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Evaluating larvicidal action of *Coriandrum sativum* (Dhania) and Mentha (Mint) plant extracts against *Aedes aegypti* and *Aedes albopictus* Larvae under laboratory conditions

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Abstract
An experiment was carried out on "evaluating larvicidal action of *Coriandrum sativum* (Dhania) and Mentha (Mint) plant extracts against *Aedes aegypti* and *Aedes albopictus* Larvae". Rural areas of Islamabad i-e Rumli, Bari-Iman, Karlot, Barakahu and Rawal town were selected for vector collection. *Aedes aegypti*, vector of dengue belongs to class insecta, Order Diptera and Culicidae family. *Aedes albopictus* is vector of dengue and also found in Pakistan. Mentha (Mint) was found to be more efficient as it starts showing mortality at 10%. A significant mortality was observed at 10 to 15% concentrations, followed by complete mortality at subsequent concentrations as compared to *Coriandrum sativum* (Dhania). No significant mortality was observed at 15% concentration of *Coriandrum sativum* (Dhania) plant extract but it was observed at 20% concentration. This study intends to evaluate larvicidal action of *Coriandrum sativum* (Dhania) and Mentha (Mint) plant extracts against *Aedes aegypti* and *Aedes albopictus* Larvae.

Keywords: *Aedes aegypti*, *Aedes albopictus*, Dengue, *Coriandrum sativum*, Mentha (Mint).

1. Introduction
Mosquitoes are the most nuisance causing insects ever known to human race. They kill more humans worldwide in five minutes, than sharks do in a year (CDC, Centers for Disease Control and Prevention, 2008) [1]. Ever since these blood sucking creatures are known to cause diseases, humans are trying to work out methods for their reduction and elimination. Diseases that have caused a serious impact on public health include Malaria, Dengue, chikungunya, Japanese Encephalitis and many more. Among all the mosquito-borne diseases, the most recent one that has threatened public is dengue. The Dengue is global public health concern. It is endemic in more than 100 countries of Africa, Southeast Asia, the Eastern Mediterranean, the Western Pacific, and the Americas. Dengue causes more illness and death than any other arbovirus. Worldwide, there are approximately 2.5 billion people at risk of infection, and the World Health Organization (WHO) estimates that there are about 50 to 100 million cases per year (2, 3). Although dengue-like symptoms had been reported earlier, the first known pandemic of dengue-like illness (Pai H, Lu Y, Hong Y, Hsu E, 2005) [4].
Pakistan has experience a number of dengue fever outbreaks since 1992 (Khan E, Hasan R. J, 2005) [5]. In Pakistan first confirmed outbreak of Dengue fever was reported in 1994 (WHO, 2011) [6]. The disease was endemic in southern parts of Sindh and Baluchistan since 1994 however since 2004-5 dengue has been spread across the country (Guidelines for Dengue Vector) [7] High disease incidence and annual epidemic trend was observed during November 2005 in Karachi, Sindh (Experience and Lessons Learned during the 2011) [6] and 4,500 dengue cases were registered and the epidemic continued to affect a large number of people in Azad Jammu & Kashmir in 2006 but went largely unreported (Khan E, Hasan R. J, 2005) [5]. Lately as a result of Lahore epidemic-2011, dengue has become an important public health issue in Pakistan (Dengue GCP Guidelines) [8] Pakistan is at high risk of being hit by large epidemics because of many over crowded cities, unsafe drinking water, inadequate sanitation, large number of refugees and low vaccination coverage. (Jahan F, 2011) [9]

Humans' being the preferred host of dengue vector gets the virus and a complex interaction develops between host and viral factors. (10, 11) Female vector mosquitoes become infective with virus after an extrinsic incubation period of 8–12 days and can then transmit DENV for the rest of their approximately 1-month life span. Dengue course follows 3 phases: febrile, critical, and convalescent. Fever typically lasts 2–7 days and can be biphasic (Dengue: Kay. M. Tomashek, HaroldS. Margolis) [12].

Aedes aegypti, the principle vector of dengue belongs to class insecta, Order Diptera and Culicidae family. The vector lives in close association with human dwellings. A little humidity is very enough for these vectors to continue their generation. It is very common in areas lacking piped water system and depends greatly on water storage containers for laying their eggs. The dengue virus undergoes Trans-ovarian transmission. This means the virus is passed to the subsequent generations. This phenomenon makes the situation worst as even without taking blood meal from an infected host, the vector can still have the virus i-e from its infected parents. *Aedes albopictus* is secondary vector of dengue and is also found in Pakistan.

Prevention and control of dengue and DHF has become more urgent with the expanding geographic distribution and increased disease incidence in the past 20 years [13-17].

Diagnosis is made usually on basis of clinical signs and symptoms. This diagnosis method is mostly adapted in dengue endemic areas as there are more chances that a patient having dengue warning signs n symptoms of will have the infection. These warning symptoms include abdominal pain, ongoing vomiting, Liver enlargement, mucosal bleeding and lethargy along with fever and chills. till now, medical scientists are not able to develop any specific dengue disease treatment. Intravenous fluid and electrolyte replacement is recommended by physicians as supportive treatment to avoid dehydration. Pain relievers such as ibuprofen, aspirin or naproxen sodium should be avoided as they contain anti-coagulants which will lead to more blood loss.

Since there is no treatment or vaccination available so focus should be on control of dengue vector. Various vector borne disease control methods are available and are used globally. List of control measures are:

- Physical control like Bed nets/physical protection through screens, Chemical control: use of insecticides/ repellents and Biological control: use of larvivorous fish, Bti strains.
- Botanicals have natural chemical that have larvicidal actions. Screening and identifying those plants, getting their extracts and evaluating their efficacy against various vector larvae will definitely be helpful in providing any alternates. This study intends to evaluate larvicidal action of *Coriandrum sativum* (Dhania) and Mentha (Mint) plant extracts against *Aedes aegypti* and *Aedes albopictus* larvae laboratory condition.

Materials and Methods

Collection was made from rural areas of Islamabad like Rumli Village, Bari-Iman, Karlot Village Barakahu and Rawal town through hand collection, Aspirator and CDC sweeper at day time because of diurnal habitat. Collection was performed using different collection methods i-e hand collection through Aspirator, through CDC sweeper. Collection was done outdoor as dengue vector is exophilic. And collection was done at day time because of vector activity habit i.e. diurnal. Adults were taken to insectary for rearing after obtaining samples. They were sorted out for target species selection after feeding the Unfed adults fed artificially then they were set for oviposition in ovi traps. Eggs were taken and placed in larval pans. Rearing was done at optimal temperature 25-27c degree Celsius and humidity conditions i-e 70-75%. Late third and early fourth instar larvae obtained were taken for bioassays. Leaves were washed, dried and later on grounded in an electric grinder. The grounded material was placed in thimble and kept in extraction tube in soxhlet apparatus with extractor ID 38mm, extractor volume 85 ml and flask volume 250 ml for the extraction of oil by steam distillation method using acetone as solvent. Solvent was evaporated at room temperature, leaving oil which was then collected. Stock solution was prepared in acetone solvent as the extraction procedure used acetone in extraction procedure. 1, 5, 10, 15, 20, 25 and 30% solutions were prepared.

Oil Conc. (in ml)	Solvent Conc. (in ml)
1 ml	99 ml
0.5 ml	49.5 ml
Oil Conc. (in ml)	Solvent Conc. (in ml)
5 ml	95 ml
1.25 ml	23.75 ml
Oil Conc. (in ml)	Solvent Conc. (in ml)
10 ml	90 ml
2.5 ml	22.5 ml
Oil Conc. (in ml)	Solvent Conc. (in ml)
15 ml	85 ml
0.812 ml	5.312 ml
Oil Conc. (in ml)	Solvent Conc. (in ml)
20 ml	80 ml
1.25 ml	5 ml
Oil Conc. (in ml)	Solvent Conc. (in ml)
25 ml	75 ml
1.56 ml	4.56 ml
Oil Conc. (in ml)	Solvent Conc. (in ml)
30 ml	70 ml
1.6 ml	4.3 ml

After preparing stock solutions through above calculations for both Mint and Dhania extracts, there dilutions were prepared, for conducting Bioassays.

2.5 Bioassays

Dilutions were prepared in glass beakers of 500 ml. in each beaker a batch of 30 late third and early fourth instar larvae were placed and kept for 24 hours. Results were observed after 24 hour of exposure.

Results and Discussion

Bioassay conducted on both target species yielded following results:

Percentage Mortality Results

Extract 1: Mentha (Mint)

Table 1: % Mortality of *Aedes aegypti* with 24 hr exposure to mint extract

Aedes *aegypti*		% Mortality After 24 hr.						
	Control	1%	5%	10%	15%	20%	25%	30%
Replicate 1	0	0	60	100	100	100	100	100
Replicate 2	0	6	73	74	99	100	100	100
Replicate 3	0	0	63	93	100	100	100	100

Table 2: %Mortality of *Aedes albopictus* with 24 hr exposure to mint extract

Aedes albopictus		% Mortality After 24 hr.						
	Control	1%	5%	10%	15%	20%	25%	30%
Replicate1	0	0	36.6	93.4	93.4	100	100	100
Replicate2	0	0	53.3	96.6	100	100	100	100
Replicate3	0	0	43.4	100	100	100	100	100

Extract 2: *Coriandrum sativum* (Dhania)

Table 3: % Mortality of *Aedes aegypti* with 24 hr exposure to *Coriandrum sativum* (Dhania) extract

Aedes aegypti		**% Mortality After 24 hr.**						
	Control	1%	5%	10%	15%	20%	25%	30%
Replicate 1	0	0	0	0	32	98	100	100
Replicate 2	0	0	0	0	22.2	99	100	100
Replicate 3	0	0	0	0	23	100	100	100

Table 4: % Mortality of *Aedes albopictus* with 24 hr exposure to *Coriandrum sativum* (Dhania) extract

Aedes albopictus		**% Mortality After 24 hr.**						
	Control	1%	5%	10%	15%	20%	25%	30%
Replicate 1	0	0	0	0	11	89.67	100	100
Replicate 2	0	0	0	0	17.7	94.56	100	100
Replicate 3	0	0	0	0	8	99	100	100

Efficacy of Dhania and mint is evidenced from above tables which show mortality at various concentrations.

The dose–response relationship, or exposure–response relationship, describes the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor (usually a chemical) after a certain exposure time. (18) This may apply to individuals (e.g.: a small amount has no significant effect, a large amount is fatal), or to populations (e.g.: how many people or organisms are affected at different levels of exposure). Studying dose response, and developing dose–response models, is central to determining "safe" and "hazardous" levels and dosages for drugs, potential pollutants, and other substances to which humans or other organisms are exposed. These conclusions are often the basis for public policy. The U.S. Environmental Protection Agency has developed extensive guidance and reports on dose-response modeling and assessment, as well as software. Dose–response relationships generally depend on the exposure time and exposure route (e.g., inhalation, dietary intake); quantifying the response after a different exposure time or for a different route leads to a different relationship and possibly different conclusions on the effects of the stressor under consideration. The B column in following tables represents the extent to which the value of that independent variable contributes to the value of the dependent variable. The t-values in the coefficients table indicate the variable's statistical significance. No significant mortality was observed at 15% concentration of *Coriandrum sativum* (Dhania) plant extract but it was observed at 20% concentration. Complete mortality was observed at 20% concentrations. Although no significant mortality was found at concentrations less than 10 in both extracts yet a very good result that can be observed was that the larvae taken for these test were not pupated. This means although the larvicide has not shown mortality, yet it has suppressed their growth and limit them to remain in their larval stage till death. This face can also be implied as useful in terms of limiting larval growth thus leading to no adult population. The results obtained from the plant extracts used in this study were satisfactory and establish the efficacy. Mortality increases with increase in plant extract dose and complete mortality is observed at 10 to 20 % concentrations. Less concentration were still useful as they limit the larval development to the larval stage with no pupal or adult emergence. So this study shows promising results and should be further tested for control of dengue vector mosquitoes on large scale

Table 5: regression analysis-*Aedes aegypti*-24 hr exposure of mint extract

Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**T**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	26.156	15.035		1.740	.133
	Dose	3.265	.891	.831	3.663	.011

Table 6: regression analysis-*Aedes albopictus*-24hr exposure of mint extract

Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**T**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	21.550	15.116		1.426	.204
	Dose	3.465	.896	.845	3.867	.008

Table 7: regression analysis-*Aedes aegypti*-24hr exposure of dhania extract

Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**T**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	-13.590	12.155		-1.118	.306
	Dose	4.089	.721	.918	5.674	.001

Table 8: regression analysis-*Aedes albopictus*-24 hr exposure dhania extract

Model	**Unstandardized Coefficients**		**Standardized Coefficients**	**T**	**Sig.**
	B	**Std. Error**	**Beta**		
(Constant)	-13.590	12.155		-1.118	.306
Dose	4.089	.721	.918	5.674	.001

Lethal concentration

Lethal concentration (LC) is the concentration of any insecticide or larvicide which kills a certain number of populations after certain exposure hours. LC is measured in dose per kilogram or per liter. Various values of LC i-e 50, 99 and 9 or any other is measured for identifying the toxicity level of that insecticide. For example LC 50 means the dose of insecticide required to kill 50% of the population under test.

Lethal concentration calculation using equation of straight line

Equation of straight line

$$Y = a + b\,x$$

Where, a and b are obtained from above coefficient tables obtained through SPSS linear regression. Here x variable is the dependent variable i-e mortality which depends upon the DOSE which is independent variable Y. By putting values of mortality we want to achieve, we can get the value of required dose that will be actually the lethal concentration.

For Mentha (Mint)

Table 9: LC values of MINT extract in ml/L

LC_1	Y=26.156+3.265(1)	29.43 ml/L
LC_{50}	Y=26.156+3.265(50)	189.4 ml/L
$LC_{99.9}$	Y=26.156+3.265(99.9)	352.3 ml/L

For *Coriandrum sativum* (Dhania)

Table 10: LC values of *Coriandrum sativum* (Dhania) extract in ml/L

LC_1	Y= -13.5+4.08(1)	ml/L
LC_{50}	Y= -13.5+4.08(50)	186.5 ml/L
$LC_{99.9}$	Y= -13.5+4.08(99.9)	386.1 ml/L

Table 11: Result Summary after 24 hr

Extract		Equation of straight Line	LC value
Mentha (MINT)	LC_1	Y=26.156+3.265(1)	29.43 ml/L
	LC_{50}	Y=26.156+3.265(50)	189.4 ml/L
	$LC_{99.9}$	Y=26.156+3.265(99.9)	352.3 ml/L
Coriandrum sativum (Dhania)	LC_1	Y= -13.5+4.08(1)	ml/L
	LC_{50}	Y= -13.5+4.08(50)	186.5 ml/L
	$LC_{99.9}$	Y= -13.5+4.08(99.9)	386.1 ml/L

5. Conclusion

From this study, it is concluded that Mentha (Mint) was found to be more efficient as it starts showing mortality at 10%. A significant mortality was observed at 10 to 15% concentrations, followed by complete mortality at subsequent concentrations while *Coriandrum sativum* (Dhania) was found to be little less efficient in terms of rate of mortality.

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