.8) of the seedlings following the method of Snell and Snell (1971). The extract containing 100 mg protein ml^{-1} was used as enzyme source and Bovine Serum albumin (BSA, Fraction V, Sigma) was used as substrate for protease. The reaction mixture contained seedling extract 1.0 ml, BSA solution 1.0 ml (equivalent to 100 mg protein) and 0.1 M $MgSO_4$, $7H_2O$, 0.1 ml. The residual protein content of the solution was determined with coomasie brilliant blue reagent (Bradford, 1976).

Amylase activity in the seedling extracts was measured by the method of Khan and Faust (1967) using starch as substrate.

RESULTS

Effect on plant growth

Calixin showed adverse effects on growth of all the three plant species as indicated by decrease of dry weight and plant height (Table 1). The effects were particularly noticeable at 50 and 100 mg kg^{-1} concentrations. At 50 mg kg^{-1} concentration, calixin caused reduction in dry weights at 30.6%, 42.9% and 43.8% in pea, mungbean and chickpea respectively. Reduction in plant height at the same concentration was 40.4%, 47.8% and 48.7% respectively. At 100 mg. kg^{-1} the decrease was 61.2%, 57.2%, 56.3% and 58.6%, 64.4%, 65.7% in dry weight and height of the plants of pea, mungbean and chickpea respectively.

Effect on germinability and seedling weight

Germinability of mungbean seeds was not influenced by presoaking for 4 h in different concentration of calixin. But fresh weights of seedlings raised from such seeds decreased with the fungicide concentration. However, the protein contents of the same seedlings increased more or less proportionately to the decrease of fresh weight (Fig. 1).

Effects on plant enzymes

The protease activity of the mungbean seedlings raised from treated seeds was found to decrease with increasing fungicide concentration (Fig. 2). Protease activity of the seedlings extract was also inhibited when the fungicide was added to the reaction mixture of protese assay using an extract of seedlings raised from untreated seeds (Fig. 3).

Dehydrogenase activity of the intact 2, 4 and 6 days old seedlings from pretreated seeds was found to decline considerably in comparison to the control (Fig. 4). The myalase activity in the extracts of the seedlings, however, remain unaffacted.

Table 1. Effect of calixin added to soil populated with *Rhizobium* species on the dry weight and plant height of *Pisum sativum*, *Vigna mungo* and *Cicer arietinum* in pot culture

Plant species	Growth parameter	Concentration of Calixin (mg. kg^{-1})			
		0	10	50	100
P. sativum	Dry weight of whole plant (g) (Mean ± S.E.)	0.36 ±0.22	0.31 ±0.15	0.25 ±0.05	0.14 ±0.09
	Dry weight of root protion (g) (Mean ± S.E.)	0.14 ±0.13	0.14 ±0.11	0.07 ±0.03	0.06 ±0.04
	Average height of plants (cm) (Mean ± S.E.)	36.9 ±2.4	31.1 ±1.4	22.0 ±2.2	15.3 ±1.9
V. mungo	Dry weight of whole plant (g) (Mean ± S.E.)	0.07 ±0.04	0.06 ±0.03	0.04 ±0.03	0.03 ±0.01
	Dry weight of root portion (g) (Mean ± S. E.)	0.012 ±0.008	0.009 ±0.0007	0.009 ±0.006	0.007 ±0.006
	Average height of plants (cm)	24.7 ±1.1	15.0 ±0.4	12.9 ±0.5	8.8 ±0.4
C. arietinum	Dry weight of whole plant (g) (Mean ± S. E.)	0.16 ±0.03	0.14 ±0.03	0.09 ±0.04	0.07 ±0.05
	Dry weight of root portion (g) (Mean ± S.E.)	0.05 ±0.03	0.06 ±0.02	0.04 ±0.02	0.03 ±0.02
	Average height of plants (cm) (Mean ± S.E.)	41.3 ±1.5	30.2 ±1.7	21.2 ±0.02	14.2 ±1.1

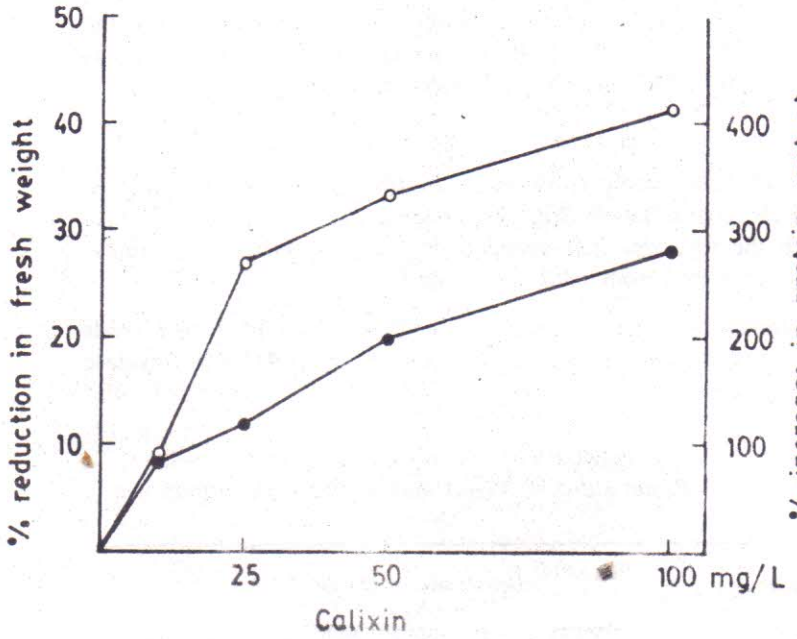

Fig. 1. Fresh weight and residual protein of 6 days-old seedlings of mungbean grown from calixin treated seeds

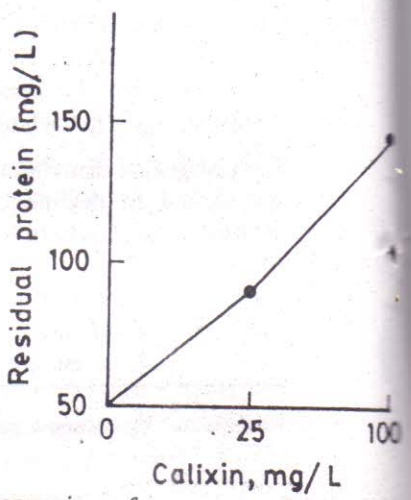

Fig. 2. Depression of protease activity in 2, 4 and 6 days. Old mungbean seedlings due to calixin

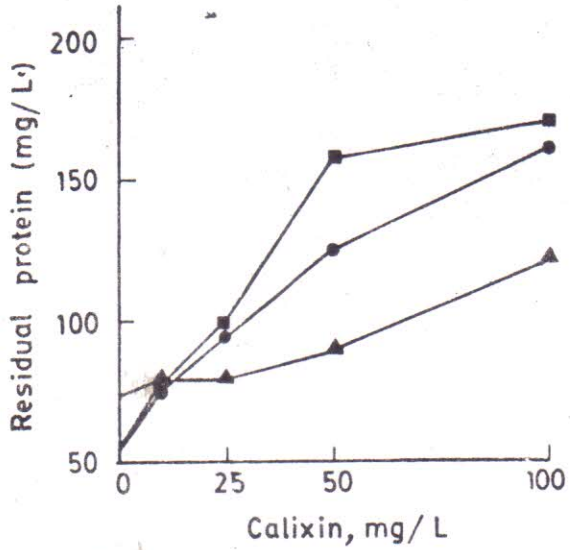

Fig. 3. Effect of calixin concentration on protease activity in assay mixture

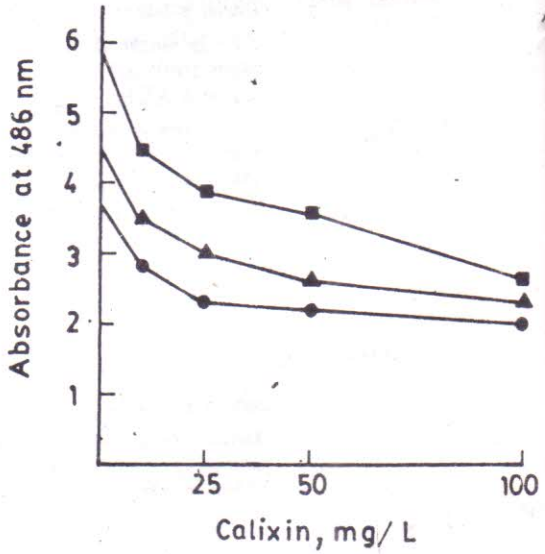

Fig. 4. Dehydrogenase activity of mung seedlings in 2, 4, and 6 days as influenced calixin in treatment of seeds

Age of plants during observation : 45 days.

Data based on the arithmetic mean of 18 plants for each treatment.

DISCUSSION

Systemic fungicide tridemorph was developed by Kradel *et al.* (1968) and was found to be a potent inhibitor of sterol biosynthesis. The fungicide exhibited some phytotoxic effects (Buchenauer, 1975; Siddiqui and Haahr, 1971; Schmitt *et al.*, 1981, 1982; Hosokawa *et al.*, 1984; Bergmann *et al.*, 1982; Cortes *et al.*, 1982). Bladocha and Benveniste (1983) observed that the height of maize seedlings treated with tridemorph decreased with increase of concentration of fungicide (1, 5 and 20 mg l^{-1}). It was observed that tridemorph significantly reduced yield of pods and haulms in ground nut particularly at low phosphate level (Salako, 1990). The present observation showed that calixin affected growth of leguminous plants, although the concentrations used (10, 50 and 100 mg. kg^{-1}) were higher than the recommended dose for field applications. The roots of the plants were also affected adversely. Formation of side branches of the tap root was inhibited and the roots become thicker. Also root nodulation after inoculation with proper rhizobia was strongly inhibited even at a concentration of 10 mg kg^{-1} (Kalam and Banerjee, unpublished). Tridemorph was found to have no effect on germination of mungbean seeds. Also it had no effect on the amylase activity indicating possibly a positive correlation. Seedling fresh weight was slightly affected, particularly in the 6 days old seedlings. At the same time, the soluble protein of the seedlings increased proportionately with the fungicide concentration, indicating possibly a lack of protein mobilization in the seedlings (Fig. 1) This was confirmed by the data on protease activity which was inhibited more or less proportionately. The dehydrogenase activity of the seedlings was similarly found to be inhibited. It was found earlier that tridemorph strongly inhibited dehydrogenase activities also of susceptible bacteria (Kalam and Banerjee, 1995).

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