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INTERACTION OF *METARHIZIUM ANISOP*LIAE AND *ACALYPHA ALNIFOLIA* ON THE MOSQUITOCIDAL AND IGR ACTIVITY OF DENGUE VECTOR, *AEDES AEGYPTI* (L.) (CULICIDAE: DIPTERA: INSECTA)

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ABSTRACT
In the mortality assessment of laboratory concern there was 98% larval (I & II instar) mortality was recorded at 10% concentration of *Acalypha alnifolia* on 24hrs duration. The % values of LC_{50} (LC_{90}) are 5.69 (9.23) in first instar and 6.00 (9.35) in second instar stages. The 3rd and 4th instar larval mortality was 94 and 92% and the % of LC_{50} (LC_{90}) values are significant in the 3rd and 4th instar larval mortality. *M. anisopliae* treatment affect the larval survival and movement, after the treatment there was 94,90,85 and 82% mortality was noted in the I,II,III and IV instar stages. The % of LC_{50} (LC_{90}) values are 7.96 (11.75) in 3rd and 8.42 (12.38) in 4th insar stages. In our combined study of plant and fungal metabolites, the % of larval mortality was 74,82,90,98 and 100 at I, II, III and IV larval instars, respectively. The % of LC_{50} (LC_{90}) are 9.28 (38.91) at $1x10^6$ conidia/ml + 1% (fungi with plant) concentration and it was increased LC_{50} (LC_{90}) values of 12.96(26.20) at $5x10^6$ conidia/ml + 5% concentration. Higher larvicidal mortality was noted in the combined treatment then in the individual treatment. Both fungi and plant are interacting with each other and control *Aedes aegypti* larvae very effectively. Similarly, we conduct the effect of *A. alnifolia* on IGR activity of *A.aegypti*. The total emergency was 15% at 10% concentration treated *A. aegypti* followed by the control total emergency was 95%. The IGR activity significantly reduced the total life span. The emergency of larval, pupal and adult duration was extended in the experiment. Finally we concluded that plant and microbial combination was greater significant role to reduced the mosquito population have potential for biological control of dengue vector, *Aedes aegypti*.

KEYWORDS: *Acalypha alnifolia. Metarhizium anisopliae. Aedes aegypti.* IGR. Mortality

INTRODUCTION

Chemical measures in public health programs were initially considered likely to decrease mosquito populations, but these have failed because the constant use of chemical insecticides has often led to disruption of natural biological control system and outbreaks of insect species ((Brown, 1986; Lee *et al*., 2001). Moreover, problems created by using synthetic insecticide include the development of mosquito resistance, environmental pollutions and undesirable effects on humans, mammals and other non target organisms (Brown, 1986; Lee *et al*., 2001). In an attempt to resolve these problems, attention to insecticide of natural origin, particularly plant derived products, has been recently revived. A considerable number of studies have emphasized the research and development of herbal substances for controlling mosquitoes (Sukumar *et al*., 1991; Jayabalan *et al*., 2003; Kamalakannan *et al*., 2010). Mosquitoes are pestiferous insects, which are responsible for the transmission of various dreadful diseases like malaria, filariasis, dengue and yellow fever, Japanese *Encephalitis* etc. *Culex quinquefasciatus* commonly known as the domestic mosquito is an obligatory ecto-parasitic vector. It plays a major role in the transmission of filariasis disease, which is transmitted by nematode parasites, *Wuchereria bancrofti*. The *Anopheles species* transmits the four parasites (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malaria* and *Plasmodium ovale*). Dengue is spread by the bite of *Aedes aegypti*, mosquito. Dengue viruses occur in most tropical areas of the world (Kamalakannan *et al*., 2010). Phytochemicals derived from various botanical sources (The most promising botanical groups are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, Aristolochiaceae and Malvaceae) (Regnault-Roger, 1997) have provided numerous beneficial uses ranging from pharmaceuticals to insecticides (Kamalakannan *et al*., 2010). The phytochemicals derived from plants sources, which is not only act as larvicidal activity and also involved many biological activity. Neem products have multiple effects in insects such as antifeedant, growth regulation, fecundity, suppression and sterilization, oviposition, repellency or attractancy and changes in biological fitness. Neem contains only traces of azadirachtin but compounds like salanin, nimbin, meliantriol etc are also present in neem, which are useful in the control of same insect species (Murugan *et al*., 2007).Similarly, *Acalypha alnifolia* is a tribal (Irulas) plant and mainly used for adult mosquito control by burning of dried plant as a smoke. Biological control at the larval stages of development of mosquitoes is one of the techniques which afford a cheap, easy to use and environmental friendly method of mosquito control. Natural insecticides are phytotoxic and do not accumulate chemical residue in the flora, fauna and soil. Phytochemicals with mosquito larval activity occurs in the oil, leaves and roots of plants (Sosan *et al*., 2001). Many

biological control agents have been evaluated against larval stages of mosquitoes, of which the most successful ones (Fillinger *et al*., 2003). Similarly, entomopathogenic fungi are considered excellent candidates for biopesticides due to their safety, relatively limited host range, ease of production and suitability of large scale production (Ferron 1978; Kamalakannan *et al*., 2008). Following successful use of an entomopathogenic fungus mosquito larvae and adult of the malarial, filarial and dengue vector. *Metarhizium* is being considered as a potential biological control agent since the species is pathogenic for the larvae of several tree hole breeding mosquito species. However, the large does required for the significant mortality rates of mosquito larvae was not considered practical (Pinnock *et al*., 1973; Chapman, 1974). Entomopathogenic fungi play an important role in the regulation of insect population. There is a diverse array of fungal insect pathogenic species from within the four different classes. Adaptations range from obligate pathogens of specific insect species to generalists capable of infecting many hosts species to species that are facultative pathogens. The early attempts in using fungi as microbial control agents of pest insects were soon overshadowed by the development of chemical insecticides. It was not until the late 1950s that attempts to use entomopathogenic fungi for insect pest management resurfaced. To date, there are many commercial products available worldwide, based on less than 10 fungal species (Shah and Goettel 1999; Copping 2001). Hence, in the present study to evaluate the fungal pathogen combined with plant compound was more significant mortality of *A. aegypti* species of mosquitoes.

MATERIALS AND METHODS

The *Metarhizium anisopliae var. anisopliae* (Metsch.) Sorokin sample were obtained from T- Stenes & Company, Research and Development (R&D) Coimbatore, Tamil Nadu, India. The *Acalypha alnifolia* Klien ex Willd collected form Kallar Biosphere reserve forest station; Coimbatore and it were botanically identified at Botanical Survey of India, Coimbatore, India.

Mosquito larval and pupal rearing and adult maintenance

The eggs of *Aedes aegypti* were collected from the local (in and around Bharathiar University Campus, Coimbatore) area with the help of "O" type brush. These eggs were kept to the laboratory and transfer to 18x 13x4cm size enamel trays containing 500ml of water and kept for larval hatching in the laboratory condition and it for cultured. The mosquito larval culture maintained in our laboratory at 27+2 °C, 75 – 85% RH, Under 14L : 10D Photoperiod cycles. The larvae will be fed with dog biscuits and yeast at 3: 1 ratio. The feeding was continuing till the larvae transformed into the pupal stage.

The pupae will be collected from the culture trays and transferred to plastic containers (12x12 cm) containing 500 ml of water with the help of a dipper. The plastic jars will be kept in 90 x 90 cm size mosquito cage for adult emergence. The cage made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part covered with a muslin cloth. The bottom of the cage fitted with 10% sugar solution for a period of three days before they will be provided an animal for blood feeding. The adult female mosquitoes are allowed to feed on the blood of a rabbit (showed on the dorsal side) for two days, to ensure adequate blood feeding for 5 days. After blood feeding enamel trays with water from the culture trays will be placed in the cage for the adults to lay eggs.

Larvicidal Bioassay (Individual and combined)

Excised Plant parts were washed with tap water and shade dried at Room Temperature. The help of an Electrical mixie powdered the dried Plant parts. From each sample ½ Kg of the powdered materials was extracted with Methanol using the apparatus (Vogel, 1978). The solvent was to evaporate through by rotary vacuum evaporator.

To make 1% Concentration, one gram of plant residue dissolved in 100 ml of methanol from the stock the desirable concentration was prepared. To make 1% one gram of sample was dissolved in 100 ml of distilled water.

Leaves of the various plants were collected during the flowering season (January to April) of the plant, shade - dried and finely ground. The finely ground plant material was extracted with petroleum ether (Boiling point range 60-80°C) and methanol by standard method of extraction (Sujatha *et al*., 1988). The residue was then made into a 1% stock solution with acetone. The stock solutions for various test concentrations were prepared and one ml of the sock solution was added to 249 ml of tap water in a 500 ml enamel bowl.

Larvicidal activities of the methanolic extracts were determined by following the standard procedure (WHO, 1975). 50 early fourth instars larvae and 50 pupae of vector of *Aedes aegypti* (obtained from the cyclic colony that has been maintained for the past 6 month at the laboratory were transferred to 249 ml of tap water taken in 500 ml bowls . Five replicates were set up for each test concentration and the plant extract was tested at concentrations ranging from (2 to 10% of *A. alnifolia* concentration and $1x10^6$ to $5x10^6$ conidia/ml of *M. anisopliae*) with two replicates of control with addition of 1 ml acetone alone to 249 ml of tap water. Bioassay was conducted at room temperature 27 ± 3°C with 85% relative humidity. In the case of experiment for determining pupicidal activity, the mouth of each bowl containing pupae was covered with muslin cloth to prevent the escape of any emerged adult mosquitoes. Mortality in larvae / pupae was recorded 24 h post – treatment.

The control mortality was corrected by using Abbott's formula (Abbott's, 1925)

Corrected Mortality = Observed mortality in treatment - Observed Mortality in control / 100 – Control Mortality X 100

Percentage of Mortality = Number of dead larvae / Number of larvae Introduced X 100

TABLE 1. Larvicidal effect of methanolic extract of *Acalypha alnifolia* on dengue vector, *Aedes aegypti*

Instars	No. of larvae 50 (Death / Hrs(24h) Concentration (%)					% of Mortality/ (Mean ± SE)	% Value of LC_{50} LC_{90}	95% confidential Limit LCL $LC_{50}(LC_{90})$	95% confidential Limit U CL $LC_{50}(LC_{90})$	Regr. Coeff. X (Y)	Chi square Value (x^2)
	2	4	6	8	10						
I	5^a	13^a	28^a	38^a	48^a	98 (26.4±0.24)	5.69 (9.23)	5.16 (8.47)	6.20 (10.31)	0.3613 (-2.0562)	0.918
II	3^{ab}	11^b	26^b	38^b	47^{ab}	97 (25±0.26)	6.00 (9.35)	5.50 (8.62)	6.50 (10.38)	0.3822 (-2.2943)	0.189
III	3^c	8^c	22^c	33^c	44^c	94 (22±0.25)	6.65 (10.37)	6.12 (9.51)	7.21 (11.63)	0.3442 (-2.2906)	0.433
IV	1^d	6^d	16^d	26^d	42^d	92 (18.2±0.29)	7.47 (11.06)	6.94 (10.15)	8.06 (12.42)	0.3566 (-2.6654)	1.033

LCL – Lower Confidential Limit, UCL- Upper Confidential Limit, S.E: Standard Error Within the column means (± S.E) followed by the same letter(s) are not significantly different at 5% level by DMRT , Chi-Square value significant at P< 0.05 level.

TABLE 2. Laboratory evaluation of M. anisopliae on dengue vector, *Aedes aegypti*

Instars	No. of larvae 50 (Death / Hrs(24h) Concentration (conidia/ml (1 to $5x10^6$)					% of Mortality/ (Mean ± SE)	% Value of LC_{50} LC_{90}	95% confidential Limit LCL $LC_{50}(LC_{90})$	95% confidential Limit UCL $LC_{50}(LC_{90})$	Regr. Coeff. X (Y)	Chi square Value (x^2)
	1	2	3	4	5						
I	2^a	6^a	20^a	32^a	42^a	94 (20.4± 0.27)	7.00 (10.65)	6.48 (7.78)	7.57 (11.92)	0.3514 (-2.4628)	0.653
II	2^a	5^{ab}	18^b	30^b	40^b	90 (19± 0.28)	7.33 (11.13)	6.79 (10.18)	7.94 (12.54)	0.3375 (-2.4765)	0.797
III	0^b	4^c	16^c	27^c	35^c	85 (16.4± 0.31)	7.96 (11.75)	7.40 (10.72)	8.62 (13.65)	0.3375 (-2.6868)	3.386
IV	0^b	3^d	14^d	24^d	32^d	82 (14.6± 0.32)	8.42 (12.38)	7.82 (11.21)	9.19 (14.25)	0.3242 (-2.7328)	3.308

LCL – Lower Confidential Limit, UCL- Upper Confidential Limit, S.E: Standard Error Within the column means (± S.E) followed by the same letter(s) are not significantly different at 5% level by DMRT , Chi-Square value significant at P< 0.05 level.

TABLE 3. Combined larvicidal effect of *M. anisopliae* and *A. alnifolia* against dengue vector, *Aedes aegypti*

Conc. (% + conidia/ml) (fungi + plant)	No. of larvae 50 (Death / Hrs) (3^{rd} and 4^{th} instars)					% of Mortality/ (Mean ± SE)	% Value of LC_{50} LC_{90}	95% confidential Limit LCL $LC_{50}(LC_{90})$	95% confidential Limit U CL $LC_{50}(LC_{90})$	Regr. Coeff. X (Y)	Chi square Value (x^2)
	2	4	8	16	24						
1 x 10^6 + 1	5^e	15^e	22^e	30^{de}	37^e	74 (21.8±0.19)	9.28 (38.91)	6.66 (23.17)	16.22(29.99)	0.141 (-1.814)	0.771
2 x 10^6 + 2	11^d	19^d	27^d	33^d	41^{cd}	82(26.2±0.18)	10.08(29.22)	8.05 (20.50)	18.53(42.31)	0.096 (-1.254)	0.156
3 x 10^6 + 3	19^c	25^c	32^c	40^c	45^c	90(32.2±0.18)	10.88(25.22)	9.05 (19.15)	15.20(41.45)	0.089 (-0.973)	0.163
4 x 10^6 +4	25^b	30^b	39^b	44^b	49^b	98(37.4±0.23)	12.80(21.85)	10.67(19.98)	16.47(63.27)	0.066 (-0.675)	0.114
5 x10^6 + 5	33^a	40^a	48^a	50^a	50^a	100(44.2±0.25)	12.96(26.20)	11.05(17.88)	41.57(355.3)	0.043 (-0.401)	0.154

LCL – Lower Confidential Limit, UCL- Upper Confidential Limit, S.E: Standard Error Within the column means (± S.E) followed by the same letter(s) are not significantly different at 5% level by DMRT, Chi-Square value significant at P< 0.05 level

Test for Insect Growth Regulatory Activity (IGR)

The *Acalypha alnifolia* were tested for larval, pupal and adult development activity against freshly emerged first instar larvae of *Aedes aegypti* followed by the standard procedure (Amalraj *et al*., 1988).Tests of the microbial pesticide for development activity were done at different concentration ranging from 2 to 10% concentration. The desired concentration of the test solution achieved by adding 1.0 ml of an appropriate stock solution to 249ml of dechlorinated water. Five replicate for each concentration were set up. All larvae were monitored till to adult emergence and were provided with larval food. Observations were done at 24h intervals and the death larvae and pupae were daily removed and counted. The developmental stages of larvae, pupae and adults were recorded. The emergence inhibition concentration (EI_{50}), (EI_{90}) was derived from the experimental data through probit analysis (Finney, 1971).

Statistical Analysis

The data gets from the bioassays subject to statistical analysis. The Dungun Multiple Range Test (DMRT) and t-test were used.The analytical data together with tables are presented in appropriate places in the report. SPSS soft ware package was computing all the data including probit analysis, correlation equation, SE and mean of the sample.

RESULTS

The data were presented in the table 1 provided the larvicidal effect of methanolic extract of *Acalypha alnifolia* on various stages (I, II, III, IV) of *A. aegypti.* The percentage of larval mortality of I instar was 98% at 10% concentration. Whereas, II instar larval mortality was 97%; III instar 94% and IV instar 92% larval mortality was observed in the laboratory test. The LC_{50} (LC_{90}) values, regression equation and chi-square values are 5.69 (9.23), Y= -2.0562 + 0.3613 at I instar larval mortality. When compare to IV instar, the LC_{50} (LC_{90}) values, regression equation and chi-square values are 7.47 (11.06), Y= 0.3566 + -2.6654 and x^2= 1.033. The percentage of larval mortality was also concentration dependent. When concentration increased the mortality also increased. The younger instars are more susceptible than older once. Similarly, in M. anisopliae fungal spores ($5x10^6$ conidia/ml) concentration, the % of larval mortality in I instar was 94% whereas, in other stages (II, III and IV) of larval mortality also increased with increasing concentration. The LC_{50} (LC_{90}) values, regression equation and chi-square values are 7.00 (10.65), Y= -2.4628 +0.3514 and x^2=0.653 at I instar mortality. Similarly, II, III and IV instar larval mortality was 90, 85, 82% and LC_{50} (LC_{90}) values, regression equation and chi-square values are noted in the table 2.

Table 3 provided the combined larvicidal (only used 3 and 4^{th} instars) effect of *A. alnifolia* and *M. anisopliae* on dengue vector, *A.aegypti*. The percentage of larval mortality was 64% at $1+1x10^6$ plant extract/conidial spores concentration. Similarly, 100% mortality was observed at $5.00 + 5x10^6$ extract/conidial spores concentration on 3 and 4^{th} instar larvae. The combined treatment of plant and fungal spores most effective against larval stages when compare to individual study or treatment. 100% mortality was observed in at 24hrs of combined treatment. The individual study there was 75% total effect of larval stages of *Aedes aegypti*. The LC_{50} (LC_{90}) values, regression equation, chi-square values are given in the table 3. Similarly, The DMRT values are significant different 5% level. The SD error was also followed in the table 3.

Table 4 provided the biology of dengue vector/ IGR activity of *A. aegypti*. The *A. alnifolia* at 2% concentration, the adult emergence was raised up to 12.5 days and in at 4% concentration the adult emerged up to 14.8 days and at 10% concentration, it was raise 20.8 days when compare to control the adult was emerged at 12.58 days. The % of EI_{50} (EI_{90}) values are 1.17 (9.74). The larval, pupal and adult emergence duration was maximum extending in treated group than in control group. The survival rate was decreased with increasing concentration of active compound and conidial spores concentration. The combination of microbial and botanical compound greatly inhibits the larval and pupal development. The larval, pupal and adult emergency was greatly inhibit due to toxin produced by the plant compound/ fungal metabolites.

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. Studies on natural plant products as larvicides have indicated that they could provide possible alternative to synthetic insecticides. Plants extract and phytochemicals have potential as products for mosquito control because many of them are selective, may often biodegradable to non toxic products, and may be applied to mosquito breeding places in the same way as conventional insecticides (Sukumar *et al*.,1991; Jayabalan *et al*., 2003; Murugan *et al*., 2007). Similarly, *Acalypha alnifolia* having potential for the control *Aedes aegypti* larvae, pupa and adult stages proved in the laboratory study. Murugan and Jeyabalan (1995) studied the effect of some indigenous properties in *Anopheles stephensi*. Babu and Murugan (2000) investigated that the larvicidal effect of resinous exudates from tender leaves of *Azadirachta indica*. Vahitha *et al*., (2002) have studied the larvicidal efficacy of *Pavonia zeylamica* L. *Acacia ferruginea* D.C. against *Culex quinquefasciatus* say. In the present study reveals that the tribal plant *Acalypha alnifolia* (Euphorbiaceae) having multiple effect on the mosquito larvae of *Aedes aegypti*. It has insect growth regulatory activity, larvicidal, pupicidal, adulcidal properties was observed in the experiment.

Murugan *et al*., (2003) studied the interactive effect of botanical and *Bacillus thuringiensis* subsp *israelensis* on *C. quinquefasciatus* say. Earlier, Jeyabalan *et al*. (2003) investigated the effects of *Pelagonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* liston. In the present study also nimbecidine and *A. alnifolia* showed to be a potent mosquitocidal effect on *Aedes aegypti* and it may be

due to the presence of active compounds. In our study mainly focus on combined effect of microbial and botanical compound are more virulent or toxicity to larval stages. There was 100% larval mortality was observed at 24 hrs duration. Senthil Nathan *et al*. (2005) tested the methanolic extracts of leaves from the Indian white cedar *Dysoxylum malabaricum* Bedd. (Meliaceae) against mature and immature *Anopheles stephensi* Liston (Diptera) mosquitoes under laboratory condition. The extract showed strong larvicidal, pupicidal, adulticidal and antiovipositional activity. The maximum leaf extract concentration tested in this study was 10%, which produced pronounced effect. In the present study the larvicidal treatment of *A. alnifolia* showed higher (98%) mortality was observed in the first and second instar than in the third and fourth instar. The plant compound treatment, the younger larvae more susceptible than the older one and is due to active compound present in the plant. The *Acalypha alnifolia* contain the active the active compound like Acalyphin etc. In the present study showed the IGR activity of *Aedes aegypti* are more susceptible to *Acalypha alnifolia*. However, relatively moderate levels of mortality were observed in female by 21 days. The mean egg hatchability was also decreased. Similarly, the adult emergence was significantly reduction in at 10% concentration of plant treatment. The oviposition test was also observed in the study area, the % of emergency inhibition (EI_{50}) was 1.17% treated *A. aegypti*, whereas, percentage of EI_{90} was 3.66%. The oviposition attractancy test was conducted the entire biology period of dengue vector. The effective concentration was given to the larvae and it allowed for developing adult stage and finally treated adult was produce eggs. In the experimental test, the active compound produced for plant insecticide was arrest the development of gonad in the mosquito reproductive system due to the toxin found in the endocrine system of insect. The search for effective mosquito pathogens that can be used in mosquito control operations has been ongoing for several decades. Both laboratory and field studies of those fungi that appeared to have potential for operational use, have been evaluated. The fungal pathogens are mainly effective against the larval stages of mosquitoes (Federici, 1995). Concerning mosquito-pathogenic fungi, three genera are generally considered important; Lagenidium, Coelomomyces and Culicinomycees (Roberts, 1974) has one or more traits useful for mosquito control, but none of them possesses the full array of properties needed for general applied and cost effective control. In our study of laboratory evaluation of *M. anisopliae* are more suitable for the control of *Aedes aegypti*. The early larval stages are more susceptible then the later once.

Many laboratory studies have shown the potential of *Metarhizium anisopliae* as a mosquito control agent. Roberts (1970) 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 (unformulated) conidia. In a laboratory experiment reported by Ramoska (1982), the fungus suppressed *Culex quinquefasciatus* larval populations for nearly a month. On the other hand, the strain used by Alves *et al*. (2002) had lost its effect on the same mosquito species after only three days. Daoust and Roberts (1982),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^2. 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 m^{-2} in small artificial ponds reduced *Culex pipiens* by 91% and 94% (Roberts, 1974). Besides larvae, also adult mosquitoes proved to be susceptible to the fungus. Recently, adult *Culex quinquefasciatus* and *Anopheles gambiae* s.s were infected in the laboratory study. Both species proved susceptible and succumbed to infection with unformulated dry, and oil-formulated conidia, with LT_{50} values ranging from 4-6 days (Scholte 2003; Kamalakannan *et al*., 2008). In the present study investigated that the M. anisopliae most virulent to *Aedes aegypti* larval stages at laboratory study. The spore concentration at $5x10^6$ conidia/ml was more effective to control larval stages. The time duration was also decreased with increasing concentration. The synergistic/interactive effect observed in bioassays using a combination of botanical extracts and different synthetic insecticides have been observed in several previous studies (Kalyanasundaram and Babu, 1982; Kalyanasundaram and Das 1985; Mulla and Su, 1999; Thangam and Kathiresan, 1991). Similarly, we investigated the combined effect of plant and microbial compound are higher toxicity to all

larval stages than the individual compound treatment. Therefore, combined studies are rapidly killed the larvae and mortality time duration was also reduced. Some extracts have also produced synergistic effects with insect growth regulators. Mulla and Su (1999) showed that neem seed kernel extract has synergistic effects when combined with the juvenile hormone analog methoprene. In our study the tribal plant *A. alnifolia* having insect growth regulatory activity. A few studies have mentioned synergism between different botanical extracts. Mwaiko (1992) reported that a mixture of the peel oil extract of three citrus species (lemon, orange and bitter orange) was much more effective than for the peel oils extract for the individual species. In the present study observed combined action of plant and microbial insecticides having strong synergism. Both compounds are suitable for plant- microbial interactive insecticide to the control of *Aedes aegypti*. Mwaiko (1992) studied the joint action and with the synthetic pyrethroid insecticides cypermethrin against *Cx. Pipiens* larvae. In the present study made to attempt on the combined effect of *M. anisopliae* with and *A. alnifolia* had showed higher toxicity and also observed higher mortality was evident in the larval and pupal and adult population. We conclude that the application of interaction of fungal pathogens and plant compound rapidly kill larva, pupa and adult dengue vector could

significantly reduce parasite transmission and therefore lead to reduced dengue risk.

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