Influence of Selected Pesticides on Germination and Associated Metabolic Changes in Wheat and Mung Bean Seeds

Ramesh R. Dalvi, Bharat Singh, and Dattaji K. Salunkhe*

Germination of wheat and mung bean seeds was used for bioassay to demonstrate the toxic effects of menazon (*S*-[(4,6-diamino-*s*-triazin-2-yl)methyl)] *O*,*O*-dimethyl phosphorodithioate), disulfoton [*O*,*O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate]), and GS-14254 (2-methoxy-4-isopropylamino-6-butylamino-*s*-triazine). At certain concentrations these pesticides suppressed germination and seedling growth of these species as a result of impaired respiration, starch, and protein degradation. Further studies revealed that the pesticides inhibited development of amylase and ATPase activities during germination. The effects were more pronounced in wheat than in mung beans.

In recent years concern over problems associated with pesticide use has often been expressed. Substantial contribution to the residues of pesticides that mainly include insecticides and herbicides derives from spraying and seed dressing. Although protection of seeds and seedlings is the prime aim of seed treatments, secondary effects on seed germination are more likely to occur from seed treatment as well as from accumulated residues resulting from the repeated use of pesticides.

It is now realized that, depending on their concentrations, soil residues of *s*-triazine herbicides (Holly and Roberts, 1963; Sheets and Shaw, 1963; Ercegovich, 1965) may cause damage to the germinating seeds in the soil. GS-14254 (2-methoxy-4-isopropylamino-6-butylamino-*s*-triazine) is a member of the *s*-triazine family and is considered to have residual phytotoxicity due to the presence of methoxy group (Harris *et al.*, 1968). Unlike organochlorine insecticides, organophosphates are relatively less persistent but can remain in the soil for several months (Edwards, 1965). As a replacement for persistent chlorinated hydrocarbons, they are finding increased use for protection of crops. However, they appear to be more phytotoxic especially when used as seed dressing (Scopes, 1969). Guyer *et al.* (1958) found that phorate, an organophosphate insecticide, when used as a seed treatment, adversely affected wheat germination. Gifford and his associates (1959) also noted that wheat germination was reduced and seedling survival was lower due to phorate treatment. From the relevant literature, it appears that there is a paucity of information about the mode-of-action studies on these pesticides in seeds or seedlings. Therefore, the studies reported herein were initiated to investigate the toxic effects of two insecticides (menazon and disulfoton) and a herbicide (GS-14254) on such parameters as rate of germination, seedling growth, respiration, degradation of food reserves, and development of α-amylase (amylase), adenosine triphosphatase (ATPase), and protease activities in the seeds of wheat and mung beans.

MATERIALS AND METHODS

Chemicals. Menazon (*S*-[(4,6-diamino-*s*-triazin-2-yl)-methyl] *O*,*O*-dimethyl phosphorodithioate) was obtained from Imperial Chemical Industries Ltd., Haslemere, Surrey, England. Disulfoton (*O*,*O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate) was procured from Chemagro Corporation, Kansas City, Mo. GS-14254 was received from Geigy Agricultural Chemicals, Ardsley, N.Y. Triton B-1956, a surfactant, was secured from Rohm & Haas Co., Philadelphia, Pa.

Germination. Mung beans (*Phaseolus mungo* L.) and wheat (*Triticum aestivum* L. cv. Lemhi) seeds were purchased from a local market. Uniform-sized seeds were soaked in running tap water for 1–2 hr and surface-sterilized by immersion in a 10% (v/v) water-diluted solution of Clorox for about 10–15 min. The seeds were stirred occasionally, rinsed thoroughly in distilled water, and then placed (ten seeds per petri dish of 5 cm diameter) on a double layer of filter paper. Menazon (0–250 ppm), disulfoton, or GS-14254 (0–100 ppm) in 0.02% Triton B-1956 or 2% DMSO solution in water was added to each petri dish. Triplicate petri dishes were placed in a humid dark germinator at 30°C.

The percent germination and length of root and shoot were determined after a period of 120 hr.

Respiration. After 24, 48, and 72 hr germination periods, both treated and untreated seeds were placed in Warburg flasks with 1 or 2 ml of distilled water to keep the respiring seeds moist. The oxygen uptake by the germinating seeds per unit of time was measured on a Gilson respirometer.

Chemical Constituents. Starch, reducing sugars, and soluble amino acids in the germinating seeds were determined using the same procedure as reported previously (Dalvi *et al.*, 1971).

Enzyme Extracts and Assays. The initial steps of preparing enzyme extracts from the germinating seeds were essentially the same as described elsewhere (Dalvi *et al.*, 1972). The enzyme assays are briefly outlined herein.

Amylase activity was measured by adding 1.0 ml of the enzyme preparation to 1.0 ml of 1% potato starch solubilized in 0.016 *M* acetate buffer (pH 4.8) and incubating the mixture at 25°C for 5 or 10 min. The increase in reducing power was determined by addition of dinitrosalicylic acid (Bernfeld, 1955). The specific activity was defined as mg of maltose produced per mg of protein in 10 min.

ATP-hydrolyzing phosphatase activity was estimated by incubating 1.0 ml of the enzyme preparation with 0.033 *M* Tris buffer (pH 7.3), 0.001 *M* $MgCl_2$, and 0.004 *M* ATP in a total volume of 3.0 ml at 38°C for 15 min. The reaction was stopped by the addition of 1.0 ml of 15% TCA, and the phosphate released was determined by the method of Sumner (1944). The specific activity was defined as μg of Pi formed per mg of protein in 15 min.

Interdepartmental Program in Toxicology, Utah State University, Logan, Utah 84321.

Table I. Influence of Pesticides on Germination of Seeds and Seedling Growth during 120 hr

Pesticides	Concentration, ppm	Percentage inhibition of control: Germination, Mung beans	Germination, Wheat	Root growth[a], Mung beans	Root growth[a], Wheat	Shoot growth[a], Mung beans	Shoot growth[a], Wheat
Menazon	50	10	3	20	16	24	14
	100	33	7	13	22	41	19
	200	67	40	64	46	77	21
	250	70	57	60	66	79	45
Disulfoton	20	0	3	8	16	9	14
	40	10	7	11	24	18	17
	80	12	10	12	37	33	29
	100	20	7	21	36	29	31
GS-14254	10	0	30	19	23	11	34
	20	0	33	24	70	17	61
	50	7	44	48	76	49	61
	100	50	74	56	79	69	80

[a] Root and shoot growth based on the length measurement of the germinated seeds following the treatment.

Proteolytic activity was determined by adding 2 ml of the enzyme preparation to 3 ml of 2% casein adjusted to pH 7.1 in 0.067 *M* phosphate buffer. The incubation was made at 38°C for 1 hr. For each sample, duplicate tubes plus a zero time control were prepared. A 2.0-ml aliquot was pipetted into 2.0 ml of 15% TCA, filtered, and the increase in optical density (OD) was measured at 280 mμ in a 1-cm cuvette. The proteolytic activity was defined as that amount of enzyme which, in 1 hr, caused an increase of 1.0 in o.d. of the filtrate at 280 mμ. The specific activity was expressed as units of proteolytic activity per mg of protein. In all cases, the activities of enzymes were based on the protein content of the enzyme extract determined by the method of Lowry *et al.* (1951).

The data were subjected to an analysis of variance and the means were compared by the least significant difference (LSD) method at 5% level (Steel and Torrie, 1960).

RESULTS

In the text, reference to "significance" is to be taken to mean a significant difference compared to untreated control at the 0.05 level.

It is evident from Table I that at various concentrations menazon, disulfoton, or GS-14254 were not only able to inhibit the germination of mung beans and wheat seeds but also were considerably effective against seedling growth. It was also noted that the seedlings surviving the pesticide treatment were distorted and weak. The degree of inhibition of germination of seeds and seedlings development depended on the concentration of the chemicals.

At their maximum concentrations, menazon, disulfoton, and GS-14254 significantly blocked the respiration of the germinating seeds at the end of 72 hr after treatment (Figure 1). However, it is interesting to note that the respiration of germinating wheat seeds was considerably more affected than that of the mung beans in all cases.

Compared to the control seeds, these pesticides in treated seeds caused significant reductions in the amounts of sugars and amino acids after 72 hr of germination period (Figure 1). It is also seen that the rate of starch degradation in seeds treated with the pesticides was significantly lower than that in the control. It is also apparent from the results that a considerably less amount of reducing sugars and amino acids was

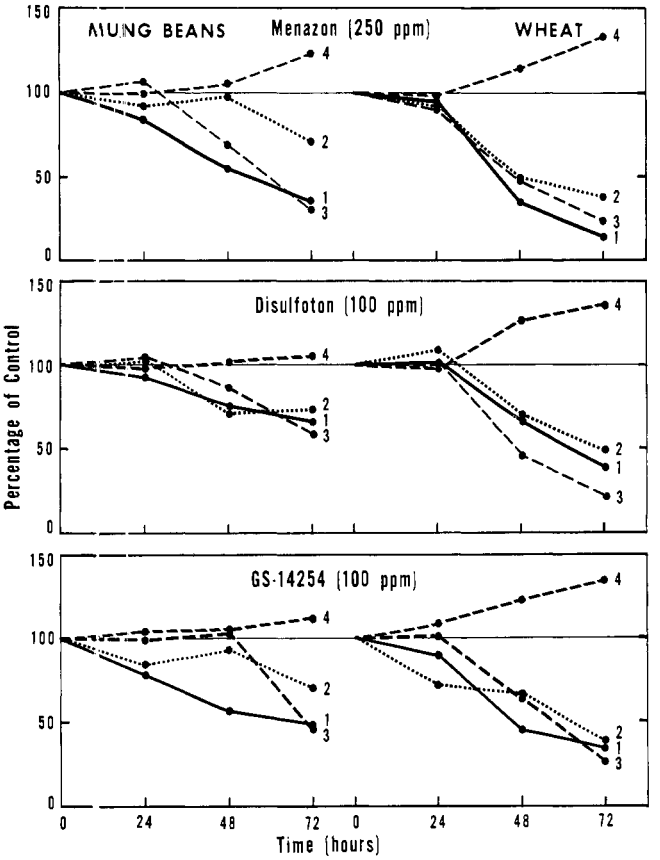

Figure 1. Effect of menazon, disulfoton, and GS-14254 on the rate of respiration (1), and the content of free amino acids (2), reducing sugars (3), and starch (4) of the germinating seeds of mung beans and wheat

formed in the treated wheat seeds than in the mung beans, as compared to their respective controls.

The effects of menazon, disulfoton, and GS-14254 on amylase, ATPase, and protease of the germinating seeds are illustrated in Figures 2, 3, and 4, respectively. It is evident that the development of amylase activity in the seeds treated with the optimum concentrations of the pesticides tended to be lower than that in the control seeds. The adverse effect of menazon on the development of amylase activity was more pronounced than that of disulfoton and GS-14254.

As germination progressed after 24 hr, it was observed that the increase in ATPase activity was considerably lower in the treated seeds than in the untreated controls at the maximum concentration of the pesticides. In contrast, the development of proteolytic activity in control and disulfoton- and menazon-treated seeds was not significantly different during the germination period. However, in the seeds treated with GS-14254, it was considerably lower at 72 hr germination period.

DISCUSSION

Suppression of germination and subsequent growth by the pesticidal treatments indicates that some of the biochemical processes taking place during germination are impaired. Adverse effects of pesticides on seed germination have been demonstrated by several workers (Scopes, 1969; Gifford *et al.*, 1959; Penner, 1968). Our results also indicated that various concentrations of menazon, disulfoton, and GS-14254 inhibited germination of mung beans and wheat seeds to varying degree. Scopes (1969) also found that 250 ppm of menazon and 100 ppm of disulfoton inhibited germination of wheat, field beans, and sugar beet seeds.

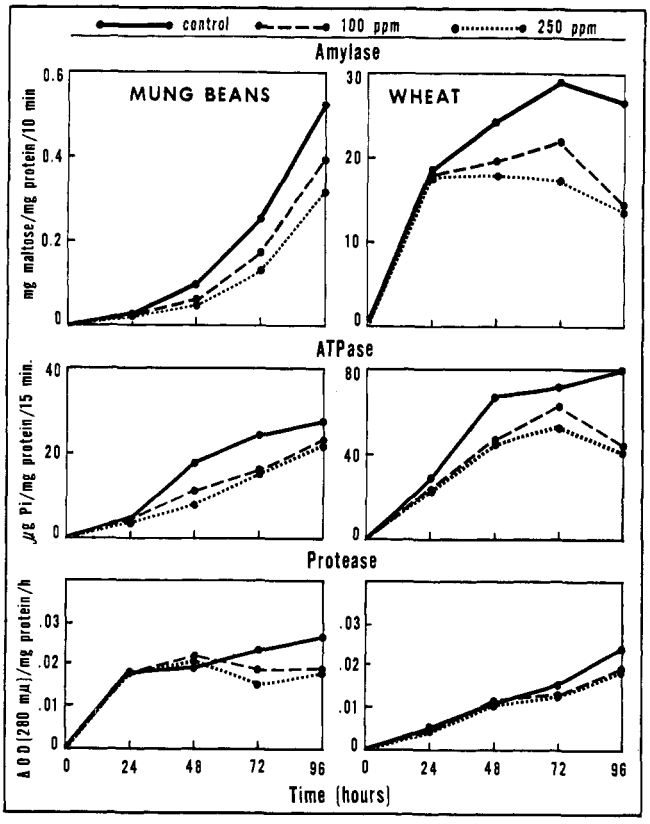

Figure 2. Effect of menazon on the amylase, ATPase, and protease activities during germination of mung bean and wheat seeds

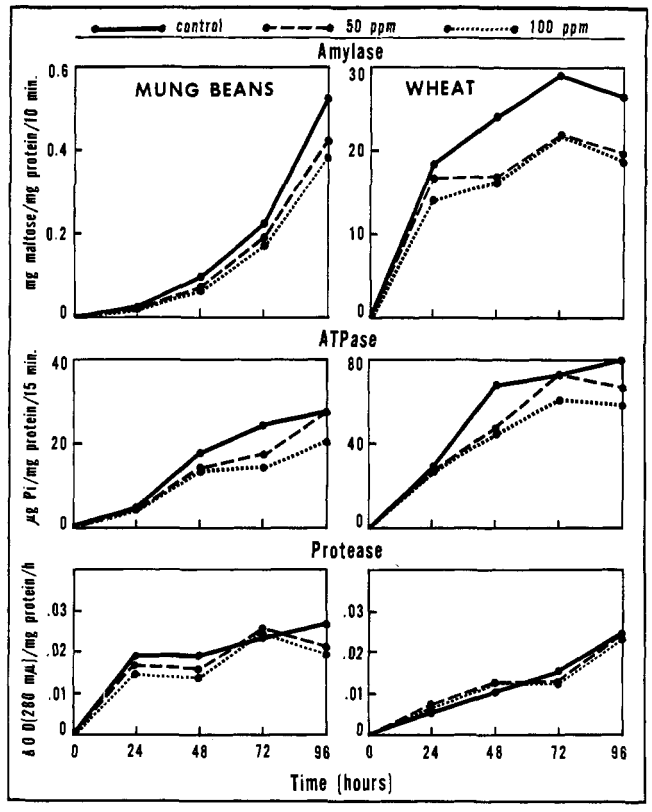

Figure 3. Effect of disulfoton on the amylase, ATPase, and protease activities during germination of mung bean and wheat seeds

Chemical analysis of the control and the treated seeds in this study indicated that there was significantly less formation of reducing sugars and free amino acids in the latter at the end of the 3-day germination period. These results are in agreement with the findings of Chopra and Nandra (1969), who reported a decrease in reducing sugars in germinating mustard seeds treated with Thiometon (*O,O*-dimethyl-*S*-ethylmercaptoethyldithiophosphate) and they attributed it to the inhibition of lipase activity in the seeds. In our results this might be related to the less starch degradation in the treated seeds.

The reduction in the rate of respiration of germinating seeds treated with the pesticides evidently suggests the blocking of biochemical processes essential for the supply of energy to the growing embryo. Similar results were noted by Wassink and van Elk (1961) where CIPC (isopropyl *N*-3-chlorophenylcarbamate) inhibited the rate of respiration of germinating pea seeds. Many other herbicides have also been reported to inhibit respiration when applied to the plants (Bishop, 1958; Simon, 1953).

The data concerning the effect of the pesticides—menazon, disulfoton, and GS-14254—on development of the activity of hydrolytic enzymes show that these pesticides inhibit amylase and ATP-hydrolyzing phosphatase activities at varying degrees in germinating mung beans and wheat seeds. Since hydrolytic enzymes such as amylase, protease, and phosphatase are produced during germination (Briggs, 1963), pesticides may have an adverse effect on their syntheses. According to Penner (1968) the inhibition of barley germination and seedling development in culture solutions containing herbicide (amiben or bromoxynil) was due to the effect of the herbicide on enzyme development or synthesis during germination. Inhibition of GA-enhanced synthesis of α-amylase by several herbicides has been reported (Jones and Foy, 1971). It seems to be likely then that the pesticides studied herein might have inhibited seed germination in a similar manner.

More pronounced inhibition of amylase activity in treated wheat seeds compared to that in mung beans may suggest the possibility that these pesticides inhibit germination by impairing degradation of carbohydrate reserves during germination since wheat seeds are dependent on starch for their energy supply. Penner (1968) noted that amiben and bromoxynil herbicides inhibited barley germination by inhibiting the degradation of storage carbohydrates but not in squash seeds, which were tolerant to the same concentrations of the herbicides. Chopra and Nandra (1969) also concluded that Thiometon insecticide slowed the breakdown of carbohydrates in germinating mustard seeds. Less inhibition of mung bean germination than that of the wheat seeds may be attributed to the comparatively large protein reserves of mung beans since (sulfhydryl groups of) proteins are known to bind and detoxify foreign chemicals in the biological systems. Alternately, certain detoxifying enzyme systems might have been more active in mung beans as a defense mechanism rather than in wheat.

In contrast to amylase and phosphatase activities in the germinating seeds treated with the pesticides, the activity of casein-hydrolyzing protease, with the exception of GS-14254, was not significantly different from that of the controls. Ashton *et al.* (1968) reported that different herbicides varied widely in their ability to inhibit increase in proteolytic activity in germinating squash seeds. Young and Varner (1959) found that the inhibitors of amylase and phosphatase syntheses did not have significant effect on proteolytic activity although *de novo* synthesis of proteolytic enzymes (Penner and Ashton, 1966) occurs in the cotyledons during germination. But the former group of workers demonstrated only small changes in

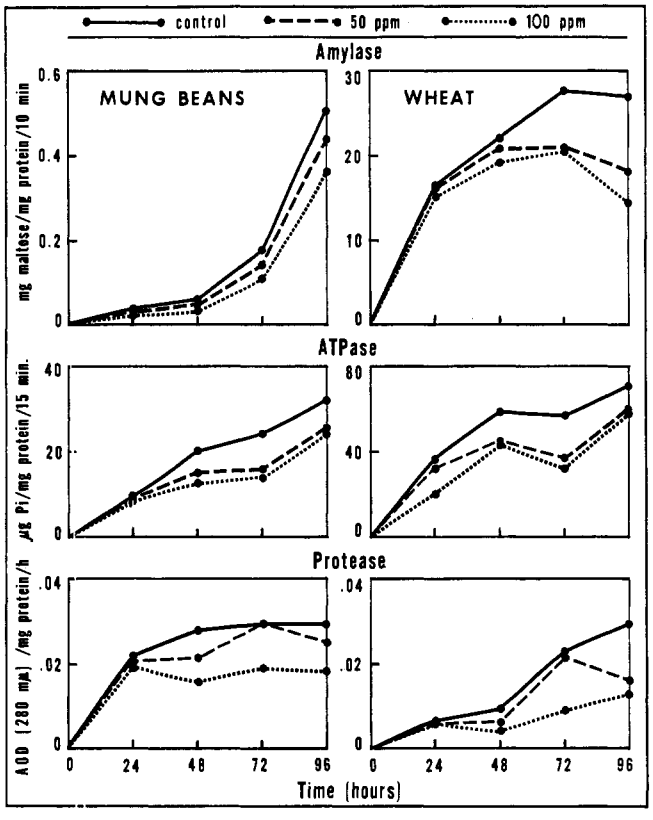

Figure 4. Effect of GS-14254 on the amylase, ATPase, and protease activities during germination of mung bean and wheat seeds

proteolytic activity as normal germination proceeded. Therefore, it is difficult to draw any conclusion regarding the effect of these pesticides on proteases of the germinating seeds.

Thus numerous factors may be involved in the inhibition of germination process by a pesticide treatment. Since the growth of both radicle and plumule in the pesticide-treated seeds was impaired, it can be predicted that the pesticides may inhibit cell division like phenylcarbamates (Sweeney and Marsh, 1971). These pesticides may interfere with protein synthesis in the germinating seeds. For instance, Litterst *et al.* (1969) reported that disulfoton affects protein synthesis in HeLa cells. Similarly, the inhibition of protein synthesis by some herbicides was reported by Mann *et al.* (1965). Synthesis of amylase in barley seeds was inhibited by inhibitors of RNA and protein synthesis (Chrispeels and Varner, 1967); therefore, these pesticides may perhaps be inhibitors of RNA and protein synthesis. The phytotoxic effects of these pesticides on oxidative phosphorylation (Ashton *et al.*, 1968) or other energy-rich compounds required for the synthesis of hydrolytic enzymes may not be ruled out in the treated seeds.

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