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## V. SUMMARY

Indoxacarb and methomyl were chosen to study their toxicity against three important different pests: cotton leafworm *Spodoptera littoralis* (Boisd.), terrestrial slugs *Lehmania marginata* (Müller) and white albino rats *Rattus norvegicus* var. *albus*.

The inhibitory effects of *in vivo* LC<sub>0</sub> treatment of indoxacarb and methomyl of the three different pests were investigated against the activities of: a) Acetylcholinesterase (AChE), b) Adenosine triphosphatase (ATP), c) Alanine aminotransferases (ALT), d) Aspartate aminotransferases (AST), and e) Blood parameters [haemoglobin, creatinine, red blood cells (RBCs and white blood cells (WBCs))] of male white albino rat.

The dissipation and distribution of methomyl were also measured using high performance liquid chromatographic analysis for terrestrial slug as well as in different tissues of male white albino rat (cerebrum, cerebellum, heart, liver and kidney).

### A. Toxicity Studies

#### 1. Laboratory studies

1.1. Cotton leafworm, *Spodoptera littoralis* (Boisd) The toxicity of indoxacarb and methomyl against the 4<sup>th</sup> instar larvae of cotton leafworm, *S. littoralis* (Boisd.) using topical application as well

as poisoned food techniques were recorded. The results revealed that:

- a) methomyl insecticide was less toxic than indoxacarb against cotton leafworm larvae using both topical application or poisoned food techniques.
- b) While the topical application treatment was more efficient against cotton leafworm in the case of indoxacarb, in contrast poisoned food treatment induced lower  $LC_{50}$  value, indicating higher insecticidal efficiency in the case of methomyl.

**1.2. Terrestrial slug, *Lehmania marginata*** The molluscicidal activity of indoxacarb against land slugs, *Lehmania marginata* was about 9.2 times lower than its insecticidal activity against cotton leafworm, using topical application treatment, the  $LC_{50}$  values were 5700 and 6222 ppm, respectively. In the contrary, methomyl insecticide exerted excellent molluscicidal activity against the land slug compared with its potentiality as insecticide against cotton leafworm, using both methods of treatments. The  $LC_{50}$  values of methomyl using poisoned food method against slug and cotton leafworm larvae were 100 and 950 ppm, respectively.

Methomyl was more effective as molluscicide compound using topical application and poisoned food techniques than indoxacarb with 13.9 and 32.7 times, respectively.

**1.3. White albino rats, *Rattus norvegicus var. albus*** The effect of indoxacarb and methomyl  $LC_0$  subcutaneously injection on

pregnant females white albino rats, *Rattus norvegicus* var. *albus* as well as their offspring are studies and the results revealed that:

1. Little effect was noticed on the body weight during gestation period, however, both methomyl and indoxacarb lowered the body weight of the pregnant rats by about 4 and 5%, respectively, indicating the insignificant effect of indoxacarb and methomyl LC<sub>0</sub> injection on the body weight of pregnant white albino rats.
2. The gestation period increased slightly due to previous treatments.
3. It was obvious that the main effect of indoxacarb and methomyl was higher and concerned on the offspring.
4. Both body weight as well as mortality percentages of the offspring were significantly noticed due to indoxacarb and methomyl LC<sub>0</sub> treatments. The lowering values in body weight were 21.25 and 13.0% for indoxacarb and methomyl, respectively. In contrast, the mortality percentages were 19.0 and 26.0% for LC<sub>0</sub> indoxacarb and methomyl, respectively.

## **2. Field studies**

**2.1. Cotton leafworm, *Spodoptera littoralis* (Boisd.)** The toxicity of indoxacarb and methomyl against cotton leafworm, *S. littoralis* in field, when applied at the field application rate was recorded. The results revealed that:

1. Indoxacarb was more effective against cotton leafworm than methomyl. However, both insecticides caused a significant reduction in total counts of larvae after 12 hrs of treatment.
2. Regarding the differences in mode of action of both insecticides, indoxacarb blocks sodium channels in nerve cells, leading to suppress feeding poor coordination, paralysis and death of the

insect. While, methomyl depends mainly as a carbamate insecticide on the acetylcholinesterase inhibition.

3. The highest degree of mortality was noticed for both insecticides after 3 days of treatments. Decline of mortality percentage was significant by increasing the time period, however, the percentage mortality values were 29.8 and 34.54 after 7 days of treatment for indoxacarb and methomyl, respectively.

**2.2. Terrestrial slug, *Lehmania marginata*** The efficiency of indoxacarb and methomyl against terrestrial slug, *Lehmania marginata* under field condition was studied. The data indicated that highest mortality of slugs was observed for both insecticides after 1 and 3 days of treatment for indoxacarb and methomyl, respectively. Methomyl was highly effective against slugs than indoxacarb.

Both insecticides lowered the percentage mortality against both pests by increasing time and this might be due to compounds decomposition by either environmental conditions or intoxication by pest system.

## **B. Biochemical Studies**

*In vivo* effects of indoxacarb and methomyl on the activities of acetylcholinesterase (AChE), adenosine triphosphatase (ATPase), alanine aminotransferases (ALT) and aspartate aminotransferases (AST) of the three different economic pests; cotton leafworm, *Spodoptera littoralis* (Boisd.), terrestrial slug, *Lehmania marginata*

(Müller) and white albino rat, *Rattus norvegicus* var. *albus* were investigated on the basis of LC<sub>0</sub> values.

### **1. Effect of indoxacarb and methomyl against acetylcholine-esterase (AChE) activity**

The results of the inhibitory effects of LC<sub>0</sub> indoxacarb and methomyl against AChE activity of cotton leafworm, slug and white albino rat indicated higher AChE activity of slug more than enzyme activity of cotton leafworm or white albino rat. The activity of the slug enzyme was higher 1.93 and 29.59 times more than AChE enzyme activity of cotton leafworm or albino rat, respectively. The highest inhibitory effect against AChE activity of the three selected animals was induced by LC<sub>0</sub> methomyl treatments. The average mean of the AChE activity values as O.D/mg protein .min were 0.059, 0.118 and 0.0052 for cotton leafworm, slug and albino rat, respectively. Slight inhibition in the AChE enzyme activity was observed due to LC<sub>0</sub> indoxacarb treatments. Enzyme activity values were 77, 64.7 and 76.4 for cotton leafworm, slug and white albino rat, respectively compared to their corresponding control.

### **2. Effect of indoxacarb and methomyl against adenosine triphosphatase (ATPase) activity**

The results of ATPase activity revealed that:

- a) Slug ATPase activity was the highest among all other selected animals. Slug ATPase activity values were 6.75 and 33.75 times higher than that of cotton leafworm and albino rat, respectively.
- b) Remarkable ATPase inhibitory effect was induced by LC<sub>0</sub> indoxacarb treatment that the lowest enzyme activity was noticed in



respect to all selected animals. Enzyme activity inhibition due to LC<sub>0</sub> indoxacarb treatments was about 50% of its original value of than cases studied.

- c) LC<sub>0</sub> methomyl treatment caused slight inhibition in the ATPase activity, indicating that ATPase enzyme is not specific target for methomyl effect.

### **3. Effect of indoxacarb and methomyl against alanine aminotransferases (ALT) activity**

The data revealed that insect ALT activity was higher than that of slug or albino rat in the absence of any treatment. The insect enzyme activity was 2.54 and 4.65 folds higher than that of slug and albino rat, respectively. Although the lowest ALT enzyme activity was noticed for albino rat, treatments with LC<sub>0</sub> of either indoxacarb or methomyl caused remarkable enzyme induction. All pesticide treatments increased the ALT enzyme activity of the three selected animals, however, the lowest increase in ALT enzyme activity was noticed in the case of cotton leafworm.

### **4. Effect of indoxacarb and methomyl against aspartate aminotransferases (AST) activity**

It was noticed that AST activity of cotton leafworm was the highest, while AST activity of white albino rat was the lowest. It was clear that all treatments induced a high degree of enzyme activity elevation. Increased AST enzyme activity due to indoxacarb and methomyl treatments was consistent at all experimental time intervals.

## **5. Haematological effects of indoxacarb and methomyl**

The haematological effects of LC<sub>0</sub> indoxacarb and methomyl subcutaneously injection on adult male white albino rat, *Rattus norvegicus* var. *albus* were investigated. Haemoglobin, creatinine, white blood cells (WBCs) counts and red blood cells (RBCs) counts are recorded. From the recorded data, the following observations could be noticed:

- a) Remarkable difference between the effect of indoxacarb and methomyl were found. While indoxacarb treatment caused an increase in the RBCs counts with an average increase of 13.3%, it decreased the WBCs counts with an average of 49.2%, methomyl decreased both blood cells counts by 46.49 and 57.69% for RBCs and WBCs, respectively.
- b) Both indoxacarb and methomyl treatments induced insignificant changes in both haemoglobin and creatinine contents.

## **C. Residue Analysis Studies**

High performance liquid chromatographic (HPLC) analysis of methomyl was used in order to measure the residue amount of methomyl as ng/g tissue of both terrestrial slug, *Lehmania marginata* and male white albino rat, *Ruttus norvegicus* var. *albus*.

The results indicated high performance liquid chromatographic methomyl response after 48 hrs of topical application and poisoned food technique methods at different concentrations against gastropod slug *L. marginata*.

Treatments by topical application and poisoned food technique showed no insignificance.

The distributed amounts of methomyl in different brain regions and organs of male white albino rat, *R. norvegicus* var. *albus* due to LC<sub>0</sub> methomyl subcutaneously injection could be summarized as follows:

- a) Cerebellum tissue contained higher amounts of methomyl than cerebrum by 1.8 folds.
- b) Kidney had the highest methomyl residue of 6.99 ng/g tissue.
- c) Both liver and heart were no insignificantly different in their amount of methomyl recovered, the values were 4.870 and 4.826 ng/g tissue for liver and heart, respectively.
- d) The organ that contained the lowest methomyl residue was the (cerebrum) of the rat brain.
- e) It was obvious that increasing time of measurements after treatments attenuated the amount of methomyl recovered remarkably, the amount of methomyl was decreased descendingly by increasing time interval.