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Correlation between proximate chemical composition and insect pests of maize cultivars in Peshawar

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Abstract
The present study was conducted at Agronomy Research Farm (ARF), The University of Agriculture, Peshawar (UAP), Pakistan during 2015. Seven maize cultivars, namely Azam, Jalal, Babar, Pahari, Iqbal, Climax 3055 and Climax 30M62 were tested. Results of the proximate chemical composition of maize cultivars showed the moisture percentage in the range of 9.59%-12.27%. Similarly ash content was recorded in the range of 0.83%-1.13%, crude fats of 2.65% -2.97%, crude protein in the range of 10.03%-1.96%, fiber of 0.89%-1.37% while carbohydrates content of 71.12%-75.31% respectively. From the correlation matrices of the insect pests densities with biochemical characters, minimum moisture percentage, minimum protein content, higher ash content were proved to have contribution for resistance in maize cultivars against the insect pest attack. From the information mentioned above, the maize cultivar Climax 3055 was proved resistant while Pahari was proved susceptible cultivar among the seven tested maize cultivars.

Keywords: Insect Pest, Resistance, Maize cultivars, Ash, Protein, Carbohydrate, Crude Fat, Crude Fiber, Moisture

1. Introduction
Corn, usually called “maize” (*Zea mays* L.) (family Poaceae) is an annual, cross pollinated, Kharif crop. Zea, makai, corn, jovar and silk corn, etc. are other synonyms used to recognize maize. Corn provides food for humans, feed for animals particularly poultry and livestock and raw materials for various industries, hence it is a multipurpose crop [1]. In Pakistan, the total area under maize cultivation in 2013-14 was more than one million hectares and it yielded 3.5 million metric tons. The total area contribution of Khyber Pakhtunkhwa province under maize was 56 percent which contributed 63 percent of the total production [2]. Maize is mostly grown in Haripur, Swabi, Mardan, Malakand, Charsadda, Peshawar, D.I. Khan, Kohat and Bannu districts of Khyber Pakhtunkhwa [3]. In Pakistan, the most dependable, profitable and staple crop after potato is maize [4].

benefits and uses are countless. Chemical proximate composition shows that maize usually contains 10 percent proteins, 3.5 percent fiber, 2 percent minerals and 80 percent carbohydrates. Vitamin B and Iron are also present in maize [5]. Culinary oil (edible oil) is also extracted from maize seeds. A chemical called Levulinc acid is an ingredient used as antifreeze is also a maize derivative. There are many factors which are responsible for low yield of maize [6] among which insect pests are major ones. Maize germinating seeds and seedlings are damaged by other insect pests such as cutworms, wireworm, false wireworms, black field earwigs, maize stem borer and beetles such as African black beetle. Invertebrate insect pests that attack the maize crop at the development and maturity stages include corn aphid, green vegetable bug, corn earworm, red shouldered leaf beetle, common army worm, maize leaf hopper, two-spotted spider mite, maize weevil and thrips [7].
An important component of Integrated Pest Management (IPM) which is environment friendly, compatible with other control methods and is packaged in seed is the Host Plant Resistance (HPR) [8]. Numbers of plant characters are involved in host plant resistance. Herbivores and their natural enemies are influenced rather positively or negatively by the

structures of the plant [9]. These plant characters can be divided on biochemical and morphological basis. The most important qualitative resistance factor against many insects of maize in corn crop is 2, 4-dihydroxy-7-methoxy-(2H)-1, hydroxamic acid, 4-benzoxazin-3-(4H)-one (DIMBOA) which reduces with the maturity of the plant. In few species of plants, silica is present in the trichomes of juvenile plant varieties that impart indigestibility to the herbivore pest [10]. The identification of different maize genotypes with other resistance mechanisms is very necessary so as to increase the levels of resistance and diversify the borer's resistance bases [11]. Many irrelevant effects on grain yield due to the presence of more than one resistance mechanism would improve the pattern of plant response and will certain the resistance durability.

2. Materials and Methods

A research work under the title "Biochemical basis of resistance in maize cultivars against their insect pests" was conducted at the ARF, UAP during 2015. RCB design was used to carry out the experiment. There were three replications and each replication had seven treatments (Climax 30M62, Climax 3055, Babar, Iqbal, Azam, Jalal and Pahari). Separate experimental units were used to sow each maize cultivar. During March 2015, these maize cultivars were sown in lines. Buffer zone of one meter was kept between the replications and a half meter between the treatments for isolation. Size of the whole experimental field was 24m×11m which was divided into 21 sub-plots each with an area of 3m×3m. Row to row and plant to plant distance was kept 75cm and 25cm, respectively. Standard agronomic practices were adopted in the field throughout the maize growing season. The experimental field was left open for natural infestation of insect pests. Data was recorded on weekly basis from germination till the maturity of cultivars.

The details of the experiments were as following:

2.1 Insect pest data collection for maize cultivars screening:

To check the comparatively resistant and susceptible responses of maize cultivars, the experimental field was observed thoroughly at weekly intervals for insect pest infestation on upper, middle and lower portion of the plant. Corn flea beetle data was recorded as number leaf^{-1} while Maize shoot fly, grass hopper, four-spotted leaf beetle, army worm and red pumpkin beetle data was recorded as number plant^{-1} on randomly selected 6 plants per treatment. The collected specimens of Grass hopper, Shoot fly, and red pumpkin beetles were killed with the help of killing jar and then properly pinned in the insect collection box. Whereas the flea beetles and four-spotted leaf beetles were mounted and army worms were preserved in 70% alcohol. All the collected insects were deposited at the Insect Museum, Department of Entomology, UAP.

2.2 Proximate Chemical composition of maize cultivars leaves:

Standard methods were used to determine the proximate chemical composition, i.e. % moisture, % ash, crude protein, crude fiber, crude fat and carbohydrates content per 100 grams of maize leaves.

Moisture content

Determination of moisture contents was carried out at the Department of Agricultural Chemistry, UAP. Well mixed sample of 1 gm. was taken in a dried and weighed china dish to determine the moisture content. The sample was then placed in oven at 105 ºC for four hours (AACC, 2004). Calculation for moisture content was as following:

$$\text{Moisture (\%)} = \frac{W1-W2}{W} \times 100$$

Where;
W1 = Sample's initial weight
W2 = Sample's final weight
W = Sample weight (1gm)

Ash

Ash content was determined by taking about 1 gm of well-mixed sample from each treatment in a cleaned and dried China dish and charred over a slow burning flame and then was placed in muffle furnace at 550 ºC for four hours until constant weight was obtained [12]. The ash content in percentage was calculated as under.

$$\text{\% Ash} = \frac{\text{Difference in weight}}{\text{Weight of the sample}} x100$$

Crude protein

Determination of crude protein was done by Kjeldahl method at the Agricultural Chemistry department, The University of Agriculture, Peshawar, Pakistan. In the presence of digestion mixture, the digestion was made by heating the samples with concentrated Sulphuric acid to find protein content. The mixture was then made alkaline. Thus Ammonium sulphate was formed and the released ammonia was collected in 2% boric acid and against standard HCl it was titrated. For total protein calculation, the amount of nitrogen was multiplied with constant factor (6.25) [12].

Formula for calculation of % protein content of the sample was:

$$\text{\%Nitrogen} = \frac{(S-B) \times N \times 0.014 \times 100 \times 100}{\text{Weight of the sample (g)} \times V \text{ (ml)}}$$

$$\text{\%N} = Y$$

Now,

$$\text{\% protein} = Y \times 6.25$$

$$\text{\% protein} = X$$

Where,

S= Sample (Titration)
B = Titration (Blank)
N = Normality of HCl used (ml)
V = Sample volume taken from distillation (ml)
0.014 = Molecular weight of nitrogen
100 = Dilution water
6.25 = the constant factor (maize) for finding % protein.

Crude fat

Soxhlet apparatus was used to perform crude fat extraction. In a clean and previously dried extraction thimble, about 2-4 gm

moisture free sample was taken. Then the thimble was placed in an extraction tube. Weight of previously cleaned and dried 200 ml round bottom flask was taken. Then with the solvent (petroleum ether 40-60), it was filled up to one third and was connected with the extraction tube. Top water and burner apparatus remained on. The extraction process took 3-4 hours. After every 5-10 minutes, siphoning occurred at the condensation rate of 3 to 4 drops per second. When the process was finished, thimble was then removed from the extractor and flask was heated to collect all the solvent for future use. The apparatus was allowed to cool down. Then at 105 °C, the flask was dried for 1 hour. Finally it was cooled and weighted again (AACC, 2004) [12]. The oil content of the sample was calculated in percentage as:

$$\% \text{ oil} = \frac{(\text{weight of flask+oil}) - (\text{weight of empty flask})}{\text{Weight of sample}} \times 100$$

Crude Fiber

Chemicals

2% NaOH =10g/500ml water solution

2% HCl= 10ml/500ml water solution

Procedure

Two gm well-mixed sample was taken at first to find crude fibre. Then it was put in a 200ml HCl containing beaker. The samples in acid were then boiled for 30 minutes by placing them on water bath. After boiling, the residue was filtered and transferred to a 200 ml NaOH containing beaker and boiled again on water bath for 30 minutes and was filtered and washed with 100ml hot water. Then the residue was transferred to crucibles and put in oven at 105 °C for four hours. It was then allowed to cool and was weighed. Then these crucibles were put in a furnace at 550 °C for four hours, again allowed to cool and reweighed [12].

$$\% \text{ Crude Fiber} = \frac{(\text{weight of oven dried residue - weight after ignition}) \times 100}{\text{Weight of sample}}$$

Carbohydrates contents

The weights of crude protein, crude fibers, fats, moisture contents and ash were subtracted from 100 for the determination of Carbohydrate contents.

100 - (Moisture + ash + fibers + fats + protein)

Statistical analysis

Each parameter's recorded data was analyzed statistically by using Statistix 8.1 Software and Fisher Protected Least Significance Difference Test was used for the separation of means at 5% level of significance [13].

Table 1: Experimental Layout

R1	R2	R3
Azam (OPV)	Climax 3055 (hybrid)	Iqbal (OPV)
Babar (hybrid)	Jalal (OPV)	Pahari (OPV)
Pahari (OPV)	Climax 30M62 (hybrid)	Babar (hybrid)
Climax 3055 (hybrid)	Azam (OPV)	Jalal (OPV)
Iqbal (OPV)	Babar (hybrid)	Climax 3055 (hybrid)
Climax 30M62 (hybrid)	Pahari (OPV)	Azam (OPV)
Jalal (OPV)	Iqbal (OPV)	Climax 30M62 (hybrid)

OPV: Open Pollinated Varieties

3. Results

The results of proximate chemical composition of maize leaves are given in Table 2. It was found that higher crude protein was present in the leaves of Pahari (11.96 g/100 gm) and lower in C-3055 (10.03 g/100 gm). Maximum amount of crude fat was recorded in the leaves of Babar (2.97 g/100 gm) and lower in Jalal (2.72 g/100 gm). The crude fiber content was obtained maximum from the leaves of Iqbal (1.37 g/100 gm) while minimum from Azam cultivar (0.89 g/100 gm). Higher percentage of moisture was noticed in the leaves of Pahari (12.27%) while lower in C-30M62 (9.59%). Maximum ash percentage was observed in the leaves of C-3055 (1.13%) and lower in Azam (0.83%). Moreover, the carbohydrate recorded was higher in the leaves of Jalal (75.31 g/100 gm) while lower in Pahari cultivar (71.12 g/100 gm).

Table 2: Proximate chemical composition of maize cultivars leaves during 2015.

Maize cultivars	Contents					
	Crude protein (mg/100 gm)	Crude fat (mg/100 gm)	Crude fiber (mg/100 gm)	Moisture (%)	Ash (%)	Carbohydrate (mg/100 gm)
Azam	11.37 c	2.81 c	0.89 d	11.20 c	0.83 cd	72.90 c
Babar	11.71 b	2.97 a	1.09 c	11.72 b	0.87 cd	71.64 d
Pahari	11.96 a	2.88 bc	0.96 d	12.27 a	0.81 d	71.12 e
C-3055	10.03 f	2.93 ab	1.11 c	9.64 e	1.13 a	75.16 a
Iqbal	10.81 d	2.65 e	1.37 a	10.16 d	0.93 bc	74.08 b
C-30M62	10.26 e	2.73 d	1.28 ab	9.59 e	1.03 ab	75.11 a
Jalal	10.07 f	2.72 de	1.19 bc	9.68 e	1.03 ab	75.31 a
LSD value	0.0828	0.0755	0.1055	0.1249	0.1160	0.2044

Means followed by same letters with in each column are not significantly different at 5% probability level (LSD test).

Correlation matrix of biochemical analysis with insect pest density

Table 3 shows the result of correlation between the population densities of shoot fly, flea beetle, grass hopper, army worm, leaf beetle and red pumpkin beetle with biochemical factors of maize cultivars leaves. Correlation of crude protein with the shoot fly, flea beetle, army worm, leaf beetle, grass hopper and red pumpkin beetle was recorded positively significant. The crude fiber was found non-significant and negatively correlated with the insect pests densities. Positively non-significant correlation was observed for the crude fat content with the shoot fly, grass hopper, leaf beetle and red pumpkin beetle but negatively non-significant with the flea beetle and army worm. Moisture content was noticed significant and positively correlated with the insect pest densities except flea beetles where correlation was positively non-significant. Negative and significant correlation was recorded for ash content with the insect pest densities but non-significant and negative effect with flea beetle population density. Moreover, correlation matrix between the carbohydrate content and insect pest densities was found positively non-significant.

Table 3: Correlation matrix of insect pest's density and biochemical factors of maize cultivars leaves during 2015.

Insect pests	Biochemical factors					
	Crude protein	Crude fat	Crude fiber	Moisture	Ash	Carbohydrate
Flea beetle	0.7406	-0.0144 ns	-0.1287 ns	0.7013 ns	-0.7340 ns	0.5968 ns
Shoot fly	0.8578	0.1218 ns	-0.2037 ns	0.7781	-0.8455	0.8232 ns
Grasshopper	0.9071	0.2856 ns	-0.6903 ns	0.9020	-0.9471	0.6115 ns
Army worm	0.9115	-0.3719 ns	-0.6216 ns	0.9369	-0.8933	0.4943 ns
Leaf beetle	0.9312	0.2058 ns	-0.6127 ns	0.9047	-0.9793	0.6809 ns
Red pumpkin beetle	0.9186	0.3349 ns	-0.5641 ns	0.9358	-0.9042	0.5126 ns

Correlation is significant at 5% level.

ns = Non-significant correlation.

4. Discussion

The present results of chemical proximate analysis of the seven maize cultivars were comparable to that of some earlier results, e.g. Ullah *et al.* [14] reported moisture percentage in the range of 9.201-10.908%, ash 0.7-1.3%, crude fats 3.21-7-71%, crude protein 7.71-14.60%, fiber 0.80-2.32% and carbohydrates 69.659-74.549%. Saeed *et al.* [15] in a study of proximate chemical composition and the mineral contents in maize, found moisture in the range of 7-11%, fat 2.87-4.50%, crude fiber 1.02-2.80% and 10.01- 12.90% protein, which were comparable to the present results. The insects are attracted and deterred in one way or the other by the biochemical composition of the plants, which has been documented by findings of many earlier researchers. Saleem *et al.* [16] observed positive and significant correlation between crude protein, lipids, moisture content, calcium, reducing sugar, zinc and magnesium with the insect densities. Parvez *et al.* [17] investigated low moisture content having contribution for resistance against *Chilo partellus* (S.) in maize cultivars. Having a deterrent property, maximum ash percentage will reduce the chance of insect pest attack. Mbaiguinam *et al.* [18] documented the concentration of secondary metabolites like lectins, non-proteinic amino acids, alkaloids and enzymes inhibitor etc. as anti-feedant, repellent and/or toxic to the insect pests. Dixon and McKay [19] stated that the soluble nitrogen level (protein content) affects the reproduction of aphids. Only the reports of Ali *et al.* [20] are in contrast to our results who stated that the percent moisture was not important in cotton cultivars regarding insect pest infestation.

5. Conclusion and Recommendation

Results of the proximate chemical composition of maize cultivars leaves showed the moisture percentage in the range of 9.59%-12.27%. Similarly ash content was recorded in the range of 0.83%-1.13%, crude fats of 2.65%-2.97%, crude protein in the range of 10.03%-11.96%, fiber of 0.89%-1.37% while carbohydrates content of 71.12%-75.31% respectively. From the correlation matrices of the insect pest densities with biochemical characters, minimum moisture percentage, minimum protein content, higher ash content were proved to have contribution for resistance in maize cultivars against the insect pest attack. From the information mentioned above, the maize cultivar Climax 3055 was proved resistant and is recommended for cultivation while Pahari was proved susceptible cultivar among the seven tested maize cultivars. Also, there might be some other biochemical like calcium, reducing sugar, zinc, magnesium, non-proteinic amino acids, alkaloids, lectins and other enzyme inhibitors which would be involved in resistance mechanism of maize against the studied insect pests.

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