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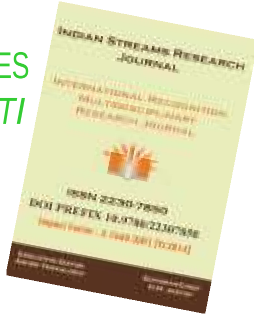
INSECT GROWTH REGULATORY ACTIVITY OF FUNGI *METARHIZIUM ANISOPLIAE* AGAINST VECTOR MOSQUITOES *AN. STEPHENSI CX. TRITAENIORHYNCHUS* AND *AE. AEGYPTI*



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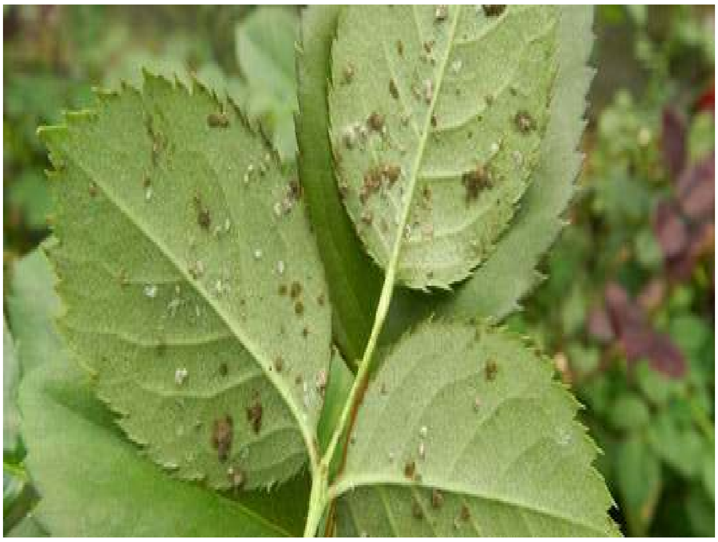
ABSTRACT

Mosquitoes are among the most important insect pests affecting the health of people and animals. In the present study, the effect of Insect Growth Regulatory (IGR) activity of fungi *Metarhizium anisopliae* (*M. anisopliae*) against the mosquitoes *An. stephensi, Cx.tritaeniorhynchus* and *Ae. aegypti*. Conidia of *M. anisopliae* were tested against eggs of the vector mosquitoes by adding fungal suspension to plastic cups containing 50 ml of distilled water with 100 larvae of mosquitoes. Each cup was inoculated with 1ml of fungal suspensions ($1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ and $1x10^9$ conidia/ml). The IGR activity of *M. anisopliae* against the malarial vector *An. stephensi* the EI_{50} values are 4.06, 3.13, 2.70 and 1.86 conidia/ml for larval age groups I, II, III and IV respectively. The bio assay with the entomopathogenic fungi of M. anisopliae against Cx. tritaeniorhynchus revealed the EI_{50} values of four larval groups were 3.01, 2.05, 1.75 and 1.42 conidia/ml I, II, III and IV age groups respectively. *M. anisopliae* as an improved act of Insect Growth Regulatory activity agents in laboratory bioassays as well as simulated field conditions suggests that it may have good potential to become part of a mosquito control program.



KEYWORDS :*IGR activity, Metarhizium anisopliae, Biological control, Anopheles stephensi, Cx. tritaeniorhynchus, Ae. aegypti.*

INTRODUCTION:-

Mosquitoes are the most powerful silent killer insects in the World. It has been carried on the dangerous diseases in human to human or animal to human. There were an estimated 198 million cases of malaria worldwide (range124 283 million) in 2013. An estimated 3.3 billion people are at risk of malaria, of which 1.2 billionare at high risk. In highrisk areas, more than one malaria case occurs per 1000 population [1]. The WHO currently estimates there may be 50 million cases of Dengue fever infection worldwide every year [2]. Filariasis caused by *Wuchereria bancrofti* is transmitted by *Cx. quinquefasciatus* mosquitoes which are widespread in the country now and lymphatic filariasis infects 80 million people annually of which 30 million cases exist in chronic infection. There are 45 million cases of lymphatic filariasis in India alone [3]. The current global strategy for the control of vector borne diseases is based on vector control, achieved through larvicidal (control of larvae), adulticidal (control of adults), and bite prevention (applications of repellents and bed nets). Chemical substance that disrupts the action of insect hormones controlling molting, maturity from pupal stage to adult, and others is called Insect Growth Regulatory (IGR) activity [4]. Majority of the chemical pesticides is harmful to man and animals, some of which are not easily degradable and spreading toxic effects recent studies stimulated the investigation of insecticidal properties of plant derived from materials or botanicals and concluded that they are environmentally safe, degradable and target specific [5]. Entomopathogenic fungi are distributed in a wide range of habitats including aquatic forest, agricultural, pasture, desert, and urban habitats [6]. More than 700 species of fungi from around 90 genera are pathogenic to insects [7]. The IGRs have an added advantage of being used at a relatively very low dose compared to the conventional insecticides. Ecdysone agonists are hormonally active insect growth regulators that disrupt development of larvae and are found to be active against *Aedes aegypti, Anopheles stephensi,* and *Cx.quinquefasciatus* [8]. The anamorphic entomopathogenic fungi *Beauveria bassiana* (Balsamo) *Vuillemin* and *Metarhizium anisopliae* (Metschnikoff) Sorokin from the order Hypocreales (Asco- mycota) are natural enemies of a wide range of insects and arachnids and both fungi have a cosmopolitan distribution [9]. Fungal species such as *M. anisopliae* and *B. bassiana* are well characterized in respect to pathogenicity to several insects and they have been used as agents for the biological control of agriculture pests worldwide. The fungus *Metarhizium anisopliae* is a well-known insect–pathogenic fungus, causing mortality in a wide range of insects, including Anopheles gambiae, for which it is currently studied as a potential biocontrol agent [10]. In the present statement, we describe Insect Growth regulatory (IGR) activity of *M. anisopliae* against the vector mosquitoes *Anopheles stephensi, Cx. tritaeniorhychus* and *Ae. aegypti.*

MATERIALS AND METHODS

Collection and laboratory maintenance of mosquito larva

Mosquito larva were collected from several neighbourhoods, including urban, rural and semi-urban regions of Annamalai Nagar, Chidambaram, India and erected in water containing glucose and yeast powder. The colonies of *An. stephensi, Cx. tritaeniorhynchus* and *Ae. aegypti* were maintained in the laboratory at a temperature of 25 °C with a relative humidity of 75% and 24 h photoperiod. The mosquito larvae were maintained in enamel containers.

PREPARATION OF FUNGUS

The fungal strains of *M. anisopliae* (MTCC: 6060) were obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. These strains were routinely maintained in our laboratory on Potato Dextrose Agar at 25 °C. Conidia of *M. anisopliae* were tested against larvae of the vector mosquitoes by adding fungal suspension to plastic cups containing 50 ml of distilled water with 100 eggs/ egg rafts of mosquitoes. Each cup was inoculated with 1ml of fungal suspensions ($1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ and $1x10^9$ conidia/ml). The control was carried out by 100ml of distilled water.

BIOASSAY

The fungi were tested for Insect growth regulatory (IGR) activity against *An. stephensi. Ae. aegypti* and *Cx. tritaeniorhynchus* by following the standard procedure [11]. Conidia of *M. anisopliae* were tested against eggs of the vector mosquitoes by adding fungal suspension to plastic cups containing 50 ml of distilled water with 100 larvae of mosquitoes. The five different test concentrations were prepared for each conidia and the test concentrations were replicated six times. The control experiments were run parallel with each replicated. Mortality counts were recorded daily. Mortality of the larvae, larval pupal intermediate, pupal mortality and adult mortality was recorded at regular intervals. Observation was continued in both treated and control bowl until the last immature pupates. Morphological abnormalities were also noted. The dead larvae and pupae were removed daily and counted. The percent of emergence at different concentrations were recorded.

Growth index was assessed by the following formula.

$$GI = \frac{\text{Total emergence \%}}{\text{Average developmental period (days)}}$$

RESULTS

The effects of *M. anisopliae* against *An. stephensi* I Instar was found to be Table 1. The total emergence days were comparatively high in other instars. The EI_{50} values of *M. anisopliae* against *An. stephensi* I instar was 4.06 conidia/ml. The EI_{50} values of *M. anisopliae* against *An. stephensi* II instar were 3.13 conidia / ml. The larval mortality was purely depending on the concentration. The EI50 value was 2.70 conidia/ml in III instar. The growth index of the III instar were 3.32, 2.23, 1.66, 1.14 and 0.65 respectively. The $1x10^9$ conidia/ml found to be more effective than other concentration. The highest concentrations of $1x10^9$ conidia/ ml were produced 77.48 percent of mortality in IV instar of An. stephensi. The growth index was 4.38, 3.76, 2.77, 2.02 and 1.40 respectively. EI_{50} value of *M. anisopliae* was 1.86 conidia/ml.

The IGR activity of *M. anisopliae* against *Cx. tritaniorhynchus* I instar EI_{50} value was 3.01 conidia/ml. The growth index was 2.80, 2.12, 1.47, 0.79 and 0.20 respectively. The total mortality at $1x10^9$ conidia/ml exerted 96.54 percent of I st instar (Table 2). The EI_{50} against *Cx. tritaeniorhynchus* II instar was 2.05 conidia/ml. The IGR activity of *M. anisopliae* against *Cx. tritaenioryhnchus* IIIrd Instar EI50 value was ranged at 1.75 conidia/ml. The average days of developmental period taken 17 days. The Growth index was examined from 4.20, 3.46, 2.94, 2.37 and 1.54. The total mortality of larva to adult observed at 73.74 percent at $1x10^9$ conidia/ml. The EI50 value of the IGR activity IV instar was 1.42 conidia/ml. The growth index was ranged from 4.87, 4.43, 3.85, 3.21 and 2.20 at different concentration from $1x10^5$ to $1x10^9$ conidia/ml.

The insect growth regulatory activity of *M. anisopliae* against *Ae. aegypti* vary with different concentrations Table 3. The EI_{50} value was I instar 2. 77 conidia/ml. The growth index was ranged from 4.03, 3.20, 2.13, 0.93 and 0.00 respectively. The growth index was reduced at higher concentration. The larval mortality was resulted in 12.18 to 30.24 percent at $1x10^5$ to $1x10^9$ conidia/ml. The total mortality was observed 99.98 percent at $1x10^9$ conidia/ml. The average day of the development was 16 to 15 days. The highest concentrations of $1x10^9$ conidia/ml of the fungus produced 84.98 percent of total mortality. The growth index was 5.14, 4.53, 3.51, 2.37 and 1.07. The current study showed that the fungi of *M anisopliae* exerted IGR activity against *Ae. aegypti* II instar. The EI_{50} value was 1.74 conidia/ml. The growth index was ranged from 6.56, 5.54, 4.43, 4.03 and 2.69 the growth index was reduced at higher concentration of $1x10^9$ conidia/ml. The larval mortality ranged from 8.08 percent at $1x10^5$ conidia/ml and 20.16 percent of higher concentration of $1x10^9$ conidia/ml. The total mortality was resulted 67.68 per cent at $1x10^9$ conidia/ml. The EI_{50} values o*f M. ansiopliae* against *Ae. aegypti* IV instar was 1.44 conidia/ml. The Growth index was decreased at higher concentration viz., 7.60, 6.10, 5.46, 4.75 and 3.66 at $1x10^{5}$ to $1x10^{9}$ conidia/ ml. The larval mortality was increased at higher concentration.

The fungi tested against the larval age groups of *An. stephensi*, *Cx. tritaeniorhynchus* and *Ae. aegypti*, the larvae of *An. stephensi* were more susceptible than the larvae of *Cx. tritaeniorhynchus* and *Ae. aegypti*. Duration of the larval period and the total development time was prolonged. The pupal mortality was increased then larval pupal intermediate mortality.

Table 1: Insect Growth Regulatory activity of *Metarhizium anisopliae* fungi against An. stephensi

Fungi	Instars	Concentration Conidia/ml	Larval mortality (%)	Larval pupal Intermediate mortality (%)	Pupal mortality (%)	Adult mortality (%)	Total mortality (%)	Total emergence (%)	Average developmental days	GI	EI 50 (Conidia /ml)
Metarhizium anisopliae	I	$1x10^{5}$	23.11 ± 0.16^{a}	19.15 ± 0.18^{a}	12.23 ± 0.14^{a}	9.13 ± 0.06^{a}	63.62±0.12	36.38±1.34	18.0	2.02±0.50	4.06
		$1x10^{6}$	25.08 ± 0.03^{b}	21.11 ± 0.11^{b}	15.08 ± 0.13^{b}	13.34 ± 0.33^{b}	74.61±0.16	25.39±0.09	18.0	1.41±0.80	
		$1x10^{7}$	28.08 ± 0.07^{c}	23.20 ± 0.18^{c}	17.10 ± 0.05^{c}	15.11 ± 0.06^{c}	83.49±0.05	16.51±0.00	18.5	0.89±0.01	
		$1x10^{8}$	31.19 ± 0.38^{d}	25.07 ± 0.04^{d}	18.07 ± 0.09^{d}	17.06 ± 0.07^{d}	91.39±0.99	8.61±.0.12	19.0	0.45±0.01	
		$1x10^{9}$	32.00 ± 0.31^{e}	26.05 ± 0.16^{e}	23.01 ± 0.17^{e}	18.00 ± 0.17^{e}	99.05±0.34	0.94±0.35	18.0	0.05±0.02	
	II	$1x10^{5}$	20.16 ± 0.09^{a}	17.07 ± 0.08^{a}	10.37 ± 0.20^{a}	7.14 ± 0.12^{a}	54.74±0.98	45.26±0.14	18.0	2.51±1.01	3.13
		$1x10^{6}$	22.08 ± 0.03^{b}	19.12 ± 0.12^{b}	12.20 ± 0.25^{b}	9.08 ± 0.16^{b}	62.48±0.08	37.52±0.16	18.0	2.08±1.09	
		$1x10^{7}$	26.07 ± 0.06^{c}	22.27 ± 0.23^{c}	15.00 ± 0.18^{c}	13.00 ± 0.08^{c}	76.34±0.11	23.66±0.05	18.5	1.27±0.09	
		$1x10^{8}$	27.17 ± 0.21^{d}	24.37 ± 0.31^{d}	20.11 ± 0.03^{d}	15.09 ± 0.12^{d}	86.74±0.90	13.26±1.21	18.0	0.73±0.02	
		$1x10^{9}$	30.10 ± 0.03^{e}	25.13 ± 0.61^{e}	22.31 ± 0.38^{e}	18.24 ± 0.20^{e}	95.78±0.15	4.22±0.03	17.0	0.24±0.11	
	III	$1x10^{5}$	16.14 ± 0.15^{a}	13.05 ± 0.05^{a}	9.26 ± 0.19^{a}	5.07 ± 0.09^{a}	43.52±0.12	56.48±0.02	17.5	3.22±1.02	2.70
		$1x10^{6}$	20.26 ± 0.18^{b}	17.28 ± 0.37^{b}	14.14 ± 0.11^{b}	10.31 ± 0.19^{b}	61.99±0.15	38.01±0.06	17.0	2.23±1.33	
		$1x10^{7}$	23.30 ± 0.23^{c}	19.11 ± 0.16^{c}	18.14 ± 0.17^{c}	11.09 ± 0.08^{c}	71.64±0.01	28.36±0.25	17.0	1.66±0.34	
		$1x10^{8}$	25.07 ± 0.06^{d}	21.24 ± 0.35^{d}	20.11 ± 0.03^{d}	14.14 ± 0.12^{d}	80.56±1.09	19.44±0.34	17.0	1.14±0.55	
		$1x10^{9}$	27.16 ± 0.17^{e}	24.18 ± 0.35^{e}	21.31 ± 0.38^{e}	16.24 ± 0.20^{e}	88.89±0.50	11.11±0.24	17.0	0.65±0.08	
	IV	$1x10^{5}$	12.32 ± 0.31^{a}	8.22 ± 0.33^{a}	5.10 ± 0.09^{a}	2.06 ± 0.04^{a}	27.70±0.03	72.30±1.02	16.5	4.38±0.06	1.86
		$1x10^{6}$	15.31 ± 0.08^{b}	11.27 ± 0.19^{b}	9.14 ± 0.06^{b}	4.12 ± 0.05^{b}	39.84±0.15	60.16±0.90	16.0	3.76±1.09	
		$1x10^{7}$	19.22 ± 0.05^{c}	16.11 ± 0.05^{c}	13.13 ± 0.09^{c}	7.14 ± 0.09^{c}	55.60±0.30	44.40±0.10	16.0	2.77±0.37	
		$1x10^{8}$	21.10 ± 0.17^{d}	20.14 ± 0.04^{d}	15.15 ± 0.09^{d}	10.21 ± 0.14^{d}	66.60±0.23	33.40±1.91	16.5	2.02±0.77	
		$1x10^{9}$	23.23 ± 0.23^{e}	22.07 ± 0.05^{e}	18.11 ± 0.09^{e}	14.07 ± 0.06^{e}	77.48±0.69	22.52±0.50	16.0	1.40±0.06	

Values in a column with a different superscript are significantly different at p <0.05% level (DMRT test). Each value X± SD represents mean of six values. EI values represented as log_{10} transformed values. EI-Emergence inhibition, GI-Growth index.

Table 2: Insect Growth Regulatory activity of *Metarhizium anisopliae* fungi against *Cx. tritaeniorhynchus*

Fungi	Instars	Concentration	Larval mortality (%)	Larval pupal Intermediate mortality (%)	Pupal mortality (%)	Adult mortality (%)	Total mortality (%)	Total emergence (%)	Average develop mental days	GI	EI $_{50}$ (Conidia /ml)
Metrhizium ansiopliae	I	$1x10^{5}$	19.15±0.06 ^{a}	15.11±0.13 ^{a}	9.09±0.08^{a}	6.11± 0.12 ^{a}	49.46±0.06	50.54±0.34	18.0	2.80±0.07	
		$1x10^{6}$	22.09±0.03 ^{b}	18.16±0.12 ^{b}	12.16±0.11^{b}	8.27±0.36 ^{b}	60.68±0.23	39.32±0.14	18.5	2.12±0.07	
		$1x10^{7}$	26.03±016 ^{c}	21.18±0.30 ^{c}	15.08±0.08^{c}	11.14±0.11 ^{c}	73.43±0.05	26.57±0.15	18.0	1.47±0.45	3.01
		$1x10^{8}$	29.20±0.20 ^{d}	24.12±0.08 ^{d}	19.09±0.10^{d}	13.37±0.39 ^{d}	85.78±0.25	14.22 ±0.60	18.0	0.79±0.12	
		$1x10^{9}$	34.15± 0.17 ^{e}	26.20±0.18 ^{e}	21.07±0.04^{e}	15.12± 0.05 ^{e}	96.54±0.80	3.46± 0.12	17.0	0.20±0.06	
	II	$1x10^{5}$	16.00±0.27 ^{a}	13.20±0.22 ^{a}	3.07±0.04 ^{a}	1.03±0.03 ^{a}	33.30±0.10	66.70±1.23	18.5	3.60±0.08	
		$1x10^{6}$	19.11±0.05 ^{b}	16.06±0.04 ^{b}	5.09 ±0.07 ^{b}	2.08±0.03 ^{b}	42.34±0.13	57.66±0.12	17.0	3.39±0.67	
		$1x10^{7}$	24.04±0.04 ^{c}	20.16±0.09 ^{c}	8.06±0.05 ^{c}	6.04±0.05 ^{c}	58.30±0.12	41.70±0.10	18.0	2.31±0.33	2.05
		$1x10^{8}$	27.28±0.25 ^{d}	23.20±0.29 ^{d}	13.15±0.16^{d}	9.07±0.32 ^{d}	72.70±0.25	27.30±0.30	18.0	1.51±0.56	
		$1x10^{9}$	30.27±0.09 ^{e}	25.13±0.12 ^{e}	17.09±0.08^{e}	11.36± 0.32 ^{e}	83.85±0.90	16.15±0.05	18.0	0.89±0.05	
	III	$1x10^{5}$	13.04±0.07 ^{a}	10.15±0.12 ^{a}	2.06±0.08 ^{a}	1.08±0.07 ^{a}	26.33±0.15	73.67±0.02	17.5	4.20±0.78	
		$1x10^{6}$	15.09±0.07 ^{b}	18.15±0.15 ^{b}	4.15±0.18 ^{b}	2.05±0.03 ^{b}	39.44±0.05	60.56±0.05	17.5	3.46±1.01	
		$1x10^{7}$	21.13±0.14 ^{c}	19.14±0.06 ^{c}	5.07±0.08 ^{c}	3.08±0.04 ^{c}	48.42±0.07	51.58±0.58	17.5	2.94±0.06	1.75
		$1x10^{8}$	25.27±0.11 ^{d}	21.21±0.30 ^{d}	7.11±0.01 ^{d}	6.07±0.05 ^{d}	59.66±1.09	40.34±1.09	17.0	2.37±0.06	
		$1x10^{9}$	28.22±0.23 ^{e}	23.16±0.14 ^{e}	12.26±0.04^{e}	10.10±0.10 ^{e}	73.74±0.02	26.26±0.03	17.0	1.54±0.13	
	IV	$1x10^{5}$	9.06±0.05 ^{a}	7.09±0.04 ^{a}	1.05±0.03 ^{a}	0.00±0.00 ^{a}	17.20±0.02	82.80±0.15	17.0	4.87±0.98	
		$1x10^{6}$	11.24±0.18 ^{b}	9.12±0.05 ^{b}	3.13±0.10 ^{b}	1.04±0.04 ^{b}	24.53±0.01	75.47±0.23	17.0	4.43±0.12	
		$1x10^{7}$	17.12±0.05 ^{c}	11.15±0.08 ^{c}	4.12±0.07 ^{c}	2.04±0.03 ^{c}	34.43±0.05	65.57±0.04	17.0	3.85±0.33	1.42
		$1x10^{8}$	20.10±0.11 ^{d}	14.14±0.08 ^{d}	6.07±0.04 ^{d}	5.09±0.04 ^{d}	45.40±.014	54.60±1.02	17.0	3.21±0.67	
		$1x10^{9}$	26.12±0.04 ^{e}	19.09±0.12 ^{e}	9.21±0.29 ^{e}	7.05±0.05 ^{e}	61.47±0.11	38.53±0.06	17.5	2.20±0.12	

Values in a column with a different superscript are significantly different at p <0.05% level (DMRT test). Each value X± SD represents mean of six values. EI values represented as log_{10} transformed values. EI- Emergence inhibition, GI- Growth index.

Table 3: Insect Growth Regulatory activity of *Metarhizium anisopliae* fungi against *Ae. aegypti*

Fungi	Instars	Concentration	Larval mortality (%)	Larval pupal Intermediate mortality (%)	Pupal mortality (%)	Adult mortality (%)	Total mortality (%)	Total emergence (%)	Average develop menttal days	GI	EI $_{50}$ (Conidia /ml)
Metarhizium anisopliae	I	$1x10^{5}$	12.18±0.35^{a}	10.07±0.05^{a}	8.11 ±0.08^{a}	5.06± 0.07^{a}	35.42±0.09	64.58±0.59	16.0	4.03±0.07	
		$1x10^{6}$	15.08 ±0.08^{b}	13.18± 0.13^{b}	11.33±0.28^{b}	9.16 ±0.04^{b}	48.75±0.77	51.25±0.12	16.0	3.20±0.99	
		$1x10^{7}$	19.23 ±0.26^{c}	17.16± 0.15^{c}	15.25±0.33^{c}	13.06±0.05^{c}	64.70±0.34	35.30±0.33	16.5	2.13±0.45	2.77
		$1x10^{8}$	26.66 ±1.25^{d}	22.29± 0.34^{d}	19.26±0.28^{d}	17.30±0.23^{d}	85.51±0.83	14.49±0.15	15.5	0.93±0.15	
		$1x10^{9}$	30.24± 0.15^{e}	27.28 ±0.32^{e}	23.31±0.34^{e}	19.15±0.06^{e}	99.98±0.23	0.02±0.02	16.0	0.00±0.07	
	II	$1x10^{5}$	10.17 ±0.06^{a}	7.10 ±0.08^{a}	6.07 ±0.05^{a}	2.04 ±0.03^{a}	25.38±0.34	74.62±0.05	14.5	5.14±0.34	
		$1x10^{6}$	14.25± 0.31^{b}	10.09 ±0.07^{b}	8.12 ±0.03^{b}	6.06± 0.04^{b}	36.52±0.07	63.48±0.12	14.0	4.53±0.15	
		$1x10^{7}$	18.28 ±0.31^{c}	12.32 ±0.29^{c}	11.15±0.07^{c}	9.10 ±0.07^{c}	50.85±0.45	49.15±0.45	14.0	3.51±0.08	1.74
		$1x10^{8}$	24.12 ±0.08^{d}	16.14 ±0.06^{d}	14.31±0.33^{d}	12.15±0.09^{d}	66.72±0.93	33.28±0.15	14.0	2.37±0.25	
		$1x10^{9}$	29.28 ±0.28^{e}	21.25± 0.32^{e}	18.15±0.15^{e}	16.23±0.34^{e}	84.91±0.19	15.09±0.33	14.0.	1.07±0.15	
	III	$1x10^{5}$	8. 08± 0.05^{a}	6.04 ±0.04^{a}	5.08 ±0.03^{a}	2.08 ±0.32a	21.28±0.14	78.72±1.04	12.0	6.56±0.07	
		$1x10^{6}$	11.14± 0.20^{b}	9.12 ±0.16^{b}	7.10 ±0.07^{b}	6.08 ±0.03b	33.44±0.23	66.56±0.50	12.0	5.54±0.16	
		$1x10^{7}$	14.27± 0.19^{c}	12.06 ±0.03^{c}	10.19±0.09^{c}	8.07± 0.08c	44.59±0.07	55.41±1.07	12.5	4.43±0.20	1.57
		$1x10^{8}$	16.08 ±0.08^{d}	15.18± 0.10^{d}	11.23±0.16^{d}	9.12 ±0.08d	51.61±0.16	48.39±0.35	12.0	4.03±0.40	
		$1x10^{9}$	20.16 ±0.24^{e}	18.21± 0.33^{e}	16.16±0.10e	13.15±0.11e	67.68±0.14	32.32±0.11	12.0	2.69±0.28	
	IV	$1x10^{5}$	7.11 ± 0.75^{a}	5.11 ± 0.07^{a}	3.07±0.04^{a}	1.06 ± 0.04^{a}	16.35±0.23	83.65±1.09	11.0	7.60±0.01	
		$1x10^{6}$	9.25± 0.28^{b}	8.20 ± 0.16^{b}	7.16±0.13^{b}	5.16 ± 0.06^{b}	29.77±1.23	70.23±1.02	11.5	6.10±0.05	
		$1x10^{7}$	12.37± 0.28^{c}	10.23± 0.18^{c}	9.17±0.15^{c}	8.13 ± 0.06^{c}	39.90±0.34	60.10±0.23	11.0	5.46±0.46	1.44
		$1x10^{8}$	15.13± 0.11^{d}	14.14 ± 2.43^{d}	10.33±0.37^{d}	8.13±0.12^{d}	47.73±0.35	52.27±0.35	11.0	4.75±0.15	
		$1x10^{9}$	18.30 ± 0.24^{e}	16.14 ± 0.06^{e}	14.13±0.08^{e}	11.16±0.10^{e}	59.73±1.09	40.27±0.67	11.0	3.66±0.90	

Values in a column with a different superscript are significantly different at p <0.05% level (DMRT test). Each value X± SD represents mean of six values. EI values represented as log_{10} transformed values. EI- Emergence inhibition, GI- Growth index.

DISCUSSION

Over the last 5 decades the indiscriminate use of synthetic insecticides in agriculture and public health programs for the control of pest species has created multifarious problems viz. insecticide resistance, environmental pollution, toxic hazards to humans and other non-target organisms. In attempt to overcome these problems, great emphasis has been recently placed on the research and development of forms of pest control using plant products and microbial origin. On account of these advantages of IGRs and the high level of activity against target species, it is likely that IGRs could play an important role in vector control programs in the future [12]. Many laboratory studies have shown the potential of *Metarhizium anisopliae* as a mosquito control agent. Roberts [13] observed effects on larvae of *Anopheles stephensi, Anopheles quadrimaculatus, Aedes aegypti, Ochlerotatus atropalpus, Ochlerotatus taeniorhynchus, Culex pipiens, Culex restuans and Culex salinarius,* and found all species susceptible to conidia. In a laboratory experiment reported by Ramoska [14], the fungus suppressed *Culex quinquefasciatus* larval populations for nearly a month. On the other hand, the strain used by [15] had lost its effect on the same mosquito species after only three days. Daoust and Roberts [16] found that over half of 52 strains from a variety of hosts taken from nine countries caused more than 50% mortality of *Culex pipiens* larvae treated with 1 mg dry conidia / 16 cm. The strains most virulent to *Culex pipiens* proved to be highly pathogenic to larvae of *Aedes aegypti* and *Anopheles stephensi* as well. In the same study it was shown that virulence of strains towards mosquitoes could increase 1.6 – 2.5 times by passage through mosquito larvae. In small scale outdoor tests, using 300 or 600 mg of conidia in small artificial ponds reduced Culex pipiens by 91% and 94% [17]. Kamalakannan et al., [18] investigated that the *M. anisopliae* more virulent to Aedes aegypti larval stages at laboratory study. The spore concentration at 5x106 conidia/ml was more effective to control larval stages. The time duration was also decreased with increasing concentration. Recently, adult *Culex quinquefasciatus* and *Anopheles gambiae* were infected in the laboratory study. Both species proved susceptible and succumbed to infection with unformulated dry, and oil formulated conidia, with LT values ranging from 4-6 days.

Insect growth regulators (IGRs) the third generation insecticides are different groups of bio pesticides that are highly active against larvae of mosquitoes and other insects. The IGRs in general have a superior margin of safety to most non-target biota including invertebrates, fish, birds and other wildlife. They are also relatively safe to man and domestic animals.

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