EFFECT OF ADDITION OF PHOSPHORUS ON THE GROWTH OF AZOLLA

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ABSTRACT

Studies on the exploitation of organic manures, especially those with the ability to fix atmospheric nitrogen, are considered very important owing to the increasing cost of chemical fertilizers manufactured by the use of non renewable sources of energy. In this study the effect of addition of concentrated superphosphate on the growth and nitrogen content of two strains of *Azolla pinnata* was determined under field conditions.

With an initial inoculum rate of 0.5t/ha. addition of 12 to 15kg P_2O_5/ha/week yielded about 11.5 tonnes fresh weight per hectare containing about 30kg N within a two week growth period. The percent nitrogen content in Azolla tissue increased linearly with the addition of phosphorus.

Only a very small fraction of the P added to the flood water was taken up by Azolla. As the rest of the phosphorus may not remain in the flood water for long, its fate should be studied in order to find out whether it could be of use to another crop grown on the same field.

The plants in some of the experimental plots developed a red colour which was most intense at lower rates of applied phosphorus.

INTRODUCTION

To overcome the burden caused by costly nitrogenous fertilizers as well as to ensure the maintenance of soil fertility, scientific investigations on many biological nitrogen fixing systems have significantly increased during the last ten years. On this aspect great interest was drawn towards the Azolla-Anabaena association, and its potential is now well documented (3, 5, 6, 8, 9, 11, 12).

Anabaena azollae, the specific heterocystous blue-green algae which is found living symbiotically within the leaves of the tiny water fern Azolla is the actual agent of nitrogen fixation (1, 5, 6, 7, 12). Azolla is found growing in the natural environment in many tropical and temperate regions of the world. The species *Azolla pinnata* is found as a member of the natural flora in Sri Lanka (4, 5, 6).

A pre-requisite for the successful practical utilization of Azolla as a nitrogen rich organic fertilizer for rice is the availability of reliable information in all important aspects of its culture. In particular, information on its nutritional requirements is essential to improve its growth in paddy fields. Phosphorus has been repeatedly seen to be one of the most critical nutrient elements for Azolla. Rapid propagation is brought about by adding phosphorus fertilizer (8, 9, 10, 13). A deficiency of this nutrient element causes reduced growth and a colour change in the fronds from green to a brownish or dark red (5, 9, 10, 12).

A knowledge of the optimum phosphorus requirement of Azolla would be useful in the rapid propagation of this plant under field conditions. This may vary under different soil conditions as well as with different strains and species of Azolla. This study was undertaken to find out the effect of different levels of P on two strains of *Azolla pinnata* in a paddy field at the Central Agricultural Research Institute, Gannoruwa, Peradeniya, in the mid country wet zone of Sri Lanka.

MATERIALS AND METHODS

A strain of *Azolla pinnata* originally collected from Bangkok, Thailand, (obtained from the Department of Microbiology, International Rice Research Institute, Philippines) and another strain of the same species collected from the irrigation reservoir Debokkawa Wewa located in the south west of Sri Lanka, were used in this study. These strains were initially multiplied in the greenhouse and then transferred for further multiplication into nursery plots 12m $\times$ 5m prepared in the field. Powdered concentrated superphosphate was added to these plots at the rate of 100kg P_2O_5/ha/week and carbofuran was added at the rate of 2kg ai/ha/week for pest control.

The two Azolla strains were combined factorially with four levels of phosphorus namely, 0, 4.16, 8.33 and 16.66kg P_2O_5/ha/week (referred to as levels 0, 1, 2 and 3 respectively) in a randomized complete block design with three replicates. The blocks ran parallel to each other separated by a 60cm wide canal which was used to irrigate the plots. Each plot was 2m×2m. The water level in the plots was maintained at 8—10cm deep. The soils of the experimental area had a pH of 5.6 and a bicarbonate extractable phosphorus content of 15 ppm.

Azolla collected from the nursery plots was washed, drained of water for about 45 minutes and added to the plots at the rate of 0.5 tonne fresh weight per hectare (200 g/plot). The appropriate amount of phosphorus and carbofuran at the above rates were added to the flood water at the beginning of the experiment and at weekly intervals thereafter.

At the end of two weeks the extent of reddening of the Azolla fronds was determined by placing a 12cm×12cm frame at two random points in each plot and visually estimating the red area within it as a percentage of the total. Following the colour assessment the Azolla was harvested, allowed to drain for about 45 minutes and the fresh weight determined. A 200 g sample of the Azolla harvested from each plot was put back to the same plot as inoculum for the second growth period. The weekly dose of phosphate and carbofuran was added and the plants were allowed to grow for two more weeks. The experiment was conducted in this way for a total of six weeks, harvesting three crops of Azolla.

A sample from the harvested Azolla from each plot at the end of the first and third crops was taken for N, P and K analysis. It was dried to constant weight in an oven at 70°C. The total N of this material was determined by micro Kjeldahl method. The plant samples were digested with a mixture of nitric, sulphuric and perchloric acids, and the P content determined by the vanado molybdate method and the K content by flame photometry.

The percent utilization of phosphorus by Azolla for a specific level of added P was calculated from the following expression.

$$\% \text{ P utilized} = \frac{A - B}{C} \times 100$$

Where A is the amount of P taken up by Azolla on addition of concentrated superphosphate, B is the amount of P taken up from plots to which no P was added, and C quantity of fertilizer phosphorus added.

The data of the colour assessment of Azolla expressed in percentages were transformed using Arcsin technique. Transformed data were subjected to statistical analysis.

RESULTS AND DISCUSSION

The fresh weight production of both Azolla strains showed a highly significant quadratic response to addition of phosphorus (Figure 1). The highest quantity produced during a two week period by Bangkok strain ranged from 9.0 to 11.5 t/ha and by Debokkawa strain from 6.4 to 8.8 t/ha. These quantities were obtained to the addition of about 12 to 15kg P_2O_5/ha/week. Bangkok strain showed a significantly higher yield than Debokkawa strain at any level of added phosphorus for all three crops. The ability of Azolla to multiply rapidly in the presence of phosphorus is seen from these results.

The percent nitrogen content in Azolla tissue increased linearly with the addition of phosphorus (Figure 2). In the first crop the two strains were not significantly different with regard to % N content, but in the third crop the % N content of Bangkok strain was significantly higher than that of Debokkawa strain at all levels of P. Further, at any given level of P, the % N content in both strains was higher in the first crop than in the third crop. In fact the N content of the first crop at higher levels of added P approached the unusually high value of 6 percent.

The percent P content in Azolla plants also increased linearly with the addition of P (Figure 3). In general the phosphorus content of Bangkok strain was higher than that of Debokkawa strain for a given level of added concentrated superphosphate.

The amounts of N, P and K present in Azolla plants at different levels of added phosphorus fertilizer are given in Table 1. With the increase in levels of fertilizer P the quantity of N and P in Azolla increased, except at P level 3 for Debokkawa strain in the third crop. Since the surface water of a rice field contains only very small quantities of nitrogen, the effect of phosphorus in significantly increasing the nitrogen content of Azolla serves to provide some indirect evidence of its role in promoting N fixation by Azolla. As far as potassium is concerned, the addition of the first level of P led to a higher K uptake, but further additions did not show corresponding increases in a consistent manner.

The percent P utilized by both strains of Azolla is very low, ranging from 7 to 14 (Table 2). The remaining phosphorus must be found in flood water, in the soil or soil solution beneath it, or elsewhere. Since the phosphate added was in powder form and water soluble, it is likely that most of it was present in the flood water immediately after addition. However, if this continued to be present there, the second Azolla crop should have more P available to it than the first crop, and the third crop more P than the previous two crops. In such an event the shape of the three graphs in Figure 1 should not have borne such similarity to each other. In point of fact the Azolla fresh weight maximum should have shifted steadily to the left with each crop. The fact that it did not do so presents indirect evidence that added P did not accumulate in flood water in significant quantities. This observation is well supported by the results of Watanabe *et al* (13), where phosphorus concentration in flood water increased with the application of phosphorus fertilizer followed by a rapid decrease to attain the normal flood water phosphorus concentration within a matter of few days.

Phosphorus can get lost from a field in the water moving laterally and from percolation. The topographic position of the experimental field may have lent itself to some losses by the former process. In puddled rice fields percolation losses are usually small owing to the presence of a hard pan. Even if percolation losses are significant, phosphorus dissolved in the moving water has the opportunity to react with soil components on its downward path. Thus it is conceivable that the majority of P losses from the system may be limited to that in water moving laterally. The P which finds its way to the solum beneath the flood water may not be fully available to Azolla, but is likely to be of use to an accompanying or a following rice crop. These considerations have important implications in the Azolla-rice cropping system, in that the P added for Azolla may be considered as fertilizer for a rice crop. This point is sometimes overlooked in working out the economics of addition of P on the growth of Azolla. Quantitative studies on the fate of the added phosphorus are required.

Although no potassium fertilizer was used in this study, Azolla plants to which phosphorus was added removed about 3.5kg K/ha/week. In comparison a high yielding rice crop of four month growth period removes about 100kg K/ha. Assuming a uniform rate of K uptake throughout the rice crop, the weekly uptake of K is about 6 kg/ha. Thus as far as Azolla is concerned, it should not be taken for granted that the natural supply of K would be adequate to achieve maximum growth. Further, the cultivation of Azolla preceding a rice crop or its growth along with rice may lead to an insufficient supply of potassium to rice. If the Azolla is incorporated in the soil, its K is put back. But there is very limited information on how soon this K can become available to a rice crop under varying soil and hydrological conditions.

At the time of initial inoculation of Azolla plants into experimental plots, the plants were green in colour, but with time some of the plants turned reddish gradually. This colour change took place within 4 to 7 days after inoculation. In each Azolla plant the change from green to red started from the centre of the frond and spread to the periphery. The colour change occurred rapidly in treatments receiving zero and level 1 of added P. One week following inoculation of the first crop, a contrasting difference in colour between some treatments was observed. The plants receiving no P turned almost completely to crimson while the plants receiving P levels 1, 2 and 3 showed the reddening mainly in the centre part of the frond. A significant negative linear response was shown by the percent red in the Azolla cover of both strains to added P (Figure 4). These observations suggest that the reddening of Azolla seen during the course of this study is mainly an effect of P nutrition of the plants. Even though the selected sample area for this red colour estimation is small compared to the size of each plot, the even spread of the red colour over the Azolla cover in each plot during the experimental period reduced the chances for erroneous estimations. Studies of the nutrition of Azolla have often shown that P deficiency, besides limiting growth, causes reddening of plants (5, 9, 10, 12). The manifestation of the red colour at all levels of added P in the third crop of both strains was higher than that in the first crop. This suggests that besides P deficiency, some other factors may have been responsible in causing reddening in Azolla. In fact it has been reported that apart from P deficiency, light intensity and temperature play a role in the reddening of Azolla (4).

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Table 1. The amounts of N, P and K present in Azolla plants at different levels of added phosphorus.

Level of P	*First crop* N	*First crop* P	*First crop* K	*Third crop* N	*Third crop* P	*Third crop* K
	kg / ha					
			BANGKOK			
0	10.53	0.30	3.93	10.82	0.18	5.03
1	17.32	0.78	6.90	22.90	0.94	7.73
2	23.15	1.22	6.56	24.86	1.47	6.63
3	23.16	1.71	8.77	36.01	2.14	8.12
			DEBOKKAWA			
0	6.43	0.19	1.87	5.64	0.08	1.89
1	14.00	0.61	4.64	16.88	0.55	4.74
2	18.92	0.86	5.32	28.95	1.38	7.30
3	21.46	1.28	6.80	27.96	1.16	6.90

Table 2. Utilization of added P by Azolla in the first crop.

Level of P	*Utilization of P* %
BANGKOK	
1	13.22
2	12.65
3	9.69
DEBOKKAWA	
1	11.57
2	9.21
3	7.49

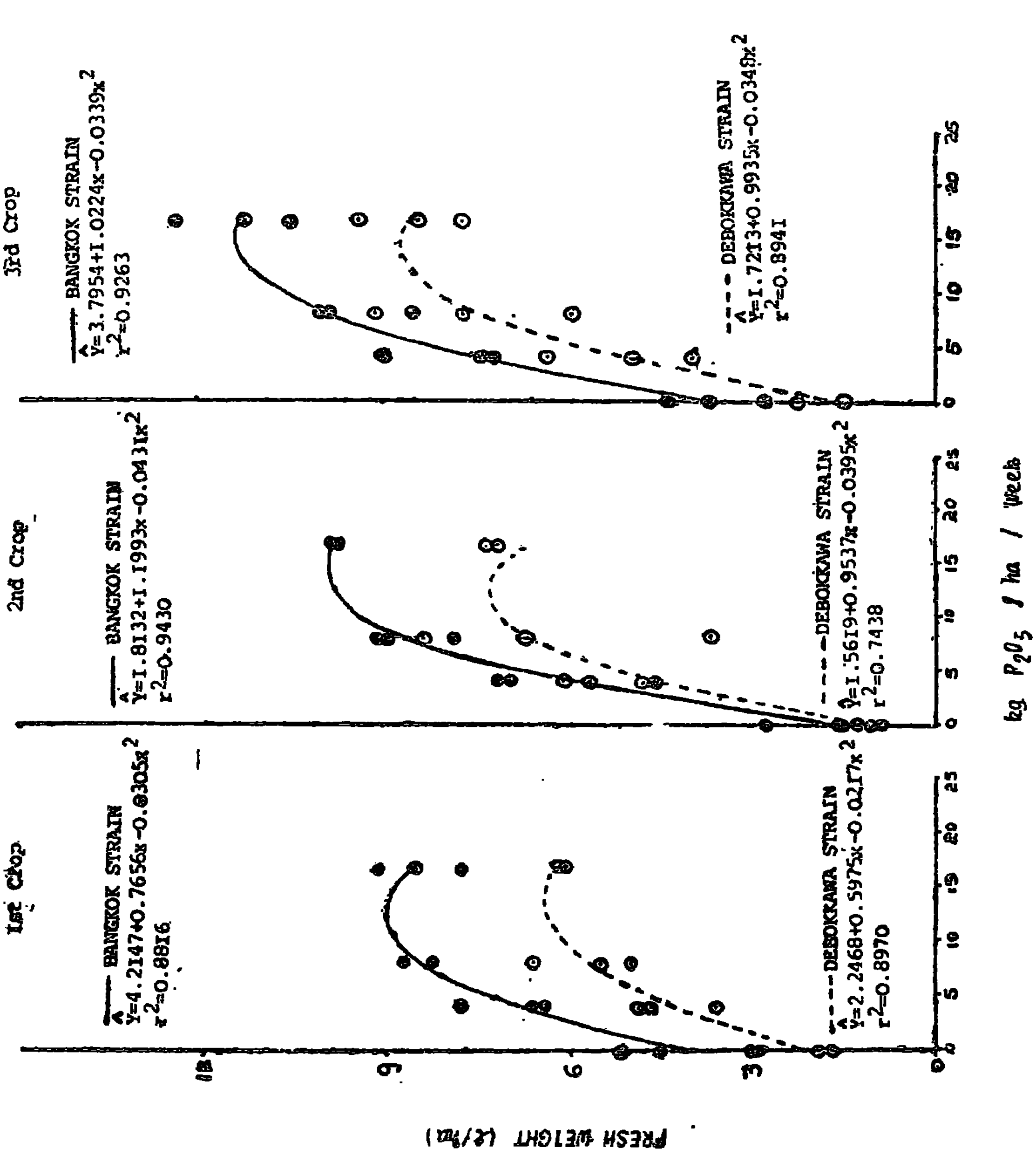

Figure 4. THE EFFECT OF ADDITION OF PHOSPHORUS ON FRESH WEIGHT OF AZOLLA.

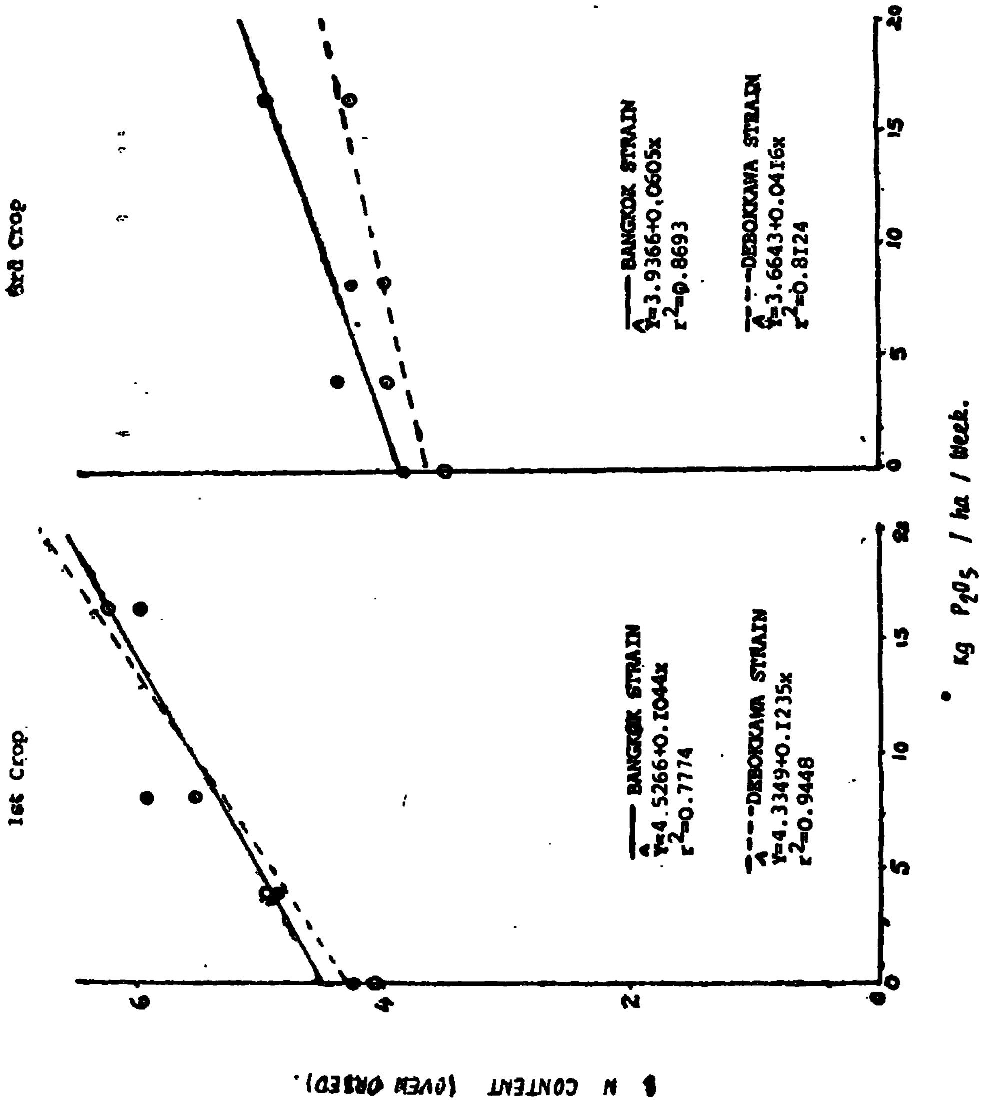

Figure 2. THE EFFECT OF ADDITION OF PHOSPHORUS ON NITROGEN CONTENT IN AZOLLA.

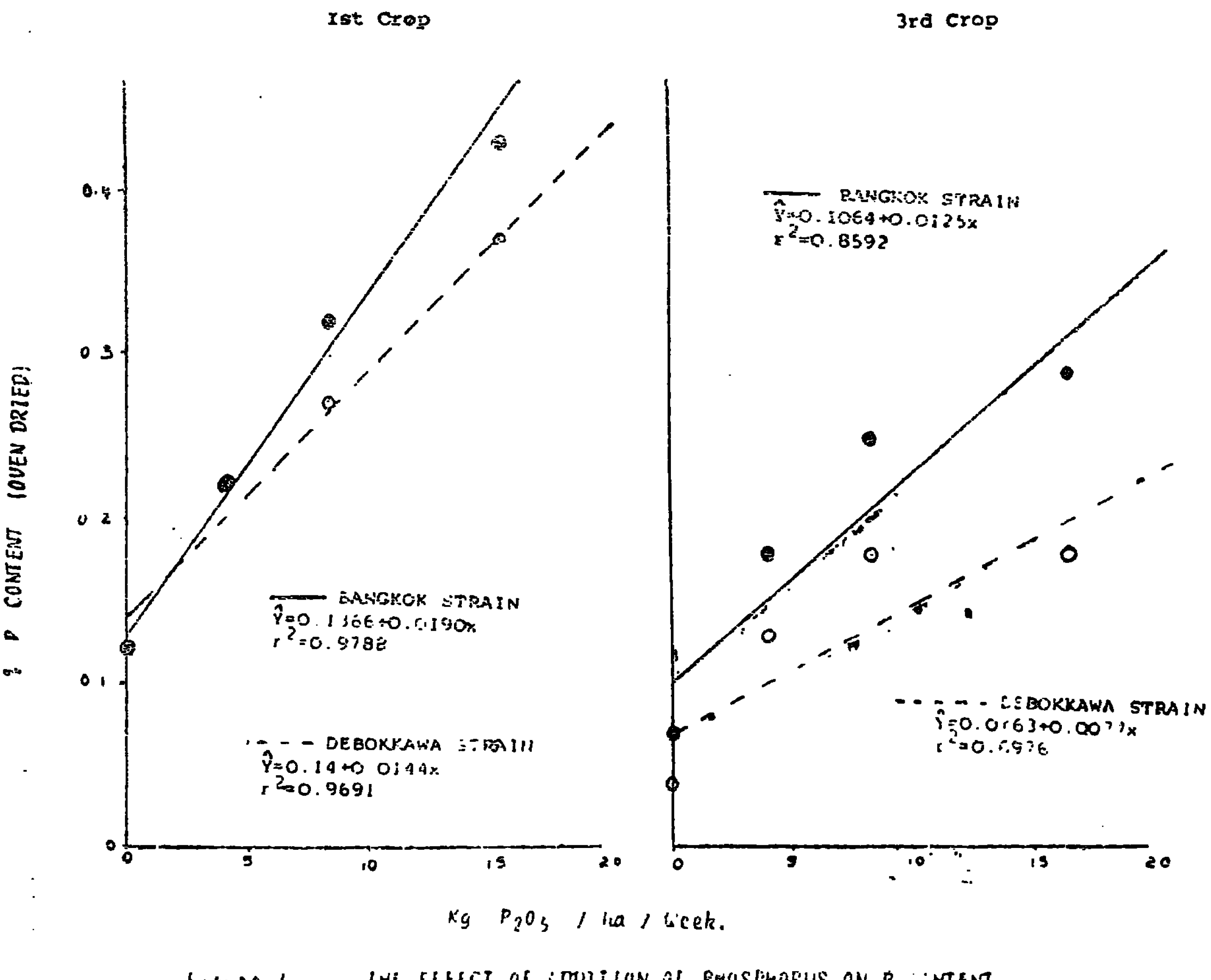

Figure 3. THE EFFECT OF ADDITION OF PHOSPHORUS ON P CONTENT IN AZOLLA.

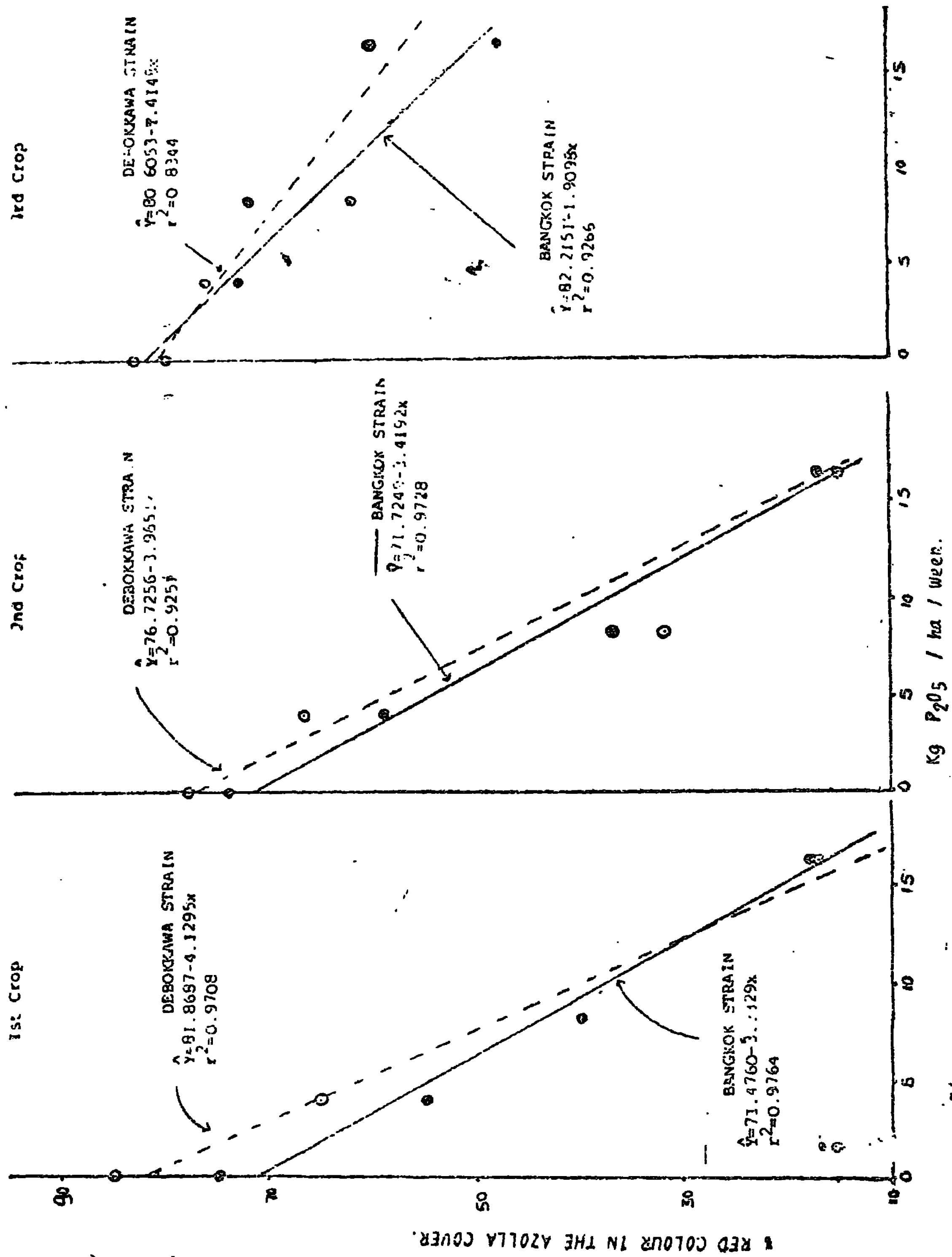

Figure 4. THE EFFECT OF ADDITION OF PHOSPHORUS ON REDDENING IN AZOLLA.