

## Influence of Leaf Leachate of *Shorea Robusta* on Growth and Nitrogen Fixing Capacity of Hybrid *Azolla*

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### Abstract

The leachate of young, mature and senescent leaves of *Shorea robusta* showed growth promotion of hybrid *Azolla* (*Azolla pinnata* × *Azolla microphylla*). The chlorophyll, total nitrogen, phosphorus contents and the heterocyst frequency of *Anabaena azollae* in hybrid *Azolla* were highest with leaf leachate of mature leaves whereas lowest with leachate of senescent leaves.

**Key words :** Leaf leachate, *Shorea robusta*, Hybrid *Azolla*.

Good quantity of inorganic and organic substances from different parts of plants, particularly from leaves are leached down due to the action of rain, dew, mist and fog (1). The effect of leaf leachate of the overhead trees may be either promotive or inhibitive to the growth of underneath ground species (2, 3). *Azolla* is a free floating fern that prefers partial shade for its sustained growth throughout the year. It has large potential as a biofertilizer in the rice culture and is being used in many countries due to its symbiotic nitrogen fixation capacity by *Anabaena azollae* living within *Azolla*. There is a large scope to identify the plant species that are promotive to the growth of hybrid *Azolla* sp. The object of the study was to analyze the effect of leaf leachate of *Shorea robusta* on the growth and nitrogen fixation of hybrid *Azolla*.

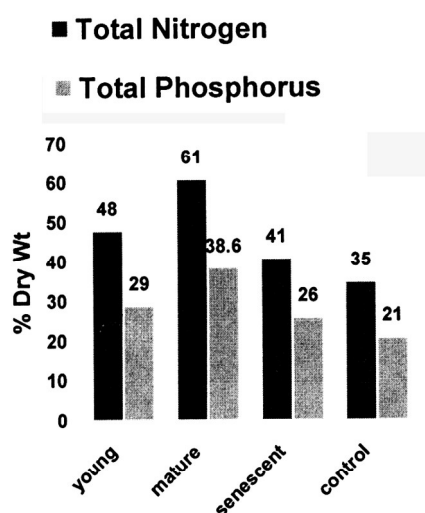
### Methods

Fresh leaves of *Shorea robusta* were collected

from Ramanabagan Deer Park, Golapbag, Burdwan. Three different kinds of leaves were collected such as young (15 to 20 days of age), mature (40 to 50 days) and senescent (showing sign of chlorosis). Leachate from these leaves was prepared by immersing the leaves of about 250 cm<sup>2</sup> area in 250 ml double distilled water for 24 hour as recommended by Tukey and Morgan (1). Hybrid *Azolla* plants (*Azolla pinnata* × *Azolla microphylla*), before incubation into the leaf leachate solution were disinfected with 0.1% HgCl<sub>2</sub> solution for 30 seconds, washed several times with distilled water and dried. In each experimental set, 2 g of biomass was inoculated into 150 ml of leaf leachate solution prepared from young, mature and senescent leaves. The whole set up was kept under partial shade which was covered with two opaque polythene sheets. The experiment was conducted during August–September, 2009. The average light intensity ranged from 70–100 w/m<sup>2</sup> and the temperature range from 22–34 C during August–

**Table 1.** Effect of leachates of different aged leaves of *Shorea robusta* on the growth of hybrid *Azolla*.

Source of leaf leachates	Fresh weight (g)	Increase in fresh weight over control (%)	Dry weight (g)	Increase in dry weight over control (%)	Chlorophyll content (mg/g dry wt)	Heterocyst frequency (%)
Young	2.35	48.73	0.25	78.57	4.25	27.25
Mature	2.48	56.96	0.31	121.42	4.83	29.76
Senescent	2.03	28.48	0.21	50	3.85	24.77
Control	1.58		0.14		2.55	22.67



**Figure 1.** Effect of leaf leachate of *Shorea robusta* on the total nitrogen and phosphorus content of hybrid *Azolla*.

September. After several days of culture, fresh weight and heterocyst frequency of *Azolla* were recorded. The fresh *Azolla* plants (0.5 g) were used to determine the chlorophyll content (4) while heterocyst frequency of *Anabaena azollae* was determined by method recommended by Konar and Kushari (3). Dried *Azolla* served in the determination of total phosphorus content (5) and estimation of the total nitrogen (6). The experiments were repeated thrice and the data were statistically analyzed.

### Results and Discussion

The leachates of young, mature and senescent leaves showed a wide variation on the growth rate of hybrid *Azolla* species. The leachate of mature leaves of *Shorea robusta* increased the growth maximum followed by young and senescent leaves (Table 1). Leaf age was a determining factor in this regard. Leachates from mature leaf had greater promoting effect than those of young leaves. The differential activity of maturity of leaves on growth and nitrogen fixation of hybrid *Azolla* might be explained for the availability of nutrients in excess of the requirement. Generally young actively growing tissue losses only small quantities of nutrients, minerals and carbohydrates whereas mature tissues are susceptible to leaching (7).

The chlorophyll content showed maximum with leaf leachate of mature leaf compared to control whereas least response was noted with senescent leaves (Table 1). There was a positive and significant effect on the heterocyst frequency of the symbiont *Anabaena azollae* by leaf leachate treatment of young, mature and senescent leaves while maximum increase was found the treatment of mature leaves. This might be explained that the promotive nutrients were available in sufficient amount of the leachates. The changes in chlorophyll content of the phytomass reflected both the effects of leaf leachates and environmental factors on growth of *Azolla* as pointed out by Leith (8).

Maximum nitrogen content was achieved by the mature leaves of *Shorea robusta* and least response was noted with the senescent leaves (Fig. 1). Same trend was noted in case of phosphorus content. The phosphorus content in hybrid *Azolla* was significantly increased by the leaf leachate of mature leaves whereas least response was observed with the leaf leachate of senescent leaves (Fig. 1). The obvious reason for distinct increase may be due to presence of growth promoters in the leachates. Leachate from young leaves had less promotive influence than mature leaves. Konar and Kushari (3) found that diosgenin content of *Costus speciosus* was highest with leachate of mature leaves of *Mangifera indica* and lowest with senescent leaves. Generally young leaves were hydrophobic and become wet with difficulties and had less leaching.

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