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In vitro and *in situ* Effects of Herbicide Thiobencarb on Rice-*Azospirillum* Association

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Abstract

The effect of different levels of thiobencarb (Saturn 50% EC) on two species of *Azospirillum* (*A. brasilense* NO40 and *A. lipoferum* 4B) was studied in pure cultures and in association with rice (*Oryza sativa* cv Giza 172) under laboratory and field conditions. In pure *in vitro* cultures, thiobencarb had no effect on *A. lipoferum* 4B whereas the growth of *A. brasilense* NO40 was inhibited. Thiobencarb also had a negative effect on the growth of aseptically grown rice plantlets. When rice plantlets were inoculated with *Azospirillum* strains in a gnotobiotic experimental model, the acetylene reduction activities measured under different thiobencarb concentrations were similar to the control without the herbicide. However, at 8 ppm, the acetylene reduction activities for both *A. brasilense* NO40 and *A. lipoferum* 4B increased significantly. In field experiments, the effect of inoculation with *A. brasilense* NO40 on yield increase was tested at thiobencarb rates of 0, 4.76 and 7.14 l ha^{-1}. Bacterial inoculation resulted in significant increases in nitrogen content and grain yield in one season (1990) both in the presence and absence of thiobencarb, indicating that the herbicide had no negative effect on the bacteria. Inoculation of rice with *Azospirillum* spp. therefore seems compatible with the herbicide treatment.

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1. Introduction

The importance of heterotrophic nitrogen fixation in paddy soils has been demonstrated by many investigators (MacRae and Castro, 1967; Yoshida and Ancajas, 1973; Rao, 1976; Charyulu and Rao, 1978; Omar et al., 1989a). Inoculation of rice with the nitrogen-fixing bacteria *Azospirillum* has shown positive effects (Heulin et al., 1991, Omar et al., 1989b; Charyulu et al., 1985). The influence of pesticides on microbial activity in pure culture and in soils with mixed populations has been reported (Mackenzie and MacRae, 1972; Wood and MacRae, 1974; Charyulu and Rao, 1978; Haahtela et al., 1988; Martinez-Toledo et al., 1988), however, the effect of pesticides on *Azospirillum* spp. has not been extensively studied. In Egypt, thiobencarb is widely used in controlling weeds in rice fields. The present study was the first to demonstrate the effect of thiobencarb on the activity of two strains of *Azospirillum* under both laboratory and field conditions.

2. Materials and Methods

Laboratory experiment

Effect of thiobencarb on Azospirillum *in pure cultures*

The bacterial strains used were *A. brasilense* strain NO40 isolated from an Egyptial soil (Omar et al., 1989a) and *A. lipoferum* strain 4B isolated from a French rice-field (Thomas-Bauzon et al., 1982). Both strains were isolated using the spermosphere model from the rhizosphere of rice (Thomas-Bauzon et al., 1982). The effect of different concentrations of thiobencarb (0, 2, 4, 8 and 20 ppm) on *Azospirillum* populations was determined by plating serial dilutions of washed *Azospirillum* cell suspensions containing 3×10^9 (strain NO40) to 5×10^7 cfu (colony forming unit) ml^{-1} (strain 4B) on nutrient agar.

Effect of thiobencarb on aseptically grown rice seedlings

The experiment was conducted using the spermosphere model (Thomas-Bauzon et al., 1982). Rice seeds (cv Giza 172), surface sterilised as previously described (Omar et al., 1989a), were pregerminated in the dark at 28°C. When the coleoptile length reached 1 cm, seedlings were transferred aseptically into test tubes containing C and N-free semi-solid (0.5% agar) mineral medium (Omar et al., 1989a). Different concentrations of thiobencarb (0, 2, 4, 8 and

20 ppm) were added and the tubes incubated in the dark in a growth chamber for 1 week at 28°C, after which shoot and root lengths and dry weights were recorded.

Effect of thiobencarb on nitrogen-fixing efficiency

After transferring the plantlets into tubes containing the semi-solid medium, 250 μl of a 10^8 cfu ml^{-1}, washed *Azospirillum* cell suspension (final concentration: 10^7 cfu ml^{-1}) were inoculated. Tubes were closed with suba-seal and 1% (v/v) acetylene (C_2H_2) and an equal amount of propane (C_3H_8 which acted as tracer) were injected. Tubes were incubated in the dark in a growth chamber for one week at 28°C after which the amount of C_2H_2 reduced to ethylene (C_2H_4) was measured by gas liquid chromatography (Pay-Unicum). Nitrogenase activity was measured by estimating the maximum nitrogenase activity (Rmax), (Heulin et al., 1989). Nitrogen content of the rice seedlings was measured by the micro-Kjeldahl method.

Field experiments

Two field experiments were conducted in the Nile delta on an alluvial clay soil at Sakha Research Station in Egypt, during two successive cropping seasons in 1989–1990. The effect of inoculation with *A. brasilense* NO40 on rice was tested at three levels of thiobencarb: 0 (weeded by hand), the recommended dose of 4.76 l ha^{-1}, and 7.14 l ha^{-1} (1.5 times the dose recommended by the Egyptian Ministry of Agriculture). Each experiment included six treatments in four 14 m^2 replicate plots. Randomized complete block design was used. Inoculation of rice was done as described previously (Omar et al., 1989b). Bacteria were grown to maximum density (10^9 cfu/ml) in nutrient broth. Seed bags were soaked in water for 24 hr then kept warm for another 24 hr. Germinated seeds were then put overnight in large basins in contact with bacterial suspension. Inoculated seeds received about 10^6 cfu/seed. Nurseries were sown on the next morning. At transplanting time, seedlings were inoculated again by dipping their roots overnight in a bacterial suspension. Nitrogen was added at the rate of 96 kg N ha^{-1} by splitting it into two equal doses of 48 kg N ha^{-1} at 30 and 60 days after transplant. Thiobencarb was applied 1 week after transplanting using a knapsack sprayer. At harvest, grain (t ha^{-1}) and nitrogen (kg ha^{-1}) yields as well as 1000-grain weights (g) were determined. Nitrogen content in the grains was measured by the micro-Kjeldahl method. Data were statistically analysed with ANOVA and Lowest Significant Difference (LSD) determined at the 5% level.

3. Results and Discussion

Laboratory experiment

Effect of thiobencarb on Azospirillum *in vitro and on rice seedlings*

In pure cultures, thiobencarb had a significant effect on the growth of *A. brasilense* NO40 (Fig. 1). With increasing levels of thiobencarb, the counts

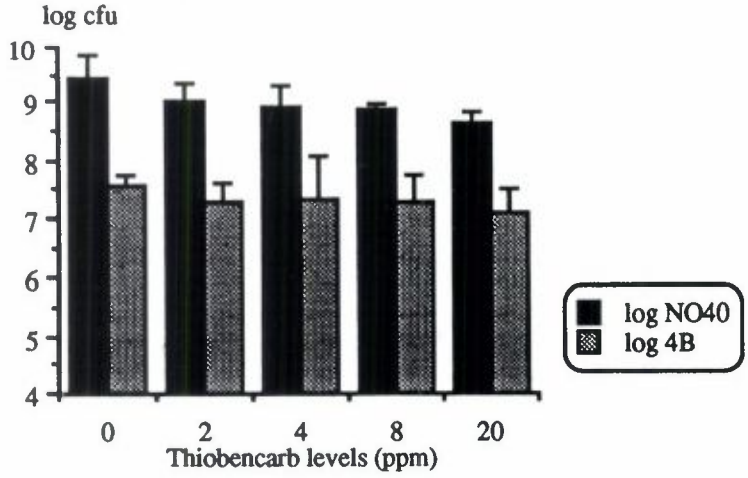

Figure 1. Effect of different thiobencarb levels on pure cultures of *Azospirillum brasilense* strain NO40 and on *A. lipoferum* 4B. Colony forming unit (cfu) were determined by plating serial dilutions of bacterial suspensions on nutrient agar. Mean ± standard deviation.

of strain NO40 decreased from 3×10^9 cfu to 4×10^8 cfu (Fig. 1). On the other hand, thiobencarb had no effect on strain 4B even at lower starting concentration (5×10^7 cfu). Strain 4B thus seemed to be more resistant to thiobencarb than strain NO40.

Aseptically grown rice plantlets were highly affected by thiobencarb (Fig. 2). Shoot biomass decreased significantly with increasing thiobencarb levels (Fig. 2a). Shoot and root lengths also decreased significantly with increasing levels of thiobencarb (Fig. 2b). Roots were less sensitive than shoots to thiobencarb under the conditions used.

Effect of thiobencarb on nitrogen-fixing efficiency

The nitrogenase activity of both strains increased significantly with respect to the control at the thiobencarb level of 8 ppm, but was not significantly different from the control at the 2, 4 and 20 ppm dose (Fig. 3). Nitrogenase activity was higher with *A. brasilense* NO40 than *A. lipoferum* 4B, but this

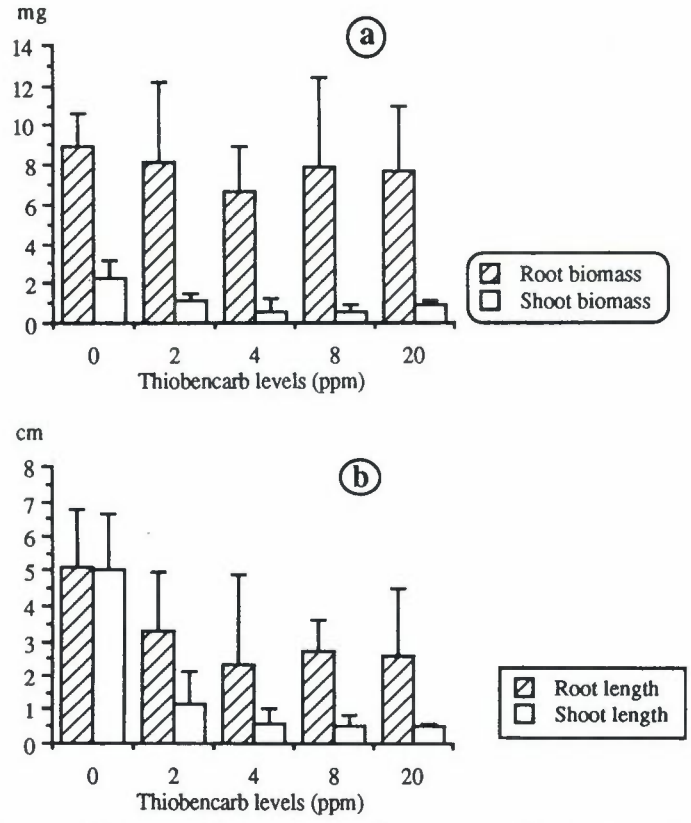

Figure 2. Effect of different thiobencarb levels on root and shoot lengths (Fig. 2a) and biomass (Fig. 2b) of rice seedlings (cv Giza 172). Plantlets were grown aseptically in the spermosphere model. Mean ± standard deviation.

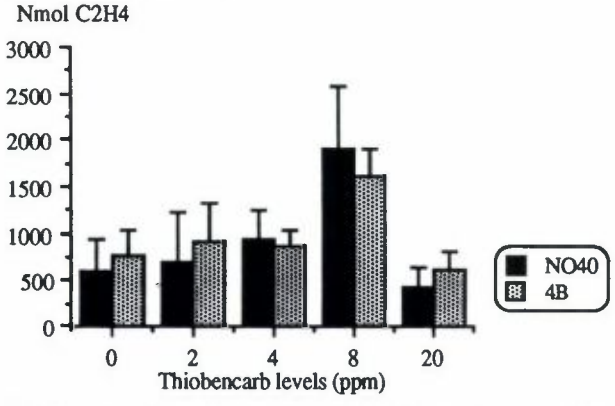

Figure 3. Effect of different levels of thiobencarb on nitrogenase activity (Nmol $C_2H_4 \times tube^{-1} \times day^{-1}$). *Azospirillum brasilense* NO40 and *A. lipoferum* 4B were inoculated to rice seedlings (cv Giza 172) in a spermosphere model. Mean ± standard deviation.

was not significant. There seemed to be an optimum concentration for the stimulatory effects of the herbicide, being between 4 and 20 ppm. This was also confirmed by the results on nitrogen content of seedlings (Fig. 4). The

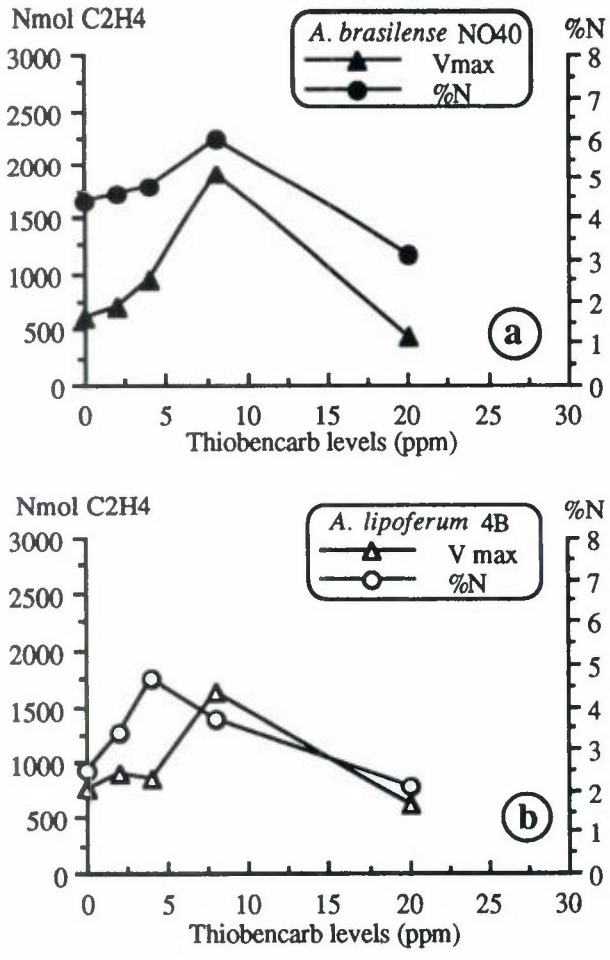

Figure 4. Effect of different levels of thiobencarb on nitrogen content of rice seedling (cv Giza 172) inoculated with *Azospirillum brasilense* NO40 (Fig. 4a) and *A. lipoferum* 4B (Fig. 4b) in a spermosphere model.

optimal thiobencarb level was 8 ppm for strain NO40 (Fig. 4a), and 4 ppm for strain 4B (Fig. 4b).

These results showed that thiobencarb affected both the growth of *Azospirillum* in pure culture and also the aseptically grown rice plantlets.

Table 1. Effect of *Azospirillum* inoculation and herbicide thiobencarb on grain yields, nitrogen yields and 1000-grain weights of rice in field trials[a]

Thiobencarb	Grain yield t ha^{-1}				Nitrogen yield kg ha^{-1}				1000-grain weight (g)			
levels	1989		1990		1989		1990		1989		1990	
l ha^{-1}	C[c]	NO40	C	NO40	C	NO40	C	NO40	C	NO40	C	NO40
0	10.3	11.0	7.8	8.7	135	134	78	93	25.7	25.9	23.4	23.7
4.76[b]	11.3	11.4	7.7	9.0	158	166	72	104	25.7	25.9	22.9	22.6
7.14	12.3	11.0	7.9	9.4	166	121	83	103	25.6	23.8	22.8	23.8
Factors	LSD (P=5%)											
Thiobencarb	NS[d]		NS		NS		NS		NS		NS	
Inoculation[e]	NS		1.1		NS		21		NS		NS	
Interaction	NS		NS		NS		NS		NS		NS	

[a] Field trial at the Sakha Research Station, Egypt, during two successive growth seasons in 1989–1990
[b] 4.76 l ha^{-1} was the level recommended by the Egyptian Ministry of Agriculture
[c] C: control, non-inoculated
[d] NS: Not significant
[e] Inoculation with *Azospirillum brasilense* NO40

However, in association, thiobencarb had a positive effect at 8 ppm on nitrogen fixation by *Azospirillum* in the spermosphere tubes, thus suggesting it could metabolize thiobencarb in the presence of seedlings.

Field experiments

The high fertility of the Nile delta was reflected by the very high yields obtained in this station, especially in 1989 (11.2 t ha^{-1}). Yields were better in the 1989 than the 1990 season (+33%) in part because of the increase in 1000-grain weights (+11%). Inoculation with NO40 increased grain yield of rice in both seasons, but the difference was significant only in the second trial (+16.6%), (Table 1). Thiobencarb application alone, at either the recommended level or at 1.5 times the dose, increased grain yields in the 1989 trial, but not significantly. These results showed that this herbicide was as efficient as hand weeding and did not decrease grain yields. The interaction effect between thiobencarb and inoculation was not significant (Table 1). This suggests that whatever the effect of inoculation, i.e. non significant as in 1989, or significant in 1990, the use of thiobencarb as herbicide did not affect the establishment of an *Azospirillum*-rice association. Nitrogen yields in inoculated rice were significantly higher than non-inoculated in the 1990 trial but not in the 1989 trial. The use of thiobencarb enhanced the nitrogen yield but not significantly. Neither the bacterial inoculation nor the thiobencarb treatment had any effect on the 1000-grain weights during the two seasons (Table 1).

4. Conclusions

Thiobencarb herbicide is toxic for *Azospirillum* in pure cultures and to aseptically grown rice, but when in association in a gnotobiotic system or in a field situation, this herbicide had no negative effects on either the bacterial activity or the rice yield. It may be concluded that the use of thiobencarb seems to be compatible with bacterial inoculation of rice cultivated under conditions of high soil fertility.

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