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A Novel Eco friendly Control of Arboviruses Vectors (*Aedes aegypti* and *Ae. albopictus*) Using *Trachyspermum ammi* seed extract -Mediated Silver Nanoparticles (AgNPs).

Shabab Nasir[1*], Muhammad Usman[1], Kashif Iqbal[1], Muhammad Ishaq[1], Ammara Batool[1] and Wajiha Arshad[1]

[1]Department of Zoology, Government College University, Faisalabad, Pakistan
* Correspondence: author: flourenceshabab@yahoo.com

Abstract: *Aedes aegypti* and *Ae. albopictus* are the key mosquito species responsible for spreading diseases of public health concern like dengue fever, West Nile virus, Zika virus and chikungunya. As these diseases are viral, so, neither proper vaccine nor treatment is available yet. Therefore, we can avoid these diseases by controlling mosquito population through chemical and biological means. Hence, for this study, acetone extract from *Trachyspermum ammi* was prepared and used as the reducing agent for the eco-friendly synthesis of silver nanoparticles (AgNPs) by using silver nitrate solution. UV-Vis spectroscopy, Powdered X-ray Diffraction (PXRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) were used for the characterization of AgNPs. The 2nd and 3rd instar larvae of *Ae. aegypti* and *Ae. albopictus* were exposed to different concentrations (50, 100, 150, 200 & 250 ppm) of plant extract and green synthesized AgNPs to calculate the percentage mortality at time intervals of 6, 12, 18, 24, 30, 36, 42 and 48hr. The Probit analysis of the data proved nanoparticles as an excellent larvicidal agents for both *Ae. aegypti* and *Ae. albopictus* with lower LC_{50} values (76.28 & 80.28 ppm and 87.02 & 89.02 ppm) as compared to the plant extract (121.44 & 127.89 ppm and 127.89 & 129.35 ppm) for 2nd and 3rd instar larvae respectively after 48 hrs exposure time. Our results suggest the extract of *T. ammi* and synthesized *T. ammi* AgNPs as excellent controlling agents for vector mosquitoes instead of pollution causing existing chemical pesticides.

Keywords: Vector mosquito; Larvicidal; *Trachyspermum ammi*; Mosquito larvae; AgNPs

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1. Introduction

Mosquito borne diseases like chikungunya, malaria, filariasis, encephalitis, dengue, and yellow fever have become the major public-health problem in the globe especially tropical and subtropical regions of the world [1]. *Aedes aegypti* and *Ae. albopictus* are the major vectors of these fatal diseases all over the world [2] and affect the lives of millions of people every year. These lethal diseases of public health concern are being combatted only by mosquito control through the applications of synthetic insecticides due to non-availability of effective vaccines at present time [3].

This approach to reduce these diseases is not good and resulted in development of insecticidal resistance in medically important vectors such as malaria and dengue fever vectors [4] and cause environmental pollution. Natural plant based phytochemicals like saponins, isoflavonoids, tannins, terpenes, steroids, etc. have been used to kill larvae of mosquito as safe alternate to synthetic insecticides [5]. In recent years trend of use of phytochemicals for the eco-friendly synthesis of silver nanoparticles (AgNPs) from silver nitrate solution is increasing. This technique has a lot of advantages such as simplicity, mild reaction conditions of pressure, temperature, energy, elimination of toxic chemicals, pollution less and cost effectiveness over chemical synthesis methods [6].

The increasing need of eco-friendly management of vector population urges the use of biological technique to synthesize nanoparticles because other methods such as electrochemical, photochemical and chemical reductions are not better and beneficial than biological method, in which the plant extract are used as reducing and capping agent for the synthesis of nanoparticles [7]. The active agents present in the plants such as polysaccharides, terpenoids, phenolics, flavones, alkaloids, alcoholic compounds, enzymes, amino acids and proteins, act both as reducing and stabilizing agent that produce stable and shape-controlled nanoparticles [8]. The properties of AgNPs can be easily analyzed by using UV–Vis spectrometer, X-Ray diffractometer (XRD), Scanning Electron Microscopy (SEM), and Fourier Transmission Infrared (FTIR) Spectrophotometer [9]. Green silver nanoparticles have the ability to penetrate through the exoskeleton into the mosquito's cells and kill them after binding to proteins or DNA. AgNPs also cause mutation in DNA, deformation of enzymes [10] and hence act as good larvicidal agents but are non-toxic to non-target animal cells like friendly arthropods and fish but highly toxic to bacteria and other microorganisms, therefore, green nanosilver particles are safe, effective and valuable to be used for mosquitocidal purpose [11].

Trachyspermum ammi belongs to the family Apiaceae, commonly known as Ajwain. It is a herbaceous annual plant found throughout Pakistan and has been used as antifungal and antibacterial drug in the form of herbal medicines [12]. *T. ammi* has flavonoids, alkaloids, fatty acids and proteins that are not only larvicidal but also help in reduction and capping of silver nano particles [13].

In current study, we made green synthesis of silver nanoparticle (AgNPs) by using the acetone extract of *Trachyspermum ammi* seed. *T. ammi* acetone seeds extract green AgNPs synthesized from this extract were evaluated for their larvicidal activity against the larvae of both *Aedes aegypti* and *Ae. albopictus* separately at different experimental conditions. This study will contribute in establishing the importance of plant sources as cidal agents and implementing green synthesis of silver nanoparticles for the future research.

2. Materials and Methods

2.1. Extraction of Acetone plant extract

Healthy and dried seeds of the *Trachyspermum ammi* plant were collected from the market Faisalabad, Pakistan and identified by a taxonomist. Seeds were washed with distilled water to make them free from dust, and then dried by placing in shady place at room temperature. Electric grinder (Anex, Germany) was used to grind the seeds into fine powder. Seeds extract was made by adopting the standard simplex centroid experiment design method adopted by Satyavani et al. [14] with slight modifications. Fifty grams of this powder along with 250 ml acetone were loaded in the inner tube of the Soxhlet apparatus and boiled at boiling point at 55.5 to 56.50 °C for 8 hrs. This extract was placed in incubator at 40°C overnight to evaporate acetone and was kept at 4ºC for further use [15].

Figure 1. *Trachyspermum ammi* (Ajwain) seed.

2.2. Phytosynthesis of T. ammi AgNPs

One mM solution of silver nitrate ($AgNO_3$) (Sigma Aldrich, USA) was prepared in 250 ml Erlenmeyer flask in the darkness to avoid action of light. Ten ml acetone plant extracts of *T. ammi* (Ajwain) and 90 ml of 1mM silver nitrate solution was put in 250 ml conical flask. Few drops (2 to 3) of 1% NaOH were mixed continuously by magnetic stirrer to maintain the pH at 8 and got a change in colour to yellowish brown. After cooling this solution, it was centrifuged 3 times at 5000 rpm for 20 minutes to obtain nanoparticle pellets. Purified suspension was prepared by dissolving the nanoparticle pellets in double distilled water and was frozen until further use [14].

UV-Vis spectroscopy, X-ray spectroscopy (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR) were used to study the characteristics of *T. ammi* synthesized AgNPs [9].

2.3. Collection and Rearing of Mosquitoes

Larvae were collected from both indoor and outdoor breeding sites by using dipper from Faisalabad district, Punjab, Pakistan (31° 25' 7.3740'' N and 73° 4' 44.7924'' E, 192 meters above the sea level). Collected larvae were brought back to the Entomology Lab, Department of Zoology, Government College University, Faisalabad, inside beakers closed with muslin cloth. These larvae were identified with the help of identification keys [16] and reared to adults in 1000 ml beakers containing water under ideal conditions at 27±2°C and 75±3% relative humidity [17]. Adults were further reared in separate glass cages. Male adults were fed with 10% sugar solution and females with blood on live white rats in separate glass cages for egg laying. Larvae emerged from the eggs were reared in batches of 300 each, in 1200 ml deionized water in stainless steel trays (35x30x5 cm) for the bioassays. Mixture of two drops of 10% sugar and 0.02% yeast suspension was given to each batch daily for 1st instar and then with finely ground fish food [18].

2.4. Bioassay

Groups of 20 actively swimming second and third instars larvae of *Ae. aegypti* and *Ae. albopictus* were released in 250ml beakers having 200ml distilled water separately (for both species and larval stage). Five concentrations viz., 50, 100, 150, 200 and 250 ppm of larvicidal solution of *T.ammi* extract and *T.ammi* AgNPs were tested for their larvicidal potential separately. The distilled water was used as control. Each treatment was consisted of five replications. Mortality rates were calculated using the WHO [19] bioassay protocol with slight changes.

The percentage mortalities were corrected by using Abbott's formula [20].

$$\text{Percentage Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \text{ X100} \quad (1)$$

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}}$$

2.5. Statistical analysis

Probit analysis using Minitab -17 statistical software (2017) was used to calculate lethal concentration of 50% (LC_{50}) and 90% (LC_{90}) of larvae and for getting dose and time mortality regression lines from experimental percentage mortality data.

3. Results

3.1. Characterization of Biosynthesized T.Ammi Silver nanoparticles (AgNPs).

3.1.1. UV-Vis spectrum of T.ammi AgNPs

Synthesis of *T. ammi* AgNPs because of Ag^{+} by the phytochemicals of *T.ammi* turned the colour of mixture reddish brown. This visible change in colour clearly showed the formation of green *T. ammi* AgNPs. This success was confirmed by UV-Visible absorption spectroscopy as shown in the figure 1, showing the maximum absorbance at 410 nm after 30 minutes of reaction time.

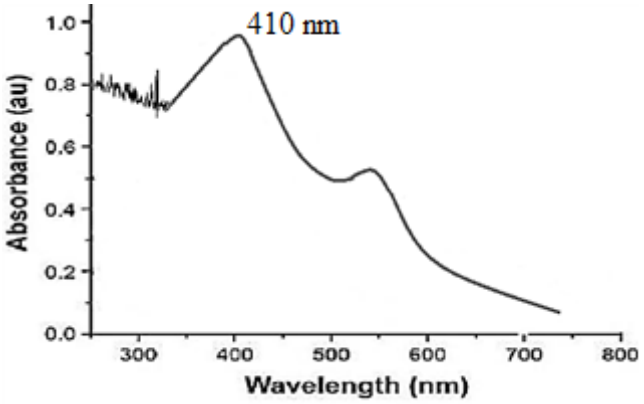

Figure 2. UV-Vis spectra of silver nanoparticles of *Trachyspermum ammi* (Ajwain).

3.1.2. Fourier Transform Infrared Radiation Spectroscopy (FTIR) Analysis of T.ammi AgNPs

The FTIR spectrum of AgNPs synthesized from extract of *Trachyspermum ammi* is shown in the Figure 2. The major sharp and well defined peaks at 3400 cm^{-1} and 3350 cm^{-1} attributed to the intra-molecular hydrogen bond in AgNPs. In addition to this, other peaks were obtained at 1694 cm^{-1} and1048 cm^{-1} were corresponding to alkene C=C stretch, alcoholic –C=O stretch. Minor peaks at 2875 cm^{-1}, 1538 cm^{-1}, and 677 cm^{-1}, are corresponding to asymmetric and symmetric stretching and vibrations of CH2 group, NH2, O-Ag-O stretching, which plays a major role in the synthesis of *T. ammi* mediated silver nanoparticles.

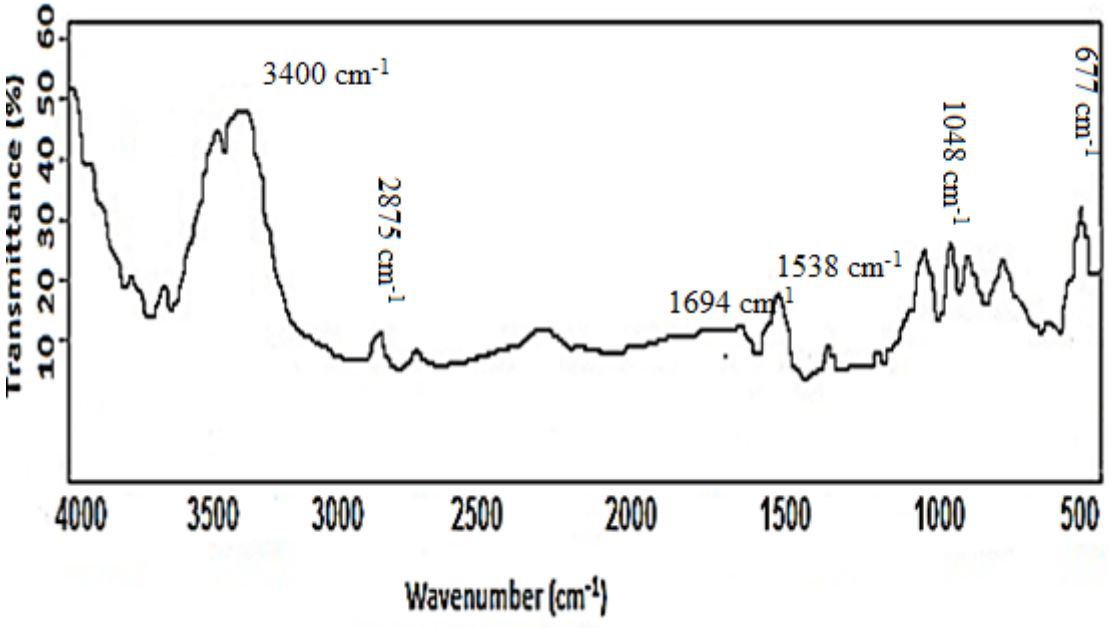

Figure 3. FTIR spectrum of silver nanoparticles of *Trachyspermum ammi* Seed extract.

3.1.3. X-ray diffraction analysis of Trachyspermum ammi AgNPs

XRD patterns of the synthesized AgNPs from *T. ammi* extract are shown by Figure 4, where four major peaks appeared. These XRD patterns correspond to the cubic crystal structure which is in agreement with the standard JCPDS file No.00-004-0783 and indicates that the synthesized nanoparticles are crystallized in a pure form without any impurities. The Braggs reflections observed in the XRD pattern at 2θ = 38°, 44°, 65° and77° which can be indexed to the (200), (220), (311) and (222) planes of pure silver, respectively. A strong diffraction peak was ascribed to the 32.23 facets of silver.

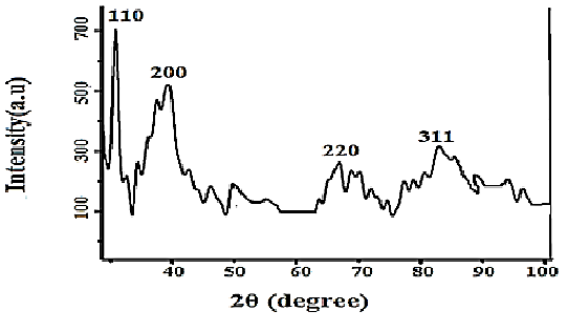

Figure 4. XRD spectrum of *Trachyspermum ammi* AgNPs seed extract.

3.1.4. SEM image of T. ammi AgNPs synthesized from seed extract

SEM image of AgNPs synthesized from *T.ammi,* showed the spherical shape with triangular tendency and 50 nm size.

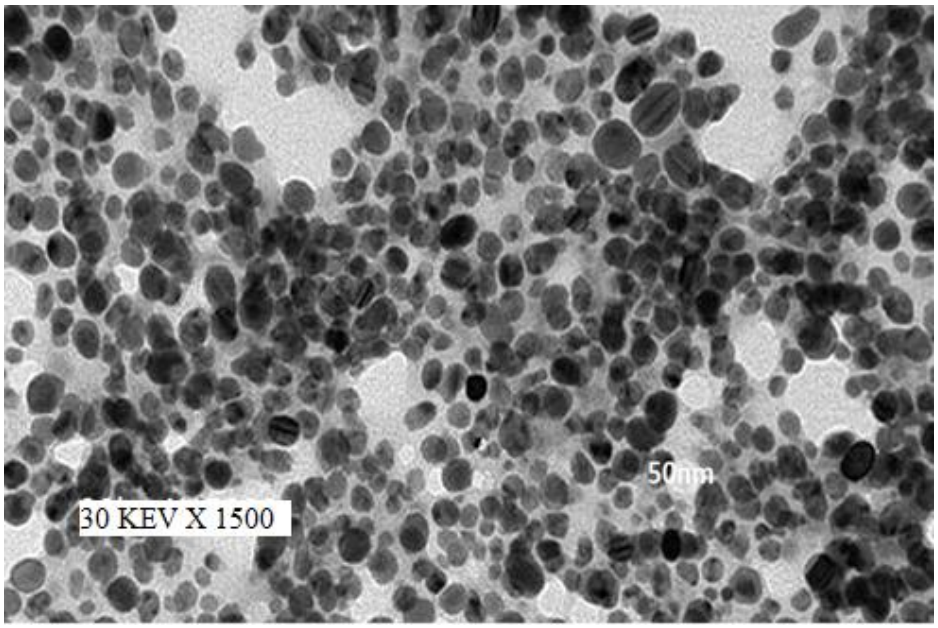

Figure 5. SEM image of *T.ammi* AgNPs.

3.2. Larvicidal activity of Trachyspermum ammi (Ajwain) acetone extract and biosynthesized AgNPs against Ae. aegypti

Results of the larvicidal activity of *T.ammi* seed extract and *T.ammi* AgNPs with varying concentrations of 50-250 ppm against 2nd and 3rd instar larvae of *Ae. aegypti* after 6,12,18, 24, 30, 36, 42 and 48h exposure times are presented in Tables 1-2. Both the acetone extract and the *T.ammi* synthesized Silver Nano particles showed a dose and time-dependent larvicidal effect. Biosynthesized *T.ammi* AgNPs showed mortality rate 100% ± 0.954 for all the exposed larvae within 36 hours at the concentration of 250 ppm (Figure

6). After 48h exposure time LC_{50}, LC_{90} values with upper and lower confidence level were 76.2847 ppm, (63.6057-86.7183 ppm) and 162.913 ppm (150.114-179.932 ppm) respectively. Regression equation was Y= -1.128+ 0.0147X with ($p<0.05$), which confirm significant result. LC_{50} and LC_{90} values against the 3rd instar larvae were 87.0217 ppm (72.0594-99.3213 ppm) and 199.620 ppm (183.566-221.229 ppm) respectively. Regression equation was Y= -1.990+ 0.0113X with p value of 0.0218 ($p<0.05$), which indicated that it was a significant result. No mortality was observed in the control groups (Table 1).

In case of *T.ammi* extract the values of LC_{50}, LC_{90} with upper and lower confidence level were 121.44 ppm (111.078-131.205ppm) and 218.85 ppm (203.804-238.502 ppm) respectively for 2nd instar larvae. Regression equation was Y= -1.591+ 0.0131X with p value of 0.001, which indicated the significant result. LC_{50} and LC_{90} values against the 3rd instar larvae were 127.89 ppm (116.406-138.665 ppm) and 239.34 ppm (221.868-262.603 ppm) respectively. Regression equation was Y= -1.33+ 0.090X with p value of 0.044which indicated a significant result. No mortality was observed in the control groups (Table 2). At the concentration of 250 ppm of *T.ammi* seed extract showed the mortality rate of 100% ± 0.168 for the 2nd and 95% ± 0.098 for the 3rd instar larvae (Figure 7).

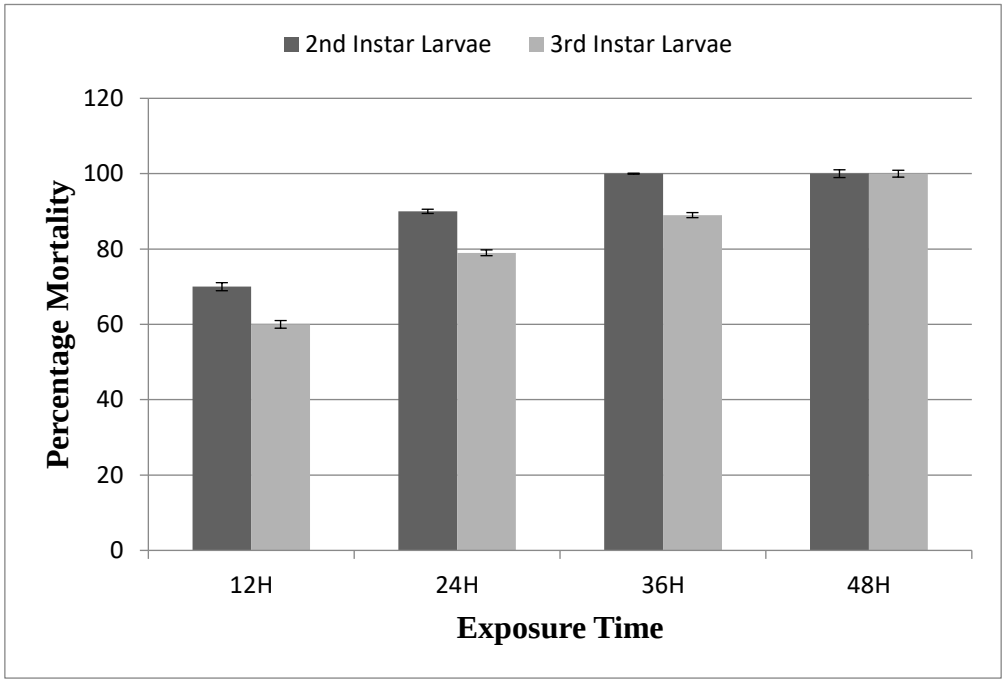

Figure 6. Percentage mortality of *Trachyspermum ammi* AgNPs against *Ae. aegypti.*

Larval instars	Time (h)	% Mortality ± SD (at 250 ppm)	Regression equation: Mortality v Treat. Conc. (ppm)	Lethal concentration (FL at 95% C.I)	
				LC_{50}	LC_{90}
2nd	6	22±0.27	Y= -1.93+ 0.0049X	368.08 (264.19-602.0)	417.0 (328.17-1035.5)
	12	70±0.22	Y= -1.639+0.0081X	200.82 (185.2-220.39)	357.83 (320.23-414.89)
	24	90±0.120	Y= -1.350+0.0107X	125.74 (113.34-137.16)	245.08 (226.3-270.4)
	36	100±0.137	Y= -1.275+0.0133X	95.76 (83.85-106.11)	191.83 (177.82-210.16)
	48	100	Y= -1.128+0.0147X	76.28 (63.61-86.72)	162.91 (150.11-179.94)
3rd	6	21±0.31	Y= -2.31+ 0.0052X	429.49 (386.87-933.08)	596.04 (448.24-1236.5)
	12	60±0.22	Y= -1.633+0.0073X	396.36 (236.32-296.49)	414.5 (351.02-477.29)
	24	79±0.119	Y= -1.372+0.077X	177.20 (162.04-194.6)	342.65 (305.82-399.02)
	36	89±0.235	Y= -1.160+0.0853X	135.95 (121.4-149.7)	286.15 (259.04-325.62)
	48	100±0.651	Y= -1.990+0.0113X	87.02 (72.06-99.32)	199.62 (183.57-221.23)

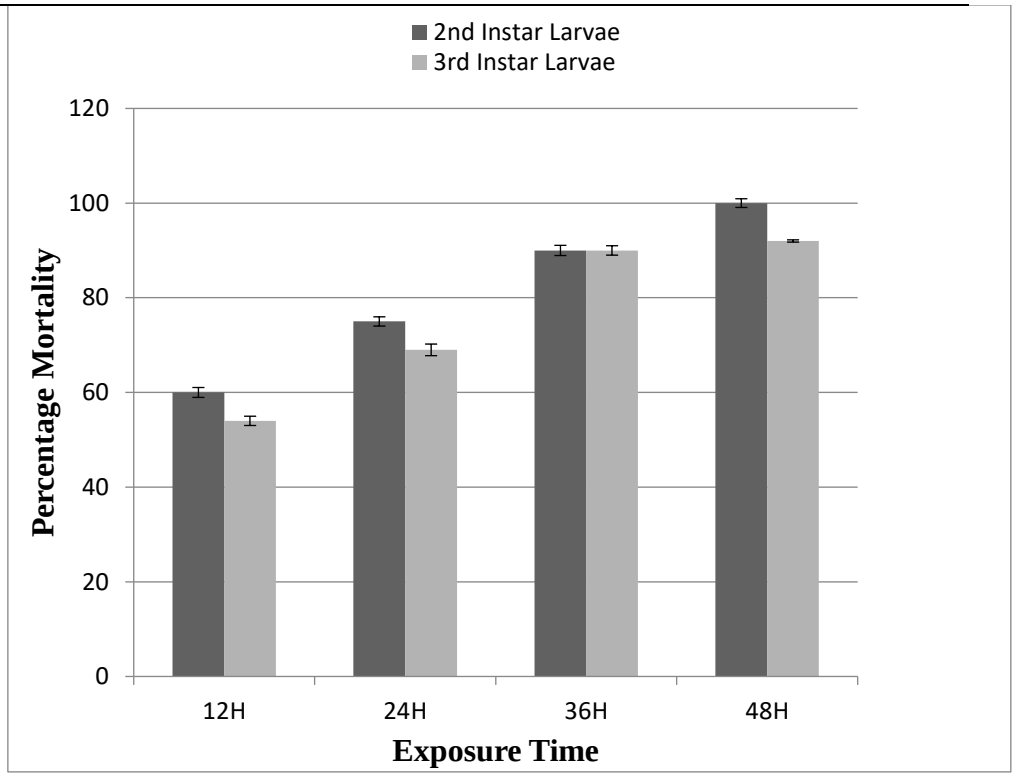

Figure 7. Percentage mortality of *Trachyspermum ammi* acetone extract against *Ae. aegypti at* the concentration of 250 ppm after 48 hrs.

Table 1. Larvicidal Activity of Green Synthesized Silver Nanoparticles with seed Extract of *Trachyspermum ammi* against 2nd and 3rd Instar larvae of *Aedes aegypti.*

Time = Time after start of exposure period; SD = Standard deviation; FL = Lower and Upper fiducial limits; C.I. = Confidence Interval.

Table 2. Larvicidal Activity of seed Extract of *Trachyspermum ammi* against 2nd and 3rd Instar larvae of *Aedes aegypti.*

Larval instars	Time (h)	% Mortality ± SD (at 250 ppm)	Regression equation: Mortality v Treat. Conc. (ppm)	Lethal concentration (FL at 95% C.I)	
				LC_{50}	LC_{90}
2nd	6	20±0.29	Y= -2.55+0.0054X	370.68 (265.14-426.94)	506.62 (418.8-953.05)
	12	60±0.22	Y= -1.57+ 0.070X	212.41(201.87-248.71)	401.93(351.92-483.37)
	24	75±0.134	Y= -1.40+ 0.082X	170.71(156.47-186.45)	36.47(293.83-375.13)
	36	90±0.199	Y= -1.44+ 0.096X	149.86(137.31-162.52)	283.15(259.02-317.07)
	48	100±0.194	Y= -1.59+ 0.0131X	121.44(111.08-131.21)	218.83(203.81-238.50)
3rd	6	18±0.21	Y= -2.52+0.0051X	390.73 (273.53-826.59)	640.2 (432.74-925.2)
	12	54±0.22	Y= -1.49+ 0.064X	233.22(210.42-267.41)	432.76(372.52-536.28)
	24	69±0.12	Y= -1.35+ 0.075X	180.66(165.04-198.97)	350.94(312.16-411.02)
	36	89±0.235	Y= -1.44+ 0.096X	160.34(143.67-197.67)	298.23(263.43-323.54)
	48	92±0.121	Y= -1.35+ 0.089X	127.89(116.41-138.67)	239.34(221.86-262.60)

3.3 Larvicidal activity of Trachyspermum ammi (Ajwain) Acetone Extract and Biosynthesized T.ammi AgNPs against Ae.albopictus

Results of the larvicidal activity of *T.ammi* seed extract and *T.ammi* AgNPs with varying concentrations of 50-250 ppm against 2nd and 3rd instar larvae of *Ae.albopictus* after 6,12, 18, 24, 30, 36, 42 and 48h exposure times are presented in Tables 3-4. Both the acetone extract and the *T.ammi* synthesized Silver Nano particles showed a dose and time-dependent larvicidal effect. Biosynthesized *T.ammi* AgNPs showed mortality rate 100%± 0.8675 for 2nd instar and 98%± 0.1635 for 3rd instar larva larvae within 48 hours at the concentration of 250 ppm (Figure 8). At the same conditions of time and concentration *T.ammi* AgNPs the LC_{50} and LC_{90} values against the 2nd larval stage were 80.2847 ppm and 170.913 ppm respectively. Regression equation was Y= -1.128+ 0.0167X with p value of 0.029, which indicated the significant result. LC_{50} and LC_{90} values against the 3rd larval stage of *Ae.albpictus* were 89.0217 ppm and 200.620 ppm respectively. Regression equation was Y= -1.990+ 0.0123X with p value of 0.0218 ($p<0.05$) which indicated that it was a significant result. No mortality was observed in the control groups (Table 3).

The larvicidal activity with LC_{50} and LC_{90} values of *T.ammi* acetone extract after 6, 12, 18, 24,30, 36, 42 and 48 hours of exposure time, against the 2nd and 3rd instar larvae of larvae of *Ae. albopictus* mosquito is presented in (Table 4). After 48 hours of exposure time the LC_{50} and LC_{90} values with lower and upper confidence level (LFL - UFL) against the 2nd larval stage were 127.89 ppm, (116.949-138.123 ppm) and 239.34 ppm, (221.304-262.537 ppm) respectively. Regression equation was Y= -1.35+ 0.089X with p value of 0.071 while for 3rd instar larvae these values were recoded as129.35 ppm (117.406-140.665 ppm) and 240.97 ppm (223.868-264.603 ppm) respectively. Regression equation was Y= -1.48+ 0.014X with p value of 0.040 ($p<0.05$) which indicated that it was a significant result. Control treatments failed to kill any larva. At the concentration of 250 ppm of *T.ammi* seed extract showed the highest mortality as 93%± 0.887 against 2nd instar larvae after 48 hours while 3rd instar larvae showed 92%±1.034 mortality in this time interval. These results showed that percentage mortality is dependent on time and concentration as shown in (Figure 9).

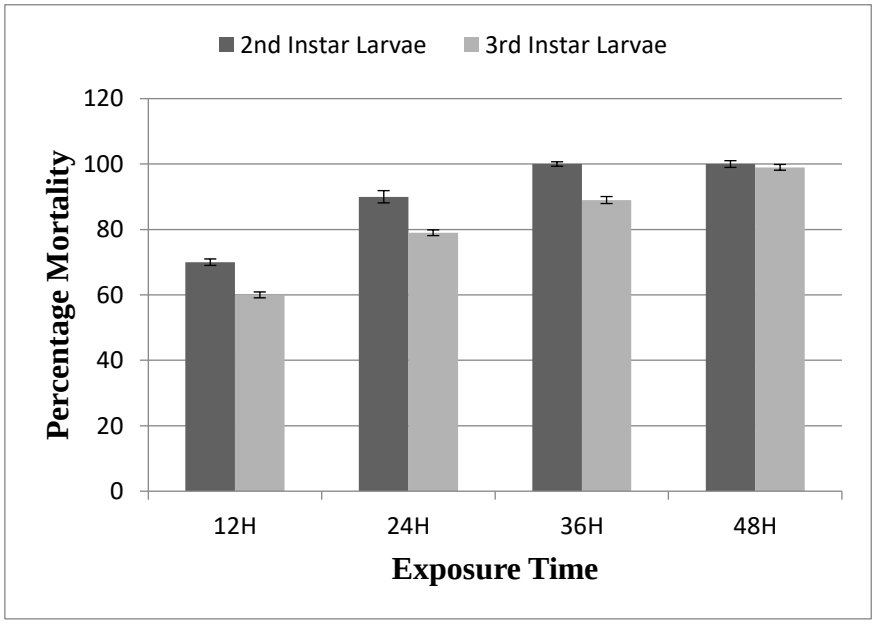

Figure 8. Percentage mortality of *Trachyspermum ammi* synthesized Silver Nanoparticles against *Ae. albopictus*.

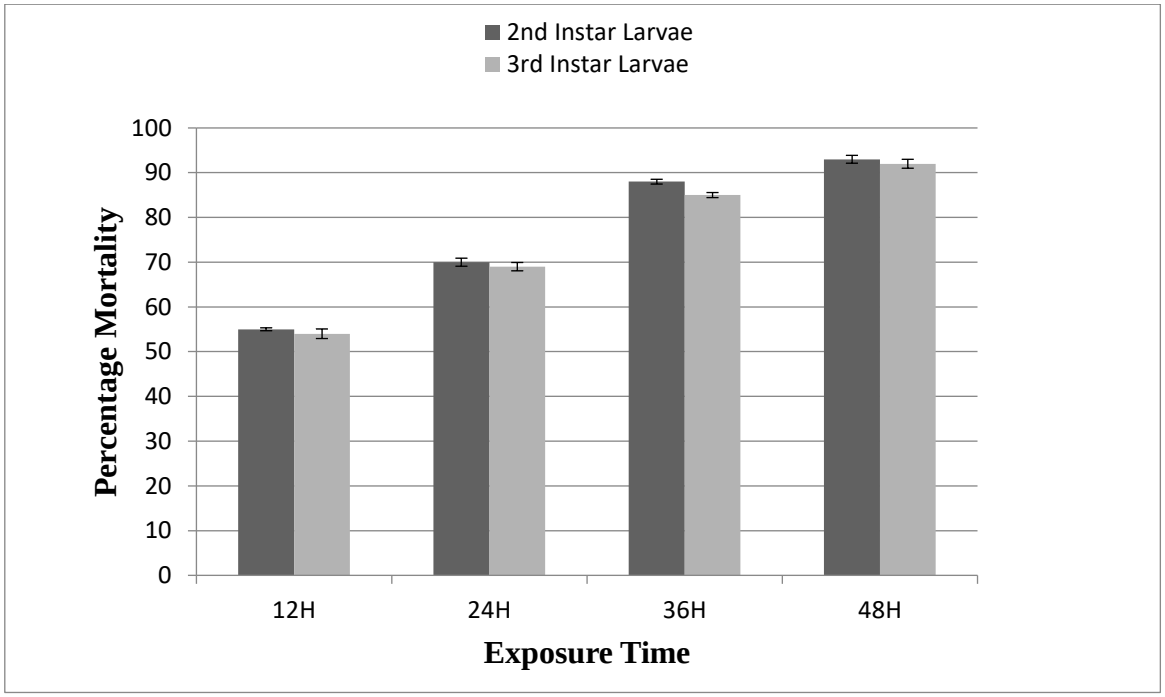

Figure 9. Percentage mortality of acetone extract of *Trachyspermum ammi* against *Ae. albopictus* at the concentration of 250 ppm after 48 hours.

Table 3. Larvicidal Activity of Green Synthesized Silver Nanoparticles with seed Extract of *Trachyspermum ammi* against 2nd and 3rd Instar larvae of *Aedes albopictus.*

Larval instars	Time (h)	% Mortality ± SD (at 250 ppm)	Regression equation: Mortality v Treat. Conc. (ppm)	Lethal concentration (FL at 95% C.I)	
				LC_{50}	LC_{90}
2nd	6	21±0.24	Y= -2.52+0.0051X	420.73(373.53-426.59)	440.22(391.32-754.34)
	12	70±0.13	Y= -1.639+0.0091X	210.82(190.22-225.39)	360.83(330.23-416.89)
	24	90±0.127	Y= -1.350+0.0017X	130.73(125.45-171.12)	250.08(213.12-362.67)
	36	100±0.099	Y= -1.275+0.0157X	96.76(89.85-115.12)	195.82(165.54-240.45)
	48	100±0.121	Y= -1.128+0.0167X	80.28(70.61-91.72)	165.34(134.32-201.21)
3rd	6	20±0.21	Y= -2.38+0.0046X	490.67(393.31-534.89)	510.54(487.32-657.45)
	12	60±0.41	Y= -1.633+0.0078X	290.36(240.34-301.49)	400.36(370.02-501.29)
	24	79±0.13	Y= -1.372+0.0078X	181.19()162.23-294.73	349.65(310.82-400.02)
	36	89±0.09	Y= -1.160+0.0088X	140.95(105.45-200.73)	290.15(257.89-345.63)
	48	98±0.121	Y= -1.990+0.0123X	99.12(78.12-167.12)	200.21(145.12-272.23)

Time = Time after start of exposure period; SD = Standard deviation; FL = Lower and Upper fiducial limits; C.I. = Confidence Interval.

Table 4. Larvicidal Activity of seed Extract of *Trachyspermum ammi* against 2nd and 3rd Instar larvae of *Aedes albopictus.*

Time = Time after start of exposure period; SD = Standard deviation; FL = Lower and Upper fiducial limits; C.I. = Confidence Interval.

Larval instars	Time (h)	% Mortality ± SD (at 250 ppm)	Regression equation: Mortality v Treat. Conc. (ppm)	Lethal concentration (FL at 95% C.I)	
				LC_{50}	LC_{90}
2nd	6	19±0.28	Y= -2.52+0.0049X	510.34(379.84-976.99)	669.12(540.01-1079.1)
	12	55±0.02	Y= -1.51+ 0.065X	231.08(199.21-263.93)	427.21(368.81-526.48)
	24	70±0.17	Y= -1.37+ 0.076X	179.50(164.14-197.39)	347.13(309.41-405.15)
	36	88±0.099	Y= -1.41+ 0.093X	150.65(137.83-167.21)	287.25(243.21-322.67)
	48	93±0.121	Y= -1.35+ 0.089X	127.89(112.94-145.23)	239.35(201.12-262.53)
3rd	6	17±0.28	Y= -2.54+0.0048X	529.48(386.87-756.45)	796.04(548.24-876.56)
	12	49±0.41	Y= -1.49+ 0.064X	233.22(189.21-267.40)	432.76(372.52-536.28)
	24	67±0.13	Y= -1.35+ 0.075X	189.21(165.04-198.98)	350.94(302.16-411.09)
	36	85±0.09	Y= -1.35+ 0.0089X	152.87(139.54-166.45)	296.17(249.12-334.95)
	48	90±0.121	Y= -1.48+ 0.014X	129.35(107.12-156.21)	240.98(203.86-276.12)

4. Discussion

Aedes aegypti and *Ae. albopictus* are the major vectors of many virulent diseases like chikungunya, dengue fever, yellow fever and zika virus all over the world [2]. These lethal diseases of public health concern are being combatting only by mosquito control and natural plant based phytochemicals are being used for killing the larvae of vector mosquito as safe alternate to synthetic insecticides [5].

Phytosynthesis of AgNPs has advantages over any other physical or chemical methods because this technique is simple, has mild reaction conditions of pressure, temperature, energy, no elimination of toxic chemicals, eco-friendly, require less time, low cost, much easy even for large scale synthesis and pollution less [21]. In this study, the silver nanoparticles are formed within the extract of *T. ammi* which act as good bio-reductant for the formation of silver nanoparticles (AgNPs). The phytochemicals found in the *T. ammi* seed extract involved in the reduction of silver ions to form silver nanoparticles (AgNPs). Formation of yellowish brown colour solution is also, the indication of the formation of *T. ammi* AgNPs, because this colour change is due to the reduction of silver ions to silver nanoparticles during reaction of silver nitrate with *T.ammi* extract. These results are in line with the results of Chouhan and Meena [22]. Values of XRD clearly confirmed the formation of pure cubic crystal structured nanoparticles which can be indexed to the (200), (220), (311) and (222) planes. These results are similar with previous research [23, 24]. Results of spectrum of FTIR also showed the formation of bio fabrication among different functional groups present in the *T. ammi* extract to stabilize the green AgNPs. Different major and minor peaks were obtained at 1694, 1048, 2875, 1538 and 677 cm^{-1} that were corresponding to different groups. These results are similar with previous studies [23, 25]. Findings of scanning electron Microscopic (SEM) analysis confirmed the size between 30-50nm and spherical shape of biosynthesized *T. ammi* AgNPs, which which confirmed that these are very fine nanoparticles [26].

In the current work, both the acetone extract and the *T. ammi* synthesized Silver nano particles showed a dose and time-dependent larvicidal effect. Biosynthesized *T. ammi*

AgNPs showed mortality rate 100% for all the exposed larvae within 36 hours at the concentration of 250 ppm. The synthesized nanoparticles showed more mortality for the both larvae of *Ae. aegypti* and *Ae. albopictus* as compared to the plant extract. It is evident from the result that the various concentrations of the plant extract, synthesized AgNPs and interval of time exposure were the main cause of mortality in *A. aegypti* and *Ae.albopictus* larvae. These results of our study might be compare with previous reports in the literature. Hanieh et al. [27] also evaluated larvicidal activity of seeds of *Trachyspermum ammi* against larvae of *Anopheles stephensi* and found excellent mosquito control. Surendran et al. [28] evaluated the fruit extract of *Sapindus emarginatus* against *A. aegypti* and calculated the LC_{50} values of 92.9 ppm. Jawale et al. [29] studied the larvicidal potential of *Cestrum nocturnum* against *Aedes aegypti* mosquito and found it outstanding as highly active larvicide, showing 100% larval mortality.

Kalu et al. [30] studied the larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. They found this plant as significant natural larvicidal agent having 100% larval mortality after 24 hr.

Our results indicated that the treatments with synthesized AgNPs resulted in higher larval mortality in both species of mosquitoes even at lower concentrations. These results are similar with the previous studies [31, 32]. These mentioned results are close but not same to our finding due to choosing different mosquito species with different larval stage, plant, concentration and different types of solvent for plant extraction. Our results strongly proved the *T.ammi* as excellent larvicidal agent against *A. aegypti* and *Ae.albopictus.*

In the present study it was concluded that the acetone extract and synthesized AgNPs from *T. ammi* had efficient larvicidal potential.

5. Conclusion

Efficient phytochemicals found in the *T. ammi* plant have ability to the synthesize Silver Nanoparticles. This biosynthesis of nanoparticles is low cost, simple and has vast range of larvicidal potential toward several types of vector mosquitoes. Since seeds of *T. ammi* is easily available throughout the region and is used in every house for cooking, its extract and active synthesized Nanoparticles synthesized from extract from these can be used in the formation of bio nanopesticides for eco-friendly control of the population of vector mosquito by applying on mosquito breeding places.

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