Influence of Different Pesticides on Azospirillum brasilense and Azotobacter chroococcum and Microbial Processes Related to the Mechanism of Detoxification

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In the last years the use of pesticides in agriculture has stimulated the study of the effects of these compounds on important soil microorganisms as nitrogen-fixing bacteria, that are able to biofertilize the soil and the culture /SIMON-SYLVESTRE and FOURNIER, 1979; DAVISON, 1988/. Nevertheless, little information is available on the mechanism of action, the fate and the detoxification processes of agrochemicals in the bacterial cell.

Studies mainly done on eucaryotic cells suggest a possible involvement of thiol compounds /as glutathione, cysteine, etc./ in antagonizing the toxicity of pesticides /SISLER, 1982; SOMERVILLE, 1986/. Recent researches on A. brasilense have indicated that glutathione and glutathione-transferase activity appeared essential to confer the resistance to fungicide captan /GALLORI et al., 1988/. It is known that the use of pesticides is not safe for the plants and injury may occur /SPOTANSKI and BURNSIDE, 1973/. To minimize the toxic activity of some herbicides, herbicides antidotes or „safeners" have been developed /HATZIOS, 1983; PARKER, 1983/. Different mechanisms have been proposed to explain the mode of action of safeners /LAY and CASIDA, 1976/, one of these is that antidotes protect plant by enhancing the herbicide metabolism, which involves principally the glutathione pathway /SOMERVILLE, 1986; GRONWALD, 1987/. No data are available up to now on the effect of antidotes on the bacterial cell.

The purpose of this study was to evaluate the biological effect of different pesticides /twelve fungicides, five herbicides and two insecticides/ and their combined use with herbicide antidote /1.8-naphtalic anhydride/, on the growth, nitrogenase activity and GSH content of two N_2-fixing bacteria A. brasilense and A. chroococcum.

Methods

Organisms and media. - A. brasilense Cd /ATCC29710/ was cultured in MSP medium /BANI et al., 1980/. BS medium /NEWTON et al., 1955/ was used in the case of A. chroococcum AzWT /from I.S.S.D.S/.

Chemicals: All pesticides used /listed in Table 1/ were obtained from S. Ehrenstorfer /Augsburg, FRG/ with the exception of 1.8-naphtalic anhydride /NAF/ obtained from Aldrich Chem. /U.S.A./.

Table 1

M.I.C. /µg/ml/ of different agrochemicals against A. brasilense Cd and A. chroococcum AzWt

Compound	A. brasilense	A. chroococcum
Fungicides:		
Benomyl	300	> 500
Captafol	75	75
Captan	25	75
Carboxin	100	> 150
Docloran	150	> 500
Folpet	50	500
Metalaxyl	> 500	> 500
Thiram	2.5	20
Triforine	> 500	> 500
Vinclozolin	500	> 500
Zineb	200	350
Ziram	5	50
Herbicides:		
Atrazine	> 200	> 200
EPTC	> 200	N.D.
Glyphosate	> 200	> 200
Metolachlor	> 200	> 200
2.4-D	> 200	> 200
Insecticides:		
Butylate	> 200	> 200
Pyretrine	> 200	> 200
Safeners:		
NAF	400	> 500

N.D. = Not determined.

Determination of M.I.C. of chemicals. - All the chemicals solubilized in DMSO were mixed with media to obtain plates at increasing concentrations of compounds. Bacterial growth was visually estimated after 48 h of incubation at 30 °C. Agar plates containing 0.1% /v/v/ DMSO were used as control.

Removal of pesticides toxicity by natural metabolites. - All natural amino acids, purine and pyrimidine bases and reduced glutathione /GSH/ were assayed for their ability to remove the growth inhibition by captan. Each metabolite was added, at different concentrations, to the solid medium plates containing inhibitory concentrations of captan and thiram. Bacterial growth was visually estimated after 2 days incubation at 30 °C.

GSH determination. Aliquots of 5 ml of cell culture of A. brasilense and A. chroococcum were collected at different times of incubation in the presence of different chemical compounds, boiled 3 min., centrifuged at 5000 rpm for 10 min. and the GSH determined in the clear supernatant by the method of AKERBOOM and SIES /1981/.

Nitrogenase assay. - Nitrogenase activity of whole cells was assayed by the acetylene reduction method /POSTGATE, 1972/. The cultures of A. brasilense and A. chroococcum were prepared as previously described /TURBANTI et al., 1988/ in MS semisolid and BS liquid media, respectively. Protein concentration was estimated by the method of BRADFORD /1976/.

Results and discussion

Determination of M.I.C. of different agrochemicals against A. brasilense and A. chroococcum

The M.I.C. values /μg/ml/ of different chemical agents on A. brasilense and A. chroococcum growth are reported in Table 1. Results obtained indicated that A. chroococcum is less sensitive than A. brasilense in respect to the toxic action of the compounds assayed; moreover dithiocarbamate /thiram and ziram/ and substituted phthalimide /captan, folpet and captafol/ fungicides are the more toxic compounds tested. Captan, which blocked the growth of A. brasilense and A. chroococcum at a concentration of 25 and 75 μg/ml, respectively, and thiram, which M.I.C.s are 2.5 and 10, respectively, were chosen as representatives of the two above-mentioned classes of fungicides and their activity has been further studied.

Effect of NAF addition on the growth of A. brasilense and A. chroococcum in the presence of captan or thiram

As shown in Table 1, NAF blocked the growth of A. brasilense and A. chroococcum at high concentrations /400 and >500 μg/ml, respectively/. Growth of A. brasilense and A. chroococcum in the presence of sublethal concentrations of NAF and captan or thiram, were determined on solid minimal medium. Results obtained, reported in Table 2, indicated that the combined use of NAF and each fungicide completely inhibited the growth of both micro-organisms.

Table 2
M.I.C. /μg/ml/ of differenc agrochemicals against A. brasilense Cd and A. chroococcum AzWt

Strain	Addition /μg/ml/	Growth
A. brasilense	NAF /200/	+++
	Captan /5/	++
	Thiram /1/	++
	NAF /200/ + Captan /5/	-
	NAF /200/ + Thiram /1/	-
A. chroococcum	NAF /500/	+++
	Captan /50/	++
	Thiram /10/	++
	NAF /500/ + Captan /50/	-
	NAF /500/ + Thiram /10/	-

+++ = Growth; ++ = reduced growth; - = no growth.

Ability of natural metabolites to remove the growth inhibition by fungidides

Previous studies on the ability of natural metabolites to remove the growth inhibition of A. brasilense by captan, indicated that only cysteine and glutathione were effective /GALLORI et al., 1988/; these thiols compounds were able to remove captan toxicity also in A. chroococcum, but GSH request was three times that of the fungicides, a concentration double of that used with A. brasilense /data not shown/.

Nitrogenase activity

The N_2-fixing ability of both microorganisms, determined as acetylene reduction activity /A.R.A./, in the presence of captan, thiram and NAF is shown in Fig. 1. Results obtained indicated that the combined use of sublethal concentrations of these chemicals reduced growth and A.R.A. of both cultures.

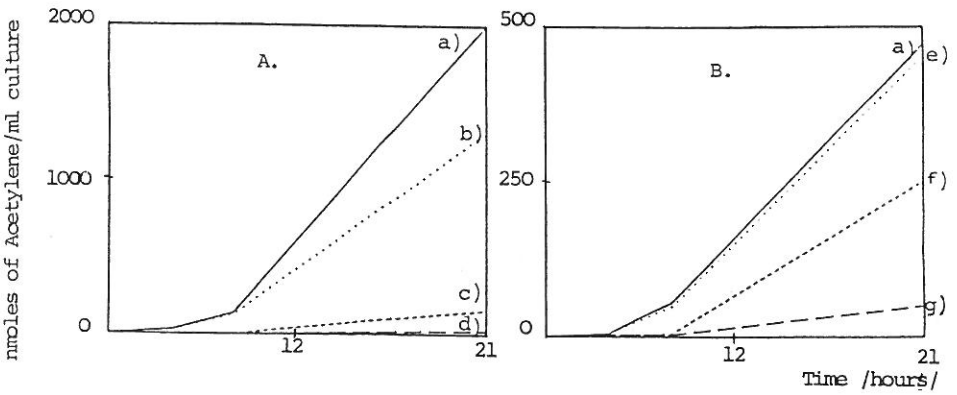

Fig. 1
A.R.A. activity of A. chroococcum AzWT /A/ and A. brasilense Cd /B/. a/ Control; b/ NAF 500 g/ml; c/ Captan 70 g/ml; d/ NAF 500 g/ml + Captan 70 g/ml; e/ NAF 200 g/ml; f/ Captan 5 g/ml; g/ NAF 200 g/ml + Captan g/ml

Determination of GSH content

Data reported in Table 3 indicated that the addition of sublethal concentration of NAF determined a GSH decrease both in Azospirillum and in Azotobacter cell cultures, without affecting the growth rate as shown by protein content. The presence of captan and thiram, at concentrations that only slightly affected the growth, determined a GSH content increase in both strains. This response was more rapid in Azotobacter than in Azospirillum; the maximum value of GSH content was at 24 and 48 h, respectively, after the addition of the fungicide. Data obtained when safener and pesticide were contemporary present in the culture, at sublethal concentrations, showed an enhancement of the toxic effect of captan and thiram on the growth of both microorganisms; the GSH content was analogously lowered.

Table 3
Glutathione and protein content of A. brasilense Cd and A. chroococcum AzWT cultures in the presence of NAF and captan or thiram

Strain	Addition /µg/ml/	GSH content /µg/mg protein/ 24h	36h	48h
A. brasilense	-	1.10 /.43/ *	0.64 /.63/	0.41 /.54/
	NAF /200/	0.00 /.25/	0.60 /.62/	0.00 /.50/
	Captan /5/	0.60 /.36/	0.90 /.42/	1.13 /.21/
	Thiram /1/	0.00 /.01/	0.30 /.05/	1.79 /.35/
	NAF /200/ + Captan /5/	0.00 /N.D./	0.00 /.01/	0.00 /N.D./
	NAF /200/ + Thiram /1/	0.07 /.01/	0.18 /.05/	0.50 /.06/
A. chroococcum	-	1.28 /.48/	2.41 /.46/	1.52 /.40/
	NAF /500/	0.55 /.51/	0.50 /.67/	0.00 /.53/
	Captan /50/	37.00 /.01/	12.70 /.04/	3.60 /.48/
	Thiram /10/	2.50 /.52/	1.00 /.38/	0.30 /.30/
	NAF /500/+Captan /50/	0.00 /.01/	0.00 /.01/	0.00 /.01/
	NAF /500/+Thiram /10/	0.10 /.03/	0.04 /.04/	0.06 /.05/

*Values in brackets represent the protein content expressed as mg/ml culture

All the data so far obtained, indicated that NAF safener, which protects crop yield against the toxic action of pesticides, acts as an inhibitor of the growth of both Azospirillum and Azotobacter cells when used in combination with captan and thiram. This inhibitory effect is also evidenced by the decrease in nitrogenase activity of both strains. If the combined use of pesticide and antidotes could have benefical effects on the crops, on the far side our results indicate a possible negative effect on some diazotrophe. The ecosystem complexicity required more detailed studies on biological effects of agrochemicals.

Summary

The effect of some pesticides /five herbicides, twelve fungicides and two insecticides/ on the growth, nitrogenase activity and glutathione content of nitrogen-fixing bacteria Azospirillum brasilense and Azotobacter chroococcum are reported. The fungicides captan and ziram appeared to be the more toxic compound assayed. Cysteine and glutathione remove the toxicity of the two fungicides when added to the medium. 1.8-Naphtalic anhydride antidote seem to enhance the fungicides toxicity when added, at sublethal concentrations, to A. brasilense and A. chroococcum cultures.

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