A novel insecticide molecule for management of insecticide resistance in major malaria vectors in India

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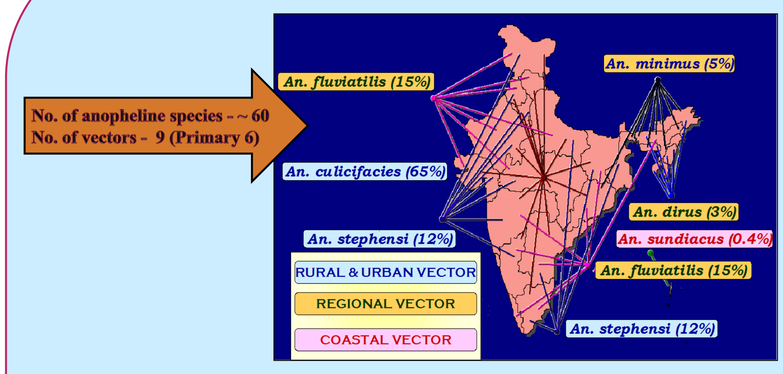
Introduction

Malaria vectors have acquired widespread resistance to many of the currently used insecticides, including synthetic pyrethroids. Hence, there is an urgent need to develop alternative strategies including development of new insecticides for effective management of insecticide resistance. In the present phase I study, chlorfenapyr, a pyrrole class of insecticide was evaluated against *Anopheles culicifacies* and *Anopheles stephensi* for its possible use for vector control.

Indian Malaria Vectors: Distribution, influence and contribution

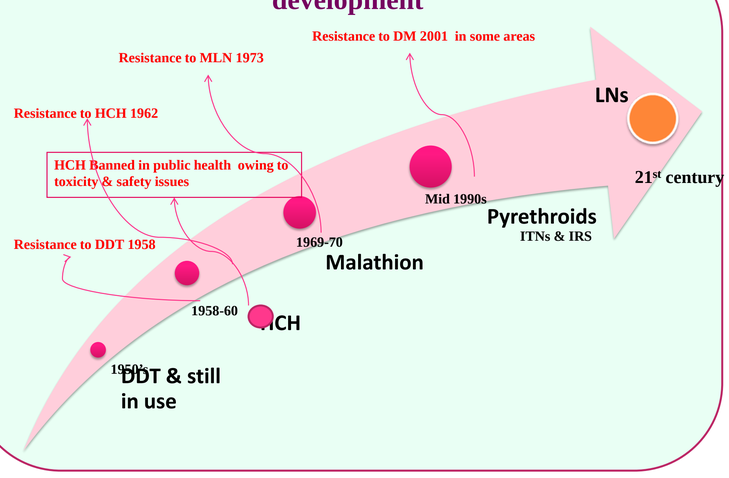


Species	Distribution	Behavior	Control Option	Remarks
An.culicifacies	Wide spread (Plains)	Endo-phagic/ philic	IRS/ ITNs/LNs	Wide spread resistance to insecticides (DDT/malathion) and to deltamethrin in some areas
An.fluviatilis	Wide spread (forest and foothills)	Endo-phagic/ -phillic/Exo-philic	IRS/ ITNs/LNs	Susceptible to insecticides
An.stephensi	Localized & urban settings	Type form -Endo-phagic/ - philic Mysorensis- Endo-phagic/ - philic	Larvicides and personal protections	No reports of resistance that can effect the interventions
An.minimus	Wide spread NE and forested areas	Endo-phagic/ - philic	IRS/ ITNs/LNs	No reports of resistance
An.sundaicus	Only in Andaman & Nicobar islands	Endo-phagic/ - philic	IRS/ ITNs/LNs	No reports of resistance that can effect the interventions
An.dirus	Localized in NE	Exo-phagic/ - philic	--	Personal protection is an option

Existing strategy of malaria control in India

- Rural areas: Adult control with indoor sprays using residual insecticides
- Urban areas: Larval control using insecticides, oils/chemicals, *Bti* and also larvivorous fishes

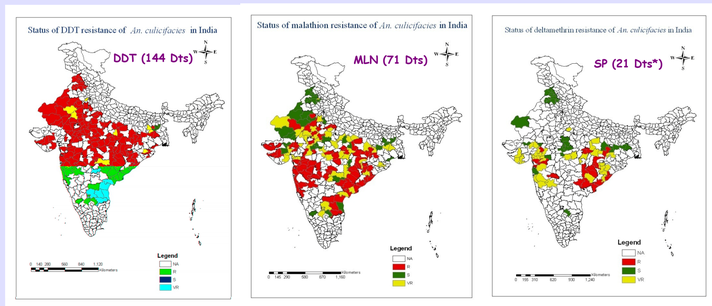
Chronology: insecticide introduction and resistance development



Physiological Mechanisms of Resistance in Vectors

Insecticide	Mechanism	Possible Cross Resistance to
DDT	Glutathione-S-Transferase	OC with DDT like structure
HCH	Gamma amino butyric acid receptor (GABA)	Cyclodienes
Malathion	Malathion carboxylesterase (MCE)	OP with carboxyl-ester bond
Pyrethroids	Mixed function oxidases Esterases kdr (?)	OP/ carbamates DDT (?)

Geographical distribution of insecticide resistance/susceptibility status in *An. culicifacies in* India(based on available data 1995 – 2011)



- Other vector species are mostly susceptible to the insecticides used in vector control
- *An. fluviatilis* - sporadic reports of resistance, but not of serious operational concern
- *An. stephensi* - urban vector - reported resistance to DDT and malathion but larvicides are used for its control to which it is susceptible

* Very low level resistance (tolerance)

New Hopes for Vector Management in India

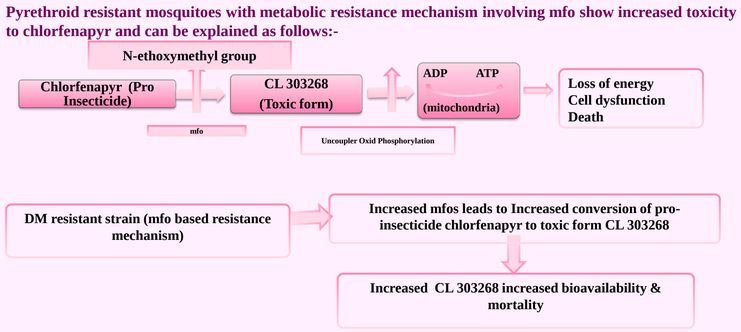
- Trial of innovative intervention - deltamethrin impregnated durable wall lining, combination LN [deltamethrin + synergist (PBO)]
- Improved insecticide formulations for increased efficacy
- IGRs and other safe insecticides (trials completed)
- New chemistries in offing and under field trials – Chlorfenapyr (pyrrole grp. Insecticide) - Phase I completed, field trials proposed (Short term-2012-2013). - Pirimiphos methyl – (short term – completed; Long term – 2012-13)

Pyrrole group insecticide molecule- Chlorfenapyr

- Phase 1 and limited field evaluation against *An. culicifacies, An. stephensi*
- Residual efficacy-400 mg a.i./m2 for 24 weeks and more.
- No cross-resistance-DDT, malathion, bendiocarb and deltamethrin
- Approved by CIB- in use in agriculture in India and neighboring countries.
- Potentiating studies using PBO showed antagonistic effect to chlorfenapyr toxicity in resistant strains.

Schematic representation of MoA of Chlorfenapyr
(Illustration based on:- Black BC et. al., Pest Biochem Physiol 1994, 50:115-128)

Pyrethroid resistant mosquitoes with metabolic resistance mechanism involving mfo show increased toxicity to chlorfenapyr and can be explained as follows:-



Thus, chlorfenapyr can be an effective insecticide for managing pyrethroid resistant mosquitoes, renewed hope for IRS for VM, field trials proposed (Short term-2012 L).

Efficacy of chlorfenapyr against *An. culicifacies* and *An. stephensi* was assessed using adult bioassay tests. In the laboratory, determination of diagnostic dose, assessment of residual activity on different substrates, cross-resistance pattern and potentiation studies using piperonyl butoxide were undertaken by following standard procedures. cross resistance patterns were also assessed in laboratory and wild populations of *An. culicifacies*.

Results

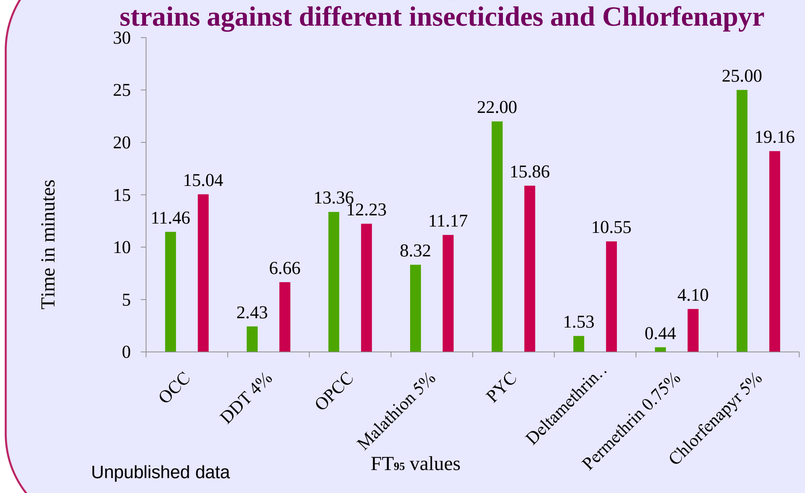
- A dose of 5.0% chlorfenapyr determined as the diagnostic concentration for adult susceptibility WHO tube test in anopheline mosquitoes with 2 h exposure and 48 h holding period.
- Residual activity of chlorfenapyr a.i. of 400 mg/m2 on five fabricated substrates, namely wood, mud, mud+lime, cement and cement + distemper effective up to 24 weeks against An. culicifacies and up to 34 weeks against An. stephensi
- No cross-resistance to DDT, malathion, bendiocarb and deltamethrin in laboratory-reared strains of *An. stephensi* and wild An. culicifacies.
- Potentiation studies demonstrated the antagonistic effect of PBO.

Insecticide susceptibility status of insecticide-susceptible (Sonepat and Nadiad) and resistant strain (Goa) of *An. stephensi* and field-collected An. culicifacies from Chhattisgarh and Gujarat states

Species	Insecticides					Control		
	DDT 4.0%	Malathion 5.0%	Bendiocarb (0.1%)	Deltamethrin 0.05%	Chlorfenapyr 5.0%	OC	OP	PY
Susceptible strains								
An. stephensi (Sonepat)	98.3 ± 2.3* (57)	100 (48)	100 (102)	100 (68)	100 (169)	0 (35)	0 (15)	4.7 (21)
An. stephensi (Nadiad)	95.9 ± 2.8* (50)	98.0 ± 2.7* (48)	100 (30)	100 (49)	100 (125)	0 (16)	0 (45)	0 (17)
Resistant strain								
An. stephensi (Goa)	10.3 ± 5.1* (77)	26.2 ± 5.9* (46)	23.4 (94)	84.9 ± 3.5* (47)	100 (116)	0 (15)	0 (36)	0 (15)
Field collected strain - Raipur								
An. culicifacies	4.2 ± 2.1* (120)	73.3 ± 3.9* (116)	80 (30)	78.2 ± 2.5* (124)	100 (211)	0(48)	0 (50)	0 (18)
Field collected strain - Panchmahals								
An. culicifacies	6.4 ± 2.6* (140)	30.1 ± 3.2* (123)	93.7 (80)	43.1 ± 3.1* (130)	100 (60)	0 (21)	0 (39)	0 (20)
Field collected strain - Vadodara								
An. culicifacies	11.6 ± 2.9* (120)	41.1 ± 3.6* (124)	93.7 (80)	59.2 ± 3.4* (130)	100 (60)	0 (20)	0 (40)	0 (20)

DDT-malathion-bendiocarb-resistant/deltamethrin-tolerant *An. stephensi (lab) and* DDT-malathion-bendiocarb-deltamethrin-resistant *An. culicifacies (field)* strains have not shown cross resistance to chlorfenapyr

Irritability level in Anopheles stephensi susceptible and resistant strains against different insecticides and Chlorfenapyr



Unpublished data

Synergistic studies with PBO in resistant and susceptible *An. stephensi*

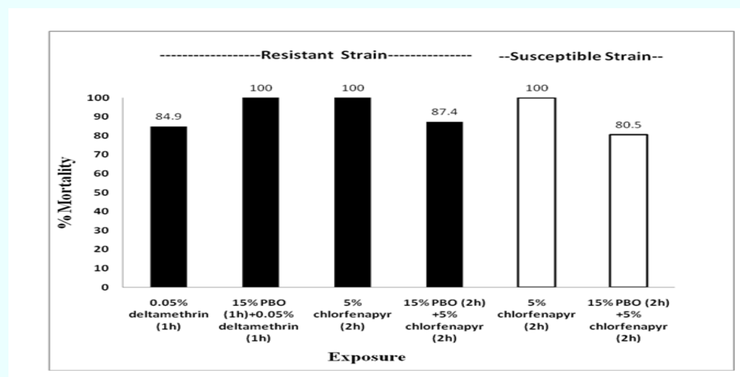


Figure 1. Potentiation studies on susceptible (Sonepat strain) and resistant (Goa strain) of *An. stephensi*. There was no mortality in pyrethroid control replicates

Resistant strain of *An. stephensi* has shown synergism (PBO) to deltamethrin toxicity, while both strains have shown antagonism to chlorfenapyr toxicity

Conclusion

Laboratory studies with susceptible and resistant strains of *An. culicifacies* and *An. stephensi*, coupled with limited field studies with wild multiple insecticide-resistant *An. culicifacies* have shown that chlorfenapyr can be a suitable insecticide for managing multiple-insecticide-resistant mosquitoes including pyrethroid resistance. Phase II trials are in progress.

Reference

Raghavendra et al. (2011). Malaria Journal 2011, 10:16, doi:10.1186/1475-2875-10

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