INTERACTIVE MEETING ON THE ROLE OF *AZOLLA* IN CONTROLLING RICE FIELD BREEDING VECTORS

Report of an Interactive Meeting
Pondicherry, India
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Introduction

Globally rice is grown in 140 million hectares of land serving as a staple food. Since rice fields are extensively irrigated they favour the prolific breeding of many disease vectors. In India about 14 species of mosquitoes belonging to 5 genera viz. *Culex, Aedes, Anopheles, Armegeres* and *Mansonia* have been suspected or incriminated from disease outbreak studies as vectors of Japanese encephalitis (JE). The principal vectors of JE, *Cx. vishnui* sub-group breeds mainly in rice fields. Over 40 viruses have been identified in rice field agro ecosystems but most important one is Japanese Encephalitis virus. Though JE was first reported in India only in fifties, in recent times its incidence has been recorded in as many as 24 states and union territories. The major and repeated outbreaks of the disease have been reported from Assam, Andhra Pradesh, Bihar, Goa, Karnataka, Kerala, Madhya Pradesh, Manipur, Pondicherry, Tamil Nadu, Uttarpradesh and West Bengal. Most of these states are principal rice growing states of the country.

The waterfern, *Azolla* is being promoted in rice cultivation to reduce the input cost of nitrogenous fertilizer and also to increase the yield. *Azolla* is also being used as feed for live stock, green manure, weed control, ornamental purposes and compost preparations. It is being used in 2-5% and 40-60% areas of rice cultivation in China and Vietnam, respectively besides India, Philippines, Japan, Korea, Thailand and USA. Besides being a green manure, *Azolla* is also known to acts as a mechanical barrier to the ovipositing female mosquitoes, since it forms a dense mat on the water surface. However, its potential as bio control agent has not been fully explored and hence an interactive meeting was organized to understand the potential of *Azolla* as a control agent of mosquito breeding. The resume of the deliberations of the interactive meeting and conclusions and recommendations are presented hereunder.

Mosquito breeding in Rice fields and incidence of Japanese Encephalitis

For rice cultivation fallow lands are flooded with water, ploughed, levelled and transplanted with rice seedlings and immediately after transplantation, mosquitoes start breeding. The density of early instars of both *Anophelines* and *Culicines* show maximum values upto 3rd week, and thereafter there is a steady decrease in the density. From the specimens collected from the field, a total of ten *Anopheline* mosquitoes viz. *An. subpictus, An. vagus, An. barbirostris, An. hyrcanus, An. splendidus, An. pallidus, An. fluvialitis, An. jamesi* and *An. tessellatus* have been recorded in the order of abundance. Five species of *Culicines* viz., *Cx. vishnui, Cx. tritaeniorhynchus, Cx. pseudovishnui, Cx. gelidus* and *Cx. bitaeniorhynchus* have also been recorded in the above study. The peak breeding of JE vectors occurs during the first two weeks after transplantation in short-term rice varieties while in long-term rice varieties it occurs during 6 to 8 weeks in addition to two peaks during first 4 weeks.

In South Arcot district of Tamil Nadu, vector abundance started raising from July and minimum infection rates (MIR) peaked in September followed by a decrease in MIR during the month of October. However, the abundance of mosquitoes remained high until March. The decrease in MIR from October onwards coincided with rising herd immunity in pigs. Although MIRs in October (0.47) and November (0.42) were lower than in September (0.92), a comparable high risk of infection for humans continued because of high vector abundance and human biting rates (Gajanana *et al.* 1999).

Azolla and its occurrence

Azolla is a cryptogenic free floating water fern (Salviniales:*Azollacea*). Although this fern is represented by 31 species, only 6 are existent, viz. *A. caroliniana, A. filiculoides, A. mexicana, A. microphylla, A. nilotica* and *A. pinnata* (Lumpkin and Plucknett, 1978). In India the dominating species is represented by *A. pinnata*, occurring in ponds, pools, reservoirs, rice fields and lakes (Majid, 1986; Ansari and Sharma, 1991). The plant body is pinnately branched with free floating horizontal stem bearing long adventitious roots on its underside leaves that are arranged alternately overlapping each other; each leaf consists of one dorsal thick green aerial lobe and a ventral thin colourless lobe, which remains submerged in water; absorption of nutrients is almost entirely through ventral lobe and the roots are practically non-functionall (Hill, 1975). *Anabaena azollae*, a cyano-bacterium lodged in the dorsal lobe cavity and is regarded as source of organic fertilizer for rice cultivation (Lumpkin and Plucknett, 1980; 1982). The *azollae* not only fulfils the nitrogen requirement of host plant *Azolla* but also liberates substantial amount of nitrogen in the medium. The carbon requirement of the soil is met from *Azolla.* The trophic independence of *Anabaena azollae* for nitrogen and that of *Azolla* for carbon, makes the system complete in all respects, for converting molecular nitrogen into ammonia.

A survey carried out to understand the natural infestation of *A. pinnata* in different water bodies (Ansari and Sharma, 1991) revealed that highest infestation occurred in pools and lowest in paddy fields. Of the 178 pools checked, 36.5% were completely covered with the fern growth while 34.8% were partially covered and no infestation was observed in 28.6%. Similarly 6.5% of the wells were completely and 9.2% partly covered with *Azolla*, while 84.2% had no *Azolla*.

Technology for mass Multiplication and Application of *Azolla*

Azolla normally reproduces vegetatively by fragmentation. Under optimum conditions doubling time of *Azolla* falls between 2 and 5.6 days (Watanabe et al. 1977, Talley et al., 1977; Kannaiyan, 1988b; 1990). The growth potential of *Azolla* is subjected to environmental variables such as humidity, temperature, light intensity, pH, and salinity. (Kannaiyan and Somporn, 1987; Kannaiyan et al., 1987 and Kannaiyan, 1988a). Since factors controlling the formation and germination of sporocarps in different species of *Azolla* are still poorly understood, production of large scale inoculum has to be done through vegetative multiplication. Further, the storage and transport of fresh, bulky material is not only cumbersome but also impracticable as *Azolla* undergoes rapid decomposition during storage under tropical conditions.

A simple nursery method for large scale multiplication of *Azolla* has been evolved for easy adoption by the farmers (Kannaiyan, 1982). The field selected for *Azolla* nursery is thoroughly prepared, and divided into one cent plots (20 X 2m) by providing suitable bunds and irrigation channels. Water is maintained to a depth of 10cm. Ten kg of fresh cattle dung mixed in 20 litres of water is sprinkled in each plot and *Azolla* inoculum at 8 kg is applied and spread uniformly. Super phosphate (100 g) is applied in three split doses at 4 days interval as fertilizer for *Azolla*. For insect pest control furadan granules at 100 g is applied 7 days after inoculation. Fifteen days after inoculation, *Azolla* is harvested and introduced into the main field as a source of primary inoculum. From one harvest 40-50 kg of Azolla is obtained from each plot. *Azolla* nursery may be planned while rice nursery is raised.

Azolla may be inoculated either before planting rice or 7-10 days after planting. The inoculum required for one hectare transplanted rice is 500 kg. The inoculated *Azolla* is found to establish and cover the rice field in 20-30 days period which is equivalent to 15-25 tonnes of biomass/ha. (Kannaiyan, 1986). This biomass is incorporated in the rice field during first weeding which decomposes in 2-3 weeks period (Subramani and Kannaiyan, 1987) and the nitrogen and other nutrients are made available to the rice crop. The left over *fronds* of *Azolla* float in water surface, multiply and cover the rice field again. Likewise, two or three incorporations of *Azolla* mat is possible for one rice crop. *Azolla* contributes 40-60 kg N ha^{-1} (Kannaiyan, 1992) besides Phosphorus, zinc and iron.

Control of Mosquito Breeding By *Azolla*

Around 1900, a strong international interest developed in the use of *Azolla* for mosquito control (Smith, 1910; Lumpkin and Plucknett, 1980). *Azolla* mats were thought to prevent mosquitoes from laying eggs and prevent larvae from coming up for air. This claim was supported by Tan chuan-chieh (1942) who found that a complete cover of *Azolla* could prevent completion of mosquitoes' life-cycle. However, he pointed out that an incomplete mat provides protection to mosquito larvae from their natural predators. Germans successfully used *Azolla* for mosquito control in the Rhine (Howard, 1910). The dense surface mat of *Azolla* was found to interfere with oviposition of mosquitoes and also prevent adult emergence (Amarsinghe and Kulasooriya, 1986). Extensive studies carried out in China to evaluate the efficacy of *Azolla* as a bio-control agent against mosquitoes in rice fields which are important breeding habitats for *Cx. tritaeniorhynchus*, principal vector of Japanese B. Encephalitis, showed that the complete coverage of *A. filiculoides* has prevented oviposition of Culicine female mosquitoes (Luo Bao-Lin, 1988). The inhibiting effect of oviposition was directly proportional to the coverage of aquatic fern on water surface. Similar results were obtained with *A. caroliniana* against *An. sinensis* a main vector of malaria. The complete coverage of *A. filiculiodes* was also detrimental to adult emergence in the case of *An. sinensis* and the average cumulative reduction was 84.7 to 98.4% against larvae and pupae respectively. Indian scientists have also reported that the complete coverage of *A. pinnata* significantly reduced the hatchability of *An. culicifacies* eggs (Rajendran and Reuben, 1988). They have also observed pupal mortality in the case of *An. culicifacies* and *Cx. quinquefasciatus* in containers completely covered with *A. pinnata*.

Field evaluation carried out with *Azolla microphylla* (Rajendran and Reuben, 1991), where *An. subpictus, Cx. pseudovishnui* and *Cx. tritaeniorhynchus* breed in rice fields predominantly indicate reduction of immature population after 13-14 days when 80% mat of *Azolla* was formed on water surface. When the density of immature stages were compared for the overall study period (13 weeks) an appreciable reduction has been achieved in the larval and pupal density of *Anopheline* and *Culicine* mosquitoes.

Another field observation indicate considerable reduction in mosquito breeding in habitats completely covered with *Azolla* (Ansari and Sharma, 1991). The suppression of breeding was more pronounced against anopheline as compared to culicine immatures. The percent reduction in anopheline immature density was 93.6 to 100% in pools, wells and paddy fields, in completely covered habitats as against 8.0 to 97.7% in partially covered habitats. The percent reduction in *Culicine* was 24.1 to 93.7% in habitats which were found completely covered with *Azolla* and in partially covered habitats the percent reduction was in the range of 27.5 to 88.5% in pools, wells, paddy fields, ponds and drains.

Discussion and Conclusion

When *Azolla* is inoculated to rice fields 7 days after transplantation, as recommended at present, it takes about 5 weeks to multiply, grow and cover the water body fully, whereas the intensity of mosquito breeding is very high during this period. Thus partial coverage of water body by *Azolla* leads to poor control of mosquito breeding, i.e., neither prevention of egg laying by wild mosquitoes nor prevention of adult emergence. If *Azolla* inoculation is done just after transplantation of rice seedlings, perhaps there is a possibility of faster coverage of the water body by the fern leading to more effective control of mosquito breeding. Further, enhancing the present application rate of *Azolla* (500 kg/ha) to 2000 kg/ha may also lead to similar situation. But, logistical problems involved in transportation and broadcasting of the large biomass need to be solved for a large scale control programme.

Azolla can multiply and grow faster during monsoon and post-monsoon periods in tropical situations and incidentally rice cultivation as well as mosquito breeding are intense during post-monsoon period. If the inoculation time and the inoculation dose of *Azolla* are optimised there is better chance for it to be used in the control of rice field breeding mosquitoes, especially, the vectors of JE virus. *In situ* production of *Azolla* along with rice nursery is advisable for inoculation to main field, as this would avoid the problems associated with transportation of inoculum produced elsewhere.

There is little information on the tolerance of *Azolla* to different pollutants, especially organics, although some information is available on salinity tolerance and data on these need to be generated. The problem of abundance of snails in *Azolla* grown fields was highlighted. Simultaneous application of neem with *Azolla* did not help much to prevent mosquito breeding.

Recommendations

- 1. More information should be generated on the potential of *Azolla* for use in mosquito control (Dr. S. Kannaiyan, TNAU and Dr. N. Balakrishnan, NICD).
- 2. Different *Azolla* species should be tested for trapping nutrients and heavy metals in polluted water bodies (VCRC).
- 3. Extracts of *Azolla* spp. should be evaluated for mosquito control potential (VCRC).
- 4. The alga, *Westellopsis* sp. should be tested for mosquito control potential by extracting bioactive components. And a research project proposal should be formulated along these lines for seeking funds from DBT (CRME).
- 5. Inoculum level of *Azolla* for effective mosquito control should be standardized (Dr. S. Kannaiyan, TNAU and Dr. N. Balakrishnan, NICD).
- 6. The oviposition behaviour of mosquitoes in *Azolla* inoculated rice fields and the detrimental effect(s) of *Azolla* mat on mosquitoes need to be studied (Dr. S. Kannaiyan, TNAU and Dr. N. Balakrishnan, NICD).
- 7. A common protocol for VCRC, CRME, TNAU & MRC, to be drawn for studies on *Azolla* in rice fields.

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8. Dr. P.K. Das, Director, VCRC, Pondicherry.

Title of papers presented

1. Studies on the use of *Azolla* sp. for the control of rice field breeding mosquitoes. Rajendran, R., Raghunatha Rao, D., and Gajanana, A.
2. Utility of *Azolla* in controlling of mosquito breeding in rice field. Ansari, M.A.
3. Growth and multiplication of the waterfern *Azolla microphylla* in wetland rice ecosystem and its role in mosquito breeding, Kannaiyan, S.
4. Studies on the role of *Azolla* in controlling rice field breeding mosquitoes in Coimbatore. Balakrishnan, N.
5. Feasibility of utilization of *Azolla* for mosquito control. Rajendran, G., and Jambulin gam, P.

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