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Evaluation of ajwain and mustard seed extract on susceptibility of *Anopheles stephensi* Liston

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ABSTRACT: The most effective method to manage mosquito population is to kill aquatic stages (egg, larva and pupa) of mosquito than the aerial adult stage. The susceptibility test was evaluated against larvae of *Anopheles stephensi* in the laboratory conditions maintaining the temperature of 27±2ºC and 70±80% Relative Humidity. The combination of the seed extracts of *Trachyspermum ammi* (ajwain), mustard oil and naphthalene were taken in the ratio 1:2:1. Results of the larval susceptibility test after 25 hours of treatment revealed that the combination of the three components are significant in killing the larval population of *Anopheles stephensi* to some extent. It has also been noticed that the rise in dose from 1ml/199ml to 7ml/99ml of water increased the larval mortality.
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KEY WORDS: Susceptibility test; *Anopheles stephensi* larvae; ajwain; mustard

Mosquito and man relationship lies on earth from millions of year ago. Major focus is on killing adult stages of mosquito but larval stages are easy to manage as they are in known areas, do not fly, do not bite, do not spread diseases, safer, easy to kill by various integrated managements. The three stages of mosquito egg, larvae and pupae are aquatic while only the adult stage is aerial. So we can manage and control three stages at a time. Three strains of *Aedes aegpti* and a single species of *Culex molestus* were studied to determine susceptibility of larvae to various insecticides by George (1957). *Anopheles stephensi* is a well known urban vector found in tropic regions of India (Dash *et al*, 2007) breeds in man-made sites such as overhead tanks, wells, masonry tanks, water coolers, barrels, discarded tyres, tins, intradomestic containers, garden pots, curing water in construction sites etc (Kumar and Thavaselvam, 1992). There are many ways of larval control like application of tablet, pellet and granular formulation of larvicidal chemicals, introduction of larvivorous fishes like *Gambusia affinis, Poecilia reticulate* in large breeding sites (ponds and pools), use of insect growth regulators, use of mineral oils and other microbial control agents such as *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (Khyami-Horani, 1995). A combination of T*rachyspermum ammi*, mustard oil and naphthalene was tested on larval susceptibility *Anopheles stephensi* Liston in the laboratory.

For conducting the test 250ml glassbeakers, dropper to transfer larvae, soxhlet apparatus for extraction of T*rachyspermum ammi*, mustard oil and naphthalene powder, third or fourth instar larvae, cooling incubator for maintain temperature were used. Larval susceptibility test was accomplished by taking the help of WHO guidelines (2005) and Khalil *et al.* (2015). Thirty larvae (basically third

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or fourth larval instar) were taken from the stock culture and transferred to the each beaker containing 199 ml of water with the help of the dropper. Five ml or gm of each of botanical oil extract of T*rachyspermum ammi* (Ajwain) seeds, oil of mustard and naphthalene was taken and mixed with 95ml of acetone to make the stock solution. These three components were mixed in 1:2:1 ratio. Variable doses 1ml, 2ml, 4ml, 5ml and 7ml were applied to the beakers starting from the lowest to highest and each experiment was conducted in triplicate. The beakers were then kept in the incubator maintaining the temperature of 28ºC. After 24 hours the larval mortality was counted with the number of moribund larvae. The mortality was also seen in control beaker having only the solvent acetone. Therefore, the mortality percentages for treated groups were corrected by Abott's formula (Abott, 1925).

Results of the larval susceptibility test are presented in Table 1 which reveals that the combination of the three components are significant in killing the larvae population of *Anopheles stephensi* and the corrected mortality percentage ranged from 29.85% to 75.42%. It had also been noticed that the rise in dose from 1ml/199ml to 7ml/199ml of water increased the larval mortality. Khalil et al. (2015), reported that corrected mortality percentage varies between 98-100% shows susceptibility; between 80-97% suggests probability of resistance and below 80% as resistant type. The mortality percentage ranged from 29.85% to 75.42% i.e resistant type.

Data was analyzed by probit analysis and the probit equation of the research work is as follows:

$$y = 4.3774 + 1.2384\ x$$

Table 1: Combination of the three components in the ratio 1:2:1

Doses in %	Replicate	Larval mortality	Average	% Mortality	Corrected Mortality
1	R1	6			
1	R2	7	6.67	33.35	29.85
1	R3	7			
2	R1	9			
2	R2	9	8.67	43.35	40.36
2	R3	8			
4	R1	8			
4	R2	10	9.33	46.65	43.84
4	R3	10			
5	R1	12			
5	R2	11	12	60	57.89
5	R3	13			
7	R1	15			
7	R2	17	15.33	76.65	75.42
7	R3	14			
Control	0	1		5	

The values for LD 50, LD 90, LD 95 are 3.1824, 34.4959 and 67.8075 with their confidence intervals ranging from 4.00 to 0.00, 8.7456 to 0.00 and 12.3287 to 0.00.

Choubey (2007) reported the insecticidal activity of *Trachyspermum ammi, Anethum graveolens and Nigella sativa* essential oils against stored product beetle *Tribolium castaneum* Herbst. Horowitz *et al*. (1998) evaluated the pesticidal resistance of *Culex pipens* against chemicals Chlorpyrifos, Cypermethrin and Permethrin. Nazni *et al*. (2005) conducted experiments on adult and larval susceptibility against *Culex quinquefasicatus* (Say) using multiple concentrations of insecticides malathion, temephos and permethrin in Malaysia. According to WHO (2013) if the test conducted with 100 larvae showing below 90% mortality need not to be confirmed for existence of resistant test.

Further test are needed to study about susceptibility of the above combination against larvae of *Anopheles stephensi* in large area. Currently malaria control is based on drugs and insecticides but the problem comes for resistance to insecticides therefore environment management offers new approach for sustainable malaria control (Lindsay *et al*., 2004). The significance of the above test is that it is a simple method to apply and all the three components are easily available at home. Vasquez *et al*. (2009) also evaluated the susceptibility test of temephos, chlorpyrifos and permethrin against *Culex pipens* which showed significant resistant ratios.

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