Adulticidal & larvicidal efficacy of three neonicotinoids against insecticide susceptible & resistant mosquito strains

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Background & objectives: Due to ever growing insecticide resistance in mosquitoes to commonly used insecticides in many parts of the globe, there is always a need for introduction of new insecticides for the control of resistant vector mosquitoes. In this study, larvicidal and adulticidal efficacies of three neonicotinoids (imidacloprid, thiacloprid and thiamethoxam) were tested against resistant and susceptible populations of *Anopheles stephensi* Liston 1901, *Aedes (Stegomyia) aegypti* Linnaeus, and *Culex quinquefasciatus* Say (Diptera: Culicidae)*.*

Methods: Laboratory-reared mosquito species were used. Insecticide susceptibility tests were done using standard WHO procedures and using diagnostic dosages of insecticide test papers and larvicides. Adulticidal efficacy of candidate insecticides was assessed using topical application method and larval bioassays were conducted using standard WHO procedure.

Results: The results of topical application on 3-5 day old female mosquitoes indicated that resistant strain of *An. stephensi* registered lower LC_{50} values than the susceptible strain. Among the three insecticides tested, thiacloprid was found more effective than the other two insecticides. *Culex quinquefasciatus* registered lowest LC_{50} for imidacloprid than the other two mosquito species tested. In larval bioassays, the LC_{50} values registered for imidacloprid were in the order of *Cx. quinquefasciatus* <*An. stephensi* (SS) <*An. stephensi* (RR) <*Ae. aegypti*. In case of thiacloprid, the order of efficacy (LC_{50}) was *Cx. quinquefasciatus* <*An. stephensi* (SS) <*An. stephensi* (RR), whereas in case of thiamethoxam, the larvicidal efficacy was in the order of *An. stephensi* (RR) <*An. stephensi* (SS) <*Cx. quinquefasciatus.*

Interpretation & conclusions: The present study indicated that insecticide resistant strains of mosquito species tested showed more susceptibility to the three neonicotinoids tested, and the possibility of using neonicotinoids for the control of resistant mosquitoes should be explored.

Insecticides of different classes, namely organochlorines, organophosphates, carbamates and synthetic pyrethroids have been in use since last 2-5 decades in vector control programmes all over the world. Due to continued use of these insecticides, the vector species have developed multiple resistances to these insecticides. There is always a need for alternative insecticides for effective control of vector mosquitoes. The neonicotinoids are systemic toxins that target acetylcholine receptors in the insect nervous system. As per the Insecticide Resistance Action Committee Mode of Action (IRAC MoA) classification these are classified as nicotinic acetylcholine receptor (nAChR) agonists and grouped in 4A[1]. Imidacloprid was the first nicotinoid registered and was found effective in agriculture[2]. Neonicotinoids are unique from any other insecticides currently available for field use[3], and have attracted attention due to their high efficacy, safety to mammals, low toxicity, no-cross resistance and unique mode of action[4]. These compounds have emerged as the fourth generation of pesticides replacing organophosphates, carbamates and pyrethroids, and have been used extensively for insect control[4-7]. Neonicotinoids cause irreversible blockage of post-synaptic nicotinergic acetylcholine receptors[8]. In general, these compounds possess low mammalian toxicity and are relatively non-toxic to non-target species[9,10]. These are highly effective in control of a wide range of insect pests[11]. Further, neonicotinoids are selective to insects because of the differential sensitivity of insect and vertebrate nACHR subtypes[9].

Imidacloprid is a systemic, chloro-nicotinyl insecticide in use in agriculture with soil, seed and foliar applications for the control of sucking insects including rice hoppers, aphids, thrips, whiteflies, termites, turf insects, soil insects and some beetles. The chemical acts by interfering with the transmission of stimuli in the insect nervous system. It blocks nicotinergic pathway leading to the accumulation of acetylcholine, which may result in paralysis, and eventually death of the insect. It is reported effective both by contact and via stomach action[12]. In a study carried out by Paul *et al*[13], imidacloprid had shown LC_{50} (lethal concentration) of 84 ng/ml against fourth instar larvae of *Aedes aegypti* Linnaeus. (Diptera: Culicidae), and in another study by Pridgeon *et al*[14], imidacloprid registered LD_{50} of 7.7×10^{-4} µg/mg of mosquito in topical application against *Ae. aegypti*, 1.2×10^{-3} µg/mg against *Culex quinquefasciatus* Say (Diptera: Culicidae) and 3.8×10^{-4} µg/mg against *Anopheles quadrimaculatus* Say (Diptera: Culicidae).

Rao *et al*[15] using different analogues of imidacloprid on the larvicidal properties against *Cx. quinquefasciatus* showed that the analogues exerted more toxic effect than the pure imidacloprid and these showed good larvicidal efficacy on *Cx. quinquefasciatus*. In another study the LC_{50} against yellow fever mosquito was 0.03 mg/l for imidacloprid, 0.06 mg/l for acetamiprid and 0.007 mg/l for thiamethoxam and 0.11 mg/l for dinotefuran[15]. Bhinder *et al*[16] compared the toxicity of imidacloprid and thiamethoxam in *An. stephensi* Liston (Diptera: Culicidae) and noticed measurable differences in internal transcribed spacer 2 (ITS2) sequences of control and treated mosquitoes indicating genetic damage. It was found that imidacloprid-treated mosquitoes had eight deletions, 29 insertions, 18 transitions and 33 transversions, whereas thiamethoxam-treated had 10 deletions, 8 insertions, 47 transitions and 68 transversions. All these studies demonstrated insecticidal efficacy of neonicotinoids in different combinations against different mosquito species. In the present study, an attempt was made to study the mosquito adulticidal and larvicidal efficacy of three neonicotinoids, namely imidacloprid, thiacloprid and thiamethoxam against insecticide resistant and susceptible mosquito adults and larvae.

Material & Methods

The study was conducted in the National Institute of Malaria Research (NIMR), New Delhi, India. The experiments were conducted at the Insecticide and Insecticide Resistance Laboratory recognized as WHO Collaborating Centre for Phase I Testing and Evaluation of Public Health Pesticides, at NIMR during January 2012 to February 2013. Technical grade imidacloprid (99.2%) and thiacloprid (98.4%) were provided gratis by M/s Bayer Crop Science Pvt. Ltd., Mumbai, and thiamethoxam (99.1%) by M/s Syngenta Corporation, Mumbai, India.

Mosquito species:

(i) Anopheles stephensi (Sonepat): DDT-malathion-deltamethrin susceptible (SS), established in 1996 and still being colonized (306 generations as on January 2, 2012)

(ii) An. stephensi (Goa): DDT-malathion-deltamethrin resistant (RR) established in 2009 and still being colonized (72 generations as on January 2, 2012)

(iii) Culex quinquefasciatus (Sonepat): DDT-malathion- resistant and tolerant to deltamethrin

established in 1999 and still being colonized (252 generations as on January 2, 2012)

(iv) Aedes aegypti (Delhi): DDT-malathion-deltamethrin susceptible, established in 2009 (128 generations as on January 2, 2012)

All these mosquito species are being maintained as cyclic colonies in the Insectary of NIMR, New Delhi, for several years. These are characterized quarterly for insecticide susceptibility status. The mosquitoes are reared in closed rooms maintained at 27±2°C temperature and 70-80 per cent relative humidity with 14:10 h light and dark photoperiods; 10 per cent glucose soaked cotton pads are provided as food source for adults regularly and vertebrate blood for reproduction. Larvae were provided ground dog biscuits and yeast powder (3:2).

Determination of insecticide susceptibility: The laboratory-reared mosquito species were exposed to diagnostic dosages of different insecticides using WHO adult susceptibility kits and methods[17] to the diagnostic dosages of DDT (4%), malathion (5%) and deltamethrin (0.05%). Insecticide test papers were procured from Vector Control Research Unit, University Sains Malaysia, Malaysia. The larval susceptibility was determined using diagnostic dosages of fenthion, malathion and temephos using WHO larval tests[18]. Diagnostic doses (in mg/l) used for different larvicides as recommended by WHO for different mosquito species are as follows: temephos, malathion and fenthion: 0.02, 3.125, 0.05, respectively for *Ae. aegypti*; 0.25, 1.0, 0.05, respectively for *An. stephensi*; and 0.02, 1.09, 0.05, respectively for *Cx. quinquefasciatus*.

Adulticidal efficacy (Topical application): These studies were carried out on the laboratory-reared DDT-malathion-deltamethrin susceptible and resistant mosquitoees. For the assays, 3-day old sugar-fed female *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* were used. Different concentrations of insecticidal solutions (0.1 to 100 ppm) were prepared in acetone. Batches of 25 mosquitoes were anaesthetized for 25-60 sec with a steam of CO_2 in an anaesthetic chamber and placed on cold plate maintained at 4ºC. The weight of the average mosquito was determined in advance. Each concentration was tested against 100 mosquitoes and acetone control was run concurrently. Insecticidal solution (0.1 µl) was placed on the pronotum of thorax of the mosquito using micropipette. After applying the insecticide, the mosquitoes were transferred to holding tubes and kept for observation for 24 h in a climatic chamber maintained at 27°C temperature and 80 per cent relative humidity. Mortality was scored after 24 h and corrected by applying Abbott's formula[19]. The toxicity of the insecticide is expressed in ng/mg of mosquito.

Larvicidal efficacy of neonicotinoids: Larvicidal efficacy was tested by exposing late III or early IV instar larvae of different mosquito species to different concentrations of insecticidal solution in ethanol (ranging from 1 to 200 ppm). Six to eight concentrations were tested against each species and a minimum of four replicates (20 larvae in each replicate) were used for each concentration. One millilitre of the insecticidal solution was added to 99 ml of boiled and cooled tap water in a paper cup of 150 ml capacity. In each cup, 20 larvae were introduced with the help of strainer carefully. The larvae were treated in four replicates against each dose and mortality, pupal emergence, and moribund larvae were recorded at 24, 48 and 72 h intervals. The mortality data were subjected to log-probit analysis to calculate lethal doses. Dosages of neonicotinoids ranging from 0.01 to 2 mg/l were used in the studies.

Statistical analysis: Log probit analysis was used to calculate lC_{50} and LC_{90} values using SPSS v 18.0 software (SPSS Inc, Chicago, USA). Chi square test was used for testing the Pearson's goodness of fit among the dosages tested.

Results

The results of insecticide susceptibility tests carried out on different strains of *Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi* mosquitoes using WHO tube test are shown in Table I. The results indicated that *An. stephensi* (Sonepat) was susceptible to DDT, malathion and deltamethrin. *An. stephensi* (Goa) was resistant to DDT, malathion and deltamethrin. *Culex quinquefasciatus* was resistant to DDT and malathion; and verification required (tolerant) to deltamethrin (T). *Aedes aegypti* (Delhi) was found susceptible to DDT, malathion and deltamethrin.

Results of larval susceptibility tests conducted on late III and early IV instar larvae of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* strains are shown in Table II. Susceptibility status of larvae of the three species tested to different WHO recommended diagnostic dosages of larvicides indicated that the larvae of *An. stephensi* (Sonepat) strain were susceptible to fenthion, malathion and temephos. The

Table I. Mortality of mosquito strains exposed to WHO specified diagnostic concentration of different insecticides in WHO adult susceptibility tests

Species and strain	Mortality (No. exposed)		
	DDT 4%	Malathion 5%	Deltamethrin 0.05%
Anopheles stephensi (Sonepat)	100 (101) S	100 (97) S	100 (99) S
An. stephensi (Goa)	26.8 (97) R	19.2 (99) R	79.5 (83) R
Culex quinquefasciatus (Sonepat)	29.5 (98) R	4 (100) R	84.3 (103) VR
Aedes aegypti (Delhi)	98 (100) S	100 (100) S	100 (100) S

S, susceptible; VR, verification required (tolerant); R, resistant
Figures in parentheses indicate no. of mosquitoes exposed in WHO Tube test

Table II. Mortality of late III or early IV instar larvae of different mosquito strains exposed to WHO specified diagnostic concentrations of different larvicides in larval susceptibility tests for 24 h

Species and strain	Mortality (No. exposed)		
	Fenthion	Malathion	Temephos
Anopheles stephensi (Sonepat)	100 (80) S	100 (80) S	100 (81) S
An. stephensi (Goa)	100 (80) S	98 (84) S	36 (77) R
Culex quinquefasciatus (Sonepat)	100 (80) S	18.7 (80) R	100 (80) S
Aedes aegypti (Delhi)	100 (74) S	100 (85) S	96.2 (81) VR

S, susceptible; VR, verification required (Tolerant); R, resistant
Figures in parentheses indicate no. of mosquito larvae exposed

larvae recorded 100 per cent mortality in 24 h exposure to diagnostic dosages. *An. stephensi* (Goa) strain registered 100 per cent mortality in case of fenthion, 98 per cent mortality to malathion and only 36 per cent mortality to temephos indicating resistance to temephos. *Culex quinquefasciatus* larvae showed 100 per cent mortality to fenthion and temephos, whereas it was found resistant to malathion (<20% mortality). In case of *Ae. aegypti,* 100 per cent mortality was reported in exposures to fenthion and malathion, whereas in case of temephos, 96.2 per cent mortality was reported indicating verification required status to this larvicide.

The results of topical application of three neonicotinoids against the three species of mosquitoes are shown in Table III. The LC_{50} values registered for *An. stephensi* susceptible strain against imidacloprid, thiacloprid and thiamethoxam were 2.217, 0.295 and 0.946 ng/mg of mosquito, respectively, whereas the resistant *An. stephensi* strain showed LC_{50} of 0.297, 0.267 and 0.302 ng/mg of mosquito, respectively to these insecticides. The results indicated that resistant strain of *An. stephensi* registered lower LC_{50}

values than the susceptible strain. Among the three insecticides, thiacloprid was found more effective than the other two insecticides tested. *Cx. quinquefasciatus* registered lowest LC_{50} for imidacloprid than the other two mosquito species tested.

The results of larvicidal efficacy tests on three mosquito species against the three insecticides are shown in Table IV. The LC_{50} values registered for imidacloprid were in the order of *Cx. quinquefasciatus* <*An. stephensi* (SS) <*An. stephensi* (RR) <*Ae. aegypti*. In case of thiacloprid, the order of efficacy (LC_{50}) was *Cx. quinquefasciatus* <*An. stephensi* (SS) <*An. stephensi* (RR). The larvicidal efficacy of thiamethoxam was in the order of *An. stephensi* (RR) < *An. stephensi* (SS) <*Cx. quinquefasciatus*.

Discussion

Chemical insecticides are the mainstay in vector control programmes. Resistance to organochlorines such as DDT, organophosphates such as malathion and synthetic pyrethroids has been reported in many malaria vectors, and also in culicine mosquitoes due to constant

Table III. Results of topical application of neonicotinoids against different mosquito strains											
Mosquito species	Susceptibility/ resistant status	Imidacloprid			Thiacloprid			Thiamethoxam		
		LC_{50}	LC_{90}	P value	LC_{50}	LC_{90}	P value	LC_{50}	LC_{90}	P value
Anopheles stephensi (SS)	Susceptible to OC, OP, PY	2.217 (n=372)	5.7	NS	0.295 (n=375)	20.636	$P<0.001$	0.946 (n=310)	241.3	NS
An. stephensi (RR)	Resistant to OP, OC, PY	0.297 (n=348)	1.847	NS	0.267 (n=375)	76.035	$P<0.01$	0.302 (n=348)	99.75	$P<0.001$
Culex quinquefasciatus	Resistant to OP, OC, tolerant to PY	0.009 (n=238)	12.17	NS	0.491 (n=274)	3.743	$P<0.001$	0.745 (n=331)	45.37	NS
Aedes aegypti	Susceptible to OC, OP, PY	0.558 (n=425)	6.135	$P<0.001$	ND	ND	ND	ND	ND	ND

ND, not done; OC, organochlorine; OP, organophosphates; PY, pyrethroids; NS, not significant
LC_{50} and LC_{90} are ng/mg of female mosquito

selection pressure of insecticide use in agriculture as well as in public health programmes. There are limited options for management of insecticide resistance and there is a need to add new insecticides or combinations of insecticides for effective control of mosquitoes especially that have developed multiple resistances to insecticides of different classes. In the present study, an effort was made to assess the adulticidal and larvicidal efficacies of three neonicotinoids, namely imidacloprid, thiacloprid and thiamethoxam. The adulticidal toxicity (LC_{50}) assessed by topical applications against both resistant and susceptible strains of *An. stephensi* was in the order of thiacloprid <thiamethoxam <imidacloprid indicating thiacloprid to be more toxic among the three neonicotinoids to adult mosquitoes. In contrast, against *Cx. quinquefasciatus*, imidacloprid showed increased toxicity followed by thiacloprid and thiamethoxam. In larvicidal bioassays, more toxicity was exhibited by imidacloprid followed by thiamethoxam and thiacloprid in *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti.*

The present study showed that the resistant strains registered lower LC_{50} values than the susceptible ones, indicating the possibility of using these to control the insecticide resistant vectors. In the study carried out by Darriet and Chandre[20] using different combinations of neonicotinoids and piperonyl butoxide (PBO)+deltamethrin, the combinations produced higher mortality in resistant mosquitoes than the neonicotinoids alone.

Insecticide resistance in mosquito vectors is a growing concern in many countries, and there is an urgent need for search of new compounds with different modes of action which do not show cross resistance to insecticides being used in the vector control programmes like organochlorines, organophosphates, carbamates and pyrethroids. Absence of cross-resistance in neonicotinoids with pyrethroids, carbamates and organophosphates, and organochlorines makes them potential candidates for use in mosquito control activities[21]. The possibility of using neonicotinoids for mosquito control should be explored and it can be a viable option to control mosquitoes that have already become resistant to the insecticides being used. The susceptibility of insect populations to insecticides with new modes of action can be influenced by the previous exposure to insecticides[22], the use of neonicotinoids for mosquito control appears to be less affected by existing resistant mechanisms because these are not being used in mosquito vector control programmes. Cross-resistance studies could not be carried out due to technical reasons and this was a limitation of the present study and certain tests could not be conducted on *Ae. aegypti* due to discontinuation of the colony owing to dengue threat in Delhi. Further investigations are required to study the toxicity of the compounds on non-target species that co-habit with mosquito larvae. Owing to their high mammalian safety the neonicotinoids could be an option for mosquito control.

Conflicts of Interest: None.

Table IV. Results of larval susceptibility tests with three neonicotinoids (72 h mortalities)

Species (n) Susceptibility status	Susceptibility/resistant status	Imidacloprid			Thiacloprid			Thiamethoxam		
		LC_{50} (mg/l)	LC_{90} (mg/l)	P value	LC_{50} (mg/l)	LC_{90} (mg/l)	P value	LC_{50} (mg/l)	LC_{90} (mg/l)	P value
Anopheles stephensi SS strain (Nadiad)	Susceptible to fenthion-temephos-malathion	0.049 (n=752)	0.086	$P<0.001$	24.232 (n=891)	97.5	$P<0.001$	0.052 (n=752)	0.155	NS
An. stephensi (Goa) RR	Susceptible to fenthion-malathion and resistant to temephos	0.066 (n=548)	0.77	NS	22.511 (n=806)	111.824	$P<0.001$	0.064 (n=638)	0.104	$P<0.05$
Culex quinquefasciatus	Susceptible to fenthion-temephos and resistant to malathion	0.02 (n=638)	0.07	NS	6.139 (n=800)	16.88	NS	0.343 (n=548)	8.53	$P<0.001$
Aedes aegypti	Susceptible to fenthion-malathion and tolerant to temephos	0.21 (n=548)	0.27	NS	ND	ND	ND	0.298 (n=540)	2.278	$P<0.001$

ND, not done; NS, not significant

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