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Effect of Gamma-Hexachlorocyclohexane on Amylolytic Microorganisms of Soil and Amylase Activity

M. Y. Zargar,[1] B. N. Johri[2]

[1]Section of Microbiology, SK University of Agricultural Sciences and Technology, Shalimar, Kashmir 191 121, India
[2]Department of Microbiology, G.B.Pant University of Agriculture and Technology, Pantnagar 263 145, India

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Agricultural and industrial wastes and by-products are produced in abundance through various day to day operations. Carbohydrates constitute a major component of these wastes. Major quantities of such wastes ultimately find their way into soil where a vast array of microorganisms, possessing carbohydrate utilising activity, use it as a substrate (Alexander 1977). However, these microorganisms are under a constant threat of persistent pesticides that are added to the soil from time to time to control various crop pests. Both stimulatory as well as adverse effects of different pesticides on various microbial populations and their enzymatic activities have been reported (Rozsypalova 1981; Tu 1981; Rai and Srivastava 1983; Zargar and Johri 1993; Zargar and Johri 1994). Inspite of considerable work conducted on pesticide-microbe interactions, information regarding the fate of amylolytic microflora is scanty. A few reports indicate a temporary inhibitory influence of pesticides on amylolytic microorganisms and their enzymatic activities (Gray and Rogers 1954; Voets et al 1974; Mahapatra and Rao 1981). Simultaneously, other workers have not observed any deleterious effect on such microorganisms (Tu 1982; Chhonkar 1985). The present investigation was, therefore, taken to assess the effect of gamma-hexachlorocyclohexane (γ-HCH) on amylolytic microorganisms and amylase activity.

MATERIALS AND METHODS

Soil used in the present investigation was collected from a site with no reported application of pesticides and was observed to possess a water holding capacity of 51.4%; organic matter, 2.43%; nitrogen, 1.14% and pH 7.3. Soil was powdered and sieved to remove root pieces and to obtain a uniform texture. It was divided into four parts and γ-HCH was applied at 1,2 and 3 kg a.i./ha to three parts and the fourth part was kept as a control. After thoroughly mixing the pesticides with soil, 1 kg pots were filled, kept in a glass house and watered intermittently so as to maintain 50% water holding capacity. Samples were drawn at an interval of 9 days for 45 days.

Quantitative enumeration of amylolytic bacteria and fungi was carried out on a medium containing peptone 5 g, beef extract 3g, soluble starch 5g and agar agar 15g (Aaronson 1970).

Correspondence to: M. Y. Zargar

The above medium was modified for amylolytic fungi by reducing the amount of peptone to 3g and adding streptomycin at 30 ug/mL. Amylolytic bacterial and fungal populations were determined after incubation at 30$\pm$ 2°C for 1 to 2 d and 25 $\pm$ 2°C for 2 to 3 d, respectively.

During the investigation, a few bacterial colonies exhibiting a maximum area of clear zone around them, were purified and analysed for amylase production. The isolate with highest enzyme production was maintained on nutrient agar slants and through various biochemical tests, was identified to be Bacillus sp.

Effect of γ-HCH on growth of Bacillus sp. was assessed by inoculating actively growing cells in nutrient broth contained in side armed flasks. γ-HCH was added at 5,10 and 15 mg/L to three sets of flasks and incubated at 30 $\pm$ 2°C on a rotary shaker. Growth was measured in terms of absorbance on a Spectronic-20 at 495 nm at an interval of 1 hr upto 11 hr.

One hundred mL conical flasks, containing nutrient broth with 1% starch, were inoculated with activated cells of Bacillus sp. to provide 10% of inoculum density. γ-HCH at 5,10 and 15mg/L was added to these flasks. Flasks without insecticides served as a control. All flasks were incubated at 30 $\pm$ 2°C on a rotary shaker and samples were drawn every 2 hr for 10 hr. Crude enzyme was prepared by centrifuging the contents of the flasks at 5000 g for 30 min, and the supernatant was used for quantification of amylase. Enzyme assay was carried out by the method of Bernfeld (1951).

RESULTS AND DISCUSSION

The data presented in figure 1 indicate a regular increase in amylolytic bacterial population to 18 days following incubation which was followed by a decline. Reduction in the population was significant at 3 kg a.i./ha as compared to controls which could be attributed to inhibition of starch hydrolysis (Gray and Rogers 1951). However, on 45th day following incubation, significant increases in the bacterial population was noticed in the soil treated with 3 kg a.i./ha. It would appear that by this time, other soil bacteria that degrade γ-HCH to non-toxic compounds might have increased in number, thus, relieving the stress on amylolytic bacteria (Reed and Forgash 1968; Reed and Forgash 1969).

Amylolytic fungi registered a significant population reduction on the 9th day at 2 and 3 kg a.i./ha suggesting a strong fungitoxic effect of γ-HCH (fig 2). This reduction continued for 18 days at which time the fungal population in control as well as insecticide-treated soils was comparable. Isomerization of γ-HCH to its α-form appears to be instrumental in the recovery of amylolytic fungi beyond 18 days of incubation (Benezet and Matsumura 1974; Sahu et al 1990).

The isolated bacterial culture, Bacillus sp. possessed a 1.2 hr lag phase, 4.5 hr logarithmic phase and beyond this a stationery phase was observed (fig 3). γ-HCH at 5 mg/L had a marginal influence on growth of the isolate, whereas considerable inhibition was recorded at 10 and 15 mg/L. Inhibition in growth of the isolate in pure culture was significant when compared with our observations on amylolytic microorganisms in soil. This could be due to greater specificity of the insecticides in pure culture, because the

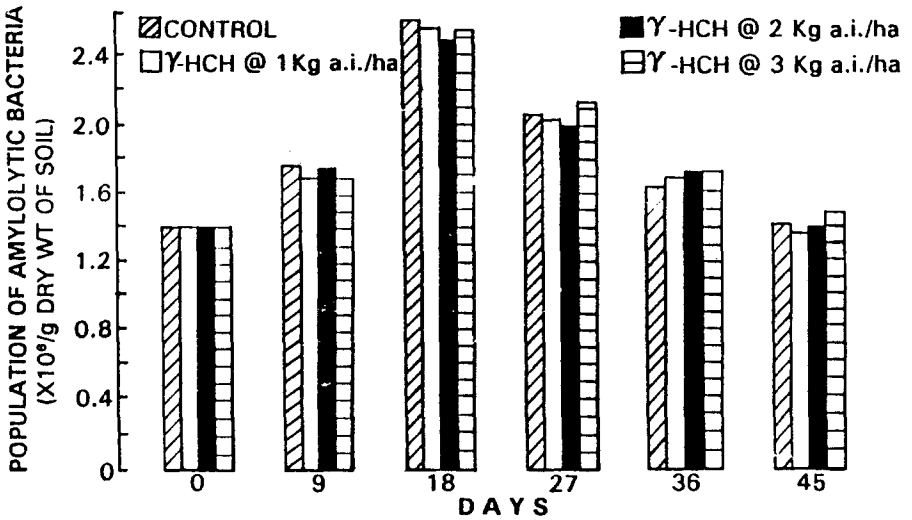

Figure 1. Effect of γ-HCH on the population of amylolytic bacteria of soil

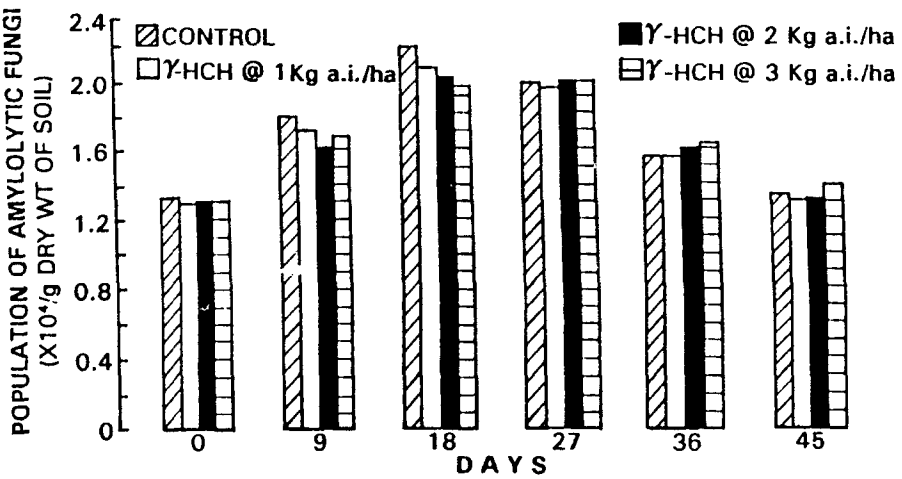

Figure 2. Effect of γ-HCH on the population of amylolytic fungi of soil

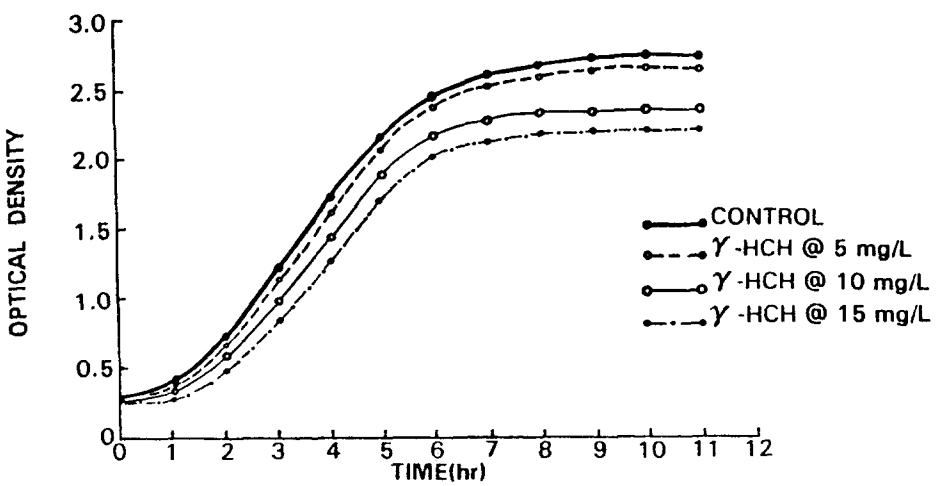

Figure 3. Effect of γ-HCH on the growth of Bacillus sp.

effective available dose is likely to be more potent when compared to a similar level in the soil due to the prevailing competition in the later case.

Pesticide-treated as well as untreated cultures exhibited maximum amylase production towards the end of log phase (table 1) and inhibition of enzyme production due to γ-HCH was concentration dependent. At 5 mg/L, the inhibitory influence was not pronounced, however, concentrations of 10 and 15 mg/L exhibited a considerable inhibition. Drastic reduction in amylase production was recorded at a concentration of 15 mg/L during the logarithmic phase where a reduction to 50% was observed. When compared with our

Table 1. Effect of γ-HCH on the production of amylase by Bacillus sp.

Time of incubation (hr)	Amylase production (µg reducing sugars/mL/min)			
	γ-HCH (mg/L)			
	0	5	10	15
2	4	3.6	3.6	3.3
4	12	10.3	8.3	6.0
6	18	16.0	15.0	13.0
8	6	6.0	5.0	4.0
10	5	5.0	4.0	4.0

earlier observations on amylolytic microorganisms, the toxic effects on enzyme production seem to be very pronounced. This could be attributed to the fact that in the later case, only one type of bacterium was under direct influence of the pesticide. However, soil possesses a consortium of microorganisms which react to the pesticide in a concerted manner. These findings corroborate with our observations on the effect of γ-HCH on pure culture and support the findings of Rozsypalova (1981) and Tu (1982).

REFERENCES

Aaronson S (1970) Experimental microbial ecology. Academic Press, New York.

Alexander M (1977) Introduction to soil microbiology. John Wiley and Sons, New York.

Benezet H J, Matsumura F (1973) Isomerization of BHC to α-BHC in the environment. Nature 243: 480-481

Bernfeld P (1951) Amylases, α-and β-. Methods in enzymology 1: 149-158

Chhonkar PK (1985) Soil enzymes, activity and agricultural significance. In: Mishra MM, Kapoor KK (ed) Proceedings of soil biology symposium, Hisar, India, pp 25-37

Gray P H H, Rogers C G (1954) Effects of benzene hexachloride on soil microorganisms. Can J Microbiol 1: 312-318

Mahapatra R N, Rao R (1981) Influence of hexachlorocyclohexane on the nitrogenase activity of rice rhizosphere. Plant and Soil 59: 473-477

Rai B, Srivastava A K (1983) Microbial decomposition of leaf litter as influenced by pesticides. Plant and Soil 74: 265-272

Reed W T, Forgash A J (1968) Lindane metabolism to a new isomer of penta-chlorocyclohexane. Science 160: 1232-1233

Reed W T, Forgash A J (1969) Metabolism of lindane to tetrachlorobenzene. J Agr Food Chem 17: 896-897

Rozsypalova Z (1981) The effect of herbicides on the enzymic activity of soil. Rost Vyroba 27: 165-171

Sahu S K, Patnaik K K, Sethunathan N (1990) Degradation of α-, β- and γ-isomers of hexachlorocyclohexane by rhizosphere soil suspension from sugarcane. Proc Ind Acad Sci (Pl Sci) 100: 165-172

Tu C M (1981) Effects of some pesticides on enzyme activities in an organic soil. Bull Environ Contam Toxicol 27: 109-114

Tu C M (1982) Influence of pesticides on activities of invertase, amylase and level of ATP in organic soil. Chemosphere 11: 909-914

Voets J P, Meerschman P, Verstraete W (1974) Soil microbiological and biochemical effects of long term atrazine applications. Soil Boil Biochem 6: 149-152

Zargar M Y, Johri B N (1993) Effect of lindane on different groups of microorganisms and soil respiration. Int J Ecol Evn Sci 19: 121-127

Zargar M Y, Johri B N (1994) Effect of γ-hexachlorocyclohexane on proteolytic microorganisms of soil and protease production. Proc Nat Acad Sci India 64(B): 65-70