III RESULTS AND DISCUSSION

A. Germination Percentage, Rate of Germination and Root, Shoot Length

The effect of Metacid and Dimecron on germination percentage of seeds and rate of germination studied in bean, okra, guar and onion is given in Table 2 and Fig. 3 respectively. The response of seed germination to both the pesticidal treatment was inhibitory at higher concentration while it was stimulatory at lower concentrations. None of the Metacid concentrations were found stimulatory in action on germination percentage of onion, okra and guar over control while in bean 0.01% and 0.02% Metacid concentrations were stimulatory in action (Table 2). On the contrary, except okra in all other three the lower concentration of Dimecron showed stimulatory effect. Among the four vegetable seeds, guar appeared to be sturdy which can clearly be seen from Table 2 where it showed its tolerance limit upto 0.015% Dimecron and was not much responded to the Metacid concentrations. The rate of germination represents typical sigmoid growth curve in all the four seeds (Fig. 3). However, the germination rate observed in guar was more at all the concentrations of Metacid and Dimecron upto 72 h of germination.

The inhibition of germination due to pesticides such as Antracol and Kitazin in *Brassica nigra* (Krishnamurty and Rao, 1980), Kitazin in *Dolichos biflorus* (Reddy and Vidyavati, 1983), Endosulfan in *Vigna radiata* (Gupta *et al.*, 1983), carbaryl in *Vigna sinensis* (Sengupta *et al.*, 1988), Thiodan-35 in pea (Agrawal and Soam, 1988) and Dithane-M 45 in crop plants like jowar, green gram, bajra, pea, maize, sunflower, ragi etc. (Somashekhar and Sreenath, 1986) have been reported. Contrary to this the insecticide Sevin has found to be beneficial in promoting the root, shoot length in *Vigna radiata* with increasing

Table 2. Effect of Metacid and Dimecron on germination percentage of four different vegetable seeds after 72 h germination.

Treatment % (v/v)	Bean	Okra	Guar	Onion
Control	76 ± 3.46	90 ± 4.09	94 ± 4.27	72 ± 3.0
<u>Dimecron</u>				
0.005	78 ± 3.55	76 ± 3.46	100 ± 4.55	76 ± 3.2
0.01	78 ± 3.45	88 ± 4.0	98 ± 4.46	64 ± 2.91
0.015	80 ± 3.64	88 ± 4.0	98 ± 4.46	60 ± 2.8
0.02	70 ± 3.46	72 ± 3.4	90 ± 4.09	56 ± 2.0
<u>Metacid</u>				
0.01	84 ± 3.82	86 ±3.91	90 ± 4.09	66 ± 3.0
0.02	80 ± 3.64	84 ± 3.82	90 ± 4.1	54 ± 2.4
0.03	68 ± 3.09	84 ± 3.82	90 ± 4.09	52 ± 2.3
0.04	52 ± 2.36	84 ± 3.82	90 ± 4.09	50 ± 2.2

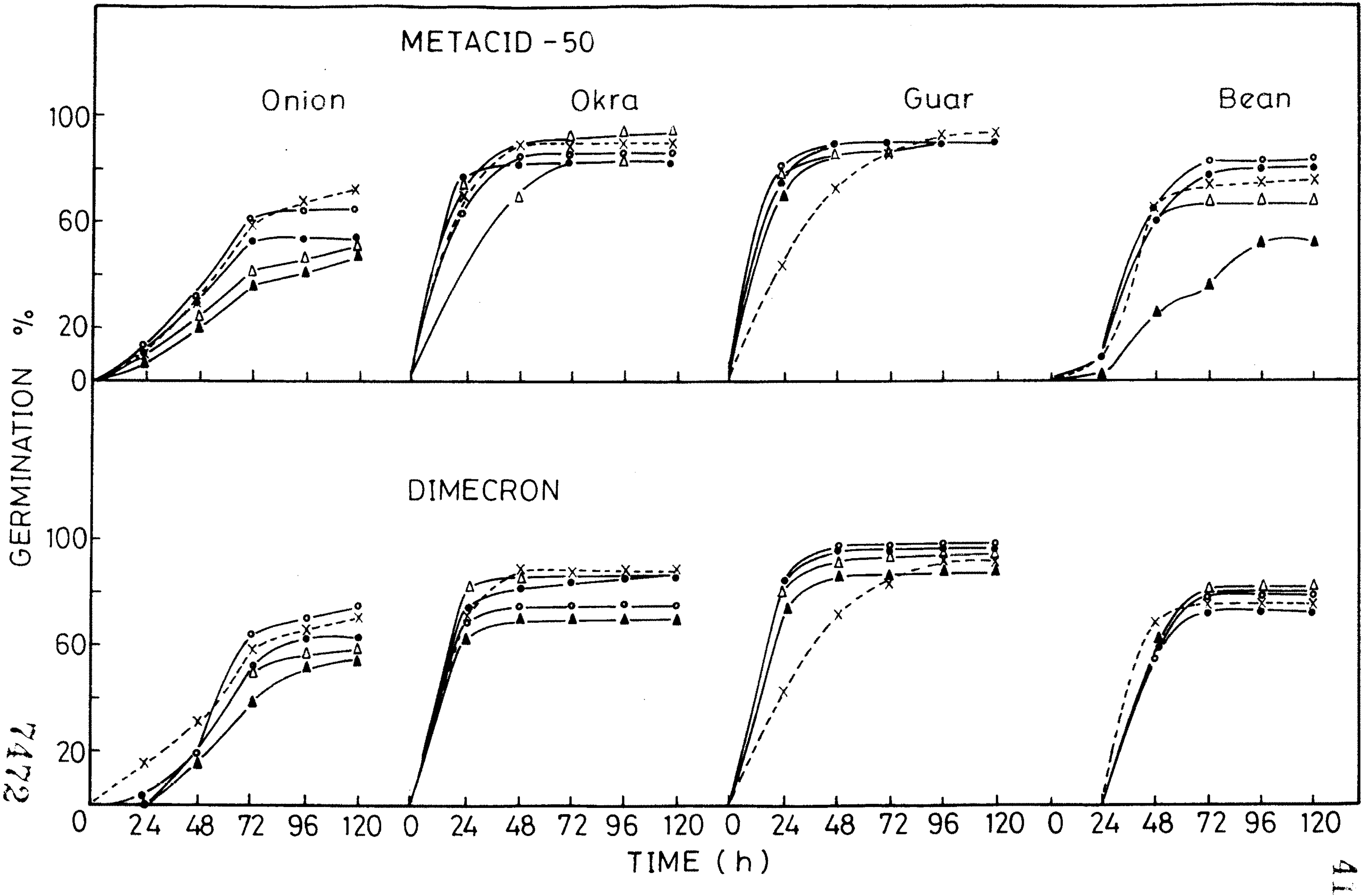


Fig. 3

7472
A

41

concentrations (Pathak and Mukherji, 1986). However, high concentrations inhibited the growth of root and shoot. According to them Sevin can be used at a concentration of 100-200 ppm. Similarly the response of seed germination varies from species to species. The treatments of Metasystox (a systemic insecticide) on seed germination of *Vigna mungo* reported to be inhibitory by Prasad and Mathur (1983), while phosphamidon (an organophosphorus insecticide) found to be stimulatory in action on seed germination of *Hordeum vulgare* (Singh *et al.* 1979).

According to Singh *et al.* (1979) generally insecticide treatments did not reduce germination but did reduce seedling height. Our results on root, shoot length of insecticide treated seeds revealed that the response of root and shoot length to insecticide differs in all the treated seeds (Fig. 4). As compared to shoot length the root length was more in all the insecticide treated seeds but it was found inhibitory with increase in insecticide concentration (Fig. 4). The shootlength was not changed much at lower concentration while inhibited at higher concentation in both Metacid and Dimecron treated seeds. However, exception is being noted in guar seeds where Dimecron favoured stimulation of root and shoot length at all the concentrations. At the same time in onion the shoot was not yet developed (Fig. 4).

B. Morphological Abnormalities

The morphological abnormalities observed during germination were photographed and presented in Fig. 5 and 6. It is very clear from the photograph that the increasing concentrations of both the pesticides *viz.* Metacid and Dimecron inhibit the seedling growth (Fig. 5). The major abnormalities observed

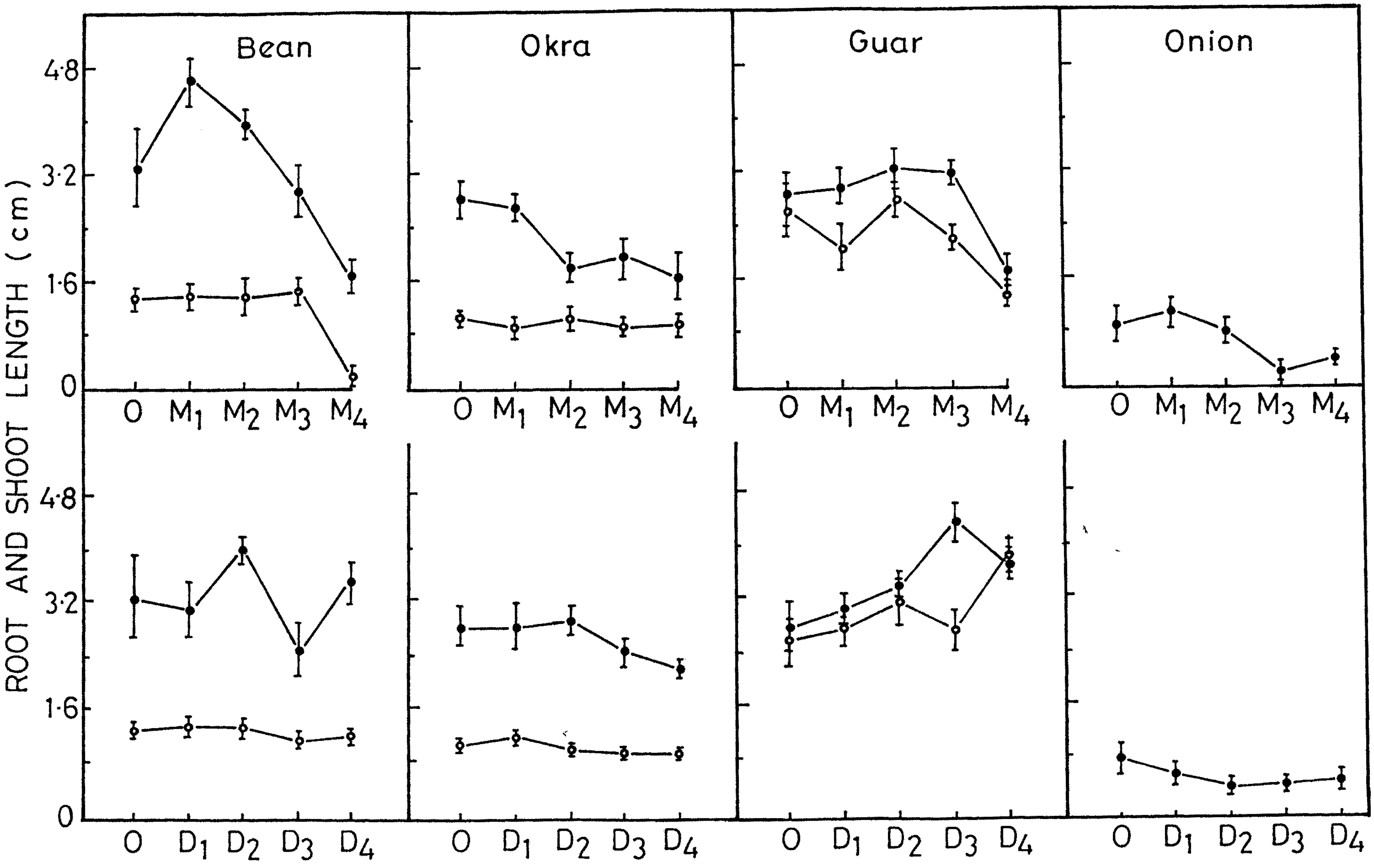
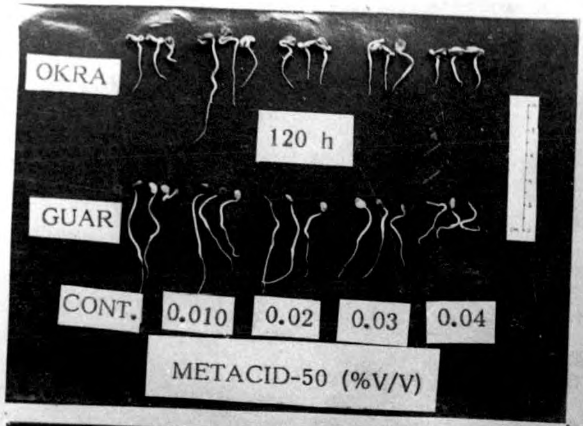

ROOT AND SHOOT LENGTH (cm)
4·8
3·2
1·6
0
Bean
Okra
Guar
Onion
O
M1
M2
M3
M4
D1
D2
D3
D4

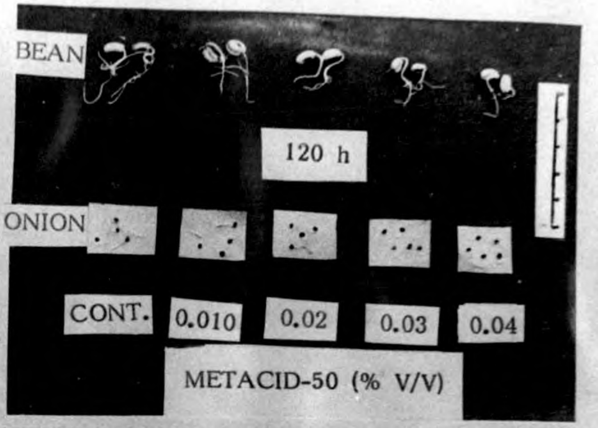
Fig. 4

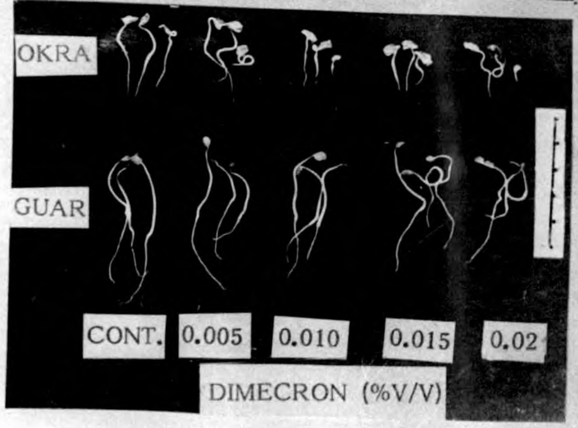
during the seedling growth were coiling, bulging and formation of constrictions in the radicle, necrosis of root tips and production of secondary roots. The coiling of the radicle was observed mainly in guar (Fig. 6) and partially in bean (Fig. 5) due to both the pesticidal treatment. Another toxic effect observed in bean and guar was bulging of the root and formation of constrictions at the tip which later on cuts off (Fig. 6). This is possibly because of the weakening of the cells due to pesticidal action.

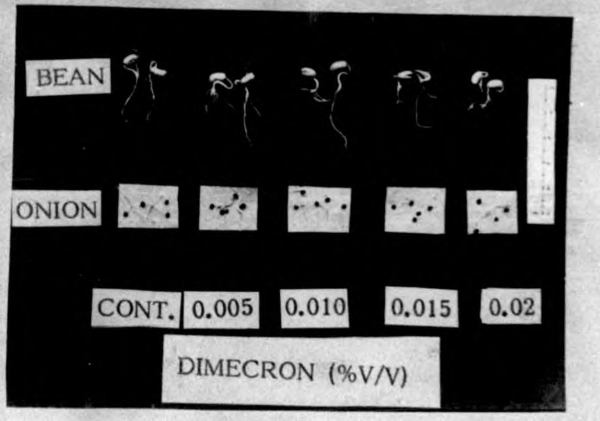
Except onion and guar both okra and bean showed formation of secondary roots when grown in different concentrations of Metacid and Dimecron. However, the formation of secondary roots was not linear in both the pesticidal treatments. In Dimecron treatment, bean showed increase in secondary roots at lower concentration and as the concentrations increased the entire seedling growth as well as lateral root development was inhibited (Fig. 5). This observation was not prominently seen in okra (Fig. 5). As against this, in Metacid treatments bean showed gradual decrease in lateral root growth with increasing concentrations. Whereas none of the concentrations of Metacid favoured formation of lateral roots in okra (Fig. 5).

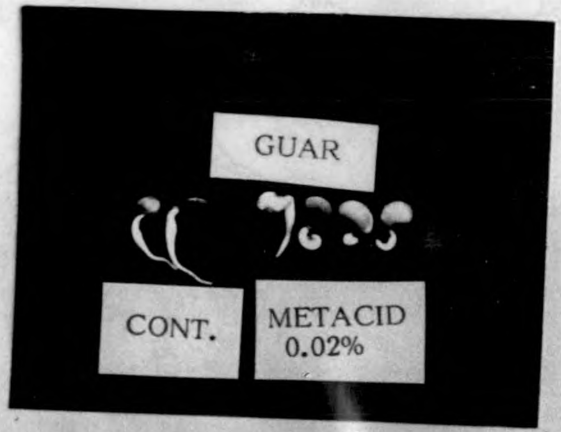
Another peculiar toxic effect observed in okra at higher concentrations of both the pesticides was sudden rupturing of seed coat and shooting of an umbrella shaped seedling (Fig. 5). This may be due to the high osmotic pressure exerted in by the pesticidal action. Apart from this blackening and cutting off of root tips were also observed at higher concentrations of Dimecron and Metacid in Allium cepa.

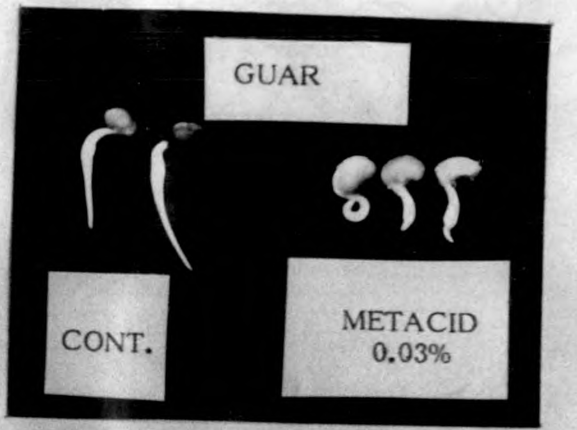

OKRA
120 h
GUAR
CONT.
0.010
0.02
0.03
0.04
METACID-50 (%V/V)

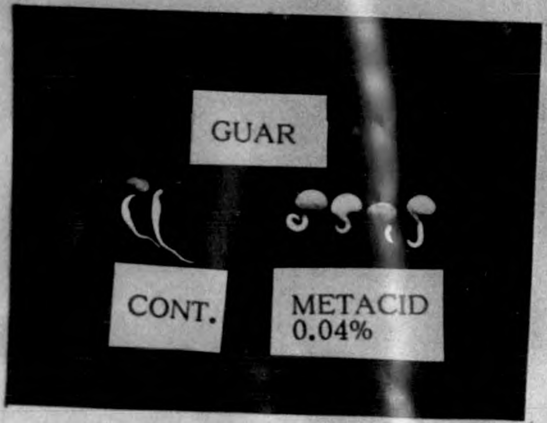

BEAN
120 h
ONION
CONT.
0.010
0.02
0.03
0.04
METACID-50 (% V/V)

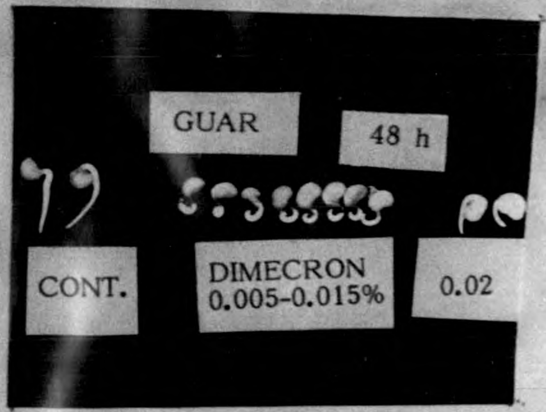

OKRA
GUAR
CONT.
0.005
0.010
0.015
0.02
DIMECRON (%V/V)


BEAN
ONION
CONT.
0.005
0.010
0.015
0.02
DIMECRON (%V/V)


GUAR
CONT.
METACID 0.02%


GUAR
CONT.
METACID 0.03%


GUAR
CONT.
METACID 0.04%


GUAR
48 h
CONT.
DIMECRON 0.005-0.015%
0.02

Gupta et al. (1983) have observed certain toxic effects such as necrosis of root tips, coiling of radicle and production of large number of lateral roots in *Vigna radiata* due to Endosulfan treatment. Rahman and Bhattacharya (1985) have also studied the effects of Sevin on *Allium cepa* and *Lathyrus sativus* and reported that necrosis and blackening of root tips were the main toxic effects induced by this insecticide. The more visible symptoms of toxicity due to Thiodan-35 on germination and growth of pea were coiling and blackening of radicle tips (Agrawal and Soam, 1988). In addition, increase in lateral root formation in *Dolichos biflorus* due to Kitazin treatment was also reported by Reddy and Vidyavati (1983). Similarly Rao et al. (1980) while working with *Abelmoschus esculentus* observed that Dithane also induces large number of lateral roots. This they have accounted for the adaptation of the seedling by way of increasing the absorbing area of the root system. But our data on *Abelmoschus esculentus* subjected to insecticidal treatment clearly indicates that the said toxic effect vary from pesticide to pesticide and species to species.

C. Effect of Pesticide Treatment on Hydrolytic Enzymes

1. Amylase

Since the enzyme α-amylase catalyses hydrolysis and solubilization of starch, its *de novo* synthesis plays a crucial role during seed germination (Ching, 1972) and hence it was studied. The treatments of Metacid and Dimecron caused considerable inhibition of the enzyme activity, however, the effect is more pronounced in Metacid rather than in Dimecron treatment (Table 3 and 4 and Fig. 7 and 8). In guar, no doubt, the amylase activity was inhibited due

Table 3. Effect of Metacid on α-amylase activity in the germinating seeds of bean, okra, guar and onion after 72 h germination.

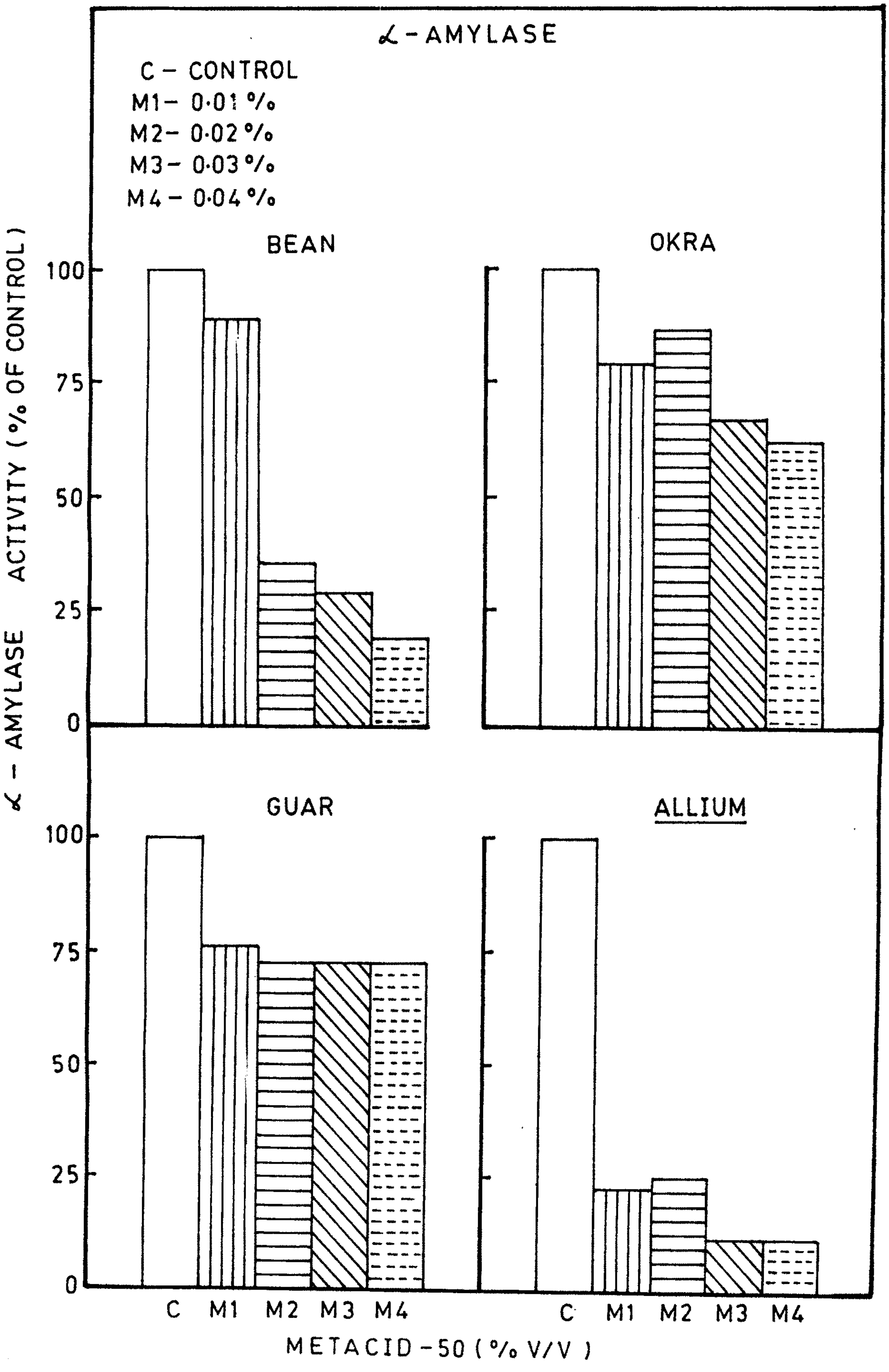
Metacid-50 % (v/v)	α-amylase ($\Delta OD_{700nm} h^{-1} g^{-1}$ fresh wt) x 10^{-2}			
	Bean	Okra	Guar	Onion
Control	7.1	4.2	2.5	7.1
0.01	6.3	3.3	1.9	1.6
0.02	2.5	3.6	1.8	1.7
0.03	2.0	2.8	1.8	0.8
0.04	1.3	2.6	1.8	0.8

Values are mean of three determinations.

Table 4. Effect of Dimecron on α-amylase activity in the germinating seeds of bean, okra, guar, and onion after 72 h germination.

Dimecron % (v/v)	α- amylase (ΔOD_{700nm} $h^{-1}g^{-1}$ fresh wt) x 10^{-2}			
	Bean	Okra	Guar	Onion
Control	7.1	4.2	2.5	7.1
0.005	5.5	4.6	3.1	3.2
0.01	6.6	4.9	2.7	3.1
0.015	6.0	3.1	2.1	2.1
0.02	2.7	1.9	1.8	1.9

Values are mean of three determinations.



∝-AMYLASE
C - CONTROL
M1- 0·01 %
M2- 0·02 %
M3- 0·03 %
M4 - 0·04 %
BEAN
OKRA
GUAR
ALLIUM
∝ - AMYLASE ACTIVITY (% OF CONTROL)
100
75
50
25
0
C
M1
M2
M3
M4
METACID -50 (% V/V)

Fig. 7

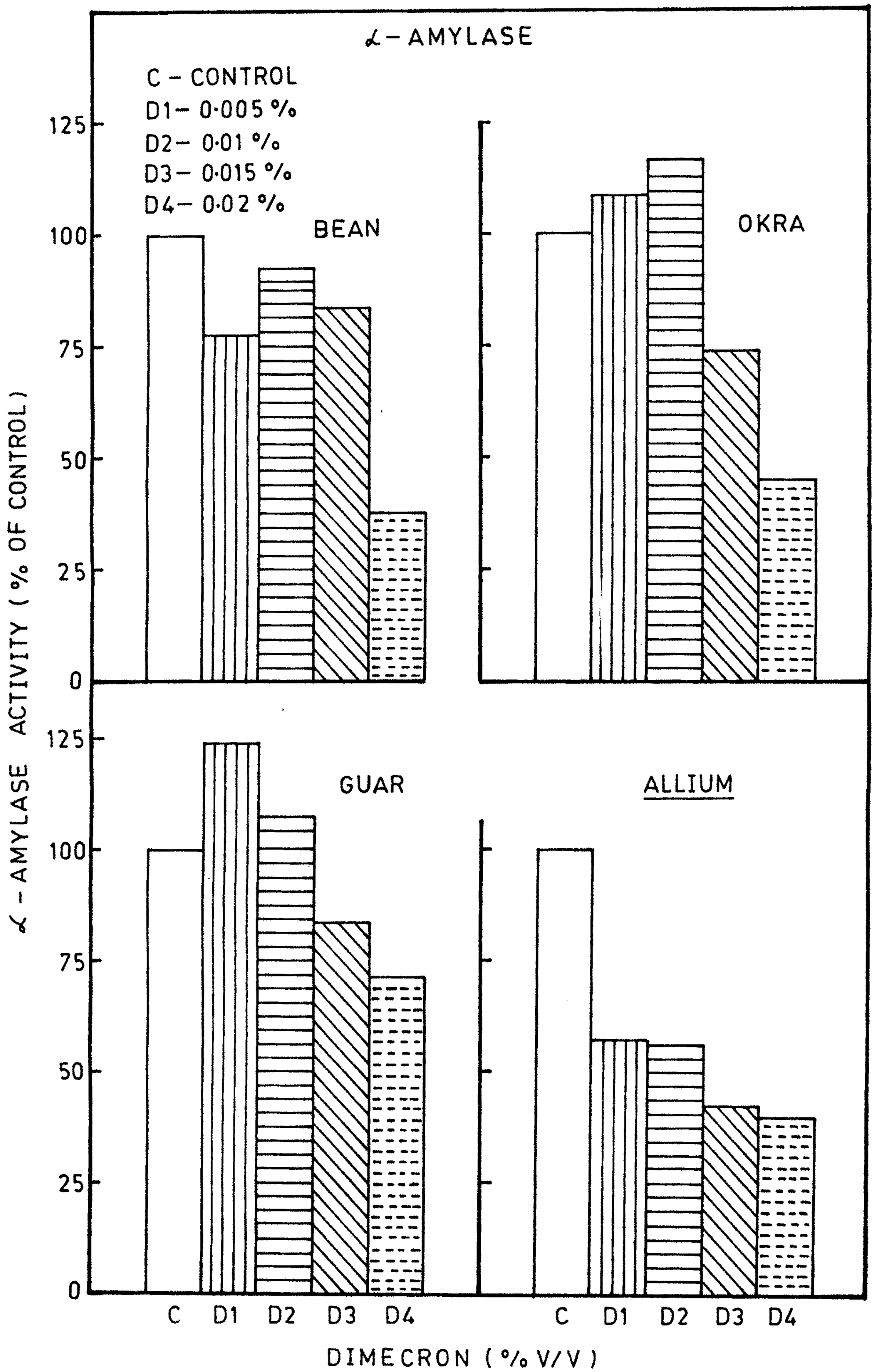

α-AMYLASE
C - CONTROL
D1- 0·005 %
D2- 0·01 %
D3- 0·015 %
D4- 0·02 %
BEAN
OKRA
GUAR
ALLIUM
α-AMYLASE ACTIVITY (% OF CONTROL)
0
25
50
75
100
125
C
D1
D2
D3
D4
DIMECRON (% V/V)

Fig. 8

to insecticide treatment but the degree of inhibition in all the concentrations of Metacid was more or less the same which can be very well attributed to the germination percentage of guar (Table 2).

Sengupta et al (1988) have shown that the activity of total amylase was significantly inhibited on exposure to toxic doses of carbaryl indicating that the breakdown of starch to readily utilizable sugar which is essential for the growth of seedling is greatly impaired due to carbaryl toxicity. Pronounced inhibition of amylase activity was also recorded by Dalvi et al (1972) when wheat seeds were germinated in the presence of Menazon and Disulfoton, the two organophosphorus pesticides. They suggested that these pesticides probably inhibit germination by impairing degradation of carbohydrate reserve during germination. The inhibition of amylase activity due to Metasystox in Vigna mungo has also been reported by Prasad and Mathur (1983). They have established a direct correlation between germination percentage, reducing sugar and amylase activity. Contrary to this Pathak and Mukherjee (1986) have observed stimulatioin of amylase activity in Vigna radiata seedlings due to Sevin treatment. However, the higher concentration (300 ppm) found to be inhibitory in action.

2. Peroxidase

The increase in peroxidase activity is always correlated with the increased respiration under stress condition (Strogonov, 1964; Aleshin et al.,1971) which can clearly be seen in Table 5 and 6 and Fig. 9 and 10 where the increase in the activity of enzyme peroxidase was observed with increasing concentrations of insecticide in guar, okra and onion. This clearly indicates

Table 5. Effect of Metacid on peroxidase activity in germinating seeds of bean, okra, guar and onion after 72 h germination.

Metacid-50	Peroxidase (ΔOD_{470nm} min^{-1} mg^{-1} protein)			
% (v/v)	Bean	Okra	Guar	Onion
Control	4.24	5.41	52.52	2.95
0.01	2.36	3.93	35.5	5.42
0.02	4.33	21.84	41.7	6.33
0.03	5.07	23.83	60.56	7.91
0.04	3.71	12.76	89.95	2.45

Values are mean of three determinations.

Table 6. Effect of Dimecron on peroxidase activity in germinating seeds of bean, okra, guar and onion after 72 h germination.

Dimecron % (v/v)	Peroxidase (ΔOD_{470nm} $min^{-1} mg^{-1}$ protein)			
	Bean	Okra	Guar	Onion
Control	4.24	5.41	52.52	2.95
0.005	1.63	12.29	72.48	2.42
0.01	2.29	8.62	74.89	5.72
0.015	3.02	10.96	83.51	6.70
0.02	2.48	17.93	63.66	17.26

Values are mean of three determinations.

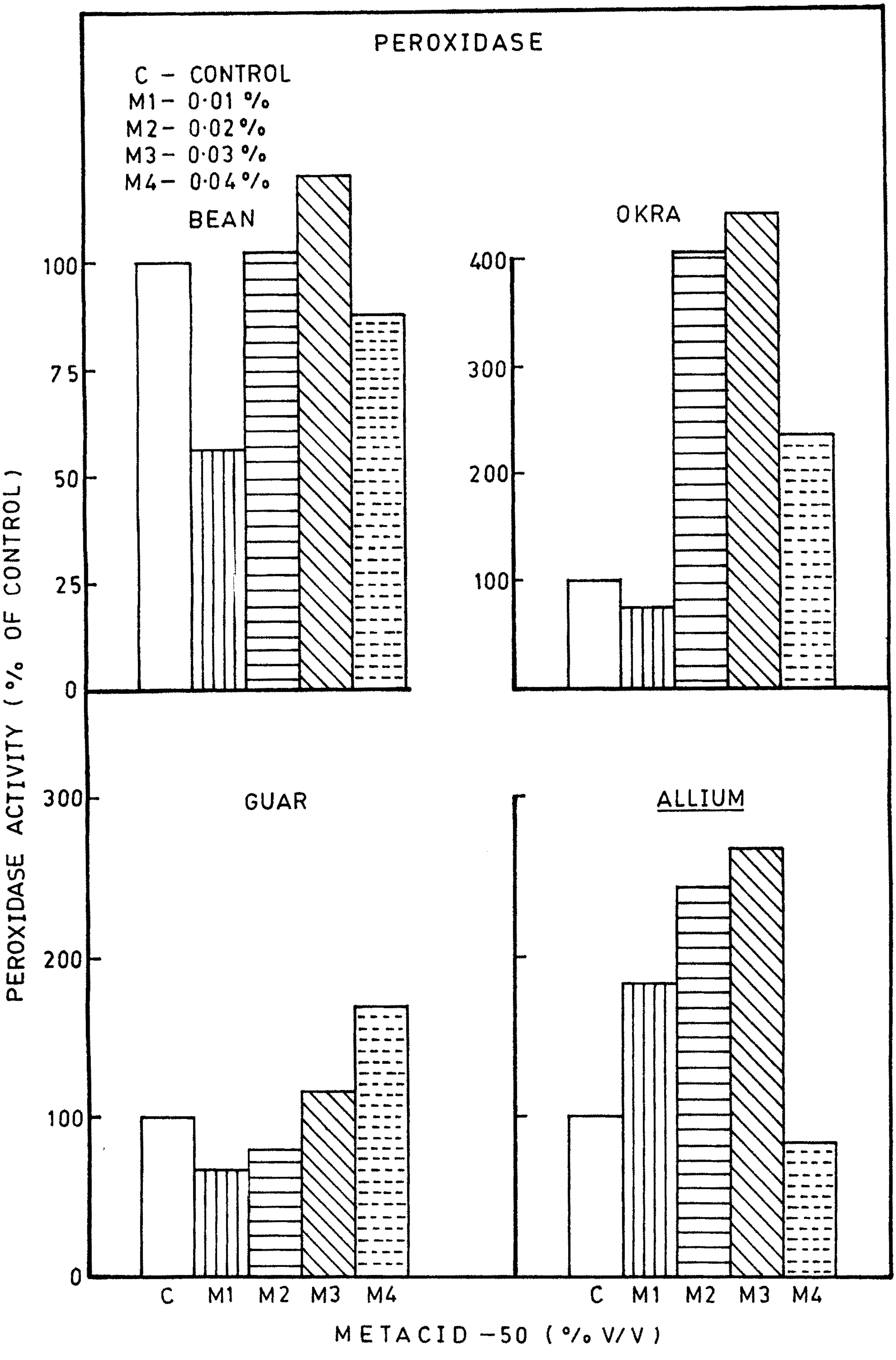

PEROXIDASE
C - CONTROL
M1- 0·01 %
M2- 0·02 %
M3- 0·03 %
M4- 0·04 %
BEAN
OKRA
GUAR
ALLIUM
PEROXIDASE ACTIVITY (% OF CONTROL)
0
25
50
75
100
100
200
300
400
0
100
200
300
C
M1
M2
M3
M4
C
M1
M2
M3
M4
METACID -50 (% V/V)

Fig. 9

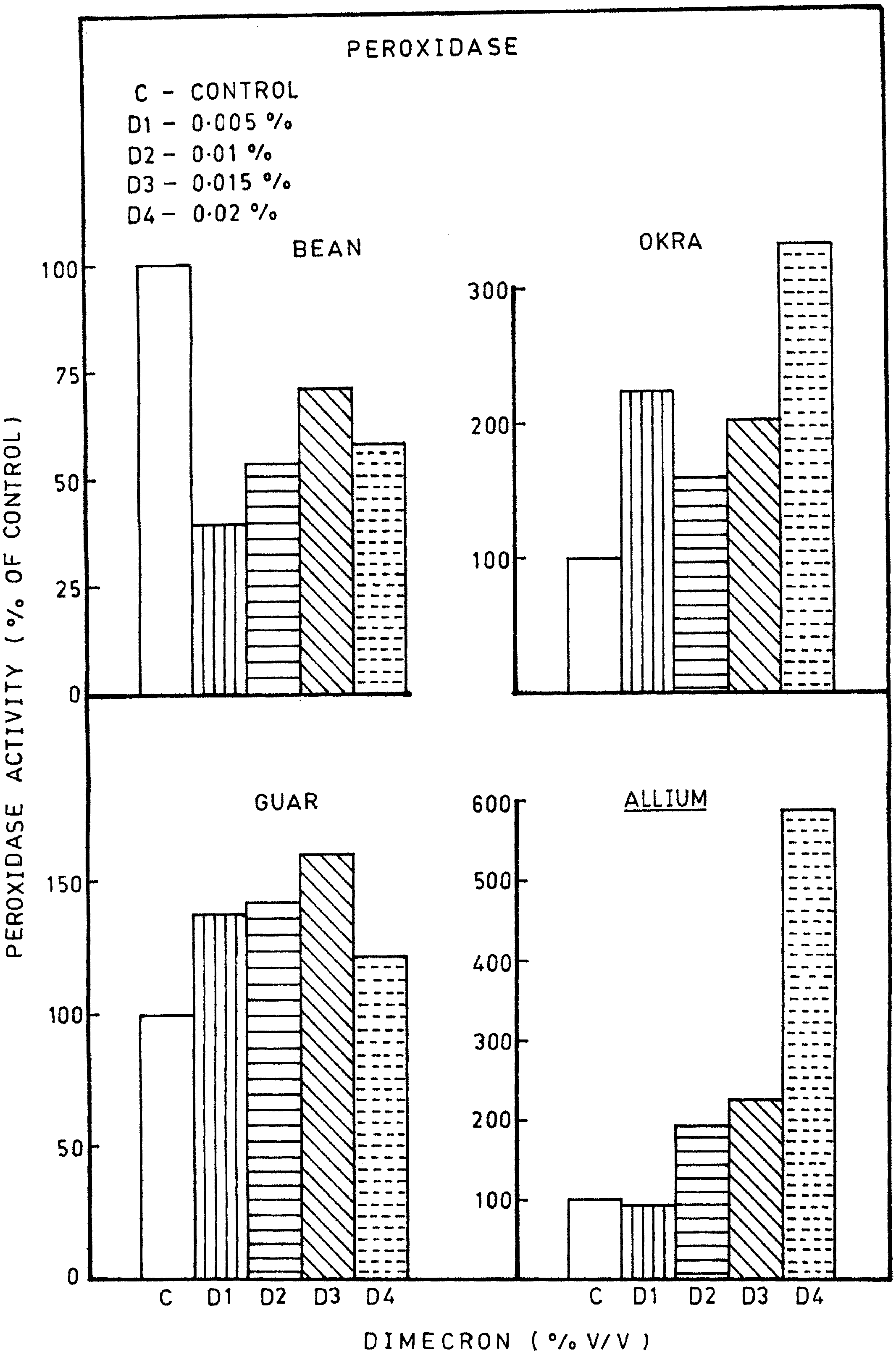

PEROXIDASE
C - CONTROL
D1 - 0·005 %
D2 - 0·01 %
D3 - 0·015 %
D4 - 0·02 %
BEAN
OKRA
GUAR
ALLIUM
PEROXIDASE ACTIVITY (% OF CONTROL)
0
25
50
75
100
150
100
200
300
400
500
600
C
D1
D2
D3
D4
DIMECRON (% V/V)

Fig.10

that the osmotic shock resulted due to the insecticide treatment increases the respiration. However, no significant increase in peroxidase activity was noticed in bean with the increase in insecticide concentrations. Possibly the increase in respiration here may be a 'Wasteful respiration' or it may be an adaptation as reported by Baba *et al.* (1964) and Aleshin *et al.* (1971) respectively in rice seedlings.

The increase in the activity of peroxidase due to fungicide (Kitazin) treatment has been reported by Reddy and Vidyavati (1983) in *Dolichos biflorus* seedlings. According to them peroxidase is inversely related with the activity of amylase and seedling growth. Contrary to this decrease in peroxidase activity in tomato leaves due to foliar application of Bavistin and Calixin has been reported by Karadge and Karne (1985). According to Aleshin *et al.* (1971) the increase in peroxidase activity reflects on its participation in heteroauxin metabolism and its active part in adaptation of plants to salt stress. This appears to be true even in case of pesticidal stress on guar which showed increased level of peroxidase and germination percentage. But it is too early to say the exact fate of peroxidase activity under pesticide treatment unless we study isoenzyme pattern. Further the peroxidase system can serve as a model system for studying the regulation of gene expression in developing plants (Van and Cairns, 1982), infact it is generally accepted that peroxidase activity and/or its isoenzyme patterns vary with changes in plant development (Hoess *et al.*, 1974). Since pesticides induce several changes in seedling development the study of peroxidase in detail is of utmost importance.

3. Catalase

Like that of peroxidase the activity of catalase is also linked with respiration (Tregubenko et al. 1973) and has also been reported to play an important role in photorespiratory glycollate pathway.

The activity of this enzyme in general found to be increased at lower concentration but decreased as the concentration of both Metacid and Dimecron increased (Table 7 and 8 and Fig.11 and 12). However, exception is being noted in okra of Metacid treatment where none of the concentrations, favoured stimulation of catalase activity. Similarly onion has shown more catalase activity even at higher concentration of Metacid (Fig. 11). However, this situation was not in case of Dimecron treatment, where both okra and onion have showed marginal or no increase in catalase activity over control (Fig. 12). Thus the stimulation of this enzyme at respective concentrations of both Metacid and Dimecron in bean, okra and guar indicates increase in respiration.

Reddy and Vidyavati (1983) and Karadge and Karne (1985) have observed increase in catalase due to fungicidal treatment in Dolichos biflorus seedlings and Lycopersicon esculentum plants respectively and Strogonov(1964) also observed an increase in catalase activity not due to pesticidal stress but in sodium chloride stressed leaves. From this observation we can safely say that increase in catalase activity by stress may be an adaptive feature in plants to regulate H_2O_2 level.

It appears from our results of pesticidal stress on catalase that bean and guar of Metacid and Dimecron and onion of Metacid treatment possess an adaptive feature to some extent, however okra of Metacid treatment did

Table 7. Effect of Metacid on catalase activity in germinating seeds of bean, okra, guar and onion after 72 h germination.

Metacid-50	Catalase (mg H_2O_2 min^{-1}g^{-1} fresh wt.)			
% (v/v)	Bean	Okra	Guar	Onion
Control	15.47	113.8	205.2	59.7
0.01	15.63	32.96	99.6	75.0
0.02	33.54	8.35	204.5	51.0
0.03	21.46	4.15	217.5	85.8
0.04	14.71	4.86	190.0	109.2

Values are mean of three determinations.

Table 8. Effect of Dimecron on catalase activity in germinating seeds of bean, okra, guar and onion after 72 h germination

Dimecron % (v/v)	Catalase (mg H_2O_2 min^{-1} g^{-1} fresh wt.)			
	Bean	Okra	Guar	Onion
Control	15.47	113.88	205.24	59.7
0.005	22.04	134.60	151.02	59.4
0.01	33.72	117.51	230.48	59.4
0.015	20.20	103.89	134.50	57.6
0.02	18.45	127.68	116.51	58.8

Values are mean of three determinations.

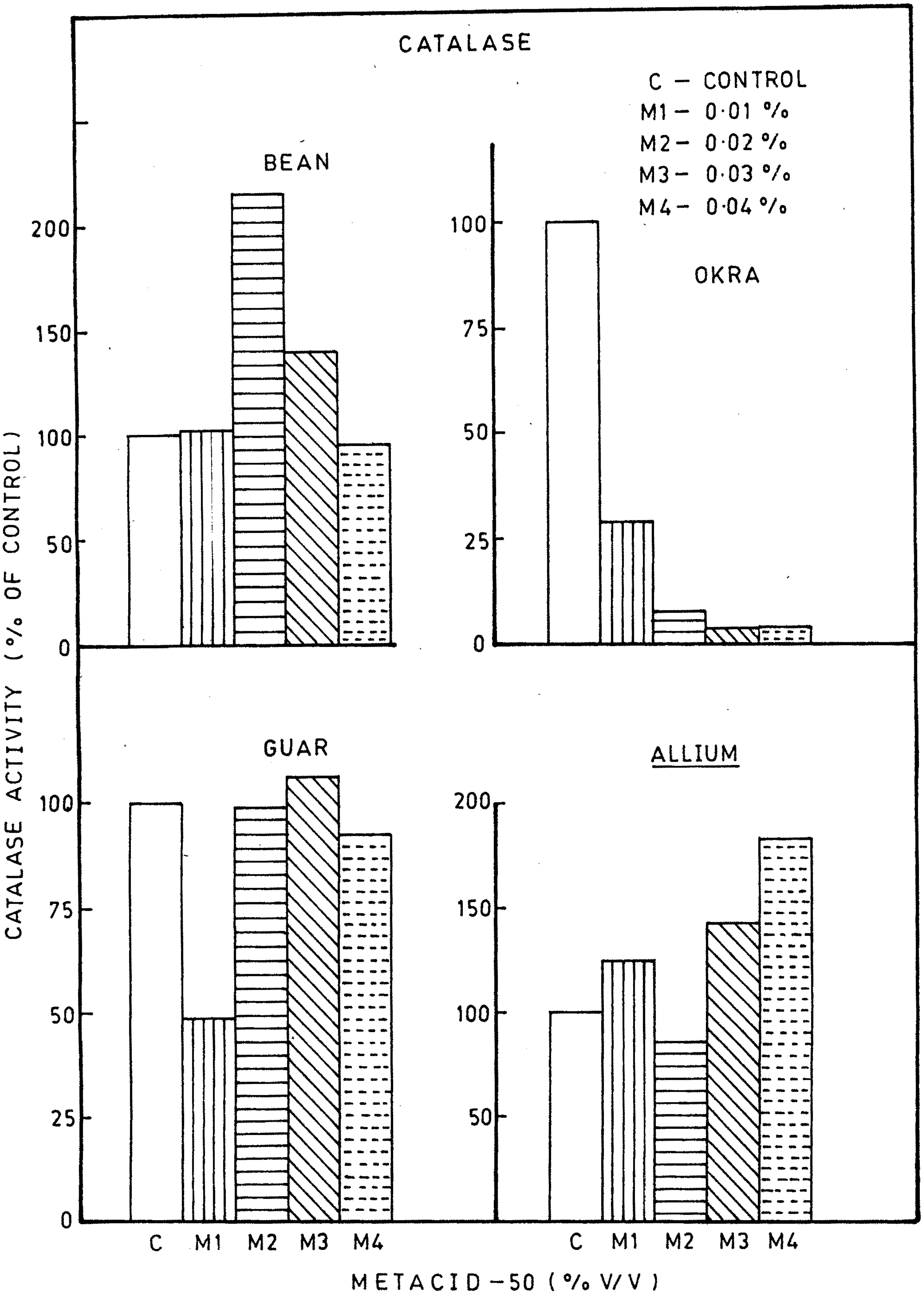

CATALASE
C – CONTROL
M1 – 0·01 %
M2 – 0·02 %
M3 – 0·03 %
M4 – 0·04 %
BEAN
OKRA
GUAR
ALLIUM
CATALASE ACTIVITY (% OF CONTROL)
0
50
100
150
200
0
25
50
75
100
0
25
50
75
100
50
100
150
200
C
M1
M2
M3
M4
C
M1
M2
M3
M4
METACID – 50 (% V/V)

Fig. 11

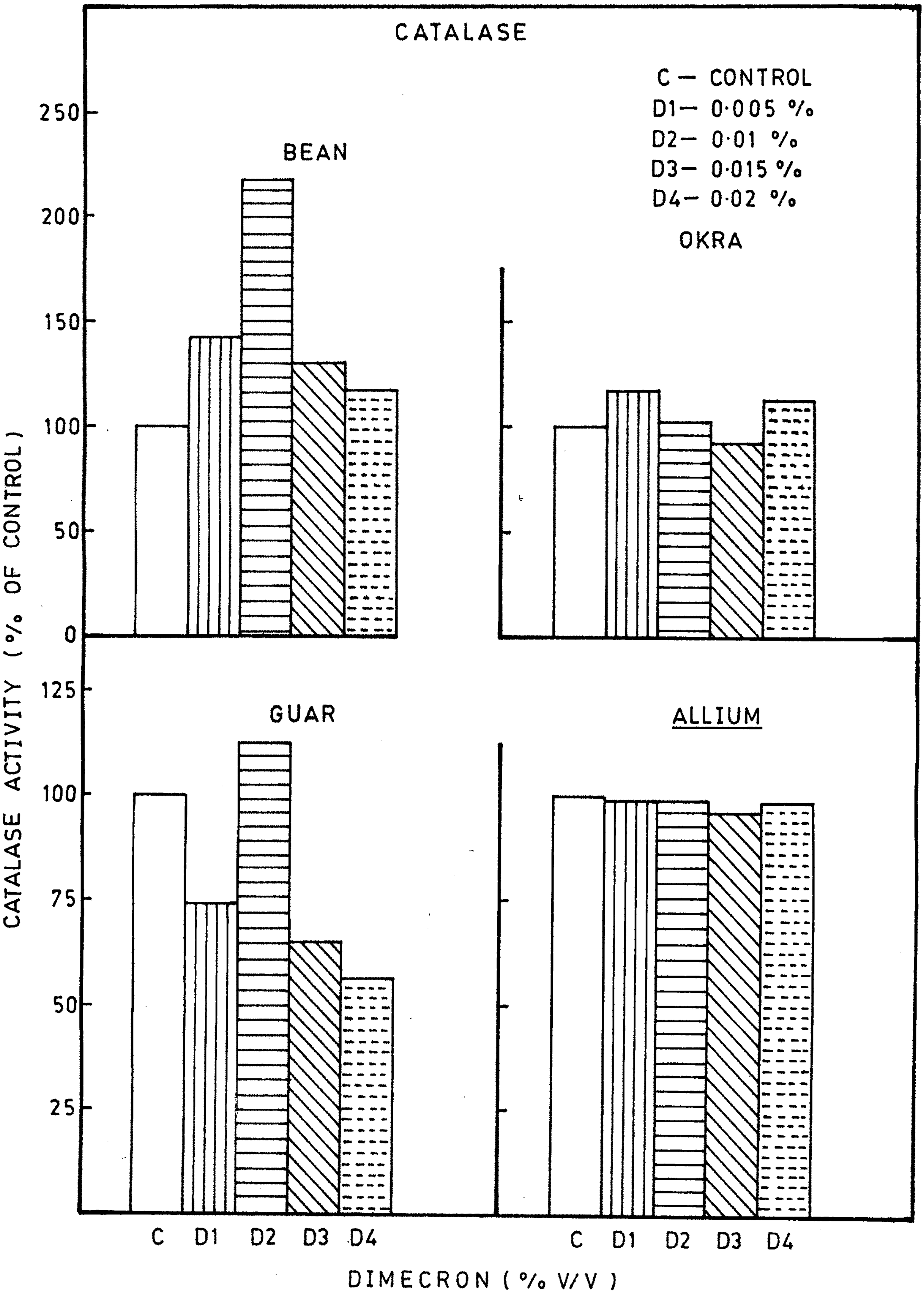

CATALASE
C — CONTROL
D1— 0·005 %
D2— 0·01 %
D3— 0·015 %
D4— 0·02 %
BEAN
OKRA
GUAR
ALLIUM
CATALASE ACTIVITY (% OF CONTROL)
250
200
150
100
50
0
125
100
75
50
25
C
D1
D2
D3
D4
DIMECRON (% V/V)

Fig.12

not. Similarly little or no response of catalase activity was noticed in Dimecron treated onion and okra. The decrease in the enzyme activity at higher concentration may be attributed to the inhibition caused by pesticide treatment on the production of the enzyme catalase in the seedlings.

4. Acid phosphatase

It is clear from the Table 9 and 10 and Fig. 13 and 14 that the activity of enzyme acid phosphatase is decreased only in okra in both the pesticidal stress over control, however very little or no change in the acid phosphatase activity was observed in bean guar and onion. As far as Metacid treatment is concerned bean, guar and onion have shown very little enhancement as the concentrations increased. This enhancement of the activity appeared to be in the range of 2 to 3% in bean and 3 to 25% in guar, while in onion no enhance ment of the activity has been observed. The situation in Dimecron treatment appears to be more or less same in bean, guar and onion whereas in okra the activity was inhibited by more than 30% and less than 60% over control.

Since nothing is known about the effect of pesticide on the activity of enzyme acid phosphatase during germination, it is not possible to correlate the results obtained. However, the decrease in the activity of acid phosphatase, in the leaves of tomato plant sprayed with fungicide Bavistin and Calixin has been reported by Karadge and Karne (1985). El-Fouly and Jung (1972) have also reported marked stimulation of acid-phosphatase in wheat seedlings under the condition of sodium chloride stress. From our results it can be said that the increase in acid phosphatase activity reflects on the increase in catabolic activities such as disturbances in phosphorus metabolism which needs further investigation.

Table 9. Effect of Metacid on acid-phosphatase activity in germinating seeds of bean, okra, guar and onion after 72 h germinatiion.

Metacid-50 % (v/v)	Acid-phosphatase (ΔOD_{420nm} h^{-1} g^{-1} fresh wt.)			
	Bean	Okra	Guar	Onion
Control	12.56	3.31	5.9	27.42
0.01	12.89	2.0	6.1	27.6
0.02	13.14	2.21	6.1	23.92
0.03	13.29	2.1	6.72	25.78
0.04	13.10	2.03	7.30	27.27

Values are mean of three determinations.

Table 10. Effect of Dimecron on acid-phosphatase activity in germinating seeds of bean, okra, guar and onion after 72 h germination.

Dimecron % (v/v)	Acid-phosphatase (ΔOD_{420nm} $h^{-1}g^{-1}$ fresh wt)			
	Bean	Okra	Guar	Onion
Control	12.56	3.31	5.9	27.42
0.005	13.72	1.33	6.63	25.41
0.01	13.77	1.2	5.83	26.85
0.015	13.83	2.22	6.19	28.17
0.02	13.57	1.67	6.1	26.74

Values are mean of three determinations.

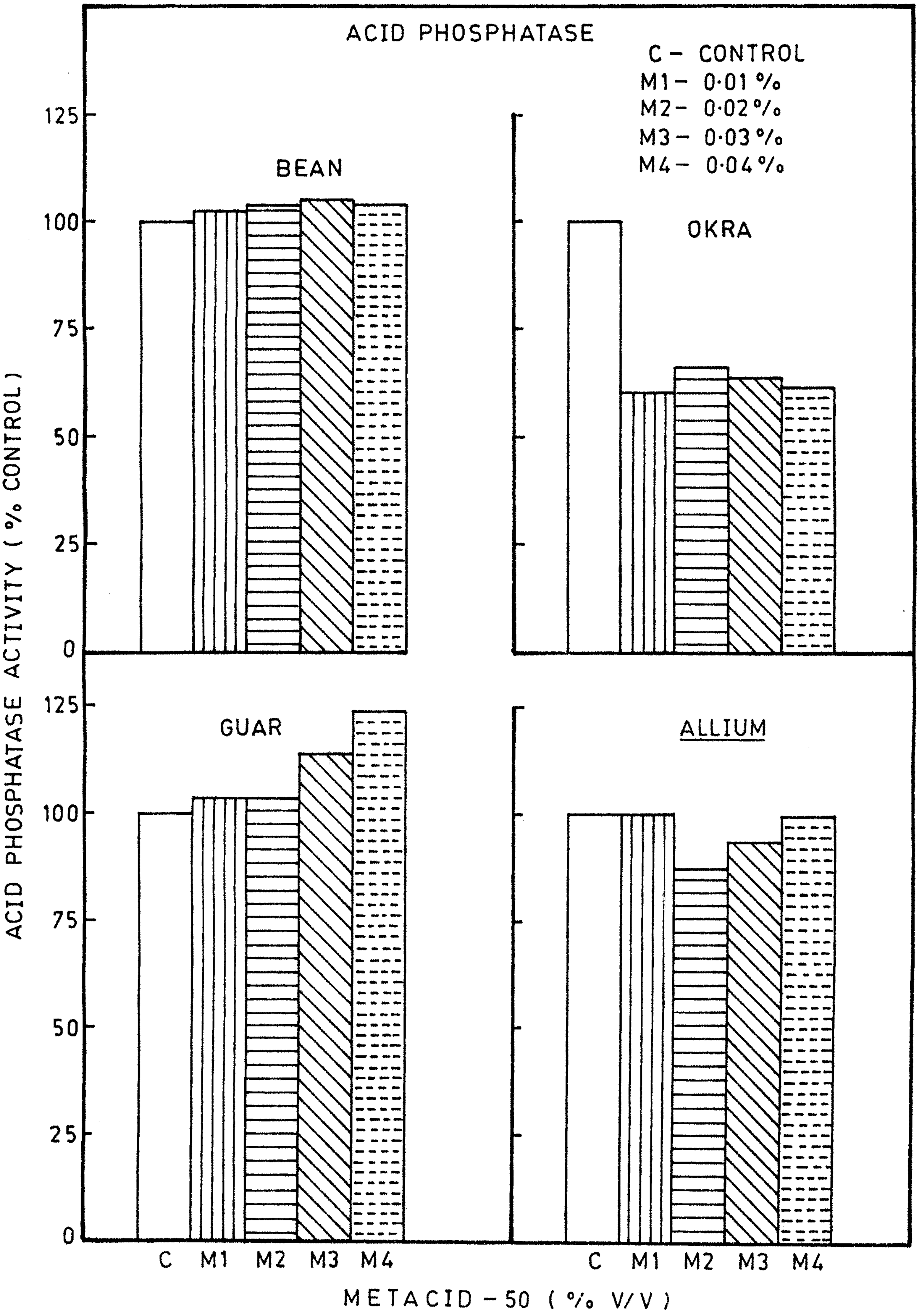

ACID PHOSPHATASE
C - CONTROL
M1 - 0·01 %
M2 - 0·02 %
M3 - 0·03 %
M4 - 0·04 %
BEAN
OKRA
GUAR
ALLIUM
ACID PHOSPHATASE ACTIVITY (% CONTROL)
0
25
50
75
100
125
C
M1
M2
M3
M4
METACID - 50 (% V/V)

Fig.13

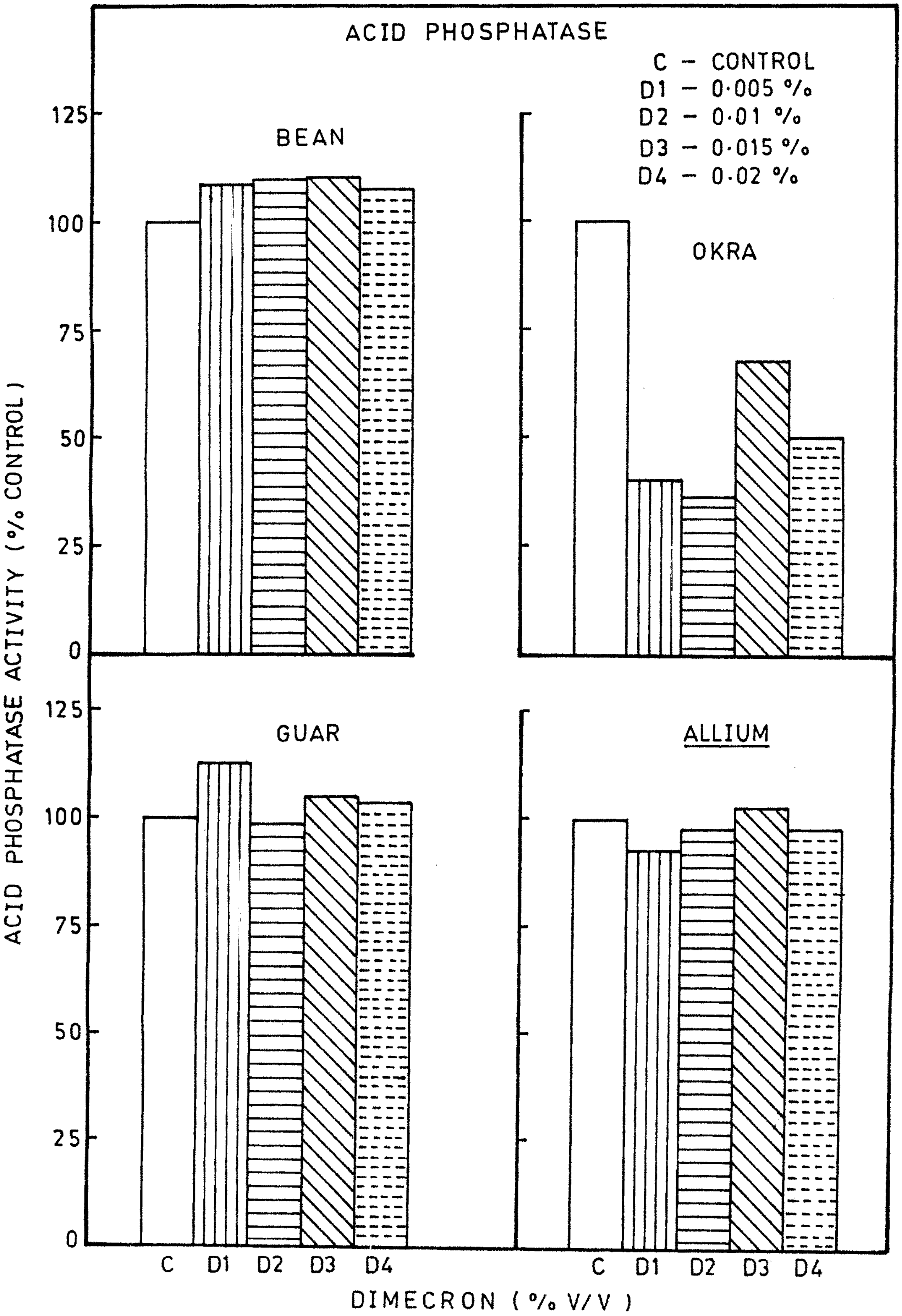

ACID PHOSPHATASE
C – CONTROL
D1 – 0·005 %
D2 – 0·01 %
D3 – 0·015 %
D4 – 0·02 %
BEAN
OKRA
GUAR
ALLIUM
ACID PHOSPHATASE ACTIVITY (% CONTROL)
0
25
50
75
100
125
C
D1
D2
D3
D4
DIMECRON (% V/V)

Fig.14

5. Protease

The effect of Metacid and Dimecron on the activity of an enzyme protease is represented histographically in Table 11 and 12 and Fig. 15 and 16. It is very clear from the histogram that there is no consistency in the protease activity with the increase in concentrations of both Metacid and Dimecron. However, one thing is clearly seen that the increase or decrease in the activity is more related with the seedling growth in the different concentrations of the pesticides. Like that of acid phosphatase, protease has also not been investigated in detail for the pesticidal effect. However, very little is known about the fate of protease under pesticidal stress. According to Reddy and Vidyavati (1983) the activity of enzyme protease was more where there was increase in growth and growth retardation was associated with the less activity of the enzyme in the seedlings of Dolichos biflorus treated with fungicide Kitazin.

Similarly the activity of protease studied by Pathak and Mukherji (1986) under Sevin stress appeared to be increased with the increasing concentrations and decreased at higher concentrations (more than 300 ppm). The antibiotics such as chloramphenicol and cyclohexamide have also been proved to be inhibitory in action on enzyme protease in the germinating maize seeds (Bose et al. 1982). The latter being more effective than the former.

Our observations on the activity of protease under insecticidal stress are more or less similar to that of above findings. However, this requires to probe in for further details to establish the exact nature of food reserve and its utilization under pesticidal stress.

Table 11. Effect of Metacid on protease activity in germinating seeds of bean, okra, guar and onion after 72 h germination.

Metacid-50	Protease (μg tyrosine liberated h^{-1} g^{-1} fresh wt)			
% (v/v)	Bean	Okra	Guar	Onion
Control	5.56	9.84	5.42	0.44
0.01	13.89	8.57	5.21	1.41
0.02	12.93	2.93	5.89	0.74
0.03	11.47	4.89	5.82	0.59
0.04	4.43	4.28	2.50	0.65

Values are mean of three determinations.

Table 12. Effect of Dimecron on protease activity in germinating seeds of bean, okra, guar and onion after 72 h germination.

Dimecron % (v/v)	Protease (μg tyrosine liberated h^{-1} g^{-1} fresh wt.)			
	Bean	Okra	Guar	Onion
Control	5.56	9.84	5.42	0.44
0.005	3.47	9.84	5.68	0.41
0.010	5.90	11.75	6.50	0.43
0.015	4.16	6.85	6.50	0.22
0.02	4.16	5.87	5.67	0.48

Values are mean of three determinations.

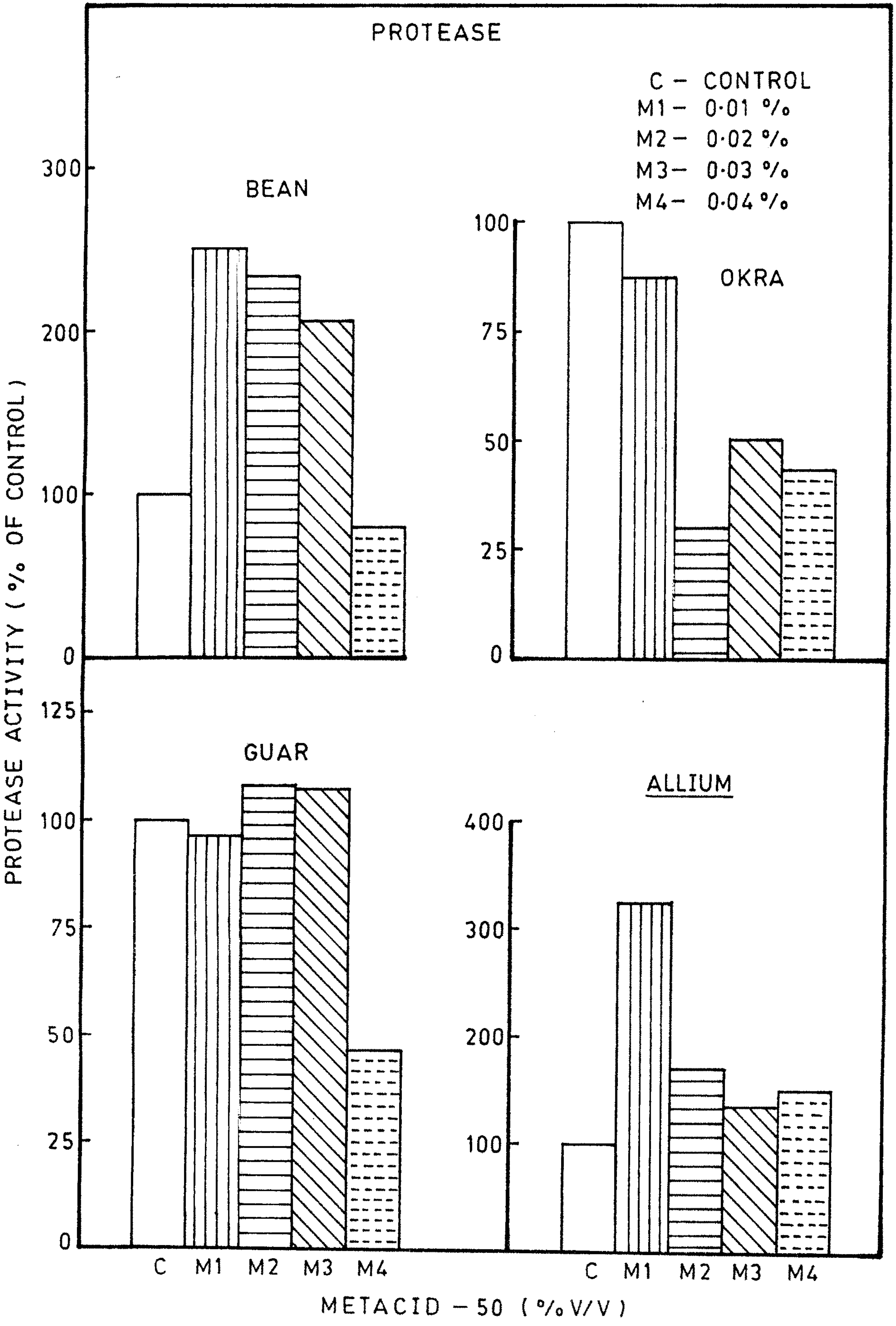

PROTEASE
C – CONTROL
M1 – 0·01 %
M2 – 0·02 %
M3 – 0·03 %
M4 – 0·04 %
BEAN
OKRA
GUAR
ALLIUM
PROTEASE ACTIVITY (% OF CONTROL)
300
200
100
0
100
75
50
25
0
125
100
75
50
25
0
400
300
200
100
C
M1
M2
M3
M4
C
M1
M2
M3
M4
METACID – 50 (% V/V)

Fig. 15

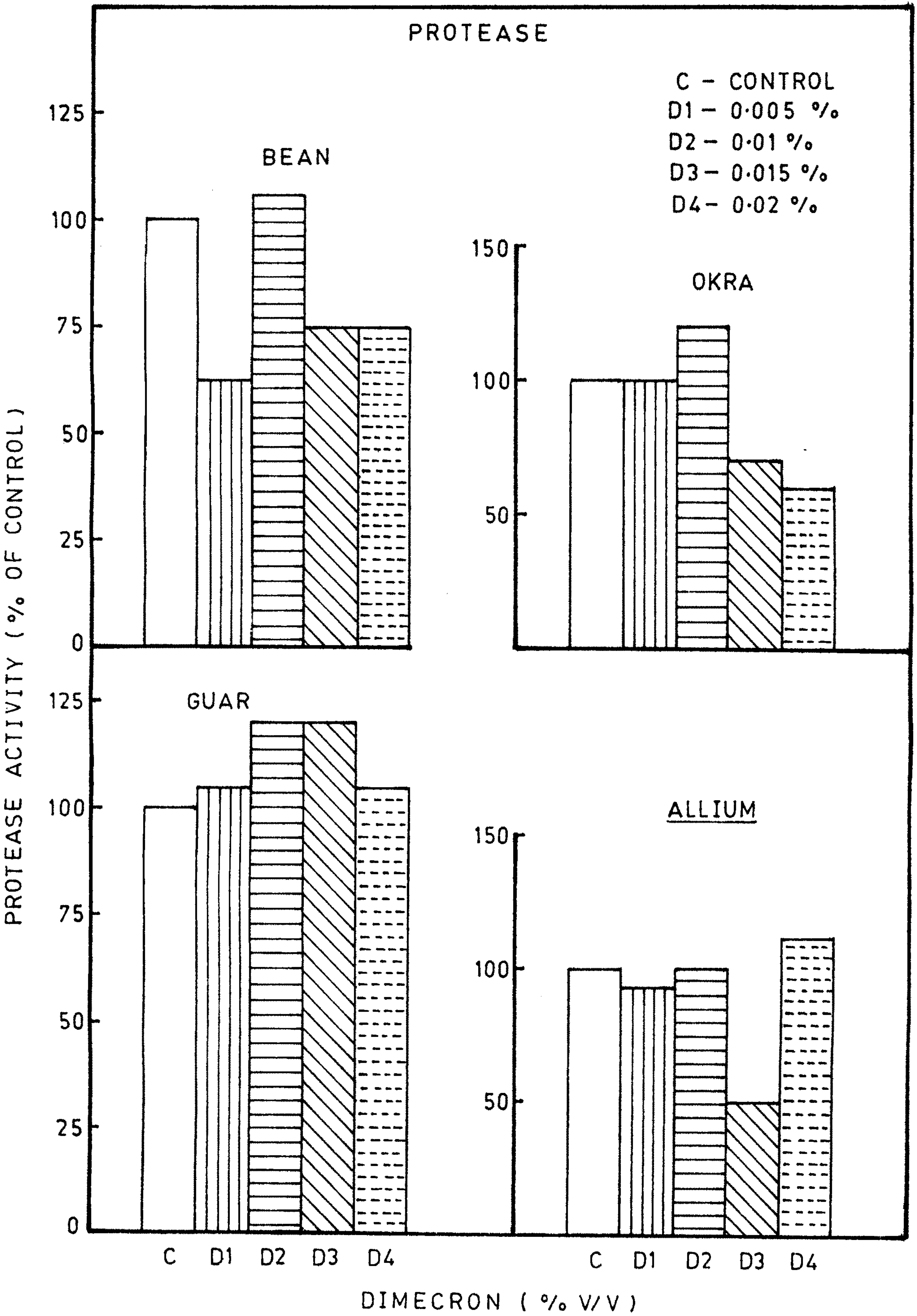

PROTEASE
C - CONTROL
D1 - 0·005 %
D2 - 0·01 %
D3 - 0·015 %
D4 - 0·02 %
BEAN
OKRA
GUAR
ALLIUM
PROTEASE ACTIVITY (% OF CONTROL)
125
100
75
50
25
0
150
100
50
C
D1
D2
D3
D4
DIMECRON (% V/V)

Fig.16

The results in general reveal that though the concentrations used in the present investigation were below the recommended doses (Dimecron : sucking insect = 0.02% a.i. chewing insects : 0.03% a.i. hardy and sucking insect = 0.04% a.i. and Metacid-50: 0.15% a.i.) for plant, as a spray, they bring constraint in the physiological events of seed germination and enzyme activity. Therefore, it is equally important to look into plant-pesticide relationship even at germination stage.

D. Effect of Metacid-50 and Dimecron on Proline Content

The data on proline content studied in bean, okra, guar and onion under pesticidal stress is depicted in Table 13-16 and in Fig. 17. The proline level of the control seedlings both in pre-treatment and continuous treatment at 24 h and 72 h seedling growth did not change much however, it varied with genus to genus. This level, increased over control by 42.8%, 8.30%, 84%, 92% respectively in bean, okra, guar and onion in the first 24 h of germination after 1 h pretreatment with Metacid (Fig. 17a). However, the situation of proline level was entirely different at 72 h of germination where no or little proline accumulation was observed in Metacid pretreated seeds (Fig.17a). The induction of proline accumulation due to Dimecron pre-treatment was 28.5%, 16.6%, 12% and 207% over control respectively in bean, okra, guar and onion at 24 h seedling growth (Fig.17c) while at 72 h seedling growth, bean and guar failed to accumulate proline whereas okra and onion did accumulate it (Fig. 17c). The seeds grown continuously in respective concentrations of Metacid-50 and Dimecron have also shown proline accumulation. This level of accumulation over control after 24 h germination in Metacid was 26.6%, 8.3%, 68% and 77.8% and after 72 h germination it was 184.8%, 9.0%, 0.0% and 44[illegible]%

Table 13. Proline content in bean, okra, guar and onion seedlings in response to Metacid 1 h pretreatment.

Metacid-50	Proline content (mg proline 100^{-1}g fresh seedling* wt)							
	Bean		Okra		Guar		Onion	
% (v/v)	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Control	10.5	16.5	6.0	22.0	12.5	50.00	5.00	13.5
0.03 %	15.0	17.5	6.5	17.5	23.0	47.0	10.0	13.5

* After 24 and 72 h of germination in distilled water

Values are mean of three determinations.

Table 14. Proline content in bean, okra, guar and onion seedlings in response to Dimecron 1 h pre-treatment.

Dimecron	Proline content (mg proline 100^{-1}g fresh seedling* wt)							
	Bean		Okra		Guar		Onion	
% (v/v)	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Control	10.5	16.5	6.0	22.0	12.5	50.0	5	13.5
0.015%	24.0	14.0	7.0	28.0	14.0	47.5	16	17

* After 24 and 72 h of germination in distilled water.

Values are mean of three determinations.

Table 15. Proline content in bean, okra, guar and onion seedlings in response to continuous treatment of Metacid.

Metacid-50	Proline content (mg proline 100^{-1}g fresh seedling* wt)							
	Bean		Okra		Guar		Onion	
% (v/v)	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Control	8	38	10	23.5	13.5	43.0	4.0	21.5
0.03 %	13.3	47.0	6.5	24.0	21.0	37.0	9.25	19.5

* Continuous germination for 24 h and 72 h in respective concentration.

Values are mean of three determinations.

Table 16. Proline content in bean, okra, guar and onion seedlings in response to continuous treatment of Dimecron.

Dimecron % (v/v)	Proline content (mg proline 100^{-1}g fresh seedling* wt)							
	Bean		Okra		Guar		Onion	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Control	8.0	38.0	10.0	23.5	13.5	43.0	4.0	21.5
0.015 %	18.0	46.0	9.0	20.5	15.5	40.5	9.0	18.5

Continuous germination for 24 h and 72 h in respective concentration.

Values are mean of three determinations.

respectively in bean, okra, guar and onion (Fig. 17b). The seeds that received continuous treatment of Dimecron have also showed proline accumulation by 71.3%, 50%, 24% and 73% over control at 24 h seedling growth while 178%, 0.0%,0.0%&37% at 72 h seedling growth respectively in bean, okra, guar and onion (Fig. 17d).

The data in general suggests that both the organophosphorus insecticides induce proline accumulation however, the time factor after the treatment matters much. Among all the four vegetable seeds bean and onion have shown more proline accumulation and least by okra. The less induction of proline accumulation at 72 h germination in 1 h pre-treatment with both the pesticides could be due to nullification of the pesticidal stress with the advancement of seedling growth. However, in continuous treatment of 72 h except bean all others have shown less induction of proline which could be accounted for inhibition of proline biosynthesis.

Deshpande and Swamy (1987) have studied the effect of methylparathion on proline accumulation in Sorghum. Their study revealed that in the case of seeds exposed to pesticides there was a pronounced increase in the level of proline and in the seedlings sprayed with twice (1000 ppm) that concentration of methylparathion, only a limited and significant increase in proline on the third day of spraying was observed. Further, their observations of vast differences in the proline levels between the plants exposed to methylparathion before seed germination and those after five days of germination indicate that the pesdticide requires a suitable physiological conditiion to induce proline accumulation. Similarly this difference in the response was exhibited even in the continuing presence of the pesticide during the seedling growth until the harvest was made for proline estimation.

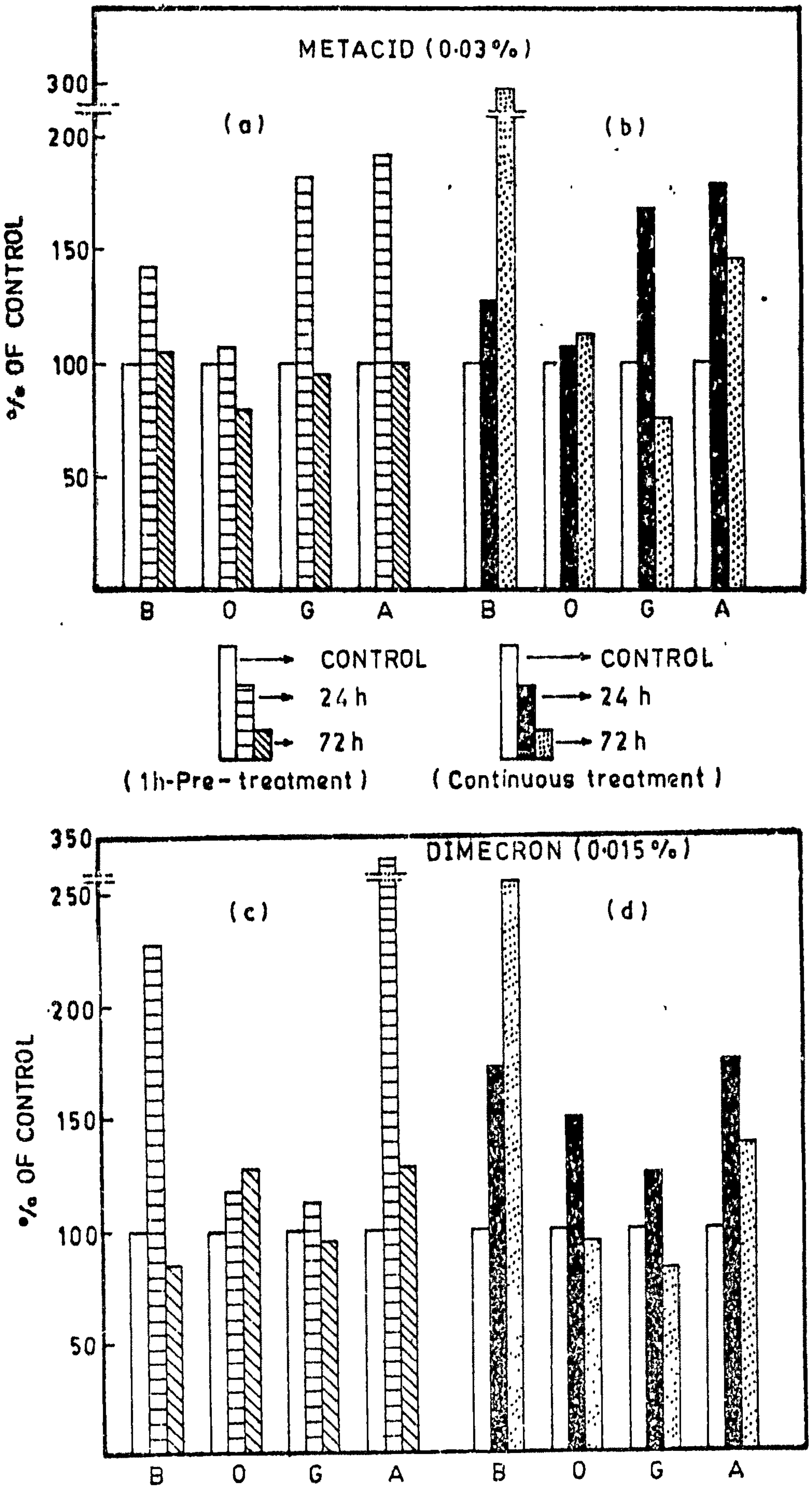

METACID (0·03%)
(a)
(b)
% OF CONTROL
300
200
150
100
50
B
O
G
A
B
O
G
A
CONTROL
24 h
72 h
(1h-Pre-treatment)
CONTROL
24 h
72 h
(Continuous treatment)
DIMECRON (0·015%)
(c)
(d)
% OF CONTROL
350
250
200
150
100
50
B
O
G
A
B
O
G
A

Fig. 17

Besides, it is evident from the literature survey that proline accumulation response to high temperature and salinity could be due to the disturbances in tissue water status (Goas et al.,1982). However, this is not the fact in the present investigation where no significant change in water content due to pesticide stress is noticed. In addition, proline accumulation in response to nutrient deficiencies (Ghildiyal et al.,1986), water logging (Wample and Bewley, 1975) low temperature (Draper, 1972; Chu et al.,1974; Withers and King 1979), air pollutants (Soldatini et al.,1978), pesticide stress (Deshpande and Swamy, 1987) and the alterations of this response caused by growth regulators (Udayakumar et al.,1976) question the argument explaining the accumulation caused by all these factors via disturbance in tissue water status. Therefore, the results warrant a search for the common mechanism of the induction of proline accumulation in plants caused by various stress factors.

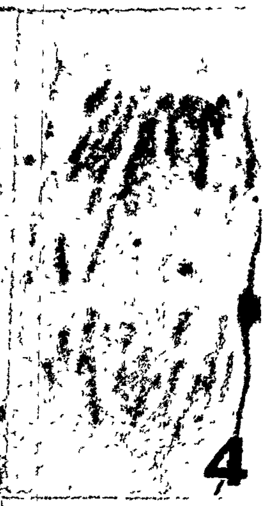
E. Effect of Metacid-50 and Dimecron on Mitotic Chromosomal Behaviour

The influence of pesticidal stress on chromosomal behaviour studied in bean, okra, guar and Allium is represented in Plate 1 Fig. 18 and in Table 17. Almost all the treated seeds have shown abnormal chromosomal behaviour (Plate I : Fig. 1-16) and the frequency of abnormality was found to be increased with increasing concentrations of both the pesticides (Fig. 18). The abnormalities comprised : non-orientation and mis-orientation of chromosomes at equator and stickiness at metaphase; stickiness, bridges (single and double), precocious movement and occurrence of laggards and fragments, diagonal spindle formation at anaphase; and bridges, diagonal arrangement of nuclei etc. were at telophase. Apart from this, formation of micronuclei, clumped nuclei and in some cases


1

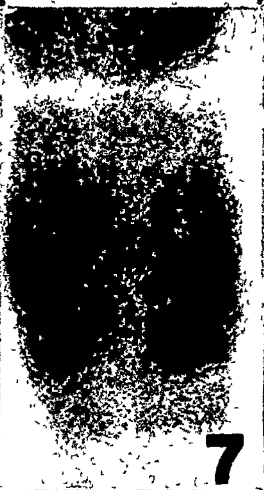

2


3


4


5


6


7


8


9


10


11


12


13


14


15


16

bi-and trinucleated cells were also observed. These abnormalities (Plate I) at metaphase, anaphase and telophase at different concentrations of pesticides are depicted in Fig. 18. It is very clear from the figure that the abnormality percentage is increased with increasing concentrations of Metacid and Dimecron

Chromosome aberrations constitute a significant portion of the genetic damage (Mann, 1977). Stickiness normally occurs due to breakage and reunion of sticky ends of chromosomes; while fragments may be formed due to interstitial deletion caused by pesticidal treatment. Ionised radiation (Lea, 1955) and organophosphorus pesticide treatments (Kaur and Grover, 1985) were also held responsible for the fragmentation. Precocious movement, non-orientation and mis-orientation of chromosomes at metaphase and anaphase were possibly due to disturbances in the formation of spindle and gene mutation respectively (Bhamburkar and Bhalla, 1980). The gene which determines the number and position of poles of the spindle may disturb the position of the spindle (Prasad, 1974). Laggards reported in the present investigation may be due to chromosome breakage or malfunction of spindle apparatus. According to Sax (1940) anaphasic bridges may be formed either due to unequal exchange or dicentric chromosomes which are pulled equally to both the poles. As such pesticides are known to induce mitotic irregularities especially anaphase bridges in plants (Grant, 1971; Epstein and Legator, 1971; Siebert and Eisenbrand, 1974). Acentric fragments, laggards or chromosome as a whole which fail to participate in the normal anaphase due to malfunction of the spindle apparatus, gets round off in cytoplasm and form micronuclei. Similarly clumped nuclei are formed due to disturbances at the cytochemical level (Evans, 1962). The formation of bi- and tri-nucleate cells observed in Metacid and Dimecron treatment indicates

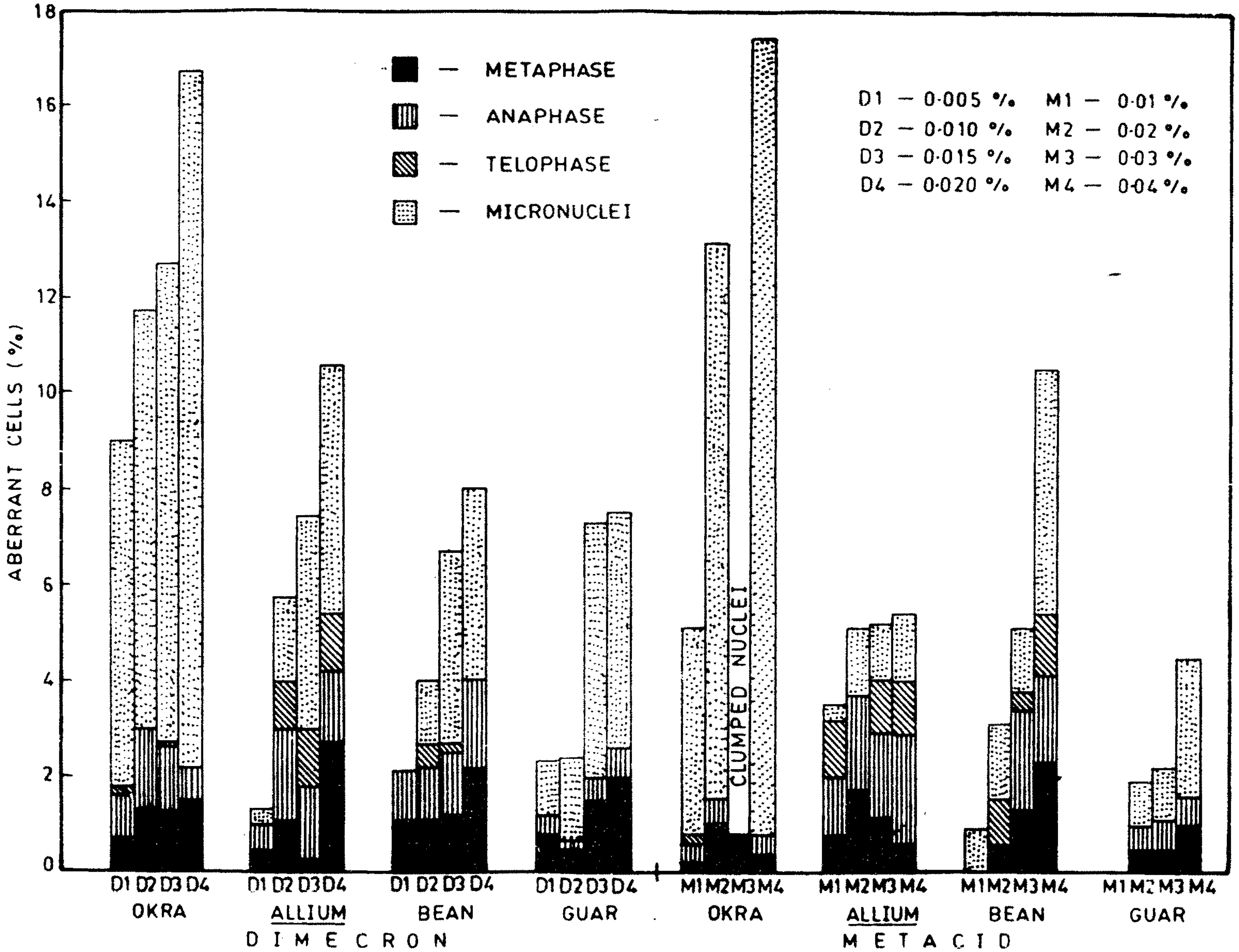

ABERRANT CELLS (%)
0
2
4
6
8
10
12
14
16
18
▪ — METAPHASE
▥ — ANAPHASE
▧ — TELOPHASE
▩ — MICRONUCLEI
D1 — 0·005 %
D2 — 0·010 %
D3 — 0·015 %
D4 — 0·020 %
M1 — 0·01 %
M2 — 0·02 %
M3 — 0·03 %
M4 — 0·04 %
CLUMPED NUCLEI
D1 D2 D3 D4
OKRA
D1 D2 D3 D4
ALLIUM
D1 D2 D3 D4
BEAN
D1 D2 D3 D4
GUAR
DIMECRON
M1 M2 M3 M4
OKRA
M1 M2 M3 M4
ALLIUM
M1 M2 M3 M4
BEAN
M1 M2 M3 M4
GUAR
METACID

Fig. 18

Table 17. Mitotic abnormalities at metaphase, anaphase and telophase following Metacid and Dimecron treatments in four vegetable seeds.

Abnormalities	Metacid (%)					Dimecron (%)				
	Contd.	0.01	0.02	0.03	0.04	Contd.	0.005	0.010	0.015	0.020
OKRA										
Metaphase	-	0.2	0.96	0.82	0.36	-	0.72	1.36	1.30	1.52
Anaphase	-	0.4	0.57	Clumped nuclei	0.36	-	0.89	1.75	1.30	0.76
Telophase	-	0.2	0.0	,,	0.0	-	0.18	0.0	0.15	0.0
Micronuclei	-	4.35	11.66	,,	16.72	-	7.17	8.58	9.98	14.43
Total Abnormalities	-	5.15	13.19	0.82	17.44	-	8.78	11.69	12.73	16.71
ALLIUM										
Metaphase	-	0.75	1.70	1.19	0.54	-	0.47	1.10	0.30	2.69
Anaphase	-	1.27	2.08	1.71	2.33	-	0.47	1.96	1.54	1.47
Telophase	-	1.19	1.33	1.03	1.10	-	0.12	0.98	1.23	1.17
Micronuclei	-	0.30	0.0	1.27	1.43	-	0.23	1.59	4.29	5.20
Total Abnormalities	-	3.51	5.11	5.20	5.40	-	1.29	5.63	7.36	10.53
BEAN										
Metaphase	-	0.0	0.56	1.26	2.32	-	1.05	1.06	1.19	2.26
Anaphase	-	0.0	0.98	2.10	1.79	-	1.05	1.06	1.33	1.79
Telophase	-	0.0	0.0	0.42	1.25	-	0.0	0.42	0.20	0.0
Micronuclei	-	0.89	1.54	1.26	5.19	-	0.0	1.40	3.98	3.94
Total Abnormalities	-	0.89	3.08	5.04	10.55	-	2.10	3.94	6.70	7.99
GUAR										
Metaphase	-	0.0	0.48	0.49	0.98	-	0.78	0.51	1.47	1.92
Anaphase	-	0.0	0.47	0.59	0.59	-	0.39	0.21	0.48	0.58
Telophase	-	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0
Micronuclei	-	0.0	0.96	1.14	2.93	-	1.17	1.53	5.37	5.02
Total Abnormalities	-	0.0	1.91	2.22	4.50	-	2.34	2.25	7.32	7.52

inhibition of cytokinesis. Padmaja _et al._ (1986) have also reported inhibition of cytokinesis in garlic (_Allium sativa_) treated with Dimecron.

Thus the seeds of four vegetables tested for cytotoxicity clearly indicate that okra exhibits more cytotoxicity while guar exhibits less cytotoxicity. It is also evident from Table 17 and Fig. 18 that maximum number of micronuclei were formed at higher concentration of Metacid and Dimecron and almost all the cells of okra root tip showed clumped nuclei at 0.03% Metacid treatment. Among all, guar appeared to be more potential vegetable to withstand pesticidal treatment with less cytotoxicity.