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Insecticidal and Larvicidal Activities of Silver Nano Particles Synthesized using Plant Bio Materials

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Abstract
Background:*Annonamuricata* is a species from the Annona genus, has many medical properties to cure diseases. The nanoparticles synthesized from the seed, leaf, fruit peel of this tree was studied for the insecticide and larvicidal activity using insects *Callosobruchus maculatus* and*Sitophilus oryzae*, larvae of the mosquitoes *Aedes aegypti*and*Anopheles stephensi*.
Methods: The silver nanoparticles are produced within 20 minutes of incubation in the silver nitrate solution the colour change and the pH of the synthesis obtained was noted. The insects and mosquito larvae were treated with the three samples for 24 hours and the percentage of mortality was noted.
Results: Among the seed, fruit peel and leaf sample the seed silver nanoparticle gave a high percentage of mortality for insects and the larvae 66 and 100 at the high concentration of the sample. The increase in concentration gave an increased percentage of mortality in the insects and larvae. Then the fruit peel silver nanoparticle gave a good percentage of mortality, next comes the leaf sample.
Conclusion: This study has proved that the *AnnonaMuricata* silver nanoparticles of seed, leaf and fruit peel can be used as natural insecticide and larvicide which will give an effective result against the insects(*Callosobruchus maculatus* and*Sitophilus oryzae*) and mosquitoes(*Aedes aegypti*and*Anopheles stephensi*).
Keyword:*Annonamuricate,* insecticidal, larvicidal, silver nano particles, soursop

Introduction

Insecticides are substance to kill insects which cause a damaging effect, used in agriculture, medicine and industries; many are toxic to humans. *Callosobruchus maculatus* belongs to the leaf beetle family Chrysomelidae, commonly called cowpea weevil and cowpea seed beetle [10]. Origination of this beetle is from West Africa, got spread globally through legumes and other crops trade. They are the pest of leguminous grains, such as cowpea, lentils, green gram, and black gram [1,8]. *Sitophilus oryzae* is also known as rice weevil from the Curculionidae family they are the distributed worldwide[2], it is the pest that attacks stored seeds, cereals and milled grains of many crops like wheat, rice, maize and split peas which causes heavy loss in the economy [9]. Larvicide belongs to insecticide which especially attacks the larval life stage of an insect they are generally used against mosquitoes. *Aedes aegypti* is a mosquito that carries four different viruses which cause disease in human-like dengue fever, chikungunya, zika fever and yellow fever [3]. Identification of these mosquitoes is by white markings on their legs and a marking on the surface of their thorax. Basically, originated from Africa and now it is found throughout the world.*Anopheles stephensi*is an urban malarial vector in India and the Middle East [4].*Annonamuricata*is a flowering evergreen tree native to America and broadly spread worldwide, belongs to the Annonaceae family. This tree is locally calledsoursop, graviola and guyabano. It

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has been studied in the last decades for its therapeutic potential, and then this species had the attention due to the bioactivity and toxicity. They also have many uses such as insecticide and parasecticide, medicinal properties are to treat fever, sedative, respiratory illness, malaria, gastrointestinal problems, heart, liver and kidney affections, to treat hyperglycemic, hypoglycemic and cancer treatment. The leaves of this plant are used to treat hypertension, diabetes and cancer[5, 6]. Because it has insecticidal properties the leaf, seed and fruit peel for this tree studied for insecticidal and larvicidal activity on *Callosobruchus maculatus, Sitophilus oryzae*and *Aedes aegypti, Anopheles stephensi* using silver nanoparticles produced from *Annonamuricata*.

Materials and Methods

Preparation of extract

The leaf, seed and fruit peel of *Annonamuricata*was collected. The samples were washed in running tap water to remove the dirt,dehydrated to remove the moisture content,powdered, sieved to fine powder and kept separately for the experiments. The aqueous extract of the three samples were prepared by adding 50ml of distilled water in the three stored samples and kept in a magnetic stirrer for 24 hours, and then it is filtered using a Whatman filter. The pH and color change was noted.

Synthesis of silver nanoparticles

The 100ml of each sample extract was mixed with 150ml of 1Mm silver nitrate solution (silver nitrate in water provides silver ions for the reaction), and then the mixture was incubated at 25℃ in the dark (to avoid phytochemical activation of silver nitrate). The observation was silvery brown precipitation at the end of 20 minutes indicates the formation of silver nanoparticles. The formed product was washed well in double distilled water, dried and stored for further experiments[11].

Insecticidal activity

Insects *Callosobruchus maculatus* and*Sitophilus oryzae* were brought from the Entomology Research Institute laboratory. An aliquot of varying concentration of sample extracts (1µl, 2µl and 3µl) made with acetone was applied on the Whatman filter paper strip of 2cm length corresponding to the dosages of 0 (as a control), 20 µl/l, 40 µl/l, 80 µl/l and 160 µl/l. The sample treated paper strips are kept in a ventilated bottle along with the 10g of cowpea for *Callosobruchus maculatus* and 10g of wheat for*Sitophilus oryzae*, 10 adult insects also released inside the bottle and allowed for 24 hours. When there was no leg or antennal movements, insects were considered dead. The percentage of viability was calculated and corrected by Abbott's formula[14, 15].

Larvicidal activity

The activity test was carried out against laboratory-reared *Aedes aegypti*and*Anopheles stephensi* mosquitoes. The life cycle of vector mosquitoes was maintained at 25℃-29℃ in the insectariums. Larval food (powdered dog food and yeast in 3:1 ratio) and 10% glucose solution was fed to the larvae and adult mosquitoes respectively. Adult mosquitoes were periodically blood-fed on restrained albino mice for egg production. The toxicity assays of the sample extract were conducted separately using the fourth instar larvae of the mosquitoes. Stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 1 ml acetone and volume raised to 100ml with distilled water.The sample extracts of varying dilutions12.5, 25, 50, 75 and 100 ppm were prepared using 1ml acetone and volume made up to 200ml using distilled water in a beaker. 25

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fourth instar larvae were released in it and the mortality is recorded after 24 hours, the beaker was kept at a controlled temperature. Each treatment was replicated five times[12, 13].

Results and discussion

The sample extract attained from the *Annonamuricata* seed (DS1), fruit peel (DS2) and leaf (DL) showed a significant colour change from white to pale yellow, orange to dark brown and green to dark brown respectively. The noted pH for the three samples were 6.58 for seed (DS1), 4.2 for fruit peel (DS2) and 5.2 for leaf (DL) which was also shown in Table1. The pH of the seed is high while comparing the other two samples then the leaf sample stands second and the fruit peel sample has the lowest pH while comparing the other.

Table 1: pH and colour change of the samples.

Sample	pH	Colour change
DS1(seed)	6.58	White to pale yellow
DS2(fruit peel)	4.2	Orange to dark brown
DL(leaf)	5.2	Green to dark brown

The silver nanoparticles production was confirmed by the precipitation of silvery brown precipitate at the end of the reaction after the incubation for 20 minutes. The silver nitrate contributes silver ions when dissolved in distilled water. It is done in dark to avoid the interaction of phytochemicals and silver nitrate. Then is silver nanoparticles were used for the further insecticidal and larvicidal activity test.

The insecticidal activity of the two insects *Callosobruchus maculatus* and*Sitophilus oryzae* was observed after 24 hours of sample extract treatment which showed no leg or antennal movements, insects were considered dead. The percentage of insect mortality was calculated and corrected by Abbott's formula [7]. For *Callosobruchus maculatus* high concentration of seed extract gave 66% mortality at 3µl concentration it is the highest while comparing the other sample extract next to seed extract, the high concentration fruit peel extract gave 63% mortality at 3µl concentration than the leaf sample with 53% mortality at 3µl concentration. At 2µl concentration the percentage mortality of the sample is low. So,as the concentration increases the mortality percentage also increases. Among the three samples extract the seed extract shows the high percentage of mortality next comes the fruit peel extract followed by the leaf extract. The LC50 and LC90 value of the three sample extracts were noted using the mortality percentage were found to be 1.21 and 2.14 for leaf, 1.50 and 2.84 for seed, 1.34 and 2.51 for the fruit peel sample respectively. From the LC50 and LC90 values, it is well known that the seed extract gives a high valuewhich is all shown in Table2.

Table 2: Callosobruchus maculatus % of Mortality, LC50 and LC90 value

Sample	Concentration (µg/ml)	%Mortality (24 hours)	LC50	LC90

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DL(leaf)	1	20	1.21	2.14
	2	30		
	3	53		
DS1(seed)	1	24	1.50	2.84
	2	40		
	3	66		
DS2(fruit peel)	1	23	1.34	2.51
	2	33		
	3	63		

Insecticidal activity for three sample extracts against *Sitophilus oryzae* insect, the seed extract shows a high percentage of mortality of 65% at high concentration next to it the fruit peel sample extract gives high mortality of about 64%. Same as that of the *Callosobruchus maculatus* insect as the concentration increases the percentage mortality also increases. The LC50 and LC90 value were also obtained from the %mortality obtained which shows that the seed extract has high LC50 and LC90 value. The values are provided in Table 3. The results of both the insects were similar and can be effectively used against the insects to protect the storage seed from them as the sample extract was natural it will not interfere with the storage seeds other than killing the insects.

Table 3:Sitophilus oryzae % of Mortality, LC50 and LC90 value

SAMPLE	Concentration(µg/ml)	%Mortality	LC50	LC90
DL(leaf)	1	21	1.18	2.13
	2	30		
	3	52		
DS1(seed)	1	23	1.48	2.81
	2	44		
	3	65		
DS2(fruit peel)	1	22	1.45	2.78
	2	39		
	3	64		

Larvicidal activity of the larvae *Aedes aegypti* and *Anopheles stephensi* was studied using *Annonamuricata* leaf, seed and fruit peel extract. As insecticide activity, the percentage of mortality increases as the concentration of the sample increases. The seed sample gives 100% mortality at 100 ppm concentration for both the larvae. Next, the fruit peel extract gives 96% for *Aedes aegypti* and 82% for *Anopheles stephensi* at 100 ppm concentration. At the low concentration than 100ppm it shows less percentage mortality again it shows that the concentration increases the percentage mortality also increases which is directly proportional to each other. The LC50 and LC90 values are calculated from the %mortality to know how well the sample work against the larvae value furnished which is shown in Table 4 and Table 5. The results obtained from the larvicidal experiment prove that the samples used can be the best solution to be against the mosquito larval growth.

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Table 4: Aedes aegypti % of Mortality, LC50 and LC90 values

Sample	Concentration(ppm)	%Mortality	LC50	LC90
DL(leaf)	100	63	132.82	344.01
	75	50		
	50	44		
	25	20		
	12.5	15		
DS1(seed)	100	100	35.65	237.19
	75	89		
	50	78		
	25	57		
	12.5	40		
DS2(fruit peel)	100	96	79.03	237.19
	75	73		
	50	60		
	25	43		
	12.5	35		

Table 5: Anopheles stephensi% of Mortality, LC50 and LC90 values

Sample	Concentration(ppm)	%Mortality	LC50	LC90
DL(leaf)	100	55	132.82	344.01
	75	35		
	50	30		
	25	24		
	12.5	15		
DS1(seed)	100	100	35.65	237.19
	75	90		
	50	70		
	25	55		
	12.5	45		
DS2(fruit peel)	100	82	79.03	237.19
	75	61		
	50	44		
	25	30		
	12.5	20		

Conclusion

Insecticidal activity on insects *Callosobruchus maculatus* and*Sitophilus oryzae*, larvicidal activity on mosquito larvae *Aedes aegypti*and*Anopheles stephensi* using silver nanoparticles produced

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from *Annonamuricata* using silver nitrate gave a good result by showing good percentage of mortality for both the insects and larvae. From this study, it is well understood that the seed of the tree *Annonamuricata* works well than the other samples. The leaf and fruit peel also show high activity against the insects and larvae.

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