Chapter - III

Results and Discussion

CHAPTER - III

-:- RESULTS AND DISCUSSION -:-

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2) Acid Phosphatase

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A) Germination Percentage :

The influence of different concentrations of NaCl on germination percentage of Dolichos biflorus, Roxb., Lens esculenta, Moench., Phaseolus aconitifolius, Jacq., Phaseolus aureus Roxb., and Trigonella foenum-graecum, Linn. has shown in Tables 1,2,3,4 and 5 respectively and figures 6,7 and 8. It is clear from the tables and figures that the final germination percentage at 120 h germination is not at all affected by the salt stress at the lower salt regimes upto 50 mM NaCl in case of horse gram (D.biflorus) and Lentil (L.esculenta). However, the process is delayed as well as inhibited in both the legumes, particularly at the higher salt concentrations (i.e. 100 mM and above). On the other hand the germination percentage remain unaffected in moth bean (P.aconitifolius) and Mung (P.aureus) under salt stress even at the higher salinity levels. However, the performance of P.aureus seems to be better than that of P.aconitifolius. Though the germination percentage under saline conditions is increased above control within 24 and 48 h of germination in fenugreek (I.foenum-graecum), upto 2.5 and 5 mM salt, it is decreased with increasing further salt concentrations. It can also be seen that the germination percentage falls down very rapidly from about 96% upto 50 mM NaCl to about 73% in 100 mM salt treatment in this species. The higher salt concentrations (200 and 300 mM) however, are strong inhibitors of germination in the species. From these results it appears that fenugreek is highly sensitive to salinity during germination.

TABLE 1 : Effect of NaCl salinity on germination in *D.biflorus*.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
0.0(Control)	73.0	78.0	91.5	96.0	97.0
2.5	71.5	87.5	96.0	97.5	97.5
5.0	63.0	83.5	83.5	90.0	90.0
10.0	72.5	80.0	94.5	96.5	96.5
25.0	67.0	71.5	85.0	94.0	94.0
50.0	54.0	72.5	82.0	91.0	91.0
100.0	18.0	49.0	49.5	60.0	60.0
200.0	09.5	28.0	33.0	40.0	40.0
300.0	-	-	-	-	-

Values are of % germination.

On the other hand P.aureus and P.aconitifolius seem to be most tolerant among the legumes studied. L.esculenta follows D. biflorus in order of salinity tolerance with an intermediate nature of salt tolerance during germination.

Usually when salt stress is increased beyond the optimal level the germination is delayed, reducing germination percentage also. The effect of salinity on germination has been studied by many workers like Wahhab (1961), Bernstein (1964), Maliwal and Paliwal (1967), Sarin and Narayanan (1968), Macke and Unger (1971), Sorour et al. (1977), Sheoran (1980), Sung (1981), Mahamoud and Hill (1981), Dubey (1982), Mukharjee et al.(1982) and Joshi and Iyengar (1982).

Six soyabean cultivars have been studied for salt tolerance during germination and later stages by Abel and Mackenzie (1964). They are of the opinion that the variations of the varieties in seed germination and emergence may be due to inherited differences as well as the difference in chloride ion concentration.

The effect of NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$ on P.antidotale seed germination has been studied by El-Mansy and Bond (1978). They have found the decrease in germination percentage with increase in salt concentration and it varies with the salt used and the specific ions involved. El-Shourbagy et al.(1979) recorded a decrease in germination percentage with increase in salinity level and percent reduction is different in different varieties of Hordeum vulgare. They observed that the addition of mixture of

TABLE 2 : Effect of NaCl salinity on germination in *L. esculenta*.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
0.0 (Control)	36.0	86.0	94.5	95.0	96.5
2.5	33.0	76.5	94.0	97.5	98.0
5.0	36.5	69.5	82.0	94.0	96.5
10.0	28.5	77.5	92.5	97.0	98.0
25.0	24.5	55.0	77.0	88.5	92.0
50.0	18.0	32.5	63.0	85.0	88.0
100.0	19.0	34.5	46.5	68.5	80.5
200.0	04.0	14.0	17.0	17.0	17.0
300.0	02.0	07.0	08.0	08.0	09.0

Values are of % germination.

amino acids enhances general growth and protein content of seedlings exposed to salt stress. Ungar et al.(1979) found inhibition of germination in Salicornia under natural saline conditions. Rizk et al.(1979) have studied germination and seedling vigour in Medicago species under saline conditions and they have also found a decline in germination percentage and even in the rate of germination under salinity stress. According to them the germination depends on the interaction between salt concentration and Medicago species. According Sheoran and Garg(1979), germination in moong is independent of the type of salinity. However, salt affects the germination by delaying it and it also affects the seedling growth. A good performance during germination of rice variety IR-20 in saline media upto 0.2 M NaCl has been recorded by Subramanium (1979). The effect of NaCl salinity on germination percentage and the seedling growth in a number of groundnut varieties have been studied by Karadge and Chavan (1980). According to them germination percentage in some varieties of groundnut is considerably influenced by salt, in some varieties it is not affected by both lower as well as higher salt concentrations, while in some other varieties salinity enhances germination and it is inhibited only at the highest salinity levels. These studies showed that groundnut has a varietal difference in response to salinity even at the germination stage.

Singh and Chandra (1980) observed a 50% decrease in germination at salinity levels beyond ECe 5 in Pennisetum

TABLE 3 : Effect of NaCl salinity on germination in *P.aconitifolius*.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
0.0 (Control)	90.0	94.0	96.0	97.5	97.5
2.5	83.0	88.0	90.0	94.5	92.5
5.0	88.5	91.5	93.0	94.0	94.0
10.0	88.0	90.0	91.5	93.0	93.0
25.0	86.0	89.0	92.5	94.5	94.5
50.0	82.0	83.5	87.5	91.5	91.5
100.0	78.5	84.5	84.5	84.5	85.5
200.0	68.5	79.0	80.0	84.5	94.5
300.0	75.0	77.0	78.0	81.5	81.5

Values are of % germination.

cultivars and also indicated a varietal difference in salinity tolerance. A decrease in germination under saline habitat in different millet cultivars has been observed by Manga and Saxena (1981). A very little effect of salinity on seedling emergence, has been observed by Mahmoud and Hill (1981) at 10 - 15°C in salt tolerant sugarbeet, however, if temperature is increased above 25°C germination declines with salt concentration. According to Sung (1981) speed of germination in barley is retarded by external NaCl but germination percentage is apparently independent of NaCl. More and Ghensikar (1982) recorded that the germination in P.aureus is delayed and decreased with increasing salt concentrations. Present observations with the same species confirm this finding. A 25% reduction in germination has been observed by Miyamoto et al.(1982) in Parthenium due to salt stress. Similarly in barley an inhibitory trend has been reported by Kumar et al.(1982), in Brassica juncea by Kumar and Malik (1984), in Melon by Nelson and Paris (1985), in Avena sativa by Verma and Yadav (1986), in pea by Setia and Narang (1986) and in paddy, maize, sorghum, cotton and tobacco by Maliwal and Paliwal (1986).

Reddy and Vora (1985) suggested while studying the effect of salinity on germination of P.typhoides seeds that the germination is generally not affected by salt, but only the process is delayed, accompanied by accumulation of proline. Similar delay due to salinity was found by Khot (1986) in Crotalaria juncea as compared to that in P.aconitifolius. It was found that salinity

TABLE 4 : Effect of NaCl salinity on germination in *P.aureus*.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
0.0 (Control)	95.5	99.5	99.5	100.0	100.0
2.5	98.5	99.5	100.0	100.0	100.0
5.0	97.5	99.0	100.0	100.0	100.0
10.0	97.0	99.5	100.0	100.0	100.0
25.0	97.5	100.0	100.0	100.0	100.0
50.0	94.0	97.0	99.5	100.0	100.0
100.0	85.5	93.5	97.0	97.0	97.5
200.0	88.0	92.0	96.0	96.5	96.5
300.0	76.0	89.5	96.0	96.0	96.0

Values are of % germination.

completely inhibits germination beyond 150 mM NaCl in C.juncea. Salinity not only affects the germination percentage but also delays the process in Chickpea (Murumkar,1986). The salinity level upto 5-6 m^S cm^{-1} is not toxic for germination in Aleuropus lagpoids (Joshi and Bhoite,1987), but the higher salinities delay and reduce the germination.

Gill and Singh (1985) suggested, on the basis of studies on germination of Oryza sativa seeds under salt stress, that tolerant varieties of paddy have faster rate of seed germination under saline conditions. Khot (1986) has found an increase in germination percentage in P.aconitifolius at low salt level concentrations. However, salinity concentrations above 100 mM upto 300 mM NaCl inhibit the germination but not to produce any harmful or toxic effects.

It is evident from the foregoing account that salinity inhibits and delays germination in most of the plants. This is probably due to the situation where the osmotic potential of seed sap is lower than that of external solution which unables the seeds to absorb the required amount of water. Similarly, accumulation of NaCl in seeds also leads to the development of metabolic disorders. However, response to salinity varies from plants to plants as well as it also depends upon the genetic makeup.

Our observations indicate that P.aconitifolius and P. aureus are tolerant as the percentage germination is not much

TABLE 5 : Effect of NaCl salinity on germination in *T. foenum-graecum*.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
0.0 (Control)	70.0	86.0	98.0	98.0	98.0
2.5	72.0	92.5	96.5	98.0	98.0
5.0	73.5	92.5	97.5	98.0	98.0
10.0	59.0	83.5	92.5	95.0	95.0
25.0	63.0	80.5	96.0	97.5	97.5
50.0	63.0	89.5	94.0	96.5	96.5
100.0	32.0	43.0	62.5	73.5	73.5
200.0	00.0	00.0	00.0	08.0	08.0
300.0	00.0	00.0	00.0	00.0	00.0

Values are of % germination.

affected. In D.biflorus there is decrease in germination percentage with increasing concentration and no germination at 300 mM NaCl. L.esculenta seems to be somewhat sensitive to salt where germination is delayed and inhibited with increasing concentration. However, it appears that D.biflorus and L.esculenta are moderately salt tolerant species and T.foenum-graecum exhibits salt sensitivity during germination.

B) Linear Growth :

1) Epicotyle length :

The effect of various concentrations of NaCl on epicotyle development in D.biflorus, L.esculenta, P.aconitifolius, P.aureus and T.foenum-graecum seedlings has been shown in Tables 6,7,8,9 and 10 and Fig 1,2,3,4 and 5 respectively. The hypocotyle development in these species has also been recorded respectively in the same tables. From these tables it is clear that P.aconitifolius seedlings have recorded the maximum length of epicotyle while, the lentil and horsegram seedlings, produce the shortest epicotyles. The epicotyle length in P.aureus and fenugreek seedlings is intermediate. It can also be seen that, in all the plants the epicotyle development takes place after about 48 to 72 h of germination and with further increase in the duration of time there is further increase in size of the epicotyle.

It is also evident that horse gram has shown a successful development of epicotyle and unfolding of leaves only upto 10 mM

Fig.1 : Photograph showing, the effect of NaCl salinity on growth and development of D.biflorus, Roxb.

1. Control (Distilled Water) 2. 25 mM NaCl
3. 50 mM NaCl 4. 100 mM NaCl
5. 200 mM NaCl

Fig.2 : Photograph showing, the effect of NaCl salinity on growth and development of Lens esculenta, Moench. seedling after 120 hours.

1. Control (Distilled Water) 2. 25 mM NaCl
3. 50 mM NaCl 4. 100 mM NaCl
5. 200 mM NaCl

Fig.3 : Photograph showing, the effect of NaCl salinity on growth and development of *Phaseolus aconitifolius*, Jacq. seedlings after 120 hours.

1. Control (Distilled Water) 2. 25 mM NaCl
3. 50 mM NaCl 4. 100 mM NaCl
5. 200 mM NaCl

Fig.4 : Photograph showing, the effect of NaCl salinity on growth and development of *Phaseolus aureus*, Roxb. seedlings after 120 hours.

1. Control (Distilled Water) 2. 25 mM NaCl
3. 50 mM NaCl 4. 100 mM NaCl
5. 200 mM NaCl

Fig.5 : Photograph showing, the effect of NaCl salinity on growth and development of *Trigonella foenum-graecum*, Linn. seedling after 120 hours.

1. Control (Distilled Water) 2. 25 mM NaCl
3. 50 mM NaCl 4. 100 mM NaCl
5. 200 mM NaCl

FIG. 1

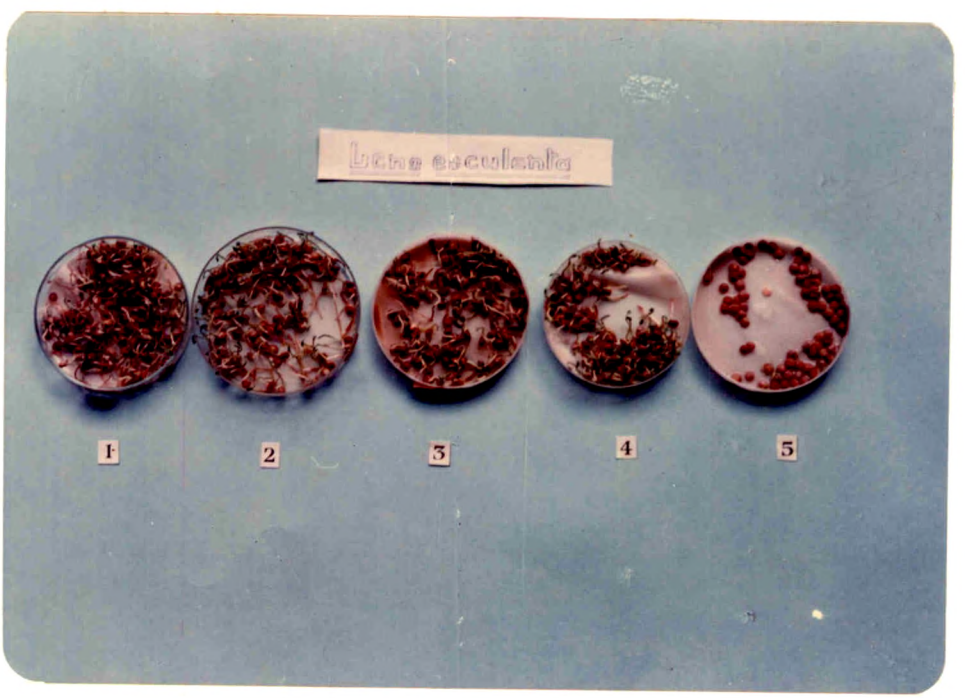

FIG. 2

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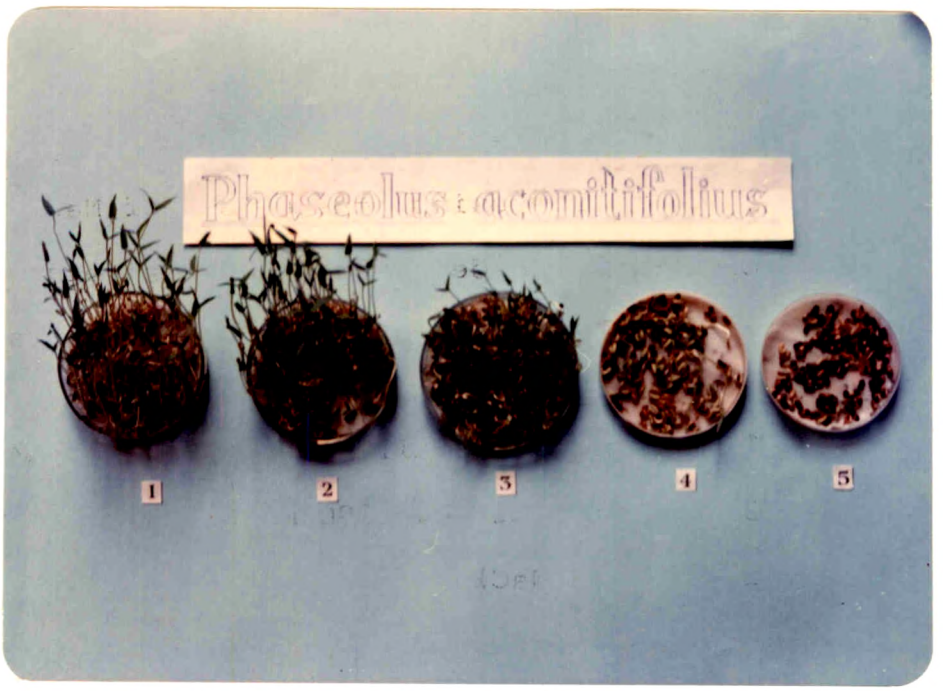

FIG. 3

FIG. 4

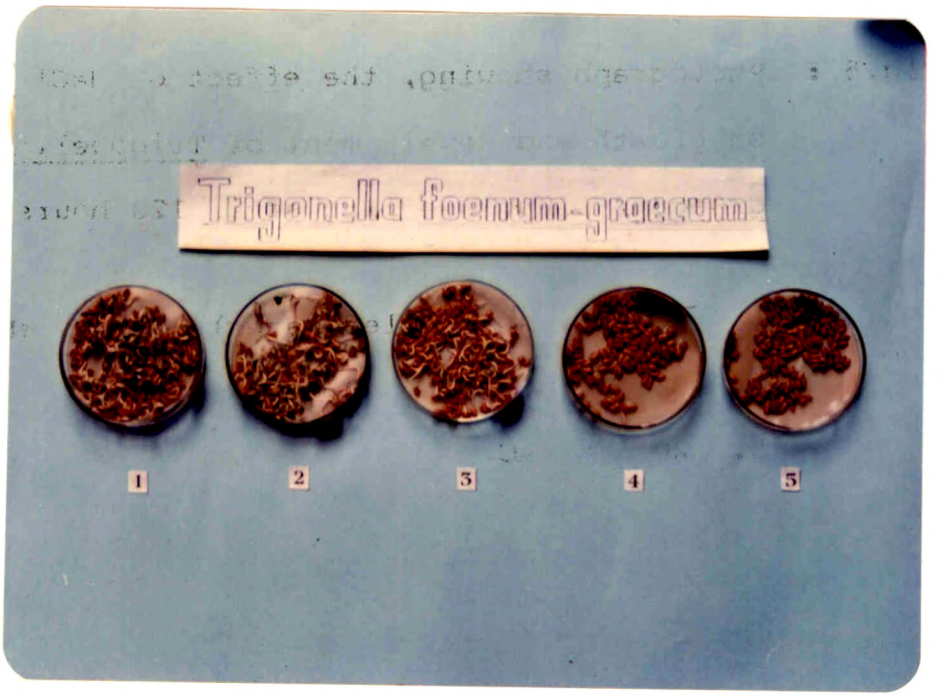

FIG. 5

NaCl level. In case of 25 mM NaCl treated seedlings the epicotyle length is equal to that in the control seedlings (1.5 cm) at 120 h. It appears that, from 50 mM NaCl onwards the epicotyle formation in this species is strongly affected. In lentil the development of epicotyle seems to be very slow with increasing the period of germination and it is decreased remarkably with increase in NaCl concentration in the medium. It can be seen that, there is more than 50% reduction in the epicotyle length of the seedlings grown at 25 mM NaCl level and at 200 and 300 mM salinity levels, the epicotyle formation is completely inhibited. Moth bean seedlings, however, show better development and unfolding of leaves in control as well as under saline conditions i.e. at different salt concentrations upto 50 mM. However, higher concentrations (100 and 200 mM) seem to be inhibitory for the development of epicotyle in moth bean. Thus, P.aconitifolius shows a better growth even under saline conditions. P.aureus has also given a good response upto 50 mM salt concentration where there is increase in size of the seedlings with increasing salt concentration (120 h growth) and the the epicotyle length exceeds that in the control. 3.5 cm is the length of epicotyle after 120 h germination under non-saline conditions and it changes to 5.6 cm which is the highest value of epicotyle at 2.5 mM NaCl . NaCl concentrations higher than this however, induce reduction in the epicotyle growth (5, 4.5 and 3.5 cm respectively at 5, 10 and 25 mM NaCl). Thus, it is apparent that, green gram probably shows better adaptations to the adverse

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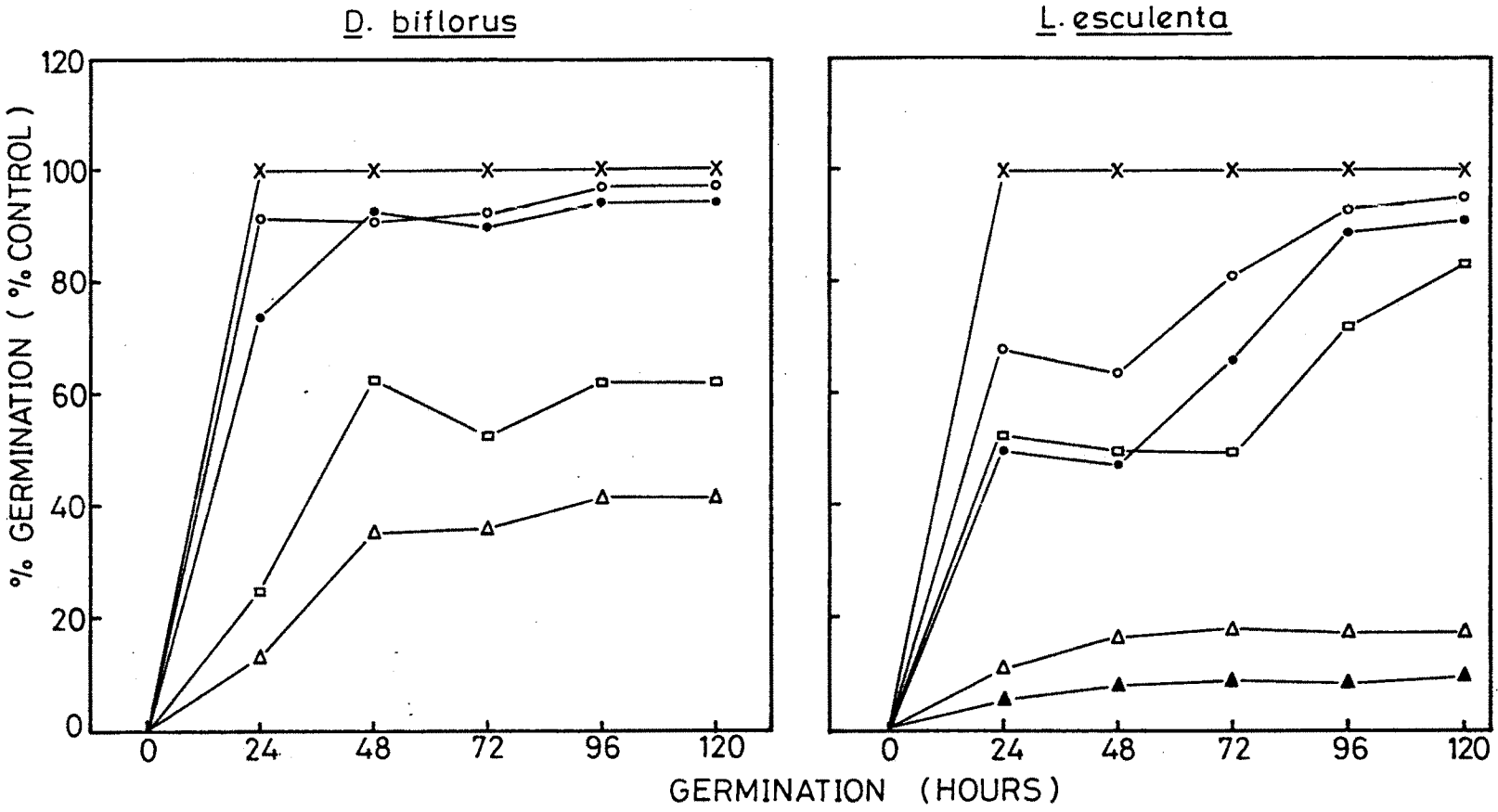


x CONTROL , o 25 mM NaCl , ● 50 mM NaCl , ▫ 100 mM NaCl , △ 200 mM NaCl , ▲ 300 mM NaCl .

Fig.6 EFFECT OF NaCl SALINITY ON % GERMINATION PERCENTAGE IN D. biflorus AND L. esculenta .

TABLE 6 : Effect of NaCl salinity on length* of the shoots (hypocotyle and epicotyle) of *D.biflorus* seedlings.

Treatment NaCl (mM)	Hours of Germination									
	24	48	72	96	120	24	48	72	96	120
	HYPOCOTYLE LENGTH (cm)					EPICOTYLE LENGTH (cm)				
0.0 (control)			2.5	5.80	6.70			0.90	1.40	1.50
2.5			1.9	4.20	6.07			0.60	1.10	1.30
5.0			2.9	4.90	5.90			1.00	1.10	1.10
10.0	NO SEEDLING FORMATION		2.2	4.60	6.10	NO SEEDLING FORMATION		0.70	0.90	1.30
25.0			2.8	3.90	6.70			0.90	1.00	1.50
50.0			1.6	3.00	5.20			0.00	1.20	1.20
100.0			1.5	2.50	4.00			-	0.70	0.90
200.0			0.7	1.08	1.30			-	-	-
300.0			-	-	-			-	-	-

* Each value is average of 10 measurements.

TABLE 7 : Effect of NaCl salinity on the length* of the shoots (hypocotyle and epicotyle) of *L.esculenta* seedlings.

Treatment NaCl (mM)	Hours of Germination									
	24	48	72	96	120	24	48	72	96	120
	HYPOCOTYLE LENGTH (cm)					EPICOTYLE LENGTH (cm)				
0.0 (control)			1.35	1.85	2.75			0.40	0.65	1.55
2.5			1.15	1.55	2.30			0.45	0.60	0.75
5.0			1.40	1.41	2.27			0.45	0.60	1.10
10.0	NO SEEDLING FORMATION		0.95	1.25	2.05	NO SEEDLING FORMATION		0.40	0.50	1.30
25.0			0.40	0.75	1.45			0.25	0.35	0.67
50.0			0.70	0.70	1.45			0.35	0.35	0.60
100.0			1.10	0.80	0.85			0.30	0.20	0.60
200.0			-	-	-			-	-	-
300.0			-	-	-			-	-	-

* Each value is average of 10 measurements.

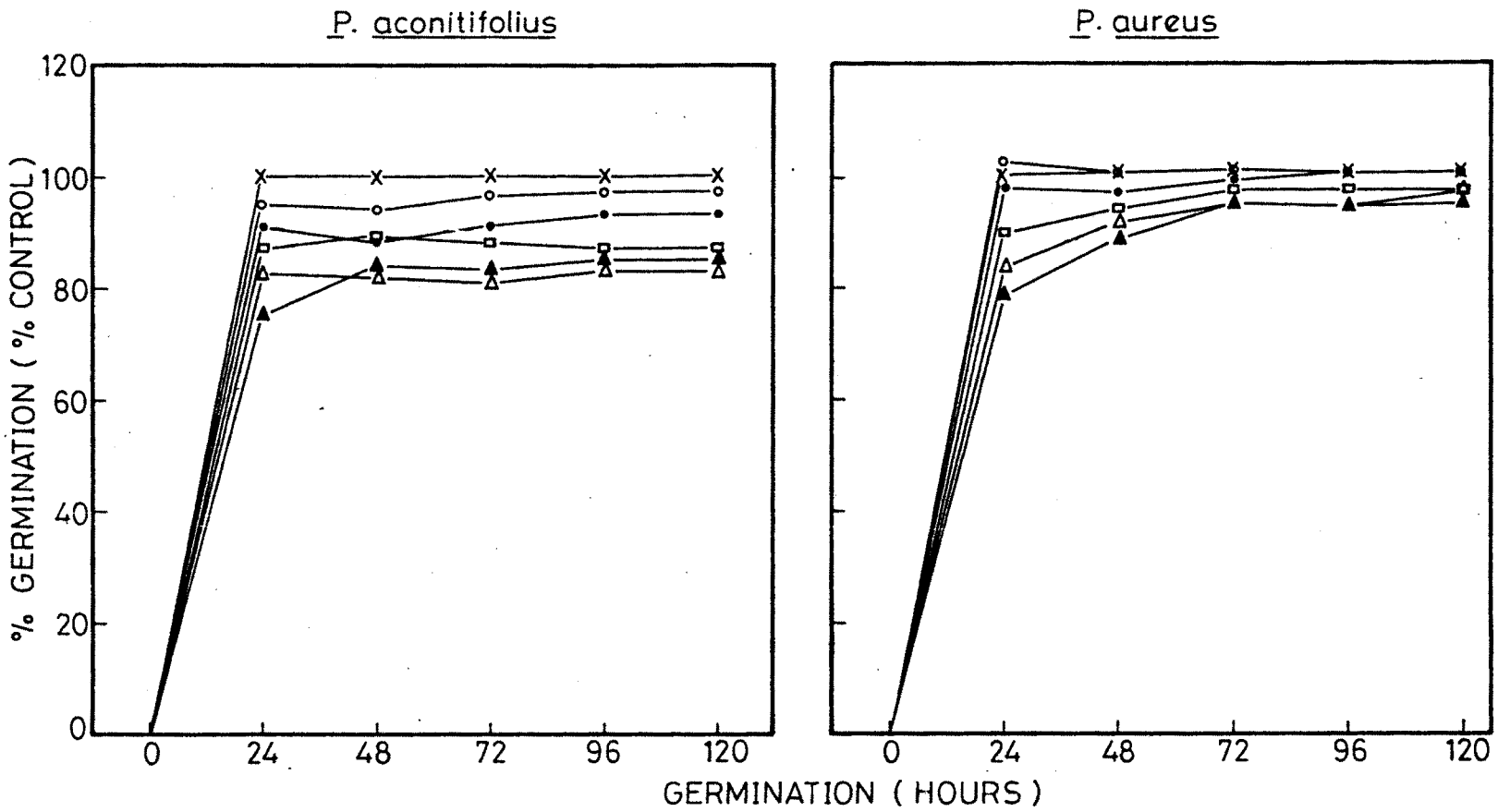
conditions of salt salinity, at least, during seedling establishment.

As there is no unfolding of leaves and hence the development of epicotyle, fenugreek seems to be very slow in development, as even in the non-saline conditions, there is no leaf formation upto 120 h germination.

2) Hypocotyle length :

From the tables 6,7,8,9 and 10 it can be seen that, in all seedlings hypocotyle is well developed as compared to epicotyle. It can be seen that, in general, in all plants the seedlings are well established before 72 h except, in P.aureus (48 h). It is also evident that, the length of hypocotyle increases with time while decreases with increase in salinity level. It can also be seen that, the maximum hypocotyle length has been recorded in P.aureus seedlings (8 to 9 cm) while, the fenugreek seedlings produce the shortest hypocotyles. The length of hypocotyle in D.biflorus and P.aconitifolius however, is almost equal.

It is evident that, upto 100 mM salt level the hypocotyle development in case of horsegram is normal. 6.5 cm is the higest length of hypocotyle which remains constant even at 25 mM salt concentration. From 25 mM onwards, however, it is decreased and at 300 mM NaCl there is no hypocotyle or seedling formation. Lentil is found to be relatively sensitive in which, the hypocotyle length is increased very slowly upto 96 h of germination and at



x CONTROL, o 25 mM NaCl, • 50 mM NaCl, ▫ 100 mM NaCl, △ 200 mM NaCl, ▲ 300 mM NaCl.

Fig.7 EFFECT OF NaCl SALINITY ON % GERMINATION PERCENTAGE IN P. aconitifolius AND P. aureus.

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TABLE 8 : Effect of NaCl salinity on the length* of the shoots (hypocotyle and epicotyle) of *P.aconitifolius* seedlings.

Treatment NaCl (mM)	Hours of Germination									
	24	48	72	96	120	24	48	72	96	120
	HYPOCOTYLE LENGTH (cm)					EPICOTYLE LENGTH (cm)				
0.0 (control)			3.1	3.70	5.75			1.2	1.45	4.10
2.5			5.7	3.22	4.41			1.1	1.73	2.25
5.0			2.7	3.80	5.85			1.1	1.55	2.50
10.0	NO SEEDLING FORMATION		3.4	3.63	4.80	NO SEEDLING FORMATION		1.2	1.60	2.95
25.0			2.7	3.00	4.65			1.4	2.10	3.25
50.0			2.3	2.55	3.05			1.1	1.50	3.50
100.0			0.9	1.35	1.70			-	1.70	0.60
200.0			0.5	0.40	0.50			-	-	-
300.0			0.5	0.50	0.50			-	-	-

* Each value is average of 10 measurements.

TABLE 9 : Effect of NaCl salinity on length* of the shoots (hypocotyle and epicotyle) of *P.aureus* seedlings.

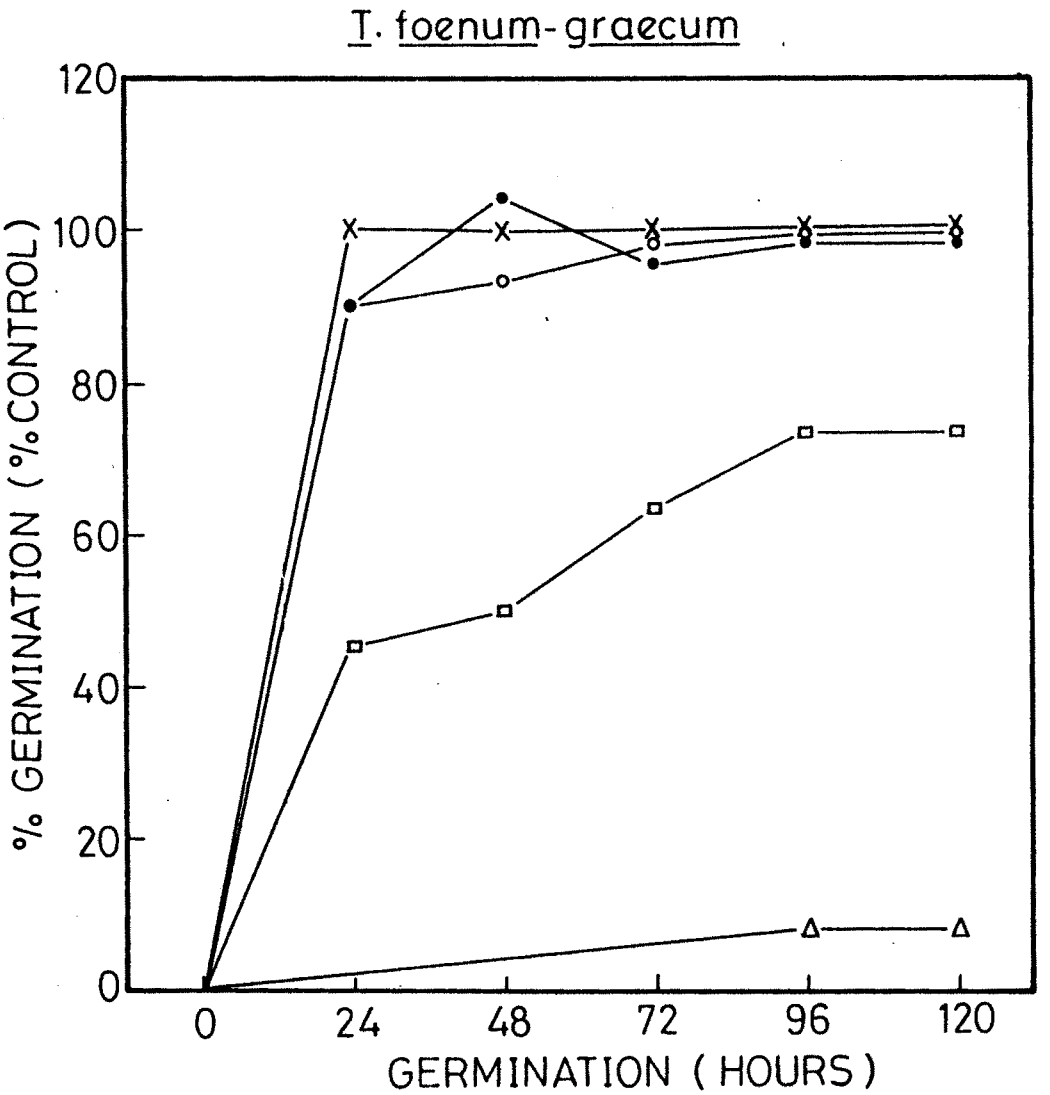
Treatment NaCl (mM)	Hours of Germination									
	24	48	72	96	120	24	48	72	96	120
	HYPOCOTYLE LENGTH (cm)					EPICOTYLE LENGTH (cm)				
0.0 (control)	NO SEEDLING FORMATION	0.55	2.35	4.50	8.85	NO SEEDLING FORMATION		1.40	2.40	3.50
2.5		1.55	3.45	7.25	9.80			1.45	2.70	5.60
5.0		1.00	3.55	4.65	9.45			1.40	2.10	5.00
10.0		0.80	2.75	6.25	8.40			1.20	2.10	4.50
25.0		0.80	2.25	5.60	7.95			0.80	2.40	3.50
50.0		0.80	1.65	4.20	7.20			0.60	1.50	2.40
100.0		0.40	0.65	1.15	1.55			0.10	0.45	0.80
200.0		0.40	0.75	0.75	0.75			0.00	0.40	0.60
300.0		0.30	0.50	0.55	0.70			-	0.50	0.70

* Each value is average of 10 measurements.

120 h it is increased considerably. 100, 200 and 300 mM salt concentrations are found to be strongly inhibitory as the seedling development is highly suppressed and even, at 200 and 300 mM there is no hypocotyle development at all.

It is also evident that, at lower NaCl levels the hypocotyle growth in P.aconitifolius is stimulated, however, the higher salt concentration supress the hypocotyle growth in this species. The seedlings at 50 mM NaCl have shown a better development of hypocotyle (5.85 cm) after 120 h of growth which is better than that in control (5.75 cm). Green gram has also given a good response with respect to seedling growth under saline conditions. It can be seen that, the growth of hypocotyle in this species is rather improved further by salinity upto 5 mM NaCl level. However, more than 50% reduction in the hypocotyle length has been found at 100 mM NaCl level.

Fenugreek seems to be rather sensitive species as, there is no seedling formation even upto 120 h at 200 and 300 mM NaCl. Thus, the seedling formation is totally inhibited by the salt at these higher concentrations. Similarly, the performance of seedlings with respect to hypocotyle length, seems to be unaffected upto about 5 mM NaCl level only. At 10 mM there is considerably decrease in the hypocotyle length. At 50 mM NaCl it is again increased and that, it is the highest of all the values. This sudden increase in size of hypocotyle in fenugreek however, cannot be explained.



x CONTROL, o 25 mM NaCl, • 50 mM NaCl,
▫ 100 mM NaCl, Δ 200 mM NaCl.

Fig. 8 EFFECT OF NaCl SALINITY ON % GERMINATION PERCENTAGE IN T. foenum-graecum

TABLE 10 : Effect of NaCl salinity on the length* of shoots (hypocotyle) of *T.foenum-graecum* seedlings.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
	HYPOCOTYLE LENGTH (cm)				
0.0 (control)			0.65	1.30	2.30
2.5			0.80	1.35	2.50
5.0			0.85	1.40	2.35
10.0	NO SEEDLING FORMATION		0.45	1.25	1.65
25.0			1.45	1.85	2.80
50.0			0.70	0.65	1.80
100.0			0.50	0.70	1.10
200.0			-	-	-
300.0			-	-	-

* Each value is average of 10 measurements.

When the root : shoot (epicotyle + hypocotyle) ratio of the seedling at 120 h of growth is considered, it can be seen that, lentil and fenugreek have this ratio higher than that in other legumes and also near unity (about 0.7 and 0.8 respectively) indicating that the root and shoot growth in these species is also at the same extent.

In other legumes, however, this ratio is almost constant (0.5) indicating more growth of shoot parts than root. It can also be seen that, due to salinity this ratio in all the legume species is markedly changed. It is observed that, in all the seedlings of all species (except in lentil) the root : shoot ratio is increased considerably due to salt treatment, indicating that salinity affect the shoot growth in the legume seedlings. In case of lentil, however, this ratio falls down remarkably due to salinity and suggest that, root growth is affected more by salt stress.

3) Root growth (length) :

The effect of different concentrations of NaCl on root growth (length) of *D.biflorus*, *L.esculenta*, *P.aconitifolius*, *P.aureus* and *T.foenum-graecum* has been shown in Tables 11, 12, 13, 14 and 15 respectively. From these observations, it is evident that, the root length of seedlings of all legume species increases with time and the maximum root length is observed after 120 h germination. In most of the species the root formation to the measurable extent takes place only after 48 h of germination

TABLE 11 : Effect of NaCl salinity on the length* of the roots of *D.biflorus* seedlings.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
	LENGTH (cm)				
0.0 (control)			1.1	2.5	4.30
2.5			1.0	2.2	3.09
5.0			1.2	2.2	3.09
10.0	NO ROOT FORMATION		1.2	2.5	3.30
25.0			1.3	1.8	4.1
50.0			0.9	1.8	3.2
100.0			0.5	1.6	2.4
200.0			0.5	0.9	1.2
300.0			-	-	-

* Each value is average of 10 measurements.

except in P.aureus where it is within 24 h of germination. This suggests that the seedling growth is very rapid in this species as compared to that in all remaining species. The seedlings of this species also show the highest value (7.25 cm), for the root length after five days growth. The shortest root produced after this period is that by fenugreek (1.80 cm). D.biflorus and P.aconitifolius have shown almost similar pattern in the rate of growth of root system.

Further, it can be seen that, NaCl salinity affects the root growth almost at all salt concentrations in all the species, except in P.aureus and T.foenum-graecum where, it is considerably affected only at higher salt concentrations (100 mM and above). It is also clear that, D.biflorus and L.esculenta show better response with respect to root growth, but only with the lower salt concentrations. Higher salt concentrations, however, inhibit the length of the root. It can be seen that, in horse gram at 300 mM NaCl there is no root emergence even upto 120 h of germination. In the same plant at 25 mM (120 h) the root length recorded is 4.1 cm and it is next to control (4.3 cm). Lentil seems to be more sensitive than horsegram where average root length of lentil is less as compared to that in horsegram. It is evident that, the root growth is affected by more than 15 % at 200 mM NaCl level in D.biflorus and P.aureus, while in case of L.esculenta and T.foenum-graecum it is at still lower salt concentration (100 mM). In P.aconitifolius it is affected

TABLE 12 : Effect of NaCl salinity on the length* of root of *L. esculenta* seedlings.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
	LENGTH (cm)				
0.0 (control)	NO ROOT FORMATION		0.90	1.65	3.40
2.5			0.65	1.30	2.80
5.0			0.85	1.40	3.00
10.0			0.45	1.00	2.75
25.0			0.35	0.69	1.85
50.0			0.45	0.45	1.90
100.0			0.40	0.30	0.35
200.0			0.40	-	-
300.0			-	-	-

* Each value is average of 10 measurements.

at the lowest salt concentration (50 mM). From these observations it can be concluded that, P.aureus and D.biflorus have a better performance under saline conditions. As far as root growth is considered, P.aconitifolius however, seems to be rather sensitive.

Lall and Sakhare (1970) have found that 0.5 atm NaCl is beneficial for the seedling growth in Sorghum and further reported that, there is a linear reduction in seedling growth with further increase in salinity level. In sugarbeet the radicle length was found to be decreased linearly when, NaCl concentration in the medium was increased from 0 to 0.2 M (Durrant et al.,1974). Robinson (1974) has also reported that, the seedlings of P.aureus become stunted when, grown in presence of NaCl. Present observations with P.aureus confirm the observations made by Robinson. Sheoran and Garg (1978), have studied the effect of chloride and sulphate salinity on germination and early seedling growth of P.aureus [illegible], have also reported that, the germination, early seedling growth and length of radicle and hypocotyle decreased due to the effect of all the salts tested.

The inhibition of seedling growth by chloride salinity has been reported by several workers (Paliwal and Maliwal,1973; Panigrahi et al.1978; Fattah and Bano, 1980; Gujarathi et al. 1981; Vora and Gopalkrishnan, 1981; Kale and Gupta 1982; Sing and Kale, 1985). Inhibitory effects of NaCl on soybean root elongation and oxygen uptake has been studied by Bejaoul (1980),

TABLE 13 : Effect of NaCl salinity on the length* of roots of *P.aconitifolius* seedlings.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
	LENGTH (cm)				
0.0 (control)	NO ROOT FORMATION		2.70	2.79	4.50
2.5			2.08	2.75	2.05
5.0			2.50	2.20	3.65
10.0			2.10	2.88	3.33
25.0			2.10	1.05	2.70
50.0			2.10	1.45	1.76
100.0			1.30	1.15	1.70
200.0			0.60	0.50	0.50
300.0			0.50	0.50	0.50

* Each value is average of 10 measurements.

and reported that, the salinity effect was significant only at the NaCl concentration higher than 50 mM. Sung (1980) have reported that, the NaCl treatment significantly inhibit the α-amylase and respiratory activity in germinating seeds which results in the reduction of plumule and radicle growth.

On the contrary Bharadwaj (1965) and Lall and Sakhare (1970) have observed stimulation of seedling growth in pea and sorghum seedlings respectively, particularly, in low regimes of NaCl salinity. An increase in growth rate of shoots of pea seedlings on exposure to -3 to -4 atm NaCl and a slight increase in the growth of roots exposed to -3 atm was observed by Hassan - Porath et al.(1972). Acharya and Sahu (1974), have reported that, the hypocotyle length in P.aureus was increased from 9.6 to 11.2 cm and that of epicotyle from 7.8 to 10.7 cm with increasing salinity from nil to 4 $mS\ cm^{-1}$ and then sharply declined after 72 h of growth in P.aureus var.Vaishakhi, with further increase in salinity. Such enhancement of seedling growth at lower doses of salt was evident in the experiments by Kumar and Bharadwaj (1981) in moong. The present observations with P.aureus local cultivar are also on the similar lines.

According to Bernstein (1974) both osmotic and specific ion effects are responsible for growth retardation. Sharma (1983) has observed adverse effect of accumulation of excess amount of sodium and chloride in the seedlings. Sheoran and Garg (1983) have reported that, the reduction in growth under chloride salinity

TABLE 14 : Effect of NaCl salinity on the length* of root of *P.aureus* seedlings.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
	LENGTH (cm)				
0.0 (control)	1.2	1.40	3.05	5.35	7.25
2.5	1.3	2.25	3.65	5.75	6.40
5.0	1.4	2.15	4.45	6.35	8.55
10.0	1.3	1.60	3.35	5.85	7.40
25.0	1.3	1.50	3.80	6.05	6.85
50.0	1.3	1.45	3.20	5.55	6.85
100.0	0.3	0.65	1.35	2.75	3.95
200.0	0.3	0.50	0.75	1.15	2.55
300.0	0.3	0.30	0.50	1.40	1.65

* Each value is average of 10 measurements.

seems to be due to accumulation of ions in the seedlings to the toxic level. Usually high salt concentrations in the saline medium create high osmotic pressure reducing the uptake of water and the inhibition of germination by salts, particularly at the higher concentrations may be due to prevention of water uptake. In the present investigation the inhibition of growth of legume seedlings observed under saline conditions, particularly, at the higher salt regimes (100 mM and above in most cases and 50 mM NaCl in some) appears to be due to inhibition of water uptake by the seeds. This may be due to relatively higher osmotic pressure in the medium, however, the response shown by the legume species differs from each other. It appears that, the species which show better performance even at the higher salt levels must have developed a mechanism for uptake of adequate amount of water during germination. In this respect *P.aureus* and *D.biflorus* seem to be more successful as compared to other legume species. Alternatively, it may be true that, the other species which show poor performance under saline conditions, may be relatively more sensitive to the toxic ion levels of sodium and chloride. However, to strengthen this statement, inorganic analysis of the seedling is required.

C) Biomass Production :

1) Fresh and dry wt :

The influence of NaCl salinity on biomass (fresh and dry wt) of *D.biflorus*, *L.esculenta*, *P.aconitifolius*, *P.aureus* ;

TABLE 15 : Effect of NaCl salinity on the length* of roots of *T. foenum-graecum*.

Treatment NaCl (mM)	Hours of germination				
	24	48	72	96	120
	LENGTH (cm)				
0.0 (control)	NO ROOT FORMATION		1.20	2.12	1.85
2.5			1.35	2.42	2.25
5.0			1.35	2.16	2.95
10.0			1.25	1.99	2.05
25.0			1.55	2.47	3.30
50.0			1.25	2.31	2.55
100.0			0.55	0.70	1.30
200.0			-	-	-
300.0			-	-	-

* Each value is average of 10 measurements.

and T.foenum-graecum seedlings has been depicted in Tables 16, 17,18,19 and 20 respectively. It is evident from the observations that in general in all the above plants with increase in the period of germination, the fresh wt of the seedlings is increased, but decreased with increasing salt concentration in the medium. P.aureus seedlings have recorded the highest fresh wt (3.74 g) at 120 h of germination at 2.5 mM NaCl. In the same plant after 24 h germination at 50 mM NaCl, the fresh wt recorded is 1.35 g and which is more than that in control. Same is the case with P.aconitifolius, where at 25 mM NaCl level fresh wt is increased throughout the period (from 24 h to 120 h) and is the highest of all concentrations and even the highest of that in control. Horse gram stands next to P.aconitifolius, where also the fresh wt slowly increases with time and decreases as per the salt concentrations. As compared to the horsegram, fenugreek ranks next to horsegram. There is very slow increment in fresh wt of the seedling of this species and it is the highest in control as well as in 25 mM NaCl level (i.e. 0.98).

As far as dry wt of a seedling is concerned it is very slowly and slightly decreases with increasing period in all the above plants except fenugreek in which at the beginning from control to 25 mM NaCl level with increasing time period it is decreased while at the 25 mM and higher salt concentrations and after 120 h germination it is found to be constant or slightly increased. It appears that the salinity has not at all influenced

TABLE 16 : Effect of NaCl salinity on the biomass* of D.biflorus seeds during germination and seedling growth .

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	FRESH WT (g)						DRY WT (g)					
0.0 (control)	0.33	0.67	0.75	1.11	1.40	1.34	0.30	0.30	0.30	0.26	0.24	0.23
2.5	0.33	0.64	0.78	1.14	1.21	1.21	0.30	0.29	0.29	0.26	0.22	0.19
5.0	0.33	0.65	0.74	1.15	1.22	1.33	0.30	0.30	0.27	0.25	0.21	0.19
10.0	0.33	0.65	0.77	1.13	1.16	1.18	0.30	0.28	0.28	0.25	0.22	0.21
25.0	0.33	0.64	0.72	1.13	1.21	0.94	0.30	0.30	0.29	0.24	0.23	0.22
50.0	0.33	0.62	0.68	0.91	0.96	1.12	0.30	0.27	0.28	0.26	0.25	0.22
100.0	0.33	0.63	0.65	0.72	0.93	0.64	0.30	0.29	0.27	0.27	0.26	0.23
200.0	0.33	0.64	0.57	0.69	0.82	0.73	0.30	0.30	0.27	0.28	0.29	0.27
300.0	0.33	0.44	0.59	0.63	0.59	0.51	0.30	0.30	0.30	0.30	0.28	0.26

* Values are of biomass per 10 seeds/seedlings.

TABLE 17 : Effect of NaCl salinity on the biomass* of L.esculenta seeds during germination and seedling growth.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	FRESH WT (g)						DRY WT (g)					
0.0 (control)	0.34	0.76	0.73	0.87	1.04	1.26	0.32	0.34	0.31	0.28	0.30	0.34
2.5	0.34	0.70	0.69	0.91	1.00	1.12	0.32	0.31	0.30	0.33	0.30	0.28
5.0	0.34	0.78	0.81	0.96	0.98	1.20	0.32	0.35	0.34	0.33	0.32	0.30
10.0	0.34	0.72	0.73	0.85	0.94	1.13	0.32	0.32	0.33	0.30	0.30	0.30
25.0	0.34	0.69	0.75	0.81	0.88	0.99	0.32	0.31	0.32	0.31	0.31	0.29
50.0	0.34	0.66	0.74	0.80	0.84	0.99	0.32	0.30	0.32	0.31	0.31	0.32
100.0	0.34	0.62	0.70	0.71	0.72	0.81	0.32	0.32	0.30	0.31	0.31	0.30
200.0	0.34	0.68	0.66	0.69	0.64	0.75	0.32	0.31	0.31	0.32	0.31	0.33
300.0	0.34	0.71	0.65	0.68	0.66	0.72	0.32	0.31	0.31	0.30	0.32	0.31

* Values are of biomass per 10 seeds/seedlings.

the dry wt of the seedlings in all plants studied. It can be seen that in all plants studied the dry wt of the seedlings grown under saline conditions is remaining unaffected. It appears that salinity probably affects the water uptake thereby affecting the fresh wt of seedlings. It is also true that salinity affects considerably the growth and differentiation of seedlings.

According to Odegbaro and Smith (1969) increasing salinity levels from 0 - 0.12 M NaCl reduced the fresh wt of young seedlings of Lactuca sativa. Similarly, Verma and Poonia (1979) found decreased root and shoot growth as well as biomass with increasing salinity in pearl millet, except at the low salt levels. Kumar and Bharadwaj (1982), observed in moong seedling a decrease in fresh wt as well as dry wt of embryo axis due to salinity. Sharma et al.(1983) found a decrease in fresh wt of 7 days old lettuce (L.sativa) seedlings with increasing salinity level. A decrease in fresh wt of Chickpea seedlings under salt stress is observed by Murumkar (1986). In the same manner under salt stress conditions a decrease in dry wt of seedlings in Medicago species is observed by Rizk et al.(1979), in sugarbeet by Mahamoud and Hill (1981), in Hibiscus cannabinus by Curtis and Lauchli (1985) in Pisum sativum by Setia and Narang (1986) and in Avena sativa by Verma and Yadava (1986). Toro (1987) observed in S.italica CV - Co - 5 a decrease in biomass production at the 50 mM salt concentration and it was decreased by 25% only at the higher salt concentration (i.e. 100 mM NaCl). According to her, it indicates the salt tolerance

TABLE 18 : Effect of NaCl salinity on the biomass* of P.aconitifolius seeds during germination and seedling growth.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	FRESH WT (g)						DRY WT (g)					
0.0 (control)	0.19	0.77	0.97	1.27	1.52	1.76	0.17	0.25	0.25	0.22	0.18	0.16
2.5	0.19	0.92	0.91	1.18	1.44	1.64	0.17	0.28	0.23	0.21	0.18	0.21
5.0	0.19	0.84	1.04	1.23	1.45	1.76	0.17	0.29	0.26	0.21	0.17	0.17
10.0	0.19	0.94	1.01	1.30	1.49	1.61	0.17	0.27	0.27	0.22	0.18	0.16
25.0	0.19	0.97	1.06	1.32	1.82	1.92	0.17	0.29	0.26	0.23	0.25	0.21
50.0	0.19	0.92	0.94	1.14	1.15	1.43	0.17	0.29	0.29	0.21	0.18	0.18
100.0	0.19	0.84	0.85	1.07	0.95	1.35	0.17	0.30	0.27	0.23	0.21	0.22
200.0	0.19	0.69	0.66	0.84	0.73	0.65	0.17	0.28	0.24	0.22	0.18	0.15
300.0	0.19	0.69	0.73	0.78	0.75	0.69	0.17	0.30	0.29	0.24	0.22	0.22

* Values are of biomass per 10 seeds/seedlings.

TABLE 19 : Effect of NaCl salinity on the biomass of *P.aureus* seeds during germination and seedling growth.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	FRESH WT (g)						DRY WT (g)					
0.0 (control)	0.29	1.26	1.63	1.83	3.00	3.41	0.26	0.39	0.38	0.26	0.32	0.24
2.5	0.29	1.28	1.72	2.00	2.88	3.73	0.26	0.41	0.38	0.32	0.27	0.26
5.0	0.29	1.18	1.64	2.21	2.60	3.51	0.26	0.36	0.36	0.32	0.27	0.25
10.0	0.29	1.19	1.56	2.20	2.64	3.61	0.26	0.37	0.37	0.33	0.28	0.28
25.0	0.29	1.23	1.57	2.11	2.68	2.98	0.26	0.39	0.37	0.33	0.29	0.24
50.0	0.29	1.35	1.67	1.94	2.66	3.12	0.26	0.47	0.41	0.35	0.34	0.31
100.0	0.29	1.00	1.31	1.60	1.94	2.06	0.26	0.40	0.39	0.34	0.38	0.29
200.0	0.29	1.20	1.21	1.29	1.65	1.68	0.26	0.49	0.39	0.36	0.37	0.31
300.0	0.29	0.95	1.14	1.18	1.52	1.59	0.26	0.38	0.37	0.33	0.35	0.31

* Values are of biomass per 10 seeds/seedlings.

TABLE 20 : Effect of NaCl salinity on the biomass* of *T.foenum-graecum* seeds during germination and seedling growth.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	FRESH WT (g)						DRY WT (g)					
0.0 (control)	0.16	0.47	0.46	0.64	0.74	0.98	0.13	0.13	0.11	0.10	0.10	0.09
2.5	0.16	0.40	0.46	0.68	0.74	0.93	0.13	0.12	0.11	0.11	0.10	0.07
5.0	0.16	0.40	0.48	0.62	0.78	0.91	0.13	0.11	0.12	0.10	0.10	0.09
10.0	0.16	0.39	0.45	0.60	0.70	0.86	0.13	0.15	0.11	0.10	0.10	0.08
25.0	0.16	0.37	0.35	0.56	0.77	0.98	0.13	0.11	0.11	0.09	0.09	0.08
50.0	0.16	0.38	0.43	0.53	0.70	0.68	0.13	0.10	0.11	0.10	0.10	0.10
100.0	0.16	0.38	0.39	0.39	0.57	0.63	0.13	0.11	0.14	0.13	0.13	0.11
200.0	0.16	0.42	0.37	0.41	0.43	0.59	0.13	0.14	0.13	0.14	0.16	0.14
300.0	0.16	0.38	0.39	0.46	0.43	0.56	0.13	0.13	0.13	0.13	0.16	0.13

* Values are of biomass per 10 seeds/seedlings.

capacity of the cultivar at the early seedling stage. Similarly she observed about 25% reduction in biomass production in cultivar SIC-1 even at the low salt concentration (i.e. 25 mM NaCl) which indicated its salt sensitivity.

On the contrary, in C.juncea and P.aconitifolius seedlings, Khot (1986) found an increase in biomass due to salinity at the low salt concentrations. However, at the higher concentrations the fresh wt of seedlings of both the species was reduced. Mahmood and Malik (1987) observed similar increase in biomass in Atriplex seedlings upto EC 15 mS cm^{-1}. However, further increase in salinity reduced the biomass production even though at 50 mS cm^{-1} concentration the plants could survive.

2) Moisture (water) uptake and dry wt of seedlings :

The effect of NaCl salinity at different concentrations of NaCl on moisture content (water uptake) and percent dry wt of seedlings of the above legume species at 72 h and 96 h of germination has been depicted in Fig.9 to 18. The moisture percentage and percent dry matter of 72 and 96 h old seedlings of D.biflorus grown under saline and non-saline conditions have been shown in Fig.9 and 10 respectively. It can be seen that the moisture level of the 72 h old seedlings increases linearly with increase in salinity level upto 25 mM NaCl where it is the highest. However, beyond this salinity level the moisture content of seedlings is severely affected and the lowest being in the seedlings treated with 200 mM NaCl. Concommitantly the dry wt of the seedlings

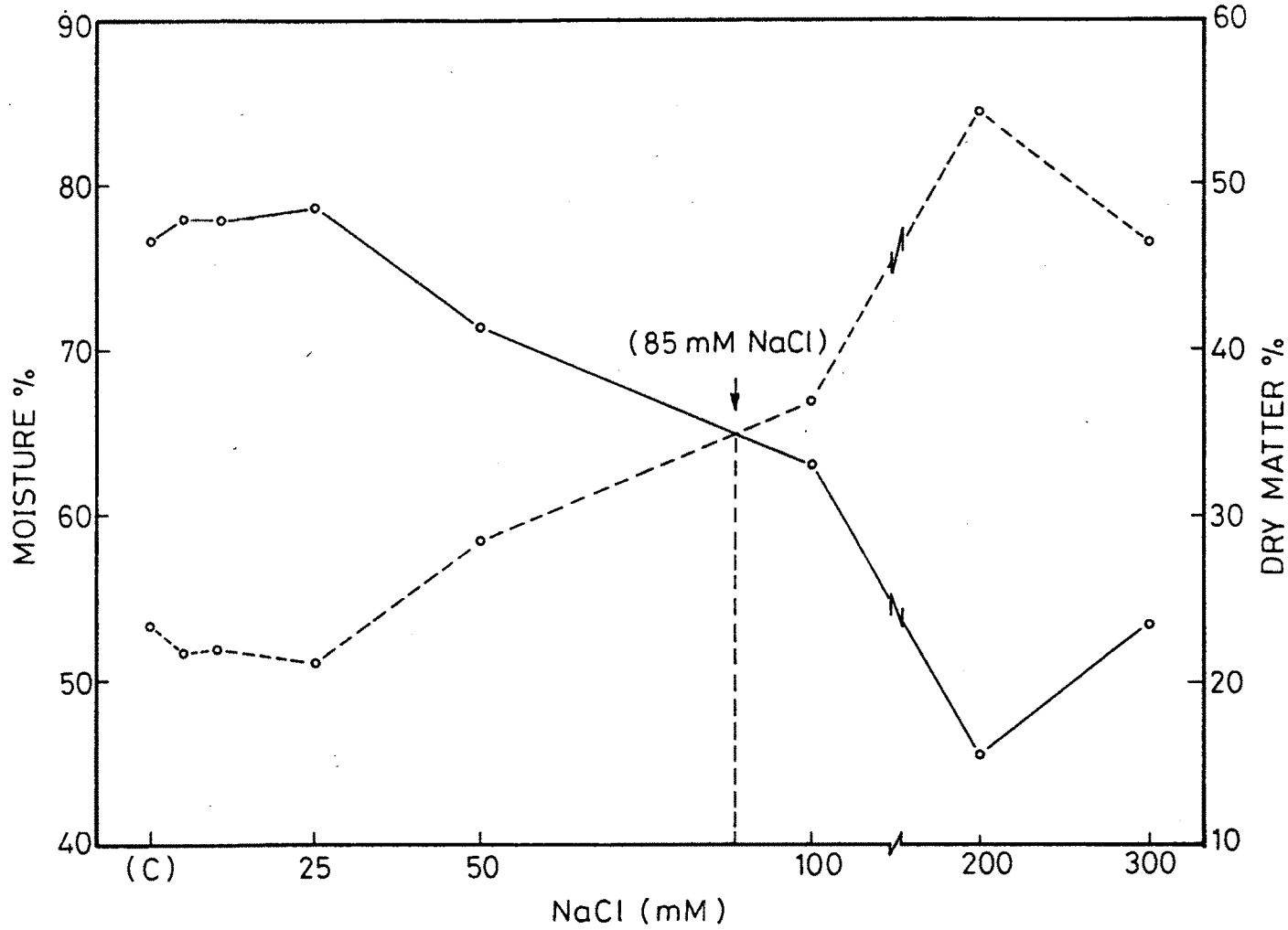


Fig.9 EFFECT OF NaCl SALINITY ON MOISTURE (—) UPTAKE AND DRY MATTER CONTENT (----) OF D. biflorus SEEDLINGS (72 HOURS GERMINATION) .

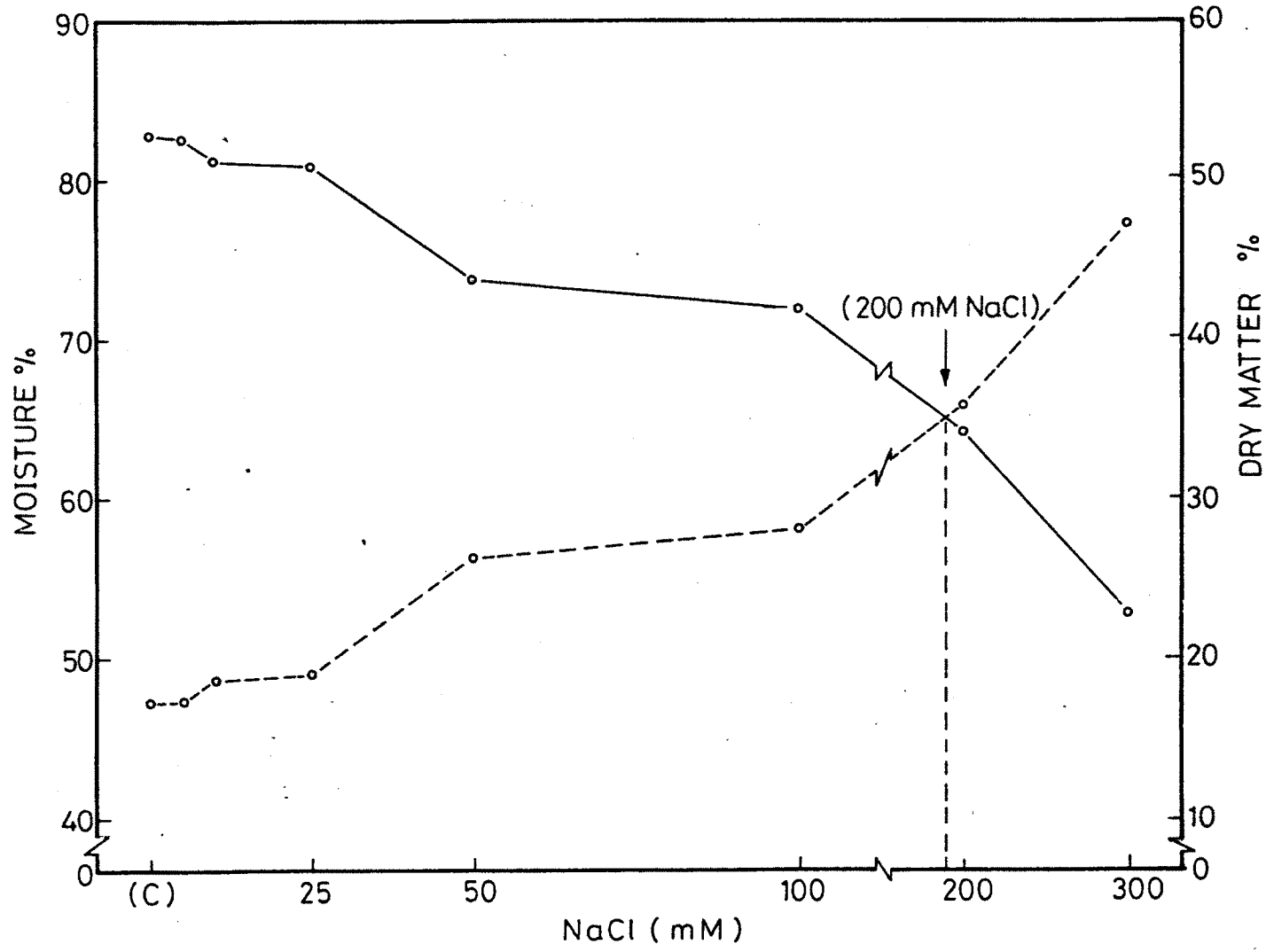


Fig. 10 EFFECT OF NaCl SALINITY ON MOITURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF D. biflorus SEEDLINGS (96 HOURS GERMINATION).

falls down upto 25 mM salt level but increases very rapidly with further increase in salinity level. The highest dry wt is recorded in the seedling grown at 200 mM NaCl level. It appears that the rate of water uptake and the dry wt of seeds or seedlings are inversally proportional to each other. However, it is interesting to observe that the axis of these two parameter intersect each other at certain point indicating certain salt concentration. In this case of D.biflorus, the 72 h old seedlings show this concentration to be about 85 mM NaCl which is obviously the first such intersecting point. 200 mM NaCl is the intersecting point in case of 96 h old seedlings of this species. It appears that water uptake, an important event in the germination of a seed, by D.biflorus seeds/seedlings is completely inhibited at the salt concentrations in the medium, indicated by the intersecting points i.e. 85 and 200 mM NaCl concentrations, exhibited respectively by 72 h and 96 h old seedlings. Thus, it can be suggested that 100-200 mM NaCl in the medium is probably the critical concentration of salt upto which level, the D.biflorus seeds can tolerate the salinity level.

The effect of different concentrations of NaCl on moisture percentage and dry matter content of L.esculenta seedlings at 72 and 96 h of germination has been depicted in Fig.11 and 12 respectively. Fig.13 and 14 show the moisture uptake and dry wt of seedlings of P.aconitifolius after 72 and 96 h of growth respectively, while, the same data for P.aureus seedling has been depicted

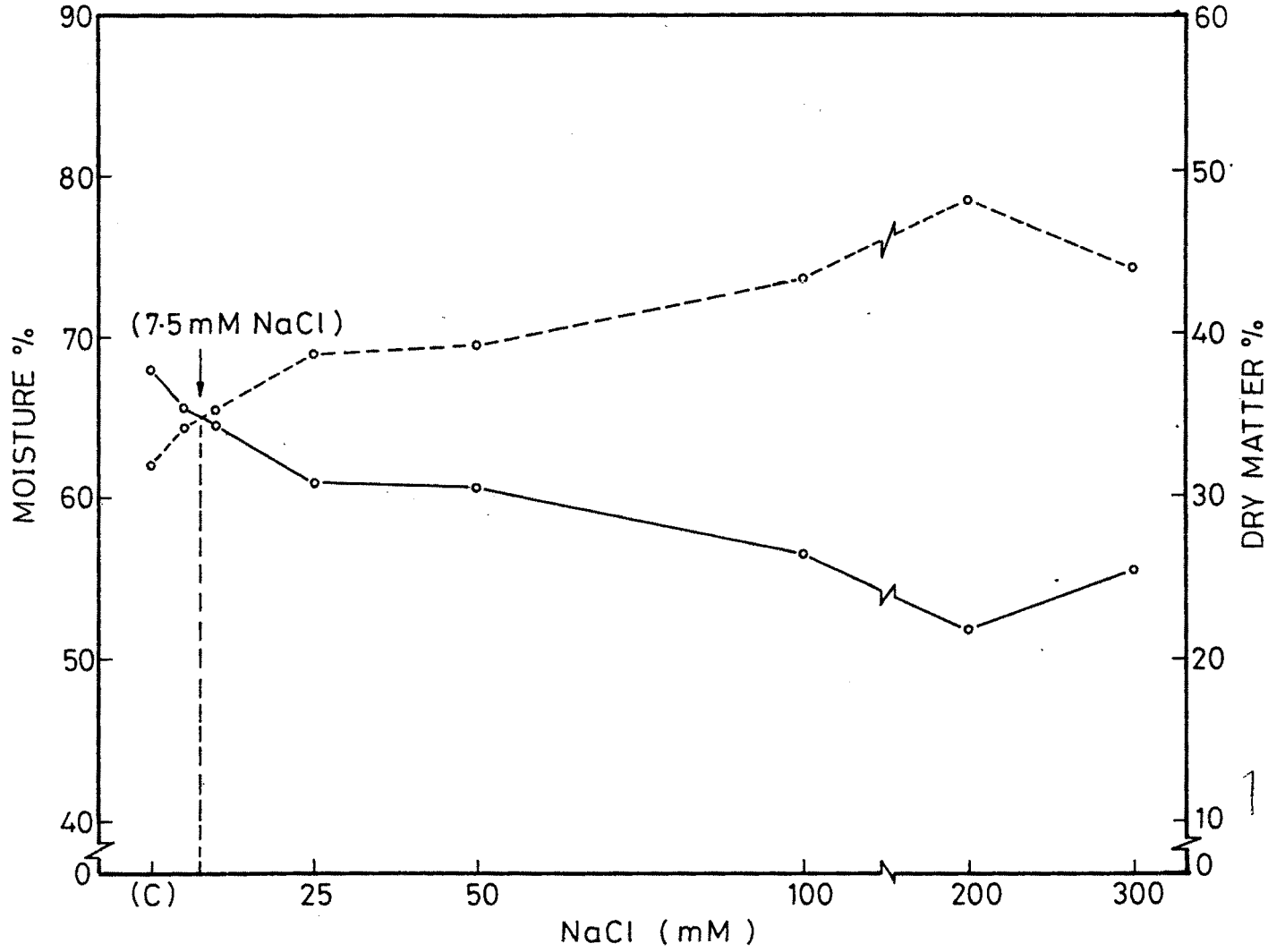


Fig. 11 EFFECT OF NaCl SALINITY ON MOISTURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF L. esculenta SEEDLINGS (72 HOURS GERMINATION).

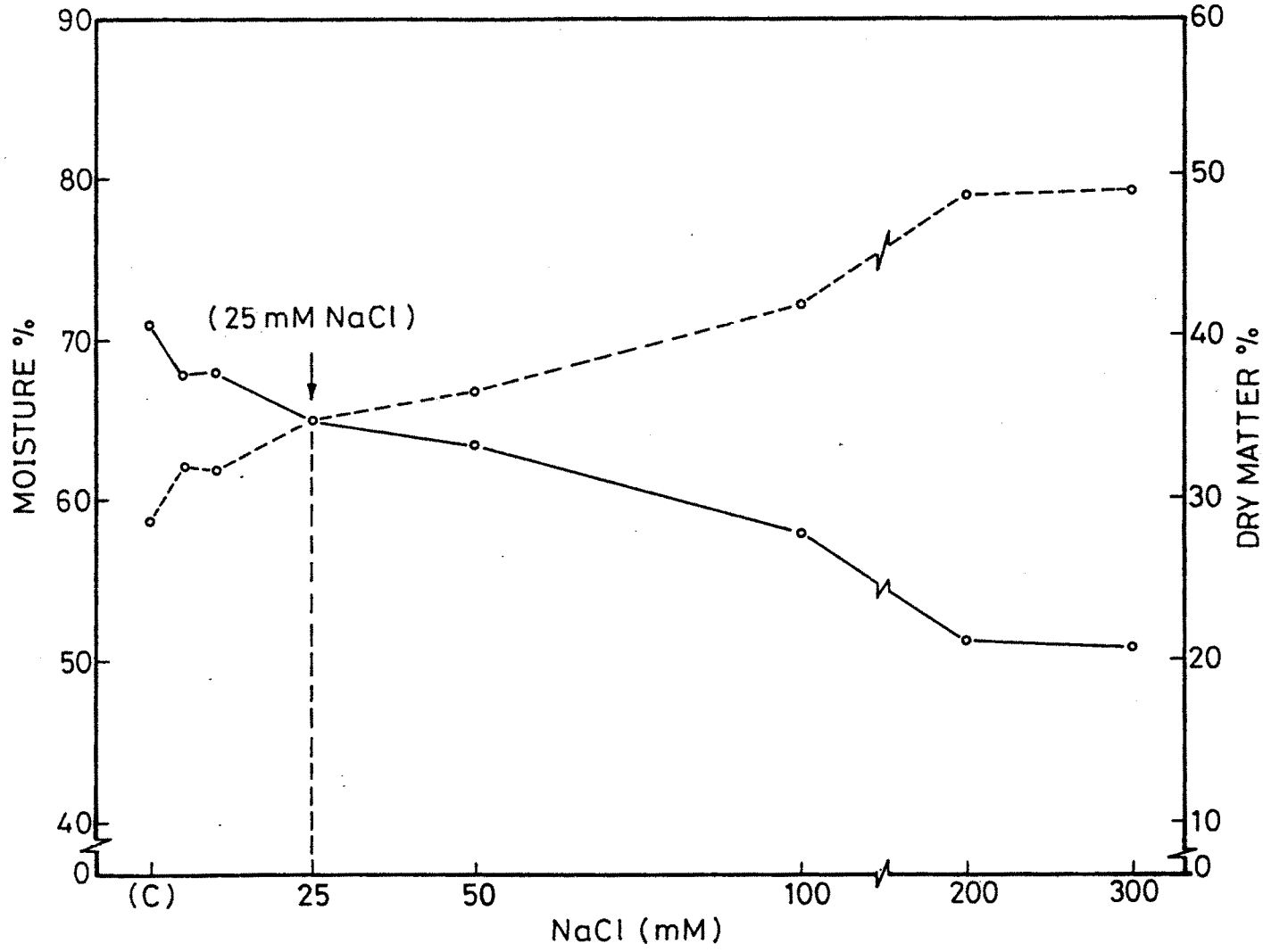


Fig.12 EFFECT OF NaCl SALINITY ON MOISTURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF L.esculenta SEEDLINGS (96 HOURS GERMINATION).

in Fig.15 and 16 for 72 and 96 h old seedlings. Fig.17 and 18 show the influence of salinity at different levels (NaCl salt concentrations) on moisture absorption and dry wt (%) of the seedlings of T.foenum-graecum after 72 and 96 h of germination respectively.

From these observations and the curves for moisture level and dry wt of seedlings it is evident that the seedlings of L. esculenta have very low level of salt which can be taken as the critical concentration for the species. It can be seen that only about 7 to 10 mM NaCl is the interesecting point or salt concentration for 72 h old (Fig.11) seedlings while it is about 25 mM in case of 96 h old seedlings (Fig.12). Thus, it appears that L.esculenta seeds are highly sensitive to salinity during germination and the salt concentration beyond 25 mM NaCl seems to be toxic or inhibitory for the process. P.aconitifolius has shown about 80 mM and 78 mM NaCl concentration as the critical concentration for seed germination (Fig.13 and 14). Thus, it can be said that P.aconitifolius seeds can tolerate the salinity level upto 100 mM NaCl, indicating its moderately salt tolerant nature. It is also evident that the water uptake during seed germination in this species is normal or not affected at all upto 10 mM salt concentration at any stage of germination. The results with another species of Phaseolus namely P.aureus are also on the similar lines. It can be seen that (Fig.15 and 16) the water uptake by the seeds during germination (upto 72 and 96 h)

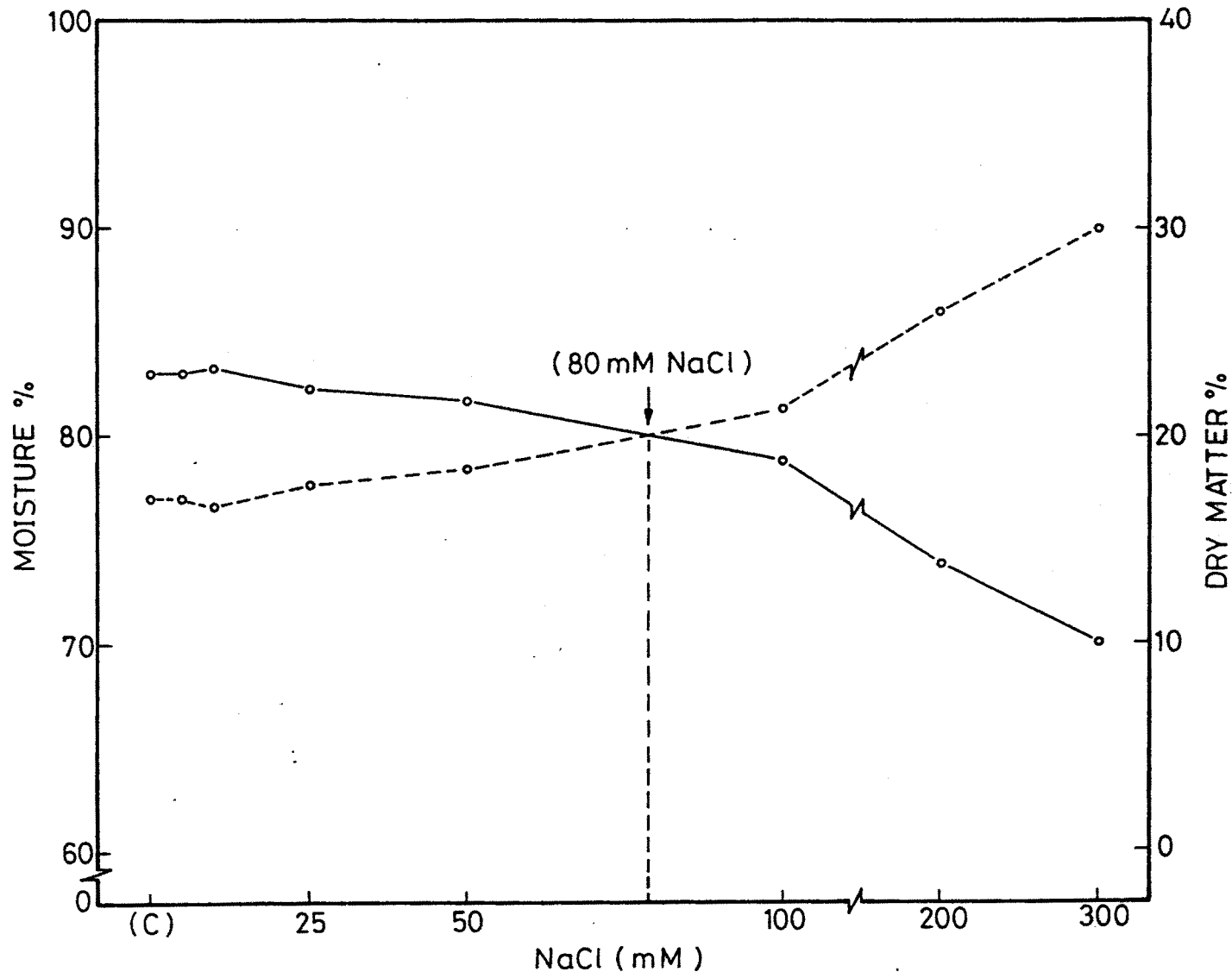


Fig. 13 EFFECT OF NaCl SALINITY ON MOISTURE (——) AND DRY MATTER CONTENT (----) OF *P. aconitifolius* SEEDLINGS (72 HOURS GERMINATION).

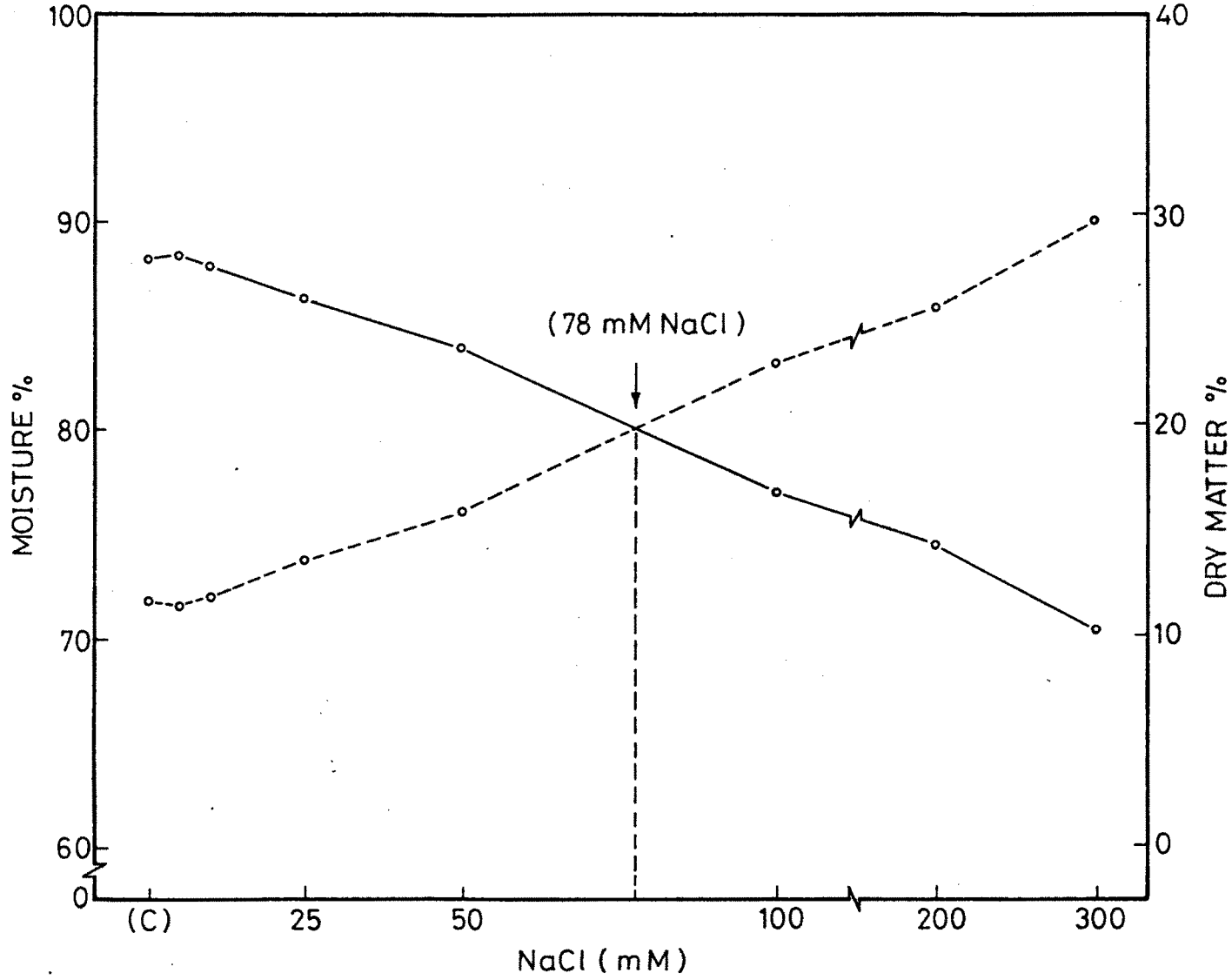


Fig. 14 EFFECT OF NaCl SALINITY ON MOISTURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF P. aconitifolius SEEDLINGS (96 HOURS GERMINATION).

is not affected (particularly in case of 96 h old seedlings) upto 10 mM NaCl level. The 72 h seedlings of the species exhibit about 80 mM NaCl as the critical concentration for seed germination. However, it is interesting to note that there are as many as three intersecting points indicating three different critical concentrations namely, 35, 100 and 120 mM NaCl (Fig.16). It is rather difficult to explain this abnormality based on the present data. However, it appears that 100 to 120 mM NaCl can be taken as the critical salt concentration in case of 96 h old seedlings. Thus, on the whole P.aureus appears to be moderately salt tolerant species particularly during germination and early seedling growth. It is also evident that 72 h old T.foenum-graecum seedlings, quite interestingly, exhibit three points of intersection between the curves of moisture percentage and dry wt percent, indicating 50, 180 and 200 mM NaCl as the critical levels (Fig.17). However, it is very difficult to interpret this. 96 h old seedlings of this species however, clearly show about 80 mM as the critical level of salt for germination (Fig.18). As far as germination and particularly the rate of water uptake is considered, this species also seems to be moderately salt tolerant. However, it can be recalled that this species has shown very poor performance under saline conditions with respect to growth (linear growth and biomass production) and development. Thus, the behaviour of T.foenum-graecum is quite different from that of the other species investigated.

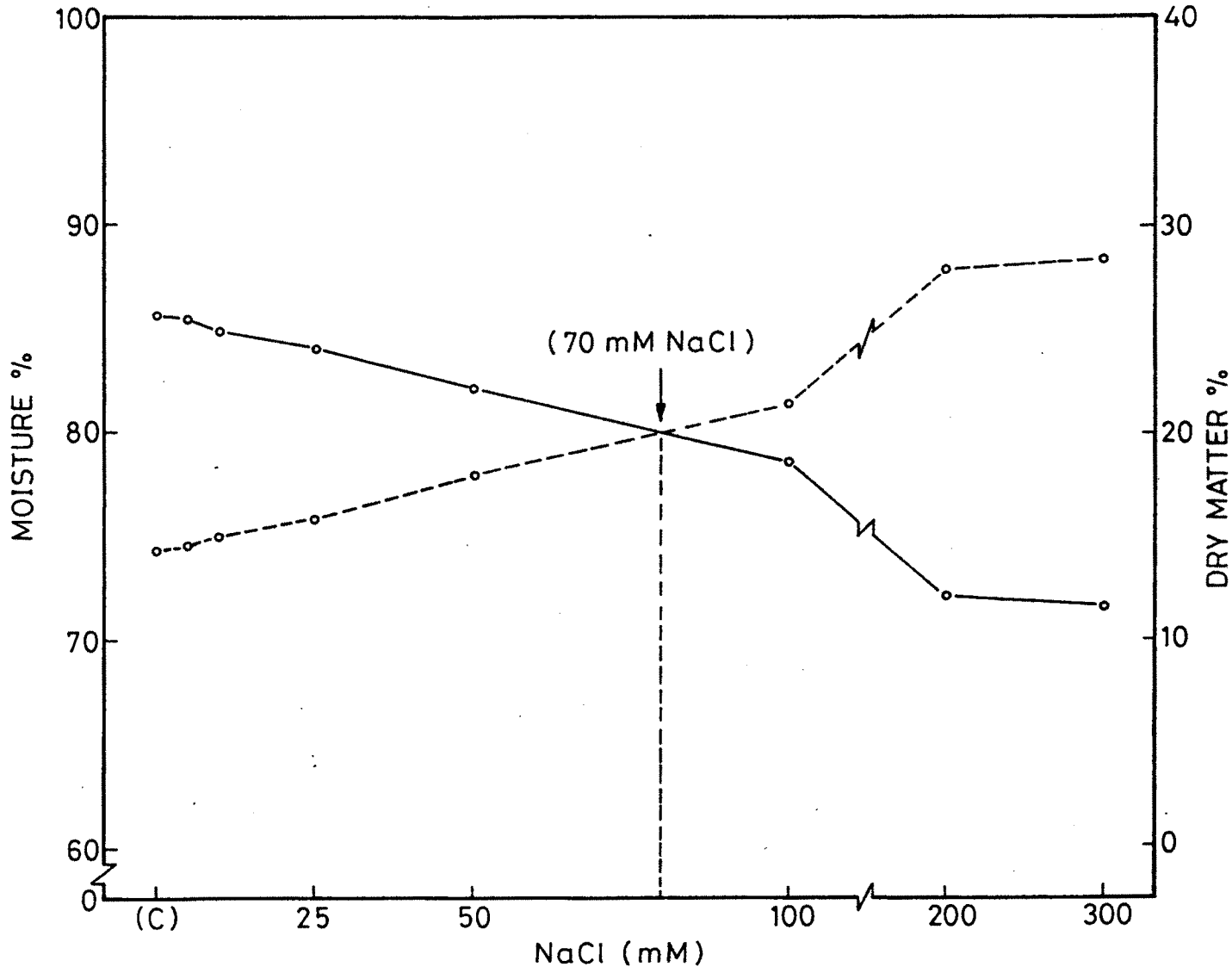


Fig. 15 EFFECT OF NaCl SALINITY ON MOISTURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF *P. aureus* SEEDLINGS (96 HOURS GERMINATION).

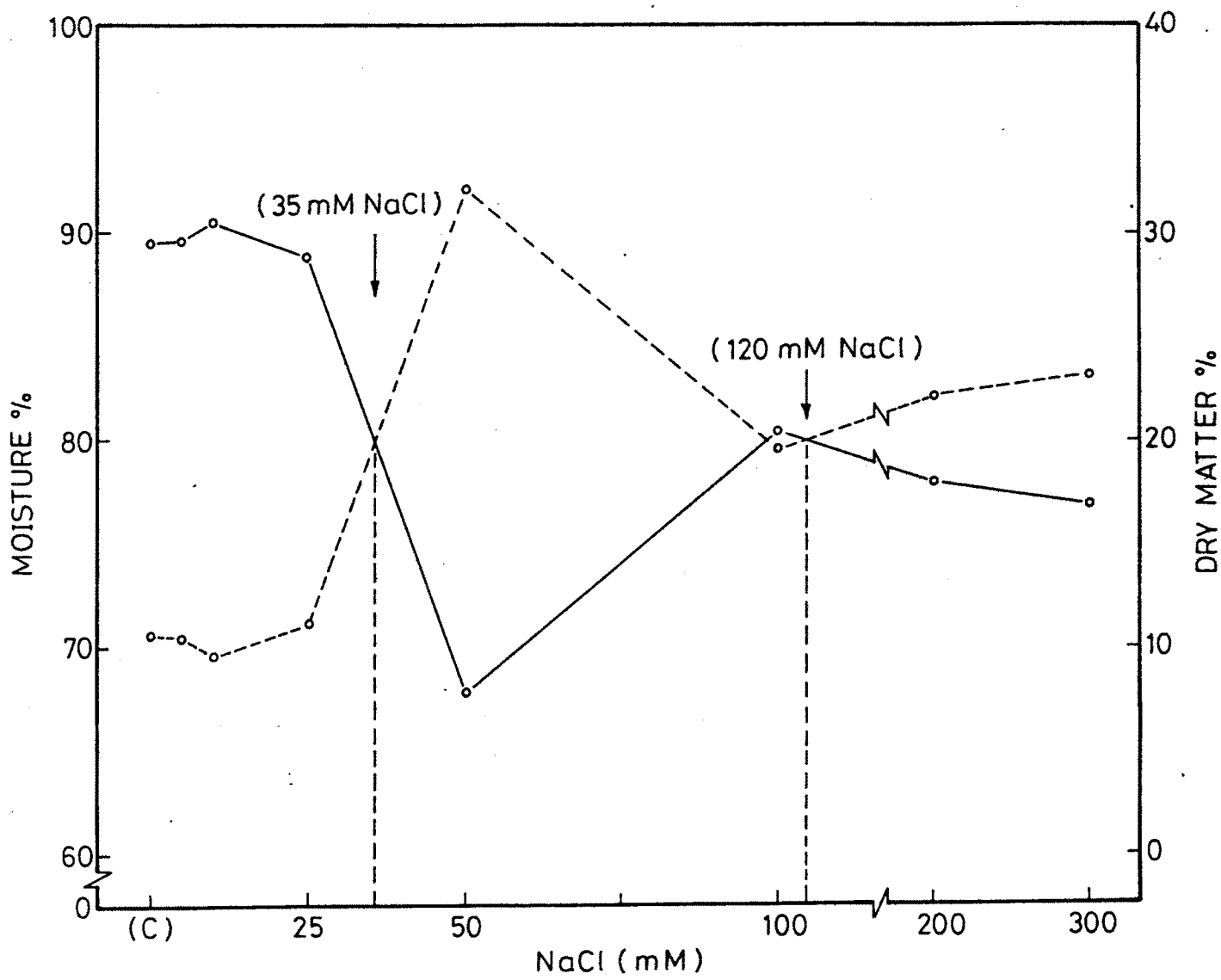


Fig. 16 EFFECT OF NaCl SALINITY ON MOISTURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF *P. aureus* SEEDLINGS (96 HOURS GERMINATION).

From such type of study, it can be proposed that, by plotting a graph of moisture content (%) and percent dry wt of a seedling at various stages of its development against the salinity levels through the point of intersection of these curves, the critical salt concentration for germination and/or seedling growth can be determined. Thus by studying the only parameters of moisture percentage and dry wt of the seedlings, it is very easy to find out the critical concentration for any type of seeds. The present proposal or suggestive conclusion, of course, requires additional data for number of such crop species.

D) Carbohydrate Metabolism :

Organic constituents during germination of seeds supply energy to the developing embryo. Among the organic constituents carbohydrates, proteins and lipids are the main substances stored as food reserve in most of the seeds. Carbohydrates play a key role in the cereal grains during their germination. Carbohydrates also have a supporting role in non starchy seeds i.e. in legume and oily seeds. Among the carbohydrates, starch is the main substance which is utilized during germination. Soluble sugars however, play a secondary role.

The effect of various concentrations of NaCl on the carbohydrate contents of the germinating seeds and seedlings of *D.biflorus*, *L.esculenta*, *P.aconitifolius*, *P.aureus* and *T.foenum-graecum* at 24, 48, 72, 96 and 120 h of developmental

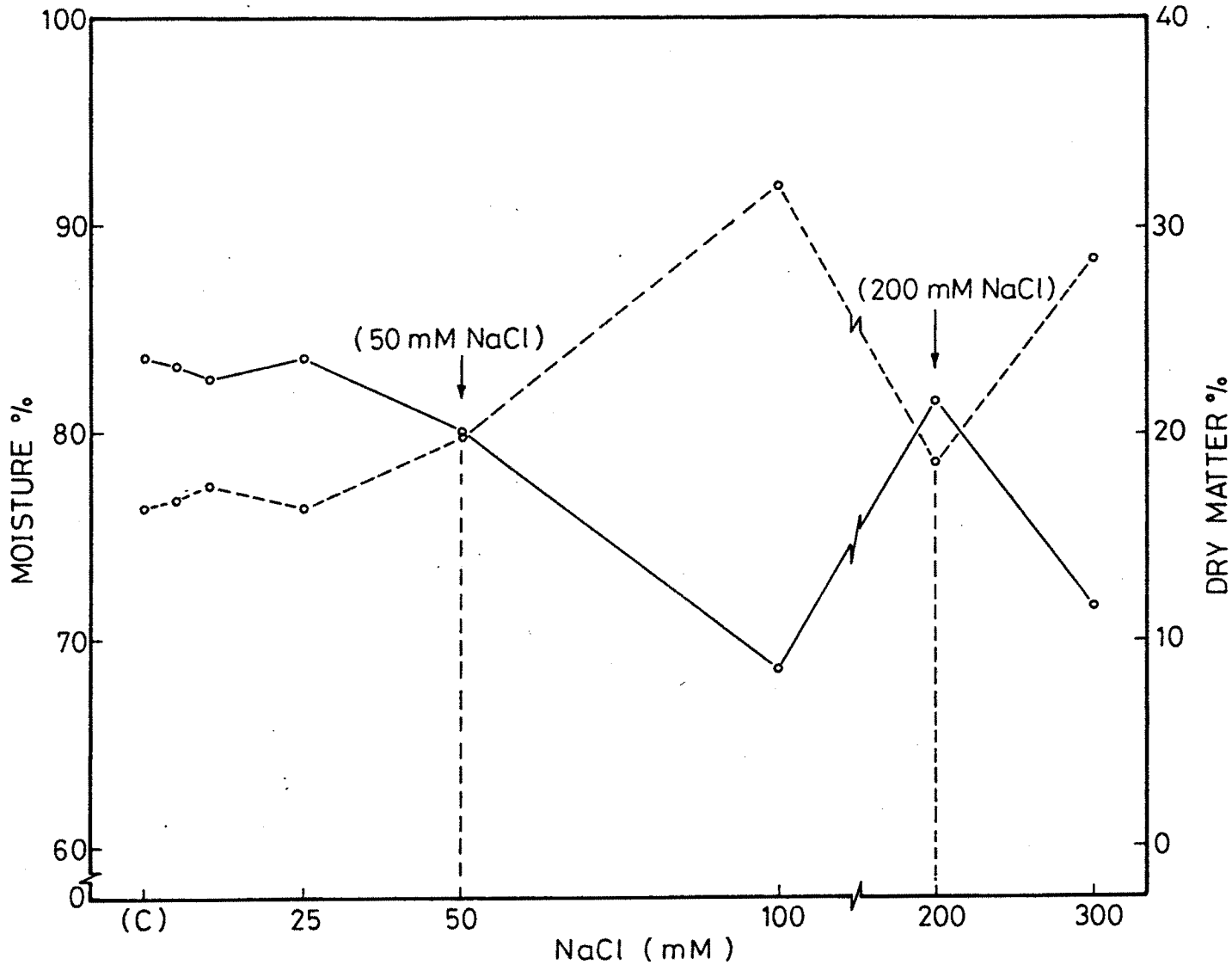


Fig. 17 EFFECT OF NaCl SALINITY ON MOISTURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF *T. foenum-graecum* SEEDLINGS (96 HOURS GERMINATION).

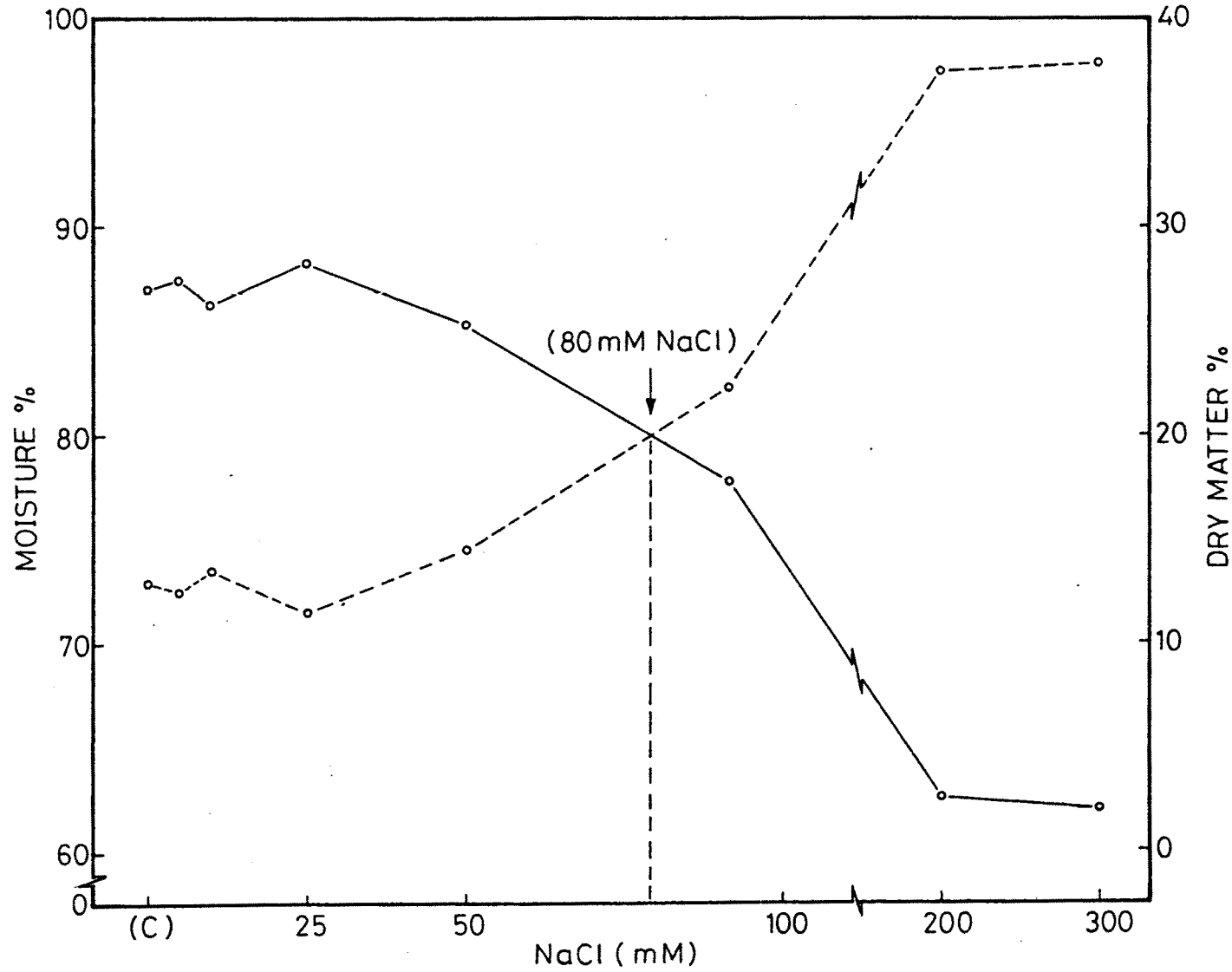


Fig. 18 EFFECT OF NaCl SALINITY ON MOISTURE (——) UPTAKE AND DRY MATTER CONTENT (----) OF *T. foenum-graecum* SEEDLINGS (96 HOURS GERMINATION).

stages is recorded in the tables 21,22,23,24 and 25 respectively. The level of soluble sugars and starch at these different developmental stages of seed germination, of the legume species grown under non-saline and saline conditions have been determined. The results as a whole, indicate that in all the species investigated, starch is utilized remarkably for the development of seedling. It can be seen that, with the advancement of stage of germination in all the species, the level of starch falls down regularly. It is also evident that, NaCl salinity profoundly influences the process of breakdown of starch, its utilization which is severly affected at the higher salt concentrations.

From table 21 it is clear that, the soluble sugar concentration in the germinating seeds of D.biflorus falls down markedly at 200 mM NaCl level. However, upto 100 mM NaCl level there is no significant change in the accumulation of soluble sugars in the seeds. It is also evident that, the highest soluble sugar content is recorded at 96 h of germination stage and again it falls down. There is continuous degradation of starch upto 96 h of germination. Thus the high level of soluble sugar at this particular stage of germination, may be as a result of maximum rate of breakdown of starch. This process of starch degradation is inhibited by salinity at all concentrations. However, it is severly affected at 200 mM NaCl level. This is particularly at 96 h of germination stage, where about 43% starch remains in the seeds. It is also clear that, the

TABLE 21 : Effect of NaCl salinity on soluble sugars and starch contents* of the seeds of *D.biflorus* during germination.

Termination NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	Soluble sugars						Starch					
0 (control)	1.57	2.75	1.05	18.47	14.22	3.33	93.05	79.51	58.94	45.58	23.82	21.31
25	1.57	1.92	1.85	09.27	14.07	6.11	93.05	83.61	53.55	47.58	27.44	19.67
50	1.57	2.02	4.65	08.40	13.77	9.11	93.05	89.27	51.86	54.51	35.55	22.29
100	1.57	2.30	7.20	11.75	14.40	8.89	93.05	90.88	53.55	47.21	37.77	20.65
200	1.57	1.80	12.10	05.42	09.97	4.22	93.05	86.80	51.27	55.10	43.45	22.94

* Values are expressed as g 100^{-1} g dry matter.

level of starch is not much affected by salinity at 120 h of germination stage. This may be due to exhaustion of reserved starch from the seeds and the starch that we record is of fully developed seedlings.

The soluble sugars and starch contents of lentil seedlings grown under saline conditions have been recorded in table 22. It is evident that, with the advancement of stage of development of seedlings, there is a linear increase in the soluble sugar contents. This is also true when, the seedlings are growing under saline conditions. The highest soluble sugar level is recorded by 120 h old seedlings, when grown under saline conditions. It is also evident that, with increase in the level of salinity in the medium, there is linear decrease in the rate of accumulation of soluble sugars in the seedlings. However, 100 mM NaCl level seems to be strongly inhibitory at 120 h stage. It is also found that, the level of starch in the seeds falls down regularly with stage of seed germination. This clearly indicates that, the utilization of starch for germination and seedling formation is continuous. It is interesting to note that, the level of starch in the germinating seeds or the seedlings is unaffected and remains almost at the same at all salinity levels. This is particularly true in the seedlings 72 h and more age. At the early stages i.e. 24 and 48 h of germination there are some fluctuations in the starch contents. Thus, the process of breakdown of starch and its probable utilization during development in lentil seems to be less affected

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TABLE 22 : Effect of NaCl salinity on soluble sugars and starch contents* of the seeds of *L.esculenta* during germination.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	Soluble sugars						Starch					
0 (control)	2.42	0.91	3.84	8.57	8.46	11.66	60.80	84.35	48.35	19.68	18.72	14.40
25	2.42	0.80	2.97	5.71	5.03	10.06	60.80	81.81	67.74	22.56	19.92	14.88
50	2.42	0.66	3.47	3.88	5.03	10.40	60.80	87.26	49.36	21.92	13.92	14.56
100	2.42	0.80	3.54	4.19	5.26	6.63	60.80	69.81	54.85	20.48	21.60	14.08
200	2.42	0.91	2.79	5.25	3.70	6.63	60.80	76.53	54.10	19.28	20.40	12.00

* Values are expressed as g 100^{-1} g dry matter.

under saline conditions, however, when the growth performance of this species during seedling development is seen, it appears that there must be some other barriers inhibiting the development under saline conditions.

In P.aconitifolius it is found that more and more sugars are released and accumulated in the seedlings with the advancement of germination phase. The highest soluble sugar content has been observed in the 120 h old seedlings (Table 23). It can be seen that, upto 72 h of stage there is no significant effect of salinity on the accumulation of soluble sugars in the seeds. However, in the seedlings 96 and 120 h old, 100 mM NaCl strongly affects the level of soluble sugars. It is also clear that, the breakdown of starch is also arrested at 100 mM salt concentration and that after 72 h of germination. However, upto this stage there is only a slight inhibitory effect on the utilization of starch. It may be true that low starch contents observed in the seedlings of 96 and 120 h old by higher salt concentration, may be due to inhibition of synthesis of starch during seedling growth. Thus, in P.aconitifolius, the utilization of starch during germination is only slightly influenced.

During germination the seeds of P.aureus also show accumulation of soluble sugars upto 72 h germination, at which stage, the seeds contain the highest amount of soluble sugars as compared to that at any other stage of development. It can

TABLE 23 : Effect of NaCl salinity on soluble sugars and starch contents* of the seeds of *P.aconitifolius* during germination.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	Soluble sugars						Starch					
0 (control)	1.57	4.10	3.72	11.86	6.92	12.18	94.29	72.82	54.35	41.02	30.76	14.35
25	1.57	4.49	6.41	7.95	5.96	14.61	94.29	78.97	66.66	36.92	25.64	22.56
50	1.57	6.22	3.85	9.23	3.97	11.54	94.29	73.84	67.69	41.02	38.46	21.53
100	1.57	4.93	5.64	7.69	2.88	3.33	94.29	61.53	53.38	56.41	43.07	41.28
200	1.57	6.28	4.57	4.49	0.08	1.15	94.29	74.35	51.28	51.28	23.07	62.56

* Values are expressed as g 100^{-1} g dry matter.

also be seen that (Table 24) the soluble sugar content of the seeds of this species is highly reduced at 200 mM NaCl at 72 and 96 h of germination. However, at 120 h stage i.e. when full seedling formation has takdn place, there is very little effect of salinity on soluble sugar content. Thus release of soluble sugars and their accumulation is affected by both 100 and 200 mM NaCl concentrations. The starch level in the seeds of P.aureus falls regularly with the advancement of stage of germination, till 96 h. At 120 h stage there is almost no change in the starch level, particularly in the seeds or seedlings grown under non-saline conditions. However, under saline conditions, particularly, at the higher salt concentrations (100 and 200 mM NaCl) there is no regular pattern of starch level in the germinating seeds, has been observed. It appears that, 100 as well as 200 mM NaCl are the toxic levels, which exhibit breakdown of starch and probably, arrest germination and seedling growth.

In fenugreek the soluble sugar contents of the germinating seeds considerably increase after 72 h of germination and then falls down to some level and remain almost constant at 96 and 120 h. It appears that, the salinity at 100 mM levels and above restrict the accumulation of soluble sugar in the germinating seeds upto 72 h of germination. However, further increase in the size of the seedlings at 96 and 120 h salinity at all concentrations shows very little effect on accumulation of

TABLE 24 : Effect of NaCl salinity on soluble sugars and starch contents* of the seeds of *P. aureus* during germination.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	Soluble sugars						Starch					
0 (control)	2.09	3.54	2.03	9.18	3.87	5.51	61.10	29.99	18.94	17.36	10.52	10.26
25	2.09	2.13	1.97	9.05	3.15	6.49	61.10	27.89	16.05	22.89	13.15	11.57
50	2.09	2.36	1.83	6.95	3.15	4.45	61.10	30.52	14.99	20.52	12.63	08.15
100	2.09	1.44	2.82	6.16	2.23	5.77	61.10	26.84	17.36	22.10	20.52	19.20
200	2.09	2.43	2.75	3.80	1.77	6.42	61.10	33.15	17.36	35.78	22.36	11.89

* Values are expressed as g 100^{-1} g dry matter.

soluble sugars. It is also evident from table 25 that, the utilization of starch during seed germination of this species seems to be rather vigorous, particularly, after 72 h of germination. This trend can also be seen in the seedlings grown in the saline conditions upto 50 mM NaCl. At 100 mM NaCl level it is considerably affected and hence the level of starch in the seeds upto 96 h of germination is increased. 200 mM NaCl also seems to be toxic for seed germination. The pattern observed for starch content of the seedlings at 120 h, however, is irregular. Brawn (1965) has mentioned that, the nutrition of the embryo depends on the conversion of insoluble reserve to soluble form. The most prominent of these substances is starch. Usually during germination, starch is hydrolysed mostly by amylases to release soluble and reducing sugars, which are utilized as the respiratory substrate for the release of energy to be utilized by the embryo for further development. This process of degradation of starch is continued till the starch reserve is completely exhaust. Here the heterotrophic mode of growth of a plant ends and now, the seedling with well developed root system and leaves starts the autotrophic mode of living.

El - Mahdy et al.(1983) has studied the effect of germination at 50°C for 96 h in the dark, on the carbohydrates of fenugreek seeds and observed that reducing and total sugars and pentosan content increased during germination. A marked

TABLE 25 : Effect of NaCl salinity on soluble sugars and starch contents* of the seeds of *T. foenum-graecum* during germination.

Treatment NaCl (mM)	Hours of germination											
	0	24	48	72	96	120	0	24	48	72	96	120
	Soluble sugars						Starch					
0 (control)	4.49	4.94	3.18	9.06	6.35	6.82	27.81	20.95	14.28	13.33	2.57	3.23
25	4.49	3.06	4.00	6.59	4.23	6.35	27.81	17.94	14.28	9.52	7.61	0.38
50	4.49	3.41	5.18	6.59	9.41	8.00	27.81	28.57	15.23	11.42	10.47	2.85
100	4.49	5.88	3.18	5.20	5.58	7.90	27.81	22.85	20.95	17.14	20.95	3.80
200	4.49	3.65	2.59	3.53	4.70	4.35	27.81	21.90	21.42	19.04	18.09	7.23

* Values are expressed as g 100^{-1} g dry matter.

increase in sucrose content was also observed after germination. Similarly maltose and fructose also appear in measurable amounts after germination. The starch content was found to be decreased. El-Shimi et al.(1984) also found that the reduction in starch content is accompanied by a gradual increase in total sugars during germination of fenugreek seeds. Ologhobo and Babatunde (1986) have reported that, starch is the major carbohydrate and about 50% of it is hydrolysed after 144 h of germination in cowpea. On the other hand, the level of reducing sugars, including glucose and fructose was increased during germination. Present studies with five different legume species also support and confirm the above findings. Paul et al.(1970) have found that starch hydrolysing enzyme, amylases, are active in P.aureus in the cotyledon and also in the axis from the start of imbibition. They (1974) have noticed the rapid depletion of starch contents in the cotyledons as soon as, the seeds of P.aureus are soaked in water. After 120 h of germination, the starch content in the cotyledon was reduced to a maximum value. Jaya and Venkatraman (1981) have found a decrease in the concentration of total carbohydrates during 96 h germination of chickpea and green gram.

It has been found that, salinity causes delay in the release of reducing sugars in gram (Bharadwaj,1965) and also in wheat (Sarin and Narayanan,1968) during germination. Hatata and Farah (1982) have studied the effects of different salts ($NaCl$, $MgCl_2$, Na_2SO_4 and $MgSO_4$) on carbohydrate metabolism in

young corn seedlings and found that, there was decrease in the total carbohydrate content with the advancement in time and increase of salt concentration. Gill and Singh (1985) have studied the effect of salinity on carbohydrate metabolism during paddy seed germination and reported that, the starch content decreased with lapse of time of sowing but less at higher salinity levels. They have further reported that, there is rapid decrease in starch level and an early and greater release of soluble sugars in the tolerant, than in the sensitive variety. Rapid germination, early induction of amylase activity and higher rate of water uptake are the characters of tolerant varieties (Gill and Singh,1985). Corchete and Guerra (1986) have studied the effect of NaCl and polyethylene glycol on solute content and glycosidase activities during germination of lentil seeds. It was found that, the radicle emergence and growth were delayed by these substances. Accumulation of soluble sugars in the embryanic axis of the species was observed under stress conditions. However, they report that, water and salt stress didn't have a marked effect on carbohydrate metabolism in intact seedlings. Sudhakar et al.(1987) have studied the biochemical changes during early seedling growth of horse gram subjected to salt stress. It was found that, the activities of hydrolytic enzymes including starch phosphorylase were enhanced by NaCl or Na_2SO_4 treatments in the cotyledons. It was also observed that, there was decrease in the levels of sugars in the salt treated plants upto 5th day, but increased in the 7th day old plant.

Rapid germination, early induction of amylase activity and higher rate of water uptake are the characters of tolerant varieties. From these observations, therefore, it appears that the response of carbohydrate metabolism to salinity varies from species to species. Higher concentrations of salts in the medium, however, inhibit the breakdown of carbohydrates probably by blocking the activity of amylases. From the present investigation it may be concluded that, the response of legume species investigated with respect to carbohydrate metabolism, to the salinity also varies from species to species.

E) Soluble Proteins :

Proteins are the main reserves in the legume seeds. Proteins are hydrolysed during germination by protease enzyme system, which is usually induced after imbibition of water. The amino acids liberated as a result of this protein brekdown, are utilized for the further development of seedlings through respiratory activities. Proteins may be either in insoluble or soluble form. Soluble proteins are readily available for utilization as well as their translocation.

The effect of NaCl salinity at different levels on soluble protein contents of the seeds of *D.biflorus*, *L.esculenta*, *P.aconitifolius*, *P.aureus* and *T.foenum-graecum* during germination at different stages (0,24,48,72,96 and 120 h) has been recorded in Tables 26,27,28,29 and 30 respectively. It can be seen that

TABLE 26 : Effect of NaCl salinity on soluble protein content* of the seeds of *D.biflorus* during germination.

Treatment NaCl (mM)	Germination hours					
	0	24	48	72	96	120
0 (control)	5.25	4.55	3.97	6.29	10.76	6.92
25	5.25	2.90	4.37	8.12	9.78	8.48
50	5.25	4.15	3.48	8.30	7.54	8.08
100	5.25	4.42	2.05	6.25	7.05	9.86
200	5.25	4.37	3.93	4.24	5.98	5.58

* Values are expressed as g 100^{-1} g dry matter.

TABLE 27 : Effect of NaCl salinity on soluble protein content* of the seeds of *L.esculenta* during germination.

Treatment NaCl (mM)	Germination hours					
	0	24	48	72	96	120
0 (control)	4.42	2.10	2.59	5.98	5.10	4.73
25	4.42	1.18	2.99	8.75	3.48	3.03
50	4.42	2.12	2.54	4.86	6.07	4.73
100	4.42	0.62	2.68	4.28	3.30	2.59
200	4.42	2.77	3.30	4.37	3.30	3.70

* Values are expressed as 9 100^{-1} g dry matter.

in all cases, except fenugreek, the soluble protein level increases after 48 h germination under normal or non-saline conditions. However, in fenugreek it is almost constant upto 120 h.

During germination of horse gram seeds, the level of soluble proteins decreases upto 72 h germination and later, there is considerable increase in it. The maximum being, after 96 h of germination. It is also evident from Table 26 that, upto 100 mM NaCl concentration, there is only a slight influence of salinity on soluble protein content of the seeds or seedlings. 200 mM NaCl treated seeds or seedlings show the low level of soluble proteins. Thus, it appears that salinity probably affects the process of availing the soluble proteins for easy and rapid utilization of the proteins for the further development. As discussed earlier the seedling growth is affected strongly by 200 mM NaCl level, in case of this species.

Table 27 records the influence of NaCl salinity on soluble protein content of lentil seeds, during their germination. The observations recorded follow, almost the same pattern, as observed in the horse gram. It can be seen that, the soluble protein level increases in the seedlings upto 72 h germination, and then falls down. The soluble protein content, in the germinating seeds of lentil are markedly affected both at 100 and 200 mM NaCl.

TABLE 28 : Effect of NaCl salinity on soluble protein content* of the seeds of *P.aconitifolius* during germination.

Treatment NaCl (mM)	Germination hours					
	0	24	48	72	96	120
0 (control)	3.10	2.10	3.93	9.15	12.90	12.36
25	3.10	1.70	3.17	5.49	12.45	11.16
50	3.10	2.14	3.84	6.43	09.95	08.16
100	3.10	1.16	3.08	3.93	08.12	07.63
200	3.10	1.61	3.75	4.42	03.88	02.81

* Values are expressed as g 100^{-1} g dry matter.

TABLE 29 : Effect of NaCl salinity on soluble protein content* of the seeds of *P. aureus* during germination.

Treatment NaCl (mM)	Germination hours					
	0	24	48	72	96	120
0 (control)	3.35	7.72	6.78	13.39	17.72	13.12
25	3.35	4.60	4.46	13.17	15.76	15.62
50	3.35	3.75	4.24	08.35	10.40	12.90
100	3.35	4.02	5.09	08.35	07.77	10.18
200	3.35	3.30	4.02	04.11	05.62	04.73

* Values are expressed as g 100^{-1} g dry matter.

The soluble protein level of P.aconitifolius seeds and seedlings, at various stages of germination, under non-saline and saline conditions has been shown in Table 28. The species behaves almost in the same manner as, horse gram and lentil behaves. It is evident that, the soluble protein level, in the seeds increases continuously with the advancement of period of germination, upto 96 h where the soluble protein content is the highest. In this species also, 100 and 200 mM NaCl concentrations, appear to be toxic as, the soluble protein content of the seeds or seedlings is severly affected by the salinity level at these higher salt concentration. Almost same pattern is evident in P.aureus. The species seems to be sensitive in this regard at higher salt concentrations. Almost same pattern is evident in P.aureus. The species seems to be sensitive in this regard at salt concentrations i.e. 100 and 200 mM NaCl.

In fenugreek the soluble protein content in the germinating seeds or seedlings remains almost at the same level except at 120 h stage at which it is strongly eleveted. From this, it appears that, probably, there is no major change in the protein reserve, even upto 120 h stage. This indicates, the process of germination in this species is very slow.

From these observations, it appears that, most of the legume species investigated, respond equally (except fenugreek) to NaCl salinity. It is also quite clear that, 200 mM NaCl is

TABLE 30 : Effect of NaCl salinity on soluble protein content* of the seeds of *T. foenum-graecum* during germination.

Treatment NaCl (mM)	Germination hours					
	0	24	48	72	96	120
0 (control)	9.57	8.26	7.95	8.26	9.11	11.34
25	9.57	8.66	7.81	8.75	9.02	10.58
50	9.57	7.23	8.70	8.35	7.86	08.79
100	9.57	8.79	7.54	8.17	6.65	09.24
200	9.57	7.50	8.93	6.38	6.70	07.90

* Values are expressed as g 100^{-1} g dry matter.

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for release of soluble proteins, in the germinating seeds of all the species investigated. This situation even, prevails at 100 mM NaCl level in lentil and horse gram indicating that, these two species are probably, more sensitive to saline conditions during germination, as compared to the remaining species.

F) Enzymes :

1) Hydroxyperoxidases :

Hydroxyperoxidases cover a wide range of oxidative type of enzyme, which play a vital role in the cells and tissues involved in very high respiratory activities. Peroxidase and catalase are the two important enzymes which play a key role, in oxidative processes. Both the enzymes have got hydrogen peroxide as the substrate which is broken-down to produce water and nascent oxygen in case of peroxidase, or molecular oxygen in case of catalase. Peroxidase is said to be a toxic enzyme, as it brings about very rapid oxidation of other substances in the cell, during its catabolic action. On the other hand, catalase is taken as a protective enzyme which destroys toxic levels of hydrogen peroxide, without oxidation of other substances. As the respiratory activities dominate the germination process, hydroxyperoxidases are remarkably induced during germination.

a) Peroxidase :

Peroxidase is involved in various metabolic pathways. Paul and Mukherji (1972) have found, a strong correlation between peroxidase and catalase activities and respiration and suggested that, these enzymes are acting as a part of respiratory mechanism of rice seedlings. Hendricks and Taylorson (1974) suggeste, possible participation of peroxidase in NADPH oxidase and electron transport during dormancy breaking in Amaranthus albus and Lactuca sativa. It is admitted that, the role of peroxidase in seed germination can be clearly defined, only after finding the location of the different isozymes within the seeds.

The effect of NaCl salinity, at different concentrations on the activity of enzyme peroxidase in the germinating seeds of D.biflorus, L.esculenta, P.aconitifolius, P.aureus and T. foenum-graecum at different developmental stage has been recorded in Tables 31,32,33,34, and 35 and Fig. 19,22,25,28 and 31 respectively. It is evident from the observations, that, the highest activity of this enzyme in the dry seeds has been recorded in lentil followed by T.foenum-graecum and the least being in both P.aureus and D.biflorus. P.aureus shows the highest activity of peroxidase in the seeds at 96 and 120 h of germination. On the other hand, the lowest activity of this enzyme in the germinating seeds at 96 and 120 h has been recorded in P.aconitifolius (Fig.25). It can also be seen that, the activity of this enzyme increased only when, the seeds start germination.

TABLE 31 : Changes in the activity of enzymes, peroxidase, catalase, and acid phosphatase in the seeds of *D.biflorus* during germination under saline conditions.

Treatment NaCl (mM)	Hours of germination					
	0	24	48	72	96	120
	Peroxidase *					
0 (control)	0.1	3	4	1	2	1
25	0.1	1	1	1	2	2
50	0.1	1	2	4	2	1
100	0.1	2	1	2	2	4
200	0.1	3	0	1	1	0
	Catalase **					
0 (control)	40	30	38	50	16	16
25	40	45	38	63	29	26
50	40	36	19	57	10	29
100	40	24	33	31	12	23
200	40	51	20	26	15	11
	Acid phosphatase ***					
0 (control)	3	3	2	1	1	2
25	3	6	1	1	1	2
50	3	4	2	1	1	2
100	3	4	1	1	1	2
200	3	6	1	1	-	1

* Δ O.D. min^{-1} mg^{-1} protein X 100

** mg H_2O_2 utilised $min^{-1} mg^{-1}$ protein X 100

*** Δ O.D. h^{-1} mg^{-1} protein X 100

Each value is mean of three determinations.

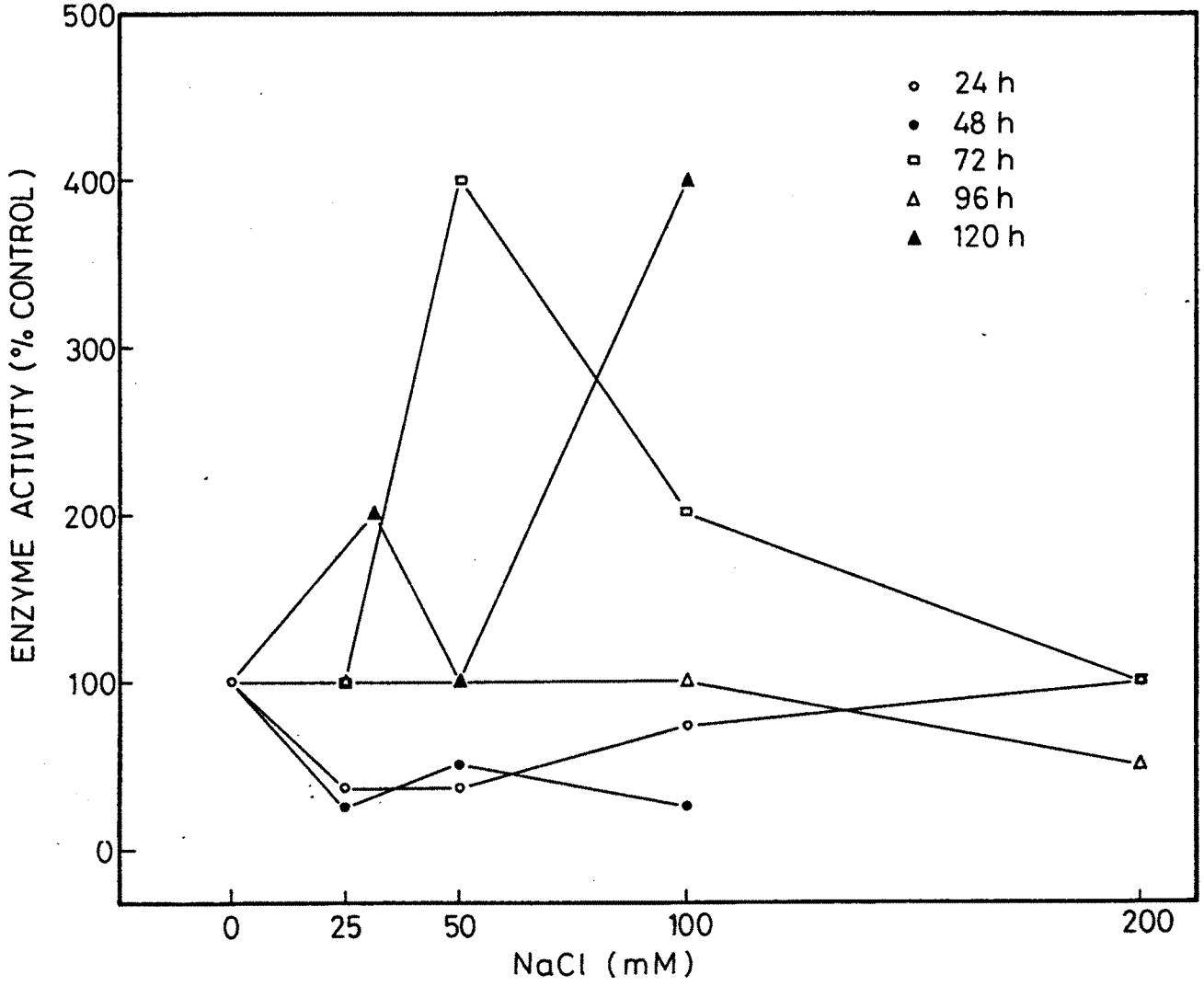

Fig. 19 CHANGES IN THE ACTIVITY OF ENZYME PEROXIDASE IN THE SEEDS OF D. biflorus DURING GERMINATION UNDER SALINE CONDITIONS.

The changes in the activity of enzyme peroxidase in the seeds of D.biflorus during germination under saline condition have been recorded in Table 31 (Fig.19). It is evident that, activity of this enzyme increases during germination of the seeds. However, NaCl salinity exerts no definite influence on this enzyme in the germinating seeds. 200 mM NaCl seems to be inhibitory, particularly, after 48 h germination. It can be seen that, activity of this enzyme is heavily reduced and even, at certain stages (48 and 120 h) it is completely suppressed by this salinity level.

The changes in the activity of peroxidase in the germinating seeds of lentil under saline conditions has been depicted in Table 32 and Fig.22. It is evident from the values recorded, that, activity of this enzyme in the seeds increases upto 24 h of germination and during later stages falls down regularly in the non-saline conditions. The lowest activity (0.6) is recorded in the seedlings, at 120 h stage. Almost similar pattern is observed in the seeds germinating under saline conditions. It is interesting to note that upto 96 h of germination, the activity of this enzyme is not all affected by low regimes of salinity (25 and 50 mM NaCl). However, 100 mM salt concentration in the medium inhibits the activity of this enzyme in the seeds. 200 mM NaCl appears to be highly toxic. It is also observed that, when the seedlings have attained the 120 h age, the effect of NaCl salinity on the

TABLE 32 : Changes in the activity of the enzymes, peroxidase catalase and acid phosphatase in the seeds of *L. esculenta* during germination under saline conditions.

Treatment NaCl (mM)	Hours of germination					
	0	24	48	72	96	120
	Peroxidase *					
0 (control)	3.0	7.7	6.5	3.6	2.9	0.6
25	3.0	7.8	4.8	4.3	2.4	1.6
50	3.0	4.4	6.0	4.2	2.6	0.9
100	3.0	4.7	3.5	2.9	2.5	1.9
200	3.0	3.2	3.3	2.6	1.4	0.9
	Catalase **					
0 (control)	17.9	8.8	23.0	37.3	14.7	18.3
25	17.9	5.5	10.0	12.9	16.8	19.3
50	17.9	11.6	12.8	13.8	13.1	20.4
100	17.9	11.4	15.0	10.2	13.0	5.9
200	17.9	10.3	21.5	6.9	9.4	0.0
	Acid phosphatase ***					
0 (control)	10.2	5.7	2.1	0.7	8.4	2.0
25	10.2	5.7	2.2	0.5	7.4	1.9
50	10.2	3.8	1.9	0.4	6.9	2.4
100	10.2	4.0	1.4	0.1	6.9	1.2
200	10.2	3.0	1.6	0.04	4.6	1.1

* ΔO.D. min^{-1} mg^{-1} protein X 100

** mg H_2O_2 utilized $min^{-1}mg^{-1}$ protein X 100

*** ΔO.D. h^{-1} mg^{-1} protein X 100

Each value is mean of three determinations.

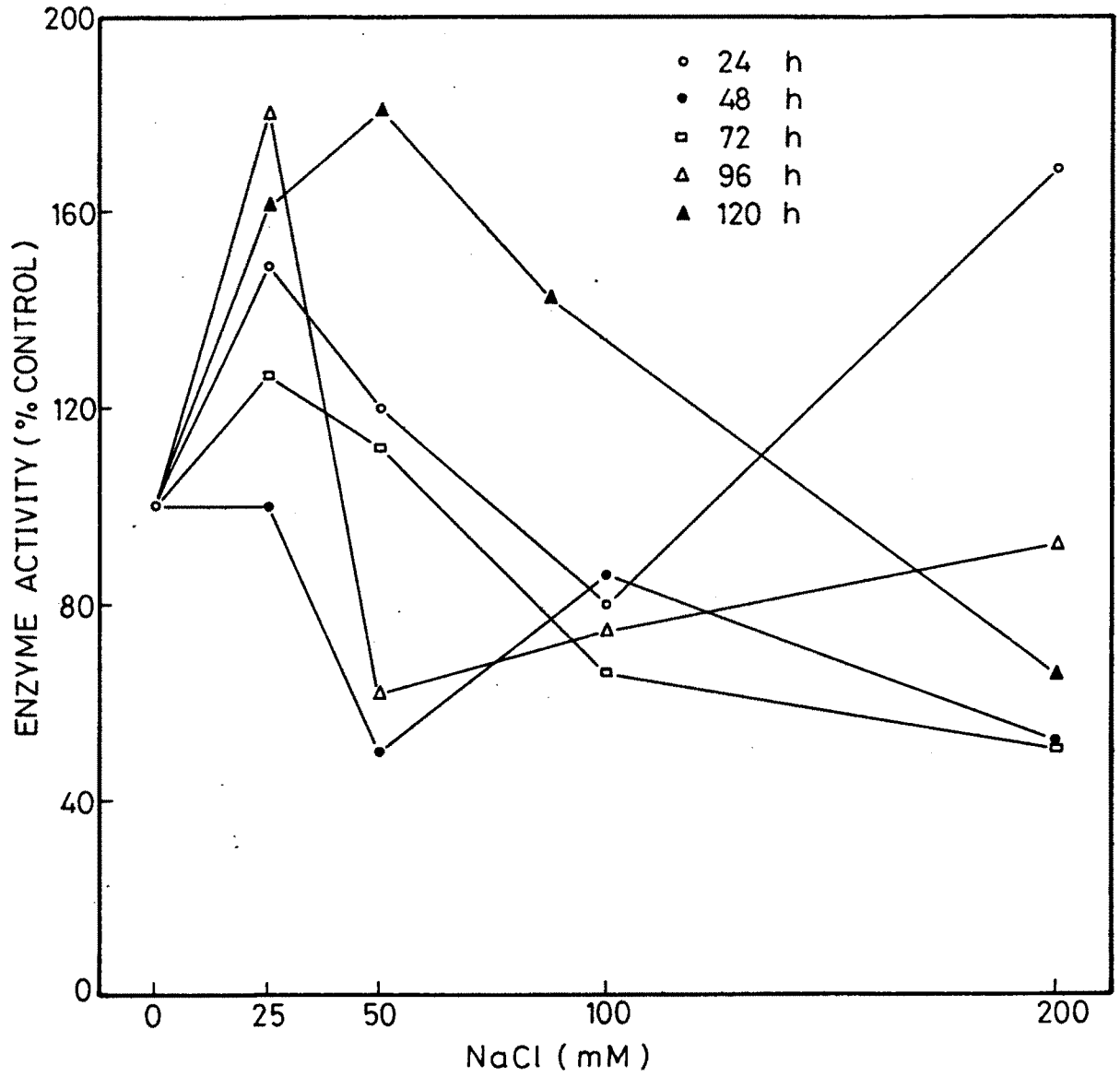

Fig. 20 CHANGES IN THE ACTIVITY OF ENZYME CATALASE IN THE SEEDS OF D. biflorus DURING GERMINATION UNDER SALINE CONDITIONS.

activity of the peroxidase in the seedlings is irregular. Behaviour of enzyme peroxidase in lentil (Table 32 and Fig. 22) appears to be quite opposite to that in P.aureus.

Peroxidase in the germinating seeds of P.aconitifolius (Table 33 and Fig.25) increases with the stage of germination and the highest activity of this enzyme is recorded at 120 h stage. From the results, however, it is apparent that, the behaviour of peroxidase in the germinating seeds of the species is irregular and indefinite under saline conditions.

It is evident from Table 34 (Fig.28), that activity of enzyme peroxidase increases with the stage of development of seedlings. This is quite remarkable, only after 72 h of germination and the highest level of this enzyme has been found in the seedlings, 120 h old, grown under non-saline conditions. Almost similar type of pattern is also observed when, the seeds are germinated under saline conditions upto 100 mM NaCl level and at 200 mM NaCl it is markedly disturbed. It is also evident from the observations that, activity of enzyme peroxidase is decreased due to the higher salt concentrations (100 and 200 mM NaCl) in the medium and quite significantly after 72 h germination.

Table 35 and Fig.31 depicts the changes in the activity of peroxidase in the seeds of fenugreek during germination under saline conditions. The observations made in this species seem to be rather interesting. It can be seen that, activity of this enzyme increases with the advancement of period of germination,

TABLE 33 : Changes in the activity of enzymes, peroxidase, catalase, and acid phosphatase in the seeds of *P. aconitifolius* during germination under saline conditions.

Treatment NaCl (mM)	Hours of germination					
	0	24	48	72	96	120
	Peroxidase *					
0 (control)	0.2	1.0	1.1	1.0	0.4	4.0
25	0.2	0.2	1.0	1.0	1.0	1.0
50	0.2	0.1	0.4	0.4	1.0	1.0
100	0.2	1.0	1.0	1.4	2.0	1.0
200	0.2	1.9	1.0	0.4	1.0	6.0
	Catalase **					
0 (control)	63	77	77	93	50	37
25	63	27	36	58	52	40
50	63	57	74	28	61	00
100	63	27	74	105	72	00
200	63	00	54	20	35	00
	Acid phosphatase ***					
0 (control)	6	11	9	2	22	4
25	6	13	11	3	24	5
50	6	13	9	2	28	7
100	6	12	11	2	16	8
200	6	9	7	2	15	4

* ΔO.D. min^{-1} mg^{-1} protein X 100

** mg H_2O_2 utilised min^{-1} mg^{-1} protein X 100

*** ΔO.D. h^{-1} mg^{-1} protein X 100

Each value is mean of three determinations.

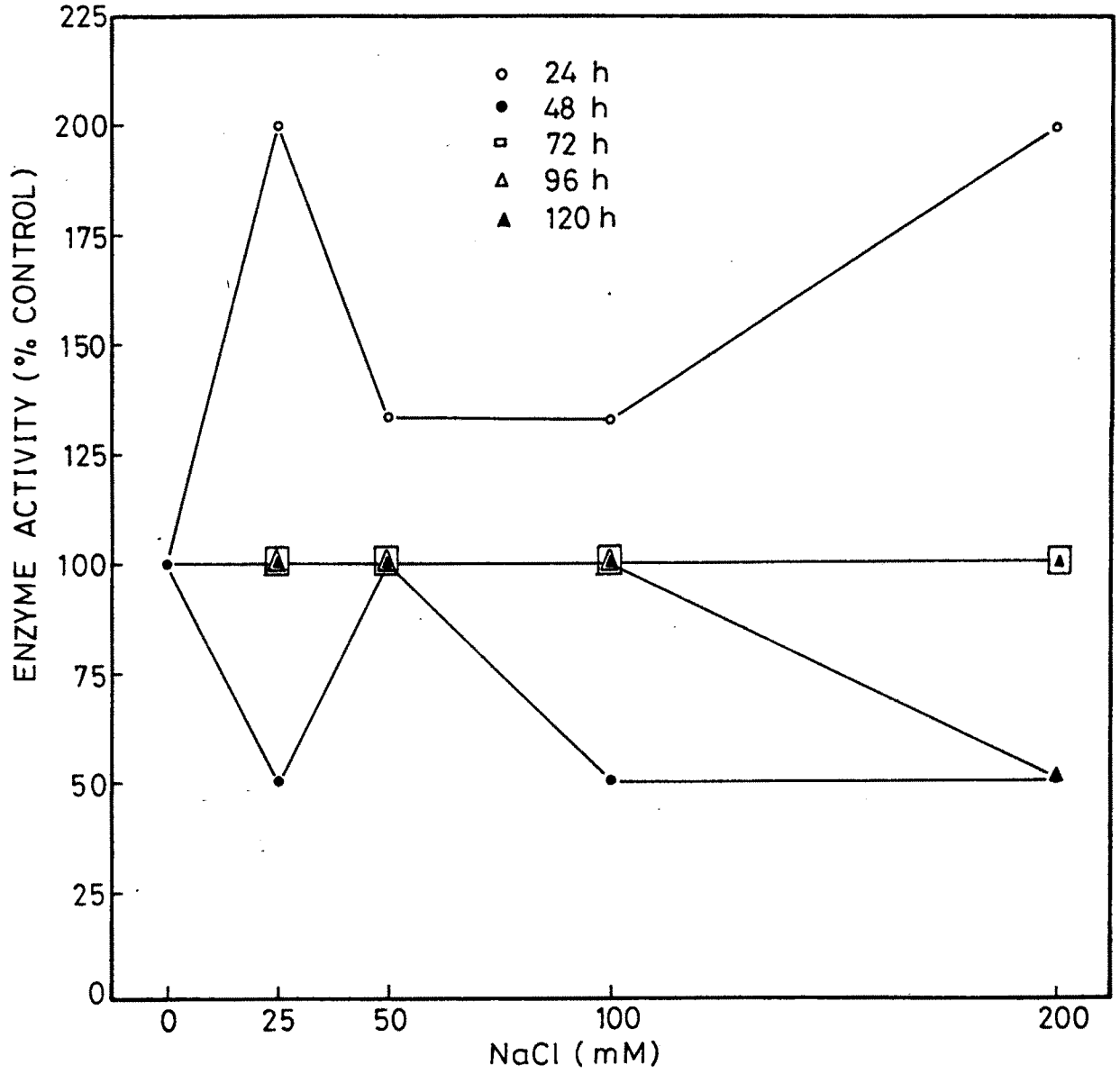

Fig. 21 CHANGES IN THE ACTIVITY OF ENZYME ACID PHOSPHATASE IN THE SEEDS OF D. biflorus DURING GERMINATION UNDER SALINE CONDITIONS.

till 48 h under non-saline conditions. At this particular stage of germination, the seeds of fenugreek exhibit, the highest level of peroxidase (12). As the period of germination or seedling growth advances further, the activity of the enzyme decreases. It is also found that, the enzyme is strongly inhibited by 200 mM NaCl level upto 48 h of germination. However, after this period, it is stimulated by 200 mM. This sudden increase in the activity of peroxidase, may produce the toxic effects in the seedlings, affecting their growth and development.

From these it appears that, the response shown by this enzyme to NaCl salinity varies from species to species. However, it is quite certain that, the highest salt concentration i.e. 200 mM NaCl seems to be toxic for all legume species investigated.

Vasile (1963) has demonstrated the inhibitory effects of high concentrations of NaCl and Na_2SO_4 on the activity of enzyme peroxidase in pea, corn and wheat. El-Fouly and Jung (1970) have observed that, peroxidase activity is not affected by lower concentrations. Maliwal and Paliwal (1972) have observed a decrease in peroxidase in okra (Abelmoschus esculentus) and spongegourd (Luffa cylindrica), with increase in salinity level in the medium. Flowers (1972) has recorded a significant inhibition of this enzyme activity by added NaCl (0.34 M) in invitro studies of peroxidase in Suaeda maritima and Pisum sativum.

TABLE 34 : Changes in the activity of enzymes, peroxidase, catalase and acid phosphatase in the seeds of *P.aureus* during germination under saline conditions.

Treatment NaCl (mM)	Hours of germination					
	0	24	48	72	96	120
	Peroxidase *					
0 (control)	0.1	0.1	2.0	05.0	12.0	13.0
25	0.1	0.1	2.0	10.0	5.0	08.0
50	0.1	0.1	1.0	12.0	11.0	10.0
100	0.1	0.1	1.0	06.0	09.0	09.0
200	0.1	0.3	1.0	02.0	02.0	02.0
	Catalase **					
0 (control)	99	67	48	48	74	98
25	99	53	58	62	83	69
50	99	37	39	83	44	105
100	99	35	39	78	91	65
200	99	45	27	46	24	16
	Acid phosphatase ***					
0 (control)	9	18	13	16	20	15
25	9	12	15	22	18	12
50	9	09	13	22	15	17
100	9	10	13	23	22	15
200	9	12	14	24	06	05

* ΔO.D. min^{-1} mg^{-1} protein X 100

** mg H_2O_2 utilised min^{-1} mg^{-1} protein X 100

*** ΔO.D. h^{-1} mg^{-1} protein X 100

Each value is mean of three determinations.

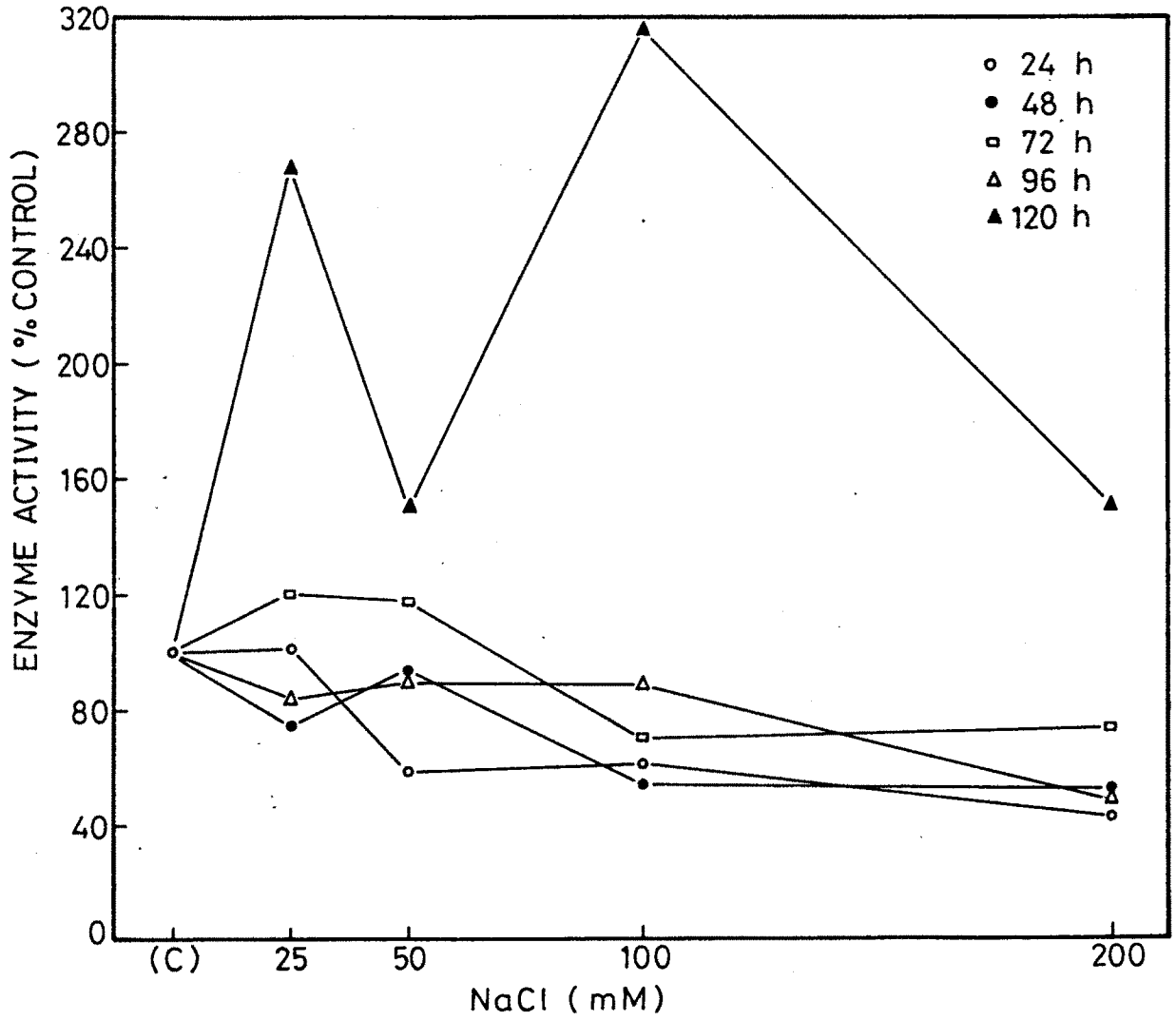

Fig. 22 CHANGES IN THE ACTIVITY OF ENZYME PEROXIDASE IN THE SEEDS OF L. esculenta DURING GERMINATION UNDER SALINE CONITIONS.

Quantitative and qualitative changes in peroxidase, during germination of mung bean under saline conditions, have been reported by Sheoran and Garg (1979). They found that activity of the enzyme increases in the embryo axis and leaves, but not in the cotyledons and roots with different salt treatments to varying degrees. They have also observed that, the number of isoenzymes of peroxidase increase with the time of germination.

Salt treatment decreases the activity of peroxidase and catalase in the germinating seeds of sorghum and maize (Tailakov, 1967). He has also noticed a difference in responses of the cultivars to the salts. Weimberg (1970) studied the levels of 18 enzymes in pea seedlings grown on highly salinized media. He has found the isozyme pattern of peroxidase from the roots of salt grown plants which was altered. Similar type of observations have also been made by number of workers. Aleshin et al.(1971) and Molokov et al.(1973) have observed insentification of peroxidase activity due to both chloride and sulphate salinities as well as changes in the isozyme pattern. Stevens et al.(1978) investigated in detail the utility of peroxidase as an indicator of salt stress in eleven cultivars of brassica. They observed that in 7 cultivars the enzyme activity was decreased due to salt stress. While, in the remaining 4 cultivars there was stimulation. From all these reports, it seems to be difficult to make any generalization about the behaviour of peroxidase

TABLE 35 : Changes in the activity of enzymes, peroxidase, catalase and acid phosphatase in the seeds of *T. foenum-graecum* during germination under saline conditions.

Treatment NaCl (mM)	Hours of germination					
	0	24	48	72	96	120
	Peroxidase *					
0 (control)	2	5	12	4	7	2
25	2	3	3	2	3	4
50	2	6	7	14	5	6
100	2	8	10	10	5	9
200	2	4	4	11	10	11
	Catalase **					
0 (control)	28	0	48	35	37	46
25	28	0	18	49	26	50
50	28	0	00	16	00	39
100	28	0	00	00	00	00
200	28	0	00	00	00	00
	Acid phosphatase ***					
0 (control)	5	7	4	2	7	6
25	5	8	4	1	9	6
50	5	8	4	1	8	4
100	5	8	4	1	7	4
200	5	7	4	1	8	3

* Δ O.D. min^{-1} mg^{-1} protein X 100

** mg H_2O_2 utilised min^{-1} mg^{-1} protein X 100

*** Δ O.D. h^{-1} mg^{-1} protein X 100

Each value is mean of three determinations.

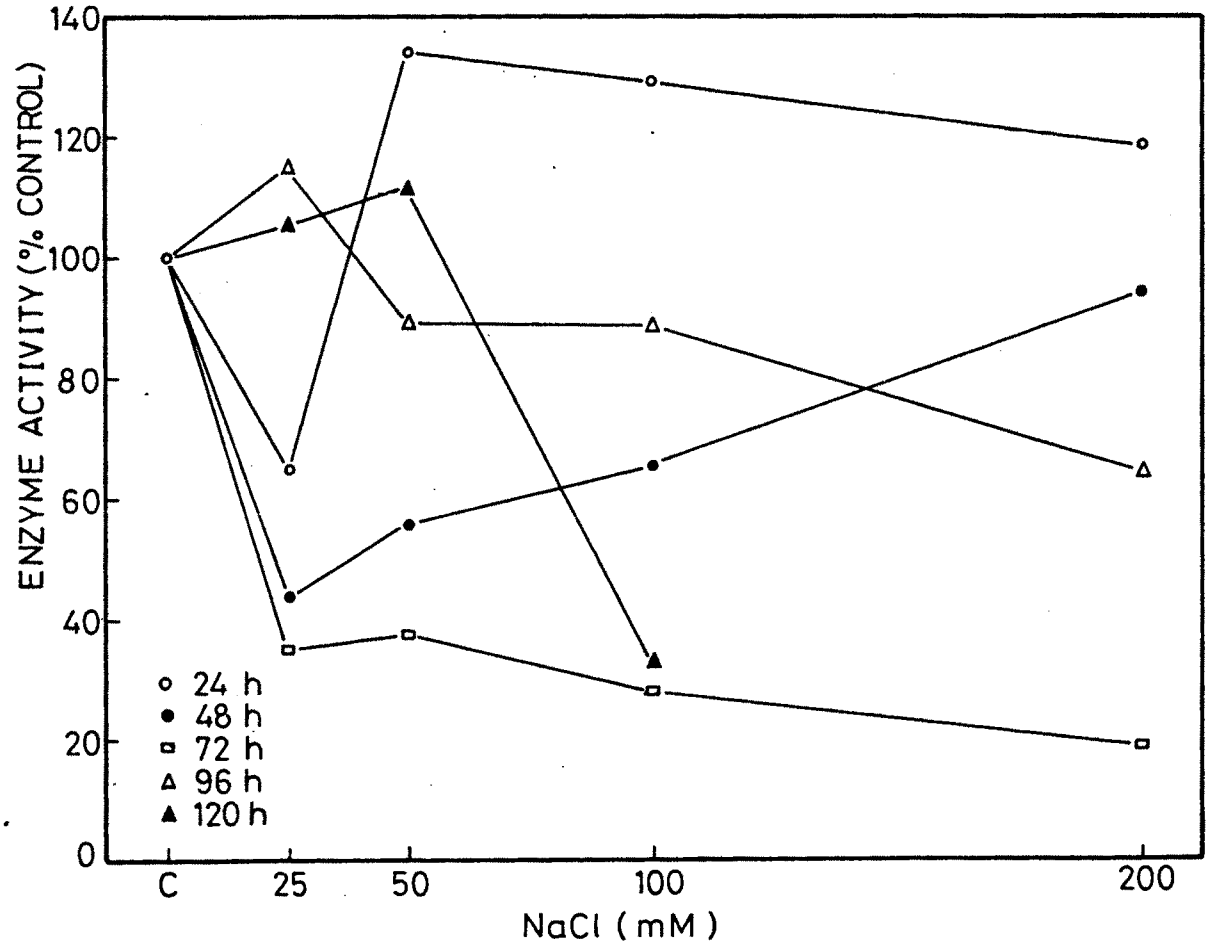

Fig. 23 CHANGES IN THE ACTIVITY OF ENZYME CATALASE IN THE SEEDS OF L. esculenta DURING GERMINATION UNDER SALINE CONDITIONS.

under saline conditions. It can be said, however that, the response of peroxidase to the salinity, varies from species to species. The present studies with five legume species also support this.

b) Catalase :

Catalase is an oxidative enzyme which catalyzes redox reactions between H_2O_2as electron acceptor and many kinds of substrates. It is mainly involved in the regulation of hydrogen peroxidase level in plant tissues. This enzyme has a peculiar role in the process of seed germination. The activity of catalase is very well correlated with germination capacity of the seeds. Mukherji and Paul (1971) have noticed a positive correlation between catalase activities and respiratory rate.

The changes in the activity of enzyme catalase in the seeds of D.biflorus, L.esculenta, P.aconitifolius, P.aureus and T.foenum-graecum during germination have been recorded in Table 31,32,33,34 and 35 respectively (and in Fig.20,23,26,29 and 32). The tables also record the level of enzyme catalase in the dry seeds of these species. It can be seen that, the highest level of this enzyme is recorded for the dry seeds of P.aureus (99) while, a lowest activity is shown by those of lentil (17.9). The legume species investigated, can be arranged in the following order of decrease activity of enzyme catalase in their dry seeds.

P.aureus > P.aconitifolius > D.biflorus > T.foenum-graecum > L.esculenta.

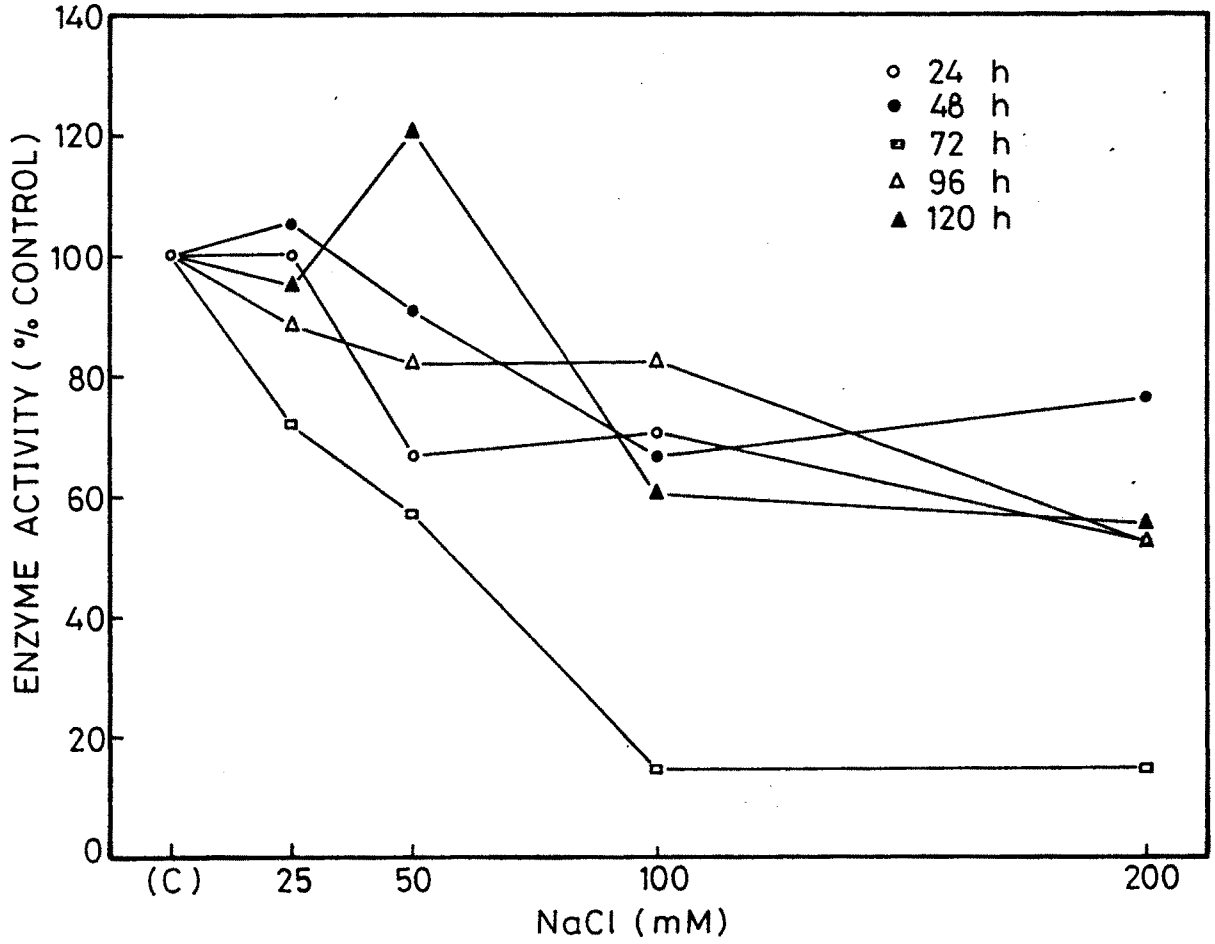

Fig.24 CHANGES IN THE ACTIVITY OF ENZYME ACID PHOSPHATASE IN THE SEEDS OF *L. esculenta* DURING GERMINATION UNDER SALINE CONDITIONS.

It has been found that in all the species investigated except P.aureus, the activity of catalase increases during germination at one or the other stages. In case of P.aureus (Table 34 and Fig.29), however, activity of this enzyme is decreased slowly, with the advancement of germination period, but it is regained after 120 h growth.

The response of enzyme catalase in the seeds of horse gram during germination to various concentrations of NaCl has been recorded in Table 31 and Fig.20. It is evident that, activity of this enzyme is increased considerably after 72 h germination under non-saline as well as saline conditions (25 and 50 mM NaCl). However, during further development of the seedlings, activity of this enzyme rapidly declines. It is also evident that, at the beginning of germination i.e. upto 24 h, salinity exerts almost no effect on the activity of catalase. During later stages of seedling development, the enzyme activity is strongly affected by higher salt concentrations (100 and 200 mM NaCl).

Activity of enzyme catalase in the germinating seeds of lentil, increases with advancement of germination period upto 72 h, when grown under non-saline conditions (Table 32 and Fig.23). During later stages, the activity is declined. It is found that, this pattern of the behaviour of catalase is not maintained under saline conditions. It can be seen that, upto 50 mM NaCl level, the activity of the catalase goes

on increasing very slowly with the period of germination. However, at the higher salt concentrations (100 and 200 mM NaCl), the activity of the enzyme is remarkably affected, particularly, during the later stages of development. It can be seen that, at 120 h of stage, the activity of catalase completely inhibited.

The level of this enzyme in the seeds of P.aconitifolius during germination, under normal conditions increases upto 72 h of germination, and declines during further stages of development (Table 33 and Fig.26). The lowest value recorded (37) for the activity of this enzyme is in the seedlings of 120 h old. It is found that upto 72 h of germination, the salinity at all concentrations inhibits the activity of this enzyme. But during later stages there is some stimulation upto 100 mM NaCl level at 96 h stage and 25 mM NaCl at 120 h. It can also be seen that, the activity of enzyme catalase in the 120 h old seedlings is fully suppressed by salt concentrations of 50 mM and above.

The Table 34 and Fig.29 depicts the values recorded for the activity of enzyme catalase, in the seeds of P.aureus during germination under non-saline and saline conditions. It can be seen that, the level of this enzyme decreases with the period of germination, till 72 h, but, during later stages of development, it is considerably increased and almost equals to that in dry seeds.

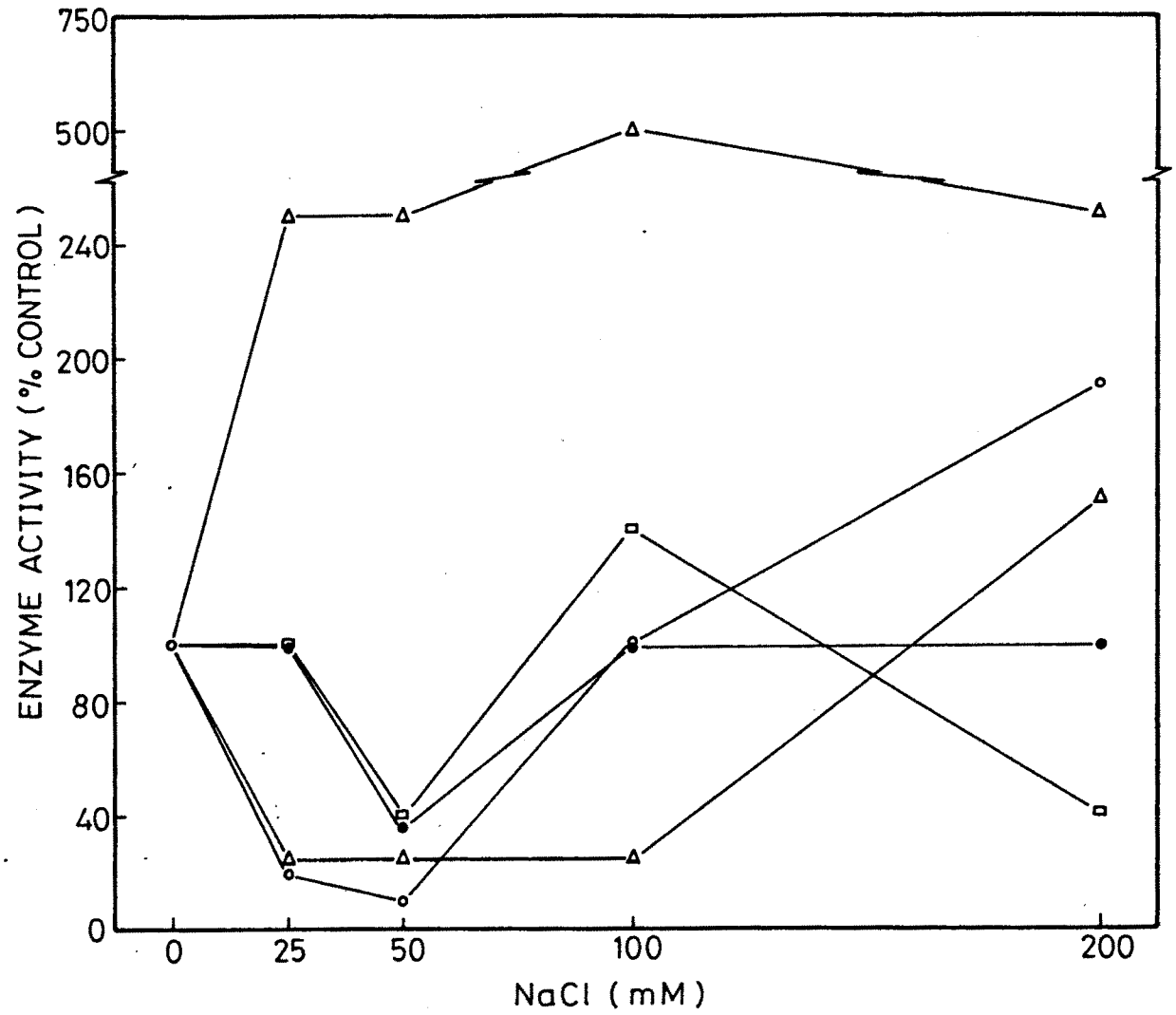

Fig. 25 CHANGES IN THE ACTIVITY OF ENZYME PEROXIDASE IN THE SEEDS OF *P. aconitifolius* DURING GERMINATION UNDER SALINE CONDITIONS.

It is also evident that, activity of this enzyme is decreased under saline conditions at all concentrations during early phase of germination (24 h). However, during later phases the effect of salinity is not consistent. There is no doubt that, the highest salt concentration (200 mM NaCl) very strongly affects the enzyme.

In case of fenugreek, there is no significant induction of the enzyme, during germination, under normal conditions, as the activity of the enzyme is almost constant at all the developmental stages (Table 35 and Fig.32). It is also evident that, the activity of this enzyme at all developmental stages starting from 24 h is strongly affected, particularly, by higher salt concentrations (100 and 200 mM NaCl). It may be recalled that, in this species activity of the enzyme, peroxidase is found to be stimulated under saline conditions.

From these observations, it appears that, enzyme catalase in most of the legume species investigated is salt sensitive and it is strongly affected, particularly, at the higher salt regimes. This is suggestive of a declination of a good germination capacity and seedling growth under stressed conditions (Nanda, 1950).

Tailakov (1967) has observed that, salt treatment decreases the activity of catalase and the respiratory rates in sorghum and maize seeds during germination. He has also

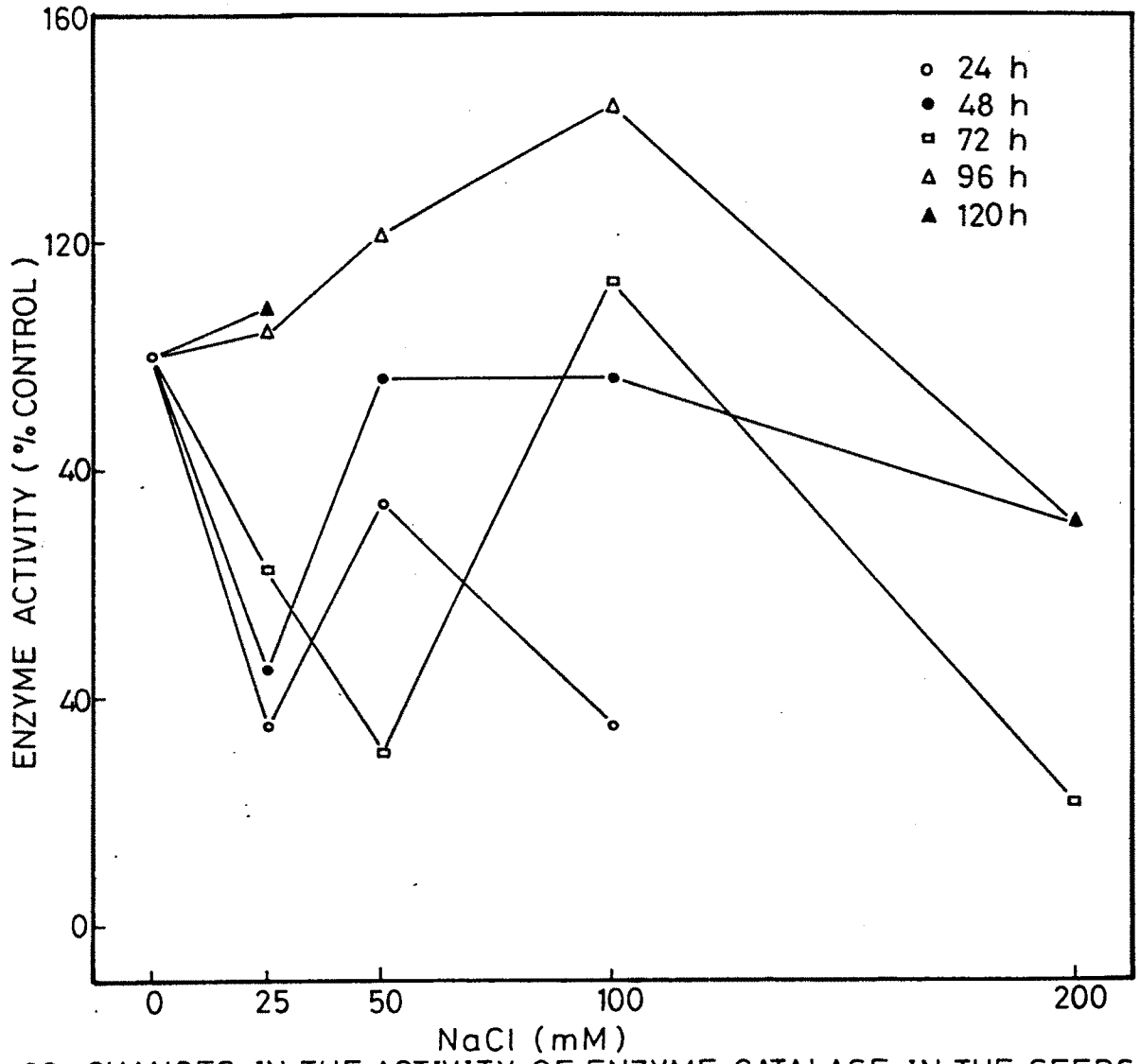

Fig. 26 CHANGES IN THE ACTIVITY OF ENZYME CATALASE IN THE SEEDS OF *P. aconitifolius* DURING GERMINATION UNDER SALINE CONDITIONS

reported that, different cultivars respond differently to the salts and there is more variation in maize than in sorghum. Parida *et al*.(1981) have studied the enzymatic changes in *Cucurbita maxima* and *Phaseolus vulgaris* cotyledons during aging. They found that, catalase activity decreases during senescence of cotyledons. It was also found that, when the cotyledon senescence was retarded by detopping, there was a marked increase in the activity of enzyme. Activity of catalase and hydrogen peroxidase content in redbean seedlings were found to be decreased in cotyledon, root fractions throughout germination whereas those of shoot fractions were increased. Thus, the catalase activity is related to the content of hydrogen peroxidase.

From the present investigation it can be concluded that, in general, activity of enzyme catalase increase with the age of seedling upto certain age (48 to 72 h), when it is maximum and falls down during the later stages of development. Catalase system in the seeds of legume species investigated seems to be highly sensitive, particularly, at the higher salinity levels of more than 100 mM. Among the species, fenugreek seems to be highly sensitive in this respect. However, in this species activity of enzyme peroxidase is stimulated by salinity which is indicative of the capability of germination of this species.

2) Acid phosphatase :

Acid phosphatase is an enzyme involved in non-specific breakdown of a variety of phosphate compounds including ATP

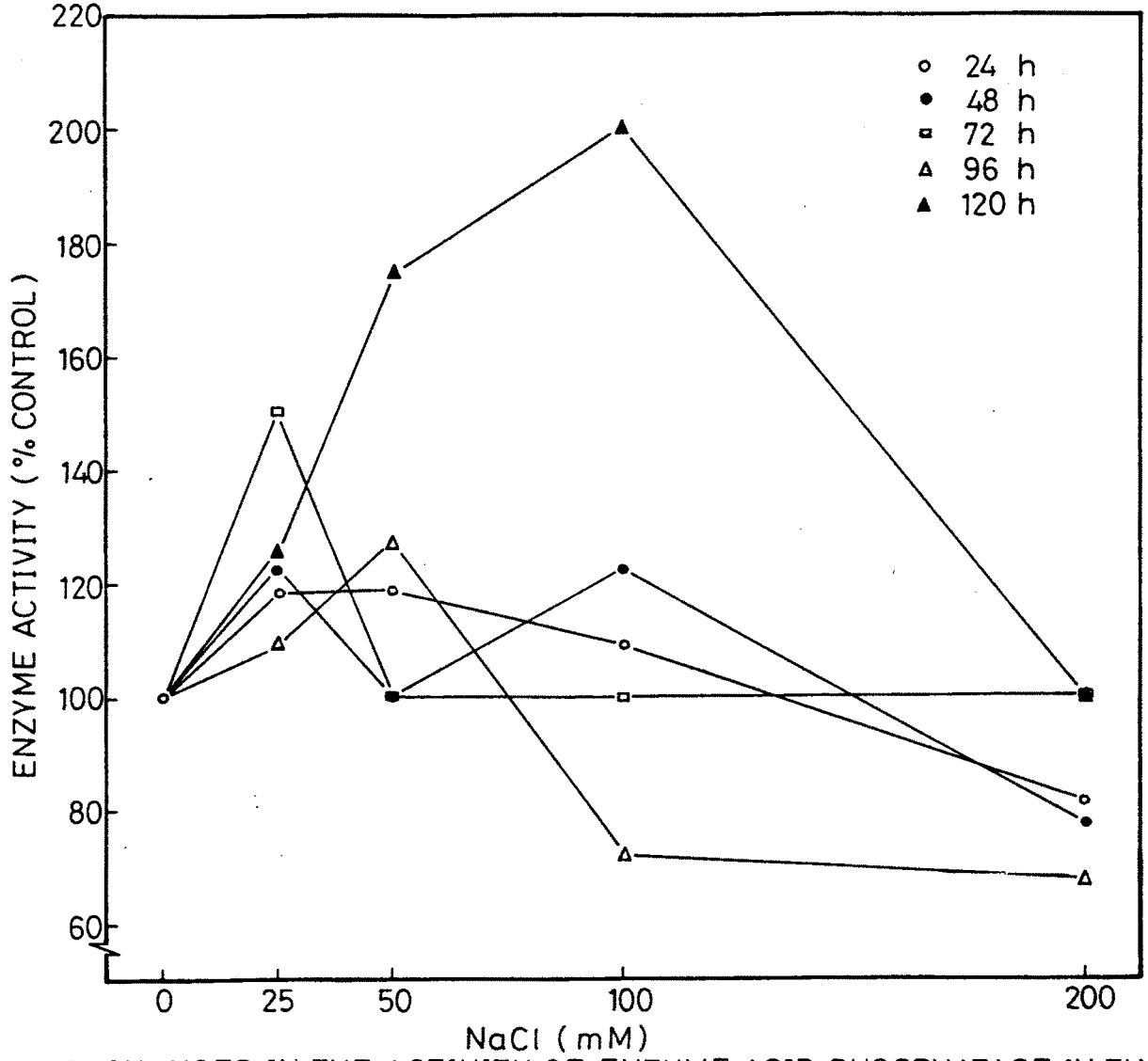

Fig. 27 CHANGES IN THE ACTIVITY OF ENZYME ACID PHOSPHATASE IN THE SEEDS OF *P. aconitifolius* DURING GERMINATION UNDER SALINE CONDITIONS.

(De Leo and Sacher, 1970). According to Flin and Smith (1967) this enzyme plays an important role in the mobilization of nutrient reserve. Besides hydrolytic activities, the enzyme is involved in several other processes in germinating seeds. Activity of this enzyme has been correlated with germination capacity by Arbestain Ribas (1977). Popov (1969) recorded stimulation of enzyme acid phosphatase, during maize seed germination. Price and Fy (1970) observed that, coleorhiza is the most prominant site of enzyme activity in dry wheat embryo. Wieczorek et al.(1975) have found the highest level of acid phosphatase on the 3rd day after barley seed germination. Biswas et al.(1979) have studied acid phosphatase activity in germinating seeds of Vigna sinensis, at various hours of germination and observed that, the activity increases with time and this has been attributed by them to denovo synthesis of enzyme protein but, not to the activity of the already synthesized protein in the dry seed. Bansal et al.(1981) have observed continuous increase in acid phosphatase activity upto 7 days in Cucumis melo seedlings.

The effect of NaCl salinity at various concentrations on the activity of enzyme acid phosphatase in the seeds of D.biflorus, L.esculenta, P.aconitifolius, P.aureus and T.foenum-graecum during germination at different developmental stages has been given in Tables 31, 32, 33, 34, 35, and Fig.21, 24, 27, 30 and 33 respectively. It is evident from these tables and

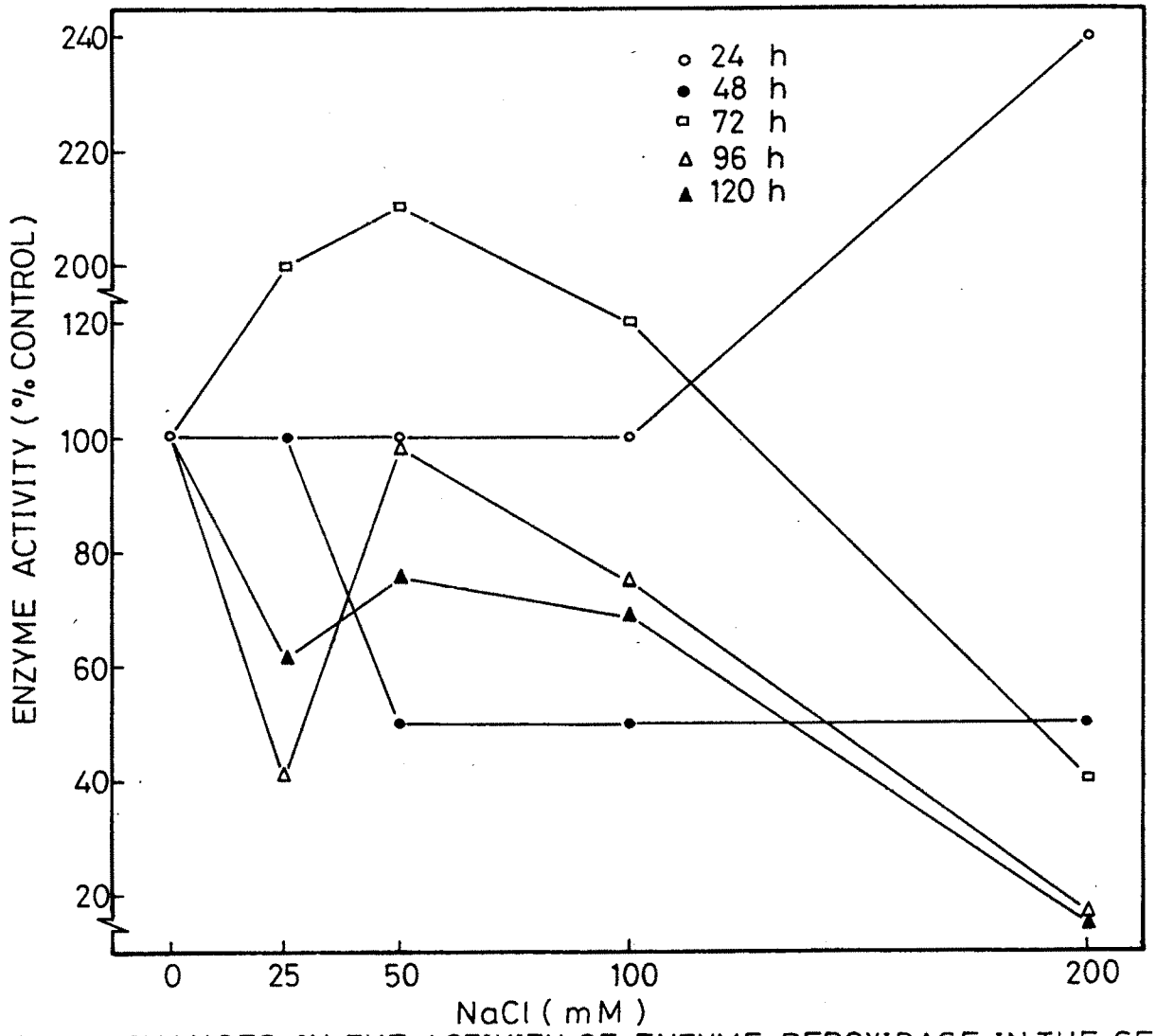

Fig. 28 CHANGES IN THE ACTIVITY OF ENZYME PEROXIDASE IN THE SEEDS OF *P. aureus* DURING GERMINATION UNDER SALINE CONDITIONS.

figs that, the activity of the enzyme is the highest in dry seeds of lentil, while, the lowest in D.biflorus. The species can be arranged in the following order with respect to decreasing level of acid phosphatase in the dry seeds.

lentil > green gram > moth bean > fenugreek > horse gram. It can also be seen that, the activity of this enzyme increases with the stage of development in case of moth bean, green gram and fenugreek. However, in other two species namely horse gram and lentil, the activity of the enzyme increases slightly after 24 h of germination (in horse gram) and after 96 h (in lentil).

From Table 31 (Fig.21) it is evident that, the activity of enzyme acid phosphatase is increased to some extent, in the germinating seeds of horse gram at 24 h of germination only. This is also true for the seeds, germinating under saline conditions at all concentrations of NaCl. However, the activity of the enzyme is at low level during all the subsequent stages of germination under both non-saline as well as saline conditions. NaCl salinity has shown no definite effect on the level of the enzyme.

The changes in the activity of acid phosphatase in the seeds of lentil during germination under normal and saline condition has been depicted in Table 32 (Fig.24). It is evident that, activity of the enzyme which is the highest in dry seeds

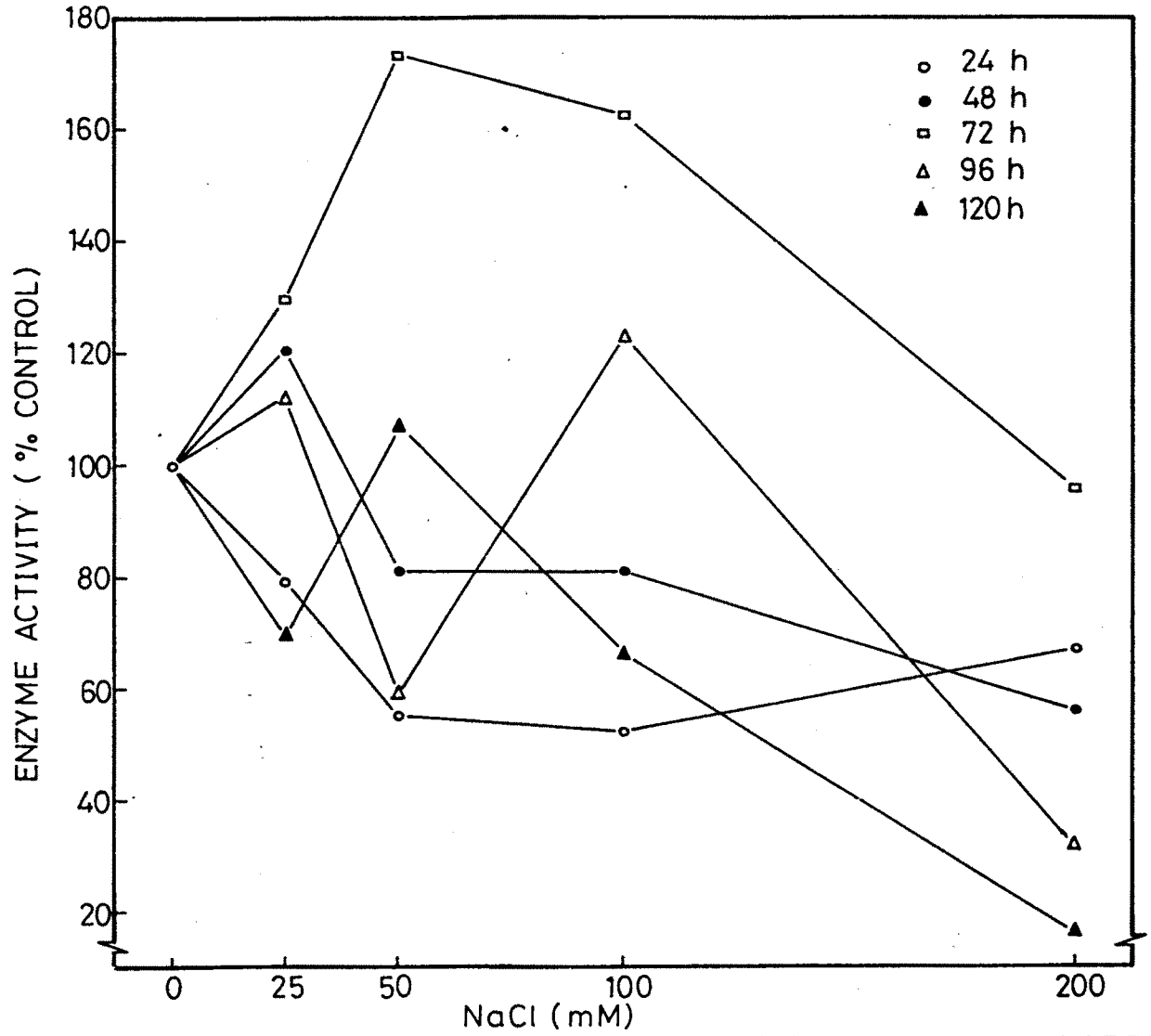

Fig. 29 CHANGES IN THE ACTIVITY OF ENZYME CATALASE IN THE SEEDS OF *P. aureus* DURING GERMINATION UNDER SALINE CONDITIONS.

decreases continuously till 72 h of germination, and increases to reach the original level during further stages of development i.e. at 96 h. However, it sharply declines at 120 h stage. It can also be seen that, the activity of the enzyme is decreased linearly with increasing salinity level in the medium. 200 mM NaCl seems to be rather highly toxic. Thus, lentil seems to be highly sensitive to NaCl salinity in this respect.

Table 33 and Fig.27 records the enzyme activity, in the seeds of moth bean during germination under salt stress. It is evident that, activity of the enzyme increases with the duration of germination till 48 h but, sharply falls down at 72 h. The highest level of acid phosphatase has been recorded for the seedlings of this species of 96 h old. The level of the enzyme again declines during further stages of development. It is also evident from the observations that, moth bean seems to be rather moderately salt tolerant with respect to the behaviour of the enzyme during germination under saline conditions. It can be seen that, activity of this enzyme is slightly affected at higher salt concentrations only (100 and 200 mM NaCl).

The changes in the level of acid phosphatase in the seeds of green gram during germination under saline condition (Table 34 and Fig.30) indicate that, level of the enzyme is remarkably increased during germination till 96 h and it is declined in 120 h old seedlings. The results also suggest that,

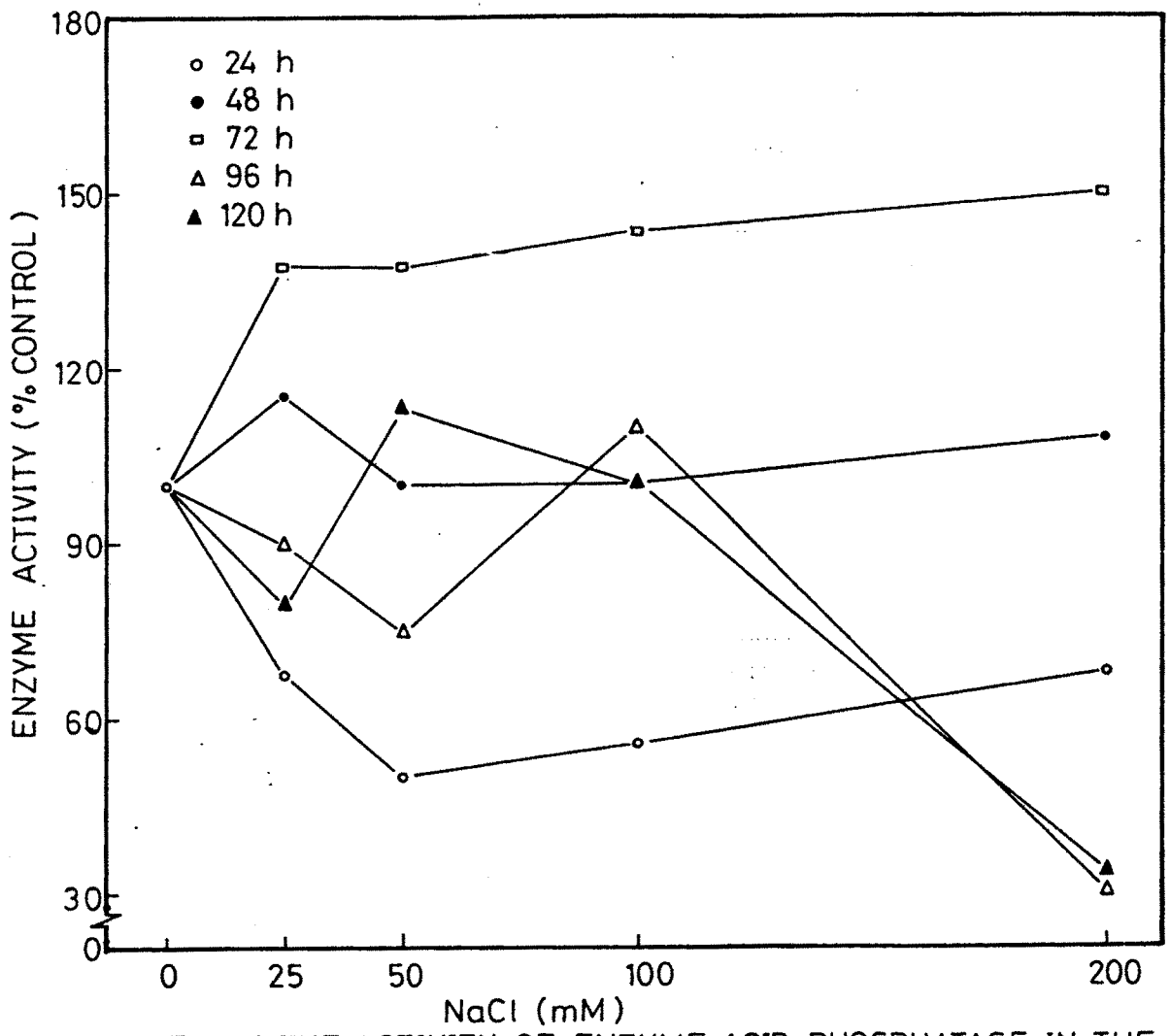

Fig. 30 CHANGES IN THE ACTIVITY OF ENZYME ACID PHOSPHATASE IN THE SEEDS OF *P. aureus* DURING GERMINATION UNDER SALINE CONDITIONS.

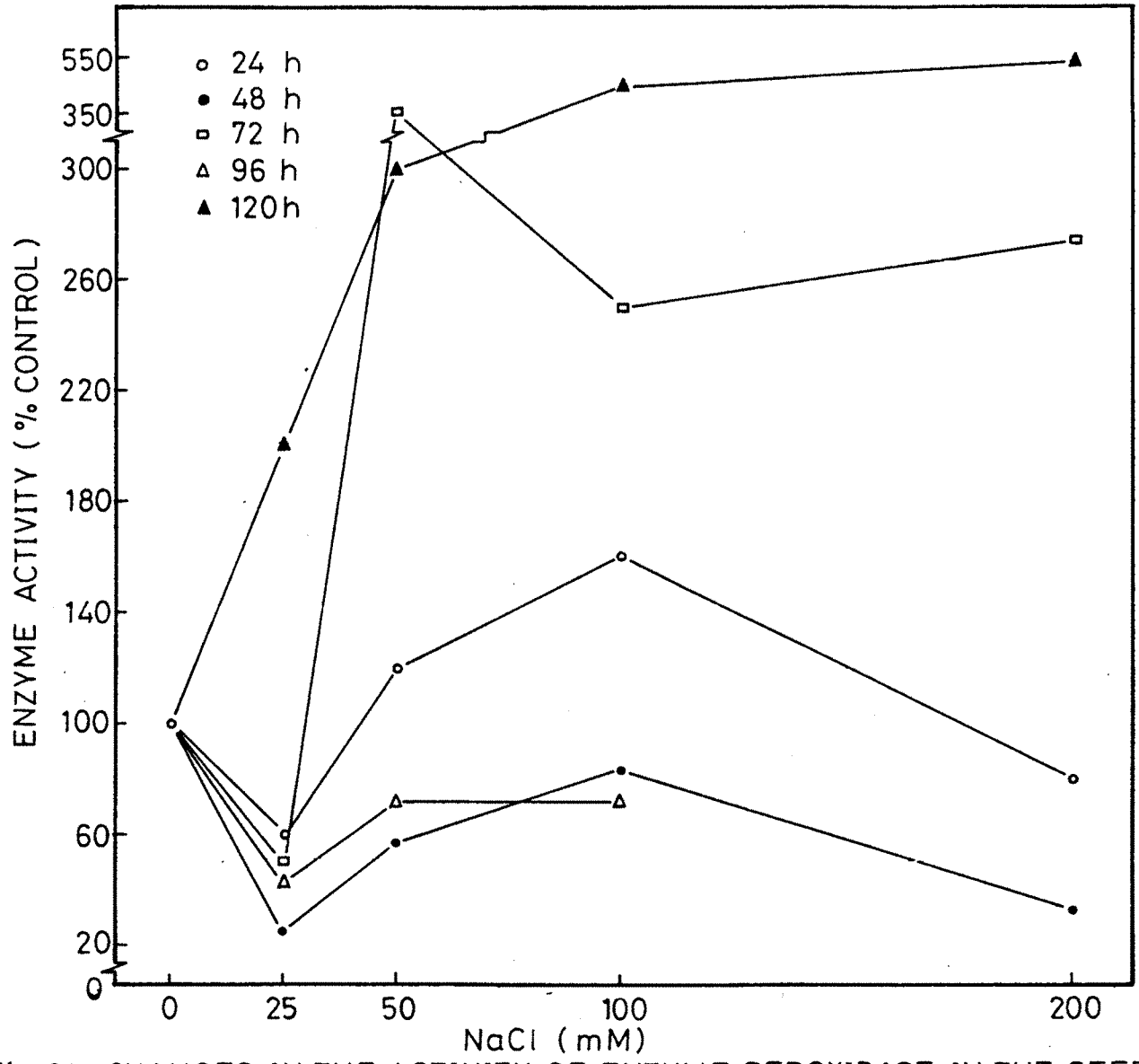

Fig.31 CHANGES IN THE ACTIVITY OF ENZYME PEROXIDASE IN THE SEEDS OF *T. foenum-graecum* DURING GERMINATION UNDER SALINE CONDITIONS.

the enzyme, during germination upto 72 h is insensitive to salinity at all concentrations. It can be seen that, the activity of acid phosphatase is unaltered upto 72 h of germination under saline conditions. 200 mM NaCl, however, strongly affects the level of this enzyme during later stages of seedling development (96 and 120 h). Thus, green gram appears to be salt tolerant, during germination but, probably becomes salt sensitive, particularly, for higher salt concentration.

Acid phosphatase in the seeds of fenugreek is induced during early phases of germination between, normal and saline conditions (Table 35, Fig.32). Activity of the enzyme, however, decreases during further stages of germination under saline as well as non-saline conditions. It is again elevated during further stages of seedling development. It is also evident from Table 35 and Fig.32 that, enzyme acid phosphatase exhibits salt tolerant nature, particularly during seed germination upto 96 h phase. However, salinity seems to affect the enzyme activity during further stages of development, and 200 mM NaCl seems to be toxic. Thus, acid phosphatase in the seeds of fenugreek seems to be moderately salt sensitive.

Zhukovskaya (1971) found that both chloride and sulphate salinities activate glucose 6 phosphate and alkaline and neutral fructose 1,6 diphosphatases in the roots and leaves of barley, millet,

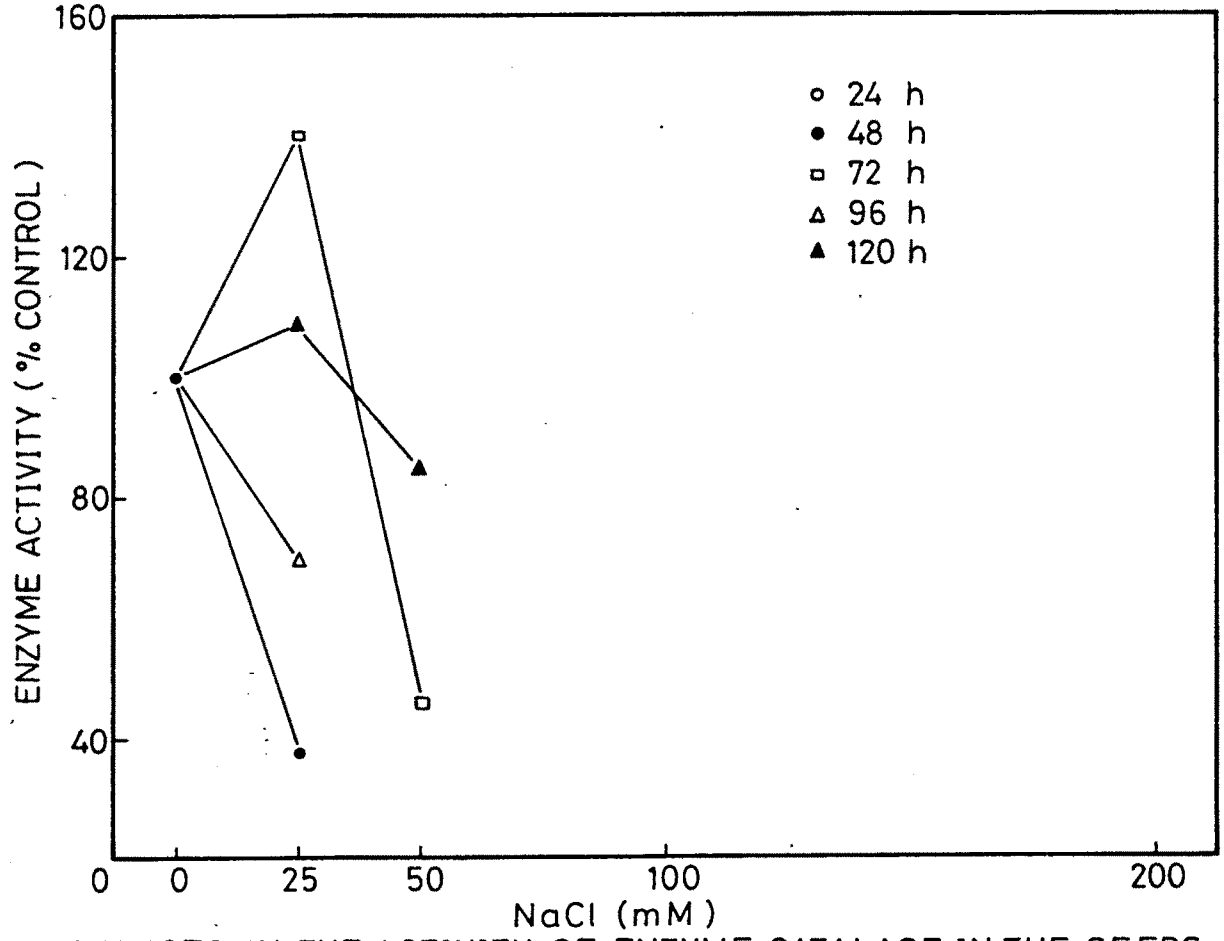

Fig. 32 CHANGES IN THE ACTIVITY OF ENZYME CATALASE IN THE SEEDS OF *T. foenum-graecum* DURING GERMINATION UNDER SALINE CONDITIONS.

sunflower and tomato. It was also reported by her that, some new phosphatases are induced by salinity which are not found active in plants grown under glykik conditions.

El-Fouly and Jung (1972) have also recorded a considerable increase in acid phosphatase in the wheat seedlings under the conditions of NaCl as well as Na_2SO_4 salinities. Ahmad and Huq (1974) have also noticed, an increase in acid phosphatase activity in the leaves of halophytic spinach under saline conditions. Thus most of these observations, indicate a shift towards more catabolic activities induced by salt stress leading to more hydrolysis of phosphate compounds facilitating phosphorus to the developing seedling or plants. Contrary to these observations Narsagaudar et al.(1979) have observed an inhibition of acid phosphatase in the seeds of sorgum during germination under saline conditions and suggested that, this may effect the hydrolysis of various reseve phosphates thereby disturbing the phosphate metabolism during germination. Khot (1986) has found that, activity of enzyme acid phosphatase continues to increase with time, upto 120 h germination in moth bean. Present observations also confirm the observations made by him. From the present investigation it can be concluded that, acid phosphatase in the legume species investigated shows a wide range of salinity tolerance and hence legume species also exhibit a differential response to salinity during germination

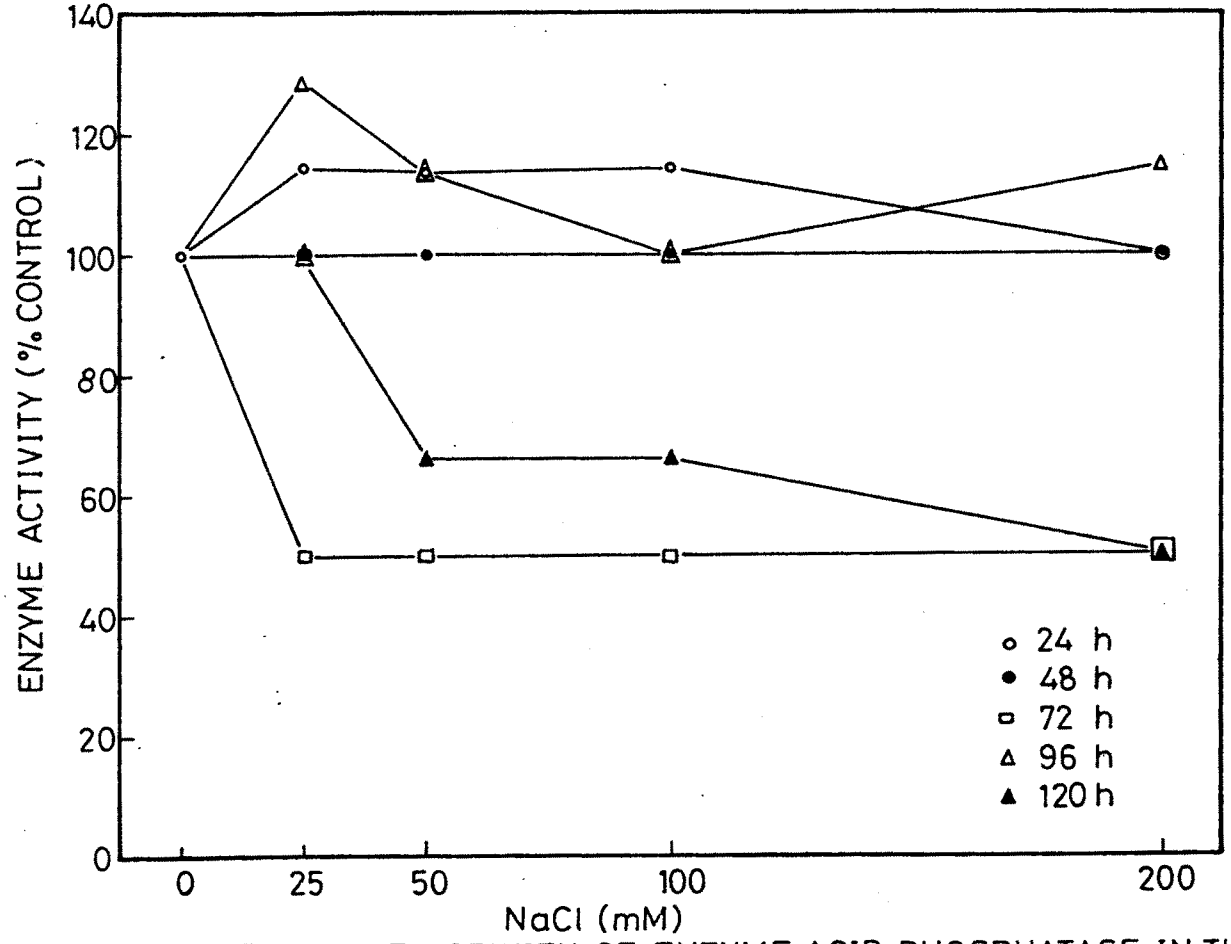

Fig. 33 CHANGES IN THE ACTIVITY OF ENZYME ACID PHOSPHATASE IN THE SEEDS OF *T. foenum-graecum* DURING GERMINATION UNDER SALINE CONDITIONS.

and seedling growth. Lentil seems to be highly sensitive, while moth bean and green gram show a tolerant nature while, horse gram and fenugreek can be considered as moderately salt tolerant.