CHAPTER - III

RESULTS AND DISCUSSION

1. Enzyme Kinetics of Hydroxyperoxidases

The dictionary meaning of kinetics is science of relations between motions of bodies and forces acting upon them. Enzyme kinetics therefore can be taken as science of relations between velocity of enzymatic reaction and the factors influencing enzyme action. Velocity of enzymatic reaction depends on or influenced by a variety of forces or factors such as substrate concentration, enzyme concentration, pH of the medium, temperature and the level of inhibitors present. Thus studies of kinetics of hydroxyperoxidases include the effect of hydrogen peroxide concentration, pH of the buffer used in the preparation of assay medium, temperature of the assay mixture for the enzyme and the concentration of other constituents of the assay mixture such as cofactors and inhibitors, particularly in the crude enzyme preparations. In the present study the effect of substrate concentration i.e. hydrogen peroxide, pH of the assay medium and temperature at which the enzymatic reaction mixture is incubated, has been investigated.

A) Peroxidase

a) Effect of substrate concentration and determination of Km

The effect of substrate concentration i.e. hydrogen peroxide, on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* and *Portulaca oleracea* L. has been recorded in tables 1 and 2 respectively and Fig.1, 3 and 5,7 respectively. The various substrate concentrations tried are from 0.05 to 4.0 µmoles. It is evident that activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* increases linearly with increase in

Table 1:- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

Sr. No.	Substrate Concentration H_2O_2 (μ moles)	Enzyme activity*	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	0.05	8.8	0.153
2	0.10	9.4	0.163
3	0.20	9.8	0.170
4	0.40	13.2	0.229
5	0.60	13.2	0.229
6	0.80	17.8	0.239
7	1.00	17.6	0.306
8	1.20	16.4	0.285
9	1.40	18.0	0.313
10	1.60	20.0	0.348
11	1.80	22.8	0.396
12	2.00	22.8	0.396
13	2.50	19.9	0.342
14	3.00	19.3	0.335
15	3.50	15.9	0.274
16	4.00	15.0	0.260

* Each value is mean of three determinations

Table 2:- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.

Sr. No.	Substrate Concentration H_2O_2 (μ moles)	Enzyme activity *	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	0.05	0.65	0.068
2	0.10	0.79	0.082
3	0.20	1.07	0.112
4	0.40	1.33	0.138
5	0.60	1.50	0.156
6	0.80	1.50	0.156
7	1.00	1.73	0.180
8	1.20	1.75	0.103
9	1.40	1.80	0.188
10	1.60	1.84	0.192
11	1.80	2.01	0.210
12	2.00	2.03	0.212
13	2.50	2.20	0.229
14	3.00	2.22	0.234
15	3.50	2.25	0.234
16	4.00	1.72	0.180

* Each value is mean of three determinations

Fig.1 :- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

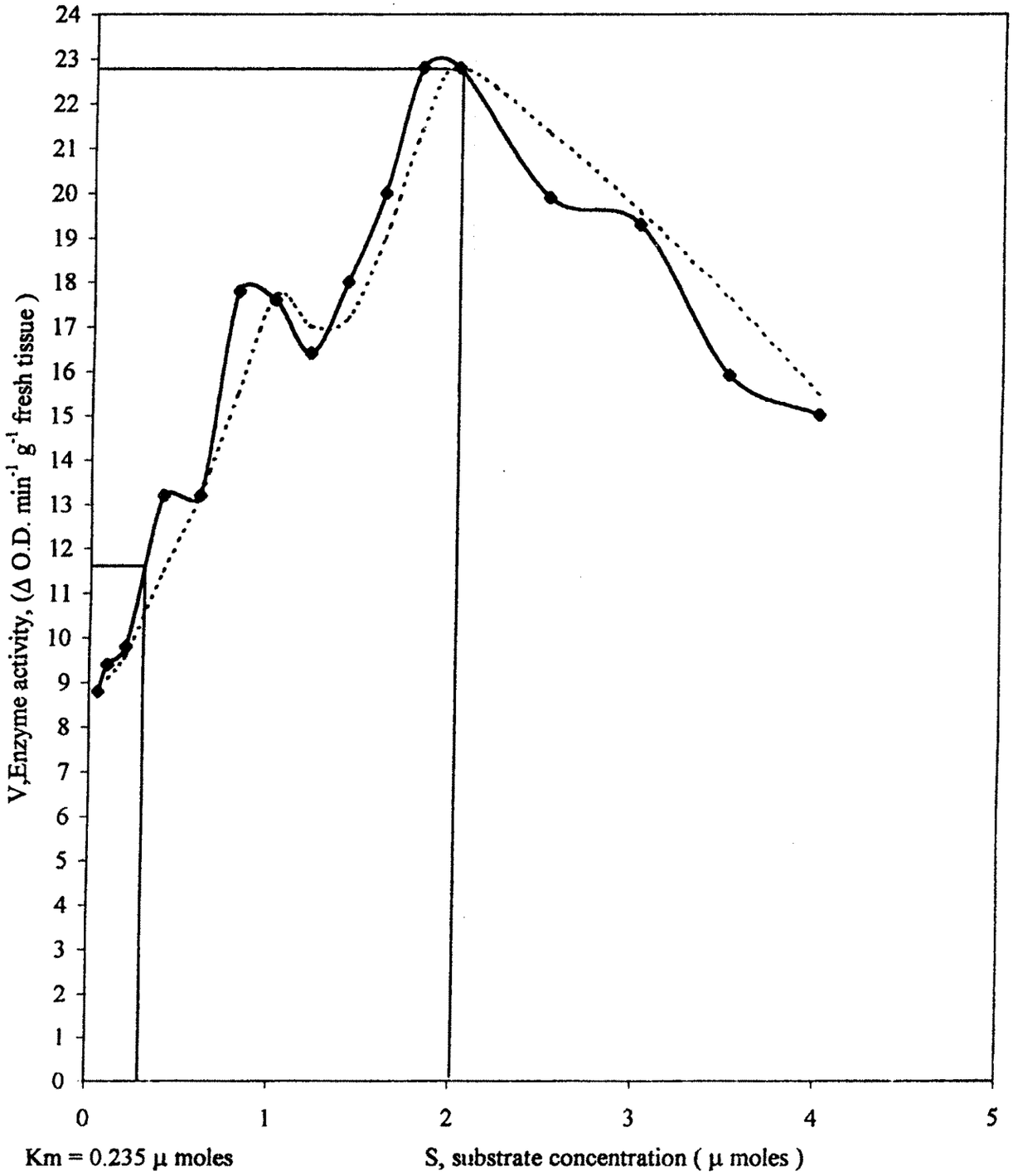


Fig 3:- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

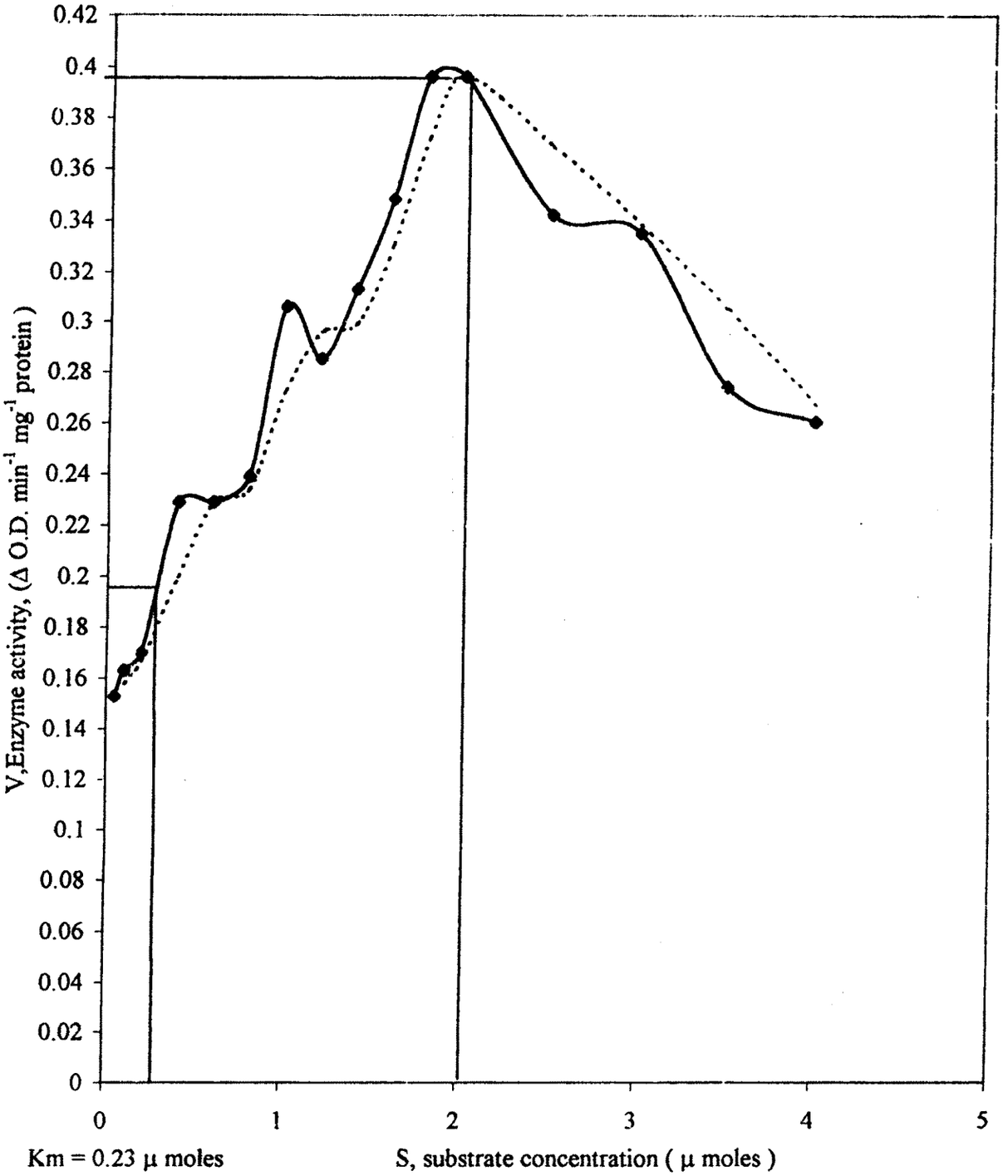


Fig. 5 :- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.

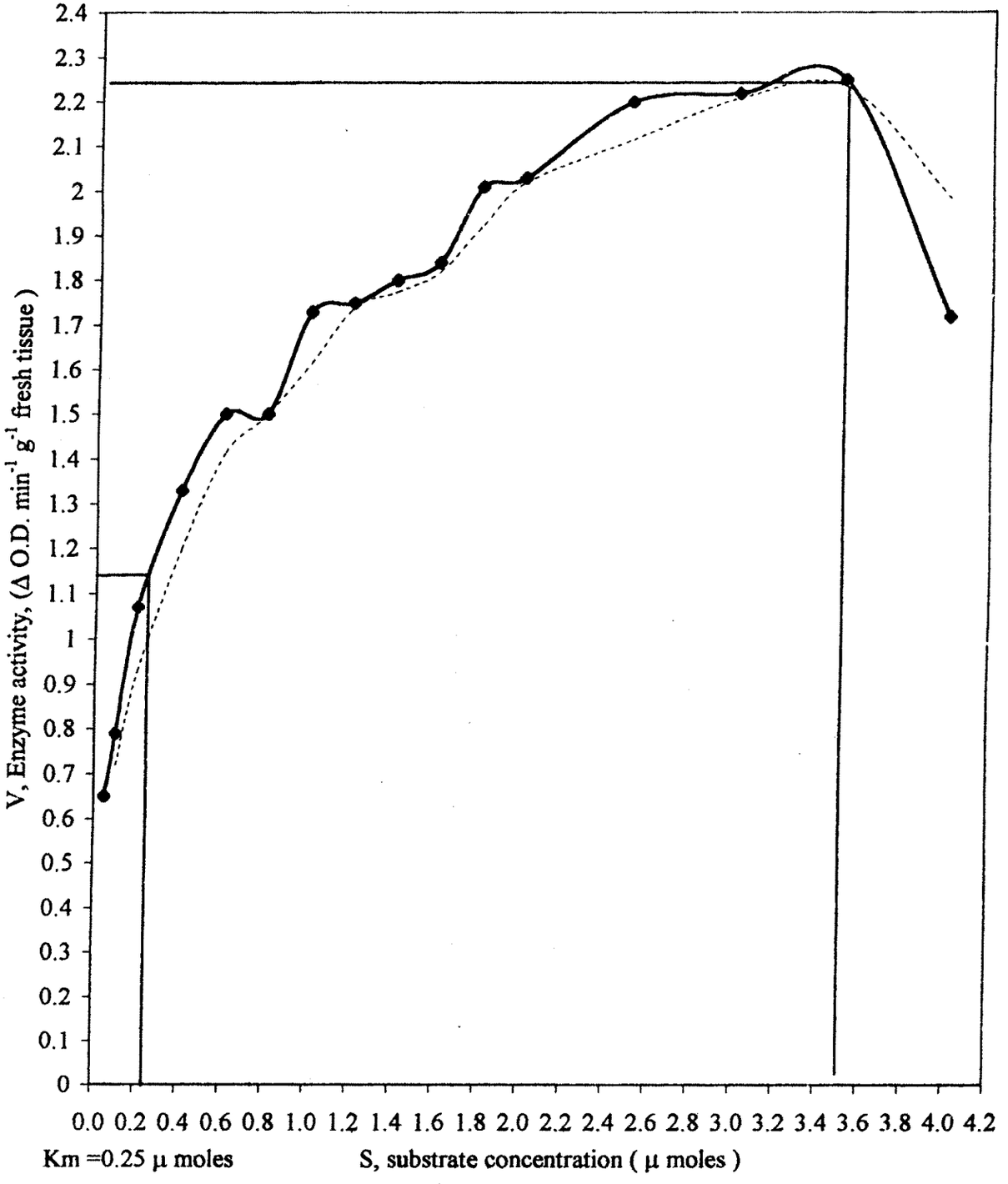
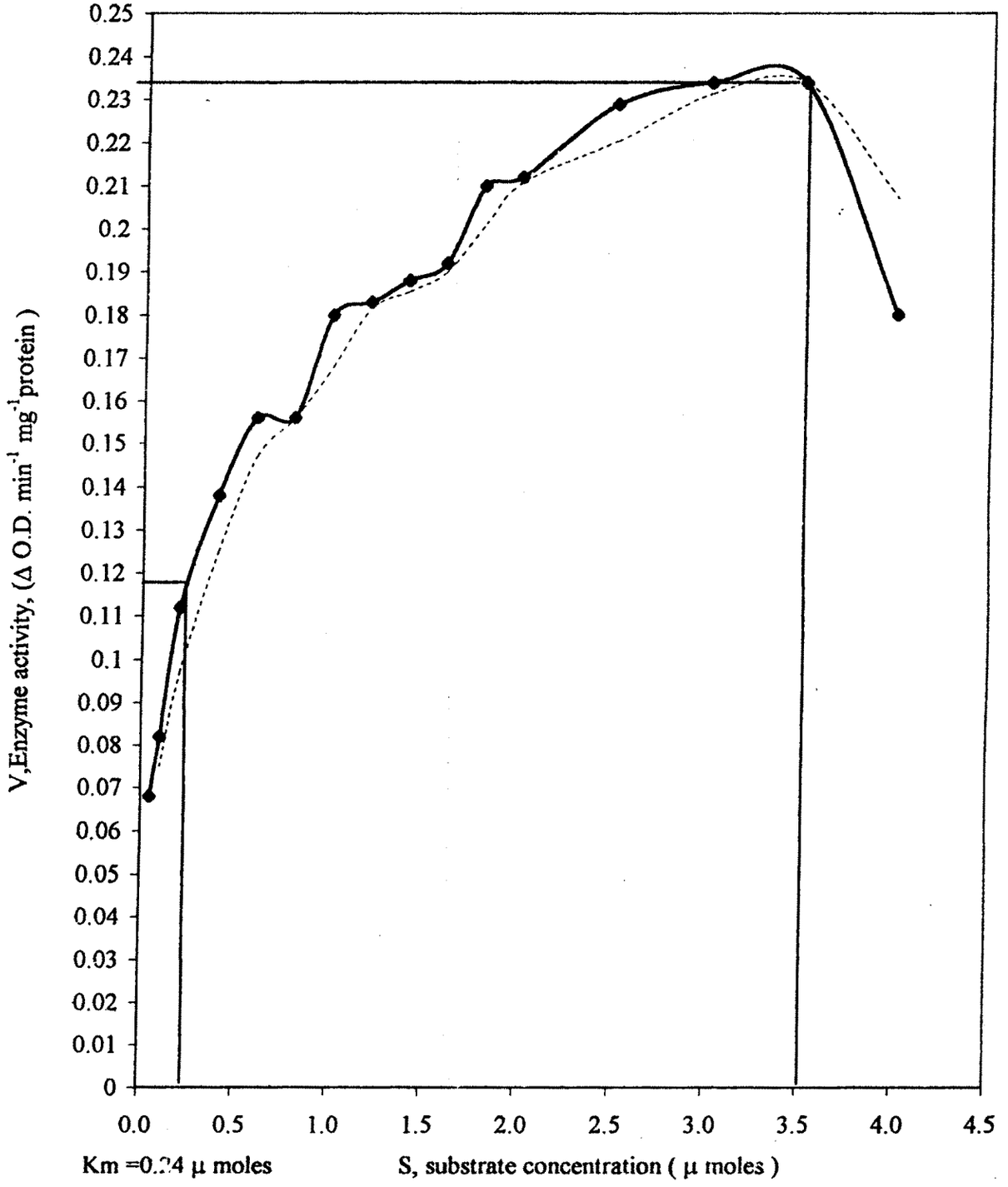


Fig. 7 :- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.



substrate concentration up to 1.8 and 2.0 µmoles of H_2O_2. The further increase in the level of substrate concentration either maintains or lowers the activity of enzyme. This is true when the activity of enzyme is expressed both on fresh tissue as well as protein bases. It can be seen that enzyme peroxidase in this plant records the maximum activity of 22.8 $min^{-1}g^{-1}$ fresh tissue or 0.396 $min^{-1}mg^{-1}$ protein. Almost similar trend can be observed in case of enzyme peroxidase in the leaves of *Portulaca oleracea*. It is observed that activity of the enzyme peroxidase increases linearly with increasing the substrate concentration, with maximum activity being at 3.0 and 3.5 µmoles H_2O_2, which is the maximum. It is also evident from Table 2 and Fig.5 that with further increase in the concentration of H_2O_2, there is a decline in the activity. This is true for enzyme activity when expressed both on fresh tissue and protein bases. It can be seen that enzyme peroxidase in this plant records the maximum activity of 2.22 $min^{-1}g^{-1}$ fresh tissue or 0.234 $min^{-1}mg^{-1}$ protein.

From these values for enzyme activity Michaelies constants (Kms) for enzyme peroxidase in *Sesbania grandiflora* and *Portulaca oleracea* (L.) have been determined. It has been shown in the form of graphs (Fig. 1,2,3 and 4 for *Sesbania grandiflora* L. while Fig. 5,6,7 and 8 for *Portulaca oleracea* L.) Fig. 1,3,5 and 7 exhibit Km. values determined with a plot of substrate concentration against velocity of enzymatic reaction. While figures 2,4,6 and 8 show Km values determined with the plots of reciprocal of substrate concentration against that of velocity of enzymatic reaction. Km i.e. Michaelis constant is the substrate concentration at which the enzymatic reaction gives half the maximum velocity of the enzymatic

Fig2 :- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

(V = Enzyme activity, Δ O.D. min^{-1} g^{-1} fresh tissue;
S = substrate concentration, μ moles.)Km = 0.235 μ moles

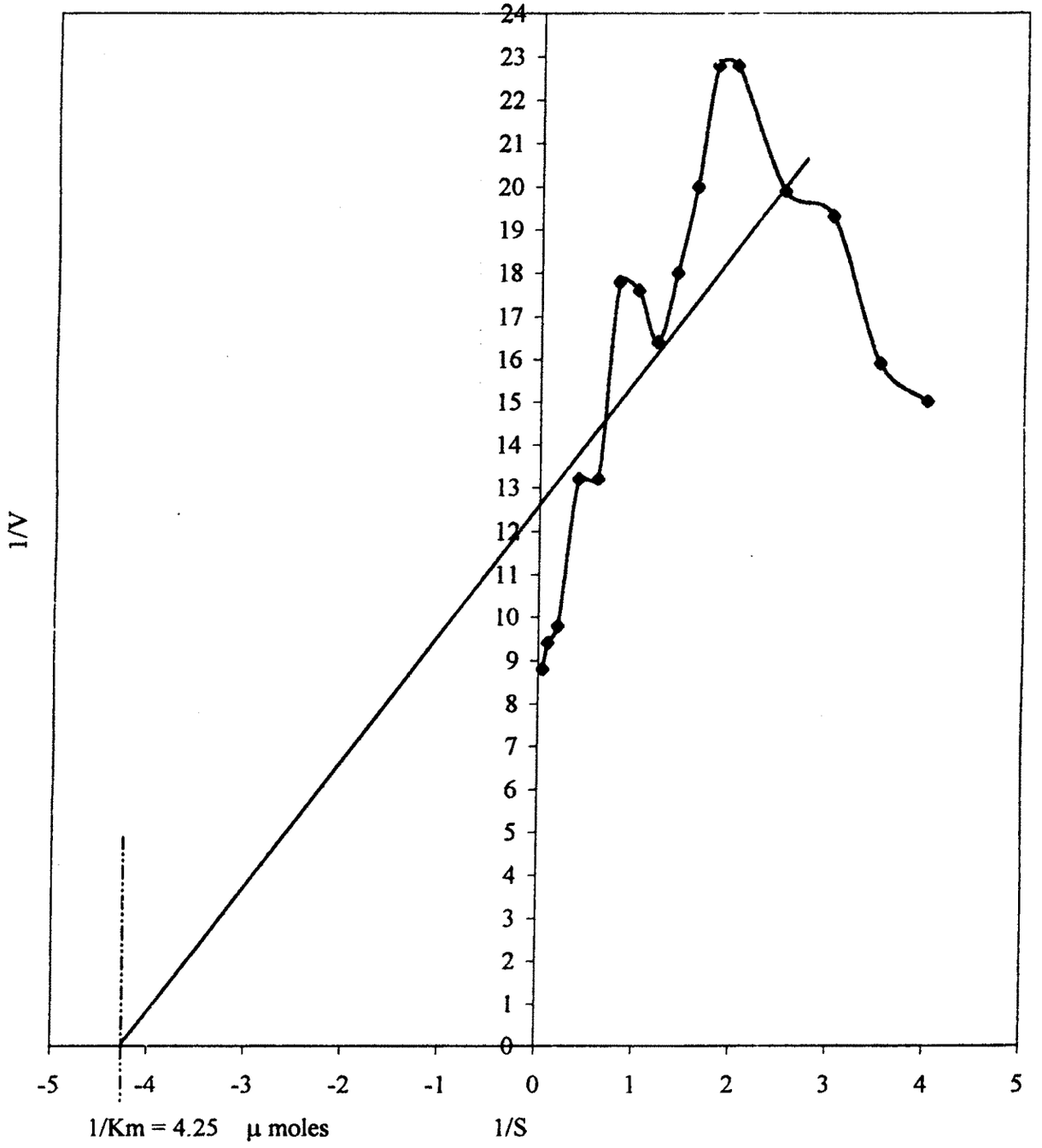


Fig 4:- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L

(V = Enzyme activity, Δ O.D. min^{-1} mg^{-1} protein;
S = substrate concentration, μ moles.)
Km = 0.230 μ moles

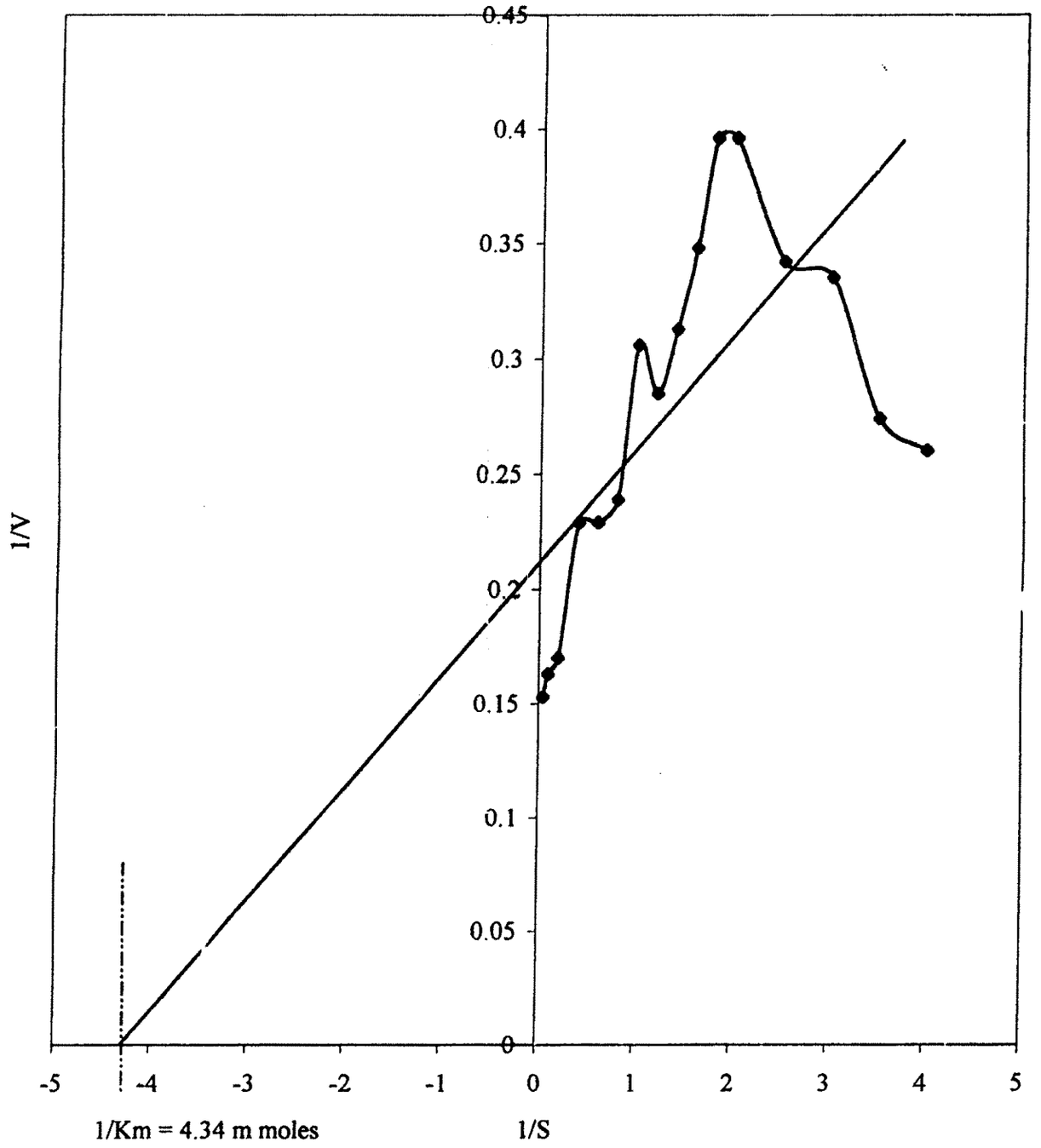


Fig. 6:- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.

(V = Enzyme activity, Δ O.D. min^{-1} g^{-1} fresh tissue;
S = substrate concentration, μ moles.)
Km = 0.25 μ moles

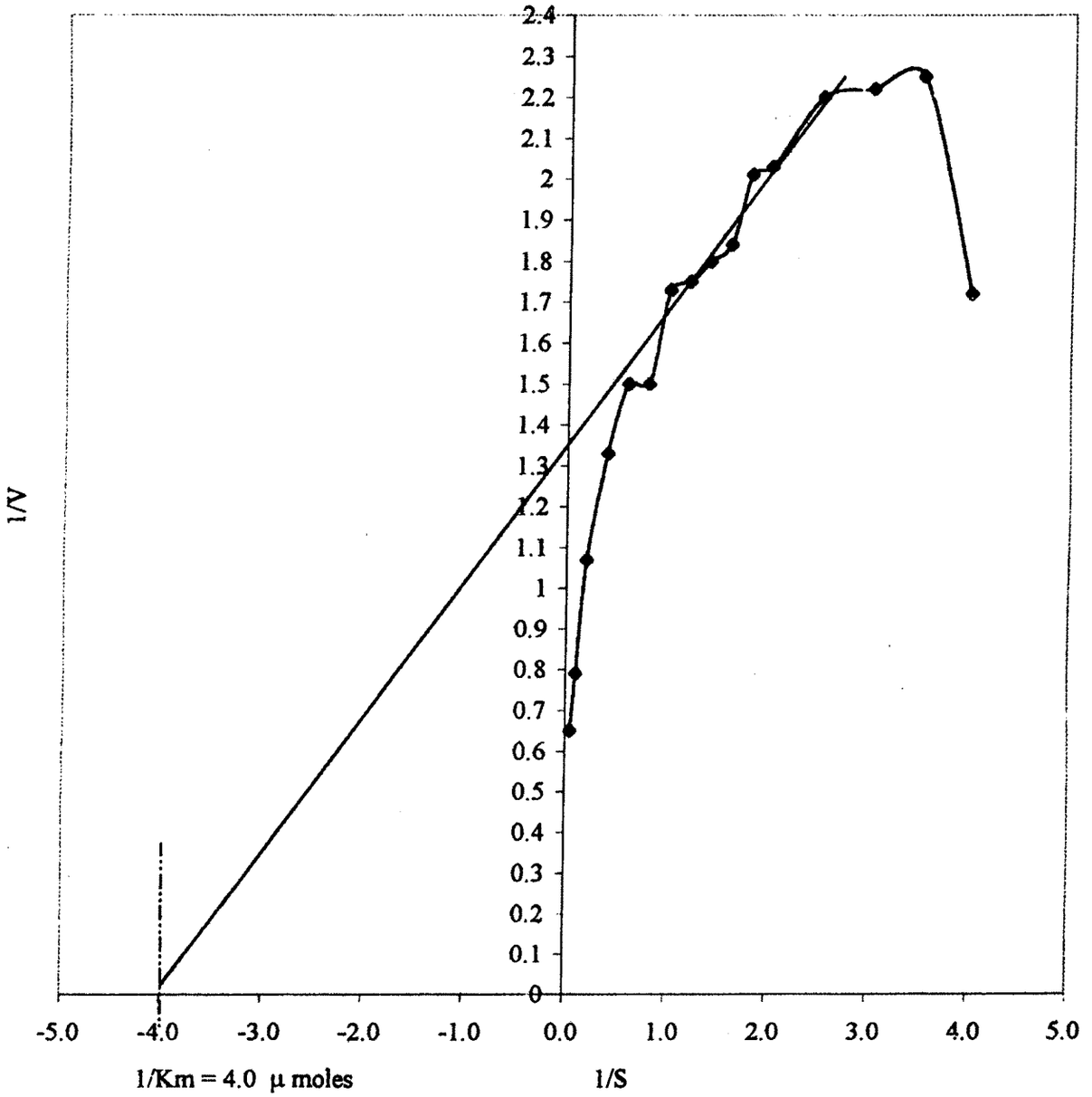
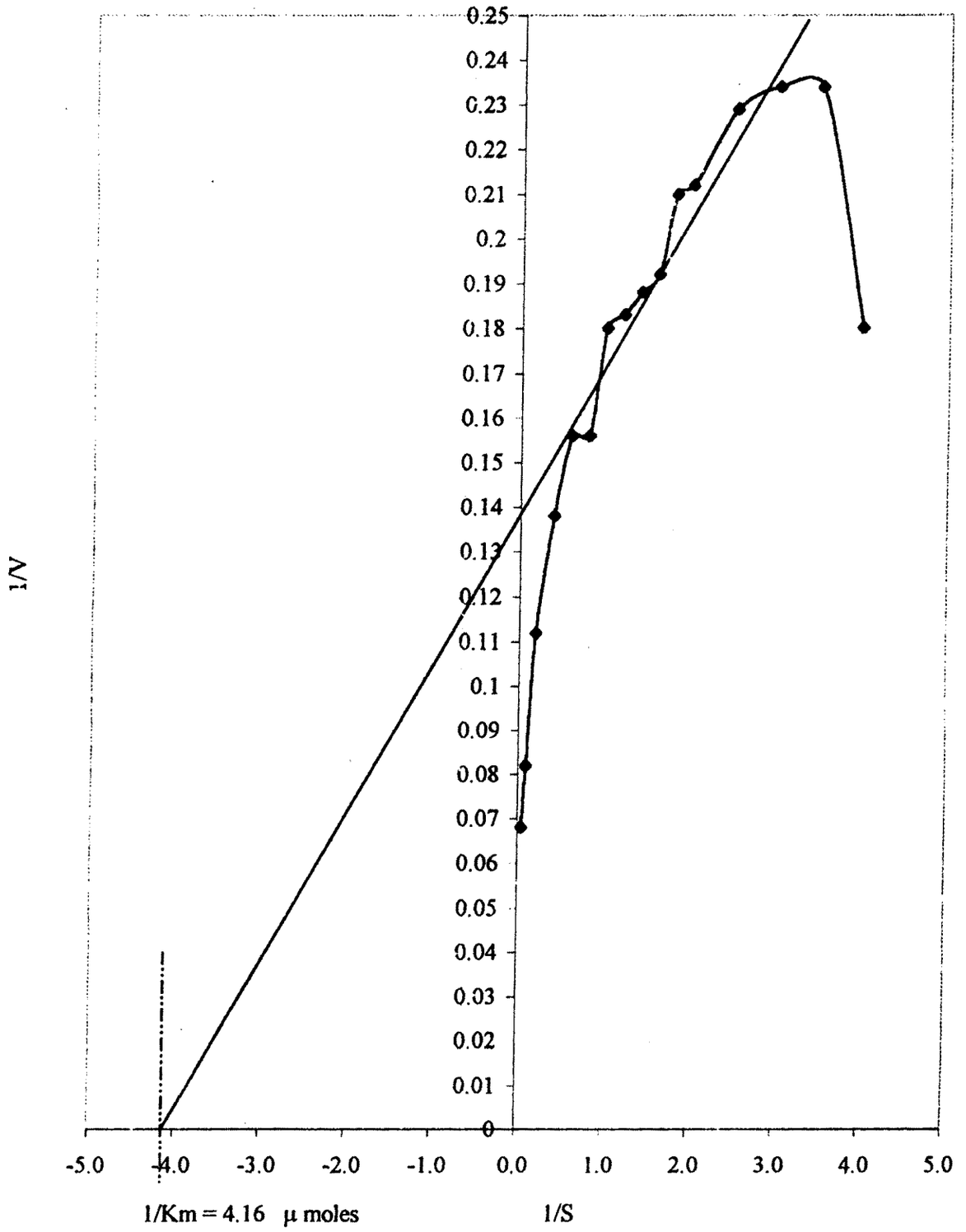


Fig. 8 :- Effect of substrate concentration on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.

(V = Enzyme activity, Δ O.D. min^{-1} mg^{-1} protein;
S = substrate concentration, μ moles.)
Km = 0.24 μ moles.



reaction. Under ideal and normal conditions each enzyme has its own Km value which may be influenced by environmental conditions. If an enzyme has only one substrate, i.e. only one substance on which enzyme acts, the enzyme has only one Km. However, if an enzyme has a number of substrates then it has more than one Km values.

From these figures it is clear that Km for enzyme peroxidase in the leaves of *Sesbania grandiflora* L. ranges between 0.230 to 0.335 µmoles. While that for enzyme peroxidase in the leaves of *Portulaca oleracea* L. ranges from 0.24 to 0.25 µmoles. It is clear therefore from these observations that Km for peroxidase in the leaves of *Portulaca oleracea* L. is slightly higher than that in case of *Sesbania grandiflora* L.

b) Effect of pH

pH is one of the important factors influencing the enzymatic reaction. Changes in the pH of an assay medium can cause a number of effects on initial velocity measurements with an enzyme. The enzyme may be irreversibly denatured by acid or base. Some of the possible effects that are caused by a change in pH are :

1. A change in the ionization of groups involved in catalyses;
2. A change in the ionization of groups involved in binding the substrate;
3. A change in the ionization of groups in the substrate;
4. A change in the ionization of other groups in the enzyme.

Changes in the ionization of groups involved in the catalytic mechanism may completely disrupt the mechanism.

When velocity of enzymatic reaction is plotted against pH, a result is a bell shaped curve for many enzymatic reactions. The point of maximal activity is the pH optimum, which is a fixed value for the enzyme under the conditions of the assay. Routine assays for activity are performed at this pH.

Effect of pH of the assay medium on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* and *Portulaca oleracea* has been recorded in the tables 3 and 4 and figures 9,10,11 and 12. The tables record pH of the assay medium before reaction as well as after the enzymatic reaction. Mean of these two values have been considered as pH of the assay medium influencing the enzymatic reaction. The media tried are from pH 5.92 to 7.53. The enzyme activity as recorded in the tables and figures is both expressed on fresh tissue as well as protein bases. It is evident from these observations that initially with increasing pH of the medium there is increase in the activity of the enzyme and later with further increase in pH, activity of the enzyme decreases linearly. This is true in case of *Sesbania grandiflora* (Table 3). It is also evident that enzyme has exhibited the highest activity at pH from 6.03 to 6.17. pH above 6.88 however affects the activity remarkably. It can be seen that at pH 7.53, it is almost half to the maximum recorded. It can be concluded, therefore, that pH 6.0 to 6.2 can be taken as pH optimum for peroxidase in the leaves of *Sesbania grandiflora* L.

The pH of the assay medium tried to study the effect of pH on peroxidase activity in case of *Portulaca oleracea* ranges from 6.08 to 7.55 (Table 4 and Fig.11 and 12). It is observed that there is an increase in the

Table 3:- Effect of pH of the assay medium on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

Sr No	pH Before Reaction	pH After Reaction	Mean pH of assay medium	Enzyme activity *	
				Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	5.8	6.05	5.92	19.25	0.34
2	6.0	6.08	6.03	23.00	0.40
3	6.2	6.15	6.17	22.75	0.40
4	6.4	6.28	6.33	20.99	0.37
5	6.6	6.43	6.51	19.00	0.33
6	6.8	6.53	6.66	18.55	0.32
7	7.0	6.78	6.88	18.05	0.31
8	7.2	6.75	6.97	16.30	0.28
9	7.4	6.90	7.15	15.50	0.27
10	7.6	7.00	7.30	14.35	0.27
11	7.8	7.05	7.42	14.35	0.25
12	8.0	7.08	7.53	12.44	0.22

* Each value is mean of three determinations

Table 4:- **Effect of pH of the assay medium on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.**

Sr No	pH Before Reaction	pH After Reaction	Mean pH of assay medium	Enzyme activity *	
				Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	5.8	6.38	6.08	2.66	0.278
2	6.0	6.43	6.21	2.91	0.304
3	6.2	6.45	6.32	2.97	0.310
4	6.4	6.55	6.47	2.81	0.293
5	6.6	6.60	6.60	2.40	0.250
6	6.8	6.72	6.76	2.29	0.238
7	7.0	6.90	6.95	1.95	0.203
8	7.2	6.88	7.06	1.68	0.177
9	7.4	7.00	7.20	1.55	0.161
10	7.6	7.10	7.35	1.44	0.150
11	7.8	7.05	7.43	1.32	0.138
12	8.0	7.10	7.55	1.29	0.135

* Each value is mean of three determinations

Fig. 9 :- Effect of pH of the assay medium on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

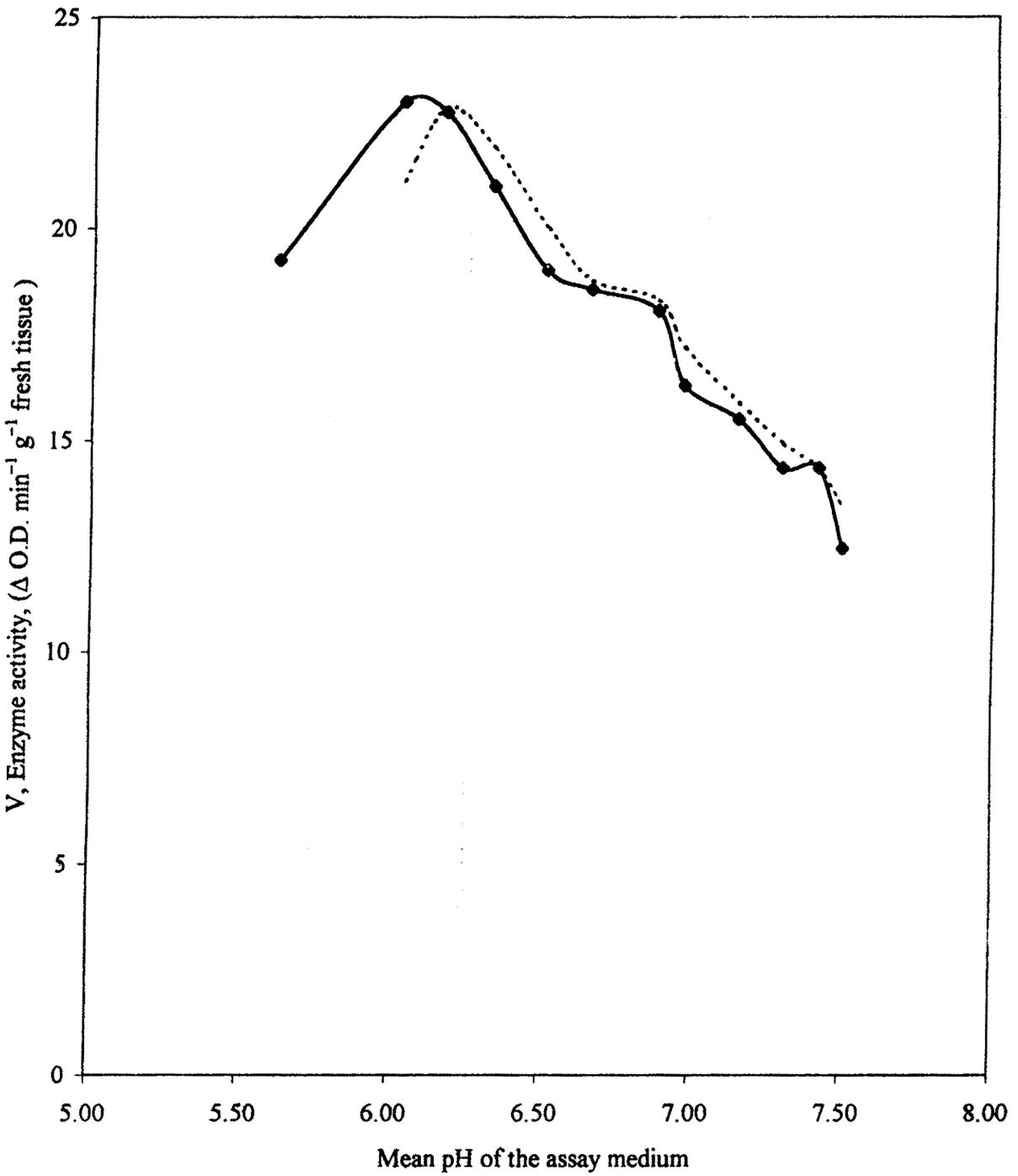


Fig 10 :- Effect of pH of the assay medium on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

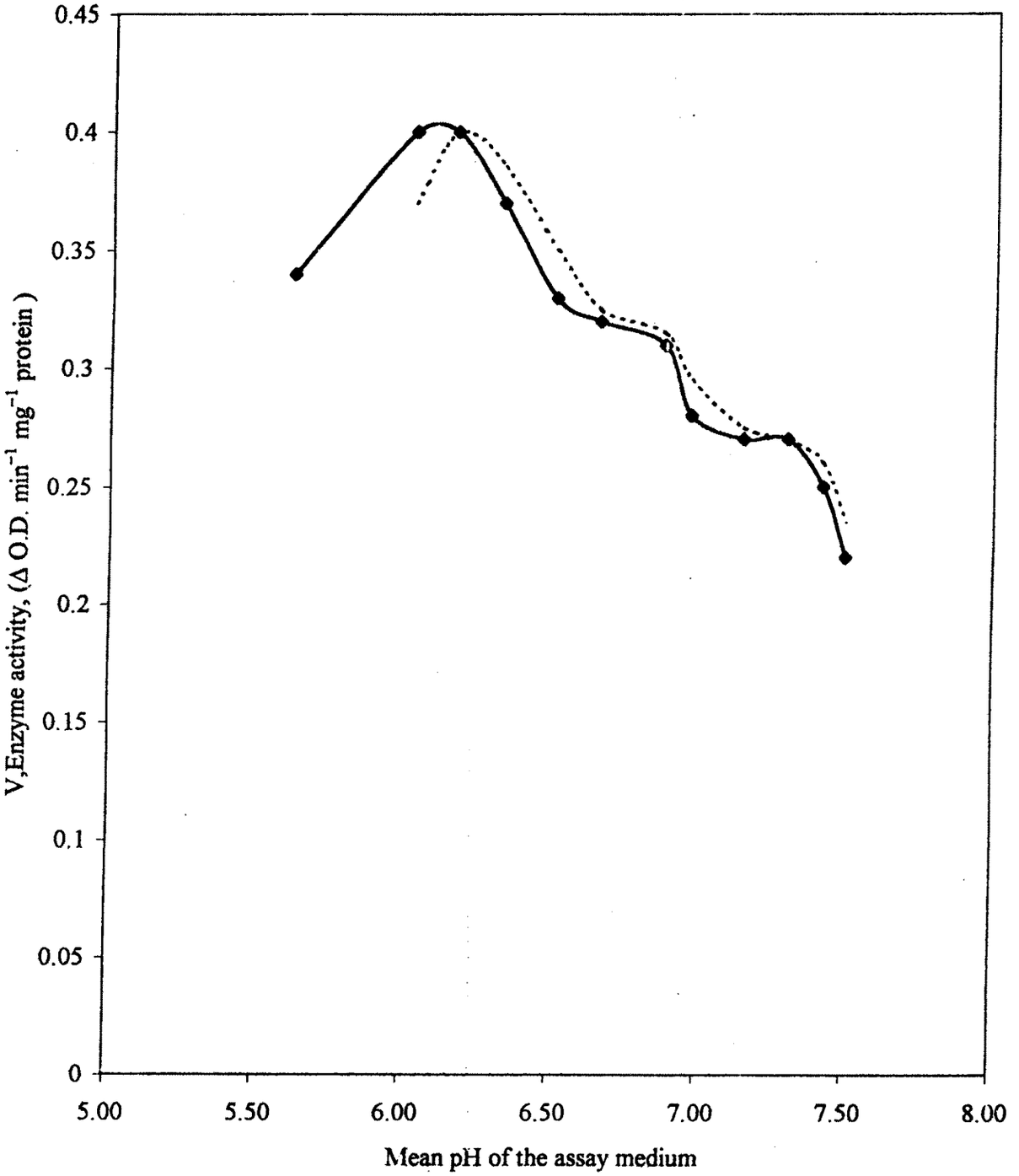


Fig.11 : Effect of pH of the assay medium on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.

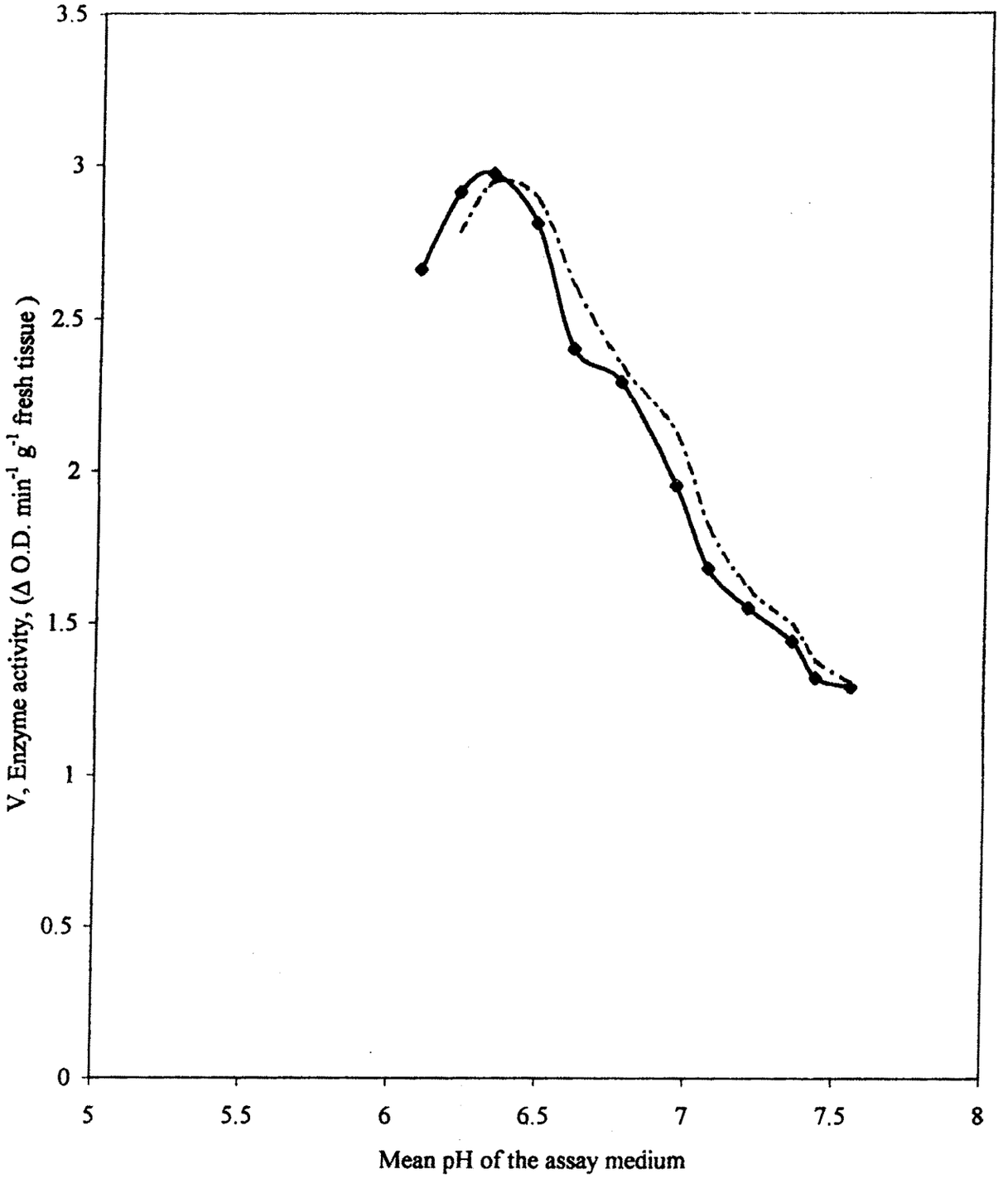
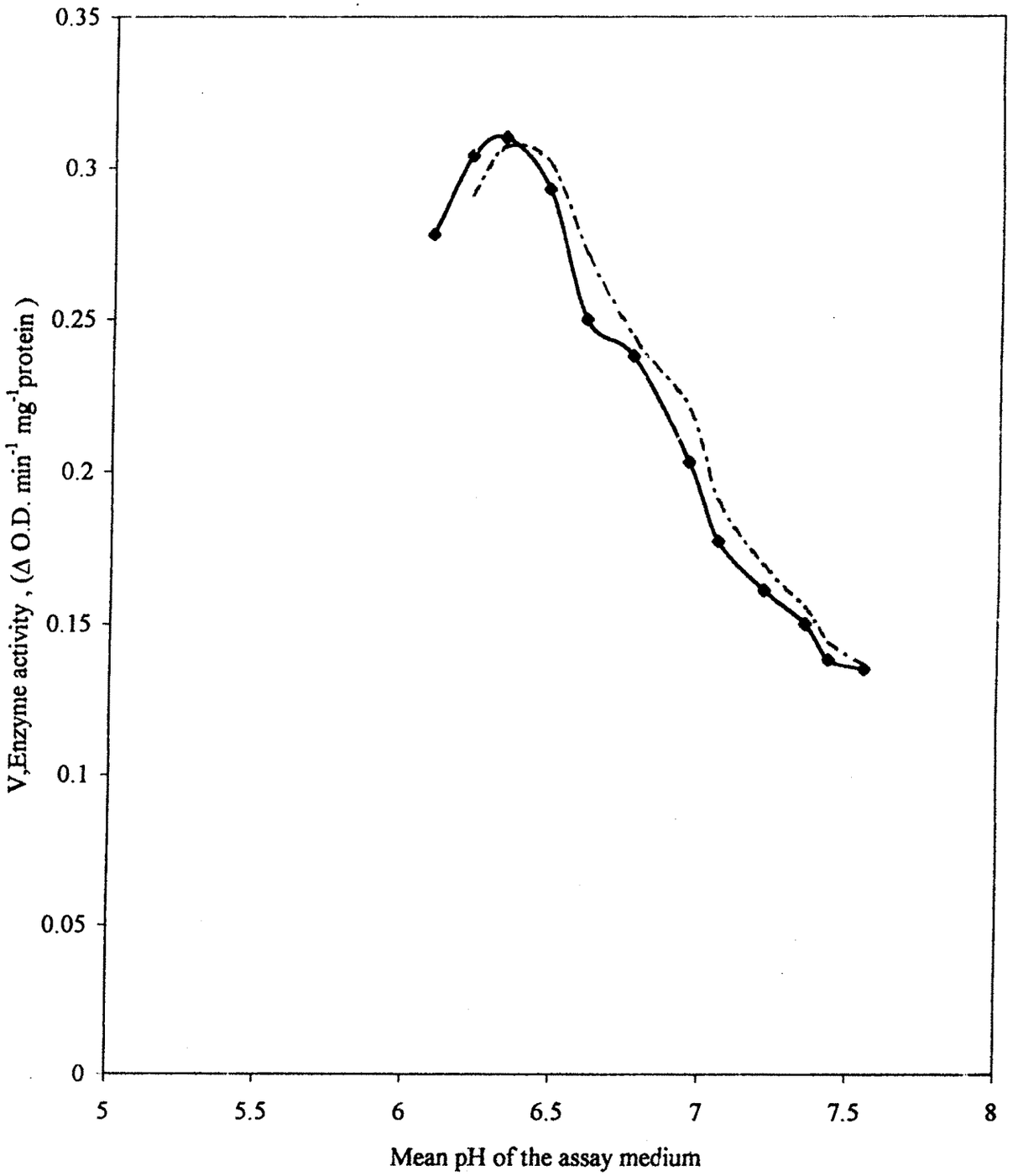


Fig.12 :- Effect of pH of the assay medium on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.



activity of peroxidase with increase in pH of the assay medium and reaches to maximum at pH 6.32. Thereafter with increase in pH, there is a constant and linear decrease in the activity of enzyme. It is reduced to almost half the maximum at pH 7.43 and 7.55. From the results it appears that 6.32 i.e. around 6.3 is the pH optimum for enzyme peroxidase in the leaves of *Portulaca oleracea* (L.).

Thus the pH optimum for enzyme peroxidase in the leaves of *Portulaca oleracea* (L.) appears to be slightly higher than that in the leaves of *Sesbania grandiflora* (L.) It is also very clear that level of this enzyme is higher in the leaves of *Sesbania grandiflora* (L.) than that from the leaves of *Portulaca oleracea.*

Hopkala et al. (1991) have studied the influence of iodide ions on the activity of soluble peroxidase from cabbage leaves. They also studied the effect of pH on the activity of this enzyme from the same source. They have recorded the highest activity of this enzyme in 0.1 M phosphate buffer at pH 6.52. However, pH 7.0 has been recorded as pH optimum for the enzyme peroxidase as CPP (Crude peroxidase preparation) from cabbage leaves. It appears that enzyme peroxidase from cabbage prefers neutral to slightly acidic pH for its maximum activity. The results obtained in the present study are also on the similar lines.

Ingham et al. (1998) have studied changes in soluble and bound forms of peroxidase during maturation and ripening of apples. They have reported that maximum peroxidase could be extracted at between pH 6 and 7 so they chose pH 6.5 for the extraction buffer. It can be concluded that

enzyme peroxidase as CPP from the leaves of *Sesbania grandiflora* L. and *Portulaca oleracea* L. has the pH optimum around 6.1 to 6.3.

c) Effect of temperature

An increase in temperature can have several effects on an enzyme catalysed reaction. The two major effects are an increase in the velocity of the reaction catalyzed and an increase in the rate of denaturation of the enzyme. Since these are both competing reactions that increase with increasing temperature, no temperature optimum can be defined for an enzyme. Enzymes have probably evolved so that they have stable structures under the physiological conditions at which they function. Thermophilic bacteria, for example contain enzymes which are stable at elevated temperatures.

The 1st step in a kinetic analysis of the effect of temperature on an enzyme catalyzed reaction is to determine the range of temperature overwhich the enzyme remains completely active. This can be achieved quite simply exposing the enzymatic reaction mixture to different temperatures for a certain duration of time and then assaying its activity. The effect of temperature on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* and *Portulaca oleracea* has been recorded in the tables respectively 5 and 6, and depicted in figures 13,14 and 15,16 respectively. Activity of enzyme has been expressed both on fresh tissue and protein bases. It is evident that with increasing temperature there is an increase in the activity of enzyme reaches to maximum and then declines with further increase in temperature. The temperature range tried is from

Table 5:- Effect of temperature on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

Sr. No.	Temperature ^0C	Enzyme activity*	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	10	3.33	0.05
2	15	3.90	0.07
3	20	6.60	0.11
4	25	5.02	0.08
5	30	4.45	0.07
6	35	4.25	0.07
7	40	3.50	0.06

* Each value is mean of three determinations

Table 6:- Effect of temperature on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L

Sr. No.	Temperature ^0C	Enzyme activity *	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	10	1.50	0.17
2	15	1.55	0.17
3	20	1.74	0.20
4	25	1.64	0.18
5	30	1.50	0.17
6	35	1.36	0.15
7	40	1.26	0.14

* Each value is mean of three determinations

Fig. 13:- Effect of temperature on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

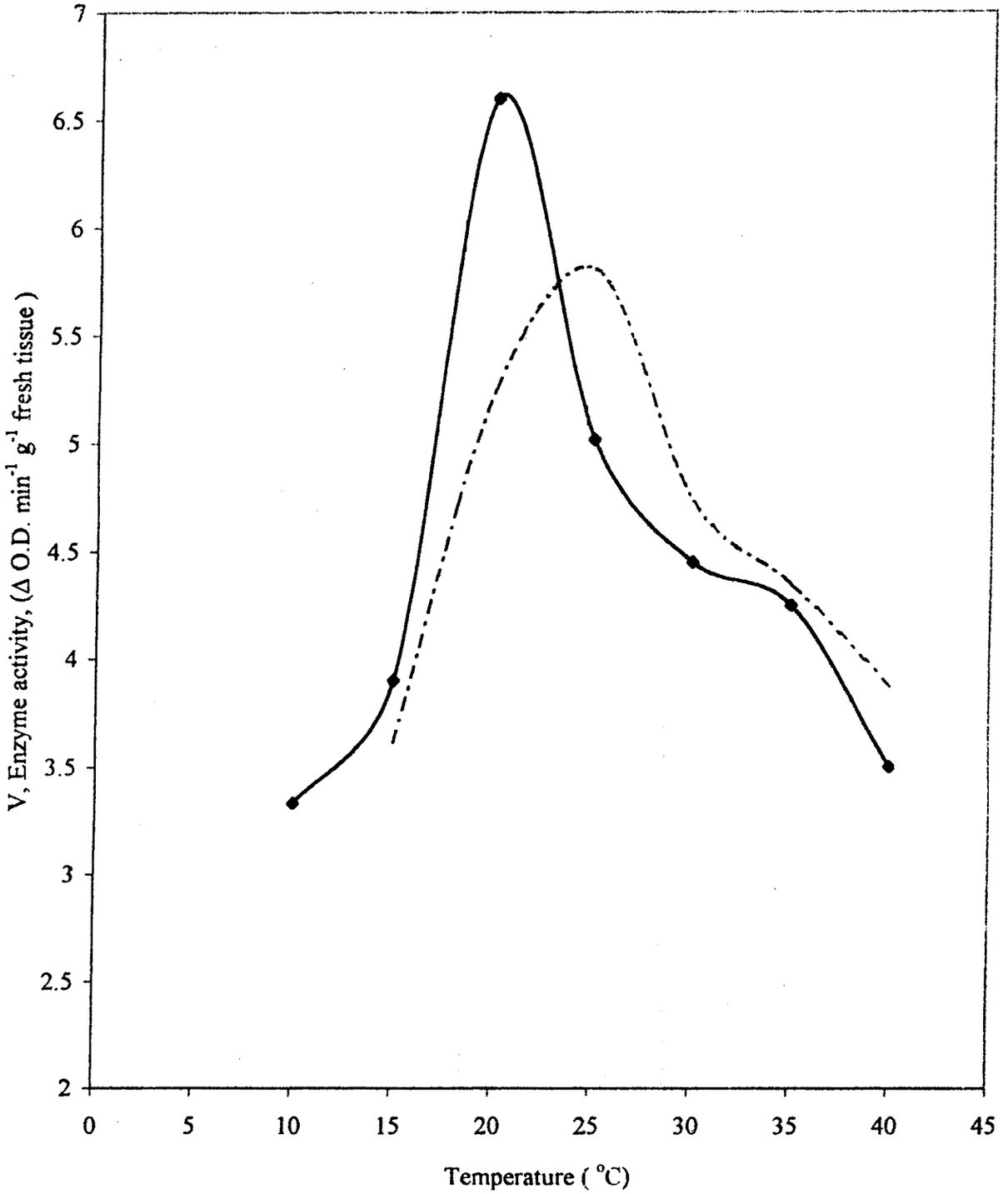


Fig. 14:- Effect of temperature on the activity of enzyme peroxidase in the leaves of *Sesbania grandiflora* L.

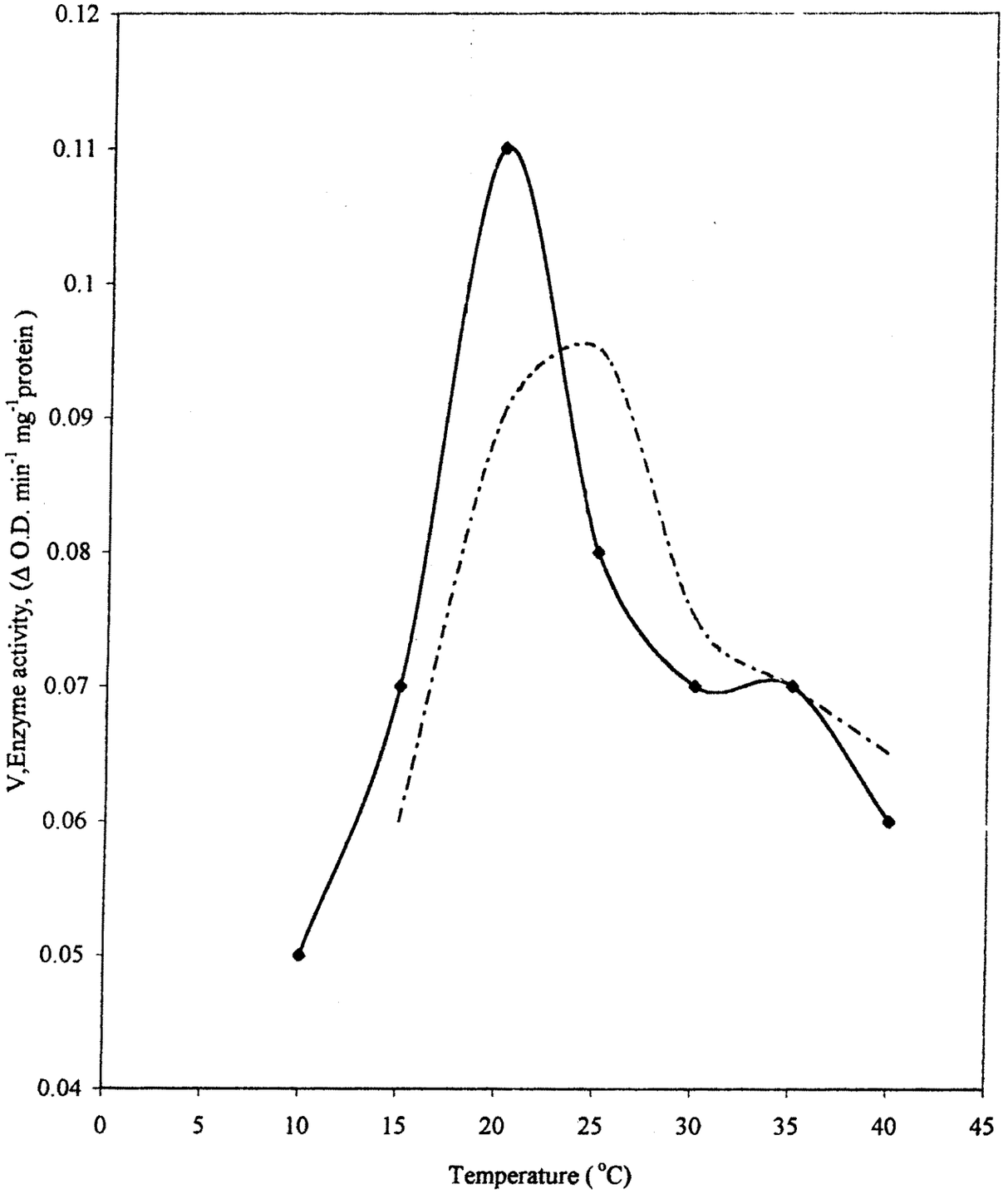


Fig. 15:- Effect of temperature on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.

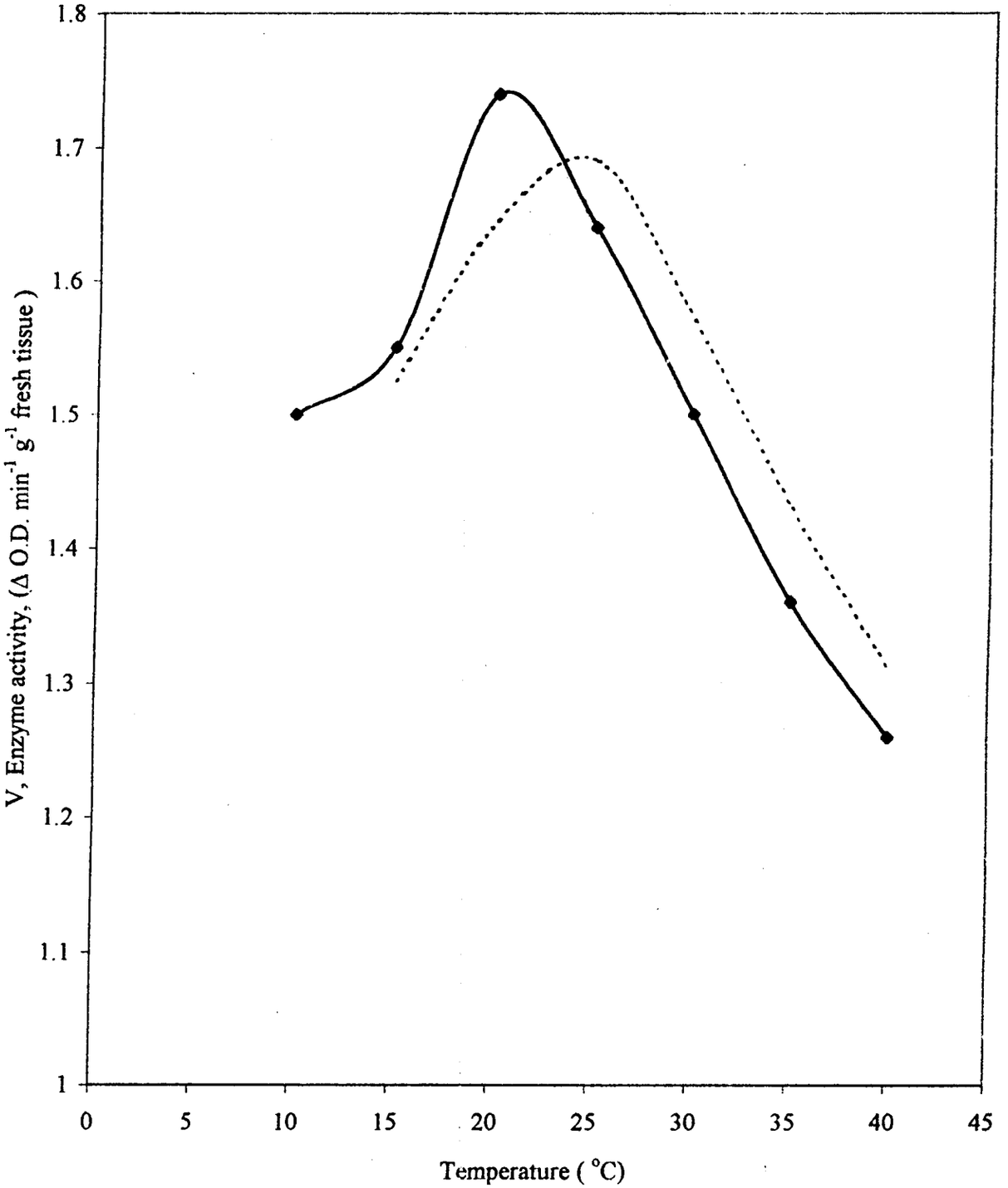
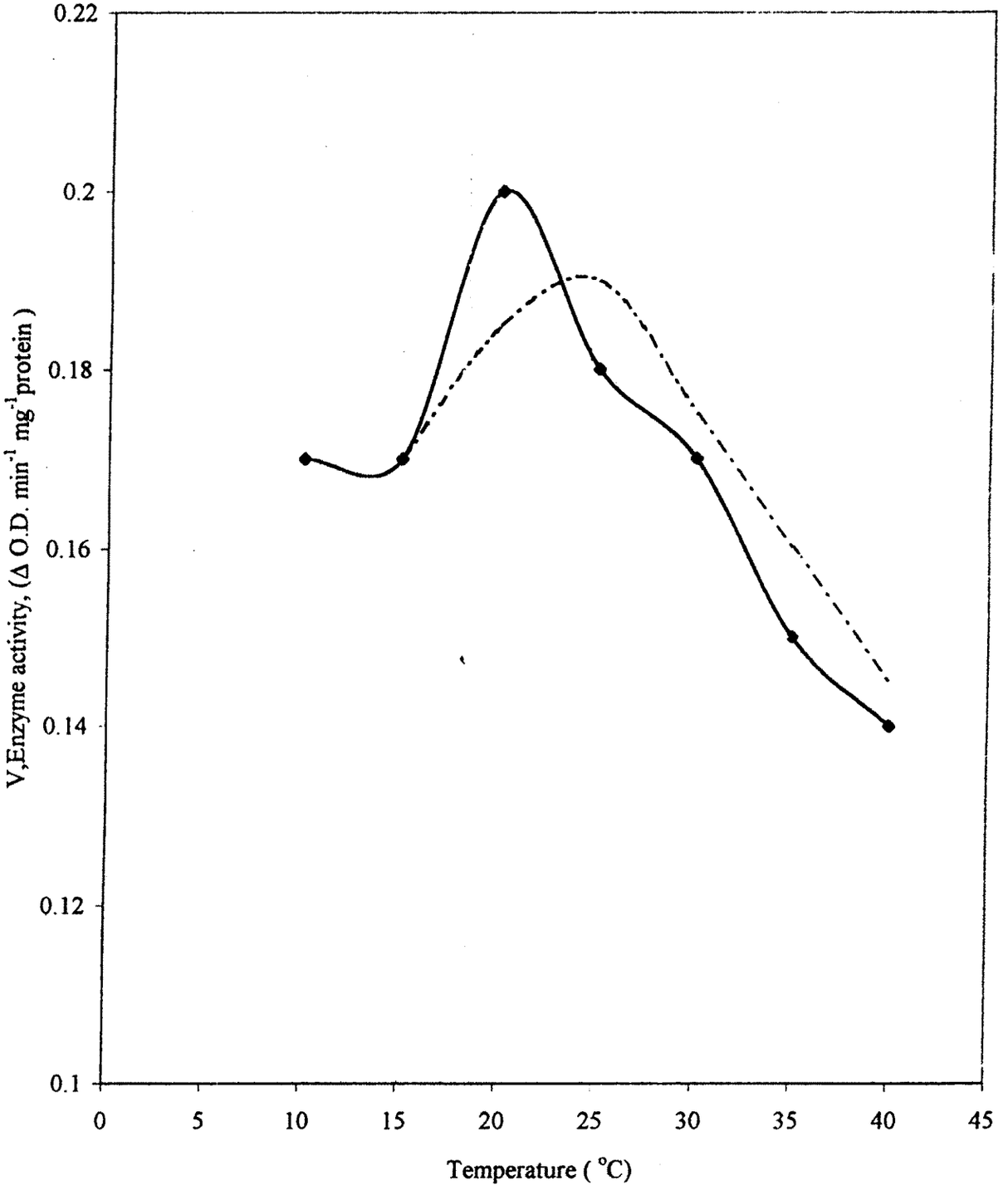


Fig.16:- Effect of temperature on the activity of enzyme peroxidase in the leaves of *Portulaca oleracea* L.



10 to 40°C. It is quite clear that enzyme peroxidase in the leaves of *Sesbania grandiflora* L. records maximum activity, 6.6 $min^{-1}g^{-1}$ fresh tissue or 0.11 $min^{-1}mg^{-1}$ of protein at 20°C. It is decreased with further increase in temperature and the lowest being at 40°C. It suggests that enzyme peroxidase from *Sesbania grandiflora*, requires still higher temperature for its inhibition or denaturation. It seems that peroxidase from this species exhibits some degree of thermotolerance. It can be considered as one of the adaptive features of this species towards stress conditions. This fast growing tree species has already been identified as moderately salt tolerant (Chavan and Karadge, 1986).

It can be seen from table 6 and figures 15 and 16 that enzyme peroxidase from the leaves of *Portulaca oleracea* L. exhibits maximum activity, 1.74 $min^{-1}g^{-1}$ fresh tissue or 0.20 $min^{-1}mg^{-1}$ protein at 20°C and almost remains constant till 25°C. Temperatures 35°C and above seem to be inhibitory as the enzyme activity recorded at 35 and 40°C temperatures is much below (1.26 $min^{-1}g^{-1}$ fresh tissue or 0.14 $min^{-1}mg^{-1}$ protein at 40°C) which is far below than that recorded at cooler conditions (1.5 $min^{-1}g^{-1}$ fresh tissue or 0.17 $min^{-1}mg^{-1}$ protein). From the results and when compared with *Sesbania grandiflora*, enzyme peroxidase from *Portulaca oleracea* appears to have less degree of thermotolerance. The behaviour of enzyme peroxidase from *Portulaca oleracea* can not be explained eventhough it is a typical C_4 plant, preferring high temperature conditions.

From the above discussion it is quite clear that enzyme peroxidase from *Sesbania grandiflora* L and *Portulaca oleracea* L. has a temperature optimum between 20 to 25°C. Hopkala et al. (1999) have reported 30°C as

the optimum temperature for peroxidase activity from the enzyme isolated from cabbage leaves.

While studying peroxidase in crassulacean acid metabolism plants namely *Kalanchoe pinnatum*, *K. tubiflorum* and *Aloe barbadensis*, Upadhye et al. (1986) have reported that despite the use of several methods and techniques eg. different assay methods, different isolation media, enzyme purification by Sephadex G. column seperation, Ammonium sulphate precipitation and polyactrylamide gel electrophoresis, activity of the enzyme could not be determined. In the present investigation leaf material of *K. pinnatum* from the plants kept growing in the dark for 10 days or more, was used for the study of enzyme peroxidase, however we failed to measure or even detect the activity of the enzyme. Besides the standard methods for isolation and assay of enzyme peroxidase from the leaves of *K. pinnatum* were used but we were unable to measure the activity. Thus present results support and confirm the observations made by Upadhye et al. (1986). They have suggested that there may be some inhibitor liberated during isolation or enzyme probably binding tightly to the enzyme molecule which interferes the activity of peroxidase in the leaf, stem and roots of *Kalanchoe*. To ressolve this further studies are needed.

B. Catalase

a) Effect of substrate concentration and determination of Km

The effect of substrate concentration i.e. hydrogen peroxide, on the activity of enzyme catalase in the leaves of *Sesbania grandiflora* L., *Portulaca oleracea* L. and *Kalanchoe pinnata* (Lamk) pers, has been

recorded in tables 7, 8 and 9 respectively and Fig. 17,19; 21,23 and 25,27 respectively. The various substrate concentrations tried are from 0.2 to 3.0µmoles. It is evident that activity of enzyme catalase in the leaves of *S.grandiflora* increases linearly with increase in substrate concentration upto 2.6 µmoles of H_2O_2. The further increase in the level of substrate concentration lowers the activity of enzyme. This is true when the activity of enzyme is expressed both on fresh tissue as well as protein bases. It can be seen that the enzyme catalase in this plant records the maximum activity of 48.5 $min^{-1}g^{-1}$ fresh tissue or 0.758 $min^{-1}mg^{-1}$ protein. Almost the similar trend can be observed in the case of enzyme catalase in the leaves of *Portulaca oleracea.* It is observed that activity of the enzyme catalase increases linearly with increasing the substrate concentration, with maximum activity being at 2.6, 2.8 and 3.0 µmoles H_2O_2, which is the maximum. It is also evident from table 8 and Fig. 21, that with further increase in the concentration of H_2O_2, there is a decline in the activity. This is true when enzyme activity is expressed on fresh tissue bases. It can be seen that enzyme catalase in this plant records the maximum activity of 1.94 $min^{-1}g^{-1}$ fresh tissue and 0.17 $min^{-1}mg^{-1}$ protein.

Almost similar trend can be observed in the case of enzyme catalase in the leaves of *K. pinnata.* It is observed that activity of enzyme calatase increases linearly with increasing the substrate concentration with maximum activity being at 1.6 µmoles of H_2O_2, which is the maximum (0.55 $min^{-1}g^{-1}$ fresh tissue or 0.081 $min^{-1}mg^{-1}$ protein). It is also evident from table 9 and Fig. 25, that with further increase in concentration of

Table 7:- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.

Sr. No.	Substrate Concentration H_2O_2 (μ moles)	Enzyme activity*	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	0.2	6.15	0.096
2	0.4	6.20	0.097
3	0.6	11.00	0.172
4	0.8	14.85	0.232
5	1.0	19.85	0.367
6	1.2	23.50	0.367
7	1.4	28.45	0.445
8	1.6	33.95	0.527
9	1.8	33.85	0.529
10	2.0	40.50	0.627
11	2.2	40.95	0.640
12	2.4	46.22	0.722
13	2.6	48.50	0.758
14	2.8	47.60	0.744
15	3.0	43.85	0.685

* Each value is mean of three determinations

Table 8:- **Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.**

Sr. No.	Substrate Concentration H_2O_2 (μ moles)	Enzyme activity*	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	0.2	0.22	0.02
2	0.4	0.45	0.04
3	0.6	0.59	0.05
4	0.8	0.90	0.08
5	1.0	0.99	0.09
6	1.2	1.14	0.10
7	1.4	1.21	0.11
8	1.6	1.14	0.12
9	1.8	1.42	0.12
10	2.0	1.64	0.14
11	2.2	1.69	0.15
12	2.4	1.78	0.16
13	2.6	1.94	0.17
14	2.8	1.94	0.17
15	3.0	1.91	0.17

* Each value is mean of three determinations

Table 9:- **Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.**

Sr. No.	Substrate Concentration H_2O_2 (μ moles)	Enzyme activity *	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	0.2	0.08	0.012
2	0.4	0.11	0.015
3	0.6	0.14	0.020
4	0.8	0.18	0.026
5	1.0	0.21	0.030
6	1.2	0.24	0.035
7	1.4	0.34	0.049
8	1.6	0.55	0.081
9	1.8	0.52	0.075
10	2.0	0.49	0.072
11	2.2	0.45	0.065
12	2.4	0.39	0.056
13	2.6	0.38	0.055
14	2.8	0.33	0.048
15	3.0	0.25	0.037

* Each value is mean of three determinations

Fig. 17 :- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.

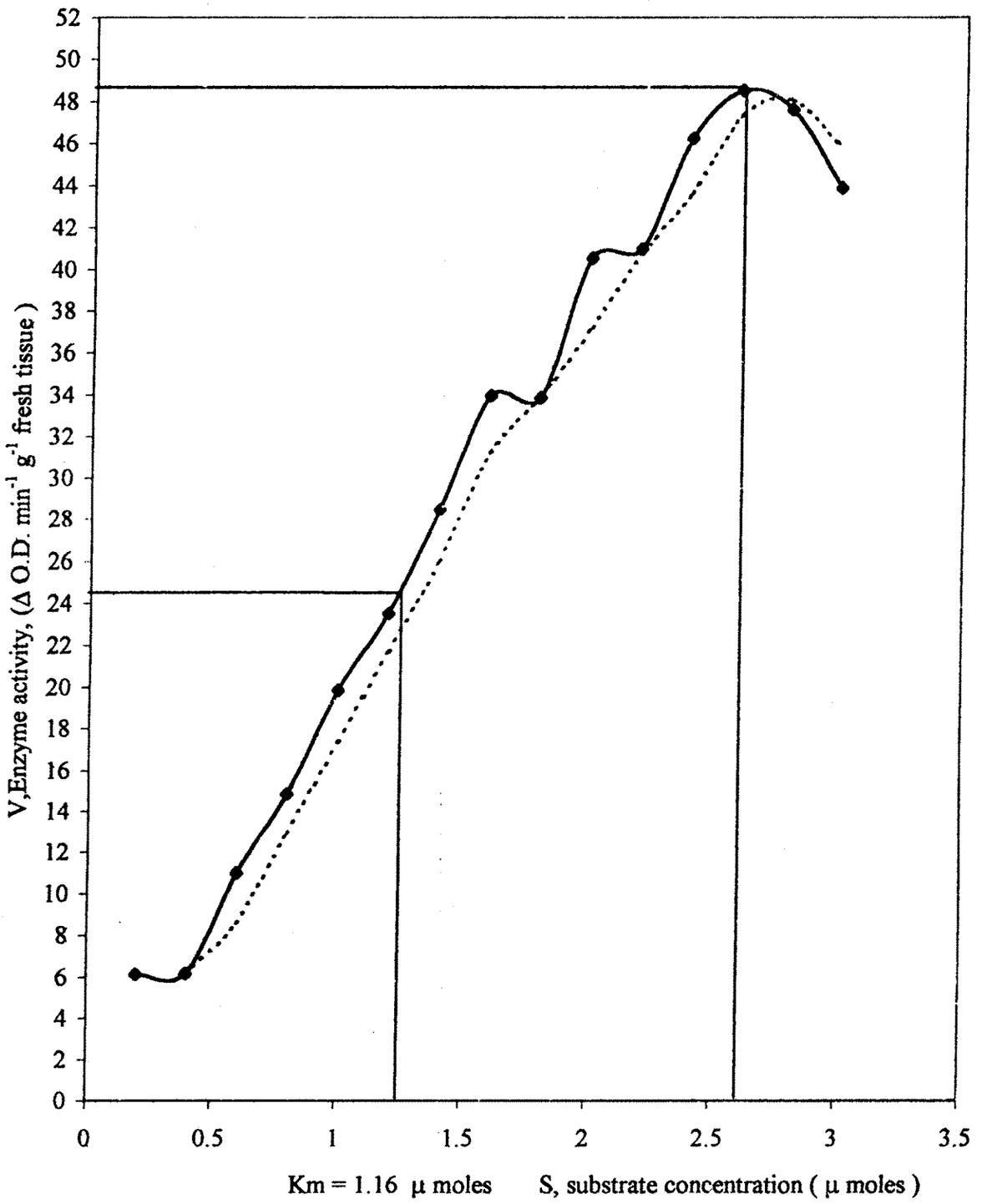


Fig. 19:- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L

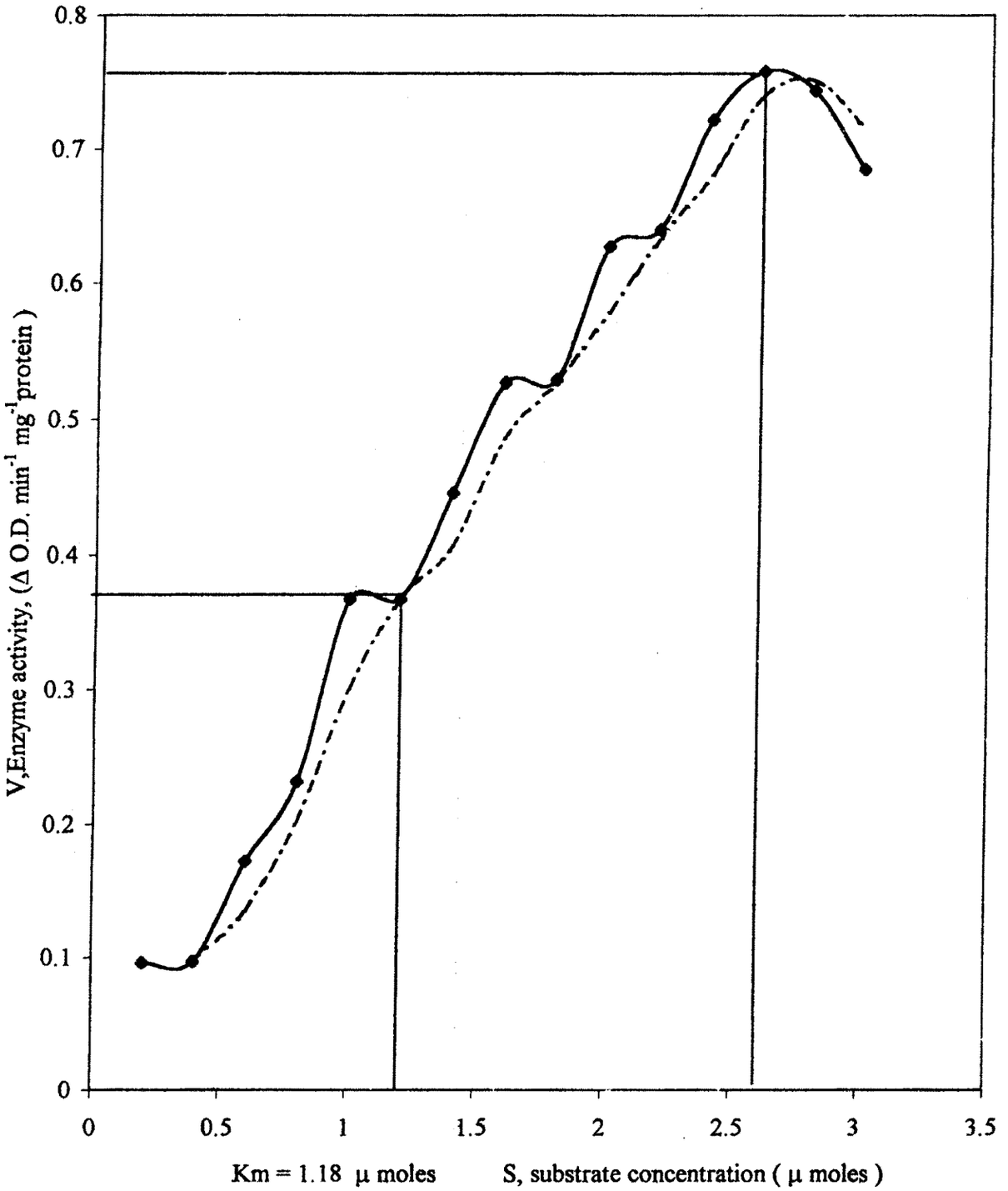


Fig 21:- **Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.**

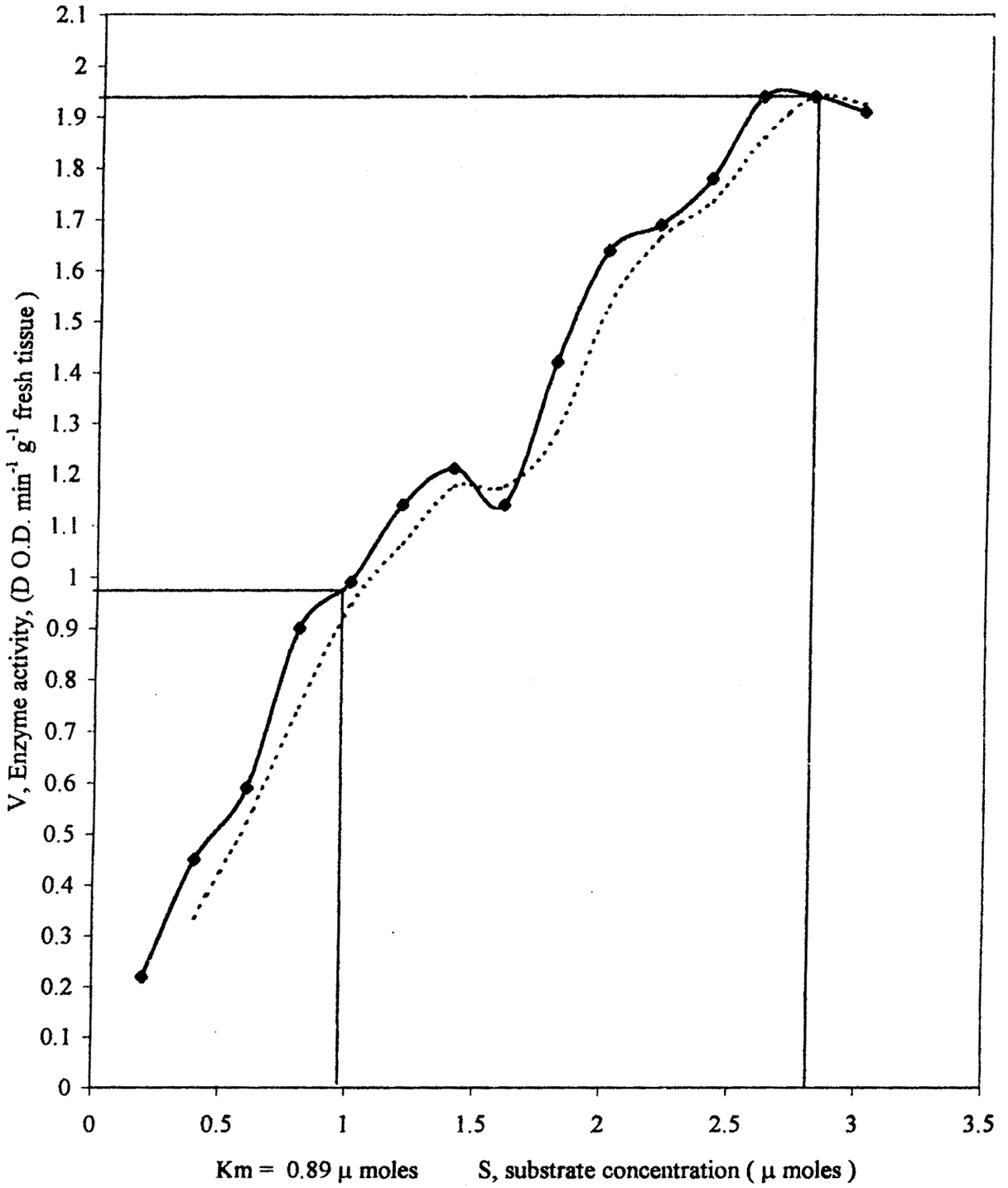


Fig.23 :- **Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.**

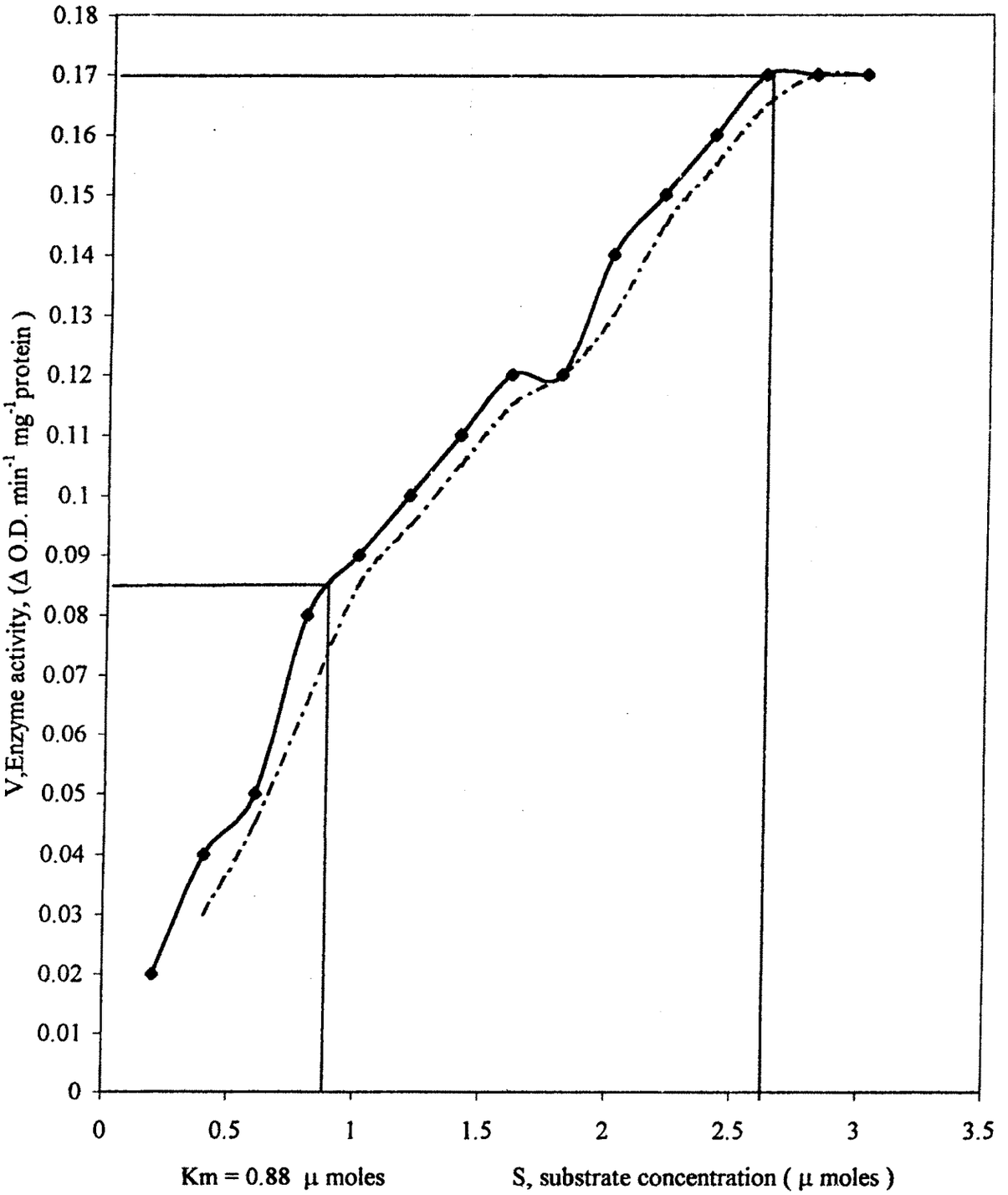


Fig.25 :- Effect of substrate concentration on the activity of enzyme Catalalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.

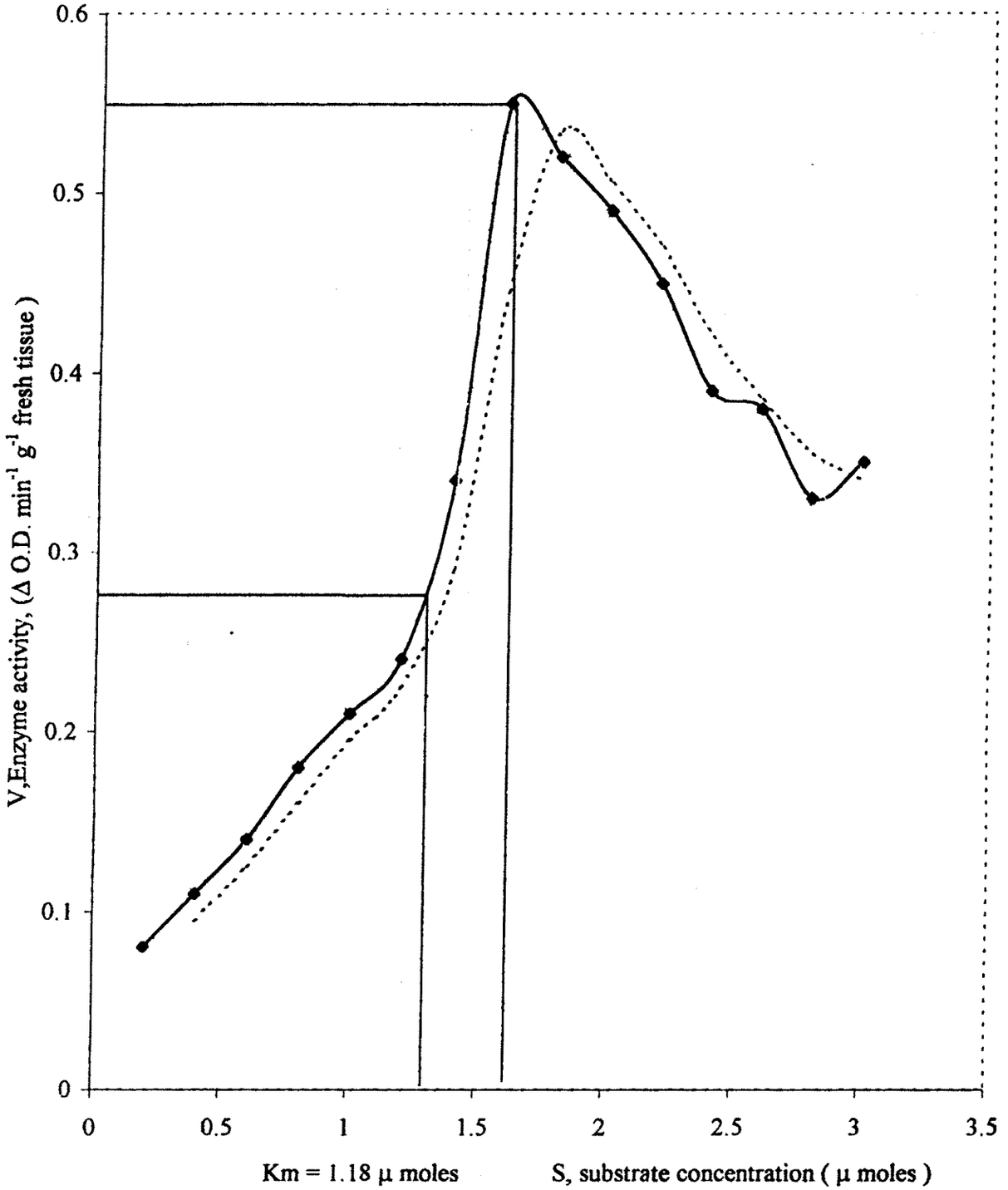


Fig 27:- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.

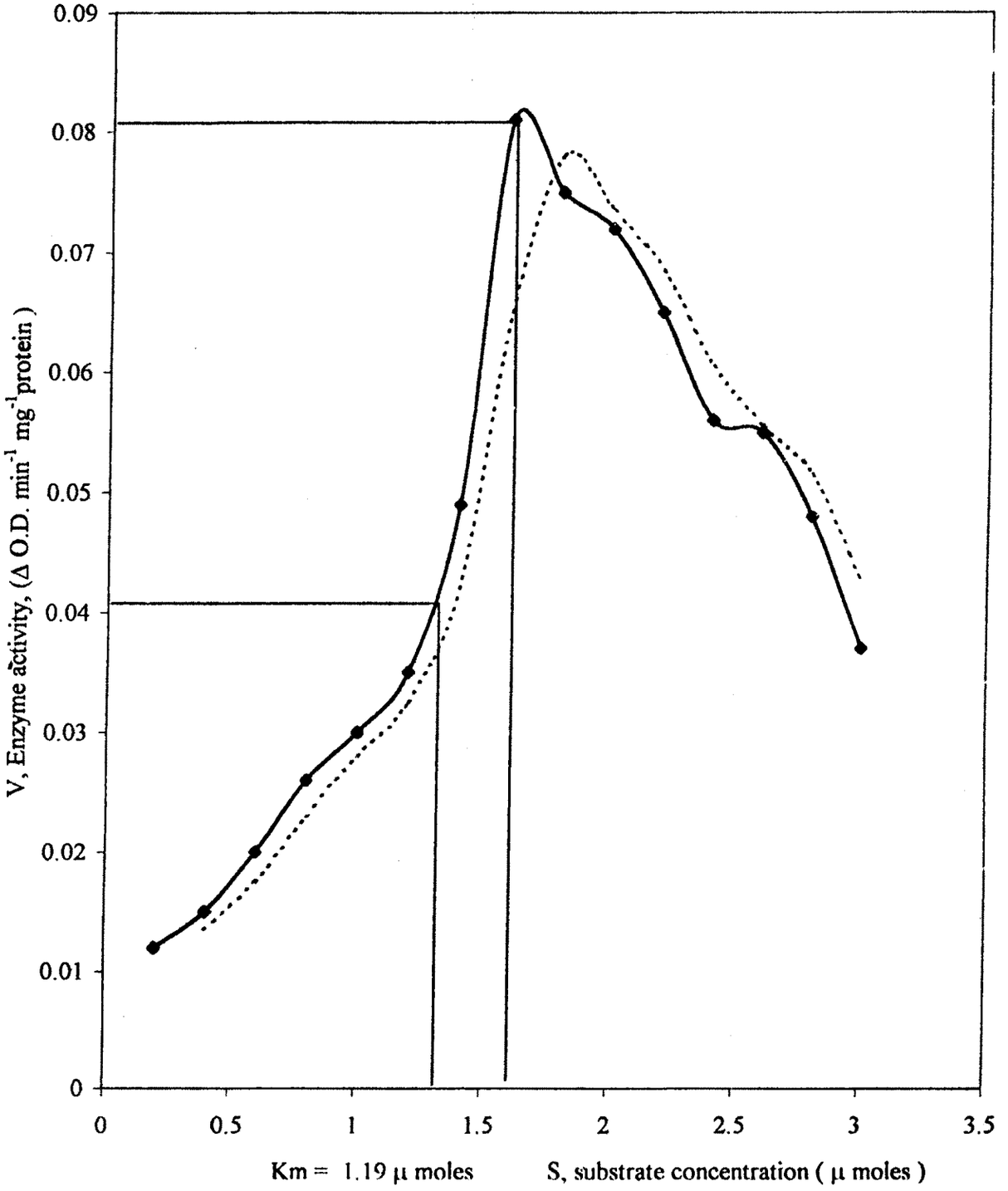


Fig18 :- **Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.**

(V = Enzyme activity, Δ O.D. min^{-1} g^{-1} fresh tissue;
S = substrate concentration, μ moles.)
Km =1.16 μ moles.

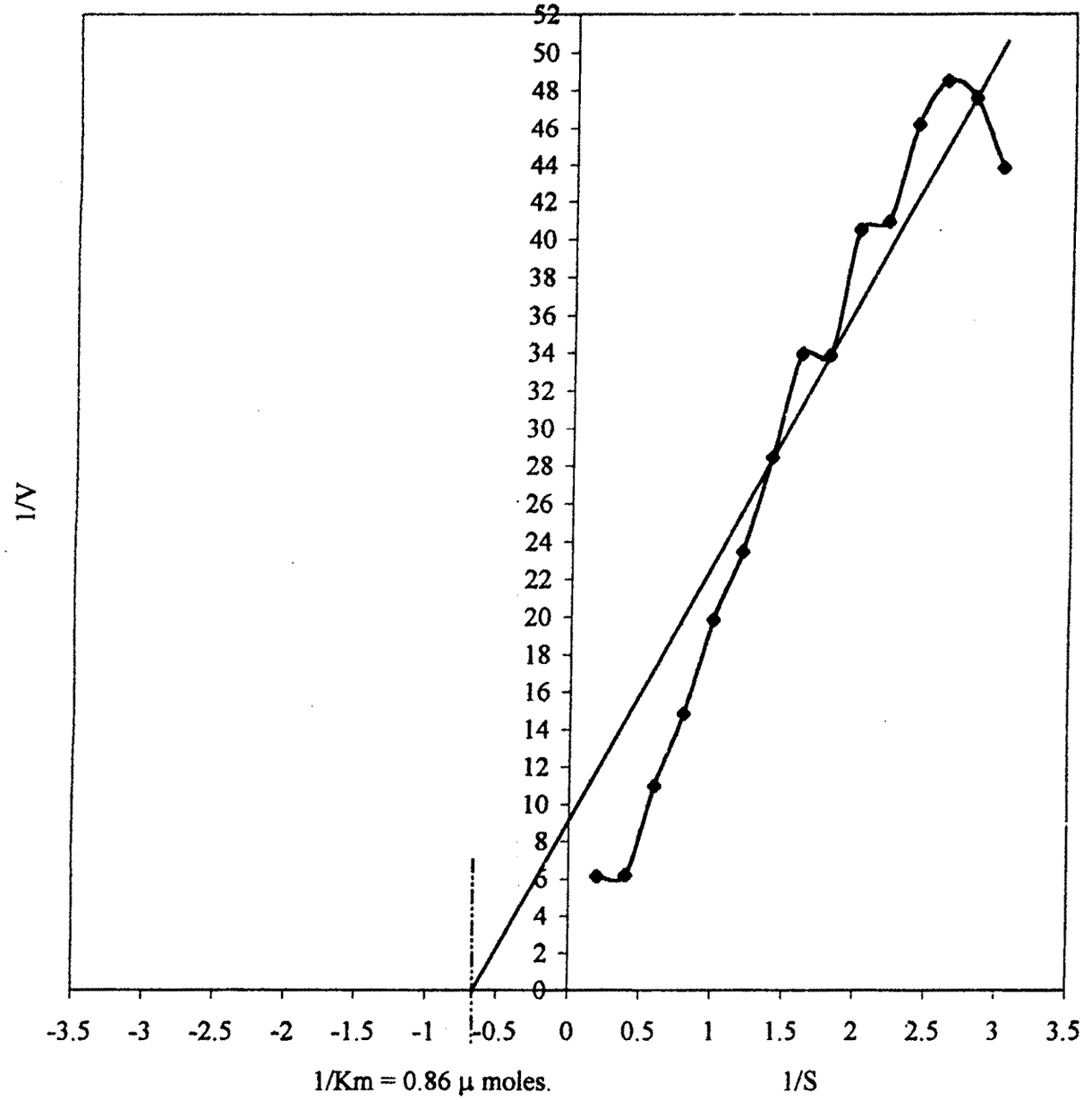


fig. 20 :- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.

(V = Enzyme activity, Δ O.D. min^{-1} mg^{-1} protein;
S = substrate concentration, μ moles.)
Km = 1.18 μ moles.

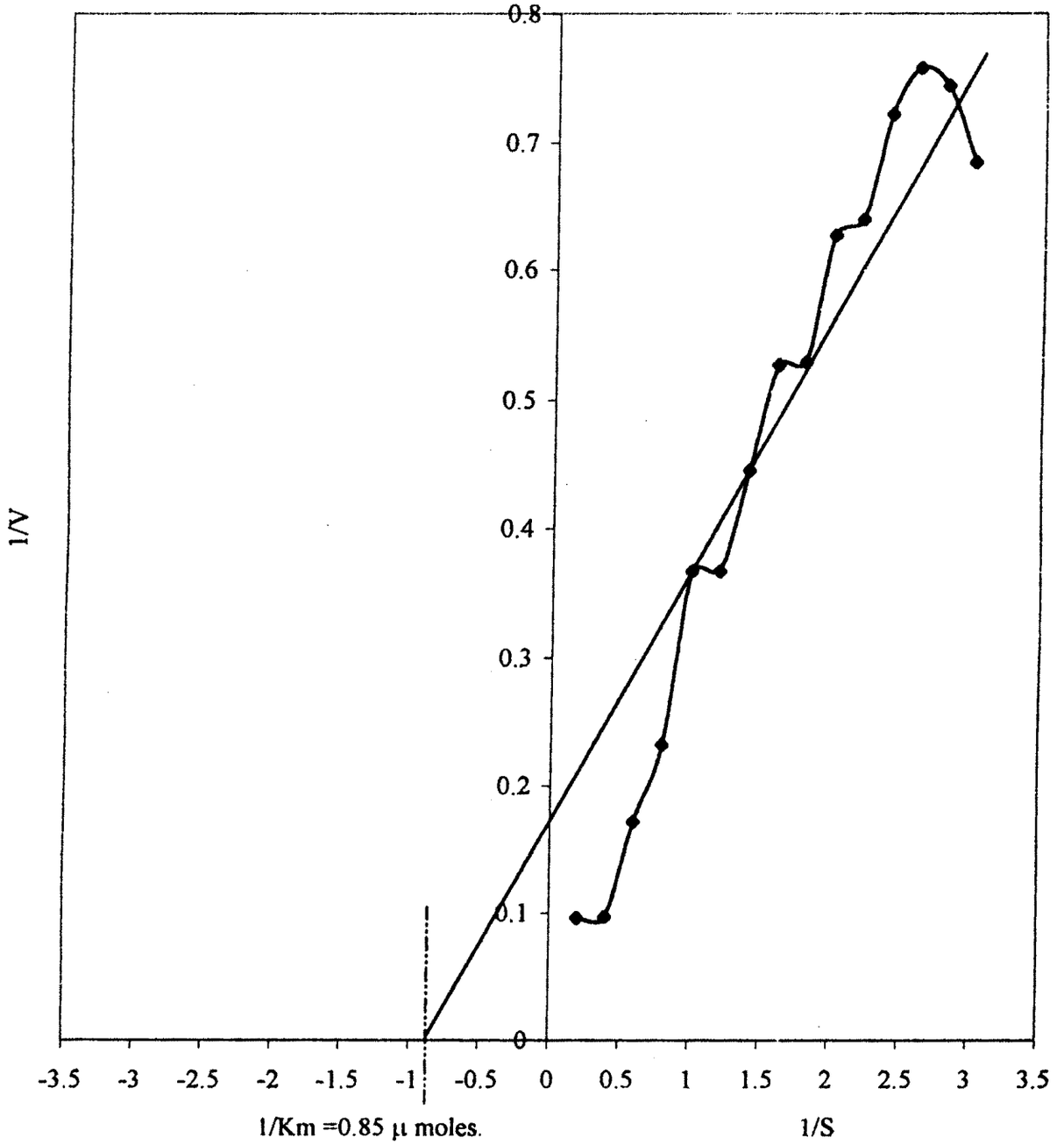


Fig.22 :- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.

(V = Enzyme activity, Δ O.D. min^{-1} g^{-1} fresh tissue;
S = substrate concentration, μ moles.)
Km = 0.89 μ moles.

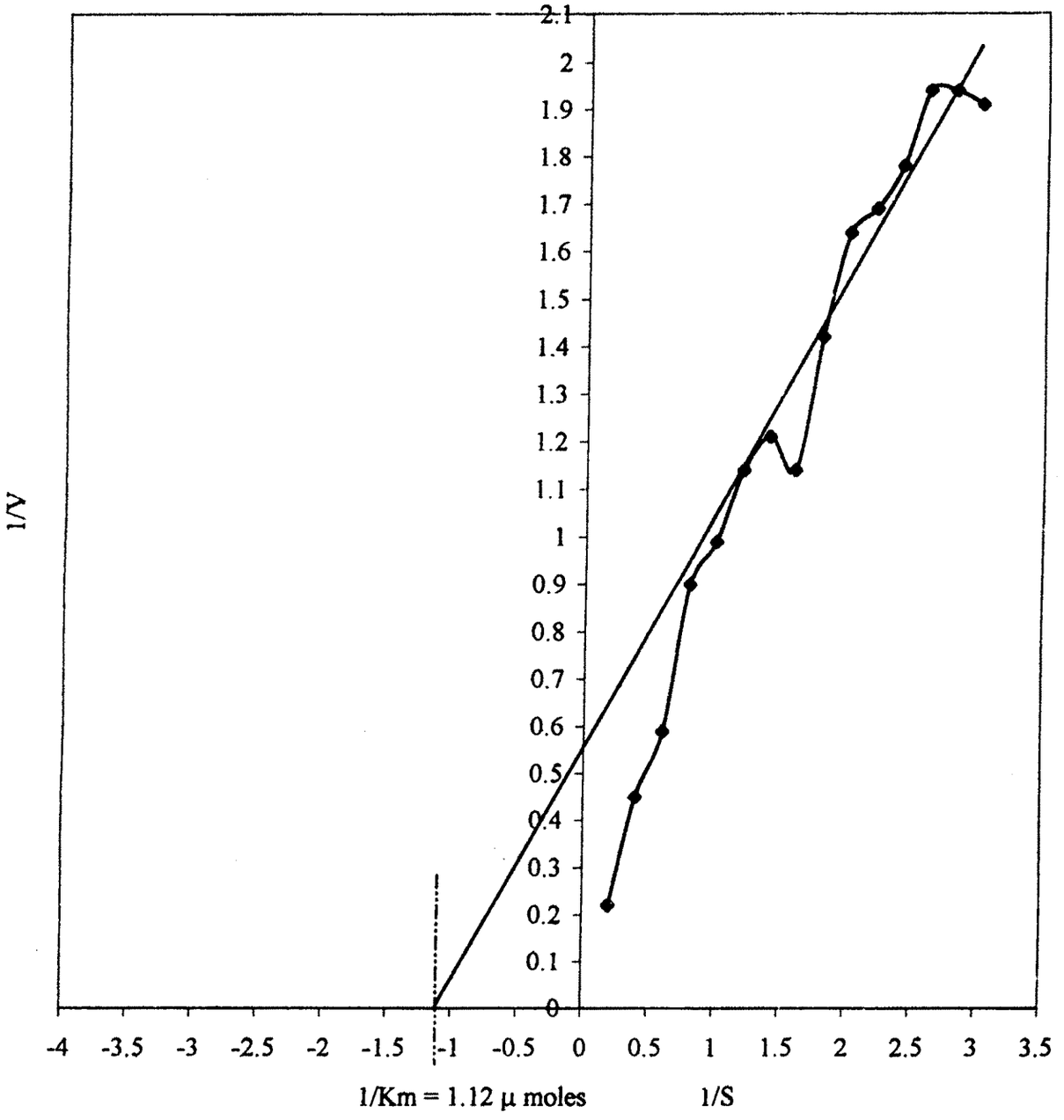


Fig 24 :- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.

(V = Enzyme activity, Δ O.D. min^{-1} mg^{-1} protein;
S = substrate concentration, μ moles.)
Km =0.88 μ moles.

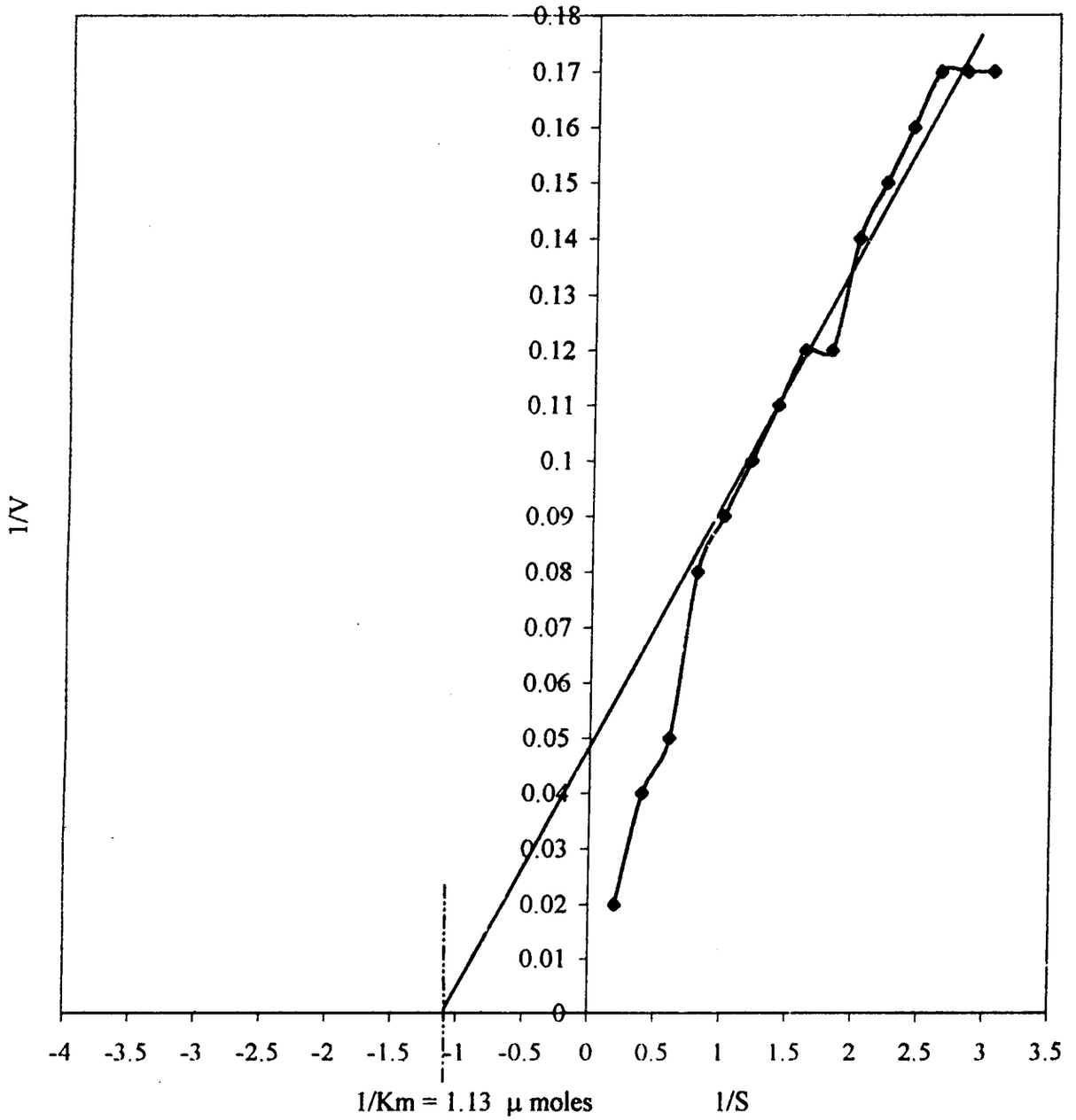


Fig.26:- Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk)

(V = Enzyme activity, Δ O.D. min^{-1} g^{-1} fresh tissue;
S = substrate concentration, μ moles.)
Km = 1.18 μ moles

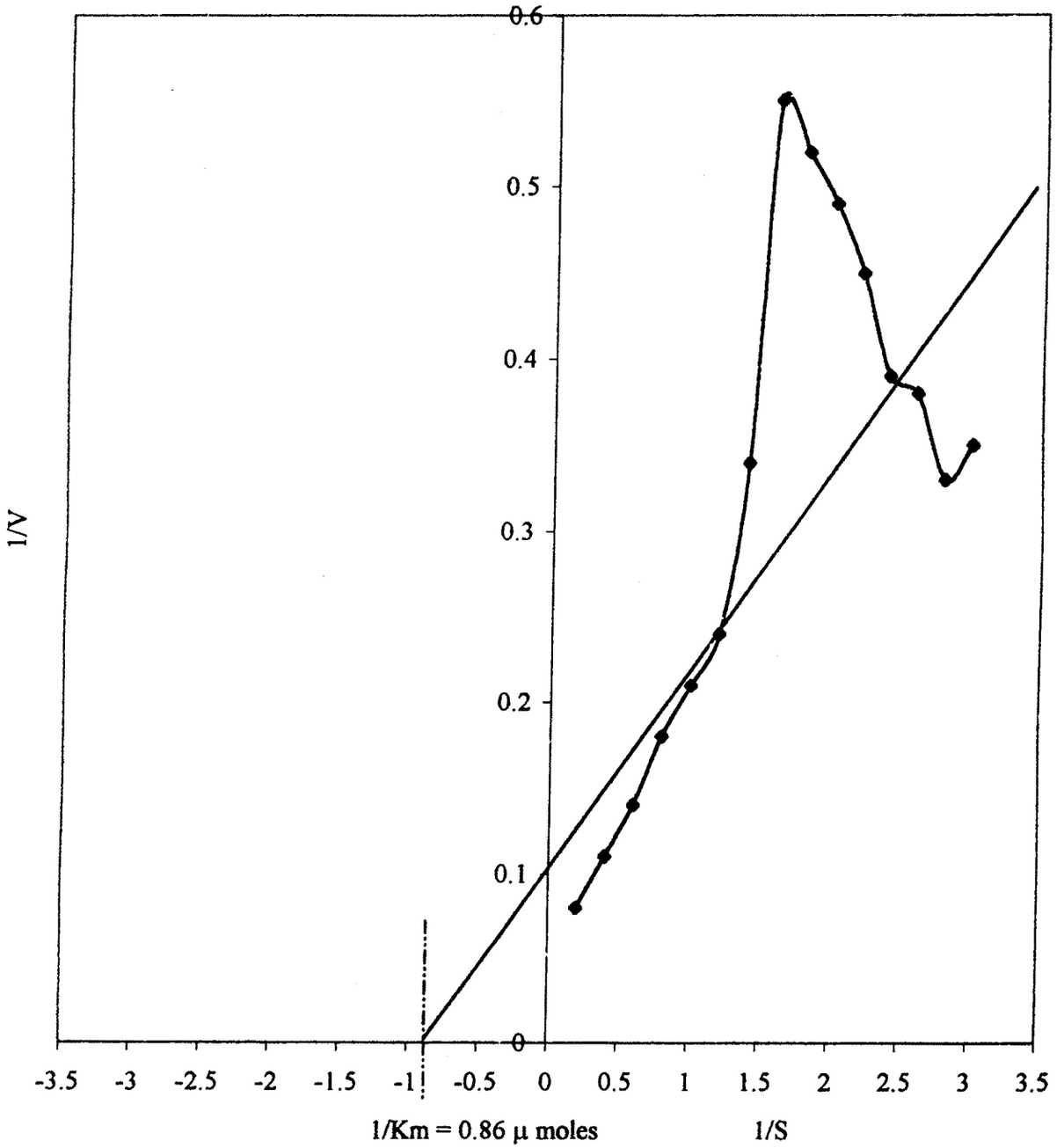
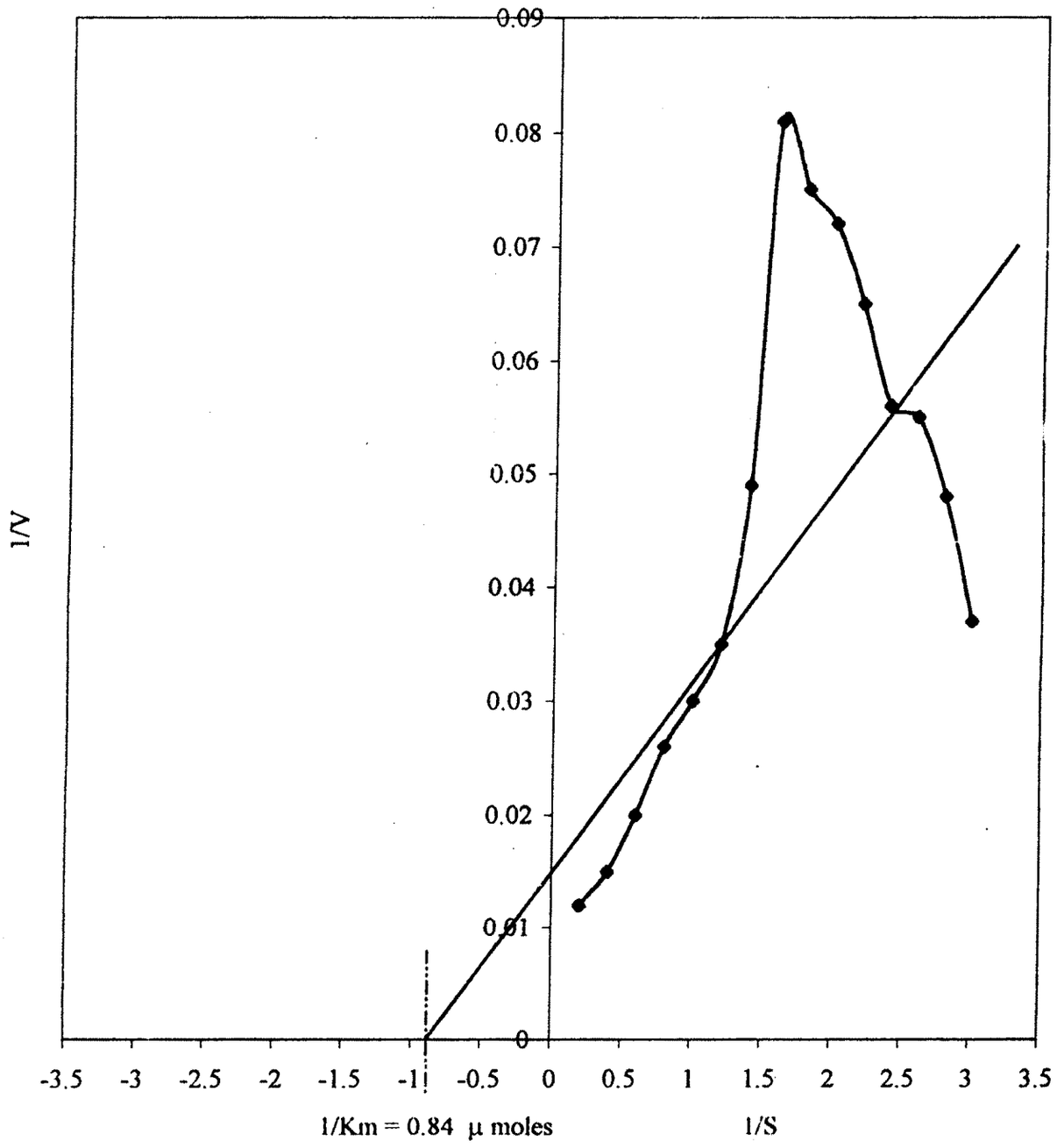


Fig.28:-Effect of substrate concentration on the activity of enzyme Catalase in the leaves of *Kalanchoe Pinnata* (Lamk).

(V = Enzyme activity, Δ O.D. min^{-1} mg^{-1} protein;
S = substrate concentration, μ moles.)
Km = 1.19 μ moles



H_2O_2, there is decline in the activity. This is true for enzyme activity when expressed both on fresh and protein bases.

From these values for enzyme activity, Michaelis constants (Kms) for enzyme catalase in *S. grandiflora* , *P. oleracea* and *K. pinnata* have been determined and have been shown in the form of graphs (Fig. 17,19,21,23,25 and 27 exhibit Km values determined with a plot of substrate concentration against velocity of enzymatic reaction, while figures 18,20,22,24,26 and 28 show Km values determined with plots of reciprocal of substrate concentration against the reciprocal of velocity of enzyme reaction)

From these figures it is clear that Km for emzyme catalase in the leaves of *S. grandiflora* ranges between 1.16 to 1.18 µmoles, for *P. oleracea* between 0.88 to 0.89 µmoles and for *K. pinnata* leaves from 1.18 to 1.19 µmoles. It is clear therefore from these observations that Km for catalase in the leaves of *Portulaca oleracea* is slightly lower than that in case of *S. grandiflora* and *K. pinnata*.

b) Effect of pH

The effect of pH of the assay medium on the activity of enzyme catalase in the leaves of *S. grandiflora*, *P. oleracea* and *K. pinnata* has been recorded in the tables – 10,11,12 and figures 29,30,31,32,33 and 34. The tables record pH of the assay medium before the reaction as well as after the enzymatic reaction. Mean of these two values has been considered as pH of the assay medium influencing the enzymatic reaction. The pH of the media tried are from 6.17 to 7.67 for the enzyme from the

Fig 29 :- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.

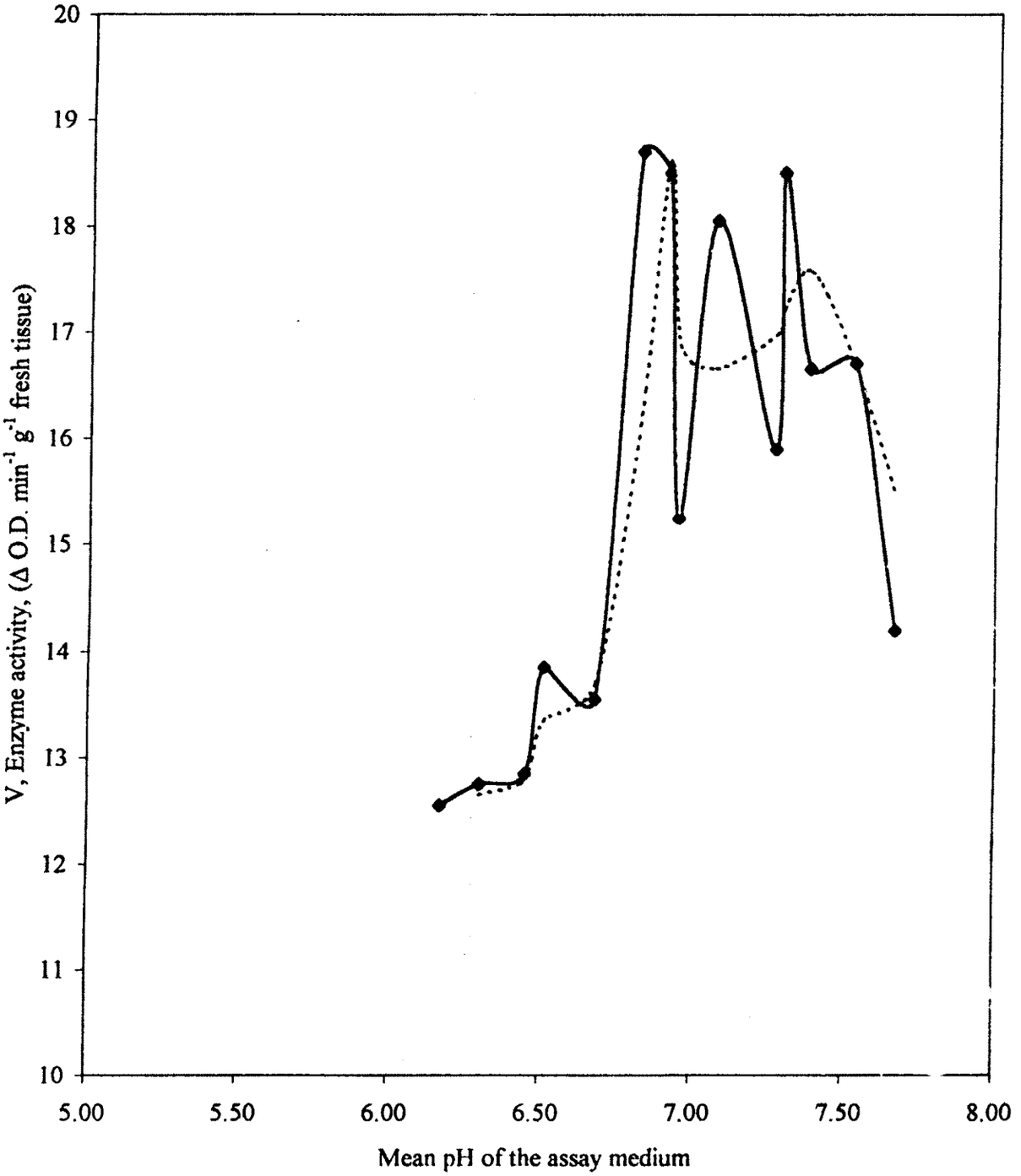


Fig.30:- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.

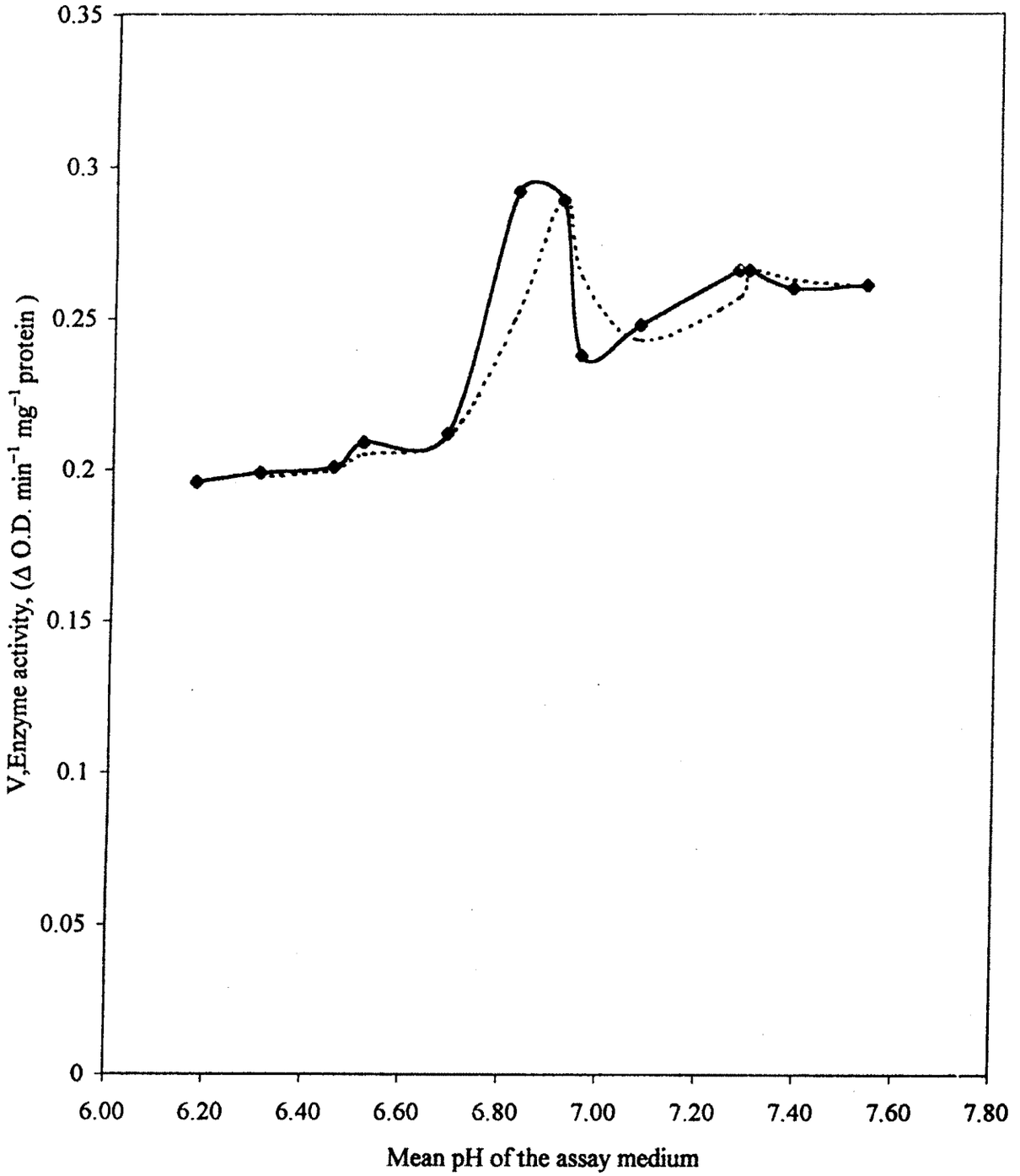


Table 10:- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L

Sr No	pH Before Reaction	pH After Reaction	Mean pH of assay medium	Enzyme activity *	
				Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	5.8	6.55	6.17	12.55	0.196
2	6.0	6.62	6.30	12.75	0.199
3	6.2	6.70	6.45	12.85	0.201
4	6.4	6.62	6.51	13.85	0.209
5	6.6	6.78	6.68	13.55	0.212
6	6.8	6.85	6.82	18.70	0.292
7	7.0	6.82	6.91	18.50	0.289
8	7.2	6.70	6.95	15.25	0.238
9	7.4	6.75	7.07	18.05	0.248
10	7.6	6.95	7.27	15.90	0.266
11	7.8	6.79	7.29	18.50	0.266
12	8.0	6.78	7.38	16.65	0.260
13	8.2	6.88	7.53	16.70	0.261
14	8.4	6.95	7.67	14.20	0.222

* Each value is mean of three determinations

Table 11 :- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.

Sr No	pH Before Reaction	pH After Reaction	Mean pH of assay medium	Enzyme activity *	
				Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	5.8	6.54	6.16	0.26	0.022
2	6.0	6.55	6.27	0.21	0.022
3	6.2	6.58	6.38	0.27	0.023
4	6.4	6.65	6.52	0.24	0.021
5	6.6	6.65	6.62	0.31	0.027
6	6.8	6.60	6.70	0.34	0.030
7	7.0	6.63	6.81	0.42	0.036
8	7.2	6.65	6.92	0.58	0.051
9	7.4	6.71	7.05	0.36	0.031
10	7.6	6.78	7.18	0.47	0.041
11	7.8	6.76	7.27	0.44	0.038
12	8.0	6.71	7.35	0.32	0.028
13	8.2	6.73	7.46	0.36	0.032
14	8.4	6.88	7.63	0.29	0.025

* Each value is mean of three determinations

Table 12:- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.

Sr No	pH Before Reaction	pH After Reaction	Mean pH of assay medium	Enzyme activity *	
				Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	5.8	6.35	6.07	0.62	0.092
2	6.0	6.38	6.18	1.06	0.157
3	6.2	6.53	6.36	0.90	0.129
4	6.4	6.71	6.55	0.84	0.120
5	6.6	6.80	6.70	0.74	0.104
6	6.8	6.85	6.82	0.66	0.097
7	7.0	7.00	7.00	0.57	0.084
8	7.2	6.95	7.07	0.62	0.091
9	7.4	7.00	7.20	0.51	0.075
10	7.6	7.00	7.30	0.49	0.072
11	7.8	7.30	7.46	0.48	0.072
12	8.0	7.30	7.56	0.41	0.061
13	8.2	7.05	7.62	0.31	0.046
14	8.4	7.15	7.77	0.12	0.018

* Each value is mean of three determinations

leaves of *S. grandiflora*. The enzyme activity recorded in the tables and figures is expressed both on fresh tissue as well protein bases. It is evident from these observations that initially with increasing pH of the medium there is increase in the activity of the enzyme and later with further increase in pH, it decreases linearly (Table 10). It is also evident that enzyme has exhibited the highest activity at pH from 6.82 to 6.91. pH above 6.91 however, has affected the activity remarkably. It can be seen that at pH 7.67, it is less than that to the maximum recorded. It can be concluded, therefore, that pH 6.8 to 7.0 can be taken as pH optimum for catalase in the leaves of *S. grandiflora*.

The pH of the assay medium tried to study the effect of pH on catalase activity in case of *P. oleracea* ranges from 6.16 to 7.63 (Table 11 and figs. 31 and 32). It is observed that there is an increase in the activity of this enzyme with increase in pH of the assay medium and reaches to maximum at pH 6.92. Thereafter with further increase in pH, there is a constant and linear decrease in the activity of enzyme. It is reduced to almost half the maximum at pH 7.63. From the results it appears that 6.92 is the pH optimum for enzyme catalase in the leaves of *Portulaca oleracea*.

The pH of the assay medium tried to study the effect of pH on the catalase activity in case of *K. pinnata* ranges from 6.07 to 7.77 (Table 12 and fig. 33 and 34). It is observed that there is an increase in the activity of catalase with increase in pH of the assay medium and reaches to the maximum at pH 6.18 (1.06 $min^{-1}g^{-1}$ fresh tissue or 0.157 $min^{-1}mg^{-1}$ protein). Thereafter with further increase in pH, there is constant and linear decrease in the activity of this enzyme. It is reduced to almost $1/9^{th}$ the

Fig.31 :- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.

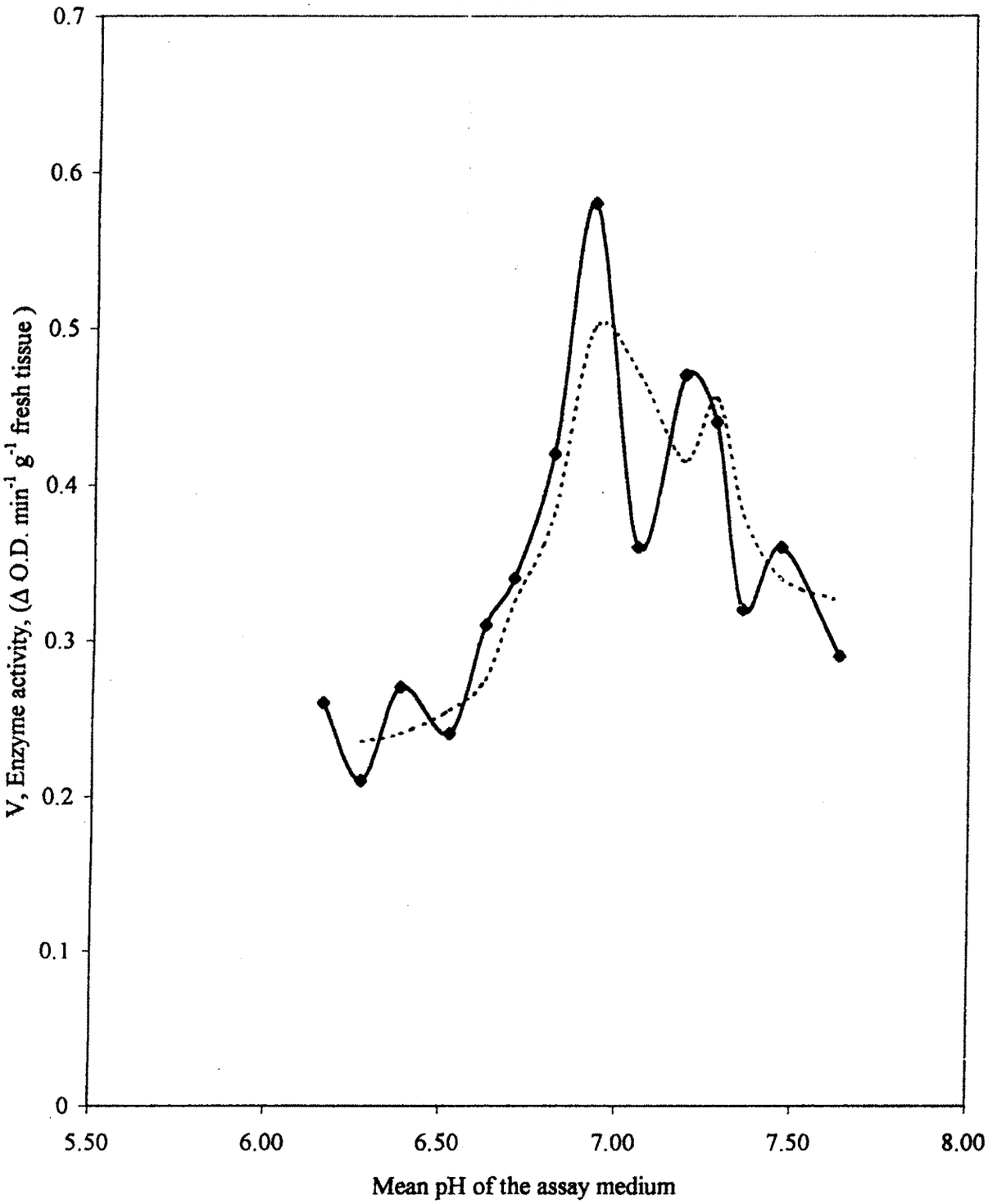


Fig 32 :- **Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.**

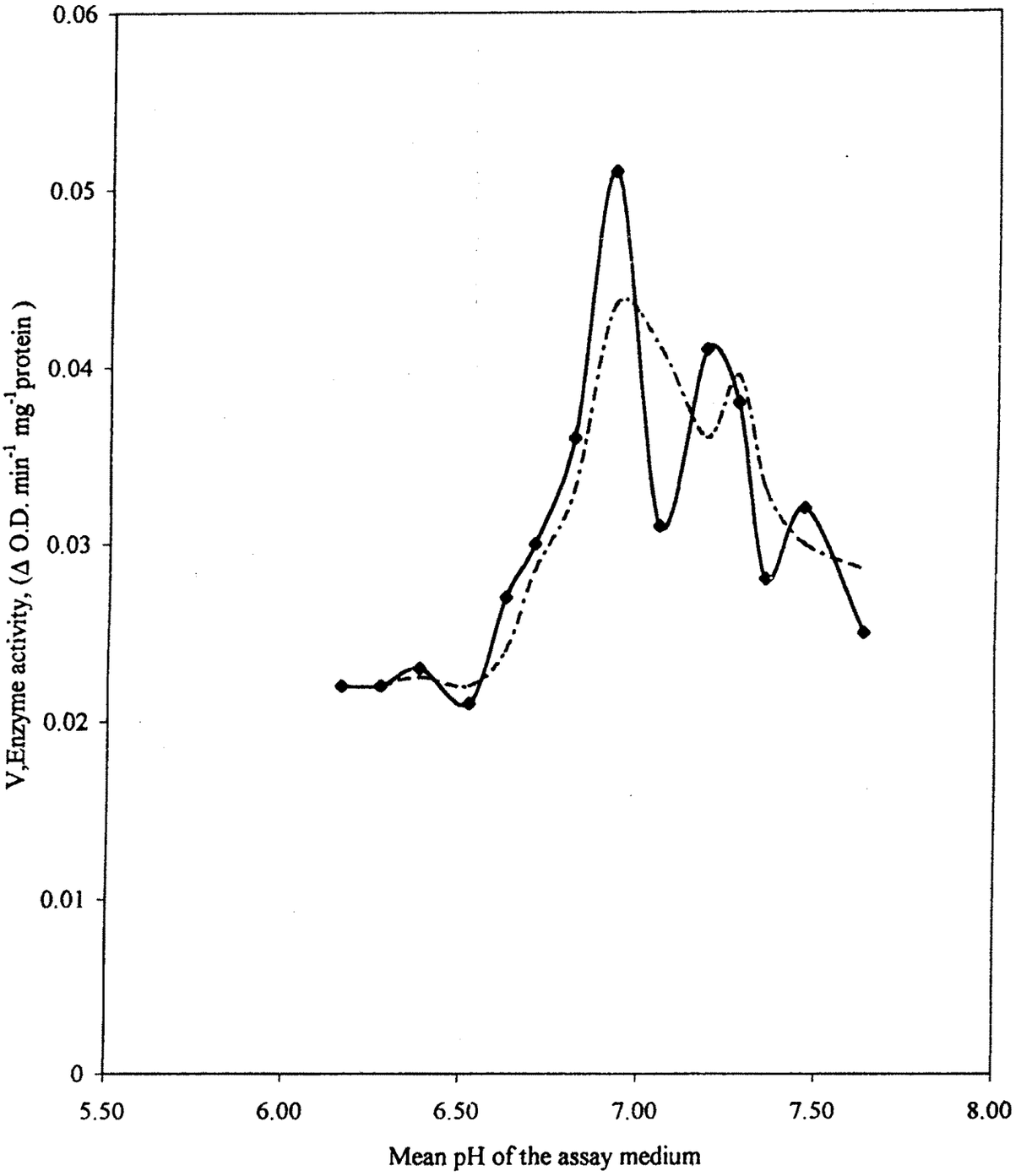


Fig.33:- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Kalanchoe Pinnata* (Lamk) Pers.

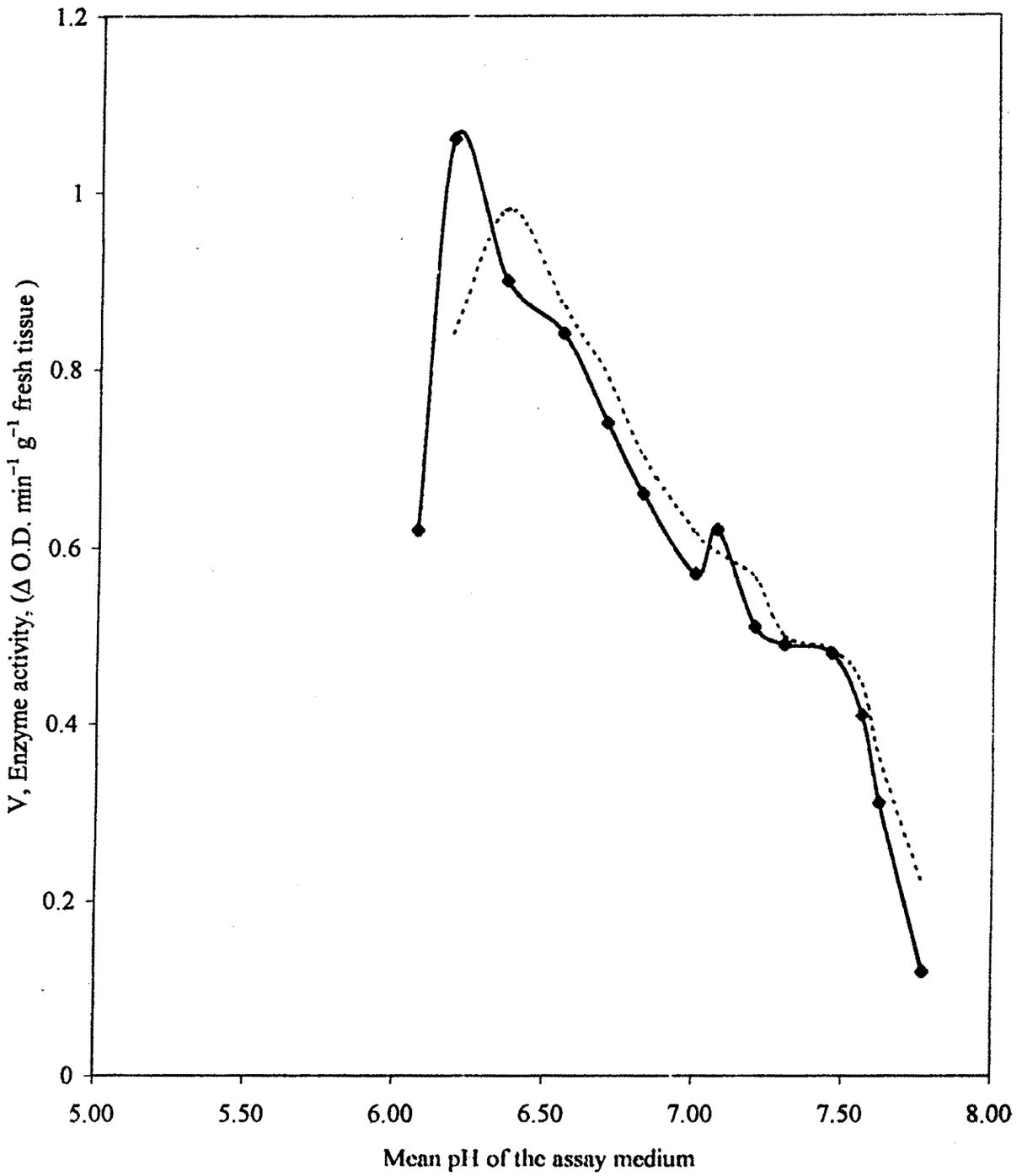
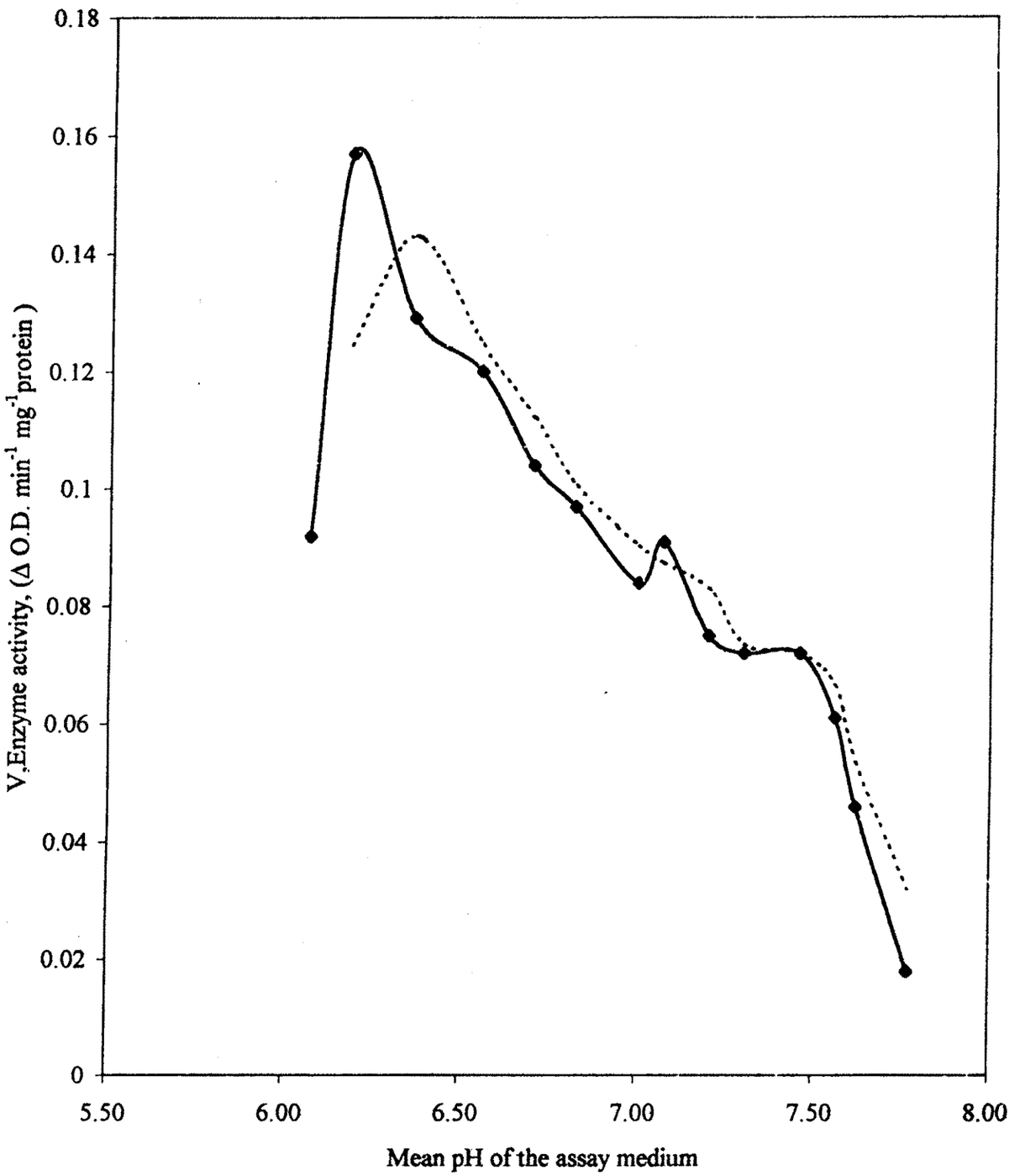


Fig.34 :- Effect of pH of the assay medium on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.



maximum value at pH 7.77. From the results it appears that 6.18 is the pH optimum for enzyme catalase in the leaves of *K. pinnata*.

Thus the pH optimum for enzyme catalase in the leaves of *P. oleracea* appears to be slightly higher than that in the leaves of *S. grandiflora* and it is near to the neutrality. However the pH optimum for the enzyme catalse in the leaves *K. pinnata* is much more acidic (6.18). It can be noted that *K. pinnatum* is a CAM plant which has an ability to synthesize, accumulate and metabolize organic acids like malic acid. It seems that enzyme catalase in this species has higher activity in the relatively acidic medium *in vivo*. This can be considered as an adaptation of this enzyme to acidic conditions. It is also very clear that this enzyme is higher in the leaves of *S. grandiflora* and *K. pinnata* than that from the leaves of *P. oleracea*.

c) Effect of temperature

The effect of temperature on the activity of enzyme catalase in the leaves of *S. grandiflora*, *P. oleracea* and *K. pinnata* has been recorded in the tables respectively 13,14,15 and figures 35-36,37-38 and 39-40 respectively. Activity of the enzyme has been expressed both on fresh tissue as well as protein bases. It is evident that with increasing temperature there is increase in the activity of the enzyme, reaches to maximum and then declines with further increase in temperature. The temperature range tried is between 10 to 40°C. It is quite clear that enzyme catalase in the leaves of *S. grandiflora* records maximum activity, 26.6 $min^{-1}g^{-1}$ fresh tissue or 0.443 $min^{-1}mg^{-1}$ protein at 15°C. It is decreased with further

Table 13:- Effect of temperature on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.

Sr. No.	Temperature ^0C	Enzyme activity *	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	10	19.65	0.328
2	15	26.6	0.443
3	20	24.55	0.409
4	25	22.10	0.350
5	30	19.30	0.322
6	35	10.85	0.181
7	40	10.15	0.158

* Each value is mean of three determinations

Table 14 :- Effect of temperature on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.

Sr. No.	Temperature ^0C	Enzyme activity *	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	10	0.59	0.066
2	15	0.72	0.081
3	20	0.69	0.077
4	25	0.59	0.066
5	30	0.32	0.036
6	35	0.21	0.024
7	40	0.16	0.006

* Each value is mean of three determinations

Table 15 :- Effect of temperature on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.

Sr. No.	Temperature ^0C	Enzyme activity *	
		Δ O.D. min^{-1} g^{-1} fresh tissue	Δ O.D. min^{-1} mg^{-1} protein
1	10	0.81	0.163
2	15	0.95	0.190
3	20	0.78	0.158
4	25	0.65	0.132
5	30	0.61	0.124
6	35	0.54	0.109
7	40	0.34	0.070

* Each value is mean of three determinations

Fig.35:- **Effect of temperature on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.**

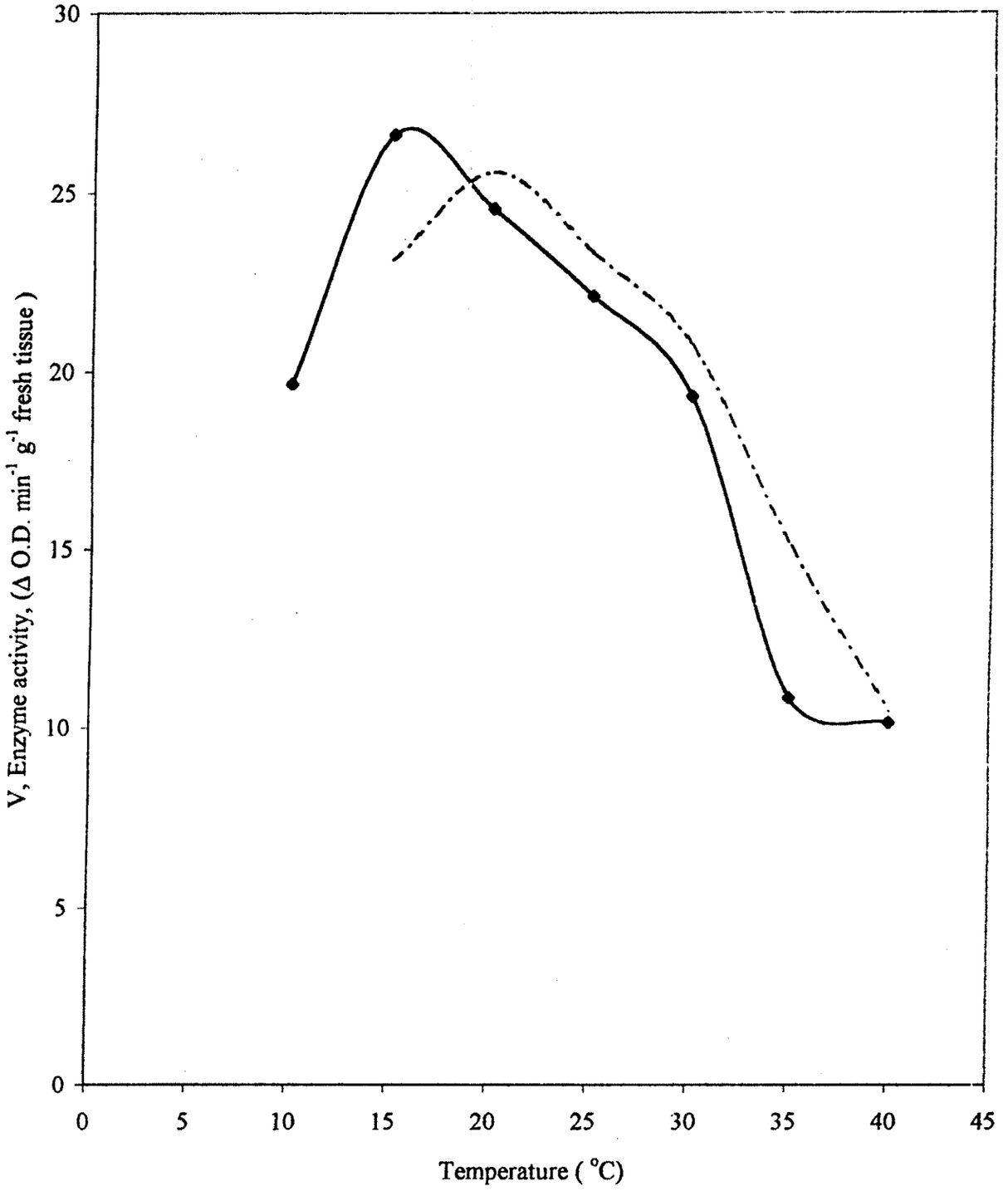
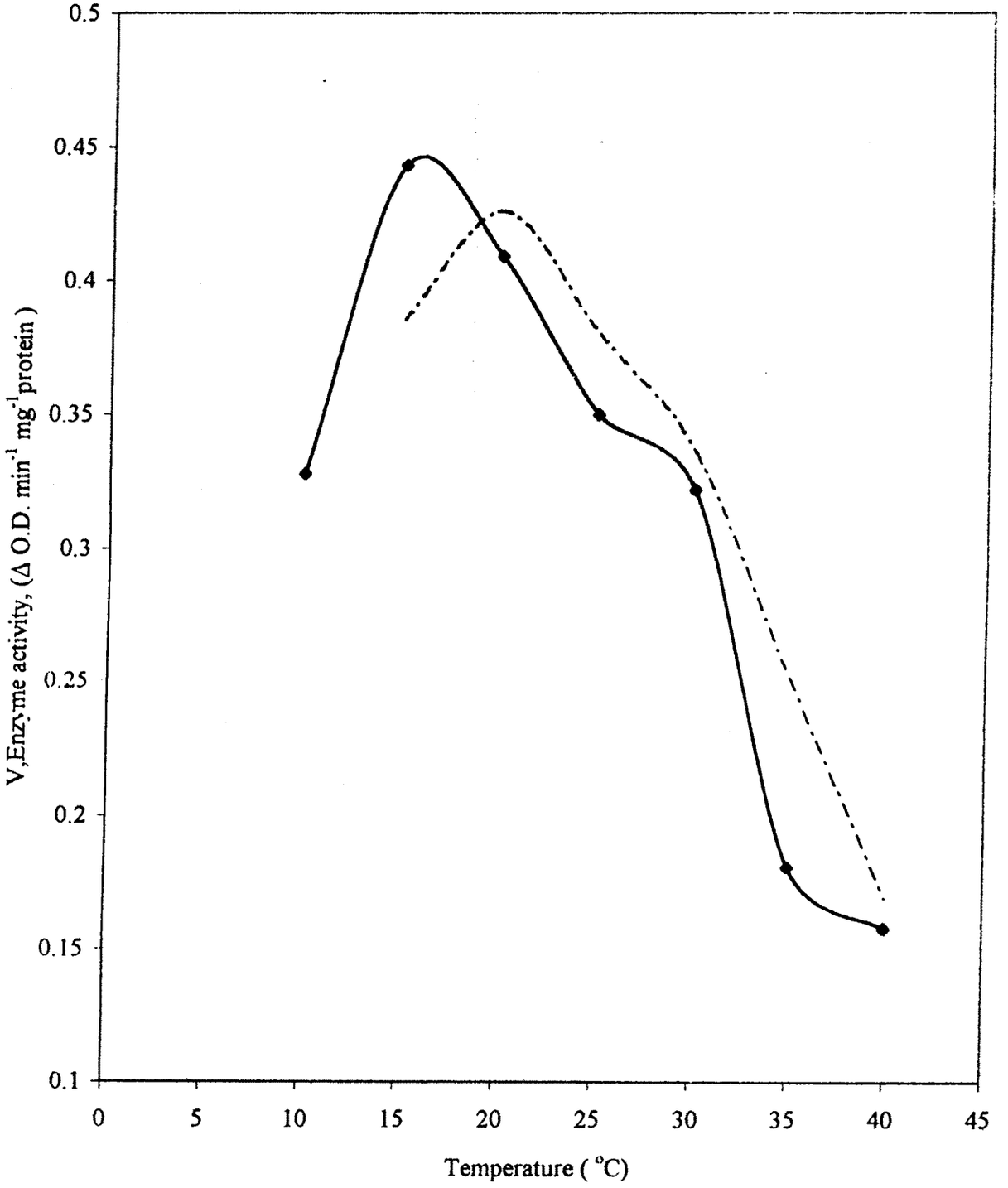


Fig. 36:- **Effect of temperature on the activity of enzyme Catalase in the leaves of *Sesbania grandiflora* L.**



increase in the temperature and the lowest activity being at 40°C temperature (10.5 $min^{-1}g^{-1}$ fresh tissue or 0.158 $min^{-1}mg^{-1}$ protein), which is however still lower than that at 10°C. It suggests that enzyme catalase from *Sesbania grandiflora* requires still lower temperatures for its inhibition or denaturation.

It can be seen from table 14 and fig. 37 and 38 that enzyme catalase from the leaves of *P. oleracea* exhibits, maximum activity, 0.72 $min^{-1}g^{-1}$ fresh tissue or 0.081 $min^{-1}mg^{-1}$ protein at 15°C and remains almost constant till 20°C. Temperature 30°C and above seems to be inhibitory as the enzyme activity recorded at 30, 35 and 40°C temperatures is much below (0.34 $min^{-1}g^{-1}$ fresh tissue or 0.070 $min^{-1}mg^{-1}$ protein at 40°C) which is far below to that recorded at cooler conditions (0.81 $min^{-1}g^{-1}$ fresh tissue or 0.163 $min^{-1}mg^{-1}$ protein at 10°C).

From the above dicussion it is clear that enzyme catalase from *S. grandiflora*, *P. oleracea* and *K. pinnata* has temperature optimum between 15 to 20°C.

Dat *et al.* (1994) have studied parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. It is reported that a heat shock at 55°C caused significant increase in endogenous H_2O_2 and reduced the catalase activity.

For comparison of activity of peroxidase and catalase from *S. grandiflora*, *P. oleracea* and *K. pinnata*, between these two enzymes in a species and among those from all the three species, an average of specific activities of these enzymes have been considered. It is found that activity

Fig.37:- **Effect of temperature on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.**

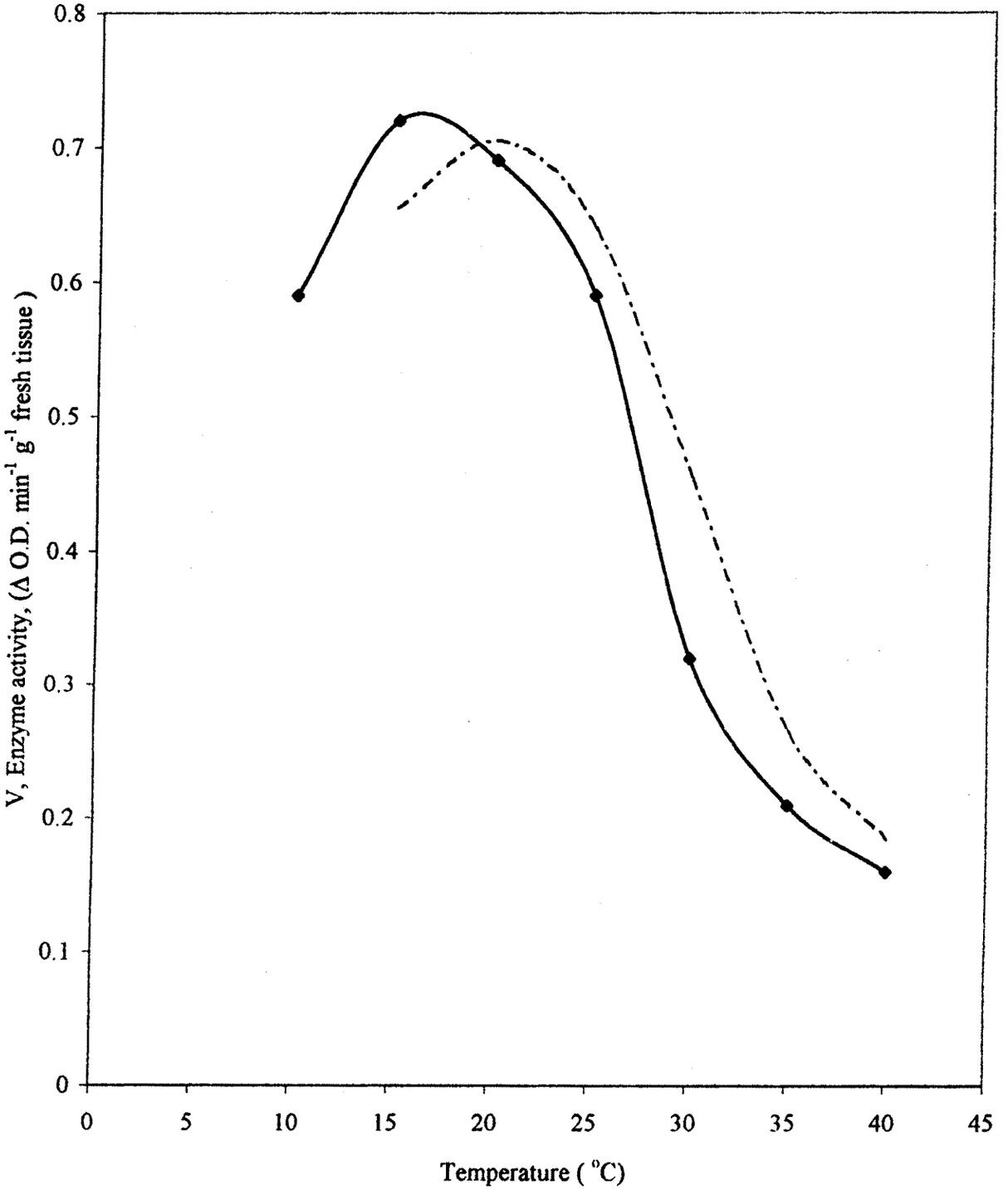
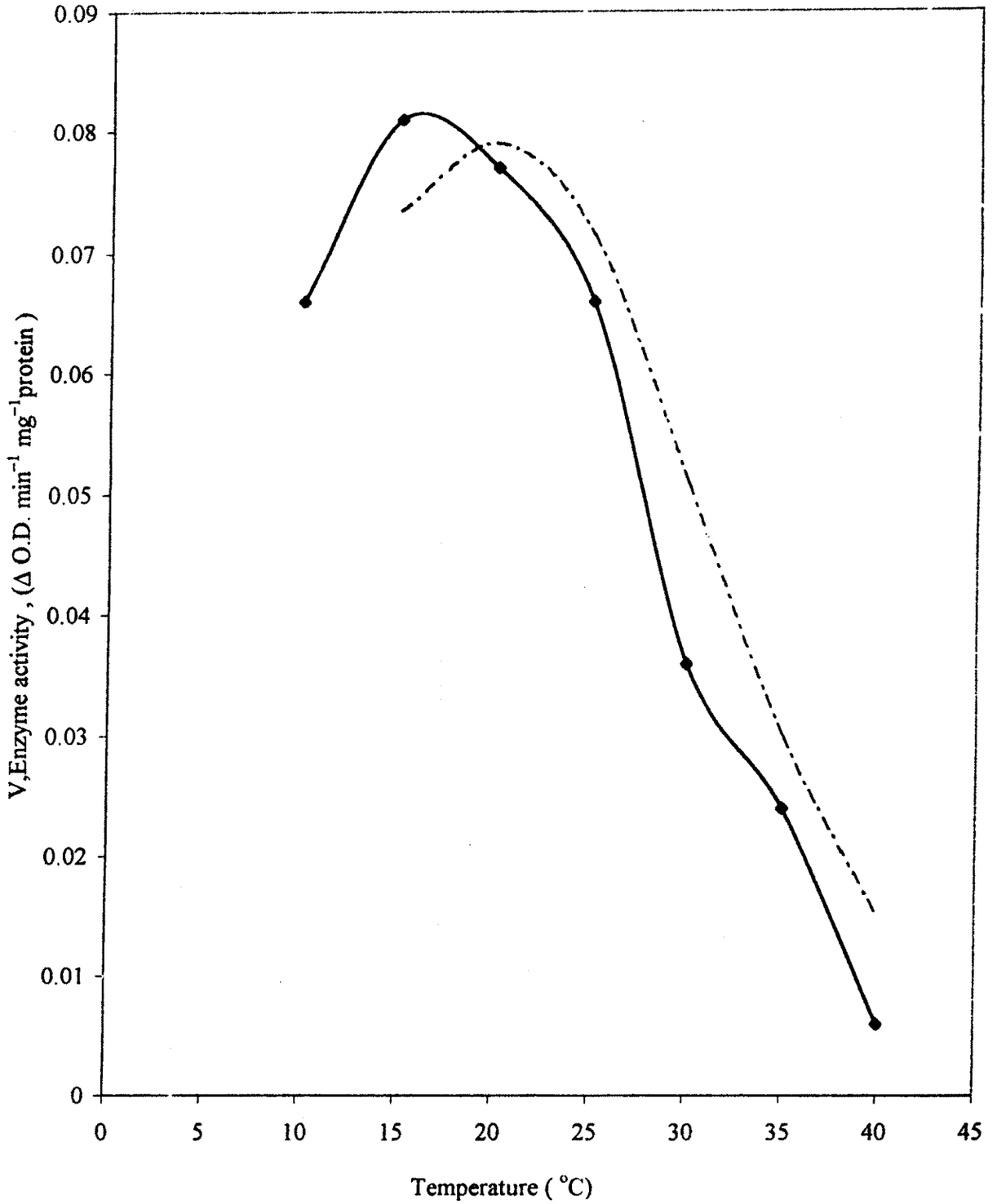


Fig.38 :- Effect of temperature on the activity of enzyme Catalase in the leaves of *Portulaca oleracea* L.



of enzyme peroxidase from *S. grandiflora* is 0.30 $min^{-1}mg^{-1}$ protein while that of catalase is 0.50 $min^{-1}mg^{-1}$ protein. It clearly indicates that enzyme catalase is much more dominent in the leaves of this legume species. On the other hand it is the peroxidase (0.25 $min^{-1}mg^{-1}$ protein) which is almost two times more active than enzyme catalase (0.10 $min^{-1}mg^{-1}$ protein) in the leaves of C_4 succulent *P. oleracea*. It is also found that the activity of enzyme peroxidase in *P. oleracea* is 0.25 $min^{-1}mg^{-1}$ protein which is lower than that in *S. grandiflora* (0.30 $min^{-1}mg^{-1}$ protein) similarly the activity of enzyme catalase in the C_4 succulent. *P. oleracea* (0.10 $min^{-1}mg^{-1}$ protein) is almost five times less than that in *S. grandiflora* (0.5 $min^{-1}mg^{-1}$ protein) while it is almost three times less than that in the CAM succulent *K. pinnata* (0.16 $min^{-1}mg^{-1}$ protein). Thus it is evident that the level of hydroxyperoxidases in the succulent seems to be less than that in many other plants, particularly the legumes like *S. grandiflora*. Generally legumes are found to be more vigorous in the metabolism involving hydroxyperoxidases, particularly catalase. Du, Shi. Hua (1983) has reported significant differences in catalase activity between C_3 and C_4 species. The average activity of this enzyme in C_3 plants was reported to be 4 fold greater than that in C_4 plants.

2. Study of other related enzymes

Peroxidase, polyphenol oxidase and IAA oxidase, having the same catalytic action have been considered, according to some authors as isozymes and interrelationships among these enzymes have been proposed (Pilet *et al.*, 1970; Hoyle 1972 and Sheen, 1969). Srivastava and Van Huystee (1977) have also studied interrelationship among these enzymes

Fig.39:- Effect of temperature on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.

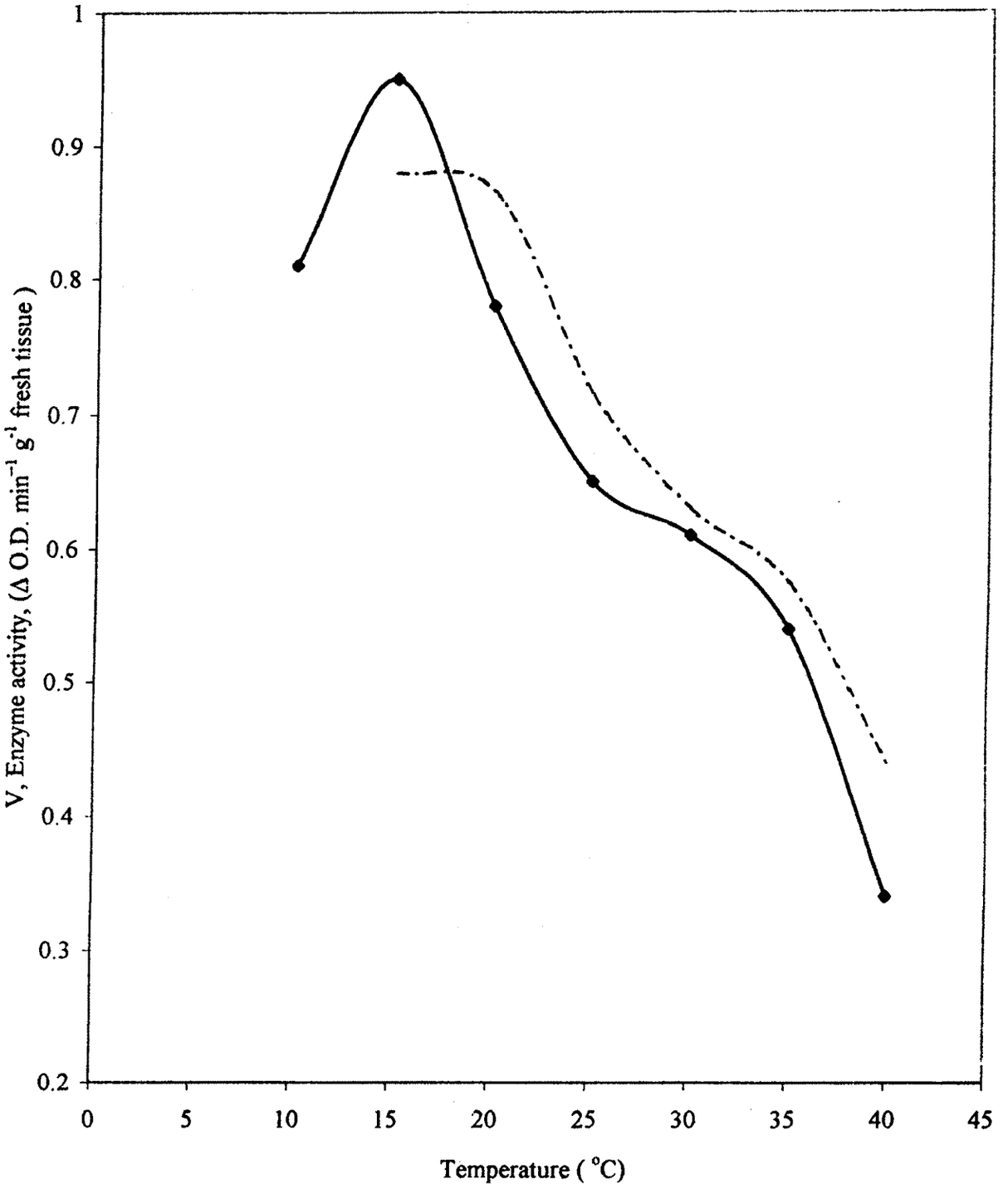
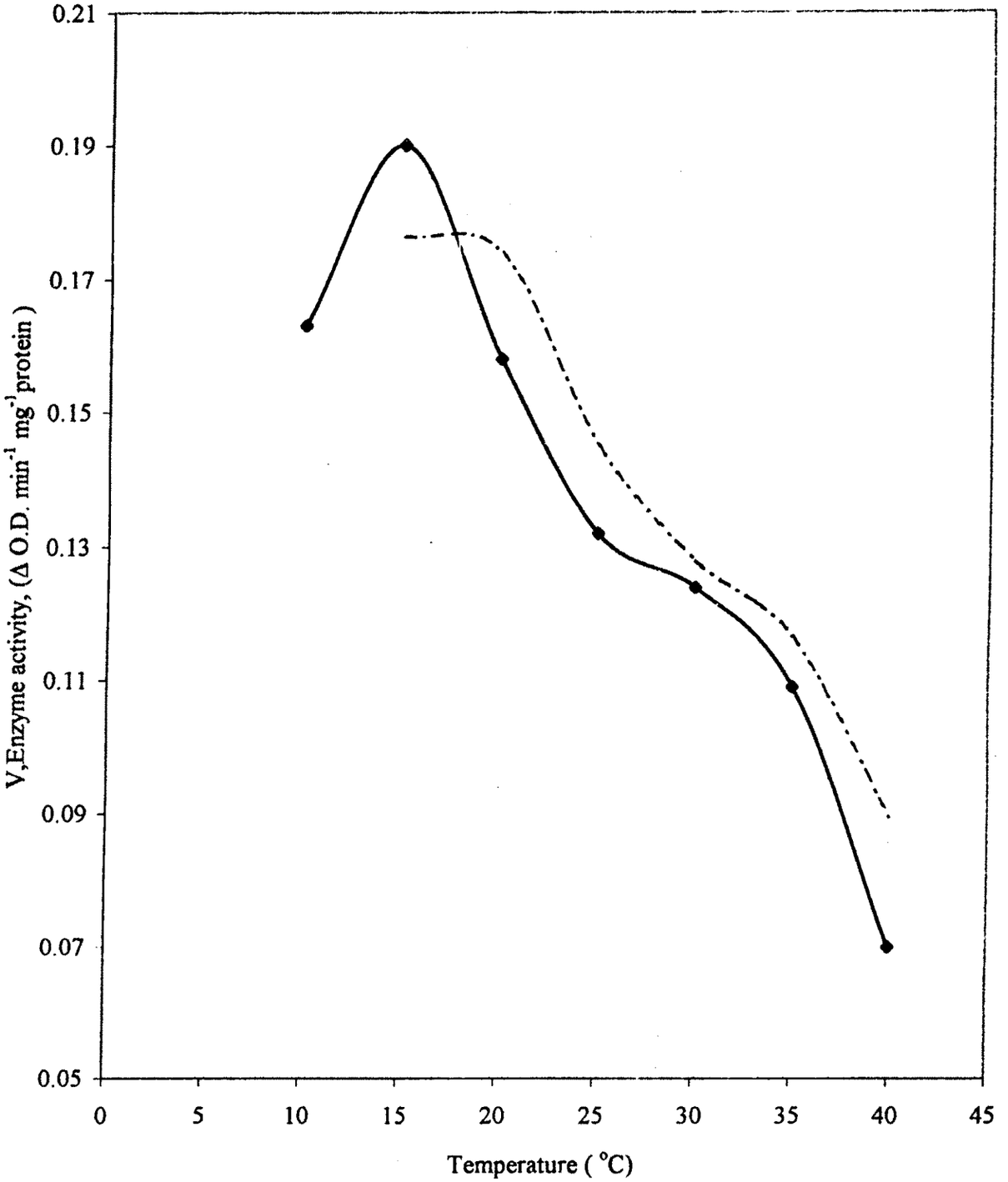


Fig. 40:- Effect of temperature on the activity of enzyme Catalase in the leaves of *Kalanchoe pinnata* (Lamk) Pers.



from peanut cells. In earlier report, Srivastava and Van Huystee (1973) proposed a close association among peroxidase, IAA oxidase and polyphenol oxidase. The three enzyme activities were found to be associated with single isozyme preparation of peroxidase (Srivastava, 1976). These studies on peanut cells revealed that polyphenol oxidase and IAA oxidase share the same active site on apoenzyme of peroxidase (Srivastava and Van Huystee, 1977). Thus peroxidase, polyphenol oxidase and IAA oxidase appear to be three forms of the same enzyme molecule. With this view in mind IAA oxidase and polyphenol oxidase along with peroxidase and catalase have been studied in the leaves of *S. grandiflora*, *P. oleracea* and *K. pinnata*.

a) IAA oxidase

Enzyme IAA oxidase oxidises indole acetic acid, thus bringing about the distruction of IAA and maintaining the level of IAA in the plant tissue. Thus IAA oxidase plays a key role in the regulation of plant growth and development which is under the control of auxins.

The activity of enzyme IAA oxidase in the leaves *S. grandiflora* and *P. oleracea* has been recorded in Table 16. It must be mentioned here that we could not measure the activity of this enzyme in the leaf tissue of *K. pinnata*. From the values of specific activities of this enzyme recorded in the Table, it is evident that the level of enzyme IAA oxidase is slightly more in the leaves of *S. grandiflora* than that in *P. oleracea*. This is in well agreement with our earlier finding that activities of peroxidase and catalase are higher in *S. grandiflora* than those in *P. oleracea* and *K. pinnata*. It

Table 16 :- **The activity of enzyme IAA oxidase in the leaves of *Sesbania grandiflora* L. and *Portulaca oleracea* L.**

Sr. No.	Name of the plant	Enzyme activity *	
		Δ O.D. hr^{-1} g^{-1} fresh tissue	Δ O.D. hr^{-1} mg^{-1} protein
1	*Sesbania grandiflora* L.	0.580	0.038
2	*Portulaca oleracea* L.	0.480	0.032

* Each value is mean of three determinations

appears that enzyme IAA oxidase in these plants may be an isozyme of peroxidase or catalase in the plants.

b) Polyphenol oxidase

According to Butt (1985) oxygenases are involved in the hydroxylation of many phenolic compounds in higher plants. A correlation between the presence of high levels of phenolic compounds and polyphenol oxidase or low levels of phenolic compounds and no polyphenol oxidase has been reported. (Vaughn and Duke, 1981; Kogima and Conn, 1982 and Bajaj *et al.* 1985).

Polyphenols are secondary metabolites. Some of these compounds play a significant role in the growth and development of plants, acting as growth promoters. They may also be involved in the defence mechanism against pathogens. The phenolic compounds inhibit the activities of several type of enzyme systems in the plant tissue. Polyphenol oxidase which oxidizes or destroyes these substances, is thus involved in regulation of level of these secondary metabolites.

The activity of enzyme polyphenol oxidase in the leaves of *S. grandiflora*, *P. oleracea* and *K. pinnata* has been recording in table 17. It is clear from the results that level of this enzyme is the highest in the leaf tissue of *S. grandiflora*. The lowest activity is recorded for this enzyme in the leaves of *P. oleracea*. Thus this enzyme from succulents records the activity lower than that in *S. grandiflora*.

It is concluded that enzymes peroxidase, IAA oxidase and polyphenol oxidase appear to be isozymes of the same enzyme with three

Table 17 :- The activity of enzyme polyphenol oxidase in the leaves of *Sesbania grandiflora* L., *Portulaca oleracea* L. and *Kalanchoe pinnata* (Lamk) Pers.

Sr. No.	Name of the Plant	Enzyme activity *	
		Δ O.D. hr^{-1} g^{-1} fresh tissue	Δ O.D. hr^{-1} mg^{-1} protein
1	*Sesbania grandiflora* L.	2.40	0.16
2	*Portulaca oleracea* L.	1.80	0.12
3	*Kalanchoe pinnata* (Lamk)	2.10	0.14

* Each value is mean of three determinations

district but similar type of catalytic actions. The level of peroxidase, IAA oxidase and polyphenol oxidase is maximum in *S. grandiflora*. The relationship among these three enzymes, however will be clear only after some further studies and enzyme kinetics for these species.

3) Isozymes of peroxidase

Isozymes or isoenzymes are multiple forms of an enzyme that differ by minor variations in aminoacid composition and some times in regulation. (De Robertis, 1987) One of the best examples of an isoenzyme is Lactic dehydrogenase (LDH), which catalyzes the conversion of pyruvate to lactate. There are five LDH isoenzymes that differ in their electrophoretic mobility in starch gels. Isoenzymes in several plant enzyme systems have been reported. The proportions of the isozymes of an enzyme change with stage of development of cells or tissues. It is also reported that expression of isozymes may be compartment specific, time specific or may be regulated by some other factors. Isozymes of PEP-carboxlase in a number of C_4 and CAM species have been reported.

Isozymes have been used as genetic markers in the genetic studies and plant breeding. Isozyme patterns for enzyme peroxidase have been widely studied and used as genetic markers.

In the present study an attempt was made to study the isozyme patterns for peroxidase from the leaf tissue of *S. grandiflora*, *P. oleracea* and *K. pinnata*. The isozymes of peroxidase are studied by gel electrophoresis technique. Polyacrylamide gel electrophoresis of peroxidase from *S. grandiflora*, *P. oleracea* and *K. pinnata* leaves has been

shown in the plates 7, 8, and 9, 10 respectively. The isozymes of peroxidase have also been recorded in Table 18 with their Rm values. It is evident from the plates and the Table that the peroxidase preparation from *S. grandiflora* leaves exhibits 5 isozymes with different Rm values. Peroxidase of *P. oleracea* leaves shows 4 isozymes while we failed to separate or find the isozymes of peroxidase from *K. pinnata.* It is also evident that some of the isozymes e.g. isozymes I and II appeared to be similar in both *S. grandiflora* and *P. oleracea.* However, the other isozymes quite differ from each other in *S. grandiflora* and *P. oleracea.* It is suggested that *S. grandiflora* has a more diversity of peroxidase isozymes. This may be one of the reasons for high level of hydroxyperoxidases in *S. grandiflora* leaves and the plants adaptibility to wide environmental conditions including soil salinity, and its rapid growth rate.

Table 18:- Isozymes of peroxidase in the leaves of *Sesbania grandiflora* L., *Portulaca oleracea* L. and *Kalanchoe pinnata* (Lamk) Pers.

Sr. No.	Name of the plant	Isozymes with their Rm values					
		1	2	3	4	5	6
1	*Sesbania grandiflora* L.	0.105	0.250	---	0.526	0.802	0.890
2	*Portulaca oleracea* L.	0.108	0.230	0.491	---	---	0.959
3	*Kalanchoe pinnata* (Lamk)	---	---	---	---	---	---

Plate - 7 : Polyacrylamide gel electrophoresis of peroxidase from
i. *S. grandiflora* leaves
ii. *P. oleracea* leaves
iii. *K. pinnata* leaves

Plate - 8 : Polyacrylamide gel electrophoresis of peroxidases from *S. grandiflora* leaves

Plate - 9 : Polyacrylamide gel electrophoresis of peroxidases from *P. oleracea* leaves

Plate - 10 : Polyacrylamide gel electrophoresis of peroxidases from *K. pinnata* leaves

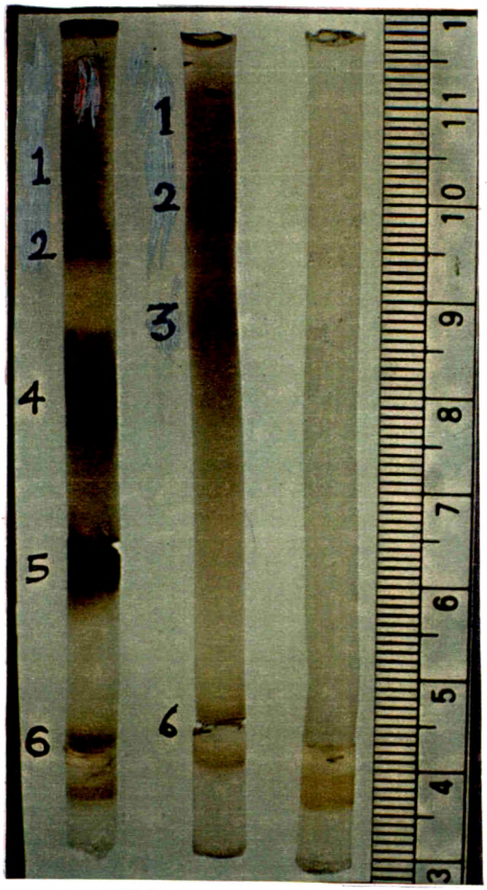


PLATE 7

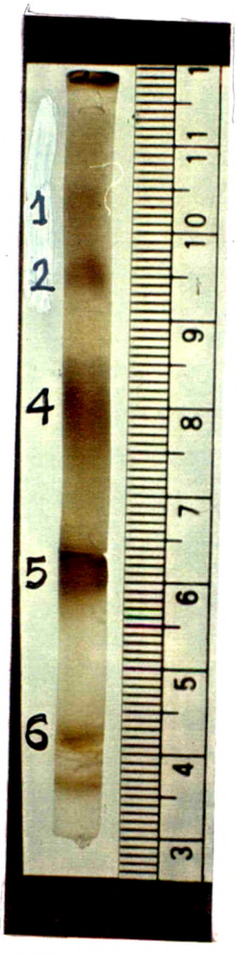


PLATE 8

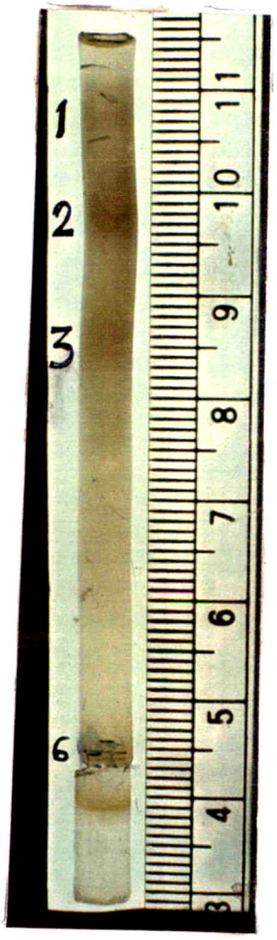


PLATE 9

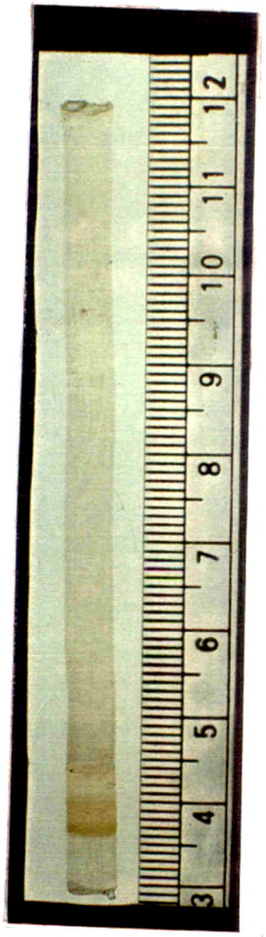


PLATE 10