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Laboratory Evaluation of the Water Fern, *Azolla pinnata* for Mosquito Control

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ABSTRACT

Preliminary laboratory evaluation of *Azolla pinnata* for control of *Culex quinquefasciatus* Say and *Anopheles culicifacies* Giles was carried out. In both the species, complete coverage of the water surface with the fern drastically reduced oviposition and adult emergence, but not larval survival. Egg hatchability was partially affected only in *A. culicifacies*.

KEY WORDS: *Azolla pinnata, Culex quinquefasciatus, Anopheles culicifacies,* suppression

Azolla, a small aquatic fern, lives in symbiotic association with blue green alga, *Anabaena azollae*. The combination of these two species makes *Azolla* a valuable source of organic nitrogen, of particular interest to rice cultivation (Lumpkin and Plucknett, 1980). *Azolla* multiplies in the paddy fields very quickly and covers the entire field in the form of a thick mat within a period of two weeks. In China, a significant reduction in mosquito larval breeding in paddy fields in which paddy was cultivated in association with *Azolla pinnata* was observed (Lu Bao Lin, 1986). Since no work has so far been carried out in India, a preliminary laboratory evaluation was carried out on the effect of a surface mat of *A. pinnata* on oviposition, immature survival, and adult emergence of two species of mosquitoes, *Culex quinquefasciatus* Say and *Anopheles culicifacies* Giles.

MATERIALS AND METHODS

A. pinnata obtained from the Department of Microbiology, Agricultural College and Research Institute, Madurai, was maintained in the laboratory in plastic tubs (21 cms diameter) containing a layer of rice field soil (4-5 cms) flooded with tap water to a level of 4-5 cms above the soil level. The tubs were kept outdoors in partially shaded places. Superphosphate and carbofuran were applied once in five days at the rate of 2 gm/m^2.

Laboratory colonies of *C. quinquefasciatus* (Madurai strain) and *A. culicifacies* (Thenpennaiyar strain) were used for these experiments. Similar methods were used for both species. To study the effect on oviposition behaviour, three ovitraps were placed in each insectary cage (23 x 23 x 23 cms) over night. Each ovitrap consisted of an enamel bowl of 150 cm^2 surface area containing 300 ml of water. In the first ovitrap, *Azolla* covered the entire surface of the water medium (10 gms wet weight of inoculum added per bowl); in the second, there was about 50 per cent coverage (5 gms wet weight/bowl) and in the third there was no *Azolla*, which served as control. Eggs/egg rafts laid by the mosquitoes in each ovitrap were counted in the morning. There were 15 - 16 replicates of the treatments.

In studies on egg hatchability and immature survival, three 500 ml beakers with 250 ml of water were used. One beaker was fully covered with *Azolla* (5 gms wet weight/beaker) and in the second 50 per cent surface was covered (2.5 gms wet weight/beaker). The third was left without *Azolla* as control. Each beaker was seeded with one egg raft of *C. quinquefasciatus* or 200 eggs of *A. culicifacies*. Hatching and development of the larvae were followed till pupation. The immatures were provided with a pinch of larval food, *viz*., dog biscuit and yeast (2:3) daily. Similarly, adult emergence was studied by introducing 50 pupae of *C. quinquefasicatus* / *A. culcifacies* in each container. Three replicates were carried out. For statistical analysis, Student's 't' test was applied.

RESULTS AND DISCUSSION

In both species of mosquitoes tested, oviposition was drastically reduced in containers containing complete coverage by *Azolla*, when compared with control (Table 1). In containers with 50 per cent coverage also there was significant reduction in the numbers of eggs laid. Thus the results clearly indicated that coverage of the water surface with *A. pinnata* significantly affected the behaviour of the two mosquito species under laboratory conditions. The *Azolla* mat forms an oviposition barrier to the mosquitoes. Almost total suppression of egg laying was observed when coverage was complete, and marked reduction in the partially covered water surface. Similar inhibitory effects on oviposition have been reported for *Culx. tritaeniorhynchus* in China (Lu Bao Lin, 1986) and for *C. quinquefasciatus* in Sri Lanka (Amerasinghe and Kulasooriya, 1986).

Partial or complete coverage of the water surface by *Azolla* did not significantly alter egg hatchability and pupal yield in case of *C. quinquefasciatus*, but the *Azolla* coverage of the water surface significantly reduced egg hatchability of *A. culicifacies* (Table 2). Since *Anopheles* species lay eggs individually, attachment of some of them to the surface of *Azolla* might be the cause for reduced hatchability in *A. culicifacies*. However, once hatched, the immatures of both species survived normally to the pupal stage in experimental containers as well as in controls. These observations suggest that *Azolla - Anabaena* complex did not have any deleterious effect on the larvae of *C. quinquefasciatus* and *A. culicifacies*. However, Mogi *et al.* (1986) have reported that *Azolla imbricata* increased mortality in the second instar of *A. peditaeniatus* and *A. sinensis* in the laboratory. They suggested that mechanical obstruction of respiration might be the cause for mortality, but did not rule out small hydra attached to the plants. Angerilli and Beirne (1974, 1982) observed that aquatic plants such as *Chara globuralis*, *Lemna minor* and *Eloda canadensis* caused heavy mortality in *Aedes aegypti* and *Culex pipiens* by chemical means. The active principles in *Chara* and *Lemna* acted as juvenile hormone mimics.

Heavy mortality was observed among pupae of both species in *Azolla* covered containers with completely covered surfaces. No significant mortality was noticed in the containers with partial coverage by the fern (Table 3). Examination of containers with complete *Azolla* coverage showed a large number of dead pupae and partially emerged adults with wing deformities. This indicated that the *Azolla* mat provided a mechanical barrier to successful emergence of adult mosquitoes. Amerasinghe and Kulasooriya (1986) have reported that most of the mortality of *C. quinquefasciatus* under thick *Azolla* cover occurred at the time of pupal-adult transformation and also suggested that *Azolla* mat would be effective in suppressing most, if not all, mosquito emergence.

The International Rice Research Institute is promoting the dissemination of *Azolla* to paddy fields in Philippines (Swaminathan, 1984). Moreover, Tamil Nadu Agricultural Department has recommended the use of *Azolla* in the paddy fields to fix nitrogen and act as fertilizer to obtain higher grain yield. Therefore, it is important to study whether *A. pinnata* could also act as a biological control agent to suppress the mosquito species such as *C. tritaeniorhynchus* and *Culex vishnui*, which are vectors of Japanese encephalitis breeding mainly in paddy field ecosystem (Pant, 1972; Bang and Pant, 1983). Field trials are in progress.

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Table 1. Effect of *Azolla pinnata* cover on oviposition of mosquitoes

	C. quinquefasciatus			*A. culicifacies*		
	Azolla coverage			*Azolla* coverage		
	Complete (100%)	Partial (50%)	Control	Complete (100%)	Partial (50%)	Control
Number of replicates	16	16	16	15	15	15
Total number of egg rafts/egg laid	4	141	382	3558	17051	59165
Mean ± SD	0.25 ± 0.77	8.81 ± 10.23	23.28 ± 21.54	237.20 ± 375.24	1136.73 ± 1272.13	3944.33 ± 2419.88
't' value	4.38 [a] 3.34 [b]	2.52 [a]	--	5.86 [a] 2.63 [b]	3.98 [a]	--
P	<0.001 [a] <0.01 [b]	<0.05 [a]	--	<0.01 [a] <0.05 [b]	<0.01 [a]	

a = comparison with control; b = comparison with 50%.

Table 2. Effect of *Azolla pinnata* on egg hatchability and pupation in *C. quinquefasciatus* and *A. culicifacies*

	C. quinquefasciatus			*A. culicifacies*		
	Azolla coverage			*Azolla* coverage		
	Complete (100%)	Partial (50%)	Control	Complete (100%)	Partial (50%)	Control
Number of replicates	6	6	5	6	5	5
Total number of egg	953	1072	592	1200	1000	1000
Number of eggs hatched	864	1027	519	678	639	876
% hatchability						
Mean ± SD	89.65 ± 7.51	95.86 ± 2.26	86.63 ± 11.62	56.5 ± 7.06	63.9 ± 5.61	87.6 ± 4.36
't' value	0.50 [a]	1.74 [a]	--	8.94 [a] 1.94 [b]	7.46 [a]	--
P	<0.05 [a]	<0.05 [a]	--	<0.01 [a] <0.05 [b]	<0.01 [a]	--
% pupal yield						
Mean ± SD	82.47 ± 6.71	70.26 ± 17.93	67.86 ± 18.86	61.21 ± 6.91	60.87 ± 10.76	68.96 ± 6.99
't' value	1.65 [a]	0.22 [a]	--	1.83 [a]	1.41 [a]	--
P	>0.05 [a]	>0.05 [a]	--	>0.05 [a]	>0.05 [a]	--

a = comparison with control; b = comparison with 50%.

Table 3. Mortality at pupal to adult stage of the mosquitoes in relation to *Azolla pinnata* coverage of the water surface

	C. quinquefasciatus			*A. culicifacies*		
	Azolla coverage			*Azolla* coverage		
	Complete (100%)	Partial (50%)	Control	Complete (100%)	Partial (50%)	Control
Number of replicates	3	3	3	3	3	3
% Mortality						
Mean ± SD	63.33 ± 6.11	48.00 ± 15.62	26.67 ± 12.05	46.00 ± 21.07	4.00 ± 3.46	5.33 ± 4.16
't' value	4.69 [a] 1.58 [b]	1.87 [a]	--	3.27 [a] 3.40 [a]	0.43 [a]	--
P	<0.01 [a] >0.05 [b]	>0.05 [a]	--	<0.05 [b] <0.05 [b]	--	--

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