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Determination of Efficacy of Cypermethrin, Regent and Carbofuran Against *Chilo partellus* Swin. and Biochemical Changes Following Their Application in Maize Plants

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ABSTRACT

In order to determine comparative efficacy of Carbofuran 3G, Cypermethrin 10EC and Regent 300EC on two maize varieties (Composite-20 and Double Top Cross) for the control of *Chilo partellus* and, changes in level of total soliuble proteion, total free amino acids, carbohydrates and crude fibres, Carbofuran, Regent and Cypermethrin were applied @ 10 kg, 150 mL, 220 mL acre^{-1}, respectively. The insecticides showed different effects on maize borer infestation only once on variety Double Top Cross, 96 h after first spray. At this interval least infestation was recorded in the plots treated with Regent while maximum was in plots treated with Cypermethrin. Increase in total soluble protein, total free amino acids, carbohydrates and crude fibres at various time points, following the application of insecticides, descended down to pre spray levels after one week of application. The relationship between changes in level of biochemical components of maize plants and insecticide application is discussed.

Key Words: Biochemical changes; Maize; *Chilo partellus;* Cyperemthrin; Carbofuran

INTRODUCTION

Maize (*Zea mays* L.) ranks third food crop in the world after the wheat and rice. Besides being a food crop, maize also provides feed for poultry and livestock. The gelatinous material from maize grains are also utilized as the raw material in paper, textile, refining, food industries and chemical industries (Chaudhary, 1983). In Pakistan, maize is grown over an area of 967 thousand hectares with an annual production of 1731 thousand tons with an average yield of 1970 kg ha^{-1}. (Government of Pakistan, 2001). Maize contributes about 6.4% of total cereal produce of the country.

The per hectare yield of maize has not increased despite the introduction of high yielding varieties. The major obstacle in achieving this goal is the attack/infestation of pest insects. The most important pest insects in maize crop are maize and jowar stem borer (*Chilo partellus* Swin,), shoot fly (*Atherigona soccata*), armyworm (*Mythimna separata*) and many species of aphid, the infestation of which ultimately results in total failure of crop (Singh & Sharma, 1984; Lisowicz, 2000).

In order to prevent infestation of maize stemborer, insecticides particularly granular formulations have been recommended as whorl treatment at 25 and 45 days after sowing (Halimie *et al*., 1989). Soil + whorl application of Furadan protected maize against maize stem borer for up to 30 and 20 days respectively. A single application of carbofuran 3G (@ 7.5 kg ha^{-1}. (in the leaf whorl) in 15 day old crop proved to be most effective in protecting against up to 6 days after application (Kalule *et al*., 1998). Besides granular application insecticide sprays are also recommended to avoid pest insect outbreak (Shams & Afzal, 1989; Talpur *et al.*, 1995; Lisowicz., 1999; Khan *et al*., 1999; Singh & Marwaha, 2001).

The use of insecticides has been demonstrated not only to kill insect pests, but also change the biochemical set up of the crop (Afifi & El-Ballal, 1980). Nothing substantial work has been done to determine the biochemical changes brought about by the insecticides in maize plant, however, some information related to this field was still available for guidance. Treatment with Methomyl and Lindane decreased the chlorophyll and soluble carbohydrates, however, treatments with Methomyl alone had no effect on the protein contents of the stem while a significant reduction was observed in leaves. The application of Butylate lipid content and cuticle were reduced, imbalance in protein contents also occurred (Lupu *et al*., 1990). Fluvalinate decreased the leaf chlorophyll content 7 days after application but starch and sugar content increased 3 day after application. Exposure to fluvalinate increased the total protein content 15 days after application (Chowdhury & Sukul, 1997). Significant increase in leaf chlorophyll content and total protein content was observed following application of fluvalinate @ 20 or 30 g a.i. ha^{-1}. (Chowdhury & Sukul, 1998).

Keeping in view the importance of maize as a cereal, the present project was, therefore, designed to determine the efficacy of Cypermethrin 10EC, Regent 300EC and Carbofuran 3G against maize stem borer (*Chilo partellus* Swin.); and biochemical changes i.e., changes in protein, carbohydrates, amino-acids and crude fibres in maize plant following the application of these insecticides on maize plants.

MATERIALS AND METHODS

The experiment on efficacy of insecticides against maize stem borer (*Chilo partellus*) was carried out at Research Area of Department of Agric. Entomology, U. A. Faisalabad, while the biochemical changes brought out by these insecticides were analyzed in Stress Physiology Lab., Nuclear Institute for Agriculture and Biology, Faisalabad.

Efficacy of insecticides. This experiment was laid down as Randomized Complete Block Design on two varieties viz., Composite-20 and Double Top Cross with three replications. Crop was sown on August 20, 2001 by following all standard agronomic practices. Plot size for each treatment in each replication was 55 m^2. In each plot, row to row and plant to plant distance was 75 cm and 22.5 cm, respectively. Cypermethrin 10EC, Regent 300EC, and Carbofuran 3G were applied @ 220 mL, 150 mL and 11 kg $acre^{-1}$, respectively, at 10% maize stem borer infestation. For recording the per cent infestation of maize borer (*Chilo partellus*) three middle rows in each plot were examined and 15 plants were randomly selected. Percent borer infestation was taken 48, 96 and 168 h following the application of insecticides and then after at weekly interval till harvesting.

Biochemical changes

To study the biochemical changes in the level of total soluble proteins, total amino-acids, carbohydrates and crude fibre, flag leaf of maize plant was assayed from each treatment in each replication at 48 h, 96 h and one week following insecticide application.

Total soluble protein. Proteins were estimated according to the method described by Lowry *et al.* (1951). Fresh leaves were chopped into tiny pieces. The pieces were freezed over night in an extracted phosphate buffer (pH 7) for extraction. Two tubes containing 0.05 mL and 0.1 mL of leaf extract were prepared for protein estimation. 0.05, 0.1, 0.2, 0.4, 0.6 and 1.0 mL of standard BSA were simultaneously used in the experiment. The volume of each tube was made up to 1.0 mL with distilled water. The blank contained only 1ml distilled water. 1ml of solution [50ml of solution (2 g Na_2CO_3 , 0.2 g NaOH, and 1 g Na-K tartrate dissolved in distilled water and volume made up to 100 mL) and 1.0 mL of another solution (0.5 g of $CuSO4.5H_2O$ dissolved in distilled water and volume made up to 100 mL) to prepare alkaline copper solution. This solution is always prepared fresh] was added to each tube. The tubes were thoroughly mixed and allowed to stand for 10 minutes at room temperature. Then 0.1 mL of 1:1 diluted Folin-phenol reagent was added, mixed well and kept for 30 min at room temperature. The optical density (O.D) was measured at 620nm on Spectronic-20 Spectrophotometer.

Total proteins were calculated with the help of following formula.

$$\text{Total soluble proteins} = \frac{O.D.\text{ of sample}}{O.D.\text{ of standard}} \times \text{Conc. of Standard} \times \frac{1}{\text{mL of sample}}$$

$$(\text{mg / g fresh weight})$$

Total free amino-acids. Total free amino–acids were determined by the method described by Hamilton and Van Slyke (1943). For the estimation of total free amino–acids 1ml of each sample as extracted for the soluble proteins were taken in culture tubes and 1 mL of 10% pyridine and 1 mL of 2% ninhydrin solution were added into each tube. The tubes were heated in boiling water bath for about 30 min. The contents of each tube were then made up to 50 mL with distilled water. The optical density of these coloured solutions were then read at 570nm using Spectrophotometer (Hitachi U- 2000) and total free amino–acids were calculated by the formula given below.

$$\text{Total free amino acids} = \frac{\text{Reading of sample} \times \text{Vol. of sample} \times \text{Dilution factor}}{\text{Weight of fresh tissues} \times 1000}$$

$$(\mu g\text{ / g fresh weight})$$

Carbohydrates. Soluble sugars were estimated following Malik and Srivastava (1985). For estimation of soluble sugars, 0.1 g of well ground dry material was homogenized and centrifuged at 1000 g × 29 $^{\circ}$C. The residue was retained which was repeatedly washed with 80% ethanol to remove all the traces of soluble sugars. The filtrate thus obtained was used for the determination of soluble sugars, then the volume of the sugar extracts was made up to 100 mL by the addition of distilled water.

Preparation of anthrone solution

1 0.4 g of anthrone and 200 mL analar H_2SO_4 were taken in 250 mL volumetric flask.

2 60 mL distilled water and 15 mL ethyl alcohol 95% was taken in 500 mL flask.

3 The large flask was placed in ice and poured the solution (1) into (2) slowly with constant stirring by means of an electromagnetic stirrer.

Preparation of glucose solution. 0.2 g glucose was taken in a volumetric flask of 100 mL. The volume was made up to 100 mL. 5 mL of this solution was diluted to 100 mL by adding 95 mL distilled water.

Preparation of blank solution. 1 mL distilled water taken in a test tube and then added 10 mL of anthrone solution. The tube was covered with cap and shaken at room temperature.

Preparation of anthrone and glucose solution. 1 mL glucose + 10 mL of anthrone solution was taken in a test tube and shaken as usual.

Preparation of sample solution. 1 mL sample solution +

10 mL of anthrone was taken in a test tube and shaken. All the tubes were heated in boiling water for 12 min, cooled and absorbance was read at 625nm.

Total soluble sugars was calculated by the following formula:

$$Total\ soluble\ sugars = \frac{Conc.of\ glucose\ sol.}{Absorbance\ of\ glucose} \times Absorbance\ of\ sample \times Dilution\ factor$$

$$(mg\ /\ g\ of\ dry\ wt.)$$

Per cent crude fibre. The crude fibre was recorded with the method described by American Association of Cereal Chemist (AACC, 1962). 1 g of dry sample and 50mL of 1.25% H_2SO_4 was boiled for 30 min in 100 mL flask, then cooled and filtered. The residues were again boiled for 30 min in 1.25% of 50mL NaOH and filtered. Filtered residues were ignited in furnace at 600ºC.

$$\%\ crude\ fibre = \frac{Weight\ of\ residues - Weight\ of\ ash}{Weight\ of\ sample}$$

RESULTS AND DISCUSSION

The comparison of per cent infestation of *Chilo partellus* at pre and post spray times i.e., 48 h, 96 h, 1, 2 and 3 week on two maize varieties (Double Top Cross and Composite 20) is given in Table I and II. After 48 h of first spray, Carbofuran, Regent and Cypermethirn were statistically at par with that of control treatment. In remaining intervals, insecticides were non-significantly different among themselves but have significant difference with control all the time intervals. Carbofuran recorded the lowest per cent infestation (0.1%) and was significantly different from Cypermthrin and Regent but latter two were non-significantly different from control at three week of the second spray on Composite 20.

Total soluble protein in both the varieties increased with the passage of time under all treatment of insecticides. However at one week post treatment interval protein in both varieties were similar to that of protein at 24 h before any treatment. Total free amino-acids were maximum at 96 h post treatment interval in both varieties. Carbohydrates increased up to 96 h after the spray of all the insecticides except in plants sprayed by Regent where these decreased after 48 h the spray of different chemicals increased fibre contents under both the sprays but in first spray the increase was up to 48 h in all chemicals except in the plants treated with Regent, however the results were some what different in second spray where induction in fibers continued up to 96 h (Table III & IV).

Table I. Comparison of per cent infestation of *C. partellus* at pre and post spray intervals on variety Double Top Cross

Treatment	Intervals					
First spray	pre spray			post spray		
	24h	48h	96h	One week	Two week	Three week
Carbofuran	15.55 n.s.	13.33 n.s.	2.22bc**	0.1 b**	2.22 b**	15.55 b
Regent	24.44	17.78	0.1 c	2.22 b	4.44 b	17.77 b
Cypermethrin	24.44	17.77	8.88b	4.44 b	11.11b	22.22 b
Control	15.55	22.22	24.44a	37.77a	37.77a	68.88 b
Second spray						
Carbofuran	15.55 b**	11.12b**	4.44 b**	4.44 b**	11.12 b*	4.44 b
Regent	17.77 b	13.33b	2.22 c	4.44 b	8.88 b	8.88 b
Cypermethrin	22.22 b	17.78b	11.12 b	11.12b	11.12 b	8.88 b
Control	68.88 a	66.66a	42.22 a	37.77a	26.66 a	26.66 b

* = significant at p> 0.01, ** = highly significant at p < 0.01, n.s. = non- significant at p> 0.05, Means sharing common letters do not differ significantly in a column.

Table II. Comparison of percent infestation of *C. partellus* at pre and post spray intervals on variety Composit-20

Treatment	Intervals					
First spray	pre spray			post spray		
	24h	48h	96h	One week	Two week	Three week
Carbofuran	15.55 n.s.	13.33 n.s.	0.1 b*	0.1 b**	8.88 b**	11.12 b**
Regent	26.66	19.99	8.88 b	2.22 b	8.88 b	2.22 b
Cypermethrin	20.00	15.55	8.88 b	6.66 b	15.55 b	8.88 b
Control	15.55	15.55	19.99a	28.88a	57.77 a	39.99 a
Second spray						
Carbofuran	13.33 b**	11.12b**	4.44 b**	4.44 b**	4.44 b**	0.1 b**
Regent	11.12 b	6.66b	4.44 b	4.44 b	6.66 b	2.22a
Cypermethrin	13.33 b	8.88b	8.88 b	0.1 b	8.88 b	6.66a
Control	46.66 a	55.55a	59.99a	19.99a	22.22a	8.88 a

* = significant at p> 0.01, ** = highly significant at p < 0.01, n.s. = non-significant at p> 0.05, Means sharing common letters do not differ significantly in a column

Table III. Comparison of total soluble protein (mg/g fresh weight), amino acids (µg/g fresh wt.), carbohydrates (mg/g dry wt.) and percent crude fiber of maize plant at pre and post spray intervals on variety Double Top Cross

Treatment	Interval			
First spray	**pre spray**		**post spray**	
	24h	**48h**	**96h**	**One week**
Total soluble protein				
Carbofuran	0.68 n.s.	0.81 b*	1.57 n.s.	0.58 n.s.
Regent	0.83	1.00 a	1.37	0.59
Cypermethrin	0.86	1.04 a	1.29	0.58
Control	0.80	0.87 b	1.02	0.44
Second spray				
Carbofuran	1.12 b*	1.69 n.s.	1.26 b**	1.53 n.s.
Regent	1.18 b	1.56	1.37 b	1.33
Cypermethrin	1.50 a	1.70	1.20 b	1.47
Control	1.54 a	1.60	2.00 a	1.40
	total amino-acids (µg/g fresh wt.)			
Carbofuran	5.53 d**	6.34 d**	12.56 n.s.	7.25 n.s.
Regent	8.00 b	9.36 b	12.52	6.74
Cypermethrin	9.29 a	10.15 a	12.53	7.00
Control	7.04 c	7.46 c	12.53	7.40
Second spray				
Carbofuran	14.81 n.s.	17.04 n.s.	13.37 n.s.	14.28 bc*
Regent	12.33	14.28	15.52	16.22 ab
Cypermethrin	10.60	12.92	17.04	18.09 a
Control	14.28	14.69	17.21	12.06 c
	carbohydrates (mg/g dry wt.)			
Carbofuran	68.37 b*	76.82 b**	87.69 b**	59.72 n.s.
Regent	77.93 ab	87.80 a	27.21 c	40.86
Cypermethrin	71.22 b	76.77 b	96.13 ab	42.47
Control	84.51 a	95.11 a	105.92 a	35.34
Second spray				
Carbofuran	65.33 a*	69.94 a*	53.72 n.s.	93.81 n.s.
Regent	32.81 b	34.87 b	34.92	81.51
Cypermethrin	42.80 b	45.80 b	27.98	78.83
Control	22.47 b	24.99 b	58.99	125.31
	percent crude fibre			
Carbofuran	16.45 a*	23.21 a**	10.62 bc**	9.23 c**
Regent	13.14 b	16.63 c	16.32 a	16.34 b
Cypermethrin	12.42 b	13.70 d	12.14 b	21.63 a
Control	14.53 ab	19.68 b	7.83 c	17.84 ab
Second spray				
Carbofuran	6.99 d**	7.26 d**	11.85 b**	5.66 b**
Regent	10.05 c	11.26 c	14.82 a	9.10 a
Cypermethrin	12.84 b	15.19 b	10.94 bc	9.66 a
Control	14.91 a	20.04 a	8.98 c	9.64 a

* = significant at p> 0.01, ** = highly significant at p < 0.01, n.s. = non-significant at p> 0.05, Means sharing common letters do not differ significantly in a column

The results of the present study showed that all the pesticides were effective in controlling the maize borer (*Chilo partellus*). In most of the cases the three of the insecticides proved similar in their effects. The control showed high percentage of infestation through out the experiment as compare to treated plots. The insecticides showed different effects on maize borer infestation only once on variety Double Top Cross, 96 h after first spray. At this interval least infestation was recorded in the plots treated with Regent while maximum was in plots treated with Cypermethrin. These results are in agreement with the findings of Halimie *et al.* (1989), Shams and Afzal (1989), Talpur *et al.* (1995), Ganguli *et al.* (1997), Kalule *et al.* (1998), Khan *et al.* (1999), Lisowicz (1999) and Singh and Marwaha (2000) who reported the effective control of maize stem borer by applying different insecticides. In brief, all the insecticides showed an equal response in decreasing the infestation of maize stem borer (*Chilo partellus*) at a significant level when compared with control. Though the insecticides were non-significantly different among themselves but these controlled the pest infestation at a significant level as against the control. These results were in partial agreement with the work of Javed *et al.* (1998).

Total soluble protein in both the varieties increased with the passage of time under all treatment of insecticides. However at one week post treatment interval protein in both varieties were similar to that of protein at 24 h before any treatment. Although trend of increase in protein in control is similar to that of insecticide treatments but total protein was significantly higher in treated plots as compare to control.

The results of present study are in confirmation of those reported by Chowdhury and Sukul (1997) and Chowdhury and Sukul (1998) but Saafan (1998) reported contrasting findings which might be due to their different set up, insecticides, time periods or may be due to the attack of different insects. The present study indicated that the spray of different insecticides increased total soluble protein which are necessary for plant growth. Proteins are the basic building block of plant body and all the metabolic enzymes are protein in nature. The results showed that increased in protein increased the metabolic activities necessary for optimum growth, but after one week the reduction in protein indicated that effect of all the insecticides deteriorated and the attack of different insects may occur after one week post treatment interval due to which reduction in plant growth took place which ultimately resulted reduction in yield.

Results also showed that recommended doses of different insecticides to enhance the protein were not suitable, their higher doses may be more effective than those used in the present study.

The results of the second spray showed that total protein were maximum after 48 h in plants treated with Carbofuran, Regent and Cypermethrin which indicated that action of all the insecticides were maximum after 48 h post treatment interval but it decreased afterward, however, a non-significant increase after 48 h post treatment interval also in carbofuran and Cypermethrin showed that the insecticides had some affect up to one week but the effect of all the insecticides deteriorated as the time interval increased after spray which might be due to the selection of plant which were going towards maturity. As at maturity the maximum protein and other products are directed towards the grains where they are stored for reserve food later on used by the human being and animals.

From the results it can be concluded that at the time of second spray a maximum activity of different insecticides was up to 48 h post spray interval. At this stage the minimum attack of insects was on stem and leaves because all the photosynthates are collected in grains so the intention

Table IV. Comparison of total soluble protein (mg/g fresh weight), amino acids (µg/g fresh wt.), carbohydrates (mg/g dry wt.) and percent crude fiber of maize plant at pre and post spray intervals on variety Composite-20

Treatment	Interval			
First spray	Pre spray		Post spray	
	24h	48h	96h	One week
Total soluble protein				
Carbofuran	0.57 n.s.	0.70 b**	0.99 c**	0.79 n.s.
Regent	0.58	0.64 c	1.47 a	0.68
Cypermethrin	0.64	0.75 a	1.18 b	0.75
Control	0.72	0.76 a	1.55 a	0.82
Second spray				
Carbofuran	1.05 b**	1.48 n.s.	1.54 n.s.	1.59 a*
Regent	1.27 b	1.69	1.61	1.69 a
Cypermethrin	1.63 a	1.76	1.82	1.66 a
Control	1.74 a	1.71	1.39	1.33 b
Total amino-acids (µg/g fresh wt.)				
Carbofuran	6.66 n.s.	8.20 n.s.	9.73 n.s.	3.46 n.s.
Regent	6.08	7.63	5.08	3.34
Cypermethrin	8.12	9.22	8.40	4.69
Control	7.60	7.77	7.21	4.82
Second spray				
Carbofuran	13.22 b**	15.46 a*	16.06 n.s.	11.95 n.s.
Regent	13.16 b	15.60 a	15.00	12.30
Cypermethrin	13.47 b	13.05 b	12.46	11.14
Control	16.85 a	17.22 a	9.85	10.34
Carbohydrates (mg/g dry wt.)				
Carbofuran	82.97 n.s.	87.93 n.s.	74.37 bc*	36.09 n.s.
Regent	81.92	89.41	96.98 ab	81.65
Cypermethrin	74.65	79.91	64.42 c	69.80
Control	73.18	76.73	111.29 a	81.67
Second spray				
Carbofuran	35.07 n.s.	36.57 n.s.	75.12 n.s.	111.87 n.s.
Regent	36.33	39.50	59.98	94.14
Cypermethrin	53.61	57.71	50.68	112.38
Control	32.17	37.61	57.81	76.97
Percent crude fibre				
Carbofuran	12.48 b*	13.51 b**	15.82n.s.	25.70 a**
Regent	12.16 b	13.58 b	13.77	19.31 b
Cypermethrin	15.61 a	18.41 a	13.79	15.22 c
Control	12.64 b	12.87 a	13.48	25.56 a
Second spray				
Carbofuran	13.00 n.s.	13.60 n.s.	10.88 c**	6.24 b**
Regent	13.35	14.20	14.50 b	12.61 a
Cypermethrin	13.05	13.33	17.93 a	13.74 a
Control	14.41	14.98	16.24 b	8.55 b

* = significant at p> 0.01, ** = highly significant at p < 0.01, n.s. = non-significant at p> 0.05, Means sharing common letters do not differ significantly in a column.

of insects may be diverted towards the grains. However the spray on cobs is dangerous for human health but the spray that have some volatile compounds may be effective at this stage.

The results of the present study indicated that amino-acids were maximum at 96 h post treatment interval in both varieties. The amino-acids are the major constituents of the proteins which are involved in plant growth. There are some reports which indicated that total amino-acids increased when the plant was facing some stress conditions (Ashraf, 1998). At this stage the maize plants treated with different insecticides may observe some environmental or insect stress (attack) due to which treated plants tried to produce more amino-acids to adjust the environmental or insect stress condition. The increase in amino-acids due to spray of different insecticides was also recorded elsewhere (Chowdhury & Sukul, 1997; Chowdhury & Sukul, 1998).

Carbohydrates are the organic compounds which plants prepare as result of photosynthesis and use them as food products for them and excess of them have been stored and used in the construction of plant body and also used by the human being and animals like maize grain contain starch used by man and animal as a food. The reduction in their synthesis may cause reduction in plant growth and yield. Data about the concentration of carbohydrates in the maize after spray showed that carbohydrates increased up to 96 h after the spray of all the insecticides except in plants sprayed by Regent where these decreased after 48 h, which might be due to its effects on some photosynthesizing organs of plant or may be due to the reduction in its effectiveness and insects attached on those organs which are involved in the carbohydrates synthesis. The results also indicated reduction in the concentration and the effectiveness of the different chemicals which were sprayed. The result of Saafan (1998) are similar to present findings. However the results of second spray showed very different results where carbohydrates increased in maize plants under all treatments up to 48 h of spray then decreased at 96 h but increased after one week which may be due to the toxicity of the sprayed chemicals. The used chemicals may change into some other form after one week which create toxicity and causes hindrance in the syntheses of carbohydrates or may be some other reasons which are unknown.

Fibres are the tissues which maintained the skeleton of the plant body, their reduction may cause the reduction in other matabolic activities. In the present study the spray of different chemicals increased fibre contents under both the sprays but in first spray the increase was up to 48 h in all chemicals except in the plants treated with Regent, however the results were some what different in second spray where induction in fibers continued up to 96 h. The increase up to 48 h may be due to their beneficial effect on photosynthesizing organ which reduced after 48 h while in second spray it remained up to 96 h which may be due the presence of some concentrations of the chemicals which busted up their effect and after one week their concentration again reduced and reduction in fibers recorded. This means a critical concentration is required to stimulate the metabolic organs which reduce after 48 h during first spray and after one week in second spray.

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