egg(s) and brings the egg-embryo complex to the growth stage at which the embryo ordinarily hatches.

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STUDIES ON CHANGES IN CONTENT AND FRACTIONATION OF TOTAL SOLUBLE PROTEIN IN THE COTYLEDON AND EMBRYO OF GERMINATING SUNFLOWER (*HELIANTHUS ANNUUS* L.) SEEDS

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ALTHOUGH studies on the multifarious aspects of germination of main crop seeds are available reports on sunflower seeds are scanty. The present work was therefore undertaken to outline the metabolic changes of protein initiated during the early stages of germination.

The transport of total soluble proteins from cotyledons to the embryonic parts increases with germinating time[1-4]. The total protein extracts in cotyledon as well as embryo of different seeds during germination after precipitation with various concentrations of ammonium sulphate differ widely and the concentration of ammonium sulphate necessary to elute the fractions of the total soluble proteins depends on the stage of germination[5-7].

'Peredovik' (EC 68415) variety obtained from the seed farm of this University was used. The seeds, immediately after harvest, were dried in sun. As the seeds were found to be most viable in a month or two after harvest, this experiment was carried out during this period.

Healthy seeds were soaked in sterile glass-distilled water for 6 h and allowed to germinate on a wet Whatman No. 1 filter paper spread on a petri dish of 15 cm diameter. About 30–35 seeds were taken in each petri dish and allowed to germinate in a BOD incubator in dark at 30°C. The seeds were taken out from incubation at 24, 48, 72, 96 and 120 h intervals of germination and the decoated seeds were dissected into the cotyledon and the embryo parts. The dissected parts were then wiped out of any adhering water and dried in an oven at 45–50°C. The dried materials were then ground in a sieve grinder at 40 mesh, packed in sealed polythene packets and stored in a desiccator at 4–5°C. When required, the dry powdered materials were used for analysis.

The protein was estimated by the colorimetric method of Lowry *et al*[8] as modified by Hartree[9]. The total soluble proteins were fractionated into three sub-fractions by the salting out technique, using ammonium sulphate as the precipitant.

Analysis of the total soluble proteins and their ammonium sulphate fractions is reported in tables 1 and 2. As the seeds pass through, from the initial to the final stage of germination, the total soluble protein decreased in the cotyledon and increased in the embryo. The percentage of increase in embryo protein was 12 and that of decrease in cotyledon protein was about 45.

Ammonium sulphate fraction reveal the following:

(a) Irrespective of the time of germination, 50% (or more) of the total soluble proteins are present as proteins precipitated by 50% saturation of ammonium sulphate (fraction A). The rest is distributed as proteins precipitated by 100% ammonium sulphate saturation (fraction B) and as proteins remaining in the supernatant solution after treatment with ammonium sulphate (fraction C). This is true for both cotyledon and embryo (tables 1 and 2).

(b) The percentage of total soluble protein, present as fraction A protein, instead of remaining the same, increases with increase of hours of germination and reaches a high value of 80% at the late hours of germination. On the other hand, the percentage of fraction B and fraction C proteins decreases with increase of hours of germination. The percentage of fraction B protein falls from 20 to 9.5 in cotyledon and from 27 to 7 in embryo. On the other hand, the percentage of fraction C protein decreases from 20 to 8 in the cotyledon part; in embryo part, however, the percentage of fraction C

Table 1 *Protein fractions of cotyledon of germinating Helianthus annuus L. seed**

Germination (h)	Total soluble	Fraction 'A'	Fraction 'B'	Fraction 'C'
0	452±0.4	270±0.4	90±0.2	90±0.3
24	420±0.5	273±0.5	80±0.2	65±0.3
	(−7.08)	(11.11)	(−11.12)	(−27.78)
48	370±0.5	259±0.6	60±0.3	50±0.4
	(−18.15)	(−4.08)	(−33.34)	(−44.45)
72	342±0.5	256±0.2	43±0.2	41±0.3
	(−24.34)	(−5.19)	(−52.23)	(−54.45)
96	290±0.6	232±0.3	28±0.1	30±0.3
	(−35.85)	(−14.08)	(−68.89)	(−66.67)
120	241±0.5	192±0.3	27±0.3	20±0.3
	(−46.69)	(−28.89)	(−70.00)	(−77.78)

*Data are expressed in μg/mg of ground dried material. Each value is mean ± SE of four observations. Values in parantheses represent per cent deviation over control.
Fractions A and B—precipitated by 50% and 100% ammonium sulphate saturation respectively.
Fraction C—remaining in the supernatant solution after treatment with ammonium sulphate.

Table 2 *Protein fractions of embryo of germinating Helianthus annuus L. seed**

Germination (h)	Total soluble	Fraction 'A'	Fraction 'B'	Fraction 'C'
0	365±0.4	183±0.3	99±0.4	81±0.3
24	362±0.6	218±0.4	82±0.2	60±0.4
	(−0.83)	(19.12)	(−17.18)	(−25.93)
48	370±0.3	295±0.5	55±0.2	20±0.4
	(1.36)	(61.20)	(−44.45)	(−75.31)
72	389±0.3	312±0.4	56±0.4	19±0.2
	(6.57)	(70.49)	(−43.44)	(−76.55)
96	394±0.3	316±0.2	45±0.4	30±0.3
	(7.94)	(72.67)	(−54.55)	(−62.97)
120	410±0.6	328±0.2	30±0.2	51±0.3
	(12.32)	(79.23)	(−69.70)	(−37.04)

*Same as table 1.

protein falls from 22 at 0 hour to about 5 at 72 h followed by a further increase to 12 at 120 h of germination.

(c) The percentages of fraction B and C proteins differ considerably among themselves at all the hours of germination in embryo but are not found to differ much in cotyledon.

(d) At all the hours of germination and in both embryo and cotyledon, the percentages of fraction A protein are found to be the highest. Fraction C proteins in cotyledons are either equal to or less than the fraction B proteins. However, in embryo the fraction C proteins are less than the fraction B proteins at all the stages, excepting at 120 h when they are higher.

Almost all the seeds contain protein reserves for the nitrogenous supplies required by the young seedlings before they are able to absorb nitrogen by roots. The overall compositional changes of the reserve protein, during germination are summarized in tables 1 and 2. The transfer of dry weight from cotyledon to the embryo at the expense of stored protein in cotyledons is evident. The reserve protein is hydrolysed during germination (by proteinases) to amino acid units and peptides depending upon their specificity, and peptidases which hydrolyse endogenous and hydrolytically produced peptides to amino acids. These amino acids then appear to be transported to the pool in seedling axis, where they again form proteins which accumulate with increase in hours of germination. As a result of the hydrolysis of protein reserve in cotyledon and increased rate of protein synthesis in the growing embryo, the concentration of the protein sharply falls in cotyledons and increases in the embryo. The increased rate of protein synthesis in the embryo may be associated with the increasing concentration of RNA and with progress of hours of germination.

Fractionation studies of proteins using ammonium sulphate indicate that most of the proteins in cotyledon as well as embryo at all stages of germination are found in the 50% saturation fraction. The per cent distribution of this protein fraction increases at the early hours of germination until it attains a very high constant value of about 80% (tables 1 and 2). This attainment is comparatively quicker in the embryo (48 h) possibly because of the higher rates of enzymatic activities. Since most enzymes belong to this fraction, the higher enzyme activities in the embryonic tissues at the later stages of germination may account for the rise in the percentage of this fraction at these stages of germination. In any case, it appears that proteins identified with the process of germination reside in the globulin fraction. Quantitative changes in the protein fraction which precipitates with 100% ammonium sulphate are also indicated in this study. The greater amount of this fraction during the earlier stages of germination suggests that these proteins may be involved in the early development associated with the germination process. As a

change in this fraction is more marked in the embryo, this may be more directly associated with the growth and development of the different embryonic parts.

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TOXICITY OF *ANACYCLUS PYRETHRUM* IN MICE

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ANACYCLUS PYRETHRUM (AP) is a medicinal plant known for its use as an aphrodisiac, sialagogue, and in hemiplegia, paralysis, epilepsy, rheumatism and tooth-ache[1,2]. It is available in all parts of the world and belongs to the Compositae family. Its therapeutic properties could be due to an alkaloid[3,4] 'pellitorin' (pyrethrin).

Aqueous root extract of this plant caused local anaesthesia with no side effects[5]. Its toxicity data are scanty, and therefore we have determined the i.p. value of LD_{50} of water extract of its dried root.

The material was identified and collected from Yogaprabha Pharmacy, Tirupati. The root was powdered and a sterile cold extract was prepared in water[6]. Its LD_{50} dose was calculated by conventional methods[7]. Four doses (400, 550, 750 and 1000 mg/kg i.p./mice) were administered in four groups (n = 18 males weighing 28–30 g) of inbred Swiss albino mice producing an effect in comparison with control (0–100% changes and mortality). Saline (0.9% NaCl) was administered (0.1 ml i.p.) in the control group. At the same time the experimental group also received AP herbal extract intraperitoneally. The LD_{50} was calculated according to the method of Litchfield and Wilcoxin[8]. Accordingly, the LD_{50} for the aqueous extract of the root of AP was 750 mg/kg i.p. dose. The graphical analysis[9] and Karber's method[10] of probit analysis were also employed for estimating the LD_{50} of the aqueous cold extract of the plant material and was found to be 800 and 758.6 mg/kg (table 1). The data were found to be significant and varied between 690 and 840 mg/kg.

Table 1 *Values of LD_{50} (determined by Karber's method)*

Group	Dose mg/kg	Number of animals	Dose difference (a)	Dead	Mean mortality (b)	Product ($a \times b$)
1	333.33	4	0	0	0	0
2	666.66	4	333.33	0	0	0
3	1000.00	6	333.34	4	2	666.68
4	1333.33	4	333.33	4	4	1333.32

LD_{50} = 800 mg/kg (approx.); The sum of the product is divided by the number of animals in a group and the resulting quotient is substrated from the least lethal dose to obtain the LD_{50} value.

However, this work enables one to have an estimate of the median effective concentration (ME_{50}) of AP and to compare the relative potency with known drugs, toxicity, rating in terms of intensity and duration to quantify its usage as a local anaesthetic as a ground-data for repetition in primates or for a pilot clinical application.

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