A STUDY ON THE CONDITIONS FAVOURING SYMBIOTIC ASSOCIATION OF *CROTOLARIA JUNCEA* WITH *RHIZOBIA*

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ABSTRACT: Some factors which influence the nodulating capacity of Sunn hemp were examined in order to obtain the maximum benefit of growing Sunn hemp as a rotation crop to improve the fertility of soil.

Ammonium nitrogen depressed plant growth more compared to nitrate and urea severely affected the nodulating capacity under laboratory conditons. Low concentration (50 ppm) of the above three forms of combined nitrogen improved the growth of seedlings in seedling agar medium, while concentrations higher than 100 ppm affected plant growth which may be due to some toxic effect.

Optimum temperature for adsorption of *Rhizobia* to the root system was fouud to be 30°C The adsorption at 25°C and 37°C was less by 25% and 35% respectively to that of 30°C. The pH range from 6.0—7.0 was found to be favourable for rhizobial adsorption to the roots.

Out of the agrochemicals tested (Brassicol, Captan and Furadan at a concentration of 2500 ppm) seed germination was reduced to 50% by Furadan and to 25% by Captan. Brassicol had no effect on the germination of seeds. Growth of the plants and the nodule formation were not affected by the application of fungicides to the soil at field concentrations.

Introduction

Demand for a high crop yield has led to the application of large amounts of commercial fertilizers to agricultural soils. This is particularly true in the case of nitrogen since the application of fertilizers is still relatively inexpensive compared to the worth of increase in crop production. But the practice of nitrogen fertilization is a potential threat to environmental quality of which pollution of surface and subsurface water is of major concern. Leaching of nitrogen occurs mainly during the period of over optimal rate of application. The observation made by Legg and Meisinger (1982) led to the use of green manure more widely in the developing countries. They stated that 50% of the applied fertilizer nitrogen is taken up by the plant 25% remains in the soil in more or less stable forms and 25% is lost by different mechanisms.

At this point of view, Sunn hemp is becoming an increasingly important green manure in Jaffna. Some major factors which influence the nodulating ability of plants such as temperature, pH, nitrogen fertilization and the application of fungicides/nematicides on soil were studied, to get the maximum benefit of growing Sunn hemp as a green manure.

Materials and Methods

Effect of inorgarnic nitrogen

Inorganic nitrogen was used in three different forms as nitrate N (KNO_3), ammonium N $(NH_4)_2SO$ and urea $CO(NH_2)_2$. Experiment was carried out on seedling agar medium incorporated with 50, 100, 150 ppm of nitrogen in the three different forms as mentioned above.

Seeds of *Crotolaria juncea* were grown on water agar plates and healthy seedlings were transferred to the plants three days after germination. Two ml of the rhizobial inoculum (1.3×10^8 cells/ml) was added to each slant after the establishment of seedlings on the slants. Required amount of distilled water was added throughout the experimental period. Dry weight of the shoots was measured four weeks after inoculation and the nodulating ability was assessed.

Effect of Temperature and pH

The adsorption assay technique (Pueppke 1984) was employed to find out the effects of temperature and pH. Ten ml portions of the bacterial inocula (10^4–10^5 cells/ml) were maintained at the appropriate temperature and pH values in order to equilibrate them. Seedlings of *Crotolaria juncea* were aseptically transferred to the inocula and maintained for one hour. After the the incubation period, root system were rinsed in rapidly flowing Jensen's nitrogen free nutrient solution and the number of rhizobia adsorped to the root system was counted by pour plate technique.

Effects of Agrochemicals

Seeds of Sunn hemp were dipped in the agrochemicals (Brassicol–Pentachloro nitrobenzene; Captan–N–trichloromethyl mercapto–4 cyclohexene–1, 2 dicarboximide; Furadan–Carbofuran at a concentration of 2500 ppm) for a minute and allowed to germinate on moist filter papers in petri dishes at room temperature. Percentage gemination was calculated after the period of two days.

Polythene bags (30 × 12 cm) were filled with untreated soil samples collected at 15 cm depth from a field at Thirunelvely. The upper 10 cm portions of the bags were filled with the soil treated with agrochemicals according to the recommended dosage (450–560 l of 2500 ppm/ha). Eight seeds were sown in each bag and the seedlings were thinned to four per bag during the first week of growth. Five replicates were maintained. Dry weight of shoot system and fresh weight of nodules/plant were measured during the seventh week of the growth of plants.

Results and Discussion

Plants showed better growth and nodulation when supplied with the combined nitrogen in the form of nitrate nitrogen (Table 1). Low concentrations of all the forms of combined nitrogen improved the growth of seedlings compared with the control plants while high concentrations seemed to affect root growth markedly in the seedling agar medium and thereby reduced the nodulating capacity. Higher concentrations may have toxic effect on young seedlings.

Plants were well nodulated in 50 ppm NO^-_3–N, 50 ppm NH_4–N, and in 100 ppm of NO_3†–N while, a few nodules were observed in the plants treated with 100 ppm $NH_{†4}$–N. The plants in other treatments failed to nodulate and the root growth was affected very much in 150 ppm–N in all treatments.

Investigations made by Chaillou *et al.* (1986) are in agreement with the results obtained in the present study i.e. ammonium nitrogen nutrition is known to depress plant growth compared with nitrate nitrogen nutrition.

Table 1. Effect of different forms of inorganic nitrogen at different concentrations on the dry matter production of Sunn hemp

Form of Inorganic Nitorgen	Mean dry weight of shoot (g)		
	50 ppm	100 ppm	150 ppm
	Inorganic Nitrogen		
Ammonium nitrogen (NH_4+)	1.666 ±0.04	0.860 ±0.02	0.960 ±0.02
Nitrate nitrogen (NO_3 –)	1.700 ±0.01	1.230 ±0.03	0.719 ±0.06
Urea $CO(NH_2)_2$	1.150 ±0.01	0.672 ±0.03	0.632 ±0.02
Control	1.004±0.007		

This was also proved in plants like tomato, mustard and buck wheat (Coic *et al.* 1961). Growth improvement shown in the lower concentrations of inorganic nitrogen also supported the study of Evans (1982). He indicated that the low concentrations of combined nitrogen had little or no effect on the normal nodulation of the plants. Urea also showed severe indication of nodulation under field conditions (Arudchandran 1989) indicating that if these plants are grown in an area where the soil is heavily fertilized with nitorgen previously, then the symbiotic activity gets affected drastically. Therefore, it is advisable to use *Crotolaria juncea* as a rotation crop where there is less combined nitrogen.

The optimum temperature for nodulation was found to be 30°C (Table 2). Rhizobia adsorption to the root was reduced to 35% by high temperature (37°C) and 25% by low temperature. The observation made by Pueppke (1984) showed that in soybean 90% reduction was in low temperature (4°C) and 65% by elevated temperature (37° C). When considering the elevated temperature, the reduction was less in the present study. This may be due to the adaptation of rhizobia strains to the tropical climate where the temperature fluctuation is less. Even though the mode of action of the effect of temperature is unknown, rhizobia differ in their survival and nodulating ability with temperature changes.

Table 2. Number of rhizobia adsorped to the root system of the seedlings of Sunn hemp kept in Jensen's nitrogen free nutrient solution at different temperatures

Temperature (°C)	Number of colonies/YMA plate
25	93±16.3
30	144±20.1*
37	109±10.8*

Values followed by * are significant: $p=0.05$

The pH range from 6.2–7.2 seemed to be favourable for the symbiotic association of *Rhizobium* sp. with *Crotolaria juncea* (Table 3). But pH 8 had an adverse effect on adsorption compared to pH 5.7 which is slightly acidic. Field assessment coincided with the laboratory result (Arudchandran and Theivendirarajah 1987), where nodulating ability of Sunn hemp was significantly retarded in soil of pH 8.

Table 3. Number of rhizobia adsorped to the root system of the seedlings of *Cortolaria juncea* kept in Jensen's nitrogen free nutrient solution at different pH values

pH value of nutrient solution	Number of rhizobia/YMA plate (Mean value of 4 plates)
5.7	127±20.6
6.2	243±50.3
6.7	172±30.7
7.2	149±27.3
8.0	72±20.1*

Values followed by * significant: p = 0.05

Seed germination was reduced nearly by 50% due to the application of Furadan; Captan reduced germination by 25% and Brassicol had no effect on seed germination.

According to Table 4, Brassicol improved the growth of the plants. Even though the improvement was not statistically significant, there was a marked increase when compared to the control. The weight of of the nodules also increased by the application of agrochemicals. Staphorst and Strijdom (1976) showed that the nodulation was improved by the application of DDT. As stated by Aggarwal (1986), the sensitivity of nodulation to agrochemicals depended on the species of *Rhizobium* as well as on the type of plant and the chemical substance. Therefore, when the above three chemicals are previously applied to soil to reduce the fungal/nematode activity, high seedling density of Sunn hemp is essential in order to obtain maximum benefit.

Table 4. Dry weight of shoot, nodule formation and % germination of *Crotolaria juncea* grown in soil treated with agrochemicals

Treatments	Germination of seeds (%)	Dry weight of shoot(g)/plant	Fresh weight of nodules(g)/plant
Water	100	0.865±0.07	0.062±0.001
Brassicol	100	0.763±0.09	0.097±0.005
Captan	77.0	0.321±0.01	0.070±0.005
Furadan	56.6	0.592±0.11	0.095±0.006

n = 20

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