Metabolism of endosulfan in the Indian honey bee

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Summary. Metabolism of topical endosulfan in the Indian honey bee, *Apis cerana indica* F., was studied. The two endosulfan isomers were found to be interconvertible in bees. The products of metabolism were endosulfan sulfate, diol, ether, hydroxyether, lactone and two unknown compounds. Endosulfan diol, hydroxyether and lactone were found to be conjugated in excreta of the treated bees.
Key words. Apis cerana indica; endosulfan metabolism.

Technical endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) is a mixture of two stereoisomers, namely endosulfan I (m.p. 109 °C) and endosulfan II (m.p. 213 °C) (fig. 1). It is a bio-degradable insecticide and is metabolized through oxidative and hydrolytic pathways[2]. Its metabolism was faster in endosulfan-resistant house flies than in susceptible ones[3].

Endosulfan is being recommended for large-scale use on fruits and vegetables in India, because it is considered to be relatively safe for bees[4,5]. Surprisingly, there is little information on its metabolism in bees. In our earlier studies, the toxicity of endosulfan for bees was found to be enhanced 4–5-fold when it was mixed with piperonyl butoxide, an inhibitor of the microsomal oxidase enzyme system. These results suggest that endosulfan is metabolized through the microsomal oxidase enzyme system in honey bees[5]. Begliomini et al. reported the presence of a metabolite of endosulfan in the field-contaminated forager of *A. mellifera*[6]. This communication is a report on the metabolism of topical endosulfan in *Apis cerana indica* F. bees. A metabolic pathway for endosulfan is being proposed on the basis of these observations.

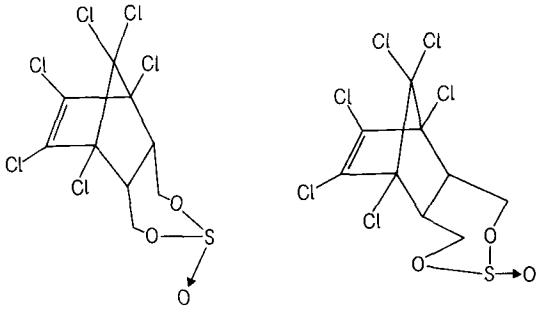
Materials and methods. The foragers of *A. c. indica* were treated topically with 2 µg each of endosulfan I and II, as already described[5]. Endosulfan and its metabolites were extracted from the tissues of surviving bees, and the excreta collected in glass jars after 2, 4, 8, 16 and 24 h of treatment were analyzed. The excreta of 15 bees were washed out from a jar with hexane and concentrated to 0.5–1 bee equivalent/ml; 1–2 µl of the extract were injected into the gas chromatograph without clean-up. Tissues were extracted by taking surviving bees in 250 ml Erlenmeyer flasks and rinsing them thrice with 15 ml batches of acetone; they were then macerated with pestle and mortar and extracted three times with 15 ml acetone each. The volume of acetone extract was reduced to three bees/ml, maintained at −45 °C overnight and filtered through high flo-supercel at −45 °C. The container and supercel were further washed with 5 ml of chilled acetone. The acetone was completely removed by blowing a gentle stream of dry air at 40–45 °C and the residue was subjected to hexane acetonitrile partitioning as described by Rao[7]. Hexane phase, 1–3 bees/ml, 1–2 µl was injected into a gas chromatograph equipped with an electron capture detector (Ni^{63}). The parameters were: detector 350 °C, oven 215 °C, injection 250 °C and gas flow 38 ml/min. Quantification of endosulfan and its metabolites was done by fortifying control extracts with different concentrations, following a similar clean-up and assay procedure. Peak areas were recorded with the integrator for comparison with unknown samples. The results of GLC were confirmed by TLC. Spots were visualized under long-wavelength UV light. A sensitivity of 50 ng for endosulfan was recorded.

For water-soluble conjugates, tissues and feces residues after solvent extraction were hydrolyzed with 150 ml of 1 N HCl for

Composition of extracts from endosulfan-treated bees 16 h after application

No.	Identity	RRT*	Rf**
1	Endosulfan ether	0.24	0.60
2	Endosulfan hydroxyether	0.43	0.03
3	Endosulfan diol	0.66	0.14
4	Endosulfan lactone	0.85	0.00
5	Endosulfan I	1.00	0.74
6	Endosulfan II	1.31	0.43
7	Endosulfan sulfate	1.78	0.20
8	Unknown I	0.32	0.51
9	Unknown II	1.18	0.89

*Hewlett Packard hp 5840 A; column 10% OV 101 (1.2 m × 0.2 cm); RT with respect to endosulfan I. **Alumina containing rhodamine B; temperature 25 ± 2°C; 4% acetone in n-heptane. Authentic samples were available for comparison in 1–7.



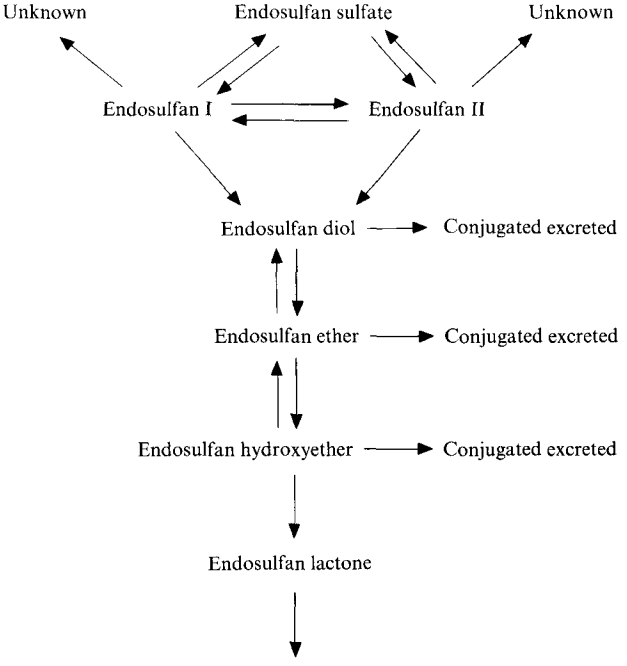


Figure 2. Proposed metabolic pathway of topical endosulfan in Indian honey bee, *A.c. indica*

2.5 h. Flasks were cooled and their contents were extracted thrice with hexane. Hexane was made acid-free by passing it through a mixture of anhydrous sodium sulfate and sodium bicarbonate. The hexane was then partitioned with acetonitrile and assayed as described. A similar technique was adopted for the study of the metabolism of endosulfan derivatives, with the difference that the time of exposure was 24 h.

Results and discussion. Analysis of endosulfan I- and II-treated bees revealed the presence of endosulfan ether, hydroxyether, lactone, endosulfan I, endosulfan II and two unknown compounds (table). The rate of metabolism of endosulfan was maximal in the first 2 h of treatment, in which 45–50% of the applied dose was metabolized. After 24 h 15–17% of the unmetabolized endosulfan could be extracted from tissues and 10–20% from excreta.

The products of endosulfan metabolism indicated both oxidative and hydrolytic pathways (fig. 2). The two isomers of endosulfan were found to be interconvertible, with the equilibrium of the reaction towards endosulfan I. The quantity of endosulfan sulfate obtained from endosulfan II-treated bees was twice that obtained from the endosulfan I-treated bees. These results support our earlier findings, that piperonyl butoxide synergized endosulfan II more than it synergized endosulfan I in bees[5]. Analysis (GLC and TLC) of endosulfan sulfate-treated bees indicated the presence of endosulfan I and II. These results are enigmatic, as earlier endosulfan sulfate could not be synergized in bees with Piperonyl butoxide[5]. Chopra and Mahfouz reported the presence of endosulfan I in endosulfan-sulfate treated tobacco leaves[8].

Endosulfan ether, diol and hydroxyether applied to bees separately broke up into the other two metabolites, showing their interconversion. In endosulfan lactone-treated bees, no metabolite was observed. However, the recovery of lactone was very poor (25%) after 24 h of treatment.

The conjugates of endosulfan diol, hydroxyether and lactone were significant in excreta and nonsignificant in tissues. It could be that the metabolites after conjugation are immediately excreted, or that the conjugation of metabolites takes place in the alimentary canal of bees rich in sugars.

The data on the rate of metabolism of endosulfan isomers in honey bees do not indicate any correlation with bee toxicity: nevertheless, the high rate of excretion of topical endosulfan suggests the possibility of its residues finding their way into honey through the honey stomach. The presence of endosulfan and its metabolites suggests that the feces could also contaminate wax in the hive and endanger the relatively susceptible brood. This important aspect needs further investigation. When residues of endosulfan in honey are estimated, the presence of its toxic metabolites produced by bees, namely endosulfan sulfate, ether and lactone, should also be taken into consideration.

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Pyridoxine application enhances nitrate reductase activity and productivity of *Vigna radiata*

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Summary. Pyridoxine, applied to seeds before sowing or by the spraying of a standing crop of moong *(Vigna radiata)* significantly enhanced leaf nitrate reductase activity (NRA). Leaf NRA levels, being significantly correlated with seed yield, can be utilized for predicting crop productivity and for adopting corrective measures, if necessary, at an early growth stage.
Key words. Vigna radiata; leaf nitrate reductase; seed yield; crop productivity.

Leguminous crops constitute a major source of vegetable proteins in tropical and subtropical countries. Simultaneously, they furnish to the soil inexpensive and nonpolluting nitrogen by symbiotic fixation. However, these crops have not received the attention they deserve in underdeveloped countries where protein malnutrition prevails on a large scale. To remedy the situation, it is desirable to increase the productivity of grain legumes in these countries and to improve the quality of their seeds.

Earlier studies in the authors' laboratory have established that pyridoxine (vitamin B_6) is effective as well as economical in increasing the yield of barley[2,3]. Pyridoxine has long been known to promote the growth of excised organs of various plant species[4–7]. Its presence in the culture medium promotes the uptake of glucose, nitrate and phosphate in excised roots of grasses[8]. Cotton seedlings have been reported to possess higher concentrations of nitrogen and phosphorus as a result of treatment of the seeds with pyridoxine[9].

Surprisingly, information on the effectiveness of pyridoxine application on the growth and yield of crops grown under field conditions is meagre[2,3,10]. However, all previous in vitro, pot culture and field studies indicate that pyridoxine invariably enhances root growth of the plants studied so far. This led us to suggest that, if this vitamin promoted root growth in legumes also, the absorption of nitrate and other nutrients would be enhanced by its application. Simultaneously, a larger surface area would be provided for *Rhizobium* infection. The consequent increase in the number of root nodules would be expected ultimately to benefit the host through enhanced nitrogen fixation. This could be of considerable practical significance for farmers as it would result in better growth and higher productivity of these hitherto neglected crops.

To test this hypothesis, two field experiments were conducted to study the effect of pyridoxine on nitrate reductase (E.C. 1.6.6.1) activity (NRA), growth and yield of *Vigna radiata* (L.) Wilczek var. K-851 (moong). The vitamin significantly enhanced NRA measured in leaves according to the method of Jaworski[11]. In addition, root growth, root nodule number and seed yield were also found to be significantly increased (table 1).