# CARBARYL TOXICITY IN GERMINATING SEEDS OF *VIGNA SINENSIS*: EFFECT OF GIBBERELLIC ACID SUPPLEMENTATION

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## ABSTRACT

The toxic effects of carbaryl (a carbamate insecticide) on the activities of different hydrolases of germinating *Vigna sinensis* seeds have been investigated. The activities of amylase and phytase from cotyledons decreased on exposure to carbaryl. But the ATPase activity extracted from seedlings was stimulated following pesticide application. Gibberellic acid, on simultaneous application with toxic doses of carbaryl, overcame the growth inhibition caused by low concentration of carbaryl. But at higher concentrations of pesticide treatment, it can only partially reverse the deleterious effects of carbaryl on growth and the levels of different hydrolases.

## INTRODUCTION

CARBARYL (l-napthyl methyl carbamate), a widely used carbamate insecticide, can inhibit plant growth and cellular respiration[1]. It also affects the phenolics and sugars in brinjal plants[2]. Our earlier studies show that carbaryl at toxic concentrations caused poor mobilization of protein and faster depletion of nucleic acids in germinating *Vigna sinensis* seedlings[3]. The activities of hydrolases of germinating seeds were affected by organochlorine and organophosphorus insecticide[4–6]. The present communication deals with the toxic effects of carbaryl on some hydrolases of germinating *V. sinensis* seeds (3-day-old). It was also investigated whether gibberellic acid ($GA_3$), a plant growth hormone, could play any role in removing the toxic influences of carbaryl on the growth and the activities of the hydrolytic enzymes.

## MATERIALS AND METHODS

Tris-ATP, sodium phytate, bovine serum albumin and $GA_3$ were purchased from Sigma Chemicals, USA. Carbaryl (99.9%) were obtained as gift from Union Carbide, India. All other chemicals were of analytical grade. *V. sinensis* seeds were purchased locally.

After surface sterilization with 0.1% mercuric chloride *V. sinensis* seeds were imbibed in water for 4 h. The seeds were then allowed to germinate in dark at 28°C in several plates for 3 days. Experimental plates contained carbaryl alone (at concentrations 75, 100 and 150 ppm respectively) and in combination with $GA_3$ ($10^{-6}$ M).

After three days of germination the seedling height, fresh weight and specific activities of different hydrolases were measured. The total amylase was extracted by homogenizing the cotyledons in unbuffered cold distilled water. The homogenate was centrifuged in the cold at 2000 *g* for 15 min and the supernatant dialysed against cold water. Total amylase activity was assayed from dialysed supernatant according to the method of Bernfeld[7] as modified by Dure[8]. ATPase was extracted from seedling by grinding in a medium consisting of 0.25 M sucrose, 0.003 M EDTA (pH 7.5). The homogenate was then centrifuged in the cold and from the supernatant enzyme activity was assayed in a reaction mixture (1 ml) containing 20 mM Tris-Cl (pH 7.5), 3 mM ATP and enzyme. $P_i$ released was determined by the method of Lowry and Lopez[9]. Phytase activity was measured from the cotyledons of the 3-day-old *V. sinensis* seeds. Cotyledons were homogenized with 10 mM Tris-Cl buffer (pH 7.0) and centrifuged at 5000 *g* for 15 min. The supernatant was assayed for phytase activity[10]. Protein was estimated[11] using bovine serum albumin as standard.

## RESULTS AND DISCUSSION

It was observed that both the elongation and the fresh weight of *V. sinensis* seedling (3-day-old) were inhibited on exposure to different concentrations of carbaryl (table 1). $GA_3$ when applied in combination with carbaryl, was able to overcome the growth inhibition completely at 75 ppm of carbaryl treatment. But above this concentration of pesticide, the plant hormone was only partially successful in reversing the toxic effects of carbaryl on seedling growth. It has been reported by Chakraborti *et al*[12] that different plant hormones

**Table 1** *Effect of different concentrations of carbaryl alone and in combination with $GA_3$ on the growth and the specific activities of different hydrolases of germinating Vigna sinensis seeds (3-day-old)*

| Condition | Seedling height (cm) | Fresh weight of seedling (mg) | Total amylase ($\mu$g maltose / $\mu$g protein/h) | ATPase ($\mu$mol/mg protein/h) | Phytase |
|---|---|---|---|---|---|
| Control | 7.22 ± 0.43 | 730 ± 35 | 5 13 ± 0.27 | 2 79 ± 0.14 | 0.223 ± 0 011 |
| + 75 ppm carbaryl | 5.04 ± 0.36* | 610 ± 32*** | 3.31 ± 0 18* | 3.97 ± 0.18* | 0.220 ± 0.010 |
| + 100 ppm carbaryl | 3.83 ± 0.28* | 445 ± 24* | 2.21 ± 0.14* | 4.82 ± 0.28* | 0.218 ± 0.013 |
| + 150 ppm carbaryl | 3.32 ± 0.22* | 426 ± 27* | 1.95 ± 0.11* | 6.46 ± 0.34* | 0.194 ± 0.010*** |
| + 75 ppm carbaryl + $10^{-6}$ M $GA_3$ | 8.07 ± 0.41** | 727 ± 25 | 4.12 ± 0.20* | 3.38 ± 0.17*** | 0.219 ± 0.016 |
| + 100 ppm carbaryl + $10^{-6}$ M $GA_3$ | 4.97 ± 0 29* | 489 ± 28* | 2 85 ± 0.16* | 4.52 ± 0.32* | 0.216 ± 0 015 |
| + 150 ppm carbaryl + $10^{-6}$ M $GA_3$ | 3.82 ± 0.38* | 458 ± 20* | 2.43 ± 0.11* | 5.84 ± 0.39* | 0.192 ± 0.011*** |

Values are mean ± S. D. of four sets of experiments; *denotes the level of significance $P < 0.001$; **$P < 0.05$; ***$P < 0.01$.

can diminish to some extent the inhibitory effects of malathion (an organophosphorus pesticide) on seedling growth of germinating *V. sinensis*. They observed that at low concentration of malathion (100 ppm) treatment, plant horomones effectively overcome the toxic effect of pesticide on the growth. But at higher concentrations of the pesticide, the plant hormones are partly successful in reversing the malathion-induced growth inhibition. Table 1 shows that the activity of total amylase was significantly inhibited on exposure to toxic doses of carbaryl indicating that the breakdown of starch to readily utilizable sugar which is essential for the growth of seedling during germination, is greatly impaired during carbaryl toxicity. Pronounced inhibition of amylase activity was recorded by Dalvi *et al*[13] when wheat seeds were germinated in the presence of menazon and disulfoton, the two organophosphorus pesticides. They suggested that these pesticides probably inhibit germination by impairing degradation of carbohydrate reserve during germination. The ATPase activity was found to be stimulated in seedlings of 3-day-old germinating *V. sinensis* seeds in the presence of growth-inhibitory concentrations of carbaryl in a dose-dependent manner. The induction of ATPase activity in roots of germinating seeds by malathion was reported earlier[6,14], The activity of phytase from cotyledons was significantly inhibited (13%) only at 150 ppm of carbaryl treatment. Phytase by acting on phytin provides inorganic phosphorus for seedling growth during germination. The present study indicates that the availability of inorganic phosphorus was significantly reduced in *V. sinensis* seeds at higher concentration (150 ppm) of carbaryl treatment. Inhibition of phytase activity in squash cotyledons by anilide herbicide propachlor was reported by Penner[15]. It is also observed that $GA_3$ at $10^{-6}$ M can partially counteract the effects of carbaryl on the enzyme systems studied. The toxic effects of carbaryl on the activities of amylase and ATPase were reversed to certain extent when $GA_3$ was supplemented with the pesticide. However, $GA_3$ at the applied concentration, failed to counteract the pesticide-induced inhibition of phytase activity. Reports are available that plant hormones can partly or completely counteract the inhibitory effects of pesticides on the levels of different hydrolases[12,16]. It was also observed that $GA_3$ was able to overcome the growth-inhibition caused by 75 ppm carbaryl treatment. But it failed to restore the normal activities of amylase and ATPase in germinating *V. sinensis* seeds at that concentration of pesticide. $GA_3$ probably reverses this growth inhibition at 75 ppm by stimulating some other biochemical processes leading to increased cellular growth.

From the foregoing discussion it is apparent that carbaryl at toxic concentration interacted with the normal metabolism of the germinating seed and the effect could somewhat be nullified by using $GA_3$ along with the pesticide.

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# NEWS

## NOBEL PRIZE VALUE RAISED

The Nobel Foundation, Stockholm has announced (March 29, 1988) an increase in the value of the Nobel Prize to 2.5 million crowns ($420,000) (15% increase over 1987). The Foundation is guardian of the Nobel honours awarded in the fields of Physics, Chemistry, Medicine, Literature, Peace and Economics. It was established in 1896.

## PENSION TO MRS. JANAKI RAMANUJAN

The Trinity College of Cambridge University has announced an annual pension of £2000 to Mrs. Janaki Ramanujan, wife of the great mathematician Srinivasa Ramanujan, FRS, who was working at the Trinity College from 1914 to 1919 with Prof. G. H. Hardy.

## SRI OMPRAKASH BHASIN FOUNDATION AWARD

Dr Amulyakumar N. Reddy, Chairman of Management Studies, Indian Institute of Science, Bangalore has been awarded the prestigious Sri Omprakash Bhasin Foundation Award for Science and Technology for the year 1987. The award carries a cash prize of Rs. 50,000/-. Prof. A. K. N. Reddy is a member of the Editorial Board for *Current Science*.