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EFFECTIVENESS OF SOME MUCILAGINOUS SEEDS AS BIOLOGICAL CONTROL AGENTS FOR MOSQUITO LARVAE

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Abstract. Thirty seeds, belonging to seventeen different families, which are commonly available in Pakistan, were tested for the production of mucilage in water and the trapping of mosquito larvae. *Ocimum pilosum*, *Ocimum gratissimum*, *Lallemantis royelleana* and *Salvia spinosa* proved to be the most effective ones. Most of the seeds tested in this experiment are nonpoisonous and of medicinal importance. These findings can be used as alternate to the chemical control agents to destroy the mosquito larvae and hence prevent water pollution.

Several workers[1–6] have reported on the potential of using seeds from certain wild plants to control mosquitoes. They have reported that the epidermal cells of the seed coats of a number of species belonging to different families contain highly hygroscopic mucilage such as the member of the family Cruciferae, Linaceae and Plantaginaceae. When such seeds are placed in water the mucilage exudes from the seed coat and in some cases the outer cell-wall also ruptures. It has been further reported that as a result of the physical contact of mosquito larvae with such mucilagenous seeds, their oral brushes become permanently attached and the larvae subsequently die as a result of drowning and exhaustion.

Material and Method

The mucilage-producing seeds have been shown in Table 1. The seeds which gave positive results were *Linum usitatissimum* Linn (Linaceae), *Mimosa pudica* Linn. (Leguminosae), *Plantago ovata* Forsk. and *Plantago major* Linn. (Plantaginaceae), *Hygrophila auriculata* Nees. and *Blepharis percica* pers. (Acanthaceae), *Matthiola incana* R. Br. (Cruciferae), *Commiphora wightii* Kunth. (Burseraceae), *Salvia spinosa* Linn. *Lallemantia royleana* Benth. Three species of Ocimum, e.g. *Ocimum bacilicum* Linn., *Ocimum sanctum* Linn. and *Ocimum americanum* wild., all belong to the family (Labiatae). *Cordia gharaf* Forsk. (Boraginaceae), *Pyrus cydonia* Linn. (Rosaceae).

The seeds which produced mucilage were bioassayed against mosquito larvae to know the trapping efficiency. The second and fourth instar of *Aedes aegypti* PCSIR-strain9 were selected for the experiment. The petri dishes of 10×35 mm dimension were used with second instar larvae and dishes of 10×40 mm dimension were used with fourth instar larvae. Six dishes were taken and 10–15 ml tap-water was filled in each dish. The screened out seeds were placed in the dishes in 5,8,10,12,15 and 20 numbers. Fifty larvae were released in each dish. The experiment was repeated five times with five replications each. A dish counting the same number of larvae but without seeds was kept as control with each set. The experiment was performed at room temperature (80±2°F) for 24 hr.

TABLE 1. SEEDS BELONGING TO 17 DIFFERENT FAMILIES, SCREENED FOR THE PRODUCTION OF MUCILAGE.

Seed	Family	Production of mucilage
Anisomeles malabarica (L) R. Br.	Labiatae	—
Ocimum bacilicum	,,	+
Ocimum sanctum Linn.	,,	+
Ocimum americanum Linn.	,,	+
Lallemantia royleana Bth.	,,	+
Salvia spinosa Linn.	,,	+
Blepharis percica (Burmi) Oktze	Acanthaceae	+
Hygrophila auriculata (Schum)	,,	+
Brassica campestris Linn.	Crucifereae	—
Sisymbrium irio Linn.	,,	—
Matthiola incana	,,	+
Commiphora wightii	Burseraceae	+
Plantago ovata (Forsk).	Plantaginaceae	+
Plantago major Linn.	,,	+
Phaseolus roxburghii Linn.	Leguminosae	—
Cassia fistula Linn.	,,	—
Mimosa pudica Linn.	,,	+
Cordia gharaf (Forsk).	Boraginaceae	+
Cordia latifolia Roxb	,,	—
Althaea officinalis Linn.	Malvaceae	—
Gossypium herbaceum Linn.	,,	—
Datura stramonium Linn.	Solanaceae	—
Pyrus cydonia Linn.	Rosaceae	+
Cydonia oblonga Mill.	Sterculiaceae	—
Linum usilatissimum Linn.	Linaceae	+
Strychnos nuxvomica Linn.	Strychnaceae	—
Trigonella foenumgraecum Linn.	Leguminosae	—
Melia sp.	Meliaceae	—
Zigyphus sp.	Rhamnaceae	—
Croton tiglium Linn.	Euphorbiaceae	—

Some seeds were found to germinate during the experiment and effected the potentiality of the seeds. To inhibit their germination the seeds were kept in an incubator at 120°C for 20 minutes.

Results and Discussion

There was considerable variation in the quality and quantity of mucilage released from the seeds of different species (Table 1). The seeds of *Linum usitatissinum* Linn., *Pyrus cydonia* Linn., *Plantago ovata* Forsk., produced sufficient amount of mucilage but did not trap larvae. *Mimosa pudica* Linn. produced copious amount of mucilage but larvae did not become attached. The surface of this mucilage appeared to function as if it were a tough membrane which prevented attachment.

The size of the seeds varied in length and breadth. The smallest *Mimosa pudica*, length 726 μ, breadth 720 μ and *Blepharis persica* is the largest, 5.5 mm in length and 0.3 mm in breadth (Table 4).

TABLE 2. PER CENT ATTACHMENT OF SECOND INSTAR LARVAE OF *Aedes aegypti* TO MUCILAGINOUS SEEDS. MEAN OF THE FIVE REPLICATION USING 5,8,10,12,15 AND 20 SEEDS PER 50 LARVAE PER CONTAINER. (observations after 24 hr at 28°C).

Seed	Family	Percentage of larvae attached to indicated no. of seeds					
		5	8	10	12	15	20
Ocimum americanum	Labiatae	74.0± 4.5	84.0±2.2	86 ±2.0	86 ± 5.0	84 ±1.7	88. ± 4.4
Lallemantia royleana	,,	82.6± 3.4	82.2±5.0	86 ±2.4	81.3± 5.0	78.4±5.0	84 ± 2.6
Ocimum sanctum	,,	76.0± 2.0	86 ±5.3	81.3±3.0	88.6± 3.0	84 ±2.5	92 ± .8
Ocimum bascilicum	,,	81.3± 3.3	80.7±4.0	80.3±4.4	80.3± 5.1	82.3±5.0	78.3± 9.0
Salvia spinosa	,,	40.0± 2.0	43.3±5.0	41.7± .8	40.2± 2.1	51.3±4.5	45,3± 5.7
Hygrophila auriculata	Acanthaceae	77.7± 0.2	86.6±2.1	78 ±1.2	78 ± 2.2	81 ±9.0	83.7± 4.0
Blepharis persica	,,	56.0± 7.0	60 ±9.0	75 ±4.0	71 ± 8.0	80 ±2.2	86 ± 1.0
Matthiola incana	Crucifereae	85.3± 6.0	81.5±6.0	63.2±4.7	66 ± 9.0	77 ±3.3	80.6± 1.1
Commiphora wightii	Burseraceae	62.0±10.0	60 ±8.0	75 ±2.3	60 ± 4.1	75 ±6.8	75 ± 1.3
Plantago major	Plantaginaceae	21.0± 0.8	20 ±9.4	42 ±6.0	52 ± 7.0	55 ±9.0	60 ±10.0

TABLE 3. PER CENT ATTACHMENT OF FOURTH INSTAR *Aedes aegypti* LARVAE TO MUCILAGINOUS SEEDS. MEAN OF FIVE REPLICATION USING 5,8,10,12,15 AND 20 SEED PER 50 LARVAE PER CONTAINER. (Observations after 24 hr reading at 28°C).

	Family	Percentage of larvae attached to indicated no. of seeds					
		5	8	10	12	15	20
Ocimum americanum	Labiatae	60 ± 1.3	60.3±3.6	70.4±3.5	70.2± 4.0	60.6±5.0	70.6± 5.0
Lallemantia royleana	,,	80.6± 6.0	85.3±7.0	79.3± 8	86.0± 9	85.4±8.0	78.6± 9.0
Ocimum sanctum	,,	68.6± .3	78 ±5.0	78.7± 4	78 ± 4.2	84.3±3.5	90.8± .3
Ocimum bascilicum	,,	62 ± 5.8	84 ±7.0	80 ±6.2	90 ±13.0	94 ±7.0	88 ± 2.0
Salvia spinosa	,,	40.7± 3.4	43 ±5.3	36 ±6.4	44.6± 9.0	32 ±6.0	46 ± .8
Hygrophila auriculata	Acanthaceae	33.6± 2.5	34.8±2.5	50 ±8.2	61 ± 2.4	61.3±2.8	56.6± 4.3
Blepharis persica	,,	40 ± 3.5	45 ±5.2	41 ±2.3	39 ± 9.0	31 ±3.1	51 ± 6.0
Matthiola incana	Cruciferae	56.5± 7.0	65.8±2.0	66.6±7.9	77.7± 3.2	58.3±7.8	53 ± 9.4
Commiphora wightii	Burseraceae	63 ± 4.0	64 ±9.1	55 ±6.0	55 ± 1.2	45 ±7.0	63 ±10.0
Plantago major	Plantaginaceae	18.3± 1.7	12.4± .2	17.6±1.8	23.1± 3.0	28 ±5.7	30 ± 1.6

TABLE 4. SIZE OF THE TEN MOST EFFECTIVE SEEDS. THE AVERAGE NUMBER OF LARVAE ATTACHED TO ONE SEED.

Seeds	Family	Size of the seeds μ	No. of seeds in 1 g	Average no. of larvae attached to one seed
Ocimum americanum	Labiatae	1221× 726	2255	5
Lallemantia royleana	,,	2805× 781	750	2
Ocimum sanctum	,,	1419× 792	1450	4
Ocimum bascilicum	,,	1386× 627	2535	4
Salvia spinosa	,,	2145×1980	355	2
Hygrophila auriculata	Acanthaceae	2772×1782	520	4
Blepharis persica	,,	5.5m×.3m	85	3
Matthiola incana	Crucifereae	2145×1353	1156	4
Commiphora wightii	Burseraceae	2409×1353	875	3
Plantago major	Plantaginaceae	990× 693	3935	2

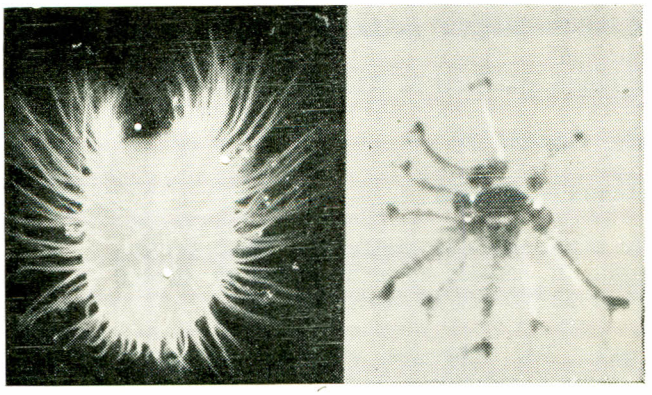

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Fig. 1. Seed of *Blepharis persica* showing mucilaginous layer and long fibers, when placed in water. Fig. 2. Fourth instar larval of *Aedes aegypti* attached to a seed of *Ocimum americanum* by mucilaginous layer.

It was observed that the seeds of *Ocimum americanum Plantago major*, *Blepharis persica* and *Salvia spinosa* produced mucilage just after the soaking in tap-water at 28°C. But the seeds of *Matthiola incana* and *Hygrophila auriculata* take time to produce mucilage.

The effectiveness of these seeds to catch second and fourth larvae in 24 hr has been shown in Tables 2 and 3 (Fig. 2). 20 seeds of each *Ocimum pilosum*, *Ocimum sanctum*, *Lallemantia royleana*, *Mathiola incana* and *Blepharis persica*, trapped more than 80% second instar larvae (Table 2). As compared to this, same seeds were found less effective to attract fourth instar larvae.

The retention of larvae did not vary uniformly with the rise in the number of seeds taken in the experiment (Tables 2 and 3). The seeds of *Salvia spinosa*, *Commiphora wightii* and *Plantago major* were found to attract very small percentage of both second and fourth instar larvae.

The minimum attachment observed by the seeds of *Blepharis persica* was in both second and fourth instar but a large number of larvae were found dead. It is very intresting that these seeds produce mucilage as well as long fibers that cover the maximum area of the dish used, and because of the net work the larvae could not come up to the surface and died due to suffocation (Fig. 1).

During the experiment it was observed that the seeds of *Ocimum americanum*, *Plantago major* and *Matthiola incana* germinated, with the result the trapping and the mucilage production was adversely effected. To overcome this problem heat treatment was given. This alternative method of eliminating-germination would also include eradication of the 'weed seeds' used to control mosquito larvae.

The heat treatment of the seed totally checked their germination but did not effect the mucilage production and trapping of mosquito larvae. The larvae reaching the moulting stage were found to escape while the old exuvium remained attached to the mucilage by oral brushes.

On the basis of these results it can be said that these seeds of *Ocimum americanum* and *Ocimum sanctum* can be utilized to destroy the mosquitoes during larval stage. This method can be applied as a suppliment to other methods of control. The advantage of the mucilagenous seeds to destroy mosquito larvae eliminates the hazards of water and air pollution.

If the extensive use of these seeds is implemented for the control of mosquito their supply will be no problem as they are abundantly available in our country. All the 30 seeds belonging to different families were purchased from the wholesale market, Karachi.

The five most effective seeds *Ocimum americanum*, *Lallementia royleana*, *Ocimum sanctum*, *Ocimum bacilicum* and *Salvia spinosa* have medicinal value, have been used as such since a long time, and are, therefore, nonpoisonous.

These seeds can be used to control the mosquito breeding in lakes and ponds specially where these are the only sources of drinking water without the hazards of water and air pollution. The efficacy of these seeds were also tested in sea water and on the basis of this test these seeds can be used to control mosquito colonies near the seashore and marshyland.

The seeds can be broadcast by hand or aeroplane and can be grown in abundance nearby the lakes from where the seeds can disperse themselves after maturity.

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References

1. L. Eldon, Reeves and G. Clement, Mosquito News, **29,** 601 (1969).
2. J.F. Bonner, *Plant Biochemistry* (Academic, New York, 1950), p. 393.
3. K. Esau, *Plant Anatomy*, (J. Wiley, New York, 1953), p. 7359
4. K. Esau, *Anatomy of Seed Plant* (J. Wiley, New York, 1960), p. 376.
5. F. Smith and R. Montgomery, *The Chemistry of Plant Gums and Mucilages and Some Related Polysaccharides* (Reinhold, New York, 1959), p. 627.
6. A.L. Winton and K.B. Winton, *The Structure and Composition of Foods* (J. Wiley, New York, 1932), vol. 1, p. 710.
7. A.K. Nadkarni, *Indian Materia Medica*, (Popular and Dhootapapeshwar, Bombay, 1954), vol. I and II, p. 861 1093.
8. Hakim Mohammod Said, *Hamdard Pharmacopoeia of Eastern Medicine* (Hamdard Academy, Karachi, 1969), p 5
9. S.H. Ashrafi, S.A. Muzaffar and M. Anwarullah, Sci. Ind., **4,** 312 (1966).