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ACCUMULATION OF FREE PROLINE IN VEGETABLE SEEDS SUBJECTED TO A PESTICIDE SHOCK

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Methylparathion and Phosphamidon insecticides induce proline accumulation in the germinating seeds of bean, okra, guar and onion. However, the time factor after the treatment matters much. Among all the four vegetable seeds, bean, guar and onion have shown more proline accumulation as compared to okra.

Keywords : Bean; Guar; Methylparathion; Okra; Onion; Phosphamidon; Proline.

Introduction

Most of the plants respond to changing osmotic potentials in their external environment by osmotic adjustments of their cellular contents [1,2]. Both inorganic ions and organic compounds are utilized for this purpose. Proline is one of the organic compounds that has been reported to accumulate in plants subjected to water stress[3, 4], salinity[5-7], nutrient deficiencies[8], water logging[9], fungal infection[10] and air pollutants[11]. Though, an osmoregulatory role for Proline at the cellular level has been suggested[12], the significance of its accumulation has not been unequivocally established[5]. Since number of stress factors known to induce proline accumulation, very little[13] or no information is available on the induction of proline accumulation by pesticidal stress. This has prompted us to study the effect of pesticidal stress on proline accumulation by employing widely used organophosphorus pesticides *viz.* Methylparathion and Phosphamidon as a seed treatment. As such the seeds when sown are exposed to the pesticides residue in the soil[13].

Materials and Methods

Seeds of kidney bean (*Phaseolus vulgaris* L.), okra (*Abelmoschus esculentus* Moench L.) Guar (*Cyamopsis tetragonolobus* L.) and onion (*Allium cepa* L.) were soaked separately for 1 hr in 0.03% Methylparathion and 0.015% Phosphamidon. After 1 hr soaking, surface washed seeds were allowed to germinate on moist filter paper (soaked in distilled water) in petriplates at room temperature (30 ± 1.5 °C). The other sets of seeds were directly allowed to germinate for 24 and 72 h in respective concentrations of Methylparathion and Phosphamidon. Proline contents were estimated after 24 and 72 hr duration of germination by the method of Bates *et al.*[14].

Results and Discussion

Proline is one of the cyclic amino acids, normally accumulates in higher plants in response to various environmental stresses[15]. The accumulation of proline in plants is linked with water relations, nitrogen and energy metabolism [16]. An accumulation of proline in a wide variety of plant species under varied kinds of stresses and it's possible role in adaptive mechanism have been reviewed[3]. The accumulation of proline in the cytoplasm is accompanied by a reduction in the concentration of less compactable solutes and an increase in cytosolic water volume [17]. In many plants proline is widely distributed as osmolyte which does not interfere with normal biochemical reactions and acts as osmoprotectant under the drought and saline conditions [18]. The proline can be considered as a storage compound supplying reductants, reduced nitrogen and carbon skeleton for post stress recovery [19].

The proline content in the germinating seeds of bean, okra, guar and onion under pesticidal stress is depicted in results (Table 1 and 2). The proline level of the control both in pre-treatment and continuous treatment at 24 h and 72 h germination did not change much. However, it varied with genus to genus. This level was found increased over control, by 42.8, 8.30, 84, and 92%, respectively in bean, okra, guar and onion in the first 24 h of germination after 1h pre-treatment with Methylparathion (Table 1). However, the situation of proline levels was entirely different after 72 h of germination where no or little proline accumulation was observed in Methylparathion pre-treated seeds (Table 1). The induction of proline accumulation due to Phosphamidon pre-treatment was 128.6, 16.6, 12 and 220% over control, respectively in bean, okra, guar and

Table 1. Effect of Methylparathion and Phosphamidon on the proline content* in the germinating seeds of bean, okra, guar and onion with response to 1 h pre-treatment.

Treatments	Proline content ($\mu g\ g^{-1}$ fresh seedlings)							
	Bean		Okra		Guar		Onion	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Control	105	165	60	220	125	500	50	135
Methylparathion % (v/v) 0.03	150	175	65	175	230	470	100	135
Phosphamidon % (v/v) 0.015	240	140	70	280	140	475	160	170

*Values are mean of three determinations.

Table 2. Effect of Methylparathion and Phosphamidon on the proline content* in the germinating seeds of bean, okra, guar and onion with response to continuous treatment.

Treatments	Proline content ($\mu g\ g^{-1}$ fresh seedlings)							
	Bean		Okra		Guar		Onion	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Control	80	380	100	235	135	430	40	215
Methylparathion % (v/v) 0.03	133	470	65	240	210	370	92.5	195
Phosphamidon % (v/v) 0.015	180	460	90	205	155	405	90	185

*Values are mean of three determinations.

onion after 24 h of germination (Table 1), while the seeds of bean and guar after72h of germination failed to accumulate proline whereas okra and onion did accumulate it (Table 1). The seeds grown continuously in respective concentrations of Methylparathion and Phosphamidon have also shown proline accumulation (Table 2). This level of accumulation over control after 24 h germination in Methylparathion was 66.22, 55.55 and 131.25 % in bean, guar and onion, respectively and after 72 h germination it was 23.68 and 2.12 % in bean and okra, respectively (Table 2). The seeds those received continuous treatment of Phosphamidon have also showed proline accumulation by 125, 14.81, and 125 % in bean, guar and onion respectively, over control after 24 h germination, while it was 21.05 % in bean after 72 h of germination (Table 2).

The result in general suggest that both the organophosphorus insecticides induce proline accumulation in the germinating seeds of all plants studied however, the time factor after the treatment matters much. Among all the four vegetable seeds, bean and onion have shown more proline accumulation and the least by okra. The less induction of proline accumulation after 72 h germination in 1 h pre-treatment with both the pesticides could be due to nullification of the pesticidal stress with the advancement of germination. However, in continuous treatment of 72 h except bean all others have shown less induction of proline which could be accounted for inhibition of proline biosynthesis.

The study of the effect of Methylparathion on proline accumulation [13] in *Sorghum* revealed that when the seeds are exposed to pesticides there was a pronounced increase in the level of proline and in the seedlings sprayed with twice (1000 ppm) that concentrations of Methylparathion, only a limited and significant increase in proline on the third day of spraying was observed. Further, their observations of vast differences in the proline levels in the plants exposed to Methylparathion before seed germination and those after five days of germination indicated that probably the pesticide requires a suitable physiological condition to induce proline accumulation. Similarly, this difference in the response was exhibited even in the continuing presence of the pesticide during the seedling growth until the harvest was made for proline estimation.

Besides, it is evident from the literature survey that proline accumulation response to high temperature and salinity could be due to the disturbances in tissue water status [5]. In addition, proline accumulation in response to nutrient deficiencies [8], water logging [9], low temperature[20-22], air pollutant [11], pesticides stress [13] and the alternations of this response caused by growth regulator[23] question the argument explaining the accumulation caused by all these factors via disturbances in tissue water status. Therefore, the results warrant a search for the common mechanism of the induction of proline accumulation in the plants caused by various stress factors. Recent studies [24] of the effect of thiocarbomate herbicide on the grass weed loose silky-bent (*Apera spica-venti* Family: Poaceae). By using a new HPTLC method they quantified amino acids in raw plant extracts. A pattern of significant changes in the content of glutamine, glycine, alanine and proline due to exposure were detected. The content of all four amino acids increased with increasing exposure rates. Quantitative analyses, 7 and 21 days after herbicide application, showed that over time the amount of proline increased.

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