Potential of *Trachyspermum ammi* (Linn.) seed extract against larva and pupa of Aedes species: A study

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Abstract: This study deals with potential of the alcoholic seed extract of *Trachyspermum ammi* (Linn.) against larva and pupa of Aedes sp. The study shows that extract is effective against different developmental stages of *Aedes* sp. Mortality rate of larva was faster than the pupa. The mortality of larva was three times faster than the pupa stage. In general, the seeds extract of *Trachyspermum ammi* is effective on larva and pupa both and can be considered to develop economic, easy to prepare, ecofriendly remedy against *Aedes* sp.
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Introduction:

Both tropical and subtropical regions of the world are marred by mosquito-borne diseases viz. Malaria, dengue, Japanese encephalitis, lymphatic filariasis (elephantiasis) and tularemia (bacterial disease). Mosquitoes of various genus viz. *Anopheles*, *Culex* and *Aedes* act as vectors of these dreaded diseases. Among the various genus of mosquitoes, *Aedes* is a day biting mosquito which acts as the vector of dengue and dengue haemorrhagic fever. It causes considerable nuisance in parks and gardens and in posh colonies with green cover (ICMR report, 2000). *Aedes* sp. breeds in clean water used for household purposes. Dengue has emerged as fast spreading disease as there has been recorded a 30-fold increase in the last 50 years. Estimation shows there occur 50 million cases of dengue infections annually and about 2.5 billion people live in dengue endemic areas (Bhattacharya *et. al,* 2013). In India, effective management of the mosquito problem, in general and Aedes, in particular has become a challenging task for the state government authorities who have to deploy the precious time and resources towards the day to today growing menace. Apart from running community awareness programs, authorities go for vector control by different methods which can be broadly classified as physical methods, chemical methods and natural methods. A number of chemicals are used to kill mosquitoes at larval, pupa or adult stage. Though these methods are powerful in controlling the species, yet they have their own damages such as development of resistance in target species, bioaccumulation, environmental toxicity etc (Liu *et. al,* 2005). N, N-Diethyl-m-toluamide (DEET) is one of the most widely used mosquito repellents. It is an effective chemical against mosquitoes. But recent research shows that *Aedes aegypti* species has developed less sensitivity towards the chemical due to decreased response of olfactory receptor neurons towards it (Stanczyk *et. al,* 2013). This side effect of chemical control has prompted researchers to develop an economical and eco friendly substitute. Plants contain a number of secondary metabolite chemicals which act as deterrent to herbivores and insects. These chemicals can be the potential pesticides scientific community chasing for (Arya *et. al,* 2014). So research of developing mosquito repellant has been turned towards the herbal remedy of the problem. Dua *et. al,* (2003) isolated some ingredients from *Lantana camara* flowers and studied their repellant properties towards Aedes species. Mosquito larvicidal activity of leaves and flower extract of *Lantana camara* against *Aedes aegypti* and *Culex quinquefasciatus* have shown promising results in a study carried out by Maneemegalai *et. al,* (2008). Aina *et. al,* (2009) have evaluated larvicidal potential of seed extracts from *Piper guineense* which gave 83% inhibition of second instar larvae of *An. gambiae.* Essential oils extracted from Piper betel have been also studied for their repellence towards mosquito. The study found it better repellent for *Anopheles stephensi* and *Culex fatigans* than the well known repellent citronella oil (Pal and Chandrashekar, 2010). A report by Holihosur *et. al* (2013) shows the efficacy of crude leaf extract of *C. inerme* against *Aedes aegypti* and its insecticidal activity against larvae and pupae of *Culex quinquefasciatus.* Literature review revels that a number of plants have the phytochemicals which show excellent mosquito repellent properties. Plants like *Litsea cubeba*, *Melaleuca leucadendron*, *Tagetes minuta*, *Viola odorata, Mentha piperita* and *Nepeta cataria* have been explored for their repellent properties against mosquito species like *Aedes aegypti*, *Anopheles annularis*, *Anopheles culicifacies,*

Anopheles stephensi and *Culex quinquefasciatus* (Sai Shankar *et. al,* 2013).

Trachyspermum ammi (L.), commonly known as Ajwain, is a medicinal plant *that* belongs to family Apiaceae. *Trachyspermum ammi* (L.) is an erect, glabrous or minutely pubescent, branched annual herbaceous plant. It bears greyish brown fruits or seeds. It is cultivated almost throughout India (Chauhan *et. al*., 2012).). Its essential oil has been found effective repellant towards growing larvae and adults of wheat flour insect pest, *Tribolium castaneum* even at very low concentration. The essential oil fumes inhibit the egg laying capacity and development of the insects (Chaubey, 2007). Its seeds are used as traditional medicine for treatment of inflammatory diseases and disorders of the digestive tract (Chatterjee *et. al*, 2013).

The present study explores the larvicidal and pupacidal potential of *Trachyspermum ammi* seeds extract against larva and pupa of *Aedes* species.

Materials and Methods:

1. Preparation of ethanolic extract of *Trachyspermum ammi* seeds: The dried seeds of *Trachyspermum ammi* were procured from local market. These were ground with the domestic mixer and the powder was sieved through a fine mesh. 100gm sieved powder was dissolved in 500 ml of pure ethanol in 1000ml capacity conical flask and was stirred in a Rotary shaker for 48 hrs at 100 rpm. The mixture was filtered through Whatman filter paper to remove undissolved seed material. Filtered extract was treated as stock solution and it was further diluted with distilled water to get different dilutions ranging from 1:4 to 1:28 (1:4, 1:8, 1:16, 1:24 and 1:28) as ad when needed. The stock solution was stored at 4°C in refrigerator.

2. Test organism:

Larvae of *Aedes* species were collected from Nekar Nagar, old Hubli, Karnataka, India. The larvae were kept in plastic boxes (30x15cm) containing declorinated tap water. These boxes were covered by fine mesh net to allow proper aeration. The boxes were maintained as per "HITSS & BOX LABORATORY ASSAY" (Training manual v. 1.0 April 2009).

3. Mosquito larvicidal bioassay

For bioassay test 20 larvae of third and fourth instars of *Aedes* species were put in 200 ml of dechlorinated tap water and appropriate amount of seed extract from the stock solution was added to it to get the desired dilutions of 1:4, 1:8, 1:16, 1:24 and 1:28.Since no mortality of larva and pupa was recorded at the concentration less than 1:24 (say 1:28), this is the liming ratio for the *Trachyspermum ammi* seeds extract to show larvicidal or pupacidal activity against *Aedes* species. This treatment with the liming concentration was done by putting 20 larvae of third and fourth instars of *Aedes* species in a box containing 240ml of declorinated tap water and adding to it 10 ml of the extract thus, making the dilution to 1:24. The control was set up with alcohol and declorinated tap water by adding 10ml ethanol in 240ml declorinated tap water to get 1:24 dilution. The number of dead larva was counted every 15 minutes till all were dead.

4. Mosquito pupa bioassay:

Observations and Method of data analysis:

Table 1: Percentage mortality of **larva** of *Aedes* sp. in *Trachyspermum ammi* seed extract and control at ratio 1:24

Time (in min)	Percentage Mortality	
	Control (ethanol & water)	T. ammi seed extract
0	0	0
15	0	0
30	0	0
45	0	0
60	0	5
75	0	15
100	0	25
115	0	40
130	0	85
145	0	100

Table 2: Mean mortality rate of **pupa** of *Aedes* spp. in *Trachyspermum ammi* seed extract and control at ratio 1:24

Time (in min)	Mortality (No.)	
	Control (ethanol & water)	T. ammi seed extract
0	0	0
30	0	10
60	0	10
90	0	20
120	0	70
150	0	70
180	0	70
210	0	70
240	0	80
270	0	80
300	0	80
330	0	80
360	0	90
390	0	100

For bioassay test 10 pupa of *Aedes* species reared in laboratory were added in 240ml of declorinated tap water in a box to that 10 ml of the extract was added making the dilution to 1:24. The control was set up

with alcohol and declorinated tap water by mixing 10ml ethanol in 240ml declorinated tap water. The number of dead pupa was counted every 30 minutes till all are dead. The experiments were replicated thrice.

The number of dead larva was counted every 15 minutes till all were dead. The number of dead pupa was counted every 30 minutes till all are dead. The experiments were replicated thrice. Percentage mortality observed in the experiment was corrected by using Abbott's Formula (Abbott, 1925).

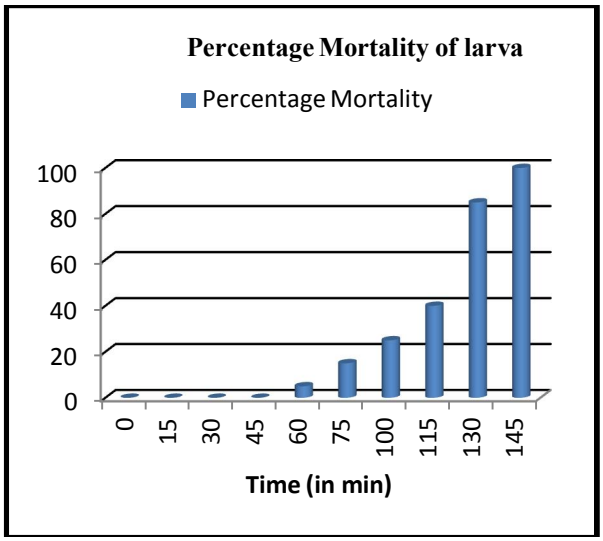

Fig. 1: Percentage mortality of **larva** of *Aedes* sp. in *Trachyspermum ammi* seed extract

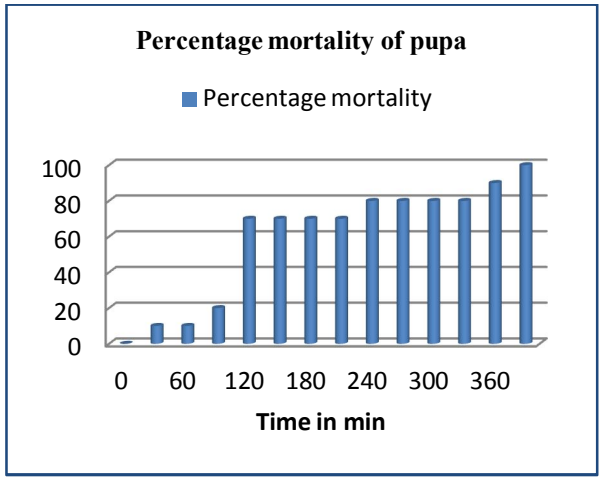

Fig. 2: Percentage mortality of **pupa** of *Aedes* sp. in *Trachyspermum ammi* seed extract

Results and discussion:

The tests were conducted in different concentration ratios from 1:4 to 1:28 (1:4, 1:8, 1:16, 1:24 and 1:28). The rate of mortality was faster in higher concentration of seed extract (viz. at 1:4 all larvae were dead in 10min).However the extract was not effective at a concentration lower than 1:24.So the concentration 1:24 was taken as limiting concentration to perform tests. The study shows that extract is effective against different developmental stages of *Aedes* sp. The result revealed that though it took 60 min to observe first mortality of larva yet all larvae were dead in 145 min from the setting up of experiment. In case of pupa first mortality was recorded in 30 min from the start of experiment and it took390 min for 100% mortality. So mortality rate of larva was faster than the pupa. The mortality of larva was three times faster than the pupa stage. In general, the seeds extract of *Trachyspermum ammi* is effective on larva and pupa both. In the meantime, no mortality in the control was observed which contained ethanol thus it showed that solvent had no effect on the mortality of larva or pupa. So the mortality may be due to the phytochemicals present in Trachyspermum *ammi.*

Literature review reveals that Trachyspermum *ammi* seeds have been analysed to contain fibre (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron and nicotinic acid (Dwivedi *et. al,* 2012).

A number of phytochemicals have been reported from essential oil of Trachyspermum *ammi* These chemicals include α-thujene, α –pinene, sabinene, β – pinene, α-phyllanderene, γ -terpinene p- cymene, β – phyllanderene, terpinene-4-ol, thymol, carvacrol, styrene and δ-3-carene.Due to these and other chemicals present in the seeds, these have been reported to show bioactivities such as analgesic effect, antibacterial activity, antifilarial activity, antifungal activity, anthelmintic Activity insecticidal activity, antioxidant activity, antihistaminic effect and antiviral activity **(**Chauhan *et. al.,* 2012).

It thus, can be concluded that as the extract is effective against both, the larva and pupa *Aedes* sp and it can be considered to develop economic, easy to prepare, ecofriendly remedy against *Aedes* sp. Also, further research can be carried out to explore its potential against other genus of mosquitoes.

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