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Research Article

Effects of Systemic Fungicides on Protein, Carbohydrate, Amino Acids and Phenolic Contents of Susceptible (Mexipak) and Resistant (Povan) Varieties of *Triticum Aestivum* L.

Zamin Shaheed SIDDIQUI, Soaliha AHMED
Stress Physiology & Environmental Pollution Lab
Department of Botany, University of Karachi, Karachi, 75270 - PAKISTAN

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Abstract: Changes in total protein, carbohydrate, phenolic content and ninhydrine positive amino acids of susceptible (MexiPak) and resistant (Povan) varieties of wheat were studied. Application of systemic fungicides caused a significant ($P<0.001$) decrease in total protein and carbohydrate content compared to the control. MexiPak (susceptible) was more adversely affected than Povan (resistant). A substantial increase in total phenol was observed in the two varieties tested. Among the amino acids, proline, methionine, tyrosine and tryptophane were found in appreciable amounts.

Key Words: Systemic fungicides, protein, carbohydrate, amino acid, phenols

Introduction

The use of systemic chemicals against harmful organisms of crop plants is an accepted application at the present time. Nowadays many modern pesticides are in use all over the world; among them, systemic fungicides are extensively used in agriculture. Benlate and calixin are systemic fungicides used for the control of diseases such as smut, grey mold, leaf spot, brown patch, downy mildew, powdery mildew, and rust in wheat (Singh, 1991). Despite its enormous application, priority should be given to the possible side effects of these chemicals on non-targeted host (plant). There are reports where the application of benlate produced chlorosis and irregular depression at the central and marginal portion of saffron leaves (Reyes, 1975). Alcholor metalaxyl induced sharp decreases in cell division (Coman et al., 1990). Triarimol inhibits the seedling growth of pea (John et al., 1975). The present study was therefore undertaken to examine the effects of systemic fungicides on total protein, amino acid, carbohydrate and phenolic contents of *Triticum aestivum* L.

Materials and Methods

Seeds of *Triticum aestivum* cv. MexiPak (susceptible) and Povan (resistant) obtained from the Pakistan Agriculture Research Council (PARC), Karachi, at the university campus were sown in plots. Twenty seeds were sown in each plot of an area of about 5x5 feet. The plots were regularly watered and seedlings were grown at a temperature of 30-35°C and 65-75% relative humidity. Fifteen-day-old seedlings were sprayed with benlate and calixin at rate of 1000, 1500 and 2000 ppm with the help of a spray machine (1000 ppm = 1 mg fungicide dissolved in 1000 ml of distilled water). The concentration of fungicides was based on the formulation of the active ingredient. Unsprayed plants served as controls. Leaf samples were collected randomly after the 15^{th} day of spraying. Changes in total protein contents in leaves were measured after the extraction in 5% TCA (Tri chloroacetic acid) and estimated using folin phenol reagent, the optical density was read at 700 nm (Lowry et al., 1951). Carbohydrate was extracted in distilled deionized water and estimated by the method described by Yemn and Willis (1956). Amino acids were extracted in 10 ml 80% ethanol by putting 1.0 g of plant material

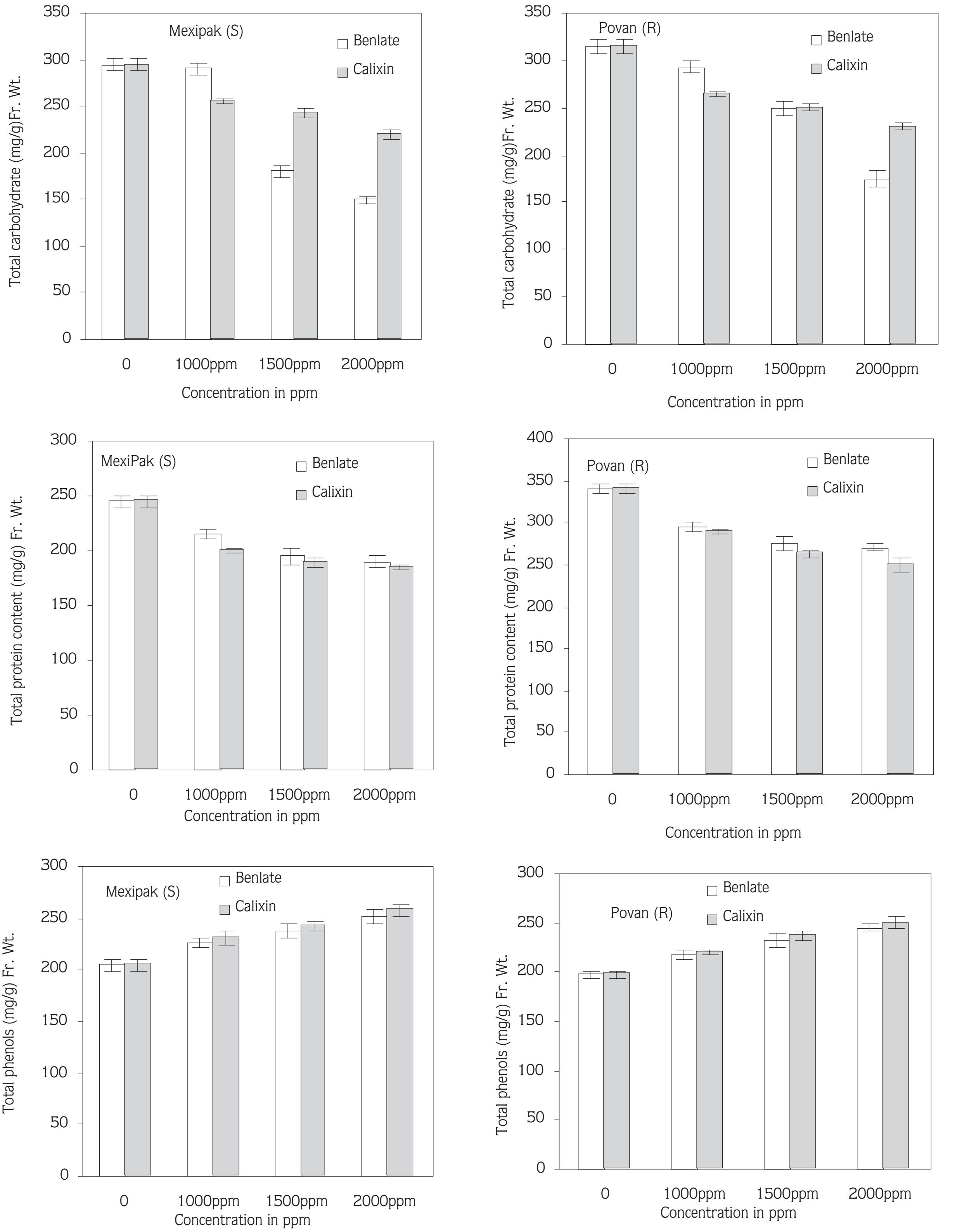

Figure 1. Effects of systemic fungicides on total protein, carbohydrate and phenolic contents of *Triticum aestivum* L. var. Mexipak and Povan. (S) susceptible, (R) resistant.

in a conical flask and boiling in a water bath for 10 minutes. Then the material was kept overnight. It was centrifuged twice at 4000 rpm. Supernatant was transferred to another tube and the volume of supernatant was reduced to 1 ml at 40°C temperature. After that 1 ml of 50% ethanol was added to the reduced extract to make the volume 2 ml and it was run in TLC by the method of Brenner et al. (Brenner et al., 1969). Total phenols were recorded in ethanol extracts and estimated using 10% folin reagent (Swain and Hillis, 1959). Earma photic 100 spectrophotometer was used for each analysis and quantity was expressed in mg/g fresh weight using standard curves. Each treatment was replicated three times. Probability analyses were determined by costat (a computer package).

Results and Discussion

The use of systemic fungicides, viz. benlate and calixin, caused a significant decrease ($p<0.001$, $F= 1345.23$) in the total protein content of resistant and susceptible varieties of *T. aestivum* (Figure 1). Benlate had a greater effect on MexiPak, reducing the total protein content as compared to the control and calixin. An osmotic shock effect of systemic fungicides results in the release of protein and loss of membrane transport ability in the leaf cells (Amar and Reinhold, 1973). It has been suggested that the toxicant produced by the application of systemic fungicides inhibits protein synthesis by binding to the larger ribosomal subunits inducing change in the enzyme system (Person et al., 1975), ceasing ATP and NADP formation (Mishra and Waywood, 1968; Siddiqui, 1997). The analyses revealed that the total carbohydrate content was significantly decreased ($p<0.001$, $F=1293.12$) by the application of benlate and calixin (Figure 1). However, the maximum decrease (24%) was found in var. MexiPak treated with calixin at a rate of 2000 ppm. The results obtained are illustrated in Table 1. Ninhydrine positive compounds were detected from the treated and control plants. Among these, neutral amino acids like glycine, leucine, alanine, phenylalanine and aspargine were present in both treated and in control samples. Methionine, tryptophane tyrosine and proline were present in appreciable amounts in treated samples. Changes were observed in the amino acid content of susceptible and resistant varieties of *T. aestivum* after the application of fungicides; a few amino acids appeared in both treated and untreated plants. Activation of enzymes

Table 1. Effect of systemic fungicides on ninhydrine positive amino acids of susceptible (Mexipak) and resistant (Povan) varieties of *Triticum aestivum* L.

		Benlate			Calixin		
Amino acids	Cont.	1000ppm	1500ppm	2000ppm	1000ppm	1500ppm	2000ppm
				Mexipak			
Glycine	++	-	+	+	-	+	+
Leucine	+	+	-	+	-	+	++
Phenylalanine	+++	-	++	+	+	-	++
Asparagine	+	++	+	+	+	+	++
Methionine	+	+++	++	++	++	++	++
Tryptophane	-	+++	-	+++	+	++	++
Tyrosine	-	++	++	+++	+	++	++
Proline	-	+	+	+++	-	++	++++
Alanine	++	+	-	+	+	-	-
				Povan			
Glycine	++	-	+	+	-	+	+
Leucine	+	+	-	+	-	+	++
Phenylalanine	+++	+	++	++	+	-	++
Asparagine	+	++	-	-	+	+	++
Methionine	+	+++	++	+++	++	++	+++
Tryptophane	-	+++	++	+++	+	++	++
Tyrosine	-	++	++	+++	+	++	++
Proline	-	+	++	+++	++	+++	++++
Alanine	++	-	-	+	+	+	-

involved in amino acid and amide biosynthesis in plants have been observed in certain cases resulting in increased levels of various amino acids and amides (Ahmed et al., 1985). The accumulation of amino acids might be the consequence of protein hydrolysis which might enter the tricarboxylic acid cycle either pyruvate by di-amination or by transamination with α ketoglutaric acid or oxalo acetic acid.

Substantial increases in total phenolic content were recorded in all treated plants (Fig. 1). The maximum increase (23%) was measured in susceptible plants when treated with calixin at a rate of 2000 ppm. It has been reported that plants treated with fungicides suffer from chemical stress (Siddiqui et al., 1997). Phenolic compounds and amino acids like proline and flavonoids, which might be produced as a result of this stress, may act as protective compounds against pests (Friend, 1977). The toxicant produced by the application of systemic fungicide inhibits respiration, photosynthesis and protein synthesis by inhibiting activity of NADH cytochrome "c" oxidases in the respiratory chain and accumulation of succinate and blocking of alternative pathway of respiration (Berger and Cwick, 1990; Pillonel, 1993). A correlation has been reported between the phenolic content of healthy tissue and resistance in various host parasite systems like potato/*Verticlium* spp. (McClean et al., 1961) and cotton/*Alternaria* in macrospora (Bashan, 1986; Prarsad and Lal, 1977). Consequently, it may be suggested that the synthesis of various metabolic products would also be affected by the application of systemic fungicides, probably at higher concentrations. Discriminate use of systemic fungicides is, therefore, recommended.

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