How to assess resistance to insecticides and acaricides

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ECTOPARASITICIDE GROUPS

- Virtually all ectoparasiticides are neurotoxins which exert their effects through nervous system. The list includes:
- Amidines (Formamidines)-Amitraz
- Macrocyclic Lactones- Abamectin, doramectin, eprinomectin, moxidectin, milbemycin oxime, selamectin- **endectocides**
- Carbamates- Carbaryl, propoxur, fenoxycarb
- Nitroguanidines- Imidacloprid and flumethrin
- Organochlorines - DDT, DDE and DDD (diclofol, methoxychlor), Cyclodines - chlordane, aldrin, dieldrin, hepatochlor, endrin and toxaphene, Hexachlorocyclohexanes (HCH) like benzene hexachloride (BHC)
- Organophosphates- malathion, sumithion, parathion, diazinon, fenthion, dichlorvos and chlorpyrifos

- **Phenylpyrazoles -Fipronyl**
- **Pyrethrins and synthetic pyrethroids - bioallethrin, cypermethrin, deltamethrin, fenvalerate, flumethrin, lambdacyhalothrin, phenothrin and permethrin**
- **Miscellaneous- Piperonyl butoxide (PBO)-it is used as synergist with pyrethroids, natural pyrethrins, jasmolines, citronella,indalone, garlic oil, DEET (N,N-diethyl-M-toluamide) and DMP (Di methyl phthalate)**
- **New category of insect control agents- IGRs the insect growth regulators, three types prevalent- Chitin synthesis inhibitors (BPU) - Diflubenzuron, Lufenuron, Flufenoxyuron, Triflumuron, Fluazuron which is the only tick development inhibitor,Chitin inhibitors (Thiazine/pyrimidine derivatives) - Dicyclanil and Cyromazine, Juvenile hormone analogues (JHA)-methoprene, fenoxycarb, Pyriproxyfen**
- **Novel insecticides- spinosad and Fluralaner**

RESISTANCE

- **WHO Definition:**

"the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within limits of tolerance of the subject"

- One of the best way to assess resistance to insecticide and acaricide resistance is to observe the effect of the insecticide / acaricide in the field conditions

- However, in vitro bioassays provide valuable inputs regarding the status of resistance.

Mechanisms of development of resistance

In general terms, resistance can arise through several mechanisms in individual ticks. Generally these mechanisms are broadly classified as target site, metabolic, or reduced penetration (cuticular penetration)

- **Target site resistance** exists when an allele of the gene coding for the target molecule attacked by the acaricide has an amino acid mutation that confers resistance to the acaricide. This resistance mechanism is common, particularly well-studied in the case of pyrethroid class of acaricides
- **Metabolic resistance** to acaricides occurs through changes in the ability of an individual to detoxify or sequester an acaricide. The enzyme families known as cytochrome P450s, esterases, and glutathione S-transferases are generally involved in metabolic resistance
- **Penetration resistance** in ticks could arise through alterations in the ability of an acaricide to penetrate or otherwise enter an individual that is treated with acaricide

Tests for detection of resistance

FAO approved tests are

- Adult immersion test (AIT)
- Larval packet test (LPT)
- Apart from this, larval immersion test (LIT), syringe immersion test, acetyl cholinesterase assay (biochemical test for detection of resistance to synthetic pyrethroid compounds) and molecular techniques like PCR have been widely employed for the detection of resistance in ticks.

➤ The enzyme assays estimating the level of metabolic enzymes in ticks which are involved in detoxification of acaricides also are done and in case of resistant ticks, the levels of these enzymes are found to be elevated thus proving that increased metabolism is the mechanism of resistance. For example, in case of pyrethroid resistance, the level of carboxyl esterase have been found to be elevated in the resistant ticks compared to the susceptible population

➤ Molecular techniques like PCR and RAPD can be employed especially to assess the mutations in target sites. The bioassays in conjunction with molecular techniques can be used to study the resistance development to acaricides in a population

Diagnostics to detect resistance

- Larval packet test (LPT)
- Larval immersion test (LIT)
- Adult immersion test (AIT)
- PCR based assays
- Enzyme assays

LARVAL PACKET TEST / LARVAL IMMERSION TEST

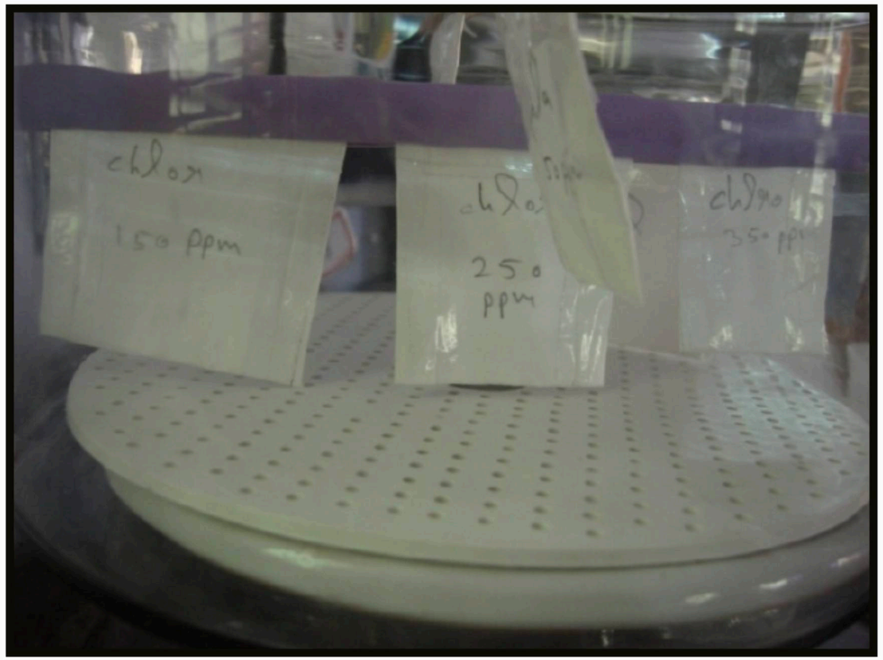
- In LPT, the tick larvae are exposed to acaricide impregnated filter papers. Results are evaluated 24 hours after each treatment by counting the number of live and dead larvae
- In LIT, seven to ten days old tick larvae are immersed in different dilutions of acaricide for 5 minutes and then the mortality is calculated
- It is an in vitro bio assay

What is required

- Whatman filter paper No. 1
- Binder clips
- Desiccator
- Petridish
- BOD incubator
- Fine painting brush

PROTOCOL

- Use whatman no. 1 filter papers for LPT
- Make packets of size 7 X 12 cm packets sealed on 3 sides and open at one side (OR) mark a rectangular area of 8.5 cm X 3.75 cm on a filter paper



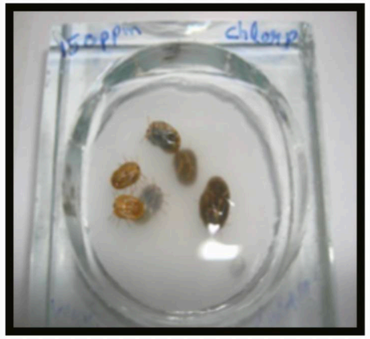
PROTOCOL

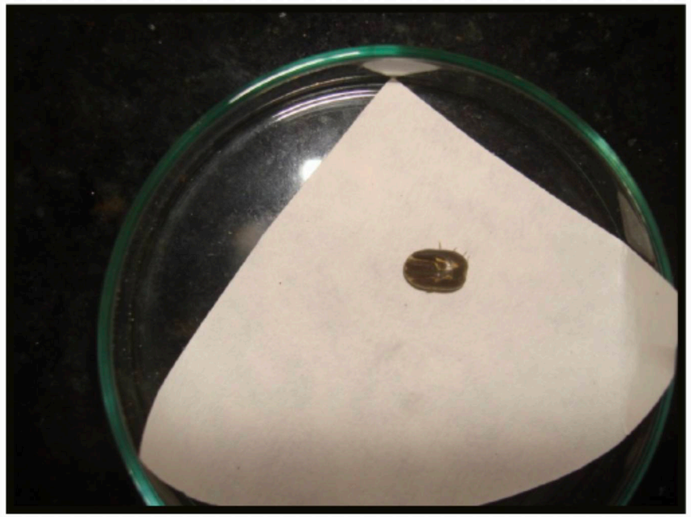
- Impregnate with 200 microlitre of acaricide in the marked rectangular area and allow it to dry
- Impregnate the control with distilled water
- Place 14 – 21 days old larvae into packets using fine paint brush and seal it
- Place approximately 100 larvae into each packet
- Incubate in desiccator at 25 º C and RH of 85% for 24 hours

ADULT IMMERSION TEST

- In AIT, dose mortality response of ticks to acaricides are determined by immersing engorged female ticks in different dilutions of an acaricide and comparing treated and untreated ticks to assess the effect of treatment on fecundity and fertility
- The weight of the engorged ticks before the trial and the weight of the eggs laid are compared between treated and untreated groups
- The test can be performed in two ways, first one is termed as classical Drummond's test which is for a period of 28 days wherein both fecundity and mortality is assessed while the modified version is for a period of 7 days to assess the mortality alone.

ADULT IMMERSION TEST





INTERPRETATION

- Compare resistant ticks hatching % with control group

- Resistance % = $(N_t / N_w) \times 100$

➤ Nt - Number of treated ticks laying eggs

➤ Nw- Number of untreated ticks laying eggs

➤ RESISTANT - Ticks ovipositing viable eggs

➤ TOLERANT - Survive more than 2 days, laying non – viable eggs

➤ SUSCEPTIBLE – Die within 24 hours

- Esterase family has numerous enzymes all of which caalyse the hydrolysis of esters. In insects, esteraces are linked with critical physiological roles such as behaviour, development, insecticide resistance as well as reproduction
- By using the esterase assay, resistant population of ticks in which the esterase levels are elevated can be visually interpreted
- Two substrates are used in this assay
  - the α and β naphthyl acetate

- Mostly the elevated esterase associated with resistance are generally active with both the substrates
- Hence,tick/larval homogenates are reacted with the substrates and they produce naphthol as the end product
- Naphthol is stained with fast blue stain solution in microtitre plates and the intensity of colour transformation is directly propotional to the amount of naphthol produced and thus the degree of elevation of esterases

Enzyme assays

Estimation of esterase enzyme activity

↓

20µl tick protein/20µl distilled water for control

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200µl alpha naphthyl acetate or beta naphthyl acetate

↓

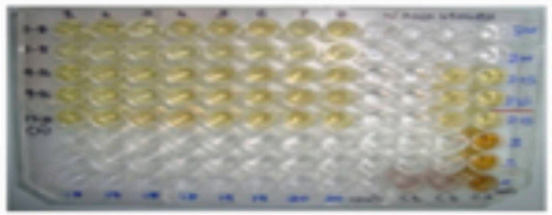
Incubate for 30 minutes at room temperature

↓

50µl of fast blue stain

↓

Measure OD at 570nm



Novel methods of control of ectoparasites of livestock and poultry

1. Dealing with fly menace in poultry units
2. Tackling flies and ticks in ruminants
3. Ectoparasites of companion animals
4. Non chemical ways to target ectoparasites
5. How to assess resistance to insecticides and acaricides

Thank you