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BIOCHEMICAL AND ELECTROPHORETICAL STUDIES OF THE EFFECT OF SOME CHEMOSTERILANTS ON THE ENZYMES OF *AEDES AEGYPTI* (L.) LARVAE

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ABSTRACT

In vivo effect of standard and prospective chemosterilants on some enzyme. is reported. Colorimetric and histochemical findings indicate that alkaline pha phatase is inhibited by shikonin, hempa, tepa and shikonin angelate. Cholinesterase is inhibited by all the compounds while diphenol oxidase is not inhibited. These findings have been discussed in the light of the earlier observations.

Introduction

Enzyme activity is affected by pesticides and consequently esterases have been shown to be inhibited (Kasai and Ogita, 1965; Voss and Matsumura, 1965; Brady and Sternberg, 1969; Naqvi *et a*l., 1969a,b; Zubairi and Naqvi, 1972; *Rashid et al*, 1973; Booth *et al.,* 1973; Gorgees *et al,* 1978; Rashan *et al,* 1979; Naqvi *et al,* 1982). While effects of neopesticides on esterases have been reported by Dawood *et al* (1975), Gorgees *et al* (1978), *Rashan et al* (1979) and Naqvi *et al.* (1982, 1984a,b) in different insects.

Although work on some aspects of the new plant products (e.g. shikonin and shikonin angelate) has been done like the toxicity and effect on enzyme pattern of *Drosophila* sp. (Dawood *et al*, 1975), toxicity against *Aedes aegypti* larvae (Sulaiman *et al*, 1978), morphological abnormalities in *Aeries aegypti* (Sulaiman and Naqvi, 1978) and affect on the phosphatases of *Aedes aegypti* pupae (Naqvi *et al,* 1982, 1984b), much remains to be done on the effects of these compounds before using them as a neopesticide.

Therefore, during the present studies, *in vivo* effect of these new naphthaquinones (shikonin and shikonin angelate) isolated *from Alkana hirsulissuma* (Afzal and Towfeeq, 1975) and the standard ones (tepa and hempa obtained by the courtesy of Dr. A.B. Borkovec) was observed on the activity of esterases, colorimetrically and electrophoretically.

Materials and Methods

A standard strain (Rockefeller susceptible strain) was reared according to Porter and Kozuchi (1961) under controlled conditions for obtaining larvae of known age and size. Two new compounds, 5,8-dihydroxy-2-(4-methyl-pent-3-enyl)-1,4-naphthaquinone (i.e. shikonin) and 1-(5,8- dihydroxynaphthaquinone-2-eyl)-4-methyl-pent-3-enyl-2-methyl crotonate (i.e. shikonin angelate) were prepared by Afza1 and Towfeeq (1975). Two known chemosterilants, hemamethyl phosphoramide or hempa and tris (1-aziridinyl) phosphine oxide or tepa were obtained by the courtesy of Dr. A.B. Borkovec, ERS, USDA, Gainesville, USA. One percent stock solution of each compound was prepared in ethanol, which was diluted by distilled water according to the requirement.

Treatment of the early 4th instar larvae was done according to WHO standard method. Doses used were 25 ppm for shikonin and shikonin angelate and 5.0 ppm for tepa and hempa and further procedure was adopted as described by Suhdman *et al* (1978).

Biochemical estimations

a) Protein quantity in all the samples was estimated according to the method of Lowry *et al.* (1951) and a standard calibration curve was prepared by using serum bovine albumin.

b) Alkaline phosphatase quantity was determined according to the method of Ashrafi *et al* (1969). Optical density was measured at 400 um on a Pye Unlearn SP 600 spectrophotometer.

c) Cholinesterase activity was measured accordⁱng to the method of Hestrin (1949) in all the samples. Standard curve of acetylcholine chloride was prepared to estimate arbitrary units of enzyme in the samples per mg protein.

d) Diphenol oxidase activity in the samples was estimated according to the method of (Carlson *et al.* (1962). Enzyme quantity is expressed in terms of arbitrary dopa units (O.D. 0.1 = 100 dopa units) released per mg protein. Histograms were prepared for all estimations by keeping activity in the control as 100%, for comparison with other samples.

Histochemical localization

Electrophoresis was performed on Joyce and Lobel electrophoresis apparatus and the procedure followed was as reported by Naqvi and (Carlson (1979) for diphenol oxidase localization (black bands) and Naqvi (1981) for alkaline phosphatase localization (red hands). 1nalization of cholinesterase was done

accord[i]ng to Guilbault *et al.* (1970) by using indoxyl acetate (green bands). Simultaneously blue protein bands were obtained by using amido black, in each case. Measurements of the bands were done immediately after the experiments and relative mobility rate was calculated for comparison, which is given in zymograms. The bands have been marked by alphabets (A-N) and intensity of the bands was noted visually. It has been expressed as high, moderate, low and poor.

Results and Discussion

During the present work, in *vivo* effect of chemosterilants of plant origin and the standard ones has been studied on non-specific esterases (alkaline phosphatase), cholinesterase and phenol oxidase (*O*-diphenol oxidase), colorimetrically and histochemically.

Biochemical estimation

Calorimetric estimations of enzymes indicate that overall the standard and the new compounds inhibit the non-specific esterase (alkaline phosphatase) to a lesser or greater extent (Fig. 1-A). Chol[i]nesterase is inhibited by all the compounds to greater extent, so much so that the activity by hempa is reduced to 10% only (Fig. 1-B). However, the diphenol oxidase activity is affected only by shikonin to some extent (Fig. 1-C). Inhibition of phosphatase by pesticides was reported by Naqvi *et al.* (1969a,b), Rashid *et at* (1973) and Leering (1973) while Naqvi *et al.* (1976) reported some inhibition of this enzyme in *Musca domestica* by tepa and thiourea (neopesticide). On the contrary, Oureshi *et al* (1983) reported almost no effect on the activity of add and alkaline phosphatase by using 11-IA's (ZR-512, ZR-515 and ZR-519), also belonging to neopesticide group. However, Shaft et al. (1986) reported inhibition of alkaline phosphatase by dimilin (IGR) and Naqvi *et al.* (1989) also by dimilin. Cholinesterase was inhibited by the chemosterilants. Inhibition of this enzyme has been reported by several workers by using pesticides (Voss and Matsumura, 1965; Brady and Sternberg, 1967). However, inhibition by chemosterilants has been reported by Naqvi *et al.* (1976). Phenol oxide was not inhibited, although it is concerned with melanization and chitin inhibition and should have some effect on it. In this group oxidases have been correlated with pesticide metabolism (Matsumura and Hayashi, 1966; Khan *et al*, 1973; Stanton and Khan, 1973). Moreover, Naqvi and Rab (1985) have reported inhibition of this enzyme in *Musca domestica* by using dimilin (IGR).

Histochemical localization

To confirm the calorimetric findings, electrophoresis was performed and the isozymes were localized in treated and non-treated samples. Histochemical findings show that there are five isozymes of phosphatase, one band of cholinesterase and one of phenol oxidase, but zymogram in Fig. 1-D shows in all 14 protein bands (A-N) of

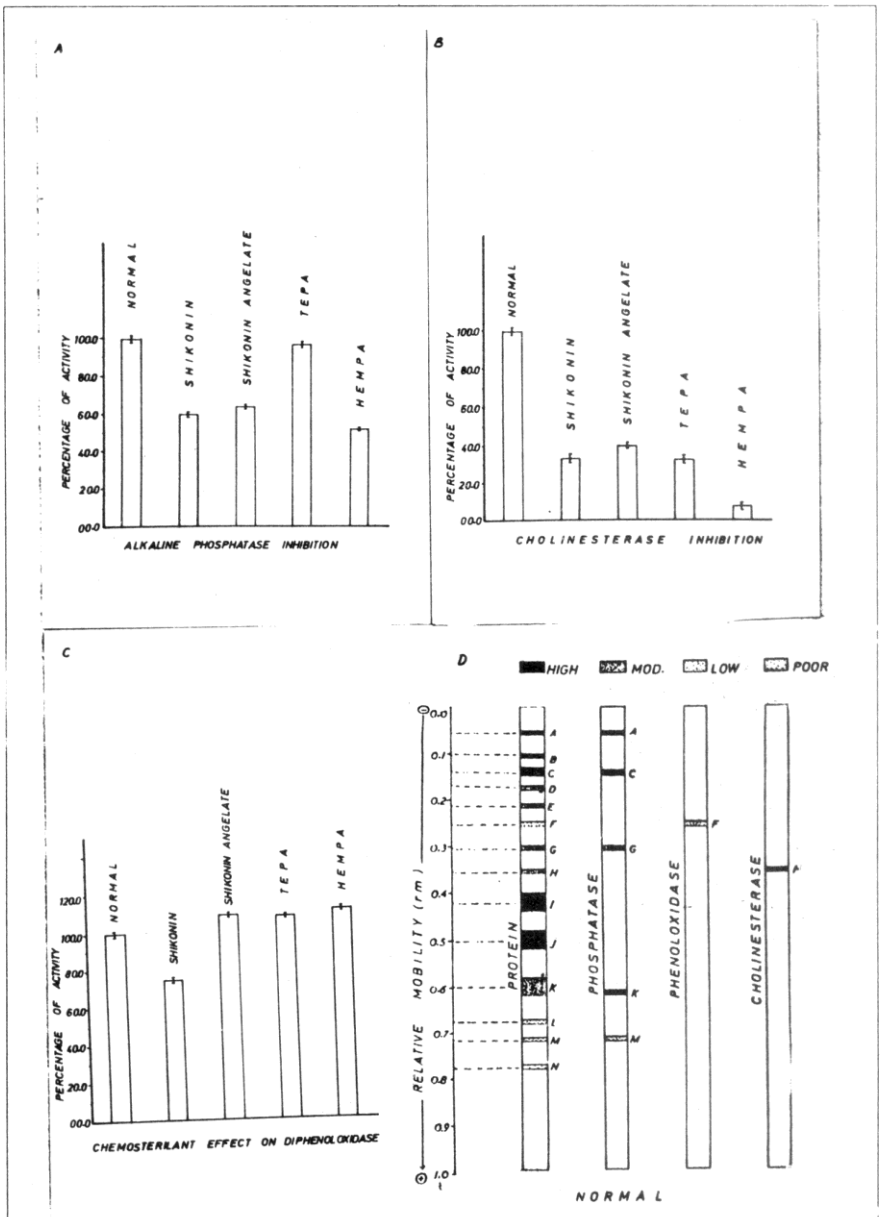

Figure 1. Effect of chemosterilants on the activity of enzymes. 1-A. Alkaline phosphatase, 1-B. Cholinesterase, 1-C. Diphenoloxidase, 1-D. Normal protein pattern.

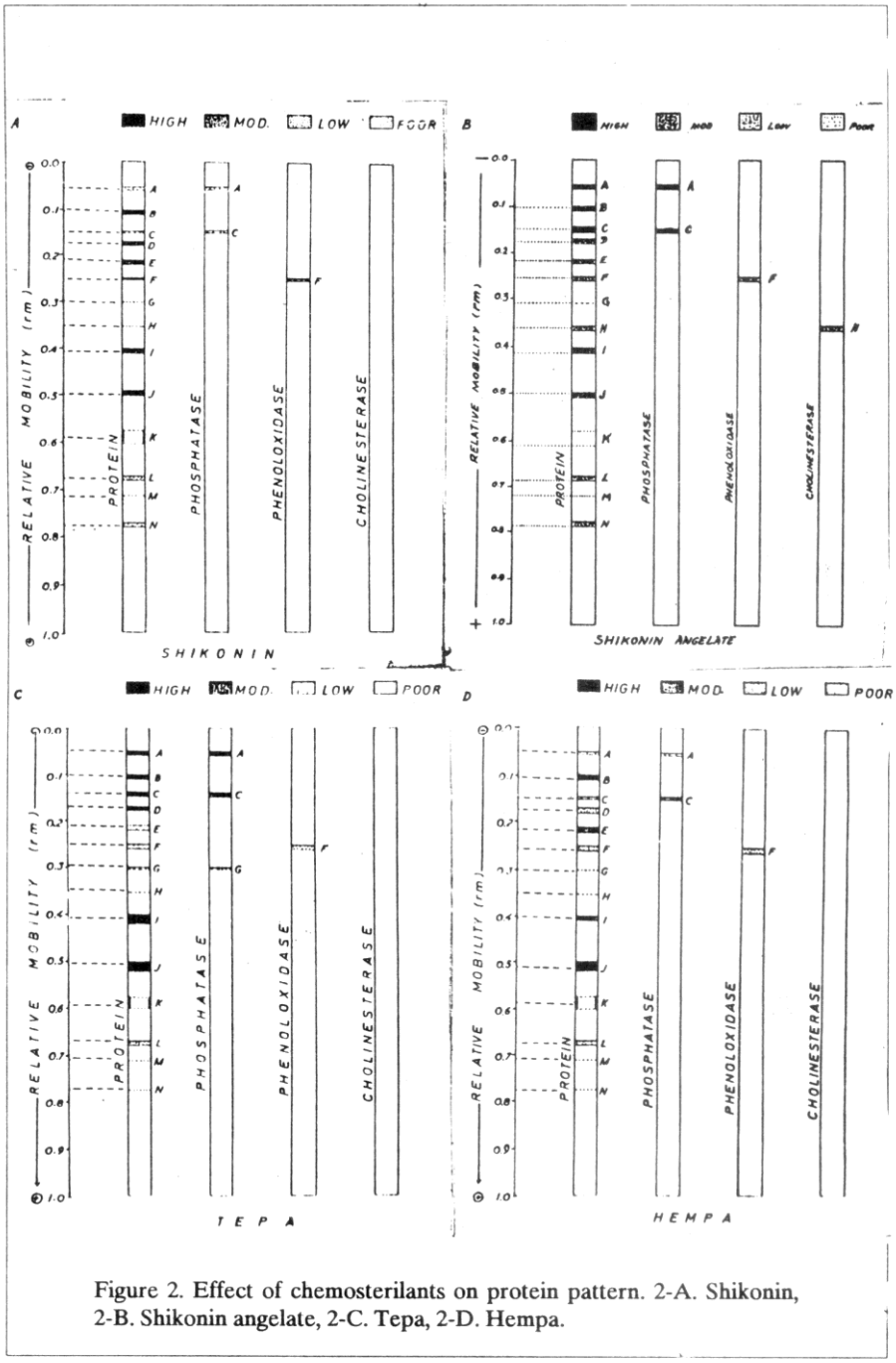

Figure 2. Effect of chemosterilants on protein pattern. 2-A. Shikonin, 2-B. Shikonin angelate, 2-C. Tepa, 2-D. Hempa.

the untreated sample, which means that there are other proteins also in addition to the above mentioned enzymes. Among them shikonin (2-A) inhibited all the isozymes of phosphatase (A and C to a lesser extent and G, K, M completely), followed by hempa having almost similar inhibition (Fig. 2-D). This confirms the colorimetric findings. Hempa was followed by shikonin angelate (Fig. 2-B) which had no effect on bands A and C but completely inhibited isozymes G, K and M, again confi[r]ming the sequence of inhibition by colorimetry. Tepa (Fig. 2-C) inhibited only two isozymes (K, M) which confirms the calorimetric results.

Cholinesterase (band H) was completely inhibited in all the cases except shikonin angelate which show slightly less inhibition (Figs. 2A-D). This again confirms the colorimetric estimations. Because there is complete inhibition of this enzyme by shikonin, tepa and hempa, the sequence of inhibitory effect is not clear. However, least inhibition by shikonin angelate is in concurrence with the colorimetric findings, which show the sequence as hempa, tepa, shikonin and shikonin angelate.

Diphenol oxidase was not inhibited except in the case of shikonin (2-A) which shows poor activity as compared to the normal gel. This again confirms the colorimetric results, although it differs from previous reports (Matsumura and Hayashi, 1966; Khan et at, 1973; Naqvi and Rob, 1985).

Inhibition of phosphatases by pesticides has been reported by several workers (Ogita, 1%1; Kasai and Ogita, 1965; Booth *et at*, 1973; Dawood *et al*, 1975). However, inhibition by chemosterilants has been reported by Dawood *et al* (1975), Naqvi *et al.* (1976, 1979), Gorgees *et al* (1978), Rashan *et* al. (1979) and Shafi *et al.* (1986). The present finding confirms earlier reports. Inhibition of cholinesterase has been reported by Naqvi *et al.* (1976), and our findings confirm these reports. Inhibition of non-specific esterase (alkaline phosphatase) and cholinesterase is evident and confirms the earlier findings. However, this point is not clear whether the inhibition of the activity is due to toxicity effect of the compounds or due to some biochemical disturbance. Moreover, it is not clear whether this inhibitory effect has some relation with some particular groups in the molecule of the compounds. This needs further investigation by using various substituted analogues of shikonin. However, one thing is clear that shikonin (a plant product) and it's analogue can act and compete as a chemosterilant.

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