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A Preliminary Study on Risk Analysis of Pesticides to Insectivorous Birds Inhabiting Cotton Based Agro-Ecosystem of Punjab, Pakistan

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Abstract: Effect of pesticides was studied by estimating the cholinesterase (ChE) enzyme activity in two insectivorous bird species *i.e*. jungle babbler *(Turdoides striatus)* and Indian wren warbler *(Prinia subjlava)* inhabiting the cotton based agro-ecosystem of Multan and the agro-ecosystem of Chakwal, an area where pesticide use is believed to be at very low scale. Morphometric data of the sampled populations revealed comparatively larger body weights in the birds captured from the control area as compared to those of the cotton area. There was suppression in the brain ChE enzyme activities in both the bird species captured during the cotton season as compared to the other sets of data *i.e.* non-cotton season and control values. The inhibition of ChE enzyme activity in jungle babbler was at 10.2% (cotton vs non-cotton), 29.6% (non-cotton vs control) and 36.8% (cotton vs control). In Indian wren warbler this inhibition was 6.2% (cotton vs non-cotton), 39.2% (non-cotton vs control) and 42.9%. (cotton vs control). The present levels of ChE inhibition in jungle babbler and Indian wren warbler provided evidence that insectivorous birds inhabiting the cotton based agro-ecosystem of Punjab are at considerable level of threat from the pesticides in use. This preliminary report suggest further investigations on determination of pesticide residues level in the avifauna, reproductive potential and viability of the insectivorous bird species in this ecosystem.

Key words: Insectivorous birds, cotton, pesticides, cholinesterase, Pakistan

INTRODUCTION

In Pakistan, the use of pesticides at commercial scale was introduced in 1954 (Jabbar and Mallick, 1994) and since then, these are increasingly applied to cotton, fruits, vegetables and cereal crops to obtain high yields. Approximately 54% of the total pesticide, being marketed, is used on cotton and the rest on other crops *i.e*. fruits, vegetables, paddy, tobacco, sugarcane etc. (Khan *et al.,* 2002). The pesticides' use, especially, in the cotton growing areas of the country has destroyed the bio-control agents and therefore the populations of natural enemies of the insect pests have declined up to 90% during the decade of 1990s (Hasnain, 1999). The high use of pesticides has contaminated the soil and water in the cotton growing areas of Punjab (Jabbar *et al.,* 1993). Studies conducted on cholinesterase (ChE) enzyme activity in women cotton pickers revealed that the inhibition of this enzyme in 25% (22 of 88) females in Multan (Masud, 1991) and in 42% (of 40) women in Multan and Bahawalpur (Tahir, 2000) was in dangerous range. The blood samples of 22 out of 25 cotton pickers from Multan area were reported to contain residues of pesticides belonging to the organophosphate, carbamate and chlorinated groups (Masud and Parveen, 1998).

There are about 55 passerine bird species found in agro-ecosystem of main cotton growing areas of Punjab. Reports based on general observations indicate that almost all the 55 species are either fully or partially insectivorous in their feeding habits. The proportion of insects in the diet of these birds varies with the seasons, growth stages of crops and development stages of the birds (Roberts, 1992). In a recent study, Hussain and Afzal (2005) recorded a total of 32 bird species including 31 Passeriformes and one Coraciiformes from the croplands of Multan, of which 23 were resident while the remaining 9 were the migrant. The total estimated bird density was 14.6 birds/ha. More than one third (37%) of the bird species exclusively derived their food from insect source. Insects belonging to the orders Hymenoptera and Hemiptera formed the major proportion of arthropod based food (Hussain and Afzal, 2005).

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Based on information generated by Hussain and Afzal (2005), the present study was designed to investigate effect of pesticides on two insectivorous bird species *i.e.* jungle babble *(Turdoides striatus)* and Indian wren warbler *(Prinia subjlava)* inhabiting the cotton based agro-ecosystem of Multan. Measurement of cholinesterase (ChE) activity in brain was used to provide the evidence of exposure of these species to the pesticides. The output of this study would provide a scientific base for further investigations of adverse effects of pesticides on bird bio-diversity in the cotton tract and would improve our knowledge about the extent to which the birds suffer due to the loading of the agro-ecosystem with pesticides.

MATERIALS AND METHODS

The ChE test samples were collected within 50 km radius in the surroundings of Multan city (30° 12'N 71°28'E), Figure 1. The area under cultivation reflects two main crops *i.e.* cotton and wheat mixed with other grain and fodder crops such as rice, maize, millet, sugarcane, barseem, lucerne, mustard etc. The mango and citrus orchard were frequently distributed and seemed an essential part of this agro-ecosystem. The control samples for the ChE study were collected from the agro-ecosystem of Chakwal (32° 56'N 72° 51'E), a central district of Pothwar plateau; about 500 km in the north of Multan (Fig. 1). The agriculture in the Pothwar area was based on wheat, groundnut and millet crops and exclusively dependent on natural rains. The use of pesticide in this area is at very low scale in comparison to the cotton belt of Multan in southern Punjab. Identification of the bird species was decided following Ali (1979) and Roberts (1992).

The birds were shot dead using air gun during cotton crop and pesticide spray season (June through September) and non-cotton season (November through April) from the Muhan district while the control samples were collected during the concurrent periods from agro-ecosystem of Chakwal district. A bird shot dead in the field was immediately dissected to separate the head from the body and the head was sealed in a small plastic bag. Each bag was tagged with information recorded on bird species, body weight, sex, habitat and date of collection. The samples were immediately stored in ice chest and were transported to the laboratory in frozen condition. In the laboratory the samples were stored in deep freezer and processed as soon as possible. A spectrophotometric method for determination of cholinesterase (ChE) activity in brain tissues of the birds (Trudeau and Cartier, 2000) was used to carry out this study.

Fig. 1. Map of Pakistan indicating (■) the study sites *i.e.* cotton based agro-ecosystem, Multan and Pothwar agro-ecosystem, Chakwal (control site).

RESULTS

The descriptions of bird species collected for estimation of cholinesterase (ChE) enzyme activity in brain tissues are summarized in Table I. The average body weight of the jungle babbler during the cotton spray season (63.3g) was less than that recorded during the non-cotton season in the same area (67.2g) and that of the control area (66.8 g). The body weights of Indian wren warblers captured from cotton area (6.4-6.7g) was less than that recorded from the Pothwar croplands (9.3g). The ratios of the body weights to brain weights in jungle babbler were estimated as: cotton season (1:0.017); non-cotton season (1:0.018); control (1:0.02). These ratios for the Indian wren warblers were: cotton season (1:0.038); non-cotton season (1:0.044); control (1:0.04).

Table I.- Level of cholinesterase (ChE) enzyme activities estimated in two bird species captured during cotton and non-cotton seasons in Multan area and agro-ecosystem of Chakwal.

Bird species	Bird sample	Sex	Sample size (n)	Body weight (g)	Brain weight (g)	AChE activity (µmol/min/g)
Jungle Babbler	Cotton season	Female	30	62.73±0.96	1.09±0.05	25.37±1.35
		Male	17	64.29±l.46	1.1l±0.06	27.10±1.49
		Both	47	63.30±0.81	1.10±0.04	25 .99± 1.0 1
	Non- cotton season	Female	12	66.83±1.39	1.20±0.08	29 .93± 1.24
		Male	6	67.83±2.41	1.28±0.07	26.93±3.09
		Both	18	67.17±1.19	1.23±0.06	28.95± 1.31
	Control	Female	6	69 .42±5.16	1.22±0.07	31.58±4.37
		Male	6	64. 17±4.07	1.35±0.08	50.72±4.75
		Both	12	66.79±3.23	1.28±0.05	41. 15±4 .22
Indian Wren Warbler	Cotton season	Female	9	6.33±0.37	0.23±0.03	27.92±2.35
		Male	11	6.36±0.15	0.25±0.02	25.27±2.27
		Both	20	6.40±0.20	0.25±0.02	26.46±l.62
	Non-cotton season	Female	16	6.75±0.21	0.30±0.01	28.25± 1.31
		Male	6	6.67±0.21	0.30±0.00	28.13± 1.73
		Both	22	6.73±l.16	0.30±0.00	28.2l±1.04
	Control	Female	4	9.25±0.14	0.35±0.03	44.65±2.04
		Male	3	9.33±0.67	0.33±0.03	48.69±7.74
		Both	7	9.29±0.26	0.34±0.02	46.38±3.23

Table II.- Statistical comparison of cholinesterase (ChE) activity values investigated in two bird species captured during cotton and non-cotton seasons in Multan area and agro-ecosystem of Chakwal.

Bird	Comparison	F –test			Level of Significance at (P<0.05)
Jungle Babbler	F (cotton vs non-cotton)	$F_{1,40}$	= 3.99;	P= 0.053	Significant
	M (cotton vs non-cotton)	$F_{1,21}$	= 0.00;	P= 0.968	Non-significant
	B (cotton vs non-cotton)	$F_{1,63}$	= 2.60;	P=0.112	Non-significant
	F (cotton vs control)	$F_{1,45}$	= 3.04;	P= 0.090	Non-significant
	M (cotton vs control)	$F_{1,21}$	= 40.63;	P= 0.000	Significant
	B (cotton vs control)	$F_{1,57}$	= 27.38;	P = 0.000	Significant
	F (non-cotton vs control)	$F_{1,16}$	= 0.23;	P= 0.642	Non-significant
	M (non-cotton vs control)	$F_{1,10}$	= 17.56;	P = 0.002	Significant
	B (non-cotton vs control)	$F_{1,63}$	= 10.46;	P = 0.003	Significant
Indian Wren Warbler	F (cotton vs non-cotton)	$F_{1,23}$	= 0.02;	P = 0.895	Non-significant
	M (cotton vs non-cotton)	$F_{1, 15}$	= 0.73;	P = 0.408	Non-significant
	B (cotton vs non-cotton)	$F_{1,40}$	= 0.86;	P = 0.359	Non-significant
	F (cotton vs control)	$F_{1,11}$	= 19.11;	P = 0.001	Significant
	M (cotton vs control)	$F_{1,12}$	= 16.75;	P = 0.001	Significant
	B (cotton vs control)	$F_{1,25}$	= 35.84;	P = 0.000	Significant
	F (non-cotton vs control)	$F_{1,16}$	= 33.73;	P = 0.000	Significant
	M (non-cotton vs control)	$F_{1,16}$	= 13.17;	P = 0.008	Significant
	B (non-cotton vs control)	$F_{1,27}$	=50.50;	P = 0.000	Significant

The overall data presented in Tables I and II and Figures 2 and 3 indicates suppression in the brain ChE enzyme activities of both the bird species (jungle babbler and Indian wren warbler) captured during the cotton season. The difference in the ChE enzyme values of the birds groups tested during the cotton season and non-cotton season of the same areas are statistically non-significant, $P>0.05$ (Table II). However, the enzyme activities in both the birds' species captured from cotton-based agro-ecosystem (Multan area) are significantly lower ($P<0.05$) than those tested from the Pothwar (control) area (Table II).

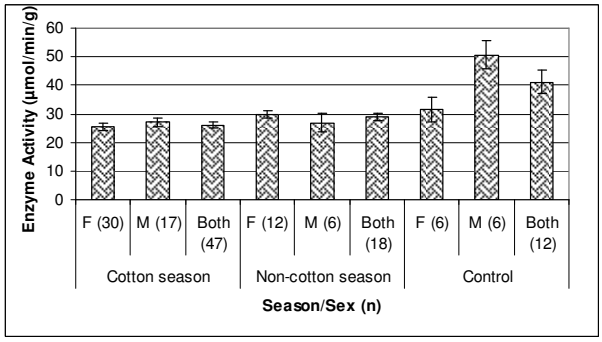

Fig. 2. Cholinesterase (ChE) activities in jungle babbler captured during cotton and non-cotton season in Multan area and from croplands of Chakwal, Pothwar Plateau (control).

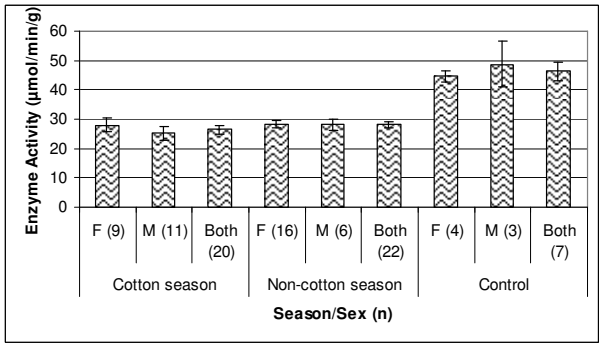

Fig. 3. Cholinesterase (ChE) activities in Indian wren warbler captured during cotton and non-cotton season in Multan area and from croplands of Chakwal, Pothwar Plateau (control).

In jungle babbler there is a 10.2% decrease in the ChE in the birds captured during the cotton season as compared to those of non-cotton season. This decrease is calculated to 36.8% when compared with the ChE values of the control group. Similarly the decrease in the ChE of jungle babbler of non-cotton season in comparison to those of the Pothwar area (control) is 29.6%.

There was a 6.2% decrease in ChE of Indian wren warbler captured during the cotton season as compared to those of the non-cotton season. In comparison to the ChE values of the control birds this suppression was estimated to 42.9%. Similarly the decrease in the ChE of the non-cotton season warblers in comparison to those of the Pothwar (control) area was 39.2%.

DISCUSSION

The study by Hussain and Afzal (2005) provided evidence that at least five bird species, jungle babbler, common babbler, common myna, Indian wren warbler and small green bee eater had an important role in predation on insects in cotton based agro-ecosystem of Punjab. Among these, the Indian wren warbler and jungle babbler were of special significance as they were feeding directly on insects. Based on assumption that these species could have higher threat from the prevailing risks of pesticides (Jabbar *et al.,* 1993; Hasnain, 1999) in this agro-ecosystem, the present study was carried out to estimate the activity of cholinesterase (ChE) enzyme in these species during the cotton and non-cotton seasons in a cotton based agro ecosystem of Punjab and in an agro-ecosystem where there was less use of pesticides (Pothwar area).

Esterases have been widely used over the last 20 years initially to assess the exposure of spray operators and then wildlife to organophosphate and carbamate agricultural pesticides. They have been used to determine whether these chemicals have been the cause of wildlife casualties. Measurement of cholinesterase (ChE) activity in plasma and brain is used in wildlife toxicology research to detect exposure to pesticides. A depression in plasma or brain ChE activity below the normal levels of the species is often used as evidence of exposure and, in the case of field mortalities, the cause of death (Ludke *et al.,* 1975).

The present study revealed suppression in the ChE enzyme activities in two bird species (jungle babbler and Indian wren warbler) from the cotton area as compared to those of the non-cotton area, where there is assumed to be less use of pesticides. Previously, there was hardly any information available on possible adverse effects of pesticide on wildlife bio-diversity in Pakistan. However, Khan (2001) had reported the opinions of six farmers from cotton growing area of southern Punjab on the loss of avifauna with the increased use of pesticides.

Masud (1991) reported in a study conducted in Multan area that out of a total of 88 female cotton pickers only one was out of risk and 74% had blood ChE inhibition between 12.5-50% while 25% were in dangerous condition with ChE inhibition between 50-87.5%. In another study of ChE activity in blood samples of 40 female cotton pickers of Multan and Bahawalpur, Tahir (2000) found ChE activity in 10% females in normal range (88-100%), 16% in mild range (76-87%), 13% in moderate range (65-75%), 19% in considerable range (51-61%) and 42% in hazardous (00-50%) range.

It could be explained from the reports of Masud (1991) and Tahir (2000) that ChE inhibition above 50% level is considered in dangerous range. The present level of ChE inhibition at the levels of 30-37% in jungle babbler and 39-42% in the Indian wren warbler are sufficient to provide evidence that insectivorous birds inhabiting the cotton based agro-ecosystem of Punjab are at considerable level of threat from the pesticides in use. The present level of exposure could be effecting: ovarian reproduction, hatching, viability of eggs, nestling growth and adult survival (Rodenhouse and Holmes, 1992; Howe *et al.,* 1996).

The present evidence of adverse effects of the pesticide on bird biodiversity along with earlier reports from this area on ChE inhibition in female cotton pickers (Masud, 1991; Tahir, 2000), pesticide residues in their blood samples (Masud and Parveen, 1998) and contamination of soil and water (Jabbar *et al.,* 1993) are sufficient to believe how the pesticides are playing havoc with human health and other non-target organisms. Farmers' perceptions reported by Khan (2001) on reduction in bird diversity in this area also support the views of negative impacts of pesticides towards the bird bio-diversity. This preliminary study suggest further investigations on the determination of pesticide residues, effect on reproductive potential and viability of the insectivorous birds biodiversity inhabiting cotton based agro-ecosystems of Pakistan.

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