Short Communications

Pakistan J. Sci. Ind. Res., Vol. 21, No. 2, April 1978

VAPORISATION AND RESIDUE OF LINDANE ON CHICKPEAS (CICER ARIENTINUM) STORED AT ABOUT 32°C IN LABORATORY

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(Received January 2, 1978; revised April 17, 1978)

Chickpeas or gram (*Cicer arientinum*) is an important source of protein in the daily diet of majority of our people in South Asia. Chickpeas under storage are, however, severely attacked by some bruchid species of insects (Coleoptera) of which *Callosobruchus analis* F. is most common in Karachi area. Parkin and Bills [1] have recommended a concentration of 1 ppm of Lindane for the protection of stored peas and beans against attack for some bruchid species. Nisa and Ahmed [2] confirmed 1 ppm concentration of lindane to be effective against *Callosobruchus analis* but only for a period of about two months under conditions of heavy cross infestation. They recommended higher dose of 5 ppm of lindane to provide a period of protection of six months and possibly more under clean conditions of storage. Dosage higher than 5 ppm may be required where the storage conditions are not very clean as is usually the case in South Asia and where a period of protection of one year or more is required. Before making such recommendations for higher dosage it will be desirable to have a first hand knowledge of the fate of lindane after application to commodities like chickpeas at temperature as are usually prevalent in our conditions. We have therefore studied residues and fate of a 0.5%-lindane dust prepared in the laboratory using fullers earth as carrier material on stored chickpeas at a temperature of about 32° up to a period of 6 months.

MATERIAL AND METHODS

A 0.5 per cent dust was prepared in the same way as reported earlier [2] adding some labelled lindane prepared (by a commercial firm) from benzene-^{14}C (U), as tracer. A 0.45-g quantity of this dust was thoroughly mixed with 100 g chickpeas in a beaker to obtain a concentration of 22.5 ppm. Treated chickpeas were transferred to a large size (1″x6″ dia) petri dish for storage at about 32°. The beaker was then rinsed with 50 ml of hexane to recover the portion of lindane dust not transferred to the chickpeas.

Samples weighing 10 g in each case were taken a different time intervals after treatment. The first sample was taken immediately and the second after 24 hr. Thereafter, samples were taken at monthly intervals for 6 months. For determining lindane dust on the surface of the seeds, each sample was first washed in 25 ml water and lindane was extracted by washing with 50 ml of hexane. The water-washed seed was then extracted with about 50 ml hexane-acetone in the ratio of 10:1 by first leaving the seed overnight in the solvent mixture and then crushing in multimixer. Some additional quantities of solvent mixture were used to compensate for losses due to evaporation. The solvent mixture was then filtered and the residue given three washings with 15 ml of the same solvent mixture. The filtrate was then washed twice, each time with 25 ml distilled water, to remove acetone from the extracts. The resultant hexane extracts were made up to 5.0 ml in volumetric flasks and 1 ml portion of each such extract was counted for radioactivity in a liquid scintillation counter. The residue of chickpeas obtained after filtration was monitored under an end-window Geiger-Muller counter to detect unextracted radioactivity (lindane) in some cases but no appreciable counts were observed.

The 0.05 g quantity of radioactive lindane dust as used in these experiments was taken up in 50 ml hexane and kept under refrigeration as standard for reference. Since all hexane extracts as well as the standard had 1 ml hexane in the counting vials, no corrections were made for the quenching effect of hexane on the liquid scintillation detection efficiency. One ml standard solution gave an average of 144 counts per minutes (cpm) per microgram lindane. The 0.45 g quantity of 0.5% dust was calculated to contain 2.25 mg of actual lindane. Of this total lindane, 0.55 mg was collected from the beaker used for treatment of seed. The actual quantity transferred to the seeds after treatment was therefore only 1.7 mg. Thus an initial concentration of 17 ppm lindane was obtained to start with instead of the intended 22.5 ppm.

RESULTS AND DISCUSSIONS

Results are presented in Table 1. Water extracts were counted for radioactivity in all cases and necessary corrections for quenching were also made but no appreciable amounts of radioactivity could be detected in the water phase. The results of radioactivity in water have therefore been ignored in the calculations. Since almost all radioactivity was encountered in the hexane phase, it is concluded that lindane did not form any water-soluble metabolites on chickpeas.

Table 1 will show that a concentration of 2.67 ppm lindane was extracted from the treated seeds immediately after application. This level rose to 4.02 ppm in 24 hr and showed only slight increase to 5.69 ppm over a period of 1 month after treatment. The amount of lindane recovered in washing correspondingly decreased rapidly from 9.09 to 3.31 ppm in 24 hr and to less than 1 ppm in all subsequent observations. This showed a rapid rate of initial peneteration which continued up to 1 month after application on lindane dust on stored chickpeas. Thereafter, there continued to be very slow but consistent fall in the level of lindane extractable from within the treated seed (Table 1). The rapid disapperance of lindane from the seed surface and its accumulation inside the seeds (extracts) is more clearly shown in the ratios of extracts/

Table 1. Recovery of radioactivity (lindane)/g of chickpeas (*Cicer arientinum*) at various time intervals after treatment with labelled lindane dust to give 17 ppm initial concentration. 144 counts/ min indicate 1 microgram lindane.

Time lapse after treatment	Radioactivity registered as counts/ min indicating lindane (ppm)		Total counts/ min/g chickpeas (ppm lindane)	Recovery %	Ratio of recovery in extracts/ washings
	In washings	In extracts			
Zero hr	1310 (9.09)	385 (2.67)	1695 (11.76)	69	0.29
24 hr	765 (5.13)	580 (4.02)	1345 (9.33)	55	0.75
1 month	130 (0.90)	820 (5.69)	950 (6.59)	39	6.32
2 months	65 (0.45)	620 (4.30)	685 (4.75)	28	9.55
3 months	57 (0.39)	575 (3.99)	632 (4.38)	26	10.23
4 months	54 (0.37)	560 (3.88)	614 (4.25)	25	10.47
5 months	35 (0.24)	475 (3.29)	510 (3.54)	21	13.70
6 months	32 (0.22)	410 (2.84)	442 (3.06)	18	12.90

washings in Table 1. The increase in this ratio clearly indicated accumulation of lindane inside the treated seed. After 1 month of initial treatment, the extractable portion (peneterated lindane) started falling but the ratio of extracts over washings continued to rise up to 5 months. This, coupled with the observation that total recoveries even at zero hour were only 69% of the total applied dose clearly indicated that the portion of lindane remaining on the seeds surface was disappearing at a faster rate compared with the portion which had peneterated into the seed. It can be argued that probably our extraction method was not efficient enough to effect complete recovery. This argument is however nullified by the fact that after the initial period of rapid disapperance of lindane from sufrace and correspondingly rapidly decreasing total recoveries up to a period of 1 month, our total recoveries were quite consistent and uniform. The rapid initial disappearance of lindane can be explained by postulating that lindane was vaporising from the seed surface. This initial vaporisation was particularly high because the seed was stored in a petri dish and at a temperature of 32°. Lindane is known to have high vapour pressure and have been used in vapour dispensers [3] in the past. The present results are therefore in conformity with this known physical property of this compound.

The postulation that lindane is vaporised rapidly from the surface of chickpeas when applied as a dust coupled with the observation that a considerable portion peneterated the seed and was less subject to such vaporisation also explained our previous experiments [2] on lindane toxicity to *Callosobruchus analis*—serious pest of chickpeas during storage. We had observed for example that lindane was 1.6 times as toxic to *C. analis* as methyl parathion when the toxicity of these compounds was compared by topical applications of a single drop directly on the head of adult insects. But when used as dust for the protection of stored chickpeas, as in the present experiments, 1 ppm concentration of lindane gave better protection of stored chickpeas against the attack of an introduced population of *C. analis* than 10 ppm concentration of methyl parathion [2]. Since the grubs of *C. analis* feed and live inside the seed chickpeas, insecticide such as lindane having high vapour pressure and peneteration would give better protection against adults which live outside the seed as well as the grubs.

In our earlier work [2] it was also argued that 5 ppm concentration of lindane is safe for human consumption assuming an average body weight of the consumer to be 50 kg and his daily requirements of chickpeas (or its split form commonly called dal) to be 100 g. This will keep the rate of his daily intake of lindane to 0.01 mg/kg which is the limit for such daily intake of this insectiside prescribed by FAO/WHO [4]. The present results indicate that even a concentration up to 17 ppm could possibly be used provided that a period of 2 months had lapsed after seeds treatment during which more than 50% of such applied dose is expected to vaporise and disappear. The need for further confirmatory experimental data is however emphasized before finalizing such recommendation.

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Pakistan J. Sci. Ind. Res., Vol. 21, No. 2, April 1975

CHANGES IN DTPA-EXTRACTABLE Zn, Cu, Fe AND Mn IN TWO ALKALINE CALCAREOUS SOILS FOLLOWED BY SUCROSE APPLICATION

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(Received February 17, 1978; revised April 23, 1978)

Micronutrient disorders have occurred in various crops grown on alkaline calcareous soils of Pakistan [1]. However, Zn disorders have been found to be more common and thus more research efforts has placed on Zn fertilization than all other micronutrient problems [1-3]. Micronutrient deficiencies have generally been attributed to their low availability and several soil conditions have been reported to promote micronutrient deficiencies in plants[4]. The reaction of our soils is alkaline and precipitation of heavy metals at these pH values seems to be an important factor which may hinder their solubilities in these soils. Total amounts may be far higher than the extractable metals but these amounts have little bearing on availability to plants. In view of widespread incidence of micronutrient deficiencies, the solubility of heavy metals in soils is becoming a subject of increasing importance. Any farm practice which increases the solubility of added or indigenous metal cations will be of great interest.

Application of farm yard manure and other organic wastes, green manuring and incorporation of crop residues in the soil are common practices followed by our farmers. The decomposition of organic manures and crop residues by microorganisms leads to the liberation of significant quantities of trace elements. In addition, many organic compounds produced in the decomposition process may bind these metals by various mechanisms[5]. Thus metals may be maintained in the soil solution through chelation whereas they would be converted to insoluble precipitates at the pH values prevalent in these soils. Moreover, reduction in soil pH due to organic acids produced in the organic matter decomposition process [6] may promote the solubilities of metal ions in soils.

The extent to which organic matter affects the solubilities of heavy metal ions have rarely been reported on the soils of Pakistan which are mostly alkaline and calcareous. The object of the present investigation was to obtain information about the effect of organic matter application on the solubilities of Zn (native plus applied), Cu, Fe and Mn (native) in two upland soils.

MATERIALS AND METHODS

The experiment was carried out in the laboratory. Two soils (0-15 cm) differing in texture were collected from Thikriwala and Kamalia towns of Faisalabad district. They were air-dried and ground to pass through a 2-mm mesh plastic sieve. Physicochemical characteristics of the soils used have been detailed elsewhere [7]. Twenty-five g portions of the soils for each treatment were taken in flat-bottomed plastic vessels. The treatments in triplicate consisted of 0, 2.5, 5.0 and 10.0 ppm Zn as $ZnSO_4.7H_2O$ and 0, 2, 4 and 8 ton/ha organic matter as sucrose. Sucrose and Zn salt were applied as aqueous solutions by adjusting the moisture level of the soil in each vessel to 75%of its field capacity. The soils maintained at this moisture level were incubated at $30\pm1°$ for 13 days— a period found sufficient for maximum fixation of Zn (data not shown). At the end of the incubation period, the soil samples were extracted with 0.005*M* DTPA (diethylenetriaminepentaacetic acid) and the concentration of Zn, Cu Fe and Mn in the soil extracts were determined by atomic absorption spectrophotometery[8].

RESULTS

Effect of Sucrose Decomposition on DTPA-Extractable Zn (Native plus Applied). Irrespective of sucrose additions, DTPA-extractable Zn of both soils increased with an increase in Zn rate ($P<0.01$, Table 1). Addition of sucrose affected extractable Zn of both soils to a lesser extent. In Thikriwala soil, Zn solubility increased slightly with sucrose application. Conversely, a minor decrease in extractable Zn of Kamalia soil was noticed with addition of sugar.

Effect of Sucrose Decomposition on DTPA extractable Cu, Fe and Mn (Native). DTPA-extractable Cu of Thikriwala soil remained unaffected at all levels of sucrose application. However, the highest level of sucrose added to Kamalia soil slightly increased Cu solubility. Sucrose effect on the Fe status of both the soils was quite marked ($P<0.01$). Extractable Fe in both soils increased with sucrose treatment. In both soils, the effect of sucrose at 2 and 4 ton/ha was almost equal and maximum increase in extractable Fe of both the soils was caused by the highest level of sucrose applied. The influence of sugar on Mn solubility of Thikriwala and Kamalia soil was also found to be significant ($P<0.01$ and $P<0.05$ respectively). In Thikriwala soil, 2 ton/ha sucrose application caused a nonsignificant increase in Mn while the increase caused by the application of sucrose at 5 ton/ha was quite considerable. A drastic increase in the extractability of this metal was recorded at the highest level of sucrose added.

DISCUSSION

The results of the present study indicated that the levels of organic matter employed in this study have little or no effect on Zn or Cu status of the soils. However, the decomposition of sucrose seems to have important practical considerations as far as the status of Fe and Mn is concerned. Solubility

Table 1. DTPA-extractable micronutrients as influenced by various levels of sucrose and zinc in two soils incubated for 13 days at 30± 1°C and 75% field capacity.

Applied		0.005*M* DTPA-extractable micronutrients							
		Thikriwala soil				Kamalia soil			
Zn (ppm)	Sucrose (ton/ha)	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn
0	0	0.28	0.62	1.81	4.42	0.71	1.64	7.44	3.96
	2	0.37	0.62	2.28	5.59	0.64	1.64	8.47	4.05
	4	0.37	0.63	2.28	6.44	0.69	1.75	8.77	4.57
	8	0.39	0.66	2.72	19.18	0.65	1.78	9.52	5.04
2.5	0	1.99	0.59	1.98	4.25	2.25	1.77	8.25	4.27
	2	2.10	0.56	2.21	4.64	2.11	1.68	8.92	3.89
	4	2.10	0.59	2.49	5.72	2.10	1.83	8.33	4.61
	8	1.96	0.57	3.30	17.77	1.97	2.22	9.96	4.25
5.0	0	3.65	0.59	1.83	4.40	3.91	1.64	7.88	3.92
	2	3.78	0.63	1.97	4.76	3.95	1.64	8.62	4.08
	4	3.87	0.63	1.98	5.99	3.78	1.68	8.92	4.24
	8	3.78	0.63	2.86	29.93	3.78	1.78	9.66	4.51
10.0	0	7.18	0.62	1.97	4.13	7.33	1.78	8.02	4.02
	2	7.22	0.60	2.21	4.97	7.33	1.78	8.76	4.01
	4	7.35	0.60	2.20	6.17	7.25	1.75	9.06	4.05
	8	7.27	0.60	2.94	13.40	7.16	1.78	9.66	4.42
LSD	(0.05) (Sucrose means)	0.06	NS	0.15	1.48	0.10	0.13	0.46	0.40
LSD	(0.05) (Zn means)	0.06	0.02	0.15	1.48	0.10	0.13	NS	NS
LSD	(0.05) (Sucrose × Zn means)	NS	NS	NS	4.18	NS	NS	NS	NS

of Fe increased considerably with sucrose additions. Mn solubility in Kamalia soil, however, increased only slightly and that too was recorded only at the highest level of sucrose treatment. This was probably due to lesser changes in pH in a heavy textured soil with high buffering capacity [9]. The increase in Mn solubility of Thikriwala soil, a light textured soil, at the highest level of sucrose addition, was of a much greater magnitude. Mn extractability at this level of sucrose application was approximately five-fold higher than the soils to which no sucrose was added. The increase in the dissolution of Fe and Mn compounds by sucrose decomposition was probably brought about by the reducing state induced by activated microbes [10,11] and by a reduction in pH due to organic acid production[6].

Consequently, results of the present study revealed that the application of organic matter to these soils may not affect Zn or Cu solubilities in these soils. The effect of organic matter decomposition on Fe and Mn status of the soils seems to be appreciable as it resulted in their increased solubilities. An increase in Fe and Mn solubility by organic matter decomposition has also been reported by earlier researchers [12,13]. Such increases may in turn affect trace element nutrition of upland plants. Thus, an increase in Fe solubility may induce or accentuate Zn deficiency in upland plants by its strong antagonistic effect on Zn absorption [14-16]. Mn salts have been found to enhance Cu absorption by upland plants [17]. Thus an increase in Mn solubility by the application of organic matter may enhance Cu absorption appreciably. The results of Somers and Shive [18] indicated that Fe and Mn are interrelated in their metabolic functions, with the effectiveness of one determined by the proportionate presence of the other. In nutrient solutions they observed typical chlorosis in soybeans due to Fe deficiency caused by higher Mn concentrations in the substrates. In the present study although extractable Fe increased in both the soils with organic matter application but an increase in Mn solubility to manifolds by liberal applications of organic matter to sandy soils may prove hazardous for Fe nutrition of upland plants.

Acknowledegments. Grateful acknowledgement is extended to Dr. S.H. Mujtaba Naqvi, Director of the Institute for providing necessary facilities for the study. Sincere thanks are due to Dr. M. Sharif, Principal Scientific Officer of this Division for his valuable suggestions and Mr. G.R. Tahir of this Institute for statistical analysis of the data.

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