Mosquito larval productivity in rice-fields infested with Azolla in Mvomero District, Tanzania

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Abstract: Azolla (Salviniales: Azollaceae) is known to reduce oviposition and adult emergence of a number of mosquito species. Several species of Azolla are reportedly indigenous to Tanzania. However, the potential of Azolla as a biocontrol agent against malaria mosquitoes has not been evaluated in the country. This cross-sectional study was carried out to assess mosquito larval productivity in irrigated rice-fields infested with Azolla in Mvomero District, Tanzania. A systematic larval sampling covering all open water bodies along designed transect was carried in rice-fields. Larval density was estimated by dipping water bodies with or without Azolla. The degree of Azolla coverage was categorized as 0%, <50%, 50%, and >80%. Larvae densities were categorised as low (<50/m^2), medium (50-500/m^2) and high (≥500/m^2) productivity. A total of 120 water bodies were surveyed and 105 (87.5%) had *Azolla microphyla* and *A. pinnata* at varying degrees of coverage. Of the total 105 water bodies with Azolla, 80 (76.2%) had a green Azolla mat, and 25 (23.8%) a brown Azolla mat. Eighty-eight (73.3%) of the sites were infested with anophelines and 109 (90.8%) with culicine larvae. Seventy percent of all water bodies contained anophelines and culicines in sympatric breeding, while 20.8% and 3.3% had only culicines and anophelines, respectively. The majority (82%) of mosquito breeding sites were found in area with Azolla substrate. Mosquito larva productivity was low in sites with highest (>80%) Azolla coverage. Seventy-two (81.8%) of the anopheline and 90 (82.6%) culicine breeding sites were infested with Azolla. Water bodies infested with green Azolla were more productive than those covered by brown coloured Azolla substrates for both culicines (13%) and anophelines (8%). Of the 1537 field collected larvae that hatched to adult stage, 646 (42.03%) were *Anopheles gambiae* s.l., 42 (2.73%) were *An. funestus* and 769 (50.03%) were *Culex quinquefasciatus.* These findings suggest that the mosquito productivity is low when the Azolla coverage is high (>80%). The promotion of Azolla in mosquito control should take into consideration the degree of Azolla coverage.

Key words: Azolla, mosquito, larval productivity, rice-fields, Tanzania

Introduction

Azolla (Salviniales: Azollaceae) is a floating water fern which commonly lives symbiotically with the blue-green algae, *Anabaena azollae* Strasburger. Azolla-Anabaena complex has been shown in many areas to fix nitrogen at high rates and increase rice harvest (Fogg *et al*., 1973; Peters, 1978; Tung & Shen, 1985; Kondo *et al*., 1989). In some parts of the world, farmers have been highly impressed by these economic gains and therefore, acceptability of Azolla is high (Rajendraan & Reuben, 1988, 1991). Besides adding nitrogen to paddy water, Azolla offers additional benefits of weed suppression (Krock *et al*., 1991) and improvement of soil structure (van Hove, 1989). In some parts of the world, and to a varying extent, Azolla has been utilised to promote rice yields (Lu Bao Lin, 1988).

Several laboratory studies have shown that complete coverage of water surface by Azolla drastically reduces oviposition and adult emergence of *Anopheles* and *Culex* mosquitoes in a number of countries in Asia (Amerasinghe & Kulasooriya, 1985; Mogi *et al*., 1986; Rajendraan & Reuben, 1988; Lu Bao Lin, 1988). Contrary to these findings, intact Azolla was not found to affect larval survival of *Culex tritaeniorhynchus* and *Anopheles sinensis* in an experiment using water containers (Annon, 1986). Similarly, *Azolla pinnata* was reported to have no effect on larval survival of *Cx quinquefasciatus* and *An. culicifacies* though in the later species, egg hatchability was partially affected (Rajendraan & Reuben, 1991).

In India, field studies showed that Azolla growths were responsible for almost complete suppression of mosquito breeding in natural habitats (Ansari & Sharma, 1991). In a rice land agro-ecosystem of South India, immature mosquito population was significantly reduced by mats of *Azolla microphylla* covering more than 80% of water surface (Rajendraan & Reuben, 1991). However, peak mosquito breeding in the area did not coincide with period of maximum coverage by Azolla, hence its utility for mosquito control was considered limited.

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Several species of Azolla are reportedly indigenous to Tanzania (Wagner, 1992). Despite the known agricultural and public health benefits of Azolla, no work has so far been done to establish evidence for the potential of this plant as a biocontrol agent against mosquitoes. Azolla fern is common in some rice farms in Mvomero District, Tanzania. The plant was introduced during the early 1980s as a bio-fertilizer in rice fields. In recent years, the use of Azolla in rice farms has been promoted (Mlozi *et al*., 2006). The objective of this study was therefore to assess the impact of Azolla coverage on the mosquito productivity in rice-fields so as to establish its potential in malaria vector control in Tanzania.

Materials and Methods

Study area

This study was carried out in Mkindo village in Mvomero District (5-8^{0}S, 37-39^{0}E) in central Tanzania. The area experiences a high and bimodal rainfall with a relatively short dry spell between June and September. Heavy rainfall is experienced in March-May and from October-December. The average annual rainfall is 1,146mm. The mean maximum temperature is 31^{0}C, which is experienced from October to March; whereas the mean minimum temperature is 19^{0}C between June and September.

Mvomero District economy depends heavily on agriculture, mainly from crop production. Mkindo village was purposively selected for this study. Rice farming is the main activity of most of its inhabitants. Since 1982, there has been an irrigation project in the area making more systematic and intensive use of water from Mkindo River. Mkindo rice farming employs the gravitational water supply technique and rice is cultivated in bunds (Mboera *et al*., 2007).

Sampling framework

This study was carried out in May-July 2006 at the end of the long rainy season. In this period mosquito density was still high and some rice fields were covered with Azolla. An area of 1 km^2 across water bodies created for rice farming purposes was selected for the study. A regular sampling strategy by using transect was employed, whereby a point was established (6˚15′22.139″S and 37˚32′20.293″E) in which two perpendicular transects were designed across the point (one from east to west and the other from north to south). Each transect was 400m^2 in size. All open water bodies along the designed transects, were searched as potential breeding sites. From each water body within the designed transect, 10 dips were taken evenly with a standard white 350 ml dipper. Each water body was visited only once in a cross sectional survey of breeding sites.

Mosquito identification and estimation of breeding site productivity

Mosquito larval density was classified as: (i) no larvae when none of the dips contained larvae; (ii) larvae at low density when a dip had an average of 1-3 larvae; (iii) larvae at medium density when a dip had 4-6 larvae; and (iv) larvae at high density when an average of more than 7 larvae per dip were found. Anopheline and culicine mosquitoes were differentiated macroscopically in the dipper based on the floating habit of the mosquito larvae.

To quantify the relative mosquito larvae productivity, a score was assigned to each breeding site according to its size and mosquito density. This classification was used to characterize anopheline and culicine mosquitoes breeding sites. Larvae densities were categorised as low (<50/m^2), medium (50-500/m^2) and high (≥500/m^2) productivity. Larvae collected from the breeding sites were reared in the laboratory and the adults identified morphologically under light microscope.

Ethical considerations

The permission to carry out this study was granted by the Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR/HQ/R.8a/Vol IX/297, March 23, 2004).

Results

A total of 120 water bodies were surveyed. Eighty-eight (73.3%) of the water bodies were infested with anophelines larvae and 109 (90.8%) with culicine larvae. Seventy percent of all water bodies contained anophelines and culicines in sympatric breeding, while 20.8% and 3.3% had only culicines and anophelines, respectively. No mosquito larvae were found in seven (5.8%) of the water bodies. Of the total water bodies, 105 (87.5%) had Azolla at varying degrees of coverage. Two species of Azolla namely *Azolla microphylla* and *A. pinnata* were identified, in most cases occurring together. About 82% and 83% of all breeding sites for anophelines and culicines, respectively, were found in rice fields with Azolla (Figure 1).

The largest proportion of the Azolla infested breeding sites for anophelines (71.6%) and culicines (55.1%) had low to medium larval densities (Table 1). Higher larval densities for both culicines and anophelines were observed in Azolla-infested breeding sites.

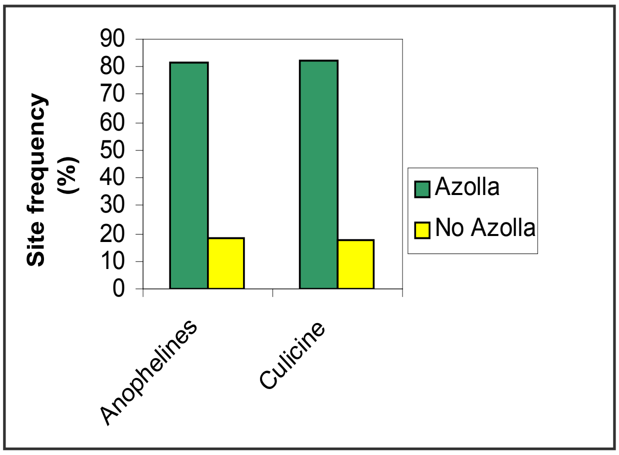

Figure 1: The percentage of breeding sites with or without Azolla

Table 1: Larval density and number of breeding sites for anopheline and culicine in Azolla infested water bodies

Presence of Azolla	**Larval density**	**Anopheline**		**Culicine**	
		No. of sites	Percent	No. of sites	Percent
Yes	Low	29	32.95	24	22.02
	Medium	34	38.63	36	33.03
	High	9	10.23	30	27.52
No	Low	12	13.64	8	7.34
	Medium	4	4.55	9	8.26
	High	0	0	2	1.83

Seventy-two (81.8%) of the anopheline breeding sites were covered with Azolla while 16 (18.2%) were not. On the other hand, 90 (82.6%) of the culicine breeding sites were covered with Azolla and 19 (17.4%) were not. Of the total 105 water bodies, 80 (76.2%) were covered by a green Azolla mat, and 25 (23.8%) by a brown Azolla mat (Table 2).

Of the 105 plots covered with Azolla, the degree of coverage was estimated as <50% in 42 (35%) sites, about 50% in 29 (24.2%) sites and more than 80% coverage in 34 (28.3%) sites. Fifteen (12.5%) sites were not covered by Azolla (Table 3).

Table 2: Mosquito larvae density and number (%) of breeding sites in green and brown coloured Azolla

Larval density	**Green Azolla**		**Brown Azolla**	
	Anopheline	Culicine	Anopheline	Culicine
Low	26 (35.1)	24 (25.5)	6 (8.1)	4 (4.3)
Medium	23 (31.1)	26 (27.7)	11 (14.9)	12 (12.8)
High	7 (9.5)	20 (21.3)	1 (1.3)	8 (8.5)

Table 3: Anopheline and culicine larval productivity in relation to Azolla coverage

Azolla coverage	Larval density	Anopheline No. of sites	Percent	Culicine No. of sites	Percent
0	Nil	2	1.67	1	0.8
	Low	9	7.5	4	3.3
	Medium	4	3.3	8	6.7
	High	0	0	2	1.7
<50%	Nil	13	10.8	2	1.7
	Low	17	14.2	16	13.3
	Medium	8	6.7	13	10.8
	High	4	3.3	11	9.2
50%	Nil	8	6.7	3	2.5
	Low	4	3.3	5	4.2
	Medium	13	10.8	12	10
	High	4	3.3	9	7.5
>80%	Nil	9	7.5	5	4.2
	Low	12	10.0	8	6.7
	Medium	13	10.8	13	10.8
	High	0	0	8	6.7

Some 12.5% of all sites surveyed had no Azolla at all and 35% were covered in less than half of the total area. Twenty-four percent of the sites were covered with Azolla by 50% while about 28% were fully or nearly fully covered with Azolla. Mosquito larval productivity was lowest in sites with the highest (>80%) Azolla coverage. The low larval productivity in relation to Azolla coverage was more pronounced in anophelines than in culicines. When breeding sites were grouped according to their productivity, sites without Azolla were less productive as compared to sites with Azolla for both anophelines and culicines. About 10% and 28% highly productive breeding sites for anophelines and culicines, respectively, were found in sites covered by Azolla.

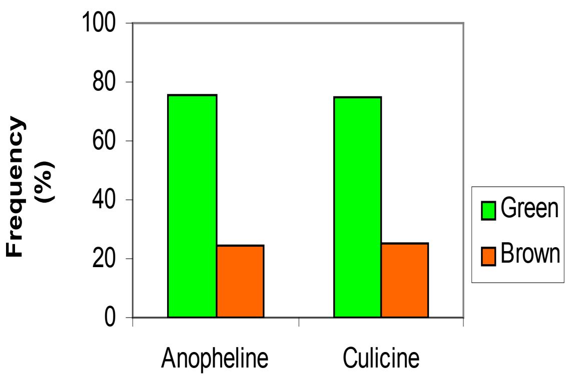

Figure 2: The proportion of plots with mosquito larvae in sites with either green or brown Azolla

Over three quarters (76%) of all water bodies were covered with green coloured Azolla while 24% had brown coloured Azolla mat. About 76% of anopheline breeding sites and 75% of all culicines breeding sites had green coloured Azolla. Only 24% of all anopheline sites and 25% of culicine sites had brown coloured Azolla.

When breeding sites were grouped according to their productivity, sites with brown Azolla were less productive than those coved by green Azolla for both anophelines and culicines. Only 1% and 9% of anophelines breeding sites covered by brown and green Azolla, respectively were highly productive. Similarly 9% and 21% of highly productive culicine breeding sites were covered by brown and green Azolla, respectively.

Of the field collected larvae reared in the insectary, 1537 hatched to adult stage. Of these 646 (42.03%) were *Anopheles gambiae* s.l., 42 (2.73%) were *An. funestus* and 769 (50.03%) were *Culex quinquefasciatus*. Other species accounted for the remaining 5.2%.

Discussion

In this study, it was observed that anopheline and culicine immature stages occur sympatrically in most breeding sites in rice-fields in Mvomero District. This suggests that breeding sites provide favourable environment and resources to support the development of the mosquito immature stages. Similar co-occurrence of *Anopheles* and *Culex* larvae has also been reported recently by Matthys *et al*. (2006) in West Africa. The majority of the sites in the rice-fields in our study areas were infested with Azolla. However, the infestation rate and intensity varied from site to site.

The reduction of larval productivity as a result of highest Azolla coverage confirms the hypothesis that maximum coverage of the plant on water surface limits mosquito productivity. That is, gravid mosquitoes are denied access to oviposit on water as well as immature being restricted from getting oxygen, light, and hence limit pupation. Studies in China showed that culicine mosquitoes cannot lay their eggs on water that is completely covered with the fern (Lu Bao Lin, 1988). However, under the same conditions, it was observed that, anophelines were not prevented from oviposition, but larval development was retarded and successful emergence of the adult mosquito from the pupa was blocked. In China, emergence studies in experimental rice fields showed that Azolla plants have little practical value in mosquito control in real life conditions (Lu Bao Lin, 1988). This is probably because the process of expanding the coverage of Azolla trails behind the mosquito breeding peak, and that even at 70% coverage, sufficient surface water remains for mosquito population densities to build up. In this study, only the highest Azolla coverage of >80% had a significant impact on the larval productivity. This observation should therefore be cautiously exploited when employing the use of Azolla as a strategy to control mosquito vectors in rice fields.

The observations that larval productivity level was hindered when the coverage of surface water with *Azolla* is >80% has also been reported in India (Rajendraan & Reuben, 1991). The high coverage of Azolla mat is likely to inhibit oviposition and cause mortality when adult mosquitoes emerge from pupae as the young adults are unable to penetrate through the physical barrier of the Azolla layers (Amerasinghe & Kulasooriya, 1985). The fact that Azolla lives in symbiotic association with the blue alga, *Anabaena azollae*, and that it fixes nitrogen from the atmosphere can be promoted as cheap alternative for chemical fertilizers in irrigated rice fields. It is important that farmers are encouraged to introduce *Azolla* into their rice fields as it is currently being done at the Mkindo Farmers Training Centre in Mvomero District (Mlozi *et al*., 2006). Community education on *Azolla* should stress its advantage of reducing weed growth, improvement of soil fertility and mosquito control. The use of Azolla as a biological control technique of rice field breeding mosquitoes and as fertilizer should therefore be promoted in both agricultural and community health education programmes.

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