ORGANOCHLORINE, ORGANOPHOSPHORUS AND SYNTHETIC PYRETHROID PESTICIDES AFFECTING AMINO ACIDS IN COTTON SEEDS AND WHEAT GRAIN DURING STORAGE

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SUMMARY: This paper presents results of the effects of studied pesticides on amino acids in cotton seeds and wheat grains stored at the room temperature (30 ± 3°C) after pesticide treatment for one month. Each sample was analyzed in triplicate along with a control sample. The influence on amino acids, was found to be variable and significant. The analytical data, obtained by employing amino acid analyzer, showed significant quantitative variations in amino acids of both the food commodities.

Key Words: Pesticide effects, amino acids.

INTRODUCTION

Many scientists have studied the effects of different pesticides on total protein in cotton seeds, wheat grains and in other crops such as maize and soybeans and found quantitative variations (1-8). The nutritive value of protein and proteinaceous foods and food products depends on their amino acids composition and essential amino acids balance. Effects of some herbicides on amino acids in soybeans have been studied and it was reported that lysine increased without reducing the amount of total protein (9). In another study, the action of different herbicides on quantitative and qualitative changes in soils was compared (10). Wolfson and Shearer (11) investigated variations in the amino acid composition in grain protein of maize grown with or without pesticides and standard commercial fertilizers. Ciszewska *et. al.* (12) tested six wheat varieties for effects of different herbicides, and found that results of protein and lysine varied depending on the year and the cultivars. Not much work has been reported about the effects of pesticides on amino acids in cotton seeds and wheat grains during storage.

Effects of certain organochlorine (OC), organophosphorus (OP), and synthetic pyrethroid (SP) pesticides, namely, p,p'-DDT, monocrotophos and cyhalothrin respectively on cotton seeds and OP compounds (chlorpyriphos-methyl and pirimiphos-methyl) and SP one compound namely permethrin on wheat grains have been studied, evaluated and their results are presented in this paper. The results were assessed using analytical data from trial application of studied pesticides directly to the aforesaid food commodities, stored for one month in sealed glass jars. Each sample was analyzed for amino acids in triplicate along with control sample by employing amino acid analyzer.

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MATERIALS AND METHODS

The analytical method of Osborne and Voogt (13) was adapted for the determination of proteins in cotton seeds and wheat grains. The sample of cotton seeds was taken in three replicates for each pesticide treatment and transferred to glass jars. Calculated amounts of monocrotophos (2 ppm), p,p'-DDT (2 ppm) and cyhalothrin (2 ppm) separately added to the glass jars and shaken for 3 hours. samples of wheat grain were also treated with three grain protectants namely chlorpyriphos-methyl, pirimiphos methyl and permethrin at recommended dosages i.e., 10, 4 and 2 ppm respectively. The fortified samples of both the food commodities were allowed to stand in the sealed glass wars for one month at room temperature (30 ± 3°C). Each fortified sample was analyzed along with the control sample. Cotton seeds and wheat grain samples containing 10-12 mg protein were accurately weighed and transferred to glass culture tubes and 10 ml 6N HCI was added. The tubes were evacuated, and sealed. The samples were hydrolyzed at 110°C for 24 hours and filtered. Filtrates were evaporated to near dryness by a rotary vacuum evaporator to remove HCI and the residues in each case were dissolved in deionized water.

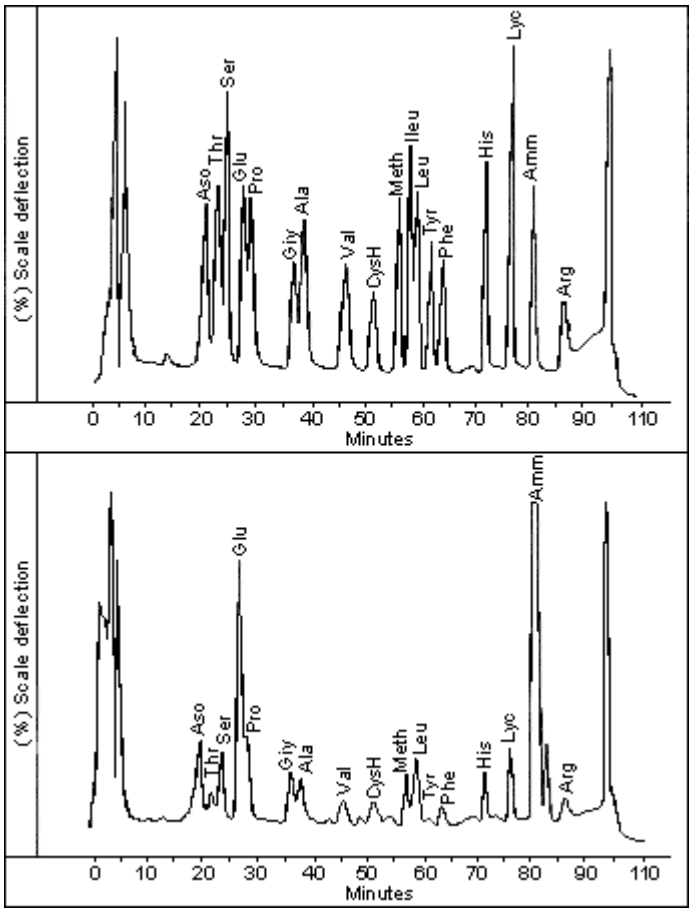
The Chromatographic glass column (10 mm i. d. x 200 mm long) was prepared with a slurry of ion exchange resin Dowex 50 W-XB (100-200 mesh) in deionized water. A mixture of studied amino acids which are commonly fond in cotton seeds and wheat grains, was prepared in deionized water and quantitatively transferred to the prepared column and allowed to settle in the column bed. The column was eluted with deionized water and then with 3.5% ammonia in deionized water. For complete elution, fractions of 2 ml each were collected and checked for the presence of amino acid with ninhydrin reagent till violet color ceased to appear. All 2 ml fractions were then combined and evaporated nearly to dryness by a rotary vacuum evaporator to remove ammonia and taken up in deionized water for amino acid analyzer. A suitable aliquot of each concentrated volume was subjected to the system of amino acid analyzed and quantitative recovery of each studied amino acid was determined.

The concentrated extracts of pesticide cotton seeds and

Table 1: %Age of studied amino acids in cotton seeds after one month treatment with pesticides.

S1 No	Name of amino acid	Cotton seed control	Cotton seed + Cyhalothrin	Cotton seed + p,p' - DDT	Cotton seed + monocrotophos
01	Alanine (Ala.)	1.20 ± 0.12	1.01 ± 0.12	0.42 ± 0.03	1.53 ± 0.27
02	Ammonia (Amm.)	0.66 ± 0.14	0.50 ± 0.03	0.12 ± 0.03	0.01 ± 0.00
03	Arginine (Arg.)	3.02 ± 0.29	1.88 ± 0.02	1.24 ± 0.10	2.48 ± 0.05
04	Aspartic acid (Asp.)	3.27 ± 0.02	2.17 ± 0.04	1.22 ± 0.02	2.24 ± 0.17
05	Cysteine (Cys.)	0.12 ± 0.04	0.07 ± 0.02	0.82 ± 0.02	0.58 ± 0.04
06	Glutamic acid (Glv.)	10.57 ± 0.10	0.11 ± 0.03	3.24 ± 0.02	12.49 ± 0.39
07	Glycine (Gyl.)	1.26 ± 0.14	1.24 ± 0.11	0.52 ± 0.03	1.02 ± 0.04
08	Histidine (Hist.)	1.26 ± 0.14	0.75 ± 0.02	0.75 ± 0.02	4.69 ± 0.23
09	Isoleucine (IIeu.)	0.71 ± 0.08	0.53 ± 0.01	NIL	0.09 ± 0.02
10	Leucine (Leu.)	1.61 ± 0.15	1.32 ± 0.06	1.03 ± 0.07	1.91 ± 0.14
11	Lycine (Lyc.)	1.25 ± 0.13	0.94 ± 0.01	7.77 ± 0.09	6.21 ± 0.21
12	Methionine (Meth.)	0.99 ± 0.10	0.17 ± 0.02	0.63 ± 0.02	0.06 ± 0.02
13	Phenylalanine (Phe.)	1.56 ± 0.09	1.15 ± 0.02	0.69 ± 0.02	1.01 ± 0.04
14	Proline (Pro.)	1.75 ± 0.04	1.75 ± 0.04	0.72 ± 0.02	NIL
15	Serine (Ser.)	1.36 ± 0.11	0.71 ± 0.03	0.40 ± 0.03	0.79 ± 0.10
16	Threonine (Thr.)	1.04 ± 0.03	0.74 ± 0.03	0.33 ± 0.02	0.92 ± 0.12
17	Tyrosine (Tyr.)	0.69 ± 0.09	0.49 ± 0.03	0.36 ± 0.03	0.87 ± 0.06
18	Valine (Val.)	1.17 ± 0.06	0.86 ± 0.07	0.03 ± 0.01	0.23 ± 0.02
	TOTAL PROTEIN:	33.49 ± 0.11	16.89 ± 0.04	20.41 ± 0.03	37.13 ± 0.11

Figure 1: i) Chromatograms of a standard mixture containing 25n-moles each amino acid.
ii) Chromatograms of extracted amino acids from cotton seeds treated with pp'-DDT.



wheat grains in deionized water were quantitatively transferred to the prepared column. Each extract was allowed to absorb into column bed and then eluted with deionized water to wash out unnecessary substances except amino acids and then proceeded exactly as mentioned in the preceding paragraph to get elute ready for amino acid analyzer. The amino acid analyzer (Biotronik LC 6001 Germany) has an automatic system with a sample injector and computing integrators for peaks. The system involved photometric detection of ninhydrin reacted amino acids. The photometer was attached to the recorder, which in turn was attached to the computing integrator. The hydrolyzed sample was dried, dissolved in the recommended buffer solution and subjected to amino acid analysis. Each of the 3 hydrolysates use analyzed using optimal parameters for amino acid analyzer. A standard solution of amino acids was used to calibrate the analyzer at least every 24 hours. The amount of amino acids in the sample was quantitatively determined by comparison with the area of the samples and the standard. Figures 1 and 2 show typical chromatograms of cotton seeds and wheat grain treated with p,p'-DDT and pirimiphos methyl respectively. Results of analysis are presented, with standard error in Tables 1 and 2.

RESULTS AND DISCUSSION

No comprehensive study of the nutritional and germination significance of different constituents in cotton seeds and wheat grains have been carried out after application of pesticides on these food commodities. The present investigation focuses on the study of proteins and particularly emphasizes the quantitative measurement of amino acids susceptible to decomposition during storage by the treatment of different types of pesticides in the studied food commodities. The

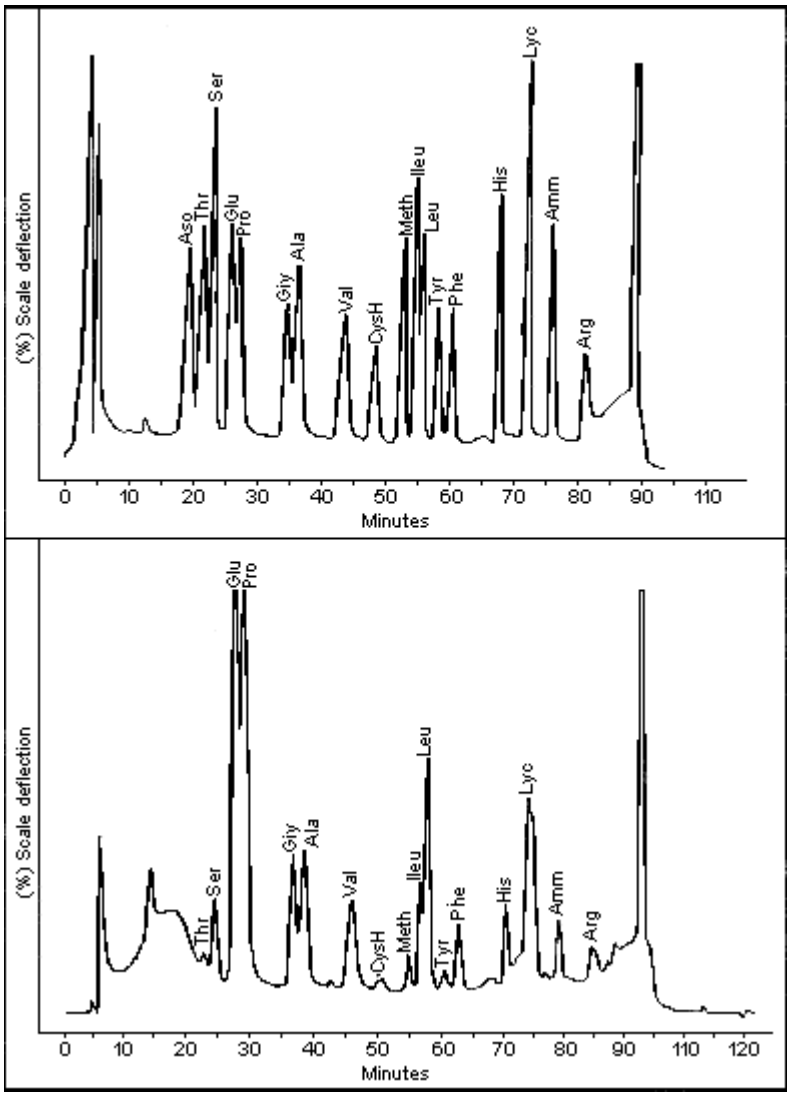
Table 2: % Age of studied amino acids in wheat grains after one month treatment with pesticides.

S1 No	Name of amino acid	Wheat control	Wheat + Chlorpyriphosmethyl	Wheat + Pirimiphosmethyl	Wheat + Permethrin
01	Alanine (Ala.)	0.51 ± 0.02	0.59 ± 0.04	0.69 ± 0.02	0.51 ± 0.02
02	Ammonia (Amm.)	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.82 ± 0.04
03	Arginine (Arg.)	0.06 ± 0.01	1.37 ± 0.04	1.04 ± 0.04	0.66 ± 0.02
04	Aspartic acid (Asp.)	0.03 ± 0.02	0.01 ± 0.01	NIL	0.10 ± 0.02
05	Cysteine (Cys.)	0.10 ± 0.02	0.21 ± 0.02	0.17 ± 0.01	0.05 ± 0.01
06	Glutamic acid (Glv.)	2.73 ± 0.10	3.55 ± 0.10	3.68 ± 0.04	2.12 ± 0.05
07	Glycine (Gyl.)	0.79 ± 0.03	0.86 ± 0.03	0.81 ± 0.03	0.76 ± 0.03
08	Histidine (Hist.)	0.45 ± 0.02	0.82 ± 0.02	0.70 ± 0.02	0.61 ± 0.02
09	Isoleucine (IIeu.)	0.38 ± 0.02	0.45 ± 0.01	0.57 ± 0.02	0.38 ± 0.02
10	Leucine (Leu.)	1.16 ± 0.04	1.59 ± 0.04	1.42 ± 0.06	1.00 ± 0.03
11	Lycine (Lyc.)	0.44 ± 0.01	1.52 ± 0.06	1.71 ± 0.02	0.82 ± 0.03
12	Methionine (Meth.)	1.79 ± 0.05	0.62 ± 0.02	0.25 ± 0.01	0.54 ± 0.02
13	Phenylalanine (Phe.)	0.59 ± 0.02	1.01 ± 0.05	0.92 ± 0.03	0.82 ± 0.03
14	Proline (Pro.)	2.72 ± 0.06	2.61 ± 0.07	2.48 ± 0.04	1.62 ± 0.05
15	Serine (Ser.)	0.10 ± 0.01	0.25 ± 0.02	0.28 ± 0.02	0.25 ± 0.03
16	Threonine (Thr.)	0.10 ± 0.01	0.21 ± 0.02	0.17 ± 0.01	TRACES
17	Tyrosine (Tyr.)	0.22 ± 0.03	0.33 ± 0.02	0.36 ± 0.03	0.67 ± 0.02
18	Valine (Val.)	0.58 ± 0.03	0.79 ± 0.04	0.86 ± 0.03	0.39 ± 0.02
	TOTAL PROTEIN:	12.83 ± 0.03	15.50 ± 0.03	16.19 ± 0.02	12.12 ± 0.03

amino acids estimated by amino acid analyzer are seventeen in number including seven essential amino acids. Tryptophan, destroyed by acid hydrolysis, could not be determined. In most of the cases, it was noted that the influence of pesticides on amino acids in both the food commodities was remarkably significant. In the treated samples under investigation in this laboratory, the amino acids arginine, aspartic acid and glutamic acid were estimated in much higher quantities than other amino acids in cotton seeds, while in wheat grain only glutamic acid and proline were present in higher concentrations as shown in Tables 1 and 2.

In cotton seed samples treated with monocrotophos (OP), proline was destroyed completely, while p,p'-DDT (OC) left the same effect on isoleucine an essential amino acid. Such effect was not observed in the samples treated with cyhalothrin (SP), but in this case a very significant influence was evident, showing marked decrease in concentration of all the seventeen detected amino acids as presented in Table 1 and Figure 1. Particularly, the decrease was very significant in glutamic acid and in an essential amino acid, methionine while negligible and no effect was observed on proline and glycine respectively. Thus, the overall decrease of amino acids corresponded total protein by reducing it to 16.6%. The aforesaid OC pesticide tends to increase essential amino acids i.e. cysteine and lycine, in considerable proportions while the rest of the amino acids and the ammonia decrease in concentration; thus 13.08% reduction was estimated in total proteins. The increasing tendency is very significant in seven amino acids, particularly in cysteine, histidine and lycine, and in appreciable amounts in alanine, glutamic acid, leucine and tyrosine in the samples treated with monocrotophos. Due to this reason, an increase of 3.64% in total proteins, was estimated in this case. Generally, a reducing effect was observed on arginine, aspartic acid, glycine, methionine, phenylalanine,

Figure 2: i) Chromatograms of a standard mixture containing 25n-moles each amino acid.
ii) Chromatograms of extracted amino acids from wheat grains treated with pirimiphos-methyl.



serine, threonine, valine and also in ammonia in the samples treated individually with aforesaid pesticide.

In the samples of wheat grain treated with two OPs chlorpyriphos-methyl and pirimiphos-methyl pesticides individually, the six essential and eight amino acids out of a total seventeen tend to rise with pesticide concentration as incorporated in analytical data in Table 2 and Figure 2. Evidently, a considerable reduction and complete destruction of aspartic acid occurred by the effects of above mentioned OP pesticides. Deficiency however, was observed only in methionine and proline. Thus the total increase in amino acids was found to be directly related to the increase of total proteins contained in control sample by 2.67 and 3.36% respectively. In case of permethrin (SP), the increase was noted to be associated with arginine, aspartic acid, histidine, serine, tyrosine, and essential amino acids lycine and phenylalanine while no influence was observed on alanine and isoleucine. The trace amount of threonine and considerable decrease in concentration of leucine, methionine, proline and valine confirms adverse effects of permethrin on these amino acids. Insignificant influ-

ence was observed on the rest of the amino acids. Due to balanced effects on the amino acids, overall reduction in total protein occurred only by 0.71%.

It may be inferred from the analytical data that the amino acids cysteine, glutamic acid, glycine, leucine, methionine and threonine have undergone similar adverse effects with SP pesticides on both the commodities. All the three studied OP pesticides left beneficial effects on alanine, cysteine, glutamic acid, histidine, leucine, lycine and tyrosine, while adverse effects were observed on aspartic acid, methionine and proline in cotton seed and wheat grain. Methionine and proline were the only two amino acids which decreased quantitatively by the effects of all the six pesticides studied in samples of both the food commodities.

The analytical data of protein is very significant for both the food commodities as the nutritive value of protein and proteinaceous foods and food products depend on their amino acid composition and essential amino acid balance. SP pesticide, cyhalothrin proved to be a very effective chemical agent as it reduced quantitatively all the detected amino acids except proline in the samples of cotton seeds. On the other hand, in wheat grain, effect of another SP pesticide namely, permethrin on amino acids was variable as it caused both decreasing and increasing affects on eight and seven amino acids respectively.

The influence of OP pesticides i.e. chlorpyriphos-methyl and pirimiphos-methyl on all the amino acids in wheat grain was found to be quite identical, while another OP pesticide, monocrotophos, produced adverse effects on majority of the amino acids in cotton seed.

The OC pesticide p,p'-DDT caused decreasing effect on all the studied amino acids present in cotton seed samples except cysteine and lycine, both of which increased quantitatively.

It may, therefore, be concluded that both studied SP pesticides leave more adverse effects than studied OC compound and cause deficiency of amino acids in both the studied food commodities while studied OP pesticides are found to be much milder in effects. Thus the significant effects described above on individual amino acids show that the studied pesticides may affect the commodities if the period of storage is extended.

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