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INFLUENCE OF DIFFERENT PHOSPHORUS MANAGEMENT STRATEGIES ON THE SPORULATION AND GROWTH OF *AZOLLA*

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SUMMARY

Phosphorus at the recommended dose of 10 kg P_2O_5 ha^{-1} divided between three equal applications, one on each of 0, 7 and 14 d after *Azolla* inoculation (DAI), increased biomass production but sporulation was invariably inhibited. Of the different options tested, lowering the phosphorus application rate to 4–8 kg P_2O_5 ha^{-1} significantly improved sporulation in *A. microphylla* (strain 202) but there was a substantial reduction in biomass production. On the other hand, changing the schedule of phosphorus application from 0, 7 and 14 DAI to 0, 3 and 6 DAI did not hamper biomass production and improved sporulation frequency and sporocarp number in *A. microphylla* (strain 202), *A. caroliniana* and *A. pinnata*. It was comparable to the no-P treatment for the number of sporocarps, with slightly lower sporulation frequency. In these species, the sporulation frequency and sporocarp number of *Azolla* enriched with 30 or 60 kg P_2O_5 ha^{-1} and then grown without any further added phosphorus were higher than those of unenriched *Azolla* grown with 10 kg P_2O_5 ha^{-1} and mostly comparable to those of *Azolla* grown without phosphorus. Foliar spray of 2.5 μg ml^{-1} gibberellic acid (GA) solution (7 DAI) along with the application of 10 kg P_2O_5 ha^{-1} (split between 0, 7 and 14 DAI) to unenriched *Azolla* increased the sporulation frequency and number of sporocarps in *A. microphylla* (strains 202 and 203), *A. caroliniana* and *A. pinnata*, not only over that of phosphorus application alone but also over the untreated control. Combining the use of P-enriched *Azolla* (*A. microphylla* strains 202 and 203) with application of GA was more effective for increasing sporulation than was the use of P-enriched *Azolla* without GA, or application of phosphorus plus GA to unenriched *Azolla*.

INTRODUCTION

Integrated nutrient management through efficient use of chemical fertilizers, organic manures and biofertilizers is important for sustaining crop productivity. The water fern *Azolla* fixes atmospheric nitrogen in symbiotic association with the cyanobacterium, *Anabaena azollae*, and serves as a biofertilzer for rice (Watanabe and Liu, 1992; Singh and Singh, 1997). Its use is not gaining popularity among farmers, however, largely because of the cumbersome practice of inoculum production through vegetative propagation, coupled with the difficulty in transporting and distributing large amounts of fresh inoculum. That these problems can be overcome to a great extent by the use of sporocarps for *Azolla*

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culture has been successfully demonstrated (Shuying, 1987; Kannaiyan, 1994). *Azolla* is a heterosporus fern and produces both the female (mega) and male (micro) sporocarps on the same plant. The factors that induce sporulation are largely unknown. Sporocarps are generally formed during a particular part of the year, though some strains are capable of producing sporocarps throughout the year (Singh, 1989; Kar *et al.*, 1999). At Cuttack (India), sporulation occurs during November–March (Singh *et al.*, 1987).

The sporocarp yield in *Azolla* depends on the biomass production and the intensity of sporulation. Application of phosphorus at 10 kg P_2O_5 ha^{-1} is necessary for maximum biomass production (Singh and Singh, 1989) but inhibits sporulation significantly (Singh *et al.*, 1987). Phosphorus deficiency favours sporulation (Kannaiyan *et al.*, 1988). Thus, there is a need to develop phosphorus management strategies that ensure maximum biomass production with little or no adverse effect on sporulation, thereby leading to high sporocarp yields.

MATERIALS AND METHODS

Azolla *strains used*

Azolla microphylla strains 202 (from Dr T. A. Lumpkin, Washington State University, Pullman, USA) and 203 (from Dr S. Kannaiyan, Tamil Nadu Agricultural University, Coimbatore, India), *A. caroliniana* (from Dr G. A. Peters, Battle Kettering Research Laboratory, Ohio, USA) and *A. pinnata* (a local collection), were used in this study. *A. caroliniana* and *A. pinnata* sporulated only during November–March (favourable period for sporulation) while *A. microphylla* produced sporocarps throughout the year, though the sporulation frequency and number of sporocarps were much lower during the unfavourable period.

Cultivation of Azolla

Exponentially growing fresh *Azolla* with no sporulation was used as inoculum in all the experiments. In treatments other than those involving phosphorus enrichment, inoculum raised with 3.3 kg P_2O_5 ha^{-1} was used. *Azolla* was grown in earthenware pots of 25 cm diameter filled with 2.5 kg soil, using 3.0 g fresh inoculum per pot. The soil was a clay-loam (Haplaquept soil) with pH 6.5, organic carbon 0.78%, total nitrogen 0.09% and available phosphorus (Olsen P) 10 mg kg^{-1} soil. The pots were kept flooded to a water depth of 3–5 cm throughout the experiment. Furadan (3% a.i. as a carbofuran) at 15 mg pot^{-1} was applied as and when required to protect *Azolla* from insect pests. Phosphorus, through single superphosphate, was applied in three equal applications 0, 7 and 14 DAI, except in treatments where a specified schedule was followed.

Details of treatments and procedures

The experiments, carried out in a nethouse at the Central Rice Research Institute, Cuttack during the period November 1997 to March 1998, followed five

different approaches: (i) lowering the rate of phosphorus application, (ii) changing the schedule of phosphorus application, (iii) P-enrichment of *Azolla* inoculum, (iv) applying gibberellic acid (GA) along with phosphorus fertilizer and (v) combining the use of P-enriched *Azolla* inoculum with the application of GA. A completely randomized design with three replications was used for all the experiments. Details of each experiment are explained below.

Rate of phosphorus application. Phosphorus at 10 kg P_2O_5 ha^{-1} split between three equal applications, one on each of 0, 7 and 14 d after *Azolla* inoculation (DAI), has been recommended (Singh, 1989). To find out whether by lowering this rate of application the inhibition of sporulation due to phosphorus could be minimized without significantly reducing the biomass production, *A. microphylla* (strain 202) was grown with 0, 2, 4, 6, 8 and 10 kg P_2O_5 ha^{-1} (0, 9.8, 19.6, 29.4, 39.2 and 49.0 mg P_2O_5 per pot). Unenriched *Azolla* inoculum was used in this study.

Schedule of phosphorus application. *A. microphylla* (strain 202), *A. caroliniana* and *A. pinnata* were used in the experiment. Phosphorus at 10 kg P_2O_5 ha^{-1} (49 mg P_2O_5 per pot) split between three equal applications was applied according to three different schedules – 0, 3 and 6 DAI (S_1); 0, 5 and 10 DAI (S_2) and 0, 7 and 14 DAI (S_3). A treatment without added phosphorus (S_0) was included for comparison. Unenriched *Azolla* inoculum was used.

Phosphorus-enrichment of Azolla *inoculum*. *A. microphylla* (strain 202), *A. caroliniana* and *A. pinnata* were used in the experiment. Treatments included: a no-P control (T_1); 10 kg P_2O_5 ha^{-1} a third of which was applied to unenriched *Azolla* on each of 0, 7 and 14 DAI (T_2); and use of *Azolla* inoculum enriched with a total of 30 (T_3) and 60 (T_4) kg P_2O_5 ha^{-1} (147 and 294 mg P_2O_5 per pot). Phosphorus-enrichment was done by applying the phosphorus fertilizer to the *Azolla* nursery in four equal applications on alternate days, as suggested by Singh and Singh (1995). Thereafter, the P-enriched *Azolla* was grown without further applications of phosphorus.

Application of gibberellic acid along with phosphorus fertilizer. *A. microphylla* (strains 202 and 203), *A. pinnata* and *A. caroliniana* were used in the experiment. Treatment combinations were: no phosphorus and no GA; phosphorus at 10 kg P_2O_5 ha^{-1} again split between three equal applications (0, 7 and 14 DAI); foliar spray of 2.5 μg ml^{-1} GA solution 7 DAI (no added phosphorus); and foliar spray of 2.5 μg ml^{-1} GA solution 7 DAI in addition to 10 kg P_2O_5 ha^{-1} split between three equal applications 0, 7 and 14 DAI. Unenriched *Azolla* inoculum was used in this study.

Use of P-enriched Azolla *inoculum and application of gibberellic acid*. The experiment was conducted using *A. microphylla* strains 202 and 203. Treatment combinations were: phosphorus at 10 kg P_2O_5 ha^{-1} split between three equal applications (0, 7

and 14 DAI) to unenriched *Azolla*; use of *Azolla* inoculum enriched with 60 kg P_2O_5 ha^{-1}; foliar spray of 2.5 μg ml^{-1} GA solution 7 DAI in addition to the application of phosphorus at 10 kg P_2O_5 ha^{-1} applied as above 0, 7 and 14 DAI to unenriched *Azolla*; and use of *Azolla* inoculum enriched with 60 kg P_2O_5 ha^{-1} followed by foliar spray of 2.5 μg ml^{-1} GA solution 7 DAI. An untreated control with unenriched *Azolla* inoculum was included for comparison.

Measurement of Azolla *growth and sporulation*

On 40 DAI, *Azolla* from each pot was collected, washed carefully to remove the adhering soil, and its fresh weight was recorded after removing excess moisture. Then, 50 plants were selected randomly and the numbers of sporulating plants and mega- and micro-sporocarps on each plant counted. The sporulation frequency (%) and percentage of mega-sporocarps to that of total sporocarps were calculated. The sporulation frequencies were transformed using the Arc Sine $\sqrt{\text{percentage}}$ transformation. The data were statistically analysed and the least significant difference at $P > 0.05$ was used for comparison.

RESULTS

Rate of phosphorus application

The increasing rates of phosphorus application gradually increased *Azolla* (*A. microphylla* strain 202) biomass production and decreased sporulation frequency and sporocarp number (Table 1). A significant reduction in the sporulation frequency and number of sporocarps per plant was observed at and above 6 and 4 kg P_2O_5 ha^{-1} respectively. At the recommended dose of 10 kg P_2O_5 ha^{-1}, the biomass production increased to 2.70 times that of the no-P treatment, while the sporulation frequency and number of sporocarps decreased by 30.0 and 27.5% respectively. Lowering the dose from 10 to 8 kg P_2O_5 ha^{-1} enhanced the number

Table 1. Effect of rate of phosphorus application on the biomass (fresh weight) production and sporulation in *Azolla microphylla* (strain 202).

Phosphorus (kg P_2O_5 ha^{-1})	*Azolla* biomass (g per pot)	Sporulation frequency (%)	Number of sporocarps per plant			Mega-sporocarp percentage
			Micro-sporocarps	Mega-sporocarps	Total	
0	67	58.9 (73.3)	4.9	3.1	8.0	38.5
2	83	56.0 (68.7)	4.6	2.9	7.5	38.3
4	97	54.4 (66.0)	4.5	2.8	7.3	38.9
6	116	52.7 (63.3)	4.5	2.8	7.3	38.5
8	143	46.9 (53.3)	4.1	2.6	6.7	38.6
10	180	45.8 (51.3)	3.7	2.1	5.8	36.2
SE	8.4	1.72	0.13	0.07	0.18	0.63

Sporulation frequency data are transformed using Arc Sine $\sqrt{\text{percentage}}$ transformation and figures in parentheses are the original values.

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of sporocarps by about 15% but showed no improvement in the sporulation frequency; biomass production decreased significantly. Lowering it further to 6 and 4 kg P_2O_5 ha^{-1} improved the sporulation frequency and sporocarp number but the biomass production was decreased substantially. The percentage of mega-sporocarps was not affected by the phosphorus treatments because of almost equal reduction in the number of micro- and mega-sporocarps.

Schedule of phosphorus application

The sporulation frequency and number of sporocarps per plant were significantly higher in *A. microphylla* (strain 202) than in *A. pinnata* and *A. caroliniana* while the reverse was true for the mega-sporocarp percentage (Table 2). Compared with the no-P treatment (S_0), the recommended practice of applying phosphorus (10 kg P_2O_5 ha^{-1}) in three equal applications on 0, 7 and 14 DAI (S_3) substantially decreased the sporulation frequency and the number of

Table 2. Effect of schedule of phosphorus (10 kg P_2O_5 ha^{-1}) application on the biomass (fresh weight) production and sporulation in *Azolla caroliniana*, *Azolla pinnata* and *Azolla microphylla* (strain 202).

Azolla spp. and treatment	*Azolla* biomass (g per pot)	Sporulation frequency (%)	Number of sporocarps per plant			Mega-sporocarp percentage
			Micro-sporocarps	Mega-sporocarps	Total	
A. caroliniana						
S_0†	80	36.1 (34.7)	3.9	3.0	6.9	43.0
S_1	130	34.8 (32.7)	4.3	3.1	7.4	41.7
S_2	135	28.4 (22.7)	3.3	2.2	5.5	39.9
S_3	150	27.0 (20.7)	3.5	2.3	5.8	39.2
A. pinnata						
S_0	75	51.6 (61.3)	4.5	2.9	7.4	38.9
S_1	145	49.2 (57.3)	4.5	3.2	7.7	41.2
S_2	160	42.7 (46.0)	3.8	2.3	6.1	37.7
S_3	170	40.0 (41.3)	3.4	2.1	5.5	38.3
A. microphylla						
S_0	90	65.5 (82.7)	6.5	3.7	10.2	36.4
S_1	160	61.6 (77.3)	6.6	3.6	10.2	35.4
S_2	170	54.0 (65.3)	4.8	2.7	7.5	36.2
S_3	170	47.7 (54.7)	4.4	2.4	6.8	35.4
SE_1‡	6.2	0.69	0.08	0.04	0.11	0.39
SE_2	7.2	0.80	0.09	0.05	0.12	0.45
SE_3	12.4	1.38	0.15	0.08	0.22	0.79

Sporulation frequency data are transformed using Arc Sine $\sqrt{\text{percentage}}$ transformation and figures in parentheses are the original values.

†S_0 – no phosphorus; S_1 – phosphorus applied 0, 3 and 6 days after *Azolla* inoculation (DAI); S_2 – phosphorus applied 0, 5 and 10 DAI; S_3 – phosphorus applied 0, 7 and 14 DAI.

‡SE_1 is for comparing the means of *Azolla* species; SE_2 is for comparing the means of phosphorus treatments; SE_3 is for comparing *Azolla* species in the same phosphorus treatment or phosphorus treatments in the same *Azolla* species.

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sporocarps per plant. Applying phosphorus on 0, 5 and 10 DAI (S_2) was significantly superior to S_3 for the sporulation frequency in all the *Azolla* species and number of sporocarps in *A. microphylla*. Phosphorus application on 0, 3 and 6 DAI (S_1) was superior to both the earlier schedules for the sporulation frequency and sporocarp number in all the *Azolla* species. It was comparable to the no-P treatment for the number of sporocarps, with marginally lower sporulation frequency. The percentage of mega-sporocarps in this treatment was comparable to that in the no-P treatment and higher than that with the other two phosphorus application schedules, except in *A. microphylla*. The different phosphorus application schedules were comparable with respect to *Azolla* biomass, and 1.6–2.3 times that of the no-P treatment.

Phosphorus-enrichment of Azolla *inoculum*

The sporulation frequency and number of sporocarps per plant were lowest in *A. caroliniana* and highest in *A. microphylla* (strain 202) while the reverse was true for the mega-sporocarp percentage (Table 3). *Azolla* enriched with 30 or 60 kg P_2O_5 ha^{-1} and then grown without any further added phosphorus produced similar biomass to the unenriched *Azolla* grown with 10 kg P_2O_5 ha^{-1} after inoculation. In all the *Azolla* species, the sporulation frequency and sporocarp number in the P-enrichment treatments were comparable to those of unenriched *Azolla* grown without any phosphorus application (control) and significantly higher than those grown with 10 kg P_2O_5 ha^{-1} after inoculation, except that *A. caroliniana* produced fewer mega-sporocarps in P-enrichment treatment using 30 kg P_2O_5 ha^{-1} than in the control. The percentage of mega-sporocarps in all the *Azolla* species was lowered due to phosphorus application after inoculation and not due to P-enrichment of *Azolla* inoculum. The effects on the biomass production and sporulation of enrichment with 30 and 60 kg P_2O_5 ha^{-1} were similar.

Application of gibberellic acid and phosphorus

The sporulation frequency and sporocarp number in most of the treatments were highest in *A. pinnata* and lowest in *A. caroliniana* (Table 4). The sporulation frequencies of *A. microphylla* strain 203 and *A. pinnata* were comparable. *A. microphylla* strain 203 produced more sporocarps than strain 202. Irrespective of *Azolla* strains, applications of phosphorus at 10 kg P_2O_5 ha^{-1} divided equally between 0, 7 and 14 DAI inhibited sporulation; GA at 2.5 μg ml^{-1} 7 DAI enhanced sporulation. Phosphorus and GA interactions were significant for both the sporulation frequency and sporocarp number. Application of GA along with phosphorus fertilizer significantly increased the sporulation frequency and sporocarp number in all the *Azolla* strains not only over phosphorus application alone but also over the untreated control. Application of GA alone recorded higher (8.3–15.8%) sporulation frequency and sporocarp number compared with phosphorus plus GA treatment. Application of phosphorus alone decreased the percentage of mega-sporocarps while their ratio in GA and phosphorus plus GA treatments was comparable to that in the untreated control. Application of GA

Table 3. Effect of phosphorus-enrichment of *Azolla* inoculum and application of phosphorus after inoculation on the biomass (fresh weight) production and sporulation in *Azolla caroliniana*, *Azolla pinnata* and *Azolla microphylla* (strain 202).

Azolla spp. and treatment	*Azolla* biomass (g per pot)	Sporulation frequency (%)	Number of sporocarps per plant			Mega-sporocarp percentage
			Micro-sporocarps	Mega-sporocarps	Total	
A. caroliniana						
T_1†	90	41.9 (44.7)	4.6	3.6	8.2	43.7
T_2	130	34.4 (32.0)	3.8	2.4	6.2	38.6
T_3	110	40.8 (42.7)	4.4	3.2	7.6	41.8
T_4	115	42.3 (45.3)	4.8	3.3	8.1	40.6
A. pinnata						
T_1	85	67.1 (84.7)	6.0	4.0	10.0	40.2
T_2	135	52.7 (63.3)	4.9	3.0	7.9	37.8
T_3	120	66.6 (84.0)	6.2	3.9	10.1	38.7
T_4	130	65.5 (82.7)	6.4	4.0	10.4	38.5
A. microphylla						
T_1	95	71.8 (90.0)	7.8	4.7	12.5	37.5
T_2	170	56.4 (69.3)	6.7	3.4	10.1	33.7
T_3	150	68.2 (86.0)	7.8	4.9	12.7	38.6
T_4	165	68.7 (86.7)	7.9	4.8	12.7	37.9
SE_1‡	5.8	0.94	0.06	0.05	0.10	0.41
SE_2	6.8	1.08	0.07	0.05	0.11	0.47
SE_3	11.6	1.89	0.12	0.10	0.19	0.82

Sporulation frequency data are transformed using Arc Sine $\sqrt{\text{percentage}}$ transformation and figures in parentheses are the original values.

†T_1 – no enrichment, no phosphorus after inoculation; T_2 – 10 kg P_2O_5 ha^{-1} after inoculation; T_3 – enrichment with 30 kg P_2O_5 ha^{-1}; T_4 – enrichment with 60 kg P_2O_5 ha^{-1}.

‡SE_1 is for comparing the means of *Azolla* species; SE_2 is for comparing the means of treatments; SE_3 is for comparing *Azolla* species in the same treatment or treatments in the same *Azolla* species.

alone had little effect on *Azolla* biomass production while phosphorus application, with or without GA, increased it substantially by similar amounts.

Phosphorus-enrichment of Azolla *inoculum and application of gibberellic acid*

The biomass production, sporulation frequency and number of sporocarps per plant in *A. microphylla* strains 202 and 203 were comparable, therefore, the results from their mean data (Table 5) are discussed. The sporulation frequency and number of sporocarps per plant were significantly higher when P-enriched *Azolla* inoculum was grown without any further application of phosphorus than when unenriched *Azolla* inoculum was grown with 10 kg P_2O_5 ha^{-1} after inoculation, irrespective of whether GA was applied or not. Application of GA substantially enhanced the sporulation frequency and sporocarp number in both the treatments. Interactions between the phosphorus and GA treatments were significant

Table 4. Effect of phosphorus and gibberellic acid (GA) application on the biomass (fresh weight) production and sporulation of *Azolla caroliniana*, *Azolla pinnata* and *Azolla microphylla* (strains 202 and 203).

Azolla spp. and phosphorus treatment	*Azolla* biomass (g per pot)		Sporulation frequency (%)		Number of sporocarps per plant						Mega-sporocarp percentage	
					Micro-sporocarps		Mega-sporocarps		Total			
	No GA	GA	No GA	GA	No GA	GA	No GA	GA	No GA	GA	No GA	GA
A. caroliniana												
No phosphorus	85	95	45.8 (51.3)	59.0 (73.3)	4.2	6.2	2.5	3.6	6.7	9.8	37.0	36.7
10 kg P_2O_5 ha^{-1}	170	190	40.4 (42.0)	52.7 (63.3)	3.6	5.0	2.0	2.9	5.6	7.9	35.5	36.8
A. pinnata												
No phosphorus	70	95	66.4 (84.0)	81.4 (97.7)	9.8	12.1	6.7	8.0	16.5	20.1	40.4	39.8
10 kg P_2O_5 ha^{-1}	170	175	58.1 (72.0)	70.9 (89.3)	8.5	10.1	5.5	6.9	14.0	17.0	39.3	40.6
A. microphylla (202)												
No phosphorus	75	95	60.7 (76.0)	79.8 (96.7)	8.9	11.4	5.1	6.4	14.0	17.8	36.6	35.9
10 kg P_2O_5 ha^{-1}	170	185	57.6 (71.3)	67.5 (85.3)	7.2	9.1	3.8	5.9	11.0	15.0	34.8	39.3
A. microphylla (203)												
No phosphorus	85	95	60.0 (78.0)	83.4 (98.0)	9.3	11.7	5.4	6.7	14.7	18.4	36.6	36.4
10 kg P_2O_5 ha^{-1}	185	190	56.4 (69.3)	67.5 (85.3)	7.6	9.7	4.0	5.9	11.6	15.6	34.7	38.0
SE_1†	4.8		0.63		0.05		0.04		0.07		0.39	
SE_2	6.8		0.89		0.06		0.05		0.10		0.55	
SE_3	9.6		1.26		0.09		0.07		0.15		0.78	
SE_4	13.6		1.78		0.13		0.11		0.21		1.11	

Sporulation frequency data are transformed using Arc Sine $\sqrt{\text{percentage}}$ transformation and figures in parentheses are the original values.
Phosphorus was applied in three equal applications 0, 7 and 14 days after *Azolla* inoculation (DAI) and GA solution (2.5 μg ml^{-1}) was sprayed 7 DAI.
†SE_1 is for comparing the means of phosphorus or GA treaments; SE_2 is for comparing *Azolla* means or phosphorus and GA interactions; SE_3 is for comparing *Azolla* and phosphorus or GA interactions; SE_4 is for comparing *Azolla*, phosphorus and GA interactions.

Table 5. Effect of gibberellic acid (GA) application on the biomass (fresh weight) production and sporulation of unenriched *Azolla* grown with 10 kg P_2O_5 ha^{-1} and phosphorus-enriched *Azolla* grown without phosphorus application (means of *Azolla microphylla* strains 202 and 203).

Type of *Azolla* and phosphorus treatment	*Azolla* biomass (g per pot)		Sporulation frequency (%)		Number of sporocarps per plant						Mega-sporocarp percentage	
					Micro-sporocarps		Mega-sporocarps		Total			
	No GA	GA	No GA	GA	No GA	GA	No GA	GA	No GA	GA	No GA	GA
Unenriched *Azolla* grown with 10 kg P_2O_5 ha^{-1}	180	178	45.8 (51.3)	53.1 (64.0)	2.9	3.5	1.6	2.2	4.5	5.7	35.4	38.9
Phosphorus-enriched *Azolla* grown without phosphorus	165	172	50.6 (59.6)	58.3 (72.3)	3.4	4.5	2.1	2.8	5.5	7.3	38.5	38.2
Control (unenriched *Azolla* grown without phosphorus)	69		50.0 (58.6)		3.4		2.1		5.5		38.2	
SE_1†	5.5		0.83		0.05		0.05		0.08		0.45	
SE_2	4.9		0.74		0.05		0.04		0.07		0.40	
SE_3	7.0		1.04		0.06		0.06		0.10		0.57	

Sporulation frequency data are transformed using Arc Sine $\sqrt{\text{percentage}}$ transformation and figures in parentheses are the original values.
Phosphorus-enriched *Azolla* was obtained by growing it in nursery with 60 kg P_2O_5 ha^{-1}; phosphorus was applied to unenriched *Azolla* in three equal applications 0, 7 and 14 days after inoculation (DAI); GA solution (2.5 μg ml^{-1}) was sprayed 7 DAI.
†SE_1 is for comparing the control vs. mean of rest; SE_2 is for comparing means of type of *Azolla* and phosphorus or GA treatments; SE_3 is for comparing type of *Azolla* and phosphorus treatment × GA interactions.

for the sporocarp number and mega-sporocarp percentage but not for the sporulation frequency. The use of P-enriched *Azolla* inoculum with no GA and application of phosphorus plus GA to unenriched *Azolla* were comparable with each other and superior to the application of phosphorus alone to unenriched *Azolla* for the sporocarp number. Combining the use of P-enriched *Azolla* inoculum with the application of GA was significantly superior to all these treatments. It increased the sporulation frequency and number of sporocarps per plant by 40.9 and 60.6% respectively over the treatment in which unenriched *Azolla* was grown with 10 kg P_2O_5 ha^{-1} after inoculation. The percentage of mega-sporocarps in these treatments was virtually the same as in the control. *Azolla* biomass in different phosphorus and GA combinations was comparable and 2.4–2.6 times that of the untreated control.

DISCUSSION

The sporocarp yield in *Azolla* depends on the biomass production, sporulation frequency and number of sporocarps per plant. To a certain extent, biomass production is directly related, and the sporulation frequency and number of sporocarps are inversely related to applied phosphorus.

Phosphorus at the recommended dose of 10 kg P_2O_5 ha^{-1} divided into three equal applications 0, 7 and 14 DAI to different *Azolla* strains in this study increased the biomass production but invariably it reduced the sporulation frequency and sporocarp number. This finding is in agreement with an earlier report on *A. pinnata* (Singh *et al.*, 1987). Lowering the phosphorus dose from 10 to 4–8 kg P_2O_5 ha^{-1} enhanced the sporulation frequency and sporocarp number but the advantage was lost due to the drastic reduction in the biomass production, suggesting that this is not a viable proposition for increasing sporocarp yield. On the other hand, changing the schedule of phosphorus applications from 0, 7 and 14 DAI to 0, 3 and 6 DAI enhanced the sporulation frequency by up to 58% and the number of sporocarps per plant by up to 50% and was comparable to the no-P treatment for the sporocarp number, with slightly lower sporulation frequency. The superior performance of this treatment over phosphorus application 0, 7 and 14 DAI resulted possibly from the lower tissue-P content in the plants during sporulation because of earlier completion of phosphorus fertilization. The sporocarps were visible only 20 DAI. Marginal reduction in the sporulation frequency with phosphorus application 0, 3 and 6 DAI over the no-P treatment is to be expected, if the maximum sporulation frequency is decided at an earlier date than the number of sporocarps. Phosphorus application 0, 5 and 10 DAI produced intermediate results. As *Azolla* biomass with the three phosphorus application schedules was comparable, and phosphorus application 0, 3 and 6 DAI ensured the highest sporocarp yield, this is the application schedule to be recommended. The higher percentage of mega-sporocarps in this treatment was an added advantage because it is the mega-sporocarps that produce sporophytes after germination.

Azolla subjected to P-enrichment accumulates phosphorus up to the maximum luxury consumption level and continues to grow until the tissue-P content gradually declines below the threshold level (Lumpkin, 1987; Watanabe *et al*., 1988). The production of similar biomass levels by the P-enriched *Azolla* grown subsequently without any phosphorus application and unenriched *Azolla* grown with 10 kg P_2O_5 ha^{-1} after inoculation in this study conformed to the earlier report (Singh and Singh, 1995). The sporulation frequency and number of sporocarps per plant were significantly higher in P-enrichment treatments than in treatments receiving phosphorus after inoculation, possibly because of lower tissue-P content in the plant during sporulation. The higher percentage of mega-sporocarps in *A. caroliniana* and *A. microphylla* due to P-enrichment resulted from greater improvement in the production of mega-sporocarps than the production of micro-sporocarps.

Kannaiyan *et al*. (1990) and Kannaiyan (1994) used 100 μg ml^{-1} GA solution to boost sporulation in *A. microphylla*. However, 2.5 μg ml^{-1} solution was used in this study based on the results of a series of experiments conducted by Kar *et al*. (1999) with different *Azolla* strains. The increased sporulation frequency and sporocarp number due to the application of GA along with phosphorus fertilizer over that of phosphorus alone and the untreated control, confirmed its stimulatory effect on *Azolla* sporulation. However, GA could not fully mitigate the adverse effects of phosphorus on sporulation, as was evident from the lower sporulation frequency and sporocarp number in the GA plus phosphorus treatment than with GA alone. As *Azolla* biomass in phosphorus and GA plus phosphorus treatments was comparable, application of GA along with phosphorus would essentially lead to the higher sporocarp yield. Combining the use of P-enriched inoculum with GA application significantly increased the sporulation frequency and number of sporocarps per plant over the use of P-enriched inoculum without GA and application of GA plus phosphorus to unenriched inoculum. This is because of the additive effects of the two individual treatments. The lower percentage of mega-sporocarps in the treatment receiving phosphorus after inoculation resulted from greater reduction in the number of mega-sporocarps than the reduction of micro-sporocarps. *A. microphylla* showed poor sporulation because this experiment was conducted towards the end of the favourable season.

In conclusion, the sporulation frequency and sporocarp number in *Azolla* could be increased substantially without any significant loss of the biomass production by changing the schedule of phosphorus application from the recommended 0, 7 and 14 DAI to 0, 3 and 6 DAI, application of GA along with phosphorus fertilizer or by the use of P-enriched inoculum and applying GA to it, further improved the sporulation frequency and sporocarp number.

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