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Effect of hexaconazole and triazophos on carbohydrate contents in germinating seeds of Spinach and Guar

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ABSTRACT

The effect of Hexaconazole 5% EC and Triazophos 40% EC on carbohydrate metabolism in spinach (Spinacea oleracea L.) and guar (Cyamopsis tetragonolobus L. Taub) had been studied in present investigation. The concentrations of both pesticides range from 0.05%, 0.1%, 0.15%, 0.2% and 0.3% were selected. Reducing sugar, total sugar, starch and total carbohydrates in germinating seeds of spinach increases as increasing concentrations of Hexaconazole but in guar it was decreases, however it affect detrimentally at the concentration of 0.30%. The Triazophos stimulates the all carbohydrates content at its lower concentration while detriments at higher concentrations in both the plants.

Key words: Germinating seeds, Carbohydrates, Hexaconazole, Triazophos, *Spinacea* , *Cyamopsis* etc.

INTRODUCTION

In modern agriculture, application of pesticides as seed treatments has become a common practice [1]. The seed treatment of systemic pesticides protects seed, seedling and young plants from attack by certain pathogens and pests. Pre-sowing seed treatment with pesticides is an efficient, economic means of plant protection [2]. The plant absorbs a certain amount of pesticides that changes the plant's metabolism [3] but the rate of change varies from crop to crop [4]. However, germinating seeds are as a model system for assessing the toxicological potential of pesticides [5]. In the present work the effect of broad spectrum systemic pesticides *viz.* Hexaconazole (5% EC) and Triazophos (40% EC) on seed reserves carbohydrates contents *viz*. reducing sugar, total sugar, starch, total carbohydrates in germinating seeds of spinach (*Spinacea oleracea* L.) and guar (*Cyamopsis tetragonolobus* L. Taub.) were studied.

MATERIALS AND METHODS

The healthy seeds of spinach and guar were treated with 0.05%, 0.1%, 0.15%, 0.2% and 0.3% concentrations of Hexaconazole 5% EC and Triazophos 40% EC pesticides for 12 h. The treated seeds were thoroughly washed with distilled water and allowed to germinate in petriplates. The germinating seedlings of 10 days were analyzed for biochemical parameters. The total sugars and starch content were estimated by method of Nelson [6] and reducing sugar by Miller [7].

RESULTS AND DISCUSSION

Qualitative and quantitative changes are involved in several metabolic pathways during seed germination and seedling growth [8]. Insecticides may cause oxidative stress in plant cells, affecting the various metabolic activities and growth components in plants [9]. The seed reserve gets hydrolyzed and change in cellular and organelles constituents such as proteins, lipids and carbohydrates takes place. However, the rate of change varies from crop to crop and species to species [10, 11]. Carbohydrates are the major storage reserves in seed hydrolyzed by the hydrolytic enzymes into glucose. These carbohydrates are utilized by developing seedling for the synthesis of various metabolic products hence the carbohydrate contents *viz*. reducing sugar, total sugar, starch and total carbohydrates were studied under influence of Hexaconazole and Triazophos pesticides in germinating seeds of spinach and guar.

Total Sugar - Sugars are the primary photosynthetic products form the building blocks for all other chemical constituents of the plants. The growth and development depend upon the availability of carbohydrates in the conductive system of plant. Sugars are the source of food for plant cells broken down chemically by respiration to supply energy for all plant functions. Nutrient availability, water supply and carbon dioxide, temperature, sunlight, and the presence of toxic substances influence the rate of photosynthesis [12]. In this concern, the effect of Hexaconazole and Triazophos on total sugar in germinating seeds of spinach and guar were studied which are depicted in Table 1 and Fig. A, B, C and D. It was found that Hexaconazole increased the total sugar with increasing its concentration in spinach and guar however maximum value was recorded for 0.20 % Hexaconazole. The Triazophos showed decreased total sugar with increasing its concentrations in spinach and guar but 0.30% Triazophos showed slightly detrimental action. Similar to these results, Kamble and Sabale [13] found total sugar content in *Trigonella* seeds increased with increasing concentrations of Carbandazim while Monocrotophos decreased total sugar with increasing concentrations. However, Santhaguru *et al*. [14] reported reduction in soluble sugars in *Cyamopsis tetragonoloba* L (taub.) with increasing concentration of rogor. Taylorson [15] found that high rates of diphenamid decreased the total sugars content in freshly seeded tomatoes. In present investigation, the Triazophos decreases the total sugar content with its increasing concentrations suggesting that the pesticides adversely affect the metabolic enzymes and inhibited the functioning of the enzymes of the photosynthetic carbon reduction (PCR) cycle, such as Rubisco, 3-PGA kinase, NADP, NAD-Glyceraldehyde-3-P-dehydrogenase, and aldolase while the Hexaconazole stimulates the activity of these enzymes returning increasing total sugar with its increasing concentration[16]. With this result the sugar contents in germinating seeds is directly related to stress factor [17] such as pesticidal action of Hexaconazole and Triazophos.

Reducing Sugar - Sugars containing the aldo or the keto group are capable of reducing copper in alkaline solutions (Fehling's solution, oxidizing agent) to produce the brick-red coloured cuprous ions are reducing sugars. The α-amylases which are found virtually in all living cells cleave the a -D-(1® 4) linkages at random and bring about conversion of the starch molecule into the reducing sugars[18]. Glucose and fructose are reducing sugars used to generate energy for seedling growth. The reducing sugar content of the seeds during germination did not exhibit a steady pattern due to oil and protein contents. This observation could be explained by the conversion of free fatty acids to sugars and the conversion of soluble sugars to reducing sugars. It is also possible that insoluble sugars were also converted to soluble and reducing sugars [19]. Considering these all in view, the effect of Hexaconazole and Triazophos on reducing sugar in germinating seeds of spinach and guar were studied (Fig. A, B, C and D). It had been observed that, Hexaconazole increased the reducing sugar content with its increasing concentration in spinach and guar. The maximum value recorded for 0.15% and 0.20% Hexaconazole. Even the highest concentration i.e. 0.30% Hexaconazole acts stimulatory effect (Fig A and C). The Triazophos decreased the reducing sugar with increasing concentration while at higher concentration 0.20% and 0.30% Triazophos showed detrimental effect in spinach and guar but in guar 0.15% and 0.20% Triazophos stimulated more reducing sugar contents as compare to untreated germinating seeds where as 0.30% Triazophos acts detrimentally (Fig. B and D). There are few reports available which described effect of pesticides on reducing sugar content in germinating seeds. These include positive as well as negative influence of pesticides. Prasad and Mathur [20] noticed considerable decrease in reducing sugar in *Vigna mungo* L. with treatment of metasystox and also Dalvi *et al.* [21] also reported a decrease in the amount of reducing sugar in wheat and mung bean seeds treated with menazon. Chopra and Nandra [22] also reported a decrease in formation of reducing sugar in germinating mustard seeds while treated with thiomenton. But carbandazium seed treatment in some plant did not affect on reducing sugar content [23]. Our results showed Hexaconazole was stimulatory at higher concentrations in germinating seeds of both vegetables while Triazophos was stimulatory at lower concentrations in germinating seeds of spinach and inhibitory in guar. The decrease in the reducing sugar content in Triazophos treatment at higher concentration might be due to some enzymatic changes which are responsible for the conversion of starch to some reducing sugars[8]. An increased level of reducing sugar in seed may be due to its non- conversion to non-reducing sugar [24]. This might be mechanism adopted by germinating seed to reduce the effect of Hexaconazole stress [25].

Starch - The principal storage carbohydrate in plants is the polysaccharide, starch. Starch is composed of two polymers, amylose and amylopectin. Starch is synthesized and stored in plastids termed as amyloplasts. Both amylose and amylopectin synthesis begins with synthesis of ADP-glucose from glucose-1-phosphate and ATP by ADP-glucose pyrophosphorylase with liberation of pyrophosphate and starch synthase catalyzes the formation of an α-1,4 linkage. Starch is catabolized by 5 enzymes: α-amylase, β-amylase, α-glucosidase, starch phosphorylase, and α-dextrin 6-glucanohydrolase (debranching enzyme). During seed germination the starch is hydrolyzed to glucose and further metabolized by glycolysis, the TCA cycle, and the electron transport chain. Starch is the major energy source for the germinating embryo. The breakdown of starch to readily utilizable sugar under amylase activity is essential for the growth of seedling [26]. During early seedling growth, starch was mobilized from the endosperm to the embryo, and as a result, the starch content of the endosperm decreased steadily and the starch content of the embryo increased during the first few days of seedling growth [27]. During these metabolic events ATP and carbon skeleton are provided for anabolic reactions essential in supporting the embryonic plant until it attains an autotrophic condition [28]. In present investigation, the effect of Hexaconazole and Triazophos on starch in germinating seeds of spinach and guar were studied (Fig. A, B, C and D) and was found that Hexaconazole increases starch with increasing its concentration in spinach and guar however 0.10%, 0.15% and 0.30 % Hexaconazole increases more starch in germinating seeds of both vegetable crops (Fig. A and C). The Triazophos shows decrease starch with increasing its concentrations in spinach while was detrimental at all concentrations in guar as compare to control (Fig. B and D). With agreements to our results, Kamble and Sabale [13] found starch content in germinating seeds of *Trigonella* increased with increasing concentrations of carbandazim while monocrotophos decreases in starch with increasing its concentrations. Our results showed the Hexaconazole was stimulatory to starch at its higher concentration in germinating seeds of both crops and Triazophos was inhibitory at higher concentrations in spinach while it act detrimentally at all concentrations in guar. The breakdown of starch at Triazophos treatment correlated with increased amylase activity during seed germination which suggesting amylase activity influence by Triazophos while Hexaconazole decreases its activity resulted in increase in starch. According to Misal and Sabale [29] the amylase activity is increases or decreases in pesticidal stress.

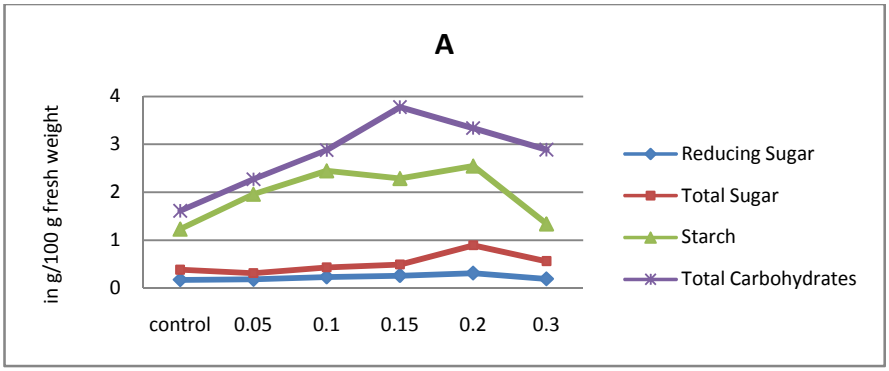
Total carbohydrates - Carbohydrates represent a broad group of substances which include the sugars, starches, gums and celluloses [18]. In higher plants these are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. However carbohydrate contents were significantly influenced by the treatment of fungicide [30]. In present investigation, the effect of Hexaconazole and Triazophos on total carbohydrates in germinating seeds of spinach and guar were demonstrated in Figure A, B, C and D and Table 1. It was observed that Hexaconazole increased total carbohydrates with increasing its concentration in spinach and guar however 0.15% and 0.30 % Hexaconazole stimulated more total carbohydrates in germinating seeds of both vegetable crops but at higher concentration it showed less stimulatory effect (Fig. A and C). The Triazophos decreased total carbohydrates in increasing concentrations in spinach while it act stimulatory with increasing concentrations in guar as compare to control. The 0.30% Triazophos showed detrimental effect on spinach and guar (Fig. B and D). Similar to our results, Avinash *et al*. [30] found positive increase in 0.1% and 0.3% carbendazim, whereas in the 0.2% concentration the carbohydrate content decreased considerably in jowar. There are few other reports describes adverse effect of pesticides on total carbohydrates content in germinating seeds. A study on *Hibiscus esculantus* and *Capsicum annum* by Ahmed and Siddiqui [31] reported an increase in carbohydrate contents in the seeds treated with benlate fungicide. Siddiqui and Ahmed [32] studied significant decrease in total carbohydrate content after the application of systemic fungicide in two varieties of wheat. Bhattacharya *et al.*, [33] investigated the effect of Carbofuran, Butachlor and Carbendazim treatments on carbohydrate contents of two summer rice cultivars and found that fungicide Carbendazim application at panicle emergence of rice produced a decreasing trend in carbohydrate content. A similar result was reported by Pablo *et al*. [34] in Carbendazim treated tobacco plants. Kishorekumar *et al.* [35] evaluated the comparative effects of different triazole compounds on carbohydrate metabolism of *Solenostemon rotundifolius.*

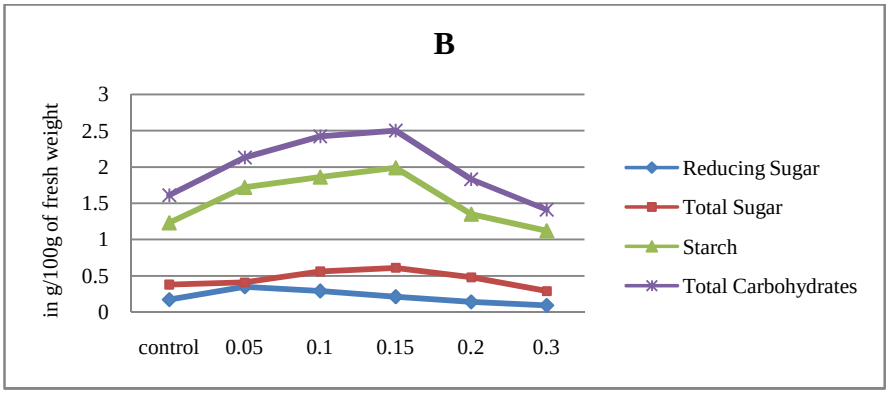
Use of systemic fungicides caused a significant decrease in carbohydrate content as compared to the control [36]. This reduction of carbohydrate contents might be the result of reduced synthesis and possibly due to damage of vascular tissues. The noticeable decrease in carbohydrates of seedlings in relation to applied pesticides might be due to the disturbing influence of such toxicants on enzymes involved in carbohydrate metabolic pathway [37]. Earlier studies suggested that toxicant produced by pesticides application retarded the carbohydrate synthesis by inducing alteration in cytochrome oxidase activity, blocking alternative respiratory pathways and accumulation of succinate [38, 39]. To agreement with all previous study, systemic fungicides like Hexaconazole and Triazophos have been found to increase NAD and NADP ratios interfering electron transport system [40] and increases ATP levels [41] increase the carbohydrates content.

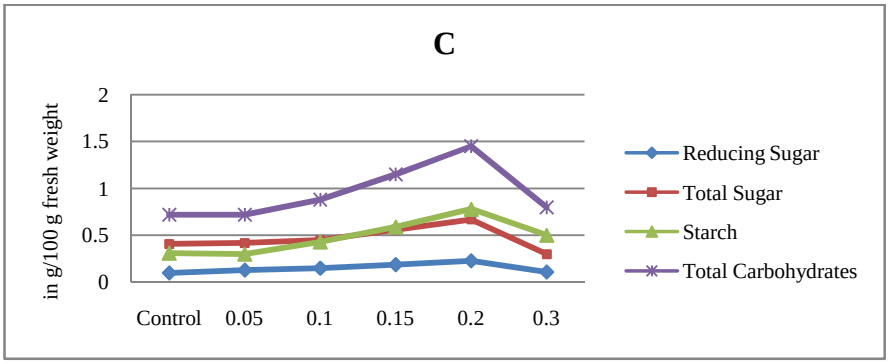
Table 1: Effect of Hexaconazole and Triazophos on Carbohydrate contents in germinating seeds of Spinach and Guar

Component Of Carbohydrates		Hexaconazole (5% EC)					Triazophos (40% EC)				
		Concentrations (%) (V/V)									
	Control	0.05	0.10	0.15	0.20	0.30	0.05	0.10	0.15	0.20	0.30
Spinach											
Total Sugar	0.38	0.31	0.43	0.49	0.89	0.56	0.41	0.56	0.61	0.48	0.29
Reducing Sugar	0.17	0.18	0.23	0.26	0.31	0.41	0.35	0.29	0.21	0.14	0.09
Starch	1.23	1.96	2.45	2.29	2.55	1.34	1.72	1.86	1.99	1.35	1.12
Total Carbohydrates	1.61	2.27	2.88	3.78	3.34	2.89	2.13	2.42	2.50	1.83	1.41
Guar											
Total Sugar	0.41	0.42	0.45	0.56	0.67	0.30	0.54	0.63	0.77	0.99	0.57
Reducing Sugar	0.10	0.13	0.15	0.19	0.23	0.11	0.12	0.16	0.19	0.18	0.09
Starch	0.31	0.30	0.43	0.59	0.78	0.50	0.30	0.29	0.21	0.15	0.11
Total Carbohydrates	0.72	0.72	0.88	1.15	1.45	0.80	0.84	0.92	0.98	1.14	0.68

All values are expressed in g/100 g fresh weight.







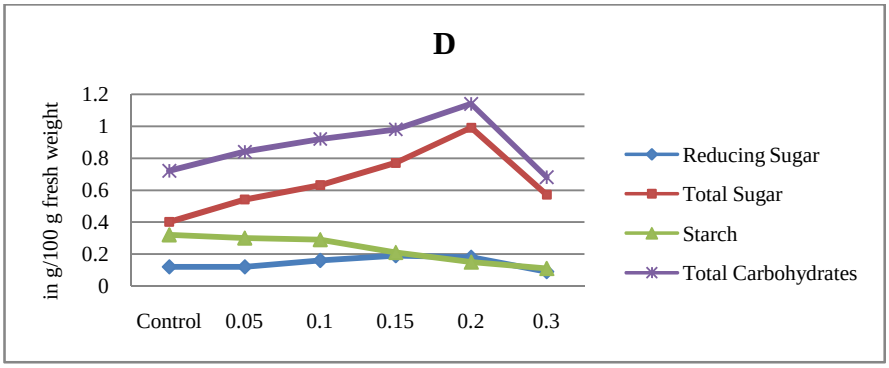


Fig (A-D) : Effect of Hexaconazole (A) and Triazophos (B) on total sugar, reducing sugar, starch and total carbohydrates in germinating seeds of spinach. While effect of Hexaconazole (C) and Triazophos (D) on total sugar, reducing sugar, starch and total carbohydrates in germinating seeds of guar

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