

## ***Azolla* Symbiotic System's Application as Biofertilizer for Green Garden Crops**

F. MILICIA and F. FAVILLI\*

*Ente di Sviluppo Agricolo della Calabria, 87100 Cosenza*

\**Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche*

*Sezione Microbiologia Applicata and Centro di Studio dei Microorganismi Autotrofi  
p.le delle Cascine 27, 50144 Firenze (Italy)*

Tel. 39 55 352051, Fax 39 55 330431

Received November 20, 1991; Accepted February 27, 1992

### **Abstract**

Experiments were carried out in field plots to evaluate the effects of *Azolla filiculoides* biomass and ammonium sulphate at quantities of 100 and 200 kg N ha<sup>-1</sup> and a combined application of the two at 50 and 100 kg N ha<sup>-1</sup> of the each as biofertilizer for tomato. *Azolla* biomass can totally substitute inorganic nitrogen without affecting crop productivity and quality.

Keywords: *Azolla*, biofertilizer, tomato cultivation

### **1. Introduction**

The water fern *Azolla* lives in a symbiotic relationship with a diazotrophic cyanobacterium, *Anabaena azollae*. Because of the high productivity and nitrogen content of its biomass, this association has been used as a biofertilizer for centuries in South East Asia, mainly in rice cultivation (Lumpkin and Plucknett, 1980; Roger and Watanabe, 1986; Whitton and Roger, 1989; Van Hove, 1989).

Research programmes dedicated to the cultivation and utilization of *Azolla* in rice farming systems were started in the mid-1970's, in Italy (Margheri et al., 1979). They confirmed the biofertilizing potential of *Azolla* for the rice and thus opened the way to studies on the utilization of the *Azolla* biomass as biofertilizer for vegetable garden crops (Favilli et al., 1988a; 1988b).

The management of vegetable gardens is characterized by the planting of a succession of different crop species over a period of a year, thus, the *Azolla* biomass is utilized as it is produced. The purpose of this paper is to report on the use of the local strain of *Azolla filiculoides* biomass in the form of green manure for tomato.

## 2. Materials and Methods

In 1990, studies were carried out on a farm of the experimental center of the ESAC (Ente di Sviluppo Agricolo per la Calabria) located in Sibari (Southern Italy). *Azolla filiculoides* biomass was produced in field level ponds of 500 m<sup>2</sup> (Favilli et al., 1988a).

Tomatoes, of the local cultivar "Mypeel 244" were grown in 21 field plots of 50 sq metres each. The *Azolla* biomass was incorporated 15 days before the transplanting of 15-day-old tomato seedlings. Each plot contained 80 tomato seedlings, 35 cm apart, in two parallel rows. Six treatments using nitrogen fertilization, (100 and 200 kg N ha<sup>-1</sup>) were applied in the form of *Azolla* biomass (6 plots), ammonium sulphate (6 plots), or a combination of the two (6 plots). Three control plots without nitrogen were also arranged. Phosphorus and K were applied respectively as superphosphate and potassium sulphate at levels of 80 kg P<sub>2</sub>O<sub>5</sub> and 40 kg K<sub>2</sub>O per hectare. The *Azolla* biomass incorporated had a N content of 3.3%, based on dry weight. Each treatment was repeated 3 times.

At harvest, total crop production and the percentage of ripe, unripe and damaged fruits were calculated for each experiment. A fruit sample of 3 kg, collected from 6 randomized plants of each plot, was utilized for the quantification of the following commercial and chemical characteristics: pH; total acidity expressed as % of citric acid; reducing sugars; total dry residue. All results are the mean values of the three experiments.

## 3. Results

The comparative effects of *Azolla* and ammonium sulphate applications on tomato yield are given in Fig. 1. All the treatments caused an increase in tomato yield while no significant differences were observed in any of the listed

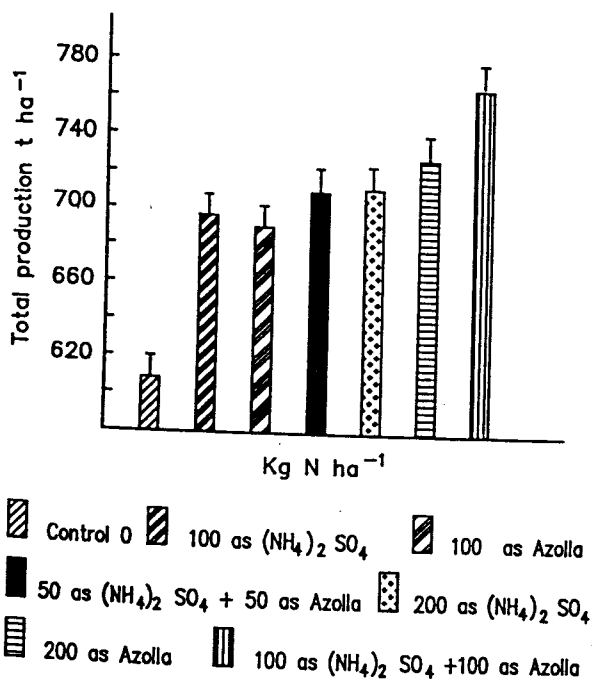


Figure 1.

above fruit chemical characteristics of the crop with the exception of total dry residue. Using 100 kg N ha<sup>-1</sup>, applied in the form of *Azolla* or ammonium sulphate alone or as the combined application of 50 kg N ha<sup>-1</sup> of each, tomato yield increases were 13.3, 14.4 and 16.9% respectively when compared to the controls. No significant differences among the yields were observed as a consequence of the different nitrogen sources.

By doubling the applications to 200 kg N ha<sup>-1</sup> (*Azolla* or ammonium sulphate, and a combined application of the two of 100 kg N ha<sup>-1</sup>), the yields increased to 21.2, 17.1 and 25.8%, respectively. At this N level the different nitrogen sources caused more marked differences in the yields. Moreover, the combined application of *Azolla* and ammonium sulphate N was more effective than each by itself. Interestingly, the use of *Azolla* appears to advance the ripening of fruit by about 10 days and results in an average 8% reduction of damaged fruits. Most of the chemical characteristics of the crop did not differ significantly from controls, i.e., pH range was 5.22 to 5.31; total acidity from 0.65 to 0.81% as citric acid; reducing sugar from 3.09 to 3.21%. Only the values of the total dry residue differed markedly, ranging from 3.76 (control) to 5.05% for combined application of *Azolla* and ammonium sulphate of 100 kg N ha<sup>-1</sup> of each.

#### 4. Discussion

The present experiments indicate that tomato yield in Southern Italy are higher in the presence of *Azolla* biomass. Other experiments have shown that other biofertilizer applications such as cyanobacterial biomass and *Azospirillum*, also enhanced tomato yields (Kaushik and Venkataraman, 1979; Rodgers et al., 1979; Bashan et al., 1989). Even in the case of naturally highly fertilized soils, such as in Sibari where crop productivity without N fertilizer has been 60.8 t ha<sup>-1</sup>, the positive effects of *Azolla* application were noted. Indeed, the combined application of *Azolla* biomass and ammonium sulphate was more effective than the application of either *Azolla* or ammonium sulphate alone. The marked effects of the *Azolla* application (alone or combined with ammonium sulphate) on the tomato yield, indicate that *Azolla* does not act simply as a nitrogen source. There are presumably other factors, such as organic matter increase which improves the chemical and physical characteristics of the soils. In addition, the use of *Azolla* could be beneficial to the crop by providing growth stimulating compounds produced by the endosymbiotic cyanobacterium. Such compounds are also actively released into the soil by living cells or after cell death or lysis. Several authors have observed the phytostimulating effects of biologically active substances produced by living photosynthetic microorganisms (Kobayashi et al., 1973; Florenzano et al., 1978; Sing, 1979; Balloni et al., 1986). Although data concerning stimulative effects of cyanobacteria and *Azolla* on rice are available (Venkataraman and Neelakantan, 1967; Liu, 1979; Sing, 1988), little is known about the response of other crops to cyanobacteria and *Azolla* application (Dadhich et al., 1969; Rodgers et al., 1979; Ferrera-Cerrato and Romero, 1982). Our experiments indicate that *Azolla* can be applied successfully as a biofertilizer with crops other than rice even in countries where agriculture is already intense and the cost of labor for the production of *Azolla* and its application would be high. The economic benefits of *Azolla* use could be estimated both as reduction in the cost of the nitrogen fertilizers and on environmental protection as a consequence of the lower requirement of chemical inorganic nitrogen.

#### Acknowledgements

This work was supported by a grant from the ESAC (Ente di Sviluppo Agricolo per la Calabria) Cosenza, Italy.

## REFERENCES

- Balloni, W., Giovannetti, L., Margheri, M.C., and Favilli, F. 1986. Interazioni fisiologico-biochimiche nella successione tra alcune specie di batteri fotosintetici anossigenici e ossigenici. *Ann. Microbiol.* **36**: 103-113.
- Bashan, Y., Ream, Y., Levanony, H., and Sade, A. 1989. Non specific responses in plant growth, yield and root colonization of non cereal crop plants to inoculation with *Azospirillum brasilense* Cd. *Canad. J. Bot.* **67**: 1317-1324.
- Dadhich, K.S., Varma, A.K., and Venkataraman, G.S. 1969. The effect of *Calothrix* inoculation on vegetable crops. *Plant and Soil* **35**: 377-379.
- Ferrera-Cerrato, R. and Romero, A.M. 1982. Propagation of an *Azolla* sp. and its potential as green manure for corn in Mexico. In: *Biological Nitrogen Fixation Technology for Tropical Agriculture*. P.H. Graham and S.C. Harris, eds. Centro International de Agricultura Tropical, Cali, Colombia, pp. 561-564.
- Favilli, F., Milicia, F., Balloni, W., Vincenzini, M., and Materassi, R. 1988a. *Azolla* biomass utilization as green manure in paddy fields in Southern Italy. In: *Proceedings of the First International Symposium on Paddy Soil Fertility II* Chang May, Thailand, pp. 841-846.
- Favilli, F., Taruntoli, S., and Balloni, W. 1988b. La biomassa di *Azolla* come biofertilizzante per frumento, girasole e mais. *Ann. Microbiol.* **38**: 109-121.
- Florenzano, G., Balloni, W., and Materassi, R. 1978. Algal organic matter and plant growth. *Zbl. Bakt. II. Abt. Bd.* **133**: 379-384.
- Kaushik, B.D. and Venkataraman, G.S. 1979. Effect of algal inoculation on the yield and vitamin C content of two varieties of tomato. *Plant and Soil* **52**: 135-137.
- Kobayashi, M. and Tchan, Y.T. 1973. Treatment of industrial waste solutions and production of useful by-products using a photosynthetic bacterial method. *Water Research* **7**: 1219-1224.
- Liu, C.C. 1979. Use of *Azolla* in rice production in China. In: *Nitrogen and Rice*. International Rice Research Institute. Los Banos, Philippines, pp. 375-394.
- Lumpkin, T.A. and Plucknett, A.I. 1980. *Azolla*: botany, physiology, and use as a green manure. *Economic Botany* **34** (2) 111-153.
- Margheri, M.C., Materassi, R., Balloni, W., and Paoletti, C. 1979. Coltura massiva di *Azolla caroliniana*: prime esperienze in Italia ed importanza agronomica del processo. *Agricoltura Italiana* **108**: 199-210.
- Rogers, G.A., Bergman, B., Henricksson, E., and Udriș, M. 1979. Utilization of blue-green algae as biofertilizers. *Plant and Soil* **52**: 99-107.
- Roger, P.A. and Watanabe, I. 1986. Technologies for utilizing biological nitrogen fixation in wetland rice: potentialities, current usage and limiting factors. *Fertilizer Research* **9**: 39-77.
- Sing, P.K. 1979. Use of *Azolla* in rice production in India. In: *Nitrogen and Rice*. International Rice Research Institute, Los Banos, Philippines, pp. 407-419.
- Sing, P.K. 1988. Biofertilization of rice crop. In: *Biofertilization Potentialities and Problems*. S.P. Sena and P. Palit, eds. Calcutta: Plant Physiology Forum, pp. 109-114.

- Van Hove, C. 1989. *Azolla* and its multiple uses with emphasis on Africa. Food and Agriculture Organization of the United Nations. FAO M/T6630E/1/10.89/2000, Rome, Italy.
- Venkataraman, G.S. and Neelakantan, S. 1967. Effects of the cellular constituents of the nitrogen-fixing blue-green alga *Cylindrospermum muscicola* on the root growth of rice plants. *J. Gen. Appl. Microbiol.* **13**: 53-62.
- Whitton, B.A. and Roger, P.A. 1989. Use of blue-green algae and *Azolla* in rice culture. In: *Microbial Inoculation of Crop Plants*. R. Campbell and R.M. Macdonald, eds. Society for General Microbiology, Vol. 25. IRL Press at Oxford University Press, Oxford, pp. 89-100.