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Efficacy of Various Insect Growth Regulators on Organophosphate Resistant Immatures of *Culex quinquefasciatus* (Diptera: Culicidae) from Different Geographical Areas of India

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Abstract: *Culex quinquefasciatus* is a primary vector of periodic lymphatic filariasis in tropical and subtropical world. In the present study, efficacy of different Insect Growth Regulators (IGRs) such as natural neem product (azadirachtin) and synthetic Chitin Synthesis Inhibitors (CSI) like diflubenzuron, triflumuron and lufenuron have been studied on organophosphate resistant late 3rd-early 4th instar larvae of *C. quinquefasciatus* belonging to four different geographical locations of India i.e., Jodhpur, Bikaner, Jamnagar and Bathinda and laboratory population. Resistance Ratio (RR) in 50% emergence inhibition (IE_{50}) has been carried out between field populations and laboratory population. Among the IGRs, the CSI (IE_{50} = 0.0002-0.0006 mg L^{-1}) have been found to be very effective at very low doses in comparison to azadirachtin (IE_{50} = 0.0171-0.0244 mg L^{-1}). Among CSI, triflumuron was most effective (IE_{50} = 0.0002 mg L^{-1}) than the others (IE_{50} = 0.0003-0.0006 mg L^{-1}), indicating no considerable resistance in any larval population against any IGR (RR = 0.6-1.5 folds). The present study indicates high efficacy of these IGRs and suggests use of these IGRs as an effective alternative source of mosquito larval control where insecticide resistance has developed for other conventional insecticides.

Key words: Insect growth regulators, susceptibility status, resistance ratio, *Culex quinquefasciatus*

INTRODUCTION

Lymphatic filariasis is a major vector borne disease making about 120 million peoples in 83 countries physically disabled (WHO, 2006a) which is transmitted by *C. quinquefasciatus* mosquito having cosmopolitan distribution. WHO (1992a) have suggested various controlling strategies to control vector transmission at different levels. Among the available vector control methods, chemical control is decisively superior over environmental and biological control strategies that have limited applicability in mitigating sporadic unpredictable outbreaks of vector borne disease. However, *C. quinquefasciatus* has also shown resistance to different insecticides used in mosquito control such as organochlorines, organophosphorous (OP), pyrethroids and microbial insecticides throughout the world (WHO, 1992b; Dorta *et al*., 1993; Wirth and Georghiou, 1996; Cheikh *et al*., 1998; Mulla *et al*., 2003; Mittal *et al*., 2004; Paul *et al*., 2005; Tikar *et al*., 2008). Among the various synthetic insecticides, Insect Growth Regulators (IGRs) are very specific in their mode of

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action for insect pests and safer to non-target organisms and environment (Mulla, 1995). Either, IGRs are interrupting the functioning of endocrine mechanism or synthesis of chitin in cuticular structures during development of insect (Mulla, 1995; Tunaz and Uygun, 2004). Various synthetic IGRs such as diflubenzuron, lufenuron, triflumuron, novaluron, methoprene have been used against mosquitoes and different insect pest (Smith, 1995; Ali *et al*., 1999; Arredondo-Jimenez and Valdez-Delgado, 2006; Cetin *et al*., 2006). WHO (2006b) also recommended the use of diflubenzuron for mosquito control. Besides emergence inhibition, these IGRs also affect the fecundity and survival of mosquitoes at sublethal concentrations (Vasuki, 1992; Ritchie *et al*., 1997). Azadirachtin, a neem component, has shown antifeeding activities and suppression in reproduction and survival in different insects including mosquitoes (Kumar and Parmar, 1998; Su and Mulla, 1998; Mulla and Su, 1999; Mittal and Subbarao, 2003; Garcia *et al*., 2006; Okumu *et al*., 2007). The development of insecticide resistance against larvicides i.e., temephos, fenthion and neem compound (azadirachtin), at various levels have been reported in *C. quinquefasciatus* populations from Jodhpur, Bikaner, Jamnagar and Bathinda of India (Suman *et al*., 2010). In the present study, efficacy of different IGRs has been evaluated in the laboratory to find out the suitable alternative insecticide for mosquito control against OP resistant larvae of *C. quinquefasciatus* collected from arid (Jodhpur and Bikaner) and semi-arid zones (Jamnagar and Bathinda).

MATERIALS AND METHODS

Insect Collection and Rearing

Larval stages of *C. quinquefasciatus* were collected during 2007 from Jodhpur and Bikaner (Rajasthan) located in Thar Desert region, Bathinda (Punjab) located in Northern semi-arid region and Jamnagar (Gujarat) is belonging to coastal region of Arabian Sea and were transported to Defence Research and Development Establishment, Gwalior, India, to study the efficacy of various insecticides (Fig. 1). These larvae were maintained in standard laboratory conditions at 27±1°C and RH 75±5% and were kept at the density of 100 larvae in 2 L de-chlorinated water in enamel bowls. Larvae were given yeast tablets as larval food. After pupation, pupae were transferred to adult cages. Male and female mosquitoes (50 pairs) were released in mosquito cage (750×650×650 mm) and were provided with 10% sugar solution *ad libitum* dispensed through a cotton wick in small petri-dishes. Chicken was offered as source for blood feeding to female mosquitoes twice in a week. Glass petri-dishes with 400 mL water were kept for oviposition. The individual egg-raft was transferred in 2 L enamel bowl for hatching and allowed for further development of larvae.

Insecticides

The following Insect Growth Regulators (IGRs) have been used for the study: Azadirachtin 1% EC (Neemarin®, Biotech International Ltd., India), diflubenzuron (Dimilin® 25% WP, Uniroyal Chemical Company, USA), triflumuron 48% EC (Bayer Ltd., Germany) and lufenuron 5% EC (Novartis India Ltd., India).

Bioassay

Larval susceptibility assays were carried out according to methods of WHO (1981). Late 3rd-early 4th instar larvae of F1 generation in 5 replicates (20 larvae/replicate) in 250 mL dechlorinated water in 400 mL glass beaker have been used for susceptibility evaluation. The 1% stock solution of various insecticidal formulations has been prepared in distilled water

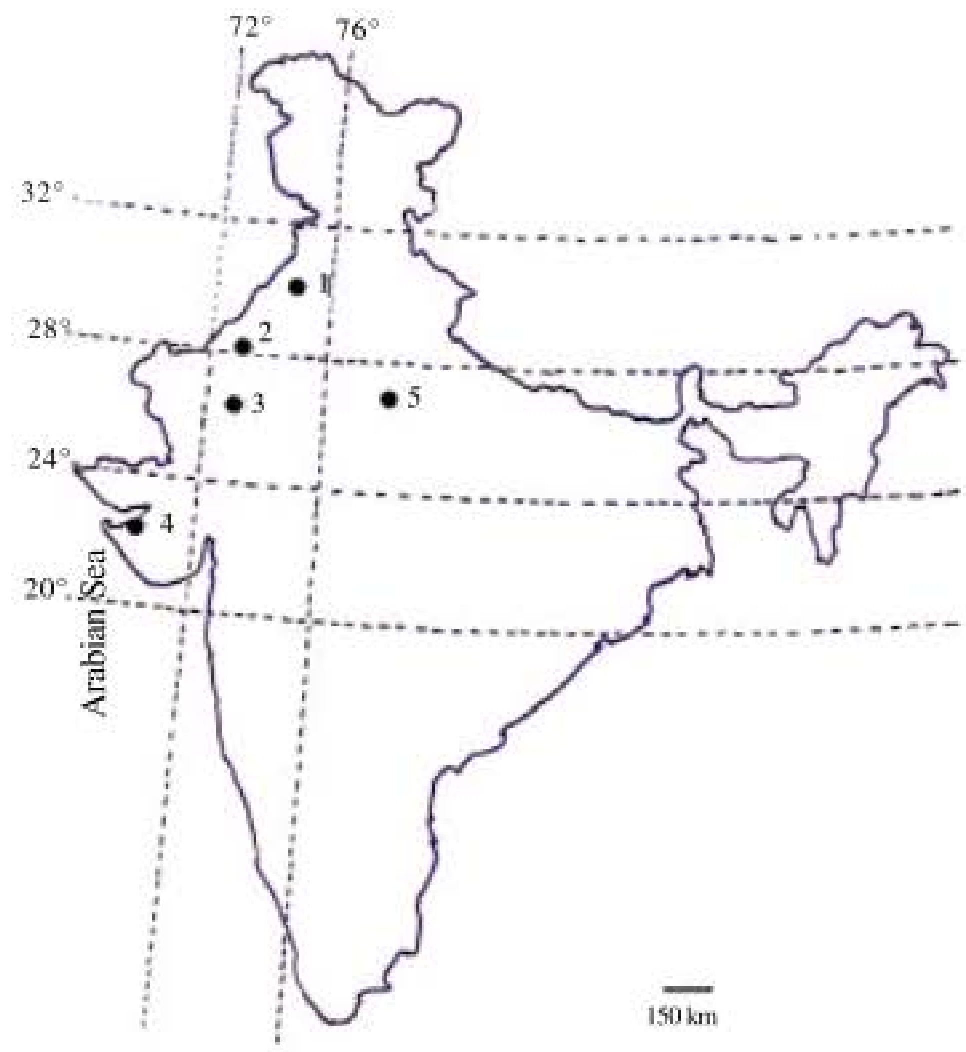

Fig. 1: Locations of collections of *Culex quinquefasciatus* in India. 1: Bathinda, 2: Bikaner, 3: Jodhpur, 4: Jamnagar and 5: Gwalior

and fresh stock solution has been used for making subsequent 5-6 serial dilutions. All experimental beakers were kept in 12:12 light:dark hours. Dead larvae, pupae or partly emerged adults were regularly removed. The observation on emergence of successful adults has been recorded daily until emergence or mortality of last individual of each treatment and control. The incomplete emergence or adult attached with pupal skin are considered as dead. Test showing mortality more than 20% in control was not considered.

Data Analysis

Data have been subjected to probit analysis (Finney, 1971) (Indostat Statistical Software, India) to determine the 50% adult emergence inhibition (IE_{50}) and 90% adult emergence inhibition (IE_{90}). If the control mortality ranged from 5 to 20%, the mortalities of treated groups are corrected according to Abbott's formula (Abbott, 1925). The Resistance Ratio (RR) has been calculated as ratio between IE_{50} of field strain and IE_{50} of laboratory strain of *C. quinquefasciatus.*

RESULTS

The results of the present study indicated that the IE_{50} and IE_{90} of diflubenzuron for different populations ranged between 0.0003 to 0.0006 mg L^{-1} and 0.0022 to 0.0033 mg L^{-1}, respectively (Table 1). Among the field populations, Jamnagar population was most susceptible while Bikaner population was found to be least susceptible to diflubenzuron. The resistance ratio for diflubenzuron ranged from 0.6 to 1.2 folds among the field populations in comparison to laboratory population (Table 1). In case of lufenuron, the IE_{50} and IE_{90} ranged from 0.0004 to 0.0006 mg L^{-1} and 0.0014 to 0.0041 mg L^{-1} among all the populations, respectively (Table 2). The efficacy of lufenuron was found to be lowest for Jodhpur and Bathinda populations and highest for Bikaner and Jamnagar populations. The resistance ratio for lufenuron among the field population ranged from 1.25 folds to 1.5 folds than laboratory population (Table 2). Triflumuron has been found to be effective between 0.0002 to 0.0003 mg L^{-1} at IE_{50} and 0.0009 to 0.0016 mg L^{-1} at IE_{90} among all the populations (Table 3). The resistance ratio for triflumuron among the field populations and laboratory population ranged from 1.0 fold to 1.5 folds indicating low efficacy against Bathinda population and high efficacy against rest of the populations in comparison to laboratory population. The efficacy of neem compound azadirachtin at IE_{50} and IE_{90} ranged from 0.0171 to 0.0244 mg L^{-1} and 0.0798 to 0.1157 mg L^{-1}, respectively, indicating inconsiderable resistance ratio (at IE_{50}) ranging from 0.9 fold to 1.3 folds among the field populations in comparison to laboratory population (Table 4). The efficacy of azadirachtin was minimum for Jamnagar population and maximum for Bikaner population.

Table 1: Inhibition of Emergence (IE) of late 3rd-early 4th instars of laboratory and various field populations of *Culex quinquefasciatus* against Diflubenzuron IGR

Populations	IE_{50} (95% FL) (mg L^{-1})	IE_{90} (95% FL) (mg L^{-1})	χ^2	Slope	RR
Laboratory	0.0005 (0.0004-0.0006)	0.0027 (0.0020-0.0035)	0.069	Y = 10.883+1.785X	-
Jodhpur	0.0005 (0.0004-0.0006)	0.0023 (0.0018-0.0029)	0.527	Y = 11.564+1.996X	1.00
Bikaner	0.0006 (0.0005-0.0007)	0.0033 (0.0023-0.0045)	6.018	Y = 10.804+1.778X	1.20
Jamnagar	0.0003 (0.0003-0.00043)	0.0022 (0.0016-0.0029)	0.483	Y = 10.703+1.651X	0.60
Bathinda	0.0005 (0.0004-0.00057)	0.0029 (0.0021-0.0040)	3.832	Y = 10.791+1.739X	1.00

RR: Resistance ratio, χ^2: Chi square

Table 2: Inhibition of Emergence (IE) of late 3rd-early 4th instars of laboratory and various field populations of *Culex quinquefasciatus* against lufenuron IGR

Populations	IE_{50} (95% FL) (mg L^{-1})	IE_{90} (95% FL) (mg L^{-1})	χ^2	Slope	RR
Laboratory	0.0004 (0.0003-0.00041)	0.0014 (0.0011-0.0018)	5.501	Y = 12.592+2.187X	-
Jodhpur	0.0006 (0.0005-0.00067)	0.0032 (0.0023-0.0044)	0.531	Y = 10.603+1.726X	1.50
Bikaner	0.0005 (0.0004-0.00057)	0.0026 (0.0019-0.0035)	1.712	Y = 10.984+1.800X	1.25
Jamnagar	0.0005 (0.0004-0.00066)	0.0041 (0.0028-0.0062)	4.522	Y = 9.915+1.504X	1.25
Bathinda	0.0006 (0.0005-0.00065)	0.0033 (0.0024-0.0046)	1.641	Y = 10.523+1.693X	1.50

RR: Resistance ratio, χ^2: Chi square

Table 3: Inhibition of Emergence (IE) of late 3rd-early 4th instars of laboratory and various field populations of *Culex quinquefasciatus* against triflumuron IGR

Populations	IE_{50} (95% FL) (mg L^{-1})	IE_{90} (95% FL) (mg L^{-1})	χ^2	Slope	RR
Laboratory	0.0002 (0.0001-0.00021)	0.0009 (0.0007-0.0012)	0.315	Y = 11.520+1.736X	-
Jodhpur	0.0002 (0.0002-0.00028)	0.0011 (0.0009-0.0015)	5.693	Y = 12.304+2.010X	1.00
Bikaner	0.0002 (0.0002-0.00027)	0.0010 (0.0008-0.0013)	0.573	Y = 12.186+1.978X	1.00
Jamnagar	0.0002 (0.0002-0.00024)	0.0012 (0.0009-0.0015)	0.209	Y = 11.234+1.689X	1.00
Bathinda	0.0003 (0.0002-0.00031)	0.0016 (0.0011-0.0022)	1.647	Y = 10.805+1.619X	1.50

RR: Resistance ratio, χ^2: Chi square

Table 4: Inhibition of Emergence (IE) of late 3rd-early 4th instars of laboratory and various field populations of *Culex quinquefasciatus* against Azadirachtin IGR

Populations	IE_{50} (95% FL) (mg L^{-1})	IE_{90} (95% FL) (mg L^{-1})	χ^2	Slope	RR
Laboratory	0.0182 (0.0156-0.0211)	0.0879 (0.0652-0.1186)	1.602	Y = 8.319+1.898X	-
Jodhpur	0.0202 (0.0171-0.0237)	0.1161 (0.0812-0.1661)	3.559	Y = 7.917+1.709X	1.11
Bikaner	0.0171 (0.0147-0.0198)	0.0798 (0.0597-0.1067)	6.185	Y = 8.559+1.997X	0.94
Jamnagar	0.0244 (0.0213-0.0279)	0.0946 (0.0726-0.1231)	1.519	Y = 8.574+2.215X	1.34
Bathinda	0.0193 (0.0164-0.0228)	0.1157 (0.0802-0.1669)	6.828	Y = 7.912+1.684X	1.06

RR: Resistance ratio, χ^2: Chi square

DISCUSSION

Culex quinquefasciatus is a prominent vector of periodic bancroftian lymphatic filariasis prevalent throughout the world besides being severe biting nuisance. This is a dominating vector species in urban or semi-urban areas and persists throughout the year. To control vector borne disease, WHO (1992b) has emphasized on destruction of vector mosquitoes involving primarily several insecticides. However, indiscriminate and continuous use of various insecticides has resulted in development of resistance in mosquitoes. Tikar *et al.* (2008) have recorded development of resistance in *A. aegypti* larvae against temephos, fenthion, malathion and DDT from different locations in India. The decrease in insecticidal susceptibility or development of insecticide resistance in populations of *C. quinquefasciatus* has been reported against temephos (upto 10.8 folds), fenthion (upto 6.94 folds), neem compound (upto 5.29 folds), cypermethrin (upto 3.19 folds), α-cypermethrin (upto 5.48 folds) and λ-cyhalothrin (upto 3.33 folds) from Bathinda, Bikaner, Jodhpur and Jamnagar in India indicating need of search for some safe and effective alternative control measures or larvicidal molecules. Besides development of insecticide resistance, insecticides are known to also be toxicity to other non-target organisms. Use of temephos and other insecticides that contaminate the human's drinking-water and food stuffs for domestic animals have also been subdued by WHO Pesticide Evaluation Scheme (WHOPES, 2002). In contrast to insecticides, IGRs are very specific in their mode of action which may be of natural or synthetic in origin. The IGRs have added advantage of being less toxic or even safe to non-target organism and environment than conventional insecticides (Mulla, 1995). The development of a new class of synthetic IGRs, benzoylphenylureas (chitin synthesis inhibitors) was a successful step forward towards non-hazardous and eco-safe Integrated Pest Management (IPM) (Tunaz and Uygun, 2004). Though, a number of IGR compounds have been evaluated against mosquitoes (Estrada and Mulla, 1986; Ansari *et al.*, 1991; Fournet *et al.*, 1993), but only methoprene, a juvenoid (JH mimic) and diflubenzuron, an ecdysoid (chitin synthesis inhibitor) have been widely used in public health.

Diflubenzuron was the first benzoylphenylurea derivative Chitin Synthesis Inhibitor (CSI) introduced in market as a novel insecticide (Miyamoto *et al.,* 1993). In present study, high efficacy of diflubenzuron (IE_{50} = 0.0003-0.0006 mg L^{-1}) has been observed against all the OP resistant field populations of *C. quinquefasciatus* in comparison to laboratory population. In other laboratory evaluations, Mulla (1995) reported LC_{90} for 4th instar larvae of *C. quinquefasciatus* to be 0.002 mg L^{-1} which was almost similar to the results of the present study. Ali *et al.* (1999) reported the high LC_{50} (0.0014 ppm) and LC_{90} (0.0034 ppm) for *C. quinquefasciatus* against diflubenzuron from Dhaka (Bangladesh) indicating low susceptibility level in comparison to present study. Perhaps, this might be due to no or negligible use of IGRs particularly CSI in studied areas of India for the control of mosquitoes or any other insect pests. Similar to present study, Ansari *et al.* (2005) studied the laboratory efficacy (LC_{50}) of formulations of diflubenzuron against different species of mosquitoes indicating slightly high efficacy of 25WP formulation against *A. stephensi* (0.0007 ppm), *A. culicifacies* (0.0008 ppm) but slightly less for *A. aegypti* (0.001) and *C. quinquefasciatus* (0.0011 ppm) in comparison to 22SL formulation (LC_{50} for *A. stephensi*-0.0009 ppm, *A. culicifacies*-0.0011 ppm, *A. aegypti*-0.0009 ppm and *C. quinquefasciatus*-0.0008 ppm). Diflubenzuron has also shown good efficacy against various mosquito species in field studies (Mulla, 1995; Ansari *et al.*, 2005). Sharma *et al.* (1979) showed effective control (80-100%) of *C. fatigans* breeding in polluted drains using 0.5 to 1 ppm of Dimilin (diflubenzuron). Ansari *et al.* (2005) showed more than 50% emergence inhibition of

C. quinquefasciatus in polluted drains and pools upto 7-9th week at 0.008 g m^{-2} of diflubenzuron. In another study, Cetin *et al*. (2006) used 0.1-0.03 mg L^{-1} of granular formulation and wettable powder of diflubenzuron in septic tank water against *C. pipiens* larvae from City of Antaliya, Turkey and noted 100% mortality up to 28 post-treatment days. This indicates successful application of diflubenzuron in polluted habitats which are the preferred breeding sites of *C. quinquefasciatus*. According to WHO (2006b), diflubenzuron has low acute and chronic toxicity to mammals with no indication of carcinogenicity, mutagenicity or teratogenicity and it can be used at the dosage of 25-100 g ha^{-1} a.i. in open water bodies. However, higher dosages are required in polluted and vegetated habitats. These various studies have shown potency of application of diflubenzuron against *C. quinquefasciatus* in various types of habitats.

Structural modifications of the IGR were to be found too more effective than parent compound. Triflumuron is a benzoylphenylurea and acts as CSI in cuticle of arthropods. It has been proved as ovicidal and larvicidal (Smith and Grigaril, 1989) and sterile agent (Howard and Wall, 1995). It has been evaluated against various agriculture and medical pests of human and veterinary importance such as mosquitoes (Miura and Takahashi, 1979; Amalraj *et al*., 1988; Sulaiman *et al*., 2004; Batra *et al*., 2005), coleopterans (Mian and Mulla, 1982), German cockroaches (Weaver *et al*., 1984; Demark and Bennett, 1990), *Locusta migratoria* (Hanrieder *et al*., 1993) and house flies (Vazirianzadeh *et al*., 2007). High efficacy of triflumuron (IE_{50}: 0.0002-0.0003 mg L^{-1}) in present study against OP resistant *C. quinquefasciatus* belonging to different geographical areas of India has been eliciting attention for effective use of this IGR. The earlier laboratory evaluation of triflumuron has shown high LC_{90} (0.007 mg L^{-1}) (Mulla, 1995) in comparison to present study (0.0009-0.0016 mg L^{-1}). Similarly, Batra *et al*. (2005) showed 50% adult emergence inhibition at 0.0003 ppm for *C. quinquefasciatus*, 0.0002 ppm for *A. aegypti* and 0.0001 ppm for *A. stephensi* in clear and polluted waters. The field efficacy of triflumuron against *C. quinquefasciatus* indicated that 0.5 and 1 ppm concentrations provided 93.3 and 100% emergence inhibition in drains upto the 6th week and 95.5 and 88.88% emergence inhibition in pools, respectively. However, these concentrations were slightly more effective (99.7 and 100% emergence inhibition) upto the 6th week in tanks (Batra *et al*., 2005). Martins *et al*. (2008) have also shown that the organophosphate resistant (resistance ratio = 13.8) population of *A. aegypti* was susceptible to triflumuron (resistance ratio = 1.3) in Brazil. These studies indicate the potency of triflumuron IGR in insecticide resistance management in mosquitoes.

Lufenuron is also a benzoylphenylurea that has similar mode of action to diflubenzuron and triflumuron. It has been used against fleas (Smith, 1995; Dean *et al*., 1999), fungus (Douglas *et al*., 2003; Richard *et al*., 2005) and *Helicoverpa armigera* (Butter *et al*., 2003). In case of dipteran insect like *Drosophila melanogaster*, lufenuron affects fecundity and production of viable eggs (Wilson and Cryan, 1997). The present study also indicated high susceptibility of *C. quinquefasciatus* populations (IE_{50}: 0.0005 to 0.0006 mg L^{-1}) collected from Jodhpur, Bikaner, Jamnagar and Bathinda against this IGR.

About 2000 plant species have been reported to possess pest control properties (Ahmed *et al*., 1984) and of these about 344 species of plants have been reported to possess bioactive materials showing some activity against mosquitoes (Sukumar *et al*., 1991). The most prominent phytochemical pesticides found in neem tree (*Azadirachta indica* A. Juss) known as azadirachtin, a steroid like tetranotriteroenoid, was isolated in 1968 from neem seeds (Butterworth and Morgan, 1971) along with about 40 bitter principles components belonging to diterpenoid, triterpenoids and flavinoid groups from different parts of neem tree.

The structural geometry of azadirachtin was revealed by Nakanishi (1975). These neem compounds have shown broad range of bioactivity against insects such as reproductive fitness, oviposition, hatchability, antifeedent, repellent, metamorphosis disruption and death (Su and Mulla, 1998; Kumar and Parmar, 1998; Mulla and Su, 1999; Mittal and Subbarao, 2003; Garcia *et al*., 2006). The efficacy of azadirachtin IGR was found to be high (IE_{50}: 0.0171-0.0244 mg L^{-1}) against OP resistant populations of *C. quinquefasciatus* from different locations of India in comparison to laboratory population in present study. Vatandoost and Vaziri (2004) showed efficacy (LC_{50}) of Neemarin (azadirachtin) to be high against *A. stephensi* (0.35 mg L^{-1}) in comparison to *C. quinquefasciatus* (0.69 mg L^{-1}). However, it is very low than the results of the present study. The ovicidal properties of azadirachtin in *C. tarsalis* and *C. quinquefasciatus* have been studied by Su and Mulla (1998) which indicates that 1 ppm azadirachtin significantly reduced egg hatching but it was non-significant at 0.5 ppm. Significant reduction in hatching of freshly laid eggs and non-significant reduction for 4 h in old eggs has been recorded with 10 ppm for 36 h. In a study, Feng and Ishman (1995) showed the development of resistance in peach potato aphid *Myzus persicae* to pure azadirachtin over 4 generations, but the same did not happen with neem extract. The multiple mode of action or complexity of mechanism may be responsible for avoiding the development of resistance in insects against mixture of neem compounds. These features of neem compound may contribute for its use in vector control.

Among all the 4 IGRs molecule studied, triflumuron (IE_{50} = 0.0002-0.0003 mg L^{-1}) is most efficient IGR with narrowest activity range among the different OP resistant populations than diflubenzuron (IE_{50} = 0.0003-0.0006 mg L^{-1}), lufenuron (IE_{50} = 0.0004-0.0006 mg L^{-1}) and natural product azadirachtin (IE_{50} = 0.0171-0.024 mg L^{-1}). However, all the studied IGRs have good efficacy than azadirachtin (Table 1-4), though the resistance ratio was less for azadirachtin and other IGRs in OP resistant field populations of *C. quinquefasciatus* than laboratory population (0.6 to 1.5 folds) indicating absence of resistance for these IGRs in all the field populations of *C. quinquefasciatus*. The other chitin synthesis inhibitor like novaluron has also been evaluated against different mosquito species from southern Chiapas, Mexico (Arredondo-Jimenez and Valdez-Delgado, 2006) which indicates highest susceptibility (LC_{50}) of 3rd instar (0.0013 mg L^{-1}) larvae of *C. quinquefasciatus* in comparison to 1st instar (0.0095 mg L^{-1}) and pupal stages (0.0096 mg L^{-1}) of this mosquito and other species of mosquitoes (0.0254-0.0326 mg L^{-1}). However, these doses are very high in comparison to present study. This variation might be due to difference in chemical structure of applied molecule and strains of mosquito. Various studies have also referred use of various IGRs for control of mosquitoes with high efficacy and in safe-manner in different habitats (Mulla and Darwazeh, 1988; Mulla, 1995; Dean *et al*., 1998; Ali *et al*., 1999; Mulla and Su, 1999; Ansari *et al*., 2005; Cetin *et al*., 2006). The present study suggests that diflubenzuron, lufenuron, triflumuron and azadirachtin have the potential IGR activity against different field populations of larval *C. quinquefasciatus* belonging to Jodhpur, Bikaner, Jamnagar and Bathinda in India which have shown development of resistance against larvicides such as temephos and fenthion (Suman *et al*., 2010). These IGRs may be used effectively as safe and ecofriendly measure against mosquito larvae in those areas where the insecticide resistance against conventional OP larvicides have emerged.

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