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Response of Surpunkha (*Tephrosia purpurea*) as a non conventional pesticide in Agriculture for eliminating unwanted fishes

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ABSTRACT

Several methods have been developed for increasing fish production; elimination of undesirable fish populations by using fish pesticides is the one method among them. The study has been conducted to replace the conventional pesticides by some herbal medicinal plant. The present experiment has been conducted to find out the effect of rotenone obtained from Surpunkha (Tephrosia purpurea) on climbing perch (Anabas testudineus). There are several plants available which are used as a source of fish poison rotenone. In the present study Surpunkha (Tephrosia purpurea) has been used as a source of rotenone. Tephrosia purpurea, commonly known as Surpunkha is a kind of herbal pesticide which can be used as a best substitute over other conventional pesticides for eliminating undesirable fishes because it is quite abundant in the subcontinent and the cheapest possible pest killer having the least hazard of pollution.

Keywords : Surpunkha (Tephorsia purpurea), Climbing perch (Anabas testudineus), fish poison, rotenone, non-conventional pesticides.

I.INTRODUCTION

Several methods have been developed for increasing fish production. By one method, undesirable fish populations are eliminated by using fish pesticides. Surpunkha (*Tephrosia purpurea*), a kind of herbal pesticide commonly known as "surpunkha" can be used as a best substitute over all other pesticides used for killing undesirable fishes. It contains rotenone which is a natural plant toxin and is acutely sensitive to fish at below 1ppm concentration. There are many reasons which favoured the selection of Surpunkha (*Tephrosia purpurea*) for the present study. It is quite abundant in sub-continent and the possible cheapest killer having the least hazard of pollution. Experiment has been conducted to find out the effect of rotenone obtained from Surpunkha (*Tephrosia purpurea*) on climbing perch (*Anabas testudineus)*. Several studies have been done on the effect of pesticide on peroxidase activity of *Anabas testudineus* (Kumar et al. 1973, Radhaih et al. 1987, Olson and Christonser 1980, Jash and Bhattacharya

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1988, Ghosh et al. 1989 and Guhathakurta and Bhattacharya 1988. The effect of pesticide on fish head kidney peroxidase activity and inhibition of thyroid hormone by pesticide was also studied (Mukherjee and Bhattacharya 1974, Bhattacharya et al. 1978).

II.MATERIALS AND METHODS

Surpunkha *(Tephrosia purpurea)* as a source of rotenone was obtained from herbal garden of Hajipur, Bihar (India) for study. These plants can also be seen in open field areas of Bhojpur or Champarana districts of Bihar. Climbing perch (*Anabas testudineus)* were regularly obtained from the local market/pond.

III.ENZYME PREPARATION:

The controlled and pollutant treated Climbing perch (*Anabas testudineus)* were sacrificed and Anterior and Posterior kidney region was dissected out, homogenized, centrifuged separately in Potter-Elvehjem and soluble supernatant fractions in each case was assayed with 0.05 M sodium phosphate buffer, pH 5.5 for the determination of the peroxidase activity. The homogenate was then subjected to 1000 g centrifugation. The supernatant was collected and used as the enzyme. Enzyme preparation from the pharyngeal region was the same as in case of Anterior and Posterior kidney region.

III.ASSAY OF THE PEROXIDASE ENZYME

The peroxidase activity was measured by following the increase in optical density (OD) at 470 nm using 1 cm light path, guaiacol as hydrogen donor. The reaction mixture contained 150 µ mole of guaiacol, H_2O_2 1:2 µ moles and enzyme preparation of suitable volume and water to make the final volume 3.0 ml. The peroxidase activity was determined by observing the change of optical density per minute per mg of protein. The results are verified by at least three experiments.

IV.PROTEIN ESTIMATION:

The protein was estimated according to the method of Lowry et al. (1951) using bovine serum albumin as the standard.

The experiment was done on the effect of Surpunkha (*Tephrosia purpurea)* on peroxide activity of fish Climbing perch (*Anabas testudineus)*. The observations and experiments were conducted for 06 hours LC_{25}, 12 hours LC_{25} and 24 hours LC_{25} at different time intervals. Different concentrations of LC_{25} were obtained in different time periods and the observations were made at LC_{25} doses.

So far as endocrine function is concerned the kidney in teleost is at great importance. It is known that the interregnal and chromaffin tissues are homologous to the mammalian adrenal cortex and medulla respectively. Oguri, (1960); Chavin and Kovacevic, (1961), and Kumar et al. (1973) demonstrated the *in vitro* peroxidase activity of a fish *Anabas testudineus.* Thyroid follicles in teleost are not assembled in a single gland but instead

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remains scattered throughout the sub-pharyngeal and para-pharyngeal area. It has also been reported that in several teleost thyroid follicles migrate extensively from the pharyngeal region, forming a large concentration in the Anterior kidney (Baker, 1958; Baker-Cohen, 1959; Chavin, 1956; Gurumani, 1971; Deshpande & Nmadkarni, 1973). Ottosen and Maunbach (1973) has worked on flounder kidney and reported Histochemical detection of peroxidase Peroxidase enzyme from teleost anaterior kidney is considered physiologically significant as reported by Kumar *et al.,* 1973. The Kidney has the ability to catalyze the synthesis of thyroid hormone (Bhattacharya et al., 1976). They (Bhattacharya et al., 1976) have also noticed an interesting phenomenon in connection with the sub cellular location of iodinating activity. Bhattacharya and Datta (1971) and Bhattacharya et al. (1976) reported Thyroid hormone synthesis by pharyngeal & Anterior kidney cell free preparation from a teleost fish *Clarias batratchus.*

V. POLLUTANTS AND PEROXIDASE ACTIVITY FROM THYROID FOLLICLES OF ANABAS TESTUDINEUS:

The present investigation was undertaken to observe the alteration of peroxidase activity due to *Tephrosia purpurea*. The peroxidase activity from three different sources pharyngeal region, Anterior and Posterior kidney region was observed and also seen the effects of *Tephrosia purpurea*. As peroxidase plays a significant role in the biosynthesis of thyroid hormone, the inhibition of thyroid function may also be mediated through the peroxidase function i.e. oxidation of iodide and incorporation of oxidized iodine into tyrosine moiety which eventually leads to the formation of thyroxin. It is therefore seems to be relevant to look into this parameter for understanding the pollutants effect on thyroid follicles in Anterior and Posterior kidney. A comparative study of peroxidase activity from anterior kidney and pharyngeal region has been done. Attempt has also been made to determine the accumulated concentration of peroxidase responsible for the inhibition and to find out certain means by which the effect of pesticide could be reversed.

VI. ALTERATIONS IN PEROXIDASE ACTIVITY OF ANABAS TESTUDINEUS DUE TO TEPHROSIA PURPUREA:

Anterior and Posterior kidney homogenate (control and treated) of *Anabas testudineus* was added to the reaction mixture as discussed above. Peroxidase activity was observed in the case of anterior kidney where as Posterior kidney showed less activity than that of the anterior kidney. Absence of peroxidase activity was noted by the addition of boiled enzymes (both the Anterior and Posterior kidney homogenate were boiled separately at 100^{0} C in a water bath for 10 min. to the respective reaction mixture). Similarly peroxidase activity of pharyngeal region was also observed.

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The results of the experiment for the determination of LC_{25} concentration have been determined. The controlled and pollutants treated *Anabas testudineus* (06h, 12h, 24h and 48 h LC_{25}) were sacrificed and Anterior and Posterior kidney region was dissected out, homogenized, centrifuged separately and soluble supernatant fractions in each case was assayed for the determination of the peroxidase activity. In the case of the Posterior kidney the inhibition was little more than that of the Anterior kidney. Inhibition of pharyngeal peroxidase activity due to the effect of *Tephrosia purpurea* was observed in similar manner as in the anterior kidney. Following results have been obtained.

Table: 1 Effect of *Tephrosia purpurea* (leaf) on pharyngeal region of *Anabas testudineus* (at 24h LC_{25})

System	Δ OD/min/mg of Protein	% of inhibition
Control	4.3	
Treated	2.5	44.86%

Table: 2 Effect of *Tephrosia purpurea* (pod) on pharyngeal region of *Anabas testudineus* (at 24h LC_{25})

System	Δ OD/min/mg of Protein	% of inhibition
Control	4.3	
Treated	2.7	39.53

VII. EFFECT ON ANTERIOR KIDNEY SUPERNATANT PEROXIDASE ACTIVITY:

In case of leaf of *Tephrosia purpurea*, no significant changes were noticed. The major difference which has been noticed was 4.87% of inhibition at 06h LC_{25}, which was quite less than *Derris elliptica*, other commonly used herbicide, where it was 17.07%. The other important aspect noticed was the highest percentage of inhibition (30.48%) at 24h LC_{25}. The percentage of inhibition observed was 4.87, 29.26, 30.48, 24.39 and 21.26 at 06h, 12h, 24h, 36h and 48h LC_{25} respectively

The similar considerable change was observed in case of pod of *Tephrosia purpurea.* Again the highest inhibition percentage (32.92%) was seen at 24h LC_{25}. Further a significant decrease in the percentage of inhibition was seen which were 29.26 and 26.82 at 36h and 48h LC_{25}.

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Table 3: Effect of *Tephrosia purpurea (leaf)* on the anterior kidney supernatant peroxidase activity.

S. No.	System		Δ OD/min/mg of Protein	% of inhibition
	Posterior Kidney			
1	Control		4.1	
2	Treated (*Tephrosia sp.)*			
	(i)	06h LC_{25}	3.90	04.87%
	(ii)	12h LC_{25}	2.90	29.26%
	(iii)	24h LC_{25}	2.75	30.48%
	(iv)	36h LC_{25}	3.10	24.39%
	(v)	48h LC_{25}	3.20	21.26%

Addition and assays were same as described for Anterior and Posterior kidney (Control and treated both)

Table 4: Effect of *Tephrosia purpurea (pod)* on the Anterior kidney supernatant peroxidase activity.

S. No.	System		Δ OD/min/mg of Protein	% of inhibition
	Posterior Kidney			
1	Control		4.1	
2	Treated (*Tephrosia sp.)*			
	(i)	06h LC_{25}	3.60	12.19%
	(ii)	12h LC_{25}	3.10	24.29%
	(iii)	24h LC_{25}	2.75	32.92%
	(iv)	36h LC_{25}	2.90	29.26%
	(v)	48h LC_{25}	3.00	26.82%

Addition and assays were same as described for Anterior and Posterior kidney (Control and treated both)

VIII. EFFECT ON POSTERIOR KIDNEY SUPERNATANT PEROXIDASE ACTIVITY:

It has been seen that the percentage of inhibition was much more in posterior kidney in comparison of anterior kidney in presence *Tephrosia purpurea*. When the same condition was applied in case of leaf of *Tephrosia purpurea*, no significant changes was noticed. The percentage of inhibition observed was 72.72, 81.81, 90.90, 81.81 and 72.72 at 06h, 12h, 24h, 36h and 48h LC_{25} respectively. The similar considerable change was observed in case of pod of *Tephrosia purpurea.* Again the highest inhibition percentage (90.90%) was seen at 24h LC_{25}. Further a significant decrease in the percentage of inhibition was seen which were 72.72 and 63.63 at 36h and 48h LC_{25}.

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Table 5: Effect of *Tephrosia purpurea (leaf)* on the Posterior kidney supernatant peroxidase activity.

S. No.	System		Δ OD/min/mg of Protein	% of inhibition
	Posterior Kidney			
1	Control		1.1	
2	Treated (*Tephrosia sp.)*			
	(i)	06h LC_{25}	0.3	72.72 %
	(ii)	12h LC_{25}	0.2	81.81%
	(iii)	24h LC_{25}	0.1	90.90 %
	(iv)	36h LC_{25}	0.2	81.81%
	(v)	48h LC_{25}	0.3	72.72%

Addition and assays were same as described for Anterior and Posterior kidney (Control and treated both)

Table 6: Effect of *Tephrosia purpurea (pod)* on the Posterior kidney supernatant peroxidase activity.

S. No.	**System**		**Δ OD/min/mg of Protein**	**% of inhibition**
	Posterior Kidney			
1	Control		1.1	
2	Treated (*Tephrosia sp.)*			
	(i)	06h LC_{25}	0.3	72.72 %
	(ii)	12h LC_{25}	0.2	81.81%
	(iii)	24h LC_{25}	0.1	90.90 %
	(iv)	36h LC_{25}	0.3	72.72%
	(v)	48h LC_{25}	0.4	63.63%

The noticeable observation was the high percentage of inhibition in case of leaves at 36h and 48h LC_{25} than that of pod of Tephrosia purpurea. In both the cases, the highest percentage of inhibition was found 90.90% at 24h LC^{25}. When a graph was plotted on the percentage of inhibition with respect to time, the following semicircle slope was formed.

IX. EFFECT OF *TEPHROSIA* PURPUREA ON PHARYNGEAL REGION OF ANABAS TESTUDINEUS*:*

When the effect on pharyngeal region was observed, it has been found that the highest percentage of inhibition was 44.86% in Tephrosia purpurea (leaf). In case of pod of Tephrosia purpurea, the percentage of inhibition noticed was 39.53%.

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X. RESULT AND DISCUSSION:

Surpunkha (*Tephrosia purpurea*) contains rotenone which is a natural plant toxin and is acutely sensitive to fish at below 1ppm concentration. For determining the sub-lethal concentration of rotenone obtained from Surpunkha on climbing perch (*Anabas testudineus)*, controlled and pollutant treated *Anabas testudineus* (06h, 12h, 24h and 48 h LC_{25}) were sacrificed and anterior and Posterior kidney region was dissected out, homogenized, centrifuged separately and soluble supernatant fractions in each case was assayed for the determination of the peroxidase activity. It has been observed that the percentage of inhibition was much more in posterior kidney in comparison of anterior kidney in the presence of *Tephrosia purpurea.* In case of anterior kidney the highest percentage of inhibition was 30.48% at 24h LC_{25}. The percentage of inhibition observed was 4.87, 29.26, 30.48, 24.39 and 21.26 at 06h, 12h, 24h, 36h and 48h LC_{25} respectively. The similar considerable change was observed in case of pod of *Tephrosia purpurea.* Again the highest inhibition percentage (32.92%) was seen at 24h LC_{25}. Further a significant decrease in the percentage of inhibition was seen which were 29.26 and 26.82 at 36h and 48h LC_{25}. In case of posterior kidney the percentage of inhibition observed was 72.72, 81.81, 90.90, 81.81 and 72.72 at 06h, 12h, 24h, 36h and 48h LC_{25} respectively. The noticeable observation was the high percentage of inhibition in case of leaves at 36h and 48h LC_{25} than that of pod of *Tephrosia purpurea.* When the effect on pharyngeal region was observed, it has been found that the highest percentage of inhibition was 44.86% in *Tephrosia purpurea* (leaf). In case of pod of *Tephrosia purpurea*, the percentage of inhibition noticed was 39.53%. Studies reveals that it is a non-competitive inhibitor in nature and the effect of this pollutant is only due to changes in the peroxidase enzyme protein. The study concludes that *Tephrosia purpurea* can be used as a best suitable, easily available and least polluting non-conventional pesticide to replace the conventional pesticide.

XI. CONCLUSION

Tephrosia purpurea is a non-competitive inhibitor in nature, so it clearly shows that the effect of this pollutant is only due to changes in the peroxidase enzyme protein. The reversal might have been resulted due to non-oxidative properties of selenium. As already discussed earlier, this study was made to suggest the use of non-conventional pesticide in the agriculture. *Tephrosia purpurea* is quite abundant in the subcontinent and the cheapest possible pest killer having the least hazard of pollution. Effect of xenobiotics is mainly focused on effects elimination, bioaccumulation and bio-concentration factors and its remedial measure. It is the chief constituent of the present day pollution research.

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