Evaluation of the effect of three different pesticides on *Azolla pinnata* growth and NPK uptake

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Abstract: Three pesticides of common use in rice fields in Egypt were used in the present work. This study was devoted to investigate the effects of different concentrations of the insecticide furadan, fungicide hinosan and herbicide saturn on the growth and NPK uptake of the aquatic fern *Azolla pinnata,* which is recommended to be applied as a biofertilizer in rice. In this respect, the results obtained showed variable effects of the three pesticides under study. Furadan and hinosan showed positive effects since each increased the growth rate of *A. pinnata* at lower concentrations (0.001, 0.002 ppm) and consequently increased its NPK content. Maximum dinitrogenase activity was also generally obtained at 0.002 ppm furadan, throughout the different incubation periods. Nitrogen, phosphorus and potassium uptake was generally increased with increasing the incubation period of the applied furadan and hinosan, at all concentrations. The highest NPK uptake by *A. pinnata* was obtained with the medium concentration (0.002 ppm) of both pesticides after 20 and 25 days of incubation. On the other hand, saturn generally showed inhibitory effects on the growth, N_2- fixation and NPK uptake even at lowest concentration (0.001 ppm).
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1. Introduction

Azolla– Anabaena symbiosis is an important N_2-fixing system between an eukaryotic fern and a prokaryotic cyanobacterium. This host-symbiont combination is exploited as a biofertilizer for many agricultural crops (Lillian 2000; Pabby et al., 2003 and Abd El- Rasoul et al., 2004). Like most plants, *Azolla* requires the macro-and micronutrients which are essential for normal growth. Nitrogen, phosphorus, potassium, calcium and magnesium are very important and produce marked effects on the fern growth (Arrora et al., 2003). It was found that application of *Azolla* appreciably improved soil fertility by increasing its total nitrogen, organic carbon and available phosphorus (Singh and Singh, 1990; Jeyabal and Kuppuswamy, 2001 and Ghoudhary and Kennedy, 2004). Investigations showed that *Azolla* is also a promising plant to be applied in controlled ecological life support systems (Xiaofeng et al., 2008). Agriculture is almost dependent on chemical pesticides (Greaves et al., 1988). However, pesticides are observed to exert determinable effects on microbial processes, which play an important role in plant growth, crop productivity and soil fertility (Nayak and Rajamamoban, 1982). The carbamates, like organochlorine insecticides, have received a moderate amount of attention in relation to their influence on cyanobacteria in paddy field ecosystems

(Hammouda, 1999). Carbofuran (furadan) is a systematic insecticide which means that it is absorbed by the plant roots and distributed to all parts of the plant and it is a member of the carbamate family which inhibits the enzyme cholinesterase by forming carbofuran -ACHE complex (Chauhan et al., 2000 and Class Resourses, 2009).

Singh et al. (1982) and Watanabe (1986) showed that mixing small amount of the insecticide carbofuran (furadan) with the *Azolla* inoculums effectively controlled most of the insects attacking *Azolla* and so increased its growth. However, addition of lower concentrations of lindane increased growth and N_2- fixation (Singh et al., 1982). The authors added that further increase of these concentrations decreased growth and N_2 fixation with all tested *Azolla* species. On the other hand, Ismail et al. (1995) found that carbofuran significantly increased *A. pinnata* chlorophyll content and dinitrogenase activity but did not affect its growth.

Rice blast is one of the most destructive diseases in rice plant (Savary et al., 2000) and is mainly controlled by application of the fungicide henosan (O- Ethyl- S- S- diphenyle-

dithiophosphate). It is considered as one of the organophosphate group (Madhaiyan et al., 2006). The phosphorothiolate (hinosan), edifenphos and iprobenfos fungicides have been used to control rice blast in rice cultivating areas (Kim et al., 2008 and Kim and Kim, 2009). On the other hand, the inhibition on the growth of *Gluconacetobacter diazotrophicus* showed no significant differences with the variation of the added doses of dithane and hinosan to the growth media (Madhaiyan et al., 2006).

Every year, major losses in paddy occur due to heavy weed infestation. There are various herbicides to control weeds but most of them have toxic residues in different parts of the rice plant (Aktar et al., 2007). Thiobencarb (S- 4-chlorobenzyle diethyl thiocarbamate) had been extensively used in modern agricultural practices (Xia, 2004). It is mainly used as a pre or post emergent herbicide (Aktar et al., 2007). One of the currently used formulations of benthocarb is saturn 50 EC. This compound is absorbed by the root systems of the herbs, translocates to the meristem and inhibits protein synthesis (Matsuo and Shibayama, 2002). On the other hand, sensitivity of cyanobacteria to herbicides varies according to the species (Sabuter and Carrasco, 1996) and the kind of herbicide but, in general, they are quite sensitive to herbicides (Irisarri et al., 2001).

Azolla growth was influenced by various herbicides. Butachlor (mashete) and benthiocarb (saturn) when applied at high concentrations showed maximum inhibitory effect on the *Azolla* growth and N_2-fixation (Singh and Mishra, 1982). Zarger and Dar (1990) also suggested that thiobencarb had inhibitory effect on nitrogen fixation and heterocyst formation in a mixed culture of *Anabaena*, *Nostoc* and *Oscillatoria.* Battah et al. (2001) added that saturn inhibited protein synthesis, which exert many secondary effects on growth. Xia (2004) added that biomass yield, protein content and photosynthetic rate were reduced only at high thiobencarb concentrations. Lales and Marte (1986) found that propanil and butachlor were most toxic to all treated ferns. On the other hand application of 2, 4-Dina stimulated the growth of *Azolla* (Singh et al., 1988).

Aim of work:

The use of insecticide furadan, fungicide hinosan and herbicide saturn has become of common practice in rice cultivation to kill the insects in culture, reduce fungal infections, and to minimize the cost involved in weeding. Algae have frequently been the subject of this investigation because of their importance as primary producers in fresh water systems. Therefore, studying the effects of elevated concentrations of these pesticides on the growth of *Azolla* is particularly important for recording its detrimental effect on different growth parameters, nitrogen- fixation and NPK uptakes by *Azolla pinnata*.

2. Material and Methods

Materials:

Azolla pinnata used in the present investigation was kindly provided by the Agricultural Microbial Department, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt.

The insecticide furadan (carbofuran; 2,3 dihydro- 2, 2- dimethylbenzofuran 7-methylcarbonate), the fungicide hinosan (O - Ethyl-S,S- diphenyl phosphorodithioste) and the herbicide saturn (S- chlorobenzyl- N, N- diethyl thiocarbamate) were kindly provided from the Central Laboratory of Pesticides, ARC, Dokki, Cairo, Egypt.

Methods:

The present experiment was carried out in the Botanical garden of the Faculty of Science, Ain Shams University, Cairo, Egypt.

Propagation of *Azolla:*

According to El- Shahat (1988), ten g *Azolla pinnata* were grown in plastic pots (32 cm in diam. and 15 cm dep.), each containing 1 Kg soil in 3 liters tap water then kept in a greenhouse till *Azolla* covered the entire water surface. *Azolla* was collected and incorporated in 0.01 mercuric chloride for 1 min. and washed gently in running tap water for several times, using a screen of 0.2 mesh, and then air dried on tissue paper for 30 min. The collected fronds were used as an inoculum for further experiments.

The effects of the insecticide furadan, the fungicide hinosan, and the herbicide Saturn on the *Azolla* growth were tested. For this purpose, modified Yoshida medium (Yoshida et al., 1976) was prepared to contain increased concentrations of $NaH_2PO4.H_2O$,$CaCl_2$, K_2SO_4 and $MgSO_4.7H_20$ (40 ppm) and trace elements 1 ml per litter. Thirteen sets of plastic pots (14 x 7 cm) were used; each pot contained 750 ml Yoshida medium. Different concentrations (0.00, 0.001, 0.002, 0.003, 0.004 ppm) of each pesticide (furadan, hinosan and saturn) were added to the Yoshida liquid medium and inoculated by one gram fresh *Azolla*. Each concentration was represented by one set and every set consists of 3 replicates for each incubation period. The pots were incubated under normal condition of light (18/6 hr.)

and temperature of 25 ºC ±2 at day time and 18 ºC ±2 at night and air humidity of about 70% for 25 days.

The Yoshida medium was changed every five days to get constant concentrations of minerals and pesticides used throughout the experiment. Developed *Azolla* culture was periodically sampled after 5, 10, 15, 20 and 25 days. Harvested *Azolla* fronds were washed by deionized water and placed under shade between two thick layers of blotting papers for approximately 1 hour before determining fresh and dry weights, doubling time, dinitrogenase activity and NPK uptake.

Calculation of doubling time:

Growth rate of *Azolla* in terms of doubling time was calculated using the following equation according to Aziz and Watanabe (1983).

Doubling time = t/r, whereas:

t = the duration of *Azolla* growth.

r = log wt /wo / 0.301

Wt = weight of *Azolla* at time t,

W0 = weight of *Azolla* at zero time i.e. weight of inoculum.

Acetylene reduction assay:

About 0.5 g fresh weight of *Azolla* of each treatment was incubated under 10% C_2H_2 in air of 500 ml flask, fitted with serum caps and containing 100 ml amended culture media.

The incubation conditions and analysis of ethylene produced were adopted as described by Kitoh et al. (1993).

NPK content:

NPK content of *Azolla* was estimated in dried plant material. Nitrogen was determined using microkjedahl method according to Black et al. (1965), phosphorus spectrophotometerically according to Olsen and Sammers (1982) and total potassium according to the method described by Jackson (1958).

Statistical analysis:

The individual data sets were subjected to the least significant differences at $p<0.05$ as calculated by Gomez and Gomez (1984).

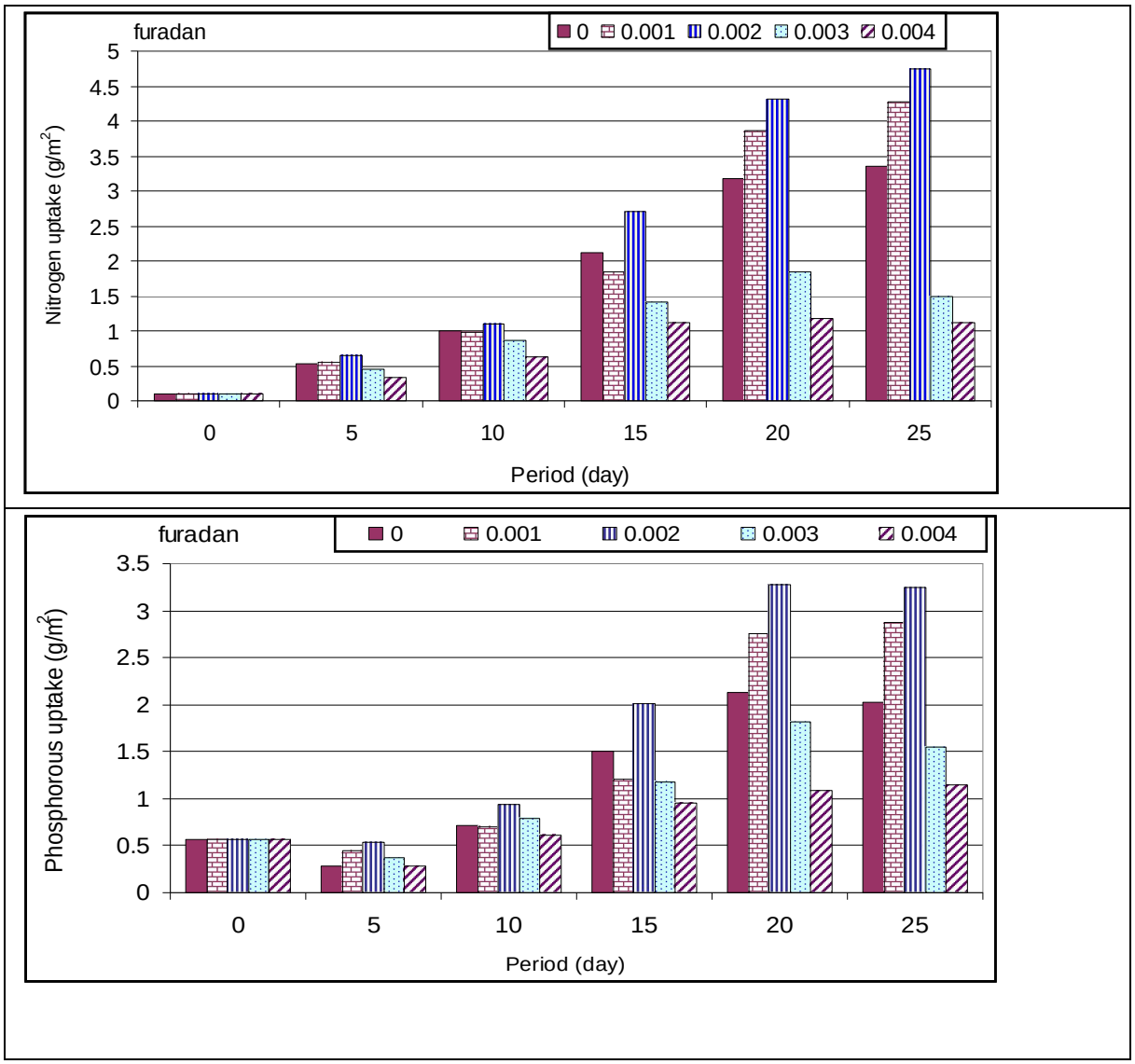
3. Results and Discussion

The different pesticides used in the present work showed varying effects on *Azolla pinnata.* A range of concentrations (0.00, 0.001, 0.002, 0.003 and 0.004 ppm) of furadan, hinosan and saturn were used to verify the optimal level which can be used without affecting the viability of Azolla.

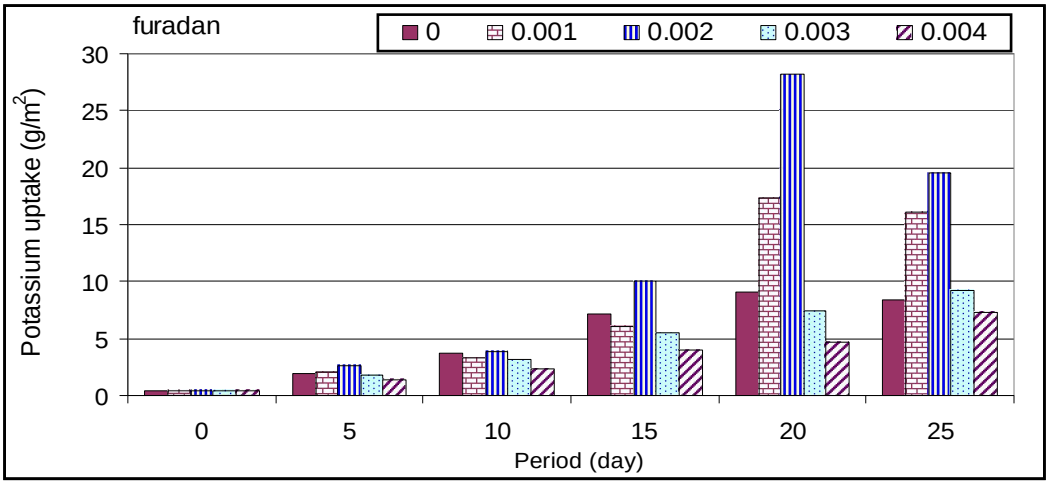
The data in Tables (1&2) showed that the fresh and dry weights of *A. pinnata* were optimal after 20 days incubation period at 0.002 ppm furadan and hinosan. A similar trend was found by Watanabe (1982) and Singh et al. (1984) who reported that mixing small amount of insecticide with *Azolla* inoculum effectively controlled most of the attacking insects and increased plant growth and N_2-fixation, while higher concentration decreased growth and N_2- fixation. On the other hand, Singh and Sethunathan (1999) found that *Azolla* could utilize carbofuran (furadan) as a sole source of carbon and nitrogen.

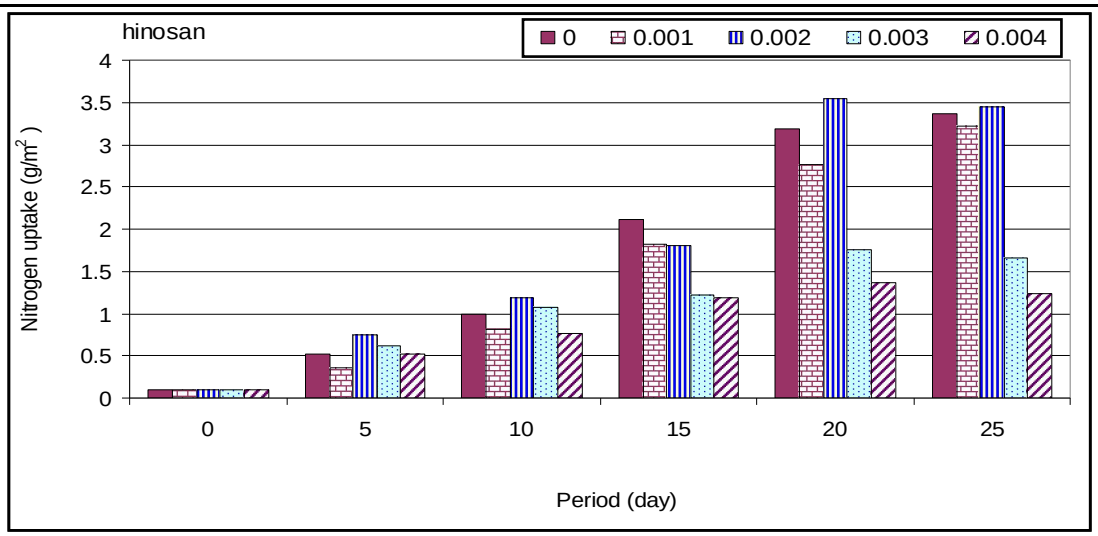
A. pinnata appeared to be sensitive to all concentrations of saturn. This observation was derived from that fronds grown in the control showed higher accumulation of fresh and dry weights than those grown in different concentrations of saturn at 5 days up to 25 days of incubation (Tables:1& 2). These results agreed with those of Lales and Marte (1986) who found that the fresh weight of *Azolla* was influenced by various herbicides. Singh and Mishra (1982) observed that benthiocarb (saturn) showed maximum inhibitory effect on *Azolla* growth and N_2-fixation. It was found that saturn has an inhibitory effect on photosynthetic CO_2 assimilation (Battah et al., 2001) and inhibited protein synthesis (Xia, 2004), which could be due to disturbances in nitrogen metabolism and photosynthetic activity (Battah et al., 2001) or due to an increase in protease activity (Bhunia et al., 1991). Such effects might exert many secondary effects on growth.

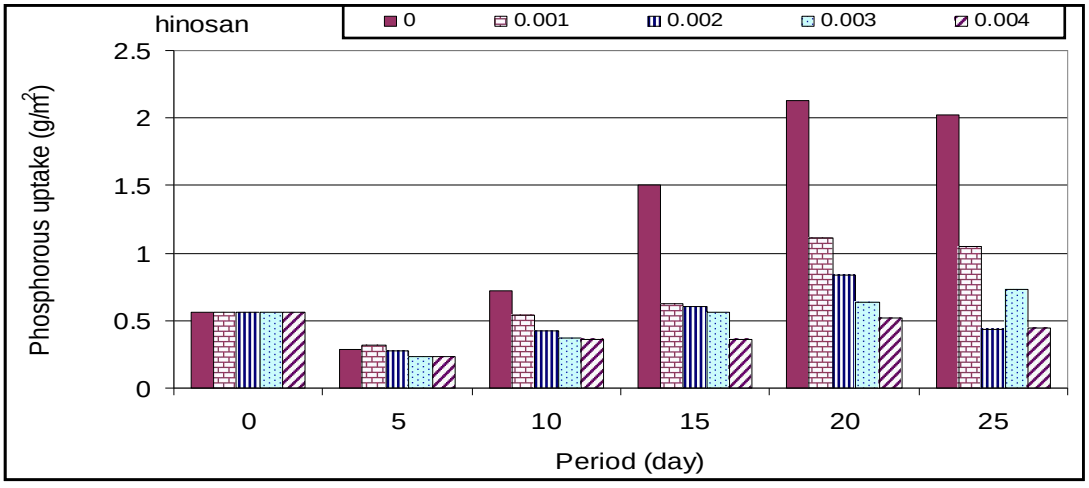
Hopkins (1998) stated that chemical herbicides have become an important management tool, but their value as a labor saving device must be carefully weighted against potentially harmful side effects.


furadan
0
0.001
0.002
0.003
0.004
Nitrogen uptake (g/m2)
Period (day)
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

furadan
0
0.001
0.002
0.003
0.004
Phosphorous uptake (g/m2)
Period (day)
0
5
10
15
20
25
0
0.5
1
1.5
2
2.5
3
3.5


furadan
0
0.001
0.002
0.003
0.004
Potassium uptake (g/m2)
30
25
20
15
10
5
0
0
5
10
15
20
25
Period (day)


hinosan
0
0.001
0.002
0.003
0.004
Nitrogen uptake (g/m2)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Period (day)


hinosan
0
0.001
0.002
0.003
0.004
Phosphorous uptake (g/m2)
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Period (day)

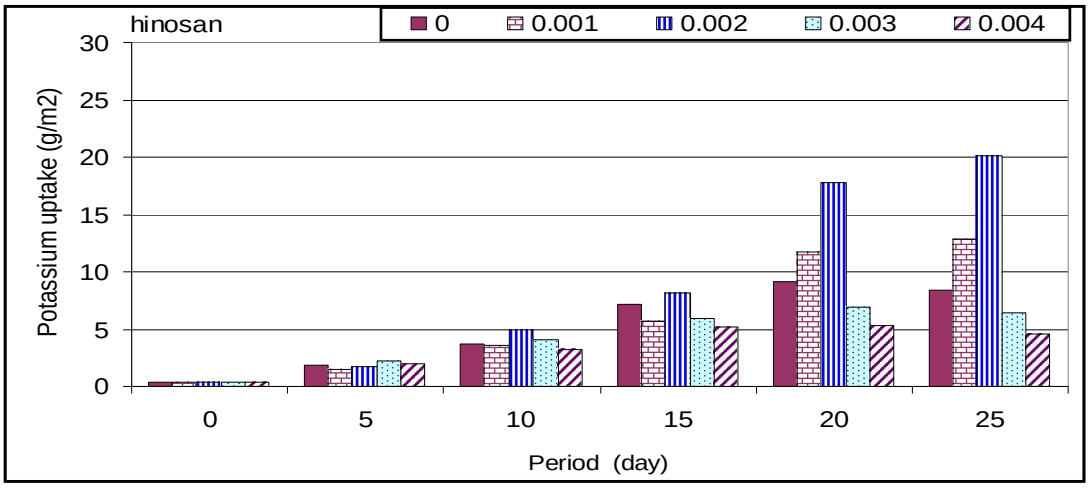
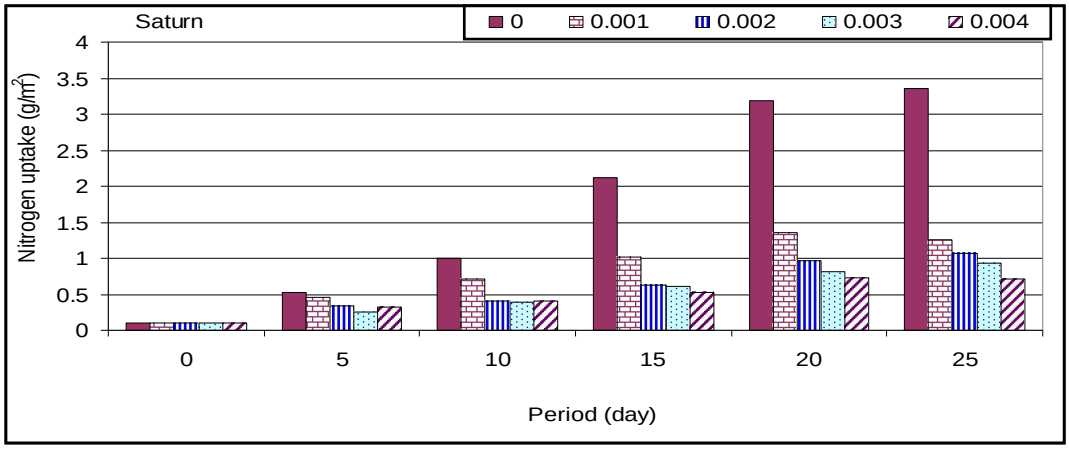
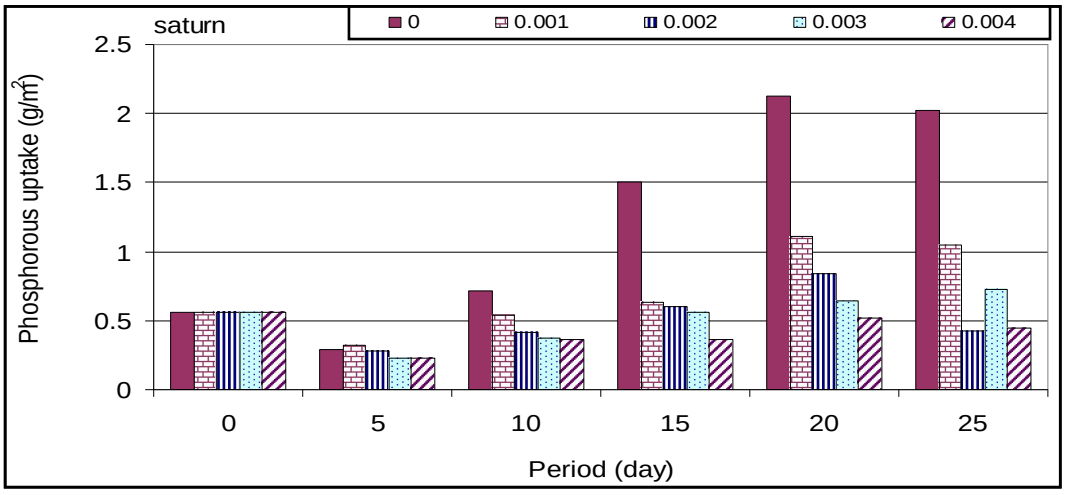


Fig. (2): Effect of different concentrations of hinosan on nitrogen, phosphorous and potassium uptake (g/m^2) by *Azolla pinnata*





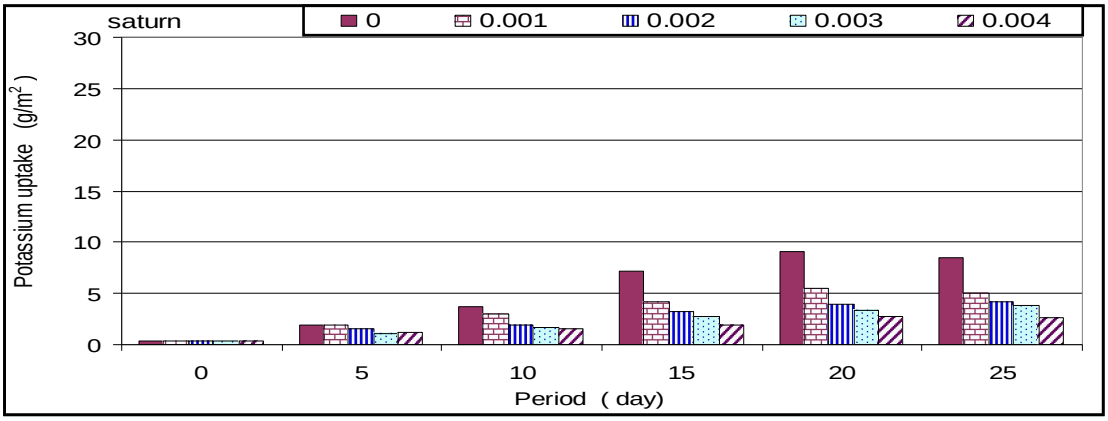


Fig. (3): Effect of different concentrations of Saturn on nitrogen, phosphorous and potassium uptake (g/m^2) by *Azolla pinnata*

Table (1) : Effect of different concentrations of furadan, hinosan and saturn on *Azolla pinnata* fresh weight (g/m^2).The values listed are the means of 3 replicated ± SD (stander deviation)

Treatment / Period (days)	control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004
0	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90±**	**90.90**
5	**586. 32** ±7.41	**404.54** ±11.41	**479.90** ±6.92	**346.65** ±7.89	**289.69** ±5.95	**270.90** ±19.90	**333.63** ±4.25	**423.63** ±14.96	**390.00** ±5.05	**378.14** ±7.41	**307.69** ±15.06	**227.24** ±41.66	**290.88** ±24.05
10	**707.20** ±39.04	**592.73** ±22.98	**667.81** ±12.37	**560.90** ±0.98	**432.72** ±15.37	**596.36** ±13.69	**745.45** ±9.50	**667.27** ±7.65	**562.73** ±8.17	**569.96** ±12.63	**354.51** ±34.41	**345.41** ±32.77	**358.51** ±38.43
15	**1221.11** ±20.33	**934.54** ±4.27	**1357.27** ±17.46	**870.00** ±1.00	**692.72** ±5.84	**966.00** ±10.52	**953.72** ±6.18	**740.00** ±5.46	**737.27** ±4.21	**752.58** ±16.37	**532.70** ±73.27	**527.22** ±30.46	**457.23** ±8.75
20	**1532.33** ±40.09	**1692.1** ±8.10	**1830.02** ±19.98	**1079.0** ±1.50	**712.41** ±8.44	**1200.0** ±18.36	**1632.7** ±8.48	**1020.40** ±13.21	**830.90** ±6.95	**987.81** ±29.22	**713.57** ±44.12	**636.3** ±81.30	**620.85** ±29.51
25	**133.63** ±30.87	**1553.4** ±18.41	**1655.15** ±15.05	**898.18** ±1.07	**687.27** ±1.64	**1279.0** ±5.80	**1559.0** ±18.33	**923.69** ±6.20	**797.27** ±12.54	**855.40** ±18.18	**756.31** ±37.56	**719.04** ±13.03	**573.58** ±8.20
L.S.D. at 0.05 P													
Conc. x time		97.741				41.590				96.621			

Table (2) : Effect of different concentrations of furadan, hinosan and saturn on *Azolla pinnata* dry weight (g/m^2). The values listed are the means of 3 replicated ± SD (stander deviation)

Treatment / Period (days)	control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004
0	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**	**5. 09**
5	**22. 64** ±1.34	**23.05** ±0.23	**26. 83** ±0.39	**19. 41** ±0.44	**14. 50** ±0.70	**15. 17** ±1.12	**18. 68** ±0.23	**23.72** ±0.83	**21. 84** ±0.27	**21. 17** ±1.81	**17. 23** ±0.72	**12.73** ±2.33	**16. 29** ±1.35
10	**39. 85** ±2.49	**33.19** ±0.23	**37. 39** ±0.66	**31. 42** ±0.06	**24. 23** ±0.86	**33. 39** ±0.78	**41.74** ±0.54	**37. 36** ±0.44	**31. 50** ±0.48	**31. 92** ±0.72	**19. 85** ±1.93	**19. 34** ±1.84	**20. 08** ±2.15
15	**68. 38** ±1.39	**52.33** ±0.08	**77.24** ±0.80	**48.72** ±0.03	**38. 23** ±0.33	**54. 09** ±0.59	**53. 40** ±0.35	**41. 44** ±0.28	**41. 29** ±0.25	**42.14** ±0.92	**29. 83** ±4.11	**29. 52** ±1.71	**25. 60** ±4.33
20	**85.14** ±1.83	**94.79** ±0.43	**102. 49** ±1.10	**60. 46** ±0.08	**39. 89** ±0.48	**67. 20** ±0.99	**91. 43** ±0.44	**57.14** ±0.75	**46. 51** ±0.36	**55. 32** ±1.62	**39. 95** ±2.47	**35. 63** ±4.65	**34.76** ±1.67
25	**74. 68** ±2.12	**86. 99** ±0.90	**92. 68** ±0.85	**50. 09** ±0.09	**38. 48** ±0.09	**71. 62** ±0.30	**87. 31** ±1.06	**51.72** ±0.35	**40. 90** ±0.58	**47. 90** ±1.02	**42. 35** ±2.11	**40. 26** ±1.47	**32.12** ±0.46
L.S.D.at 0 .05 P Conc. x time		*4.151*				2.013				3.465			

Table (3): Effect of different concentrations of furadan, hinosan and Saturn on doubling time (day) of *Azolla pinnata*. The values listed are the means of 3 replicated ± SD (stander deviation)

Treatment / Period (days)	Control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0. 004	0.001	0.002	0.003	0.004
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
5	**2.3** ± 0.10	**2.3** ± 0.11	**2.1** ±0.06	**2.6** ± 0.1	**3.0** ±0.26	**3.2** ± 0.15	**2.7** ±0.12	**2.3** ±0.1	**2.4** ±0.06	**2.4** ±0.15	**2.8** ±0.10	**3.9** ±0.7	**3.0** ±0.26
10	**3.4** ± 0.10	**3.7** ± 0.06	**3.5** ± 0.10	**3.8** ± 0.2	**4.4** ±0.10	**3.7** ±0.06	**3.3** ±0.06	**3.5** ±0.1	**3.8** ±0.10	**3.7** ±0.06	**5.1** ±0.38	**5.2** ±0.4	**5.1** ±0.40
15	**4.4** ± 0.06	**4.5** ± 0.10	**3.8** ± 0.17	**4.6** ± 0.1	**5.1** ± 0.38	**4.4** ± 0.06	**4.4** ± 0.06	**4.9** ± 0.1	**4.9** ± 0.10	**4.9** ± 0.00	**5.9** ± 0.45	**5.9** ± 0.2	**6.6** ± 0.10
20	**6.0** ± 0.17	**4.7** ± 0.10	**4.6** ± 0.10	**5.6** ± 0.1	**6.8** ± 0.06	**5.4** ± 0.15	**4.8** ± 0.15	**5.7** ± 0.1	**6.2** ± **0.15**	**5.8** ± 0.06	**6.7** ± 0.23	**7.1** ± 0.5	**7.2** ± 0.15
25	**7.9** ± 0.23	**6.1** ± 0.17	**5.9** ±0.45	**7.6** ±0.1	**8.6** ±0.10	**6.6** ± 0.10	**6.1** ± 0.17	**7.5** ± 0.2	**8.4** ± 0.05	**7.7** ± 0.06	**8.2** ± 0.20	**10.1** ± 0.4	**9.4** ± 0.06
L.S.D. at 0 .05 P Conc. x time		0.257				0.143				0.239			

Conc. x time

Table (4) : Effect of different concentrations of furadan , hinosan and saturn on nitrogenas activity (µ mol C_2H_4 /g/dry wt./hr^{-1}) of *Azolla pinnata* . The values listed are the means of 3 replicated ± SD (stander Deviation)

Treatment / Period (days)	Control	furadan				hinosan				saturn			
	Concentration (ppm)												
	0.00	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004	0.001	0.002	0.003	0.004
0	**5. 13**	**5.13**	**5. 13**	**5. 13**	**5.13**	**5.13**	**5.13**	**5. 13**	**5.13**	**5.13**	**5. 13**	**5.13**	**5.13**
5	**19. 50** ± 0.51	**14.15** ± 0.93	**18. 05** ± 0.24	**16. 00** ± 0.50	**15. 22** ± 0.38	**11. 84** ± 0.17	**10. 57** ± 0.18	**13. 00** ± 0.17	**7. 14** ± 0.26	**10. 51** ± 0.87	**12. 34** ± 0.38	**8. 14** ± 0.76	**7. 04** ± **0.21**
10	**12. 51** ± 0.59	**11.71** ± 0.15	**21.78** ± 0.23	**17. 22** ± 0.34	**11. 17** ± 0.25	**12. 13** ± 0.17	**13.78** ± 0.08	**12. 11** ± 0.26	**6. 23** ± 0.29	**5. 22** ±0.32	**6. 24** ± 0.57	**7. 17** ± 0.31	**5. 00** ± 0.26
15	**22.14** ± 0.92	**13. 50** ± 0.33	**15.75** ± 0.14	**14.13** **± 0.12**	**14. 10** ± 0.23	**7. 15** ± 0.40	**8. 25** ± 0.10	**7. 52** ± 0.35	**8. 00** ± 0.17	**7.18** ± 0.45	**9. 52** ± 0.52	**5. 23** ± 0.08	**5. 47** ± 0.40
20	**9. 41** ± 0.66	**11. 66** 0.09	**22. 50** ± 0.28	**11. 05** ± 0.17	**6. 78** ± 0.33	**7.75** ± 0.18	**11. 35** ± 0.05	**8. 18** ± 0.20	**5.14** ± 0.20	**4. 35** ± 0.17	**7. 42** ± 0.33	**4. 60** ± 0.42	**3. 79** ± 0.33
25	**17.24** ± 0.69	**7.74** ± 0.10	**19. 60** ± 0.11	**6. 50** ± 0.15	**7. 13** ± 0.25	**6. 50** ±0.53	**7. 82** ± 0.08	**6. 55** ± 0.22	**3. 33** ± 0.13	**6. 40** ± 0.55	**4. 51** ± 0.10	**3. 35** ± 0.13	**4. 22** ± 0.28
L.S.D.at 0.05 P Conc.xtime		0.6092				0.7370				0.5651			

Doubling time

The results obtained (Table 3) showed that the doubling time at lower concentrations (0.001, 0.002 ppm) of furadan and hinosan were significantly decreased than the control, especially after 20 and 25 days. However, the herbicide saturn has an inhibitory effect on *Azolla* doubling time even at low concentration (0.001ppm) since it showed a significant increase in doubling time, as compared with the control and the other two pesticides. Singh et al. (1988) and Madhaiyan et al. (2006) suggested that the application of various insecticides and fungicides showed low toxicity affects on the doubling time of *Azolla*, compared with herbicides. Xiaofeng et al. (2008) added that *Azolla* doubling time would be clearly shortened when grown in artificial controlled environmental condition and so its biomass increased.

Dinitrogenase activity

The data represented in Table (4) showed that dinitrogenase activity of *Azolla* treated with the tested pesticides gradually increased with time, till 10 or 15 days incubation, then an inconsistent decrease was obtained after 20 and 25 days.

Moreover, maximum dinitrogenase activity was generally obtained at 0.002 ppm furadan, throughout the incubation periods. These values were higher than those of hinosan and saturn at different concentrations and also than the control. A similar trend was reported by Holst et al. (1982) who found that the insecticide carbofuran (furadan) significantly increased dinitrogenase activity of *A. pinnata*. Moreover, it was increased by application of a low concentration of lindan (Singh et al., 1984). On the other hand, Ismail et al. (1995) found that the herbicide saturn reduced the growth and dinitrogenase activity of *A. pinnata*. On the other hand, Madhaiyan et al. (2006) reported that addition of pesticides to the growth media substantially reduced the dinitrogenase activity of pure cultures of *G. Diazotrophicus.*

NPK uptake

The data in Figures (1), (2), (3) showed that nitrogen, phosphorus and potassium uptake was generally increased with increasing the incubation period of the applied furadan and hinosan, nearly at all concentrations. The highest NPK uptake by *A. pinnata* was obtained with the medium concentration (0.002 ppm) of both pesticides after 20 and 25 days of

incubation. *Azolla* fronds grown in a medium supplemented by saturn at different levels showed inconsistent decrease in NPK content with lapse of time. It was obvious that NPK values at different saturn concentrations, were significantly lower than those of the control and the other two pesticides (furadan and hinosan). These results agreed with those of Arrora et al. (2003), who reported that NPK, calcium and magnesium are very important and produce marked effects on the fern growth. It was found that dry weight and total nitrogen had a higher concentration with P (Madhaiyan et al., 2006), nitrogen (Wettern, 1985 and Hechler and Dawson, 1995) and K supply (Liu, 1987). It was obvious from the results obtained throughout this experiment that *A. pinnata* showed higher tolerance to furadan and hinosan at the different concentration used, as compared with the lower concentrations of saturn.

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