REVIEW OF LITERATURE
The review of literature of the present dissertation covers four different plants. They are *Anethum sowa* Roxb., *Trigonella foenum-graecum* Linn., *Adhatoda vasica* Nees. and *Datura innoxia* Mill. As these plants belong to different families and contain entirely different groups of chemical constituents, the related literature of each plant is discussed individually.

*Anethum sowa* Roxb. :

*Anethum sowa* is an Umbelliferous herb cultivated in India as a cold weather crop. Locally it is known by different names in different languages. They are: Sans.–Satapushpi; Eng.–Indian dill; Hind. & Beng.– Sowa; Guj.–Surva; Tam.–Sata kuppi; Kan.–Sabsige. The plant mainly contains odorous volatile oils.

The use of *Anethum sowa* has been recognised since the earliest days of human civilization. The herb is valued as a vegetable and for culinary purposes. The Ayurvedic and Unani systems of medicine use the fruits mainly as carminative, stomachic, aromatic stimulant and effective as an anthelmintic in hookworm infestation and in the treatment of abdominal pain, flatulence and cholic pain in children (Waring, 1883; Shastri, 1961). The fruits and oil were found effective to reduce gripping due to purgatives and in tormina of dysentery (Nadkarni, 1954; Shastri, 1961). An infusion of the fruits is usually given as a cordial drink to women after confinement, as a uterine stimulant to remove abdominal pain and to increase the yield of milk (Shastri, 1961). The dill oil, dill water, gripe waters and other such preparations form popular remedies in India for the treatment
of indigestion and other stomach disorders of infants.

In general, the family Umbelliferae, to which Anethum sowa belongs, is a rich source of volatile oil containing plants. In India, the family is represented by about 40 genera. The members of this family exhibit fistular, furrowed stems and usually divided or dissected large leaves which are often fragrant. The flowers are small and are white, pink, yellow or blue in colour and are arranged in simple or compound umbels (Krishna and Badhwar, 1952). The fruits are usually cremocarps or mericarps. Between the primary ridges of the mericarps the schizogenous oil canals called vittae are present.

The Umbelliferous plants are generally carminative, stimulant and tonic and include several medicinal and kitchen herbs (Krishna et al., 1952). The plants of this family yield many medicinally important compounds like the essential oils of ajowan, anise, caraway, coriander, cumin, dill and fennel, oleogumresins like asafoetida, galbanum and many different groups of substances.

Volatile oils are the odoriferous principles mainly obtained from the plants. A majority of them occur as liquids at room temperature and evaporate without decomposition. They are secreted as such in the plants. The exact mechanism of their formation and utilization in the plants still
remains obscure. Most of the volatile oils generally are mixtures of hydrocarbons and oxygenated compounds. The odour and taste of volatile oils are mainly due to the presence of oxygenated constituents.

Generally, volatile oils contain some of the following chemical components:

(a) Unsaturated hydrocarbons known as terpenes:

1. Monoterpenes \((C_{10}H_{16})\) like limonene, myrcene, ocimene, pinene etc.
2. Sesquiterpenes \((C_{15}H_{24})\) like bisabolene, cadinene, zingiberene etc.

(b) Oxygenated compounds which include:

1. Alcohols like borneol, citronellol, geranool, linalool, menthol, santanol, terpineol etc.
2. Aldehydes like anisic, cinnamic, citral, citronellol, piperonal etc.
3. Esters like bornylacetate, geranylacetate, linalylacetate etc.
4. Ketones like camphor, carvone, fenchone, pulegone, thujone, menthone etc.
5. Phenols like carvacrol, chavicol, eugenol, thymol etc.
6. Phenol ethers like anethole, apiole, dillapiole, estragole, myristicin, safrol etc.
(c) Complex compounds like coumarins, glycoside derivatives, indole, peroxides etc.

**European dill and Indian dill:**

The dill plants growing in Europe are known as *Anethum graveolens* Linn. and are official in many Pharmacopoeias (British Pharmacopoeia, 1958; Indian Pharmacopoeia, 1966) because of the absence of dillapiole which is considered toxic. The Indian dill plant, *Anethum sowa* contains dillapiole and hence not used in medicinal preparations.

*Anethum graveolens* Linn. is an annual plant, usually growing to a height of 3-4 feet and possesses tripinnate leaves with linear leaflets and has yellow flowers in compound umbels. The pinnae of the leaves of *Anethum sowa* Roxb. plants are longer, slender and distantly located (Gupta, 1969) and their inflorescence consists of short peduncles, short primary rays, less number of umbels and less number of flowers per umbel in comparison to these characters of the European dill plants (Adhikari, 1965). The mericarps of European dill are brown, broadly oval, compressed, 3 to 4 mm. long, 2 to 3 mm. broad and 1 mm. thick. They have 5 ridges, three dorsal being inconspicuous and brown and the two lateral yellowish and winglike (Krishna and Badhwar, 1952). The fruits of sowa are usually in the form of cremocarps, are longer, strongly convex and narrowly winged (Gupta, 1969) and their volatile oil
contains a heavier fraction called dillapiole, which is usually absent in their herb oil (Gupta et al., 1958)

Shah et al. (1971a, 1971b, 1972a) described three varieties of Indian dill available in the local market. They are the cremocarp variety (Variyali sowo), the mericarp variety (Ghoda sowo) and the mixture of both (Vizag sowo). Excepting for some minor differences in their morphological and microscopical characters all the three varieties are similar in their general appearance. Under cultivation the vizag sowo fruits yield the tallest plants which possess more number of branches and flowers. Ghoda sowo plants differ from Vizag and Variyali sowo plants in having linear leaves, the pinnae of which are 3 to 4 times longer and are nearly 5 times less in number in each leaf. Variyali sowo herbs flowered one month earlier than the herbs of the other two varieties. Vizag sowo oil was completely devoid of dillapiole while the ratio of carvone and dihydrocarvone in the fruits of Variyali sowo, Ghoda sowo and Vizag sowo was 1:2;2:1 and 5:1 respectively. European dill fruits were found to contain only one flavonoid kaempferol, while the Indian sowo contains three flavonoids quercetin, kaempferol and isorhamnetin (Horborne and Williams, 1972).
Dark and Pale Variyali sowa:

Shah et al. (1975) reported further two varieties in the cramocarp variety and named them as black Variyali sowa and Pale variyali sowa. As these two varieties were selected for the present investigation, a detailed account of the botanical description, morphological and microscopic characters and chemical composition of the oils are discussed here in detail. The fruits of dark Variyali and Pale Variyali sowa are shown in figure 1. The appearance and morphological characters of dark Variyali sowa and pale Variyali sowa plants are shown in figure 2 and figure 3 respectively.

Pale Variyali sowa fruits give taller plants with more number of branches which possess linear leaves, pinnae of which are two times longer and three times less in number in each leaf in comparison with the dark Variyali sowa. Dark Variyali sowa herbs flower one month earlier than the pale Variyali sowa. Some morphological differences of the two plants are shown in table 1.

Dark Variyali sowa fruits are dark brown in colour having narrow wings whereas the pale Variyali sowa fruits are pale brown in colour with broader wings. Morphological differences between these two fruits are listed in table 2. (Jain, 1975).
FIG. 1

Dark and pale Variyali sowa fruits
FIG. 1
FIG.2: Whole plant of dark Variyali sowa

FIG.3: Whole plant of pale Variyali sowa
FIGS. 2-3
TABLE 2

Morphological differences of the fruits of dark variyali sowa and pale variyali sowa:

<table>
<thead>
<tr>
<th>Observations</th>
<th>Dark Variyali sowa</th>
<th>Pale Variyali sowa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shape</strong></td>
<td>Ovate, oblong, dorsally strongly convex, cremocarp with or without pedicel.</td>
<td>Void, lanceolate, slightly dorsally compressed, cremocarp with or without pedicel.</td>
</tr>
<tr>
<td><strong>Size: Length</strong></td>
<td>4.4 - 6.0 mm.</td>
<td>4.5 - 6.2 mm.</td>
</tr>
<tr>
<td><strong>Breadth</strong></td>
<td>1.6 - 2.3 mm.</td>
<td>1.9 - 2.9 mm.</td>
</tr>
<tr>
<td><strong>Breadth of wing</strong></td>
<td>0.1 - 0.2 mm.</td>
<td>0.2 - 0.4 mm.</td>
</tr>
<tr>
<td><strong>Colour</strong></td>
<td>Dark brown with yellowish narrow wings and ridges</td>
<td>Pale brown, yellowish with broader wings and ridges</td>
</tr>
<tr>
<td><strong>Weight of 100 cremocarps</strong></td>
<td>600 - 700 mg.</td>
<td>650 - 730 mg.</td>
</tr>
</tbody>
</table>
Even though some differences between the two varieties of Variyali sowa do exist, they are not helpful in clearly distinguishing the two varieties. The chemical composition of their volatile oils is more useful in distinguishing them. Pale Variyali sowa contains 4.5 to 5.0% v/w volatile oil as against 2.0 to 2.1% v/w of volatile oil present in the dark Variyali sowa. The oil of the former variety contains 39% limonene, 5% dihydrocarvone, 42% carvone and 14% dillapiole whereas the oil of the latter variety contains 24% limonene, 35% dihydrocarvone, 23% carvone and 18% dillapiole (Shah et al., 1975).

The fruits of all the varieties of Indian dill were found to contain dillapiole with the exception of Vizag sowa (Shah et al., 1971a, 1971b, 1972a, 1975). Moreover, all the varieties contained the three flavonoids, i.e. quercetin, kaempferol and isorhamnetin (Chauhan, 1974; Jain, 1975). This indicates that all the varieties are of Indian origin only and might be derived from Anethum sowa Roxb.

Sowa fruits are available throughout India and on an average, about 1350 tons of sowa fruits are sold in Indian market annually (Gupta, 1969). India's annual requirement of fruits of Anethum graveolens was estimated at about 10 tons (Virmani and Datta, 1970). About 400 tons of the Indian dill fruits are exported annually from India (Basias
et al., 1971a). An annual export of over 600 tons of dill fruits was reported by Gupta (1971).

Cultivation:

Dill is grown from seed. In cooler climates, the seed is sown in early spring (March to April). In the plains, where the summer is very hot, sowing is done in September to October. As the seeds are of small size, the soil should be made smooth and free of clods. The seed is sown in rows, usually 30 cm. apart, spaced less, if the soil is free of weeds. A fertile sandy loam soil is quite suitable for the cultivation of dill. As the yield of seed is better when the plants are not crowded too much, the plants are thinned at the proper time (Guenther, 1953). The field is irrigated according to the requirement, generally eight to nine irrigations are quite adequate for the proper maturity of the plants (R.R.L. Circular leaflet, 1961).

The crop is susceptible to weather hazards such as heavy hail, strong winds and heavy rains. Extreme heat during the critical periods of maturity is unfavourable to herbs, causing the reduction in the yield of their fruits and hence in oil considerably (Guenther, 1953).

The plants are usually attacked by Fusarium species which cause root rot and wilting (Janson, 1950).
Fruits obtained from plants grown on low nitrogen soils gave only a normal yield of oil, but the carvone content of the oil was higher than that of the fruits grown in nitrogen rich soils (El-Hinnawy, 1956). It was reported by Laszlo (1979) that for cultivation of green dill herb and its essential oil, large quantity of N and medium level of PK is optimal, whereas for dill seed and seed oil, a greater dose of P and a moderate NK fertilizer is optimal. Gibberellic acid when applied at the preflowering stage, produced early flowering, larger and thicker stems. With specific dose, volatile oil in the seed increased upto 50%. There was no significant increase in the carvone content of the volatile oil with lower concentrations of gibberellic acid, but with high concentrations there appeared to be slight increase in the carvone (Kaul and Kapoor, 1962).

Guenther (1953) suggested that when the crop is grown for fruit, the plants should be harvested when the earliest fruit is ripe. Kapoor and Abrol (1961) found that if the dill fruit is harvested at a stage when it is fully developed but still green in colour, i.e. seven to nine days after dropping of the petals (oil content 2.6 to 3.7), the oil and carvone content of the fruit are within the Pharmacopoeial limits (oil content 2 to 4% w/w, carvone content 43 to 63% w/w) and no shedding of fruits occurs, resulting in increase in the yield of the fruit. When the colour changes from
green to grey and dark grey, oil content tends to decrease and reaches the minimum level of 1.7%. At this stage profuse shedding takes place resulting in substantial decrease in the yield.

European dill is cultivated in India as a winter crop in Jammu and as a summer crop in Kashmir (Handa et al., 1955). Baslas et al. (1971) successfully cultivated *Anethum graveolens* in Indian conditions at Haldwani, Nainital district, India. The main difference of Indian and European dills is that the seeds of the former contain a heavy fraction 'dill-apiole' (Guenther, 1953) which is considered to be toxic (Wallis, 1967). Dill-apiole is absent in the fruits of European dill (Guenther, 1953). Dill herb oil from European dill contains dill-apiole (Guenther, 1953) whereas it is absent from the herb oil of Indian dill (Baslas and Gupta, 1971).

Dill-apiole was reported in *Anethum graveolens* grown in India by Baslas et al. (1971) and Chaudhry et al. (1957). Later Betts (1969) reported that dill-apiole was absent from dill grown even in India and present in the fruits of Indian dill even if they were grown in Europe.

Sowa fruits are sown in winter and the crop is harvested in the month of April (Menon, 1960). The important districts of India where sowa is cultivated are: West Khan-
desh, Surat and Nasik. About 1000 hectares of land in India, on an average is used for the cultivation of sowa, of which larger part of young plants are coppiced and used as fresh leafy aromatics (Gupta, 1969a).

For the production of herb oil, the plant should be cut immediately after the blooming period, when the seed has just started to ripen but not yet fully developed (Guenther, 1953). But Malaviya and Dutt (1940) studied the herb oil of Indian sowa before the plants started flowering and fruiting.

Dill oil :

Dill fruit contains from 2 to 4 per cent of volatile oil (sp.gr. 0.900 to 0.915; optical rotation + 70° to + 80°) consisting of carvone (40 to 60%). It is a colourless liquid with strong dill odour and limonene associated with phyllandrene and other terpenes (Wallis, 1967).

Some of the physical constants and chemical composition of the oil Anethum sowa herb and fruit are recorded in tables 3 and 4 respectively. The physical constants and percentage of the individual components of the oils from the fruits of different chemical races of Anethum sowa are presented in table 5. Standards laid out for the European dill oil are given in table 6. Khafagy and Mnajed (1968) examined the seed oil of Anethum sowa plants grown in Egypt. The seeds yielded 3% volatile oil. As constituents, limo-
### TABLE - 3

Physical constants and chemical composition of *Anethum sowa* herb oil and fruit oil:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plant part</th>
<th>% oil v/w</th>
<th>Specific gravity</th>
<th>Optical rotation</th>
<th>Refractive index</th>
<th>Phellandrene</th>
<th>Limonene</th>
<th>Carvone</th>
<th>Dihydrocarvone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rao et al., Fruit</td>
<td>3.19</td>
<td>0.9785</td>
<td>+47.60(^\circ)</td>
<td>1.4943</td>
<td>-</td>
<td>-</td>
<td>19.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malaviya and Dutt,</td>
<td>Herb</td>
<td>0.062</td>
<td>0.8726</td>
<td>286.6(^\circ)</td>
<td>1.4867</td>
<td>74.6</td>
<td>-</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>1940</td>
<td></td>
<td>(15(^\circ)C)</td>
<td>(20(^\circ)C)</td>
<td>(20(^\circ)C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>not mentioned</td>
</tr>
<tr>
<td>Malaviya and Dutt,</td>
<td>Fruit</td>
<td>0.474</td>
<td>1.0573</td>
<td>+23.6(^\circ)</td>
<td>1.5385</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1940</td>
<td>(heavy fraction)</td>
<td>(20(^\circ)C)</td>
<td>(20(^\circ)C)</td>
<td>(20(^\circ)C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaviya and Dutt,</td>
<td>Fruit</td>
<td>0.825</td>
<td>0.9719</td>
<td>+38.5(^\circ)</td>
<td>1.4905</td>
<td>-</td>
<td>9.0</td>
<td>19.5</td>
<td>not mentioned</td>
</tr>
<tr>
<td>1940</td>
<td>(light fraction)</td>
<td>(20(^\circ)C)</td>
<td>(20(^\circ)C)</td>
<td>(20(^\circ)C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gupta, 1958</td>
<td>Fruit</td>
<td>2.50</td>
<td>0.9341</td>
<td>+54.0(^\circ)</td>
<td>1.4854</td>
<td>-</td>
<td>41.70</td>
<td>7.2</td>
<td>15.6</td>
</tr>
<tr>
<td>Adhikari, 1965</td>
<td>Fruit</td>
<td>2.04-</td>
<td>0.9763</td>
<td>+40.13(^\circ)</td>
<td>1.4963-</td>
<td>-</td>
<td>14.50-</td>
<td>3.5-</td>
<td>40.0-</td>
</tr>
<tr>
<td></td>
<td>3.78</td>
<td>1.0192</td>
<td>46.51(^\circ)</td>
<td>1.5044</td>
<td></td>
<td></td>
<td>30.00</td>
<td>11.6</td>
<td>50.0</td>
</tr>
</tbody>
</table>
TABLE 4

Percentage and chemical composition of the fruit oil of Anethum sowa: (Percentage of individual constituents in the oil v/v)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Phellandrene</th>
<th>Limonene</th>
<th>Carvone</th>
<th>Dihydrocarvone</th>
<th>Dillapiole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braniga, 1946</td>
<td>traces</td>
<td>40.0-60.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30kravarty and Bhattacharya, 1954</td>
<td>-</td>
<td>34.4</td>
<td>30.0</td>
<td>9.0</td>
<td>18.9</td>
</tr>
<tr>
<td>Gupta et al., 1958</td>
<td>-</td>
<td>28.7</td>
<td>41.70</td>
<td>7.2</td>
<td>15.6</td>
</tr>
<tr>
<td>Sethi et al., 1965</td>
<td>-</td>
<td>19.7</td>
<td>19.08</td>
<td>4.38</td>
<td>-</td>
</tr>
<tr>
<td>Misra and Nigam, 1969</td>
<td>-</td>
<td>-</td>
<td>51.40</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Baslas and Gupta, 1971</td>
<td>11.4</td>
<td>21.4</td>
<td>20.70</td>
<td>14.30</td>
<td>8.6</td>
</tr>
<tr>
<td>Miyazawa and Kameoka, 1974</td>
<td>-</td>
<td>5.9</td>
<td>20.9</td>
<td>16.6</td>
<td>52.5</td>
</tr>
<tr>
<td>Asharaf et al., 1977</td>
<td>-</td>
<td>9.3</td>
<td>-</td>
<td>-</td>
<td>20.3</td>
</tr>
<tr>
<td>Lawrence, 1980</td>
<td>0.1</td>
<td>45.0</td>
<td>23.1</td>
<td>4.2</td>
<td>20.7</td>
</tr>
</tbody>
</table>
### TABLE 5

Physical constants and Chemical Constituents of different Chemical races of Indian Dill
(Anethum sowa)

(Percentage of individual constituent in oil)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Chemical race</th>
<th>% oil</th>
<th>v'w</th>
<th>Specific gravity</th>
<th>Optical rotation</th>
<th>Refractive index</th>
<th>Limonene</th>
<th>Carvone</th>
<th>Dihydrocarvone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shah et al., 1971</td>
<td>Ghoda sowa</td>
<td>2.7-3.5</td>
<td>0.9523</td>
<td>0-9801</td>
<td>+47.58° - +45.52°</td>
<td>1.4860 - 34.93</td>
<td>36.67</td>
<td>15.60</td>
<td>26.00</td>
</tr>
<tr>
<td>Shah et al., 1972</td>
<td>Vizag sowa</td>
<td>5.0-6.0</td>
<td>0.9554</td>
<td>+60.01°</td>
<td>1.4805 - 45.25</td>
<td>49.01</td>
<td>9.32</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Shah et al., 1971</td>
<td>Vizag sowa</td>
<td>5.0-6.0</td>
<td>0.9547</td>
<td>0.9726</td>
<td>+26.26° - +20.30°</td>
<td>1.4900 - 20.20</td>
<td>16.65</td>
<td>31.35</td>
<td>26.50</td>
</tr>
<tr>
<td>Shah et al., 1975</td>
<td>Black sowa</td>
<td>2.07-2.12</td>
<td>0.9440</td>
<td>+26.26°</td>
<td>1.4850 - 24.25</td>
<td>22.61</td>
<td>35.23</td>
<td>17.91</td>
<td></td>
</tr>
<tr>
<td>Shah et al., 1975</td>
<td>Pale sowa</td>
<td>4.50-5.00</td>
<td>0.9271</td>
<td>+69.30°</td>
<td>1.4900 - 39.09</td>
<td>41.62</td>
<td>5.15</td>
<td>14.14</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE - 6

Some pharmacopoeial standards of European Dill oils:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Specific gravity</th>
<th>Optical rotation</th>
<th>Refractive index (at)</th>
<th>Carvone content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian Pharmacopoeia, 1966</td>
<td>0.916 - 0.942</td>
<td>+70° - 80°</td>
<td>1.4848 - 1.4868</td>
<td>minimum: 43</td>
</tr>
<tr>
<td></td>
<td>0.942</td>
<td></td>
<td></td>
<td>maximum: 63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(at 25°)</td>
</tr>
<tr>
<td>British Pharmacopoeia, 1958</td>
<td>0.895 - 0.910</td>
<td>+70° - 80°</td>
<td>1.4849 - 1.4868</td>
<td>minimum: 43</td>
</tr>
<tr>
<td></td>
<td>0.910</td>
<td></td>
<td></td>
<td>Maximum: 63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(at 20°)</td>
</tr>
<tr>
<td>Guenther, 1950</td>
<td>0.918 - 0.918</td>
<td>+71° 10' - 76° 25'</td>
<td>1.4881 - 1.4882</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>0.910</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
nene, traces of \( \Delta ^{2} \) -pinene, carvone (37\%) and dihydrocarvone were identified; dillapiole was present in an amount of 27\%. Betts (1965) reported that dillapiole when present, occurred in about twice the amount of carvone. Stahl and Herting (1976) found that some fruits from *Anethum sowa* contained no carvone at all.

The accumulation of carvone in the developing fruits has been studied by Kalitzki (1954). He reported an increase of carvone (from 4\% to 50\%) from the inflorescence to the stage of green fruits as well as during ripening of the fruits (60\%). Ihloff (1956) observed 70\% of phellandrene in the flower buds which depleted after fertilization with a concomitant increase in carvone content. But Luyendijk (1954, 1957) ruled out the possibility of carvone being derived from phellandrene. Kapoor and Abrol (1961) also observed a gradual increase in the carvone content from fertilized flower to the mature fruit of *Anethum graveolens*. Betts (1965) reported that carvone amount increased from traces until a specific level was reached which took place about four weeks after pollination.

Bandopadhyay et al. (1972) reported coumarins and flavonoids petroselinic acid and \( \beta \)-sitosterolglucoside from dill fruits. Recently Thomar and Mukerjee (1981)
reported a new compound known as dillapional from the fruits of *Anethum sowa* Roxb.

**Biosynthesis of volatile oils:**

The biosynthesis of the monoterpenes and their oxygenated derivatives proceeds via the acetate mevalonate pathway (Stanley, 1958; 1961; Birch et al., 1959; Reitsema et al., 1960; Sandermann and Schweers, 1962a; 1962b; Sandermann and Bruns, 1962, 1965; Banthorpe and Turnbull, 1966; Hefendehl, 1966; Schantz and Huhtikangis, 1971; Akhila et al., 1980). They observed that incorporation of mevalonate into monoterpenes is so low that it might suggest the existence of an alternate pathway. The simplest monoterpenes are derived by head-to-tail condensation of the isoprene units (Ruzicka, 1953). Ruzicka (1953) in a discussion of the isoprene rule suggested mechanisms by which the various monoterpenes might be derived from geraniol as a starting point (fig.4). Murray (1966 a,b), Lincoln et al. (1971), Hefendehl and Murray (1972). Murray and Hefendehl (1972, 1973) and Sabti (1973) have clearly demonstrated the genetic control of various terpene constituents in the essential oils of *Mentha* species.

The *Mentha* plants with dominant gene C in homozygous form along with dominant form i.e. genotype Cc/CC produce carvone and dihydrocarvone. The gene I/i controls the conversion of linalool to -terpineol and further derived
FIG. 4: BIOSYNTHESIS OF CARVONE AND MENTHONE FROM GERANYL PYROPHOSPHATE

ISOPENTENYL PYROPHOSPHATE

DIMETHYLLALYL PYROPHOSPHATE

LINEAR OR CYCLIC INTERMEDIATES

REITSEMA DESIGN (1958):

FUJITA DESIGN: (1960)

LOOMIS DESIGN: (1967)

ACYCLIC TYPES AS CITRAL AND LINALOOL

CIRCULAR TYPES AS CITRAL AND LINALOOL

2 - OXYGENATED SERIES

3 - OXYGENATED SERIES
constituents from it. The dominant gene Lm prevents the conversion of L. limonene to isopiperitenone as postulated by Fujito (1960) or terpineol to terpineolene as postulated by Loomis (1967) in the presence of recessive genotype cc which prevents the conversion of limonene to carvone.

The conversions of geraniol to other monoterpenes are mainly based on postulated mechanisms but direct evidence is available to substantiate the conversion of one compound to the other. Reitsema (1958) expressed his opinion that the addition of oxygen to a terpene such as limonene to produce carvone is a known autooxidation reaction which should not be ignored, but it does not explain the absence of carvone in all but a few limonene containing oils. In the experiment with labeled $^{14}$CO$_2$ in Carum carvi, it became evident that the bio-synthesis of carvone does not involve limonene as a precursor (Schantz and Hutikangas, 1971). They postulated that these two terpenes must be independently formed from a common precursor in a living cell. Akhila et al. (1980) working with $^3$H and $^{14}$C labeled geranil and mevalonate on Mentha spicata came to the conclusion that oxidation of limonene of its biogenetic equivalent to form carvone involves shift of the endocyclic double bond and that limonene and carvone are biogenetically related and are probably formed on divergent pathways from a single common intermediate.
Luyendijk (1954) did not find any evidence for a secondary transformation of limonene to carvone. The formation of essential oils is promoted under anaerobic conditions and it is an energy furnishing process as given by the equation

$$3 \text{C}_6\text{H}_{12}\text{O}_6 + 4 \text{O}_2 \rightarrow \text{C}_{10}\text{H}_{16} + 10 \text{H}_2\text{O} + 8 \text{CO}_2 + 549 \text{cal}$$  
(Burmiester and Guttenberg, 1960).

It has been shown that high photoperiods promote the formation of oxygenated monoterpenes (Birch, 1977).

In elucidating the configuration of the carveomenthols, carveols, and related compounds, Schraats and Eliel (1965) gave a degradation scheme which is shown in fig. 5.

The biogenetic pathways proposed for phenolic and terpenes are quite different and independent of each other (Hoagen-Smit, 1953; Neish, 1960). The principal precursors for phenylpropanoid compounds are cinnamic acid and p-hydroxyacinamic acid (fig. 6). In plants these compounds arise from the aromatic amino acids phenylalanine and tyrosine respectively. Work on Ocimum species by Sobti (1976) showed that though the two pathways are independent of each other to a larger extent, appear to be connected before the formation of phenylalanine from which the aromatic ring of phenolics is considered to be derived. It was presumed that the two enzymatic systems responsible for the two
FIG. 5: DIAGRAM SHOWING THE CONNECTION OF THE CARVEOLS WITH THE CARVOMENTHOLS
FIG. 6: SOME p-HYDROXY CINNAMIC ACID DERIVATIVES

- Asarone  \( \rightarrow \) p-Methoxy cinnamic acid  \( \rightarrow \) Anethol
- \( p \)-Coumaric acid  \( \rightarrow \) Estragol
- Chavicol  \( \rightarrow \) Eugenol
- Apiole  \( \rightarrow \) Allyl catechol  \( \rightarrow \) Methyleugenol
- Dillapiole  \( \rightarrow \) Elemycin  \( \rightarrow \) Myristicin
biosynthetic pathways, although independent of each other, are linked through phosphoenol-pyruvate and pyruvate by breakdown of carbohydrates from which isopentane units responsible for the formation of terpenes and phenylalanine the precursors of phenols are derived. The author found an inverse correlation between linalool and methyl-cinnamate (Sobti, 1976).

**Extraction of the volatile oils:**

There are mainly four methods employed in the production of volatile oils. They are the distillation with steam, distillation *per se*, expression and extraction.

The term 'steam distillation' is used for three different types of hydrodistillations. They are the water distillation in which the plant material is added to the boiling water and the vapours liberated are condensed and collected. In another method of steam distillation the plant material is placed on a screen which is placed slightly above the water level of the still, thus exposing the material to the saturated steam. In direct steam distillation the steam is produced in a separate boiler and is introduced through perforated coils in the packed plant material in another vessel. A method of distillation of oil using modified Clevenger apparatus is described in Indian Pharmacopoeia (1966). Kalitzki (1954) noticed that during the first two hours of distillation of the oil,
mainly of the oil, mainly carvone was obtained and subsequently, the carvone content decreased proportionately to the duration of distillation. This observation was in agreement with the comment of Guenther (1949) who stated that quite the opposite was found for limonene. The finding that carvone appeared in the first fractions during distillation, whereas limonene appeared later, were supported by the results of Chubey and Dorrell (1976) and Balinova-Tsvetkova et al. (1976). However, hydrodistillation was found to be affecting the ratios of carvone and dihydrocarvone (Scheffer et al., 1977). Extraction with organic solvents like pentane/diethyl ether was proved to be more satisfactory (Koedam et al., 1979).

Column Chromatography of the volatile oils:

In column chromatography of the volatile oils containing monoterpenes hydrocarbons and oxygen containing monoterpenes, Garg and Nigam (1970) used silica gel and kieselguhr (4:1) eluting with petroleum ether followed by benzene, chloroform and ethyl acetate. Scheffer (1978) suggested a lot of modifications in the liquid solid chromatography of the volatile oils. He used silica gel (acid-base washed) containing 5% water and eluted with pentane at 10°C followed by elution with pentane/diethyl ether (1:1). He was able to obtain a good separation of the monoterpenes hydrocarbons as well as oxygen containing monoterpenes.
Thin Layer Chromatography:

Stahl (1965) suggested a number of solvent systems for thin layer chromatography of the volatile oils. Atal and Shah (1964) employed silica gel and a solvent system containing 5% ethyl acetate in benzene, 1% vanillin in phosphoric acid was used as the spray reagent wherein the coloured spots were visualized after heating the sprayed plates at 110°C for ten minutes. Betts (1964) used silicic acid containing fluorescein for investigation of the dill by thin layer chromatography.

Gas Liquid Chromatography:

For the gas liquid chromatography of the volatile oils, PEG columns were proved to be more suitable (Scheffer, 1978). A column temperature from 60°C-180°C gave satisfactory results. 10% silicone SF 96 columns and 10% B,B'-oxydipropionitrile (ODPN) columns were also employed. Analysis of the volatile oils from dill was also described by Betts (1969) and Chauhan (1974) and that for caraway oil by Solveson and Baerheim Svendsen (1976). A prefractionation by liquid solid chromatography followed by G.L.C. analysis was recommended by Scheffer (1978).

Other procedures for the estimation of total carbonyl compounds and carvone, in addition to the G.L.C. method
are also available. Carvone content was determined by using neutral sodium sulphate (National Formulary, 1975). A titrimetric procedure using hydroxylamine was given by I.P. (1966) and B.P. (1958). Mohan Verma (1960) determined carvone content spectroscopically by measuring the absorbance at 320 nm and 235 nm. Betts (1965) estimated the amount of carvone in the oils of *Carum carvi* and dill spectrophotometrically.

Dillapiole content was determined spectroscopically by taking the oil in spectrophotically pure methanol and measuring the absorbance at 288 nm (Chauhan, 1974).

**Pharmacology of dill oil:**

An antimoulting effect of dill oil in syrups was determined by Lord and Husa (1954). Kellner and Kober (1954, 1955, 1956) tested dill oil as well as carvone against a number of microorganisms. The growth of many bacterial species tested was inhibited. Maruzzella and Lichtenstein (1956) reported the antimicrobial activity of dill oil. Dill seed oil inhibited the growth of *Bacillus subtilis, Serratia marcescens* and *Escherichia coli*, which was tested by the filter paper disk method. The oil also showed an antifungal activity, especially against *Ustilago avenae* (Maruzzella and Liguori, 1958; Maruzzella, 1960; Maruzzella et al., 1960). Dhar and
co-workers (1968) reported that the oil of *Anethum sowa* gave positive effects for hypoglycemic properties, effect on blood pressure and on isolated intestinal tissues. The use of dill oil for flavouring and seasoning food, beverages and similar products was dealt with by many authors (Branigan, 1946; Lindemann, 1967; Karow, 1969, Lichtenstein et al., 1974) and also its use in soap and perfumery products instead of caraway oil was suggested (Virmani and Datta, 1970). Shah et al. (1972b) suggested that Indian dill can be used in place of European dill as its acute toxicity was very low. The FAO/WHO Expert Committee on Food Additives established a conditionally acceptable human daily intake of carvone up to 1.25 mg./kg. body weight (Anonymous, 1974).

*Trigonella foenum-graecum* Linn. :-

*Trigonella foenum-graecum* Linn. is a common household spice which belongs to the sub-family Papilionaceae. The seeds are used in perfumery, pickles, condiments and preparations of vegetables. The seeds are hot with a sharp bitter taste, tonic, anti-pyretic, anthelamnetic, increase the appetite, astringent to the bowels, cure leprosy, 'vata', vomiting, bronchitis, remove bad taste from the mouth, useful in heart diseases (Chopra et al., 1958). Fenugreek seeds are considered carminative and aphrodisiac, several confections made with this article are recommended for use
in dyspepsia with loss of appetite, in the diarrhoea, and in rheumatism. An infusion of the seeds is given to smallpox patients. The plant is considered a useful crop with commercial potential for extraction of diosgenin. This plant is known by different names in different languages. They are: Sans. Madhika; Hindi, Bengali, Sindi, Guj., Marath-Methi; English-Fenugreek; Arab-Hulabaha; Pers.-Shamlite; Tel.-Menthulu; Tel.-Vandayam; Mal.-Uluve/Vantayam; Kan.-Menthin; Kon.-Metthi.

*Trigonella foenum-graecum* Linn. is an annual herbaceous plant widely cultivated in many parts of Asia, Africa and Europe. This crop is grown in India traditionally as winter crop (Singh and Kewala Nand, 1984). Out of many species studied, only *Trigonella foenum-graecum* showed good amount of diosgenin (0.5-0.8% as reported by Hohannon et al., 1974).

In India it is distributed in Kashmir, Punjab and the Upper Gangetic Plain and it is widely cultivated in many parts of the country (The Wealth of India, 1976). Because of its diversified uses it has very high export value. The export data reveals that 5.3 thousand tonnes of fenugreek seeds valuing 188.86 lakhs of rupees, were exported during 1978-79 (Singh and Kewal Anand, 1984). Fenugreek is an easily cultivable plant which takes nearly
three months to complete its life cycle. Advancement of the sowing date October 15 to November 15 resulted in significant increase in the yield. A closer row spacing of 15 cm. outyielded over remaining wider row spacings. High grain yields were also recorded with the application of 45 kg./ha of nitrogen (Singh and Kewal Anand, 1984).

*Trigonella foenum-graecum* plant is a small smooth and erect aromatic herb growing to a height of 40-60 cm. The stem is hollow. The main stem gives 3 to 5 branches. The internodal length at the lower part of the herb is much lesser compared to the upper portion, which usually measures 3-4 cm. The stem is usually covered by covering and glandular hairs the former of which are two celled in nature, the lower one small and the upper one long with a thick wall tapering towards the tip. Some plants show purplish stem colouration throughout the length of the plant whereas some others show faint purple colour at the branches near the node. Leaves are trifoliolate with 3 to 4 mm. long stipules and about 1 mm. long petioles. Leaflets are nearly sessile or sub-sessile. Leaflets vary from 5 to 8 cm. in length and 5 to 8 mm. They possess toothed margin. Surface of the leaves is smooth. Epidermis shows Ranunculus stomata. Calcium oxalate crystals are absent from the leaves. Flowers are white, 1 or 2, axillary, pods 3-15 cm. long, 10-20 seeded; seeds greenish
brown, 2.5 - 5.0 x 2.0 to 3.5 mm., oblong with a deep groove across one corner giving the seed a hooked appearance. The general appearance of the plant is shown in fig. 7.

Fazli and Hardman (1971) reported 0.83% and 0.92% diosgenin in seeds obtained from Pakistan and Morocco respectively. But Bakshi and Hamed (1971) reported very low content of diosgenin in Algerian (0.35%), Morocco (0.25%) and Indian (0.1%) seeds of *Trigonella foenum-graecum*. With Infra-red spectroscopic analysis, Hardman and Fazli (1972a) found *Trigonella foenum-graecum* to contain 1.41% of diosgenin. The callus developed in *Trigonella foenum-graecum* tissue culture was reported to contain as high as 1.82% of diosgenin (Khanna and Jain, 1973). Incorporation of cholesterol in the culture medium further raised the diosgenin to 3.5% (Khanna et al., 1975). The seeds from Kangra (Himachal Pradesh) contain 1.02% of total sapogenins as diosgenin and yamogenin (Puri et al., 1976).

**Other sources of diosgenin:**

Besides *Trigonella foenum-graecum* there are other and even more valued sources of diosgenin in the plant kingdom. Of all known sources *Dioscorea* which comprises nearly 50 species occupies the foremost position as a
FIG. 7

Whole plant of *Trigonella foenum-graecum*
reliable sources of diosgenin (The health of India, 1952). But a number of problems arise in its cultivation and utilization because of the high cost involved and long life cycles of the plant. Hence extensive investigations were carried out to find some alternative sources which could yield diosgenin in workable quantities. This had become necessary because of the complexity and high cost involved in the chemical synthesis of diosgenin. Of the many plants screened so far, Costus speciosus, Balanites Roxburgii, Kallstroemia pubescens and Trigonella foenum-graecum yielded diosgenin in amounts worth considering for commercial exploitation.

Costus speciosus which occurs wild in many parts of India showed diosgenin between 0.32 to 3.37% (Bedi et al., 1976). The leaves of Kallstroemia pubescens contain 1.78 to 1.92% diosgenin in free and glycosidal form (Chakravarti et al., 1976). The pericarp of unripe but full grown fruit of Balanites aegyptiaca afforded 4.08% diosgenin/yamogenin on dry weight basis (Hardman and Sofowora, 1972).

It was established that high yields of diosgenin could be achieved from the plants through post-harvest incubation of the plant material in water and solutions. A 200% increase in diosgenin yield was noticed by
Blunden and Hardman (1963) and Hardman (1969a) after incubating *Dioscorea floribunda* tubers in distilled water. Incubation of leaves with distilled water resulted in 29-127% higher yield in Yucca glauca (Blunden et al., 1965). Hormonal solutions like IAA, NAA, GA and 2,4-Dichlorophenoxyacetic acid also exerted profound influence on the diosgenin yield when plant materials were incubated in these solutions. Hardman and Fazli (1972b) attributed that this increase of diosgenin is due to the fresh synthesis or release of hydrolytic enzymes that are capable of releasing the diosgenin bound to the cell wall of plant cells. Diosgenin precursors like squalene, when supplied to the incubation media, got readily incorporated into diosgenin and increased its yield (Hardman and Brain, 1971b). Hence it is also possible that incubation with water or hormone solutions liberates or activates such enzymes which are capable of converting the available precursors into diosgenin (Hardman and Brain, 1971b).

From their experimental observations Selvaraj and Subashchander (1982) expressed the opinion that cellulolytic and macerating enzymes do play a role in releasing additional saponin/genin formally held by adsorption and the major contribution towards increased yield was the result of biosynthesis of diosgenin by enzyme system capable of acting on available and/or added precursors. Shah et al.
(1978) observed an increase of 270% in diosgenin content from *Costus speciosus* incubated with 2,4-D.

Hardman and Brain (1971a) incubated *Trigonella foenum-graecum* seeds with IAA, and GA and could obtain 35% more yield of diosgenin. *Trigonella* seeds, when incubated with ascorbic acid, cholesterol and ascorbic acid plus cholesterol, yielded 16.5% and 29.5% more diosgenin as compared to the unincubated seeds (Bhavsar et al., 1980). Gamma irradiated fenugreek seeds when incubated in sodium azide solution afforded 111.55% higher yield of diosgenin after 24 hours (Bhusari et al., 1982).

Diosgenin, the main constituent of *Trigonella foenum-graecum*, belongs to a class of chemical compounds known as steroids. Steroids are pharmacologically active substances which include sex hormones, corticosteroids, bile acids, sterols, certain sapogenins and some glucoalkaloids like solasodine. They have a perhydrocyclopentanophenanthrene nucleus in common (fig.8). The utility of the plant steroids as a source of corticosteroids, oestrogens, progesterones, androgens, anabolic agents and oral contraceptives was recognised by Hardman (1969a) Diosgenin, first discovered by Fujii and Matsukawa (1936), was shown to be an important starting material in the production of corticosteroids (Marker, 1943). Diosgenin (25R-spirosta-5 en-3
FIG. 8: PERHYDROCYCLOPENTANOPHENANTHRENE
B-ol), occurs generally in combined form as glycoside and rarely in free state (Fieser and Fieser, 1959; Das Gupta and Pandey, 1970; Chakravarti et al., 1976). It is present as rhamno-rhamno-glucoside called dioscin (25 R-spirosta 5-2n-3-β-yl-O-6-deoxy-α-L mannopyranosyl (1→4) B-D-glucopyranoside OR diosgenin bis α-L-rhamnopyranosyl (1→2 and 1→4) B-D-glucopyranoside in tubers of commercial Dioscorea species (Hardman, 1969b).

**Biosynthesis of Diosgenin:**

Acetyl-CoA is the common precursor for the biosynthesis of both steroidal and triterpenoidal sapogenins. It is converted to mevalonate which, through further conversions, forms squalene. The steps involved in the conversion of squalene to cholesterol are well established (fig.9).

Bennett and Heftmann (1964) have demonstrated the metabolic conversion of cholesterol to diosgenin by the administration of 4-14C to the leaves of Dioscorea spiculiflora seedlings and subsequent isolation of radioactive diosgenin. Probably kryptogenin is an intermediate in this conversion. Kryptogenin contains more keto groups and it is easily converted to spiroketal ring of the sapogenins (Luckner, 1969). Bennett et al. (1970) found that 26-hydroxy cholesterol 26-14C was converted to diosgenin in Dioscorea floribunda and suggested that C-26 oxygenation is not the last step in biosynthetic pathway. The
FIG. 9: BIOSYNTHESIS OF CHOLESTEROL
conversion of cholesterol to diosgenin is shown in fig. 10.

During its conversion to other steroidal drugs, diosgenin is first converted to 16-dehydropregnanolone acetate (16-DPA). The conversions which are based on Marker's degradation method are affected by chemical and microbiological means. Production yield of 16-DPA from diosgenin is estimated to be above 60%. Further conversion of 16-DPA to different hormones is carried out by several chemical and microbiological reactions.

Methods of Extraction of Diosgenin:

Diosgenin can be extracted from crude plant material by three methods. (i) Alcoholic extraction of the glycoside from the plant material followed by acid hydrolysis (Peal, 1957). It was observed that during the acid hydrolysis, part of the diosgenin was lost by formation of 25 R-spirosta-3,5 diene due to the dehydration in ring A. (ii) The acid hydrolysis method involves the break down of the glycosidic linkage, and extraction of the released genins with a hydrocarbon solvent (Rothrock et al., 1957; Hardman and Fazli, 1972b). Suitable conditions for the hydrolysis of the dioscin were realized to be 15 lb. pressure, lower acid concentration (2 N H₂SO₄) and one hour duration. The hydrolysis should be followed by thorough washing with a base and subsequently with distilled water.
CHOLESTEROL $\rightarrow$ KRYPTOCOGENIN $\rightarrow$ DIOSGENIN

FIG. 10
The formation of diene can be minimised by aqueous incubation of the plant material before hydrolysis (Hardman and Wood, 1971; Hardman et al., 1972). (iii) Fermentation-cum-acid hydrolysis method was given by Hardman and Brain (1971b) and Hardman (1969b).

Methods of Analysis of Diosgenin:

Different methods are available ranging from gravimetric to High Performance Liquid Chromatographic for the analysis of diosgenin in the plant materials. The gravimetric method was developed by Morris et al. (1958). Yamagishi and Nakamura (1959) described a colorimetric procedure for the estimation of pure diosgenin using a solution of antimony trichloride in a mixture of nitrobenzene and methanol. Libermann's modified reagent was employed for colour development by Mu-Chou-Sand and Tung-Hui Chou (1964) whereas 0.5% formaldehyde in 60% sulphuric acid was used by Panina et al. (1963). Akira-Akahori et al. (1966) found ferric chloride-phosphoric acid to be a good reagent for determination of saturated or 5 sapogenins. When 5 sapogenin such as diosgenin was shaken with a 25% solution of antimony trichloride in 60% perchloric acid, a pink colour (\( \lambda_{\text{max}} \) 500 nm) is produced. The colour reaction follows Beer's law at room temperature (Sofowora and Hardman, 1974). A rapid Spectrophotometric method for quantitative determination of
Diosgenin from *Dioscorea* tubers was developed by Rishi et al. (1976).

Blunden et al. (1967) developed densitometric thin layer chromatography technique for the determination of diosgenin from *Dioscorea* tubers. Silica gel 'G' was the adsorbant and Hexane: Ethyl acetate (4:1) was the solvent system. Visualization was done with antimony trichloride. A linear relationship was noticed between Log of weight of diosgenin applied and square root of the absorbance of the spot produced. Quantitative evaluation was achieved by using photoelectric densitometer coupled with an integrating logarithmic recorder which computed the absorbance of the spot. One of the major sources of error in quantitative T.L.C. is the spotting procedure. Brain and Hardman (1968) improved the spotting procedure. They used a template and syringe assembly for sample application. Gas liquid chromatography was applied for the quantitative estimation of diosgenin by Kaul et al. (1969); Cooke (1970) and Dixit et al. (1977). A pyrex glass column 30 inches x 1/8 inch was packed with acid washed DMCS treated chromosorb G (80-100 mesh) coated with S.E. 30. Operating temperatures were 240°C for oven and injector block and 250°C for detector. Gas flow rates were 28 ml./minute for nitrogen, 26 ml./minute for hydrogen and 250 ml./minute for air. The total steroidal residue is
Estimation of diosgenin using infra-red spectrophotometry was carried out by Wall et al. (1952), Brain et al. (1968) and Jeffries and Hardman (1968). The separation of diosgenin and yamogenin on T.L.C. was obtained by Bennett and Heftmann (1966) where the distinct zone between the epimer bands was achieved with dichloromethane: diethylether (196:4) for 16 hours and a sample loading of 25 mg. per plate. The epimers were detected at 366 nm. as pale blue fluorescence on a yellow background.

Hardman and Jeffries (1972) developed a routine procedure involving the use of silica gel column for separating diosgenin and yamogenin and further analysing the samples by IR spectrophotometry. They further improved the column chromatographic technique by using water containing solvents (Hardman and Jeffries, 1976). Water saturated solvent mixtures of hexane and ethyl acetate in proportion of (9 + 1), (2 + 3) and (3 + 1) used.

The application of H.P.L.C. in the quantitative analysis of diosgenin and other sapogenins viz. hecogenin, tigogenin and stigmasteryl from Dioscorea deltoidea was reported by Tal and Goldberg (1981).
Pharmacology of Trigonella foenum-graecum:

Trigonella foenum-graecum show oxytocin like activity (Abdo and Alkajawi, 1969). Fenugreekine was found to have antiviral action and hence its traditional use in small pox is justified (Ghosal et al., 1974). Fenugreek also acts as insect repellent (Singh and Mehra, 1970). They fatty oil and unsaponifiable matter of Trigonella foenum-graecum possess antibacterial and antifungal properties against all the gram +ve and gram -ve bacteria and fungi tested. The antimicrobial activities, however, are less than those of known standard substances (Rathes et al., 1980). B-sitosterol was proved to have anti-inflammatory and antipyretic activity (Gupta et al., 1980). Fenugreek lowered the serum cholesterol in rats (Singhal et al., 1982).

Adhatoda vasica Nees.:

Adhatoda vasica Nees. (family Acanthaceae) is a well known Indian medicinal plant which has been in the treatment of asthma, inflammation of the respiratory tract and as anti spasmodic (Kirtikar and Basu, 1975). This drug has been put to use by traditional midwives during delivery to check post-partum haemorrhage (Nadkarni, 1954). This plant is known by different vernacular names in India. They are Beng.-Bakas, Vasaka; Bombay-Adalsa, Adorse, Adulasa, Adulaso; Dec.-Aratora;
Vasaka is a stiff, perennial, small gregarious evergreen shrub with a fetid smell. It is found all over the plains of India and in lower Himalayan ranges up to an altitude of 250 meters. It grows in wastelands in a variety of habitat and soil. *Adhatoda vasica* is the main source of quinazoline alkaloid, vasicine supposed to be an active principle (Jain, 1983).

The genus *Adhatoda* comprises of about 6 species of which only two, *Adhatoda vasica* Nees. and *Adhatoda beddomei* Clarke are found in India.

*Adhatoda vasica* Nees. is an evergreen shrub, growing to a height of 3 to 8 feet and often found gregarious in nature. Besides in India, it is also found in Ceylon, Burma and Malaya Peninsula. The shape of the stem is cylindrical excepting at the nodes where it is somewhat flattened and swollen and angular. The outer surface of the stem in the lower region is smooth excepting a few scattered lenticels, but in the upper region it is minutely puberulour. Externally the bark has a greyish-
white colour which becomes light brown in older stems and internally it has a light yellow colour. The internodes vary from 2.8 cms. in length particularly in the upper half part of the stem. At the nodes the leaves are arranged opposite and decussate and the branches are ascending.

The leaves are simple, petiolate and extipulate. Young leaves are about 5 to 12 cm. long and 1.5 to 2.0 cm. broad with a petiole 5.5 to 2.0 cm. in length. They are lanceolate, light green in colour and puberulous. The mature leaves are, however, somewhat glabrous and mostly elliptic lanceolate to ovate lanceolate. They are slightly crenate or entire margin, a tapering base and acuminate apex. There are 8 to 12 pairs of lateral veins which are reticulate. Petioles are 2 to 8 cm. long, slightly grooved on the ventral side but becoming more grooved towards the basal part of the lamina.

Flowers are found in short, dense, axillary peduncled spikes, peduncles being 3 to 9 cm. long, stout and arranged almost towards the ends of the branches. The flowers are subsessile and each is accompanied by one big bract and two small bracteoles. The size of the bract and the bracteoles diminishes towards the apical part of the spike. The bracts attached to the lowermost flowers are ovate, sub-acute, 1.5 to 4.5 cm.
by 1.0 to 3.0 cm., 5 to 7 nerved which are reticulated and the bracteoles are 0.5 to 1 cm. by 0.3 to 0.6 cm., ovate-lanceolate, acute, one-nerved, puberulous with ciliate hairs. The lowermost flowers numbering 2 to 4 are open on the spike, while the rest are small and in the bud condition. Accordingly, the size of the bracts and bracteoles, accompanying the upper flowers is smaller.

Calyx is 6-12 mm. long, campanulate, 5-fid, slightly pubescent, oblong lanceolate, acute and 3-nerved. Corolla is 2.0 to 3.0 cm. long, white with pink or purple stripes on the throat. Corolla tube is short, 6-12 mm. long with its lower half cylindrical, 3-4 mm. in diameter and the upper half laterally inflated and the limb is 2-labiate, the posterior being erect and the anterior broad and recurved. Filaments hairy at the base, long and stout, each with 2 large diverging anther cells, one much higher than the other, and the lower one being apiculate at the base. Ovary and style base are pubescent (Prasad and Prabhu, 1950).

**Adhatoda beddomei** Clarke. is closely allied to **Adhatoda vasica** Nees. from which it can be easily distinguished by its smaller and less dense habit, smaller leaves having 8 to 10 pairs of secondary nerves, shorter spikes, obscurely 5 ribbed ovate bracts and smaller flowers without the purple bars and spots at the
Chromosome biotypes of *Adhatoda vasica* were found in different ecological conditions of Eastern India. They differed in number and length of chromosomes and position of constriction. Some significant differences in the anatomical and pharmacognostic characters among these biotypes were also noted (Datta and Samanta, 1974). So far nine cytotypes have been reported in vasaka (Datta, 1968, 1978). The diploid number of chromosomes in these cytotypes varied from 34 to 50.

The microscopic details of *Adhatoda vasica* were described by Prasad and Prabhu (1950). Adulteration of vasaka with *Alitanthus excelsa* was mentioned by Satkopan and Thomas (1970).

We came across two types of vasaka plants which showed close resemblances to the description given for *Adhatoda vasica*. One variety is growing to a height of 7-10 feet, with very big leaves and giving the appearance of a shrub with stout and sturdy stem. The other one is much smaller, growing to a height of 4-5 feet with small leaves. We designated them as small leaf variety and big leaf variety. The general appearance of the two plants can be seen in fig.11 and fig.12.
FIG. 11

*Adhatoda vasica* (small leaf variety) plant
**FIG. 12**

*Adhatoda vasica* (big leaf variety) plant
Alkaloid content in *Adhatoda vasica* varies in different parts of the plant. The amount of alkaloid is quite high in roots in comparison to stem and leaf. Alkaloid percentages were found to vary from 1.0 to 1.6 per cent in leaves and from 1.6 to 1.8 per cent in roots when plants were raised from the stem cuttings.

According to Greger and Johne (1965a) alkaloid content varies in leaf according to its age. They reported that the first pair of leaves from below contain 1.07 to 1.1 per cent alkaloid whereas the second, third and fourth pair from below contain 1.2 to 1.6%, 1.9 to 1.7% and 2.5 to 2.8% of total alkaloids respectively. Datta and Samant (1978) reported 0.01 to 0.06 per cent of the total alkaloids in the barks of different cytotypes of *vasaka* plants.

The work of Pandita et al. (1983) revealed that the percentage of the various alkaloids in *Adhatoda vasica* varies with the season. The plant is rich in its alkaloidal content in the months of August and October, during which it amounts up to 2.0%. The total alkaloid content decreases after October and reaches to a minimum level of 0.4 to 0.6% in March.

So far no attempt was made to enrich the alkaloidal yield of the plants by cytogenetical manipulations,
hormone treatments or by treatment with any other chemical substances.

Vasaka contains mainly the quinazoline alkaloids. Other chemical compounds were also isolated from this plant in addition to the alkaloids. Mithal and Schroff (1954) isolated vasicinine which was later found to be identical with betaine by Mithal and Matheson (1957). Inamdar et al. (1965), Modak and Rajaramarao (1966) isolated a steroidal compound known as vasakin. Rangaswami and Seshadri (1971) reported flavonoid glycosides from this plant. Bhartiya and Gupta (1982) reported 2,4-dihydroxy chalcone 4-glucoside from vasaka plants.

Vasaka Alkaloids:

Vasaka mainly contains the quinazoline alkaloids. So far nine alkaloids have been isolated from this plant and their structures and formulae are given in fig. 13. Quinazoline alkaloids are found in only seven plant families, Acanthaceae, Leguminosae, Malvaceae, Rutaceae, Saxifragaceae, Scrophulariaceae and Zygophyllaceae (Anand Prakash and Ghosal, 1983).

The alkaloid vasicine was first isolated by Hooper in 1888 and it was shown to be identical with peganine (Spath and Kuffner, 1935). It forms colourless crystals which are sparingly soluble in water,
FIG. 13: ALKALOIDS OF ADHATODA VASICA NEES.

VASICINE \( \text{C}_{11}\text{H}_{12}\text{ON}_{2} \)
M.P. 198°C (Dec.)

VASICINONE \( \text{C}_{11}\text{H}_{10}\text{O}_{2}\text{N}_{2} \)
N.P. 200-201°C

AMISOTINE \( \text{C}_{20}\text{H}_{19}\text{O}_{3}\text{N}_{3} \)
N.P. 159-190°C
ALKALOIDS OF ADHATODA VASICA NEES. (CONTINUED)

ADHATODINE \( \text{C}_{20}\text{H}_{21}\text{O}_{2}\text{N}_{3} \)
M.P. 183°C

ADHATONINE \( \text{C}_{13}\text{H}_{12}\text{N}_{2}\text{O} \)
M.P. 165°C

VASICOLINONE \( \text{C}_{19}\text{H}_{19}\text{O}_{3}\text{N}_{3} \)
M.P. 152°C
ALKALOIDS OF ADHATODA VASICA NEES. (CONTINUED)

VASICOLINE (C_{19}H_{21}N_{3})
M.P. 135°C

VASICINOL (C_{11}H_{12}O_{2}N_{2})
M.P. 260°C

VASICOL (C_{11}H_{14}O_{2}N_{2})
M.P. 204 - 205°C
benzene, diethyl ether, acetone and alcohol. It is readily soluble in chloroform. Another alkaloid of particular interest is vasicinone which is a much weaker base having bronchodilatory activity and it is an autooxidation product of vasicine (Amin and Mehta, 1959; Amin, 1961; Amin et al., 1983). Recently the epoxides and glycosides of vasicine and vasicinone were reported (Pandita et al., 1983). Rajagopalan et al. (1983) isolated vasicinol from the roots of vasaka. A novel nor-harmal alkaloid \( C_{13}H_{12}N_2O, \text{m.p. 165-66°C} \) and a galactoside \( C_{35}H_{60}O, \text{m.p. 278-80°C} \) were obtained from the roots of this plant (Jain et al., 1980).

From the alcoholic extract of the roots vasicol was obtained which has been characterised as 1,2,3,4,9,11 hexahydropryrolo(2,1-b)quinazoline-3,11-diol (Dhar et al., 1981).

**Biosynthesis of quinazoline alkaloids:**

Anthranilic acid has been shown to be the main building block in the synthesis of quinazoline alkaloids in *Adhatoda vasica* and *Peganum harmala*. Most of the indication of quinazoline alkaloid synthesis is based on the work of Schoff and Oechlar (1936) where they obtained a quarternary base by reacting O-aminobenzaldehyde and diethyl acetal of \(-\text{aminobutyraldehyde at pH} 5\). This quarternary base on shaking with palladium under hydrogen for several days, resulted in the shift of hydrogen leading to the formation of desoxyvasicine (fig.14). A homogenate from
FIG. 14
FORMATION OF DESOXYVASICINE

\[ \text{Aminobuteraldehyde} \quad \overset{\text{pH 5.0}}{\longrightarrow} \quad \text{Desoxyvasicine} \]

\[ \text{Desoxyvasicine} \quad \overset{-\text{H}_2\text{O}}{\longrightarrow} \quad \text{Quaternary Base} \]

\[ \text{OH}^- \]
peas catalysed oxidation of a synthetic quinazolium salt to the alkaloid deoxyvasicinone (Skursky, 1965). Fitzgerald et al. (1966) proposed that combination of anthranilic acid with ornithine derivative would give vasicine type alkaloids and combination with a lysine derivative would give the homologous mackinlaya alkaloids.

Groger and his co-workers while working with *Adhatoda vasica* found that when glutamic acid derivatives were fed, nearly 50% of the radioactivity was obtained in the anthranilic acid. It was assumed that these precursors get metabolised before they are incorporated into peganine resulting in a random labelling (Groger et al., 1966, 1967; Johne and Groger, 1968; Johne et al., 1968). Radioactive aspartic acid was fed to *Adhatoda vasica* to find out its incorporation into the non-anthranilic acid moiety of peganine (Groger et al., 1967). Hatanaka (1962) observed that after feeding aspartic acid along with inactive anthranilic acid the major part of the activity (80%) was found in glycine which was obtained from C-1 and C-2 of vasicine after degradation. From this finding it appears that aspartic acid is a direct precursor of pyrrolidine ring. The biosynthesis of vasicine is shown in fig. 15.
FIG. 15: POSSIBLE PRECURSORS OF VASICINE

ANTHRANILIC ACID + ACIDIC ACID + \text{H}_2\text{N}-\text{CH}-\text{CH}_2\text{COOH} \rightarrow \text{VASA}^\prime\text{A} \text{ALKALOIDS}

L-ASPARTIC ACID
The work of Liljegren (1968) on *Peganum harmala* revealed that this plant could incorporate $^{14}$C ornithine into peganine (vasicine) wherein 84% of the radioactivity was located at C-1 and C-10 of peganine. Further, tryptophan, in small quantities, was also incorporated into anthranilic acid (Liljegren, 1971). Thus, it appears that two different pathways do exist in the biosynthesis of vasicine in *Adhatoda vasica* (Groger et al., 1967) and *Peganum harmala* (Liljegren, 1968; 1971). So far no experimental evidence could be furnished to demonstrate beyond any ambiguity the interconversion of one alkaloid to another (Liljegren, 1971).

**Extraction and isolation of vasicine and related alkaloids:**

Openshaw (1953) described the extraction procedure for the total alkaloids of vasaka. Later Mehta et al. (1963) developed a reliable technique for the isolation of vasicine and vasicinone. In their method, the residue of the alcoholic extract was extracted with hot distilled water extract made alkaline with caustic soda and alkaloids taken into chloroform, which on evaporation left a brown powder of crude total alkaloids. This powder gave two types of crystals in alcohol (95%) which melted at about 180°C. These crystals were dissolved in 95% hot alcohol and after a little cooling ethereal hydrochloride was added. Colourless vasicinone hydrochloride crystals
which crystallized cut were filtered and dried. The mother liquor, when treated with dry ether till turbidity and upon keeping at 15°C for three days, gave brown crystals of vasicine hydrochloride. From this, pure vasicine hydrochloride could be obtained after recrystallization from 95% alcohol using animal charcoal. Melting point of anhydrous vasicine hydrochloride is 208-209°C.

For the isolation of the quinazoline alkaloids Groger and Johne (1965b) Liljegren (1971), Anand Prakash et al. (1981) and Bhalla et al. (1982) tried the column chromatographic technique. Of all these methods the one detailed by Bhalla et al. (1982) is the most simple and accurate. In this the alcoholic extract is taken into acetic acid (5%) and the non-basic materials were removed by shaking the aqueous extract with petroleum ether (60-80°C) and chloroform. Dilute ammonia was added to be aqueous extract and the liberated alkaloids taken into chloroform, which on evaporation under vacuum yielded the crude alkaloidal mixture which was further subjected to column chromatography over a silica gel column by eluting with chloroform: methanol : ethyl acetate (8 : 2 : 1). The different alkaloids of vasaka were conveniently resolved on this column.
Resolution of various alkaloids of *Adhatoda vasica* was achieved by different workers using thin layer chromatography. Groger (1965b) used silica gel 'G' plates impregnated with 0.5% potassium hydroxide solution and eluted with dichloromethane : methanol (3 : 1) and separated vasicine ($R_f$ 0.6) and vasicinone ($R_f$ 0.9). Munshi et al. (1978) using n-butanol : acetic acid : water (6 : 1 : 3) on silica gel plates separated both the compounds. Bhalla et al. (1982) used chloroform : methanol : ethyl acetate (6 : 2 : 1) on silica gel 'G' plates and successfully separated vasicine ($R_f$ 0.32) and vasicinone ($R_f$ 0.62). Recently Pandita et al. (1983) succeeded in separating the alkaloids of vasaka by using chloroform : methanol (9 : 1) mixture.

Mehta et al. (1963) used Whatman No.1 filter paper and n-butanol : acetic acid : water (10 : 1 : 5). $R_f$ values for vasicine and vasicinone were found to be 0.58 and 0.79 respectively. Liljegren (1971) employed Whatman filter paper No.1 and n-butanol saturated with 5% acetic acid for the alkaloid resolution in *Peganum harmala*.

**Quantitative Estimation of the Alkaloids of Vasaka:**

The gravimetric method for the estimation of total alkaloids was reported by Atal (1980).
Titrimetric analysis based on acid-base reactions was adopted by Kannan et al. (1959) and Gupta and Jain (1979). Colorimetric method using tropeolin '00' as described by Groger (1965b) was employed in our studies and the details of the procedure are in the experimental part.

Spectrophotometric determination of the individual alkaloids, vasicine and vasicinone was carried out by Bhalla et al. (1982). The alkaloids were taken in 0.5 N hydrochloric acid and the absorbance read at 233 nm. for vasicinone and at 285 nm. for vasicine.

Pharmacology of vasaka and its alkaloids:

The volatile oil was reported to be an effective expectorant (Rajarama Rao, 1961). Vasicine, as such, was shown to be bronchoconstrictor (Amin et al., 1963) but later it was observed to be getting converted into vasicinone during its passage in the liver and eliciting the bronchodilatory action similar to that of aminophyllin (Atal, 1980). Vasicine has got good uterine stimulant and abortifacient activities. These actions were proved to be mediated through the release or synthesis of prostaglandins (Gupta et al., 1977; 1978). In this respect the effect of vasicine is similar to oxytocin and methylergometrine. Vasicine causes sterility of insects of many species. Female insects treated with vasicine in
small doses, 10.1% lay eggs which apparently looked normal but were incapable of fertilizing. Number of eggs by these insects were also reduced to 50% (News letter, R.R.L., 1980). Vasicine hydrochloride was found to be effective against Giardia trophozites. 200 μg./ml. of vasicine hydrochloride supressed the growth of Entamoeba histolytica (Bhutani et al., 1982). Ester derivatives of vasicine like the stearate ester and some of the Ayurvedic preparations like trikalu are reported to enhance the bioavailability of vasicine by preventing its oxidation (Ushe Zutshi, 1982).

Vasicinone produced bronchodilatory activity on lungs. This action was more pronounced against histamine induced bronchial constriction vasicinone exhibited positive ionotropic and chronotropic effect and increased flow in coronary vessels. It did not alter normal E.C.G. of the dog's heart (Amin, 1961) but it showed transient fall in blood pressure in dogs. Cambridge et al. (1962) could not obtain the bronchodilatory effect of vasicinone in guinea pigs in vivo though they observed the activity guinea pigs' tracheal chain. This difference of action was attributed to the rapid metabolism of vasicinone. The pharmacological action of vasicinol was reported by Lahiri and Pradhan (1964). It showed a transient fall in blood pressure in cats,
contraction of isolated guinea pig intestine and depression of isolated guinea pig heart all of which could be blocked by atropine.

Thrombopoietic activity of vasaka was reported by Atel et al. (1982) in normal rats, rabbits and dogs.

**Datura innoxia Miller:**

The genus *Datura* belongs to the family Solanaceae and grows wild in the North-West region of India. The plant mainly contains tropane alkaloids, and has been in use in the traditional systems of medicine since a very long time. The seeds have an acrid, bitter, sharp taste; heating, tonic, febrifuge, anthelmintic, alexiteric, emetic; useful in leucoderma, skin diseases, ulcers, itching, bronchitis, biliousness, jaundice and piles. The whole plant is toxic, narcotic, aphrodisiac; applied topically to remove the pain of tumours and piles. The leaves after roasting are applied locally to relieve eye pain, headache, nose trouble, enlargement of the testicle and boils. The root is useful in reducing inflammation. The leaves and the seeds are antispasmodic, anodyne, and narcotic. The inhalation of the smoke from the burning leaves is recommended for relieving attacks of asthma. It is a better cough remedy than opium, as it does not arrest secretions. The young fruits are said to be sedative.
and slightly intoxicating (Kirtikar and Basu, 1975).

In India, the genus *Datura* is represented by four species. Of these, *Datura stramonium* Linn. occurs in temperate localities and yields mainly hyoscynamine. The remaining three species, *D.innoxia* Mill., *D.metel* Linn. and *D.fastuosa* Linn. occur in the sub-Himalayan tract and plains and are rich in hyoscine which constitutes between 70 to 80% of the total alkaloids present (Prabhakar et al., 1971).

*Datura innoxia* is a native of Mexico, now found growing in Western Himalayas, the hilly regions of the Western parts of Deccan Peninsula and arid regions of Punjab, Rajasthan and Peninsular regions of Andhra Pradesh and Tamil Nadu.

*Datura innoxia* is a perennial herb with a thick fleshy and hairy stem. The leaves are petiolate, ovate or somewhat cordate, about 12 cm. long and 7 cm. wide but may reach a length of 26 cm. and a width of 18 cm., thick and pubescent. The margin is entire or may show a few small teeth, the apex is acute. There are about seven to ten secondary veins on either side of the midrib. Dichasially branched stems are pubescent and much twisted, fruits are often present in the forks. The corolla is single, white, 10 toothed and calyx inflated.
Fruit is a capsule with prominent spines. The herb, flower and fruit of this plant are shown in fig. 16 and fig. 17.

*Datura innoxia* can also be considered as a source of tropane alkaloids for commercial exploitation. The raw material is presently collected from Andhra Pradesh, Tamil Nadu and Rajasthan with an annual output turn of around 50 tonnes of dry herb. An annual availability of around 200 tonnes of dry herb is estimated from Andhra Pradesh, Tamil Nadu and Karnataka states of India. (Prabhakar et al., 1971). *Daturas* prefer rich clay loam soil and sunny situations. *Datura innoxia* is grown as a winter or spring crop and seeds are sown in October or in late January respectively. Germination of seeds is low and irregular due to the presence of an inhibitor (Zutshi and Atal, 1970). Seed germination can be improved by soaking the seed overnight in water and washing the seed repeatedly two or three times with fresh water before sowing. The seed starts germinating within a fortnight (Sarin, 1982). The possibilities of exploiting wild Daturas for commercial purposes have been surveyed by Prabhakar et al. (1971).

*Datura* mainly contains Hyoscyamine and Scopolamine, the tropane alkaloids. The various minor alkaloids found
FIG. 16

Datura innoxia plant
FIG. 16
FIG. 17

*Datura innoxia* fruits
in *Datura* are represented in table No. 7 and the structures of some of the alkaloids are shown in fig. 18. The percentage of total alkaloids and the major individual alkaloid reported in *Datura innoxia* are enlisted in table No. 8. The total alkaloid content is highest at the time of flower bud initiation and before the peak blooming period. Singh and Sharma (1976) observed translocation of the alkaloids from the pericarp into the developing seeds. Plants grown during the summer months contained more alkaloids compared to those grown in the monsoon season. Stress conditions are favourable for more accumulation of the alkaloids (Evenari, 1960). Long exposures to intense light bring about an increase in scopolamine content (Cosson et al., 1966; Nandi and Chatterjae, 1982). A gradual increase of hyoscyamine and a decrease of scopolamine was observed by Cucu and Paun (1968).

Efforts were made to enhance the alkaloid content in *Datura* by external application of different plant hormones and chemical substances and the observations of various workers are compiled in table No. 9. Verzar-Petri (1967) reported that differences occur in the time of appearance of the alkaloid components as well as their relation to one another. Rowland and Gibson (1966) observed that ascorbic acid oxidase activity was not involved in the alkaloid biosynthesis in *Datura innoxia*. 
FIG. 18: SOME ALKALOIDS OF THE TROPANE GROUP

Hyoscyamine

Scopolamine

Littorine

Meteloidine

Cocaine

Cochlearine

Belendine $R=H$

Ferrugine $R=Me$

$2\alpha$-Benzoyltropane

$2\beta$-Benzyl-$3\beta$-acetoxy-tropane
TABLE - 7

Different minor alkaloids isolated from *Datura*:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Alkaloid</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans and Partridge, 1948 and 1953; Evans and Mellendorf, 1959, Evans and Woolley, 1965; Griffin, 1966.</td>
<td>Meteloidine</td>
<td>3-tiglyloxytropane and tigloxytropane were also reported by Evans and Mellendorf, 1959.</td>
</tr>
<tr>
<td>Haga, 1954</td>
<td>Cuscuhygrine</td>
<td>---</td>
</tr>
<tr>
<td>Verzar-Petri and Sarkany, 1961</td>
<td>7-hydroxy-3-6-ditiglyloxytropane</td>
<td>Obtained from the leaves.</td>
</tr>
<tr>
<td>Evans and Griffin, 1963</td>
<td>3-hydroxy-6-tigloxytropane</td>
<td>---</td>
</tr>
<tr>
<td>Evans and PeThan, 1962; Evans and Woolley, 1965; Griffin, 1966</td>
<td>Noratropine</td>
<td>---</td>
</tr>
<tr>
<td>Evans and Volley, 1964, 1965; Evans et al., 1965</td>
<td>Aposcopolamine</td>
<td>Obtained from the leaves of <em>Datura</em> species having high scopolamine content.</td>
</tr>
<tr>
<td>Evans, 1966</td>
<td>Tropine pseudotropine</td>
<td>Mono and ditiglylesters of the hydroxy tropanes as minor components were also isolated.</td>
</tr>
<tr>
<td>Evans and Major, 1966</td>
<td>3-tiglyloxyacetoxytropane</td>
<td>---</td>
</tr>
</tbody>
</table>
**TABLE - B**

Percentage of total alkaloids in different parts of *Datura innoxia* plants as reported by various workers:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plant Part</th>
<th>% total alkaloid</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Wealth of India, 1952</td>
<td>Leaves</td>
<td>0.52</td>
<td>From plants grown in Latin America</td>
</tr>
<tr>
<td></td>
<td>Stems</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fruits</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>The Wealth of India, 1952</td>
<td>Leaves</td>
<td>0.25</td>
<td>From plants grown in Punjab</td>
</tr>
<tr>
<td></td>
<td>Fruits</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>0.23-0.26</td>
<td></td>
</tr>
<tr>
<td>Khanna, 1965</td>
<td>Leaves</td>
<td>0.564</td>
<td>The plants are collected from Ahmedabad</td>
</tr>
<tr>
<td></td>
<td>Flowers</td>
<td>0.517</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Herb</td>
<td>0.610</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pericarp</td>
<td>0.146</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.319</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>0.610</td>
<td></td>
</tr>
<tr>
<td>Evans, 1966</td>
<td>Aerial parts</td>
<td>--</td>
<td>Scopolamine is the major alkaloid</td>
</tr>
<tr>
<td>References</td>
<td>Plant part</td>
<td>% total alkaloid</td>
<td>Remark</td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
<td>------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Griffin, 1966</td>
<td>Roots</td>
<td>-</td>
<td>Hyoscyamine major alkaloid</td>
</tr>
<tr>
<td>Prabhokar et al., 1971</td>
<td>Leaves</td>
<td>0.120</td>
<td>Plants collected from Jammu-Tawi</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.217</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.078</td>
<td>Plants collected from Jammu-Contt.</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.184</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.082</td>
<td>Katre (Jammu)</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.143</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.088</td>
<td>Pathankot (H.P.)</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.188</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.114</td>
<td>Kangra (H.P.)</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.106</td>
<td>Dasholi (J &amp; K)</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.201</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.064</td>
<td>Hoshiarpur (Punjab)</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.226</td>
<td></td>
</tr>
<tr>
<td>Sarin, 1982</td>
<td>Dried herb</td>
<td>0.23% (average)</td>
<td>In herb hyoscyne constitutes 66% of total alkaloids whereas it is only 20-30% in seeds.</td>
</tr>
<tr>
<td>Sarin, 1982</td>
<td>Herb</td>
<td>0.12-0.40</td>
<td>Hyoscyamine more than hyoscyne. Plants were collected from Ahmedabad.</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>0.10-0.33</td>
<td></td>
</tr>
</tbody>
</table>
Effect of various chemical and physical treatments on the alkaloids of *Datura innoxia* Mill.:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment</th>
<th>Reported observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>French and Gibson, 1957</td>
<td>Glutamic acid</td>
<td>No significant change in the alkaloids</td>
</tr>
<tr>
<td>Shpilenya, 1962</td>
<td>Decreasing moisture content of soil</td>
<td>Percentage of alkaloids increased</td>
</tr>
<tr>
<td>Sciuchetti, 1964</td>
<td>Gibberellic acid and kinin</td>
<td>GA markedly reduced the alkaloid content whereas no change was observed with kinetin treatment</td>
</tr>
<tr>
<td>Gibson and French, 1964</td>
<td>60 ppm. gibberellic acid</td>
<td>Alkaloids increased</td>
</tr>
<tr>
<td>James and Sciuchetti, 1964</td>
<td>Studied the interaction of gibberellic acid and B 995</td>
<td>Lowered alkaloid content</td>
</tr>
<tr>
<td>Dallolio, 1965</td>
<td>Growth retardants</td>
<td>No effect</td>
</tr>
<tr>
<td>Cosson, 1966</td>
<td>Intense light for 16 hours a day</td>
<td>Sharp increase in scopolamine content</td>
</tr>
<tr>
<td>Sinha and Varma, 1965</td>
<td>Indole-3-acetic acid, Indole-3-propionic acid, 2,4-dichlorophenoxy acetic acid and gibberellic acid</td>
<td>Better vegetative growth and high alkaloid content</td>
</tr>
<tr>
<td>Reference</td>
<td>Treatment</td>
<td>Reported observation</td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sinha and Verma, 1966</td>
<td>Ammonium sulphate, superphosphate, mixture of both (1:1), potassium dihydrogen phosphate and potassium nitrate.</td>
<td>Only plants treated with potassium nitrate showed significantly higher dry weight and alkaloidal content per plant</td>
</tr>
<tr>
<td>El-hamidi et al., 1966</td>
<td>Irradiation</td>
<td>Irradiation with 16,000 rad showed an increase of 54-86% in total alkaloid</td>
</tr>
<tr>
<td>Shah and Saoji, 1967</td>
<td>Gibberellic acid, Indole-3-yl-acetic acid and Indole-3-yl propionic acid in 100 ppm concentrations</td>
<td>The alkaloid content was more in each group of the treated plants</td>
</tr>
<tr>
<td>Sinha and Verma, 1968</td>
<td>A double spray with 100 and 1000 ppm of indole acetic acid</td>
<td>Increased markedly the yield of the alkaloids</td>
</tr>
<tr>
<td>Ammal and Zutshi, 1970</td>
<td>0.8% colchicine treatment for 48 hours</td>
<td>The study suggested that possibly a number of genes are responsible for the production of alkaloids</td>
</tr>
<tr>
<td>Khanna and Nag, 1972</td>
<td>Phenylalanine and tyrosine</td>
<td>Increased alkaloid content of callus cultures of <em>Datura metel</em>.</td>
</tr>
</tbody>
</table>
Verzor-Petri (1967) further noticed that total alkaloid content had an inverse correlation with the amino acids especially arginine, ornithine, proline, glutamic acid etc.

A survey of the literature on Datura revealed that no attempt has so far been made to study the effect of foliar spray application of ascorbic acid on the alkaloids of the plant.

**Tropane alkaloids:**

The tropane alkaloids are a group of chemical compounds which are found in the plant families Solanaeceae, Convolvulaceae and Erythroxylaceae (Romeike, 1966). They are esters of organic acids (atropic, benzoic, cinnamic, isovaleric, d-\(\alpha\)-methylbutyric, tiglic, tropic truxillic and veratric acids). The esterification of these acids takes place with the bicyclic alkamines such as methylecgonine, nortropine, pseudotropine, scopine, tropine and others. The basic ring moiety, tropane, is characterised by the presence of a \(\alpha\)-azobicyclic (3,2,1) octene ring system. The Solanaceous group of the alkaloids includes those alkaloids found in the genera Atropa, Hyoscyamus, Scopolia, Mandragora, Physochlaina, Datura, Solandra, Duboisia and Anthocercis.
Biosynthesis of Tropane Alkaloids:

Ornithine and related aminoacids were shown to be the precursors of the pyrrolidine ring of tropine (Leete et al., 1954; Leete, 1962; Leete, 1962; Leete, 1964; Liebisch and Ramin, 1965; Liebisch and Schutte, 1967). Carbons 2,3 and 4 of tropine are derived from acetic acid (Kaczkowski et al., 1961) and it is assumed that the acetate is incorporated via acetoacetic acid or some suitably activated derivative such as its coenzyme A ester. The pyrrolidine ring of meteloidine is derived from ornithine (Leete and Nelson, 1969) and the hydroxyls at the 6 and 7-positions are apparently introduced at a later stage in the biosynthetic sequence, after formation of the tropine ring system (Leete, 1972). Scopolamine was also reported to have formed in a similar way. It was reported to be a metabolite of hyoscyamine (Romeike, 1955; Foder and Romeike, 1959; Romeike, 1959; Romeike and Foder, 1960; Romeike, 1962) and the reaction proceeds via 6-hydroxyhyoscyamine and 6,7-dehydrohyoscyamine. An intramolecular rearrangement in the side chain of phenylalanine leads to the formation of tropic acid (Leete et al., 1975). Tiglic acid is formed from 1-isolucine in Datura species (Woolley, 1966; Leete and Murrill, 1967).
Isolation of Tropane Alkaloids:

Much effort has been expended by the school of Evans to standardise the procedure for the isolation of different individual alkaloids from the crude mixture of total alkaloids in various species of *Datura* (Evans and Partridge, 1948, 1952; Evans and Jellendorf, 1959; Evans and Pethan, 1962). They used kieselgur column buffered with 0.5 M phosphate buffer and different non-polar solvents like carbon tetrachloride, ether, petroleum ether etc. Drey and Foster (1953) and French and Gibson (1957) estimated tropane alkaloids in vegetable drugs by paper chromatography. Alami et al. (1955) attempted the separation and estimation of hyoscyamine and hyoscine in *D. stramonium*, *D. stramonium* var. *inermis* and *D. tntula* by paper electrophoresis. Rowland and Gibson (1966) estimated hyoscine and hyoscyamine in *Datura innaxia* by thin layer chromatography. Saoji (1969) made some modifications in the procedure of Evans and Pethan (1962) for the isolation of the alkaloids using column chromatography. The technique employed by Bombardelli (1977) is very simple and can be employed in commercial isolation of hyoscyamine and hyoscine. The drug is extracted with acid water at low temperature, the percolates neutralized and extracted with a chlorinated solvent. The two alkaloids are separated by counter-extraction of hyoscyamine from the
organic phase of pH 6.5 with a phosphate buffer; hyoscyamine passes into the buffer and is reextracted after alkanisation, once again with a chlorinated solvent. Concentrating the final organic phase and treating the residues with benzene gives hyoscyamine and with hydrobromic acid gives scopolamine hydrobromine.

Quantitative Determination of the Tropane Alkaloids:

The determination of the tropane alkaloids involves extraction, purification and assay of the total alkaloids. The extraction may be carried out by maceration or continuous extraction in a percolator. The residue containing the total alkaloids is partially purified by passing it through aqueous acidic solution, basifying it and then taking the alkaloids into an organic solvent.

Titrmetric methods have been employed for a long time in the determination of the total alkaloidal content in the drug (Graf, 1965; Indian Pharmacopoeia, 1966; Leary, 1970; United States Pharmacopoeia XVIII, 1970; National Formulary, 1970; British Pharmaceutical Codex, 1973).

A number of reagents were used to form a colour complex with the alkaloids and the resulting colour complex measured colorimetrically. Gottlieb (1950) could obtain a colour complex with the use of p-dimethyl-
aminobenzaldehyde reagent. Halisler (1957) used tropolol in '00' indicator where the alkaloidal pH was adjusted to 4.6 with acetate buffer. Addition of saturated aqueous solution of tropolin '00' and extraction with chloroform yields a colour whose absorbance can be easily measured. Von Nuci Cham and John (1965) developed a method based on Vitali Morin Reaction. The application of acid dye technique was reported by Maery and Saleh (1973).

Photodensitometric techniques were employed to measure the intensity of the alkaloidal spots separated on paper and thin layer chromatograms (Shipalov et al., 1966; Gros Lebon and Debelmas, 1972).

Gas Liquid Chromatographic method was efficiently utilized by Achari and Newcombe (1971) for the quantitative determination of the various individual alkaloids in the tropane group using Datura ferox and Datura innoxia plants. The columns tried were those of 10% XE-60 and 2.5% XE-60 and 2.5% QF-1, oven temperature 220°C; carrier gas flow 50 ml/min.; carrier gas inlet pressure 30 lb./sq. in.; attenuation 5 x 10^3 (f.s.d. 5 x 10^9 amp.)

Paper chromatographic separation of the tropane alkaloids was worked out by Brindle et al. (1951), Gore
and Adshead (1952), Shipelov et al. (1966) and Evans and Treagust (1973).

In the study of thin layer chromatographic separation of the belladonna alkaloids Baumler and Rippstein (1961) used the following adsorbents and solvent systems:

1. Neutral silica gel 'G' as adsorbant.
   - Dimethyl formamide:diethyleamine:ethanol:ethyl acetate (1 : 1 : 6 : 12)
2. Alkaline silica gel 'G' layer.
   - 70\% ethanol: 25\% ammonia (99 : 1)

Bhavsar (1971) employed silica gel G plates and chloroform:methanol:ammonia (80:20:1) as solvent system. Padhan and Rao (1971) used silica gel layers with methanol:benzene (6:4) as developing solvent. Stahl (1973) has suggested silica gel GF 254 as the stationary phase and acetone:water:25\% ammonia solution (90:7:3) as the mobile phase. Detection was achieved by spraying with Dragendorff reagent and then with 0.1 N sulphuric acid. Evans and Treagust (1973) tried the following adsorbants and solvent systems:

<table>
<thead>
<tr>
<th>Adsorbant</th>
<th>Solvent system</th>
<th>Spray Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alumina</td>
<td>Ethanol</td>
<td>I₂ in CCl₄</td>
</tr>
<tr>
<td>Alumina</td>
<td>Ether:ethanol (1:1)</td>
<td>I₂ in CCl₄</td>
</tr>
<tr>
<td>Silica gel G</td>
<td>Me₂CO: Strong ammonia (4:1)</td>
<td>Iodoplatinate reagent</td>
</tr>
</tbody>
</table>
Pharmacological actions of Atropine and Scopolamine :-

Hyoscine, hyoscyamine and its racemic form atropine are used as sedative, antispasmodic and mydriatic agents. Hyoscyamine is relatively more active in stimulating the central nervous system. Hyoscyamine and its salts are employed in parkinsonianism, delirium, tremours and menic. Hyoscine, in the form of its salts is mainly used as preanaesthetic in surgery and childbirth and in prevention of motion sickness (Atal and Kapur, 1982). Atropine is therapeutically used in peptic ulcer, urinary incontinence and bronchial asthma. It is also used in the treatment of gastric hypermotility, spasticity of the small intestine and colon in treating poisoning of anticholinesterase compounds Gadum, 1959; Krantz et al., 1965; Goodman and Gilman, 1965).

Hyoscine has a purely depressant action and hyoscyamine has an initial stimulant action which is followed by depression. Atropine and scopolamine are competitive with acetyl-choline at the post-ganglionic synapses (muscarnic site of the parasympathetic nervous system). These two alkaloids are principally used to relieve spasms of the bowels in the treatment of spastic colitis, gastro-enteritis, and peptic ulcer; the antisecretory action utilized to reduce respiratory secretions in anaesthesia (Tyler et al., 1981). Toxic symptoms of atropine,
Scopolamine and belladonna tincture are skin rash, skin flushing, mouth dryness, difficult urination, eye pain, blurred vision and light sensitivity.

Atropine exhibited antifertility activity (Ratnasoiriyer, 1984). Atropine in a dose of 2 mg/ml. caused circling behaviour while administration of 5 mg./ml. caused immobilization of rat epididymal spermatozoa in vitro. A single vaginal instillation of 2 mg. of atropine just before coitus produced depression in libido. A significant reduction in fertility was evident when the same dose was instilled into the uterine cavity 10-12 hour post coitally.

Ascorbic acid:-

Ascorbic acid is an organic molecule synthesized from D-glucose or D-galactose in both plants and many animals (Isherwood et al, 1953). It is universally present in the plants both in green and non-green tissues. Ascorbic acid was found to translocate in all directions in the plants (Khudairi, 1968).

Stimulation of growth by dehydroascorbic acid was reported by Raadts and Soding (1947) in Avena coleoptile sections and they concluded that the oxidised form of ascorbic acid activated the conversion of endogenous
auxin precursors to active auxin. Low concentrations of ascorbic acid were reported to increase growth and yield of *Trigonella foenum-graecum* and *Brassica chinensis* (Chinoy et al., 1957; Garg et al., 1958). Different concentrations of ascorbic acid have been shown to retard or accelerate growth of *Avena* coleoptile cylinders (Grover et al., 1958; Khudairi, 1968). Chinoy et al. (1957) found increase in ascorbic acid levels during cell elongation and suggested that ascorbic acid has the same effect on *Avena* coleoptile as that of auxin. They further observed that the addition of growth regulators like IAA and GA₃ increased the biosynthesis of ascorbic acid from sucrose (Chinoy et al., 1965). It was suggested that the effect of gibberellic acid is manifested through the new synthesis of ascorbic acid. Similar findings were also noted by Michnickaowicz (1960), Brugovitzky and Popovici (1966).

On the basis of the above observations and other experimental evidences, Khudairi (1968) termed ascorbic acid as 'Photophytohormone'.

Ascorbic acid is stored in the bound form in plants as ascorbigen (Guha and Sen Gupta, 1938). The dienolic group consisting of hydroxyls on 2nd and 3rd carbons with a double bond between them invests the
ascorbic acid with redox property. Exogenous application of ascorbic acid accelerates its own biosynthesis (Abraham et al., 1970). A number of workers have demonstrated the formation of free radical of ascorbic acid called mono-dehydroascorbic acid (MDHA) during its peroxidative oxidation (Krasnovskii and Brin, 1946; Piette et al., 1961; Yamazaki and Piette, 1961; Yamazaki, 1962). Yamazaki and Piette (1961) have come to the conclusion that a free radical mechanism is the main pathway involved in the ascorbic acid oxidase and peroxidase reaction. Gurevich (1963) discovered a special peroxidase enzyme in wheat and corn seedlings which brought about the transfer of hydrogen from ascorbic acid to even a difficulty reducible hydrogen acceptor of a very low oxidation-reduction potential like O-dinitrobenzene.

With the imbibition of water by the embryo the major redox-cycle of the plant begins to operate by the initiation of the biosynthesis of ascorbic acid from soluble carbohydrates (Mapson, 1958; Chinoy, 1962). A special peroxidase enzyme known as ascorbic acid free radical peroxidase is produced which converts ascorbic acid into its free radical monodehydroascorbic acid (Gurevich, 1963) by the removal of one electron from 2 C or 3 C. This monodehydroascorbic acid is a more powerful reducing agent than ascorbic acid by virtue
of its unpaired electron which also imparts it the ability of spontaneous reactivity with macromolecules. The MDHA has been shown to act not only as reductant but also as an oxidant. Further oxidase and reductase enzyme systems will set the pace of conversion of MDHA ______ DHA back to ascorbic acid (Mapson, 1958; Carter and Pace, 1964). Figure 19 shows ascorbic acid and dehydroascorbic acid (DHA).

The involvement of ascorbic acid in the energy system of respiration was suggested by a number of workers (James, 1946; Waygood, 1950; Chayen, 1953; Mapson, 1958; 1960; Beevers, 1961). It is probable that the unpaired electron of MDHA, while cascading down, gives up its electron energy for oxidative phosphorylation thus augmenting the ATP pool.

Arnon et al. (1954) and Arnon (1956, 57) ascribed the role of electron carrier to ascorbic acid in the oxidative chain of photosynthetic phosphorylation in illuminated chloroplast or as a factor stabilising the activity of the chloroplast. Substantial evidence is also available from the work of Mapson and Moustaffa (1956) that reduced glutathione and ascorbic acid act as respiratory electron carriers in an enzyme system which transfers hydrogen to molecular oxygen from substrates of TPN-linked dehydrogenase.
Ascorbic acid

Dehydroascorbic acid

FIG. 19
Shakhov and Bidzilya (1966) are of the opinion that increase in free radical content at any stage of growth and development helps in enhancing the rates of physicochemical processes. This in turn results in better growth and increased productivity.

Reducing agents tend to donate and oxidants to accept electrons. The potential of such electron transfers between reductant and oxidant (the redox potential) is a measure of the energy change involved (one electron volt = 23 KCal). In such reactions, involving organic molecules, it is usual for an electron pair to be transferred from one molecule to the other, two new closed shell molecules being formed. The two molecules then part, the one having become richer, the other poorer by two electrons (Street, 1963). This situation is not altered by the discovery of L. Michaelis that "all oxidations of organic molecules although they are bivalent, proceed in two successive steps; the intermediate state being a free radical". However, the discovery did raise the question of whether electron transfer can occur between organic molecules which are not oxidising or reducing agents in the sense that they do not have two stable states differing by two electrons. Szent-Gyorgyi (1960) has drawn the attention of biologists to such systems, systems in which single electrons can be transferred between closely...
associated molecules and has stressed the possible biologi­cal importance of such charge transfer within and bet­ween molecules.

Charge transfer can take place between molecules sufficiently close so that their electron clouds overlap and involves movement of an electron from a donor to a vacant orbital of an acceptor molecule. The molecules usually stay together, if they do separate, they do so as free radicals with an unpaired electron. The electron transfer occurs without major loss of energy because it does not involve rearrangement of molecular structure. If the accepting orbital is lower than the donating one, the transfer can occur spontaneously without any outside source of energy.

As ascorbic acid readily forms free radicals which may overlap biological macromolecules causing biological semi-conduction leading to energy transfer in biological systems. The exogenous as well as endogenously added ascorbic acid is not only utilized by enzyme systems but forms a complex with macromolecules like proteins and nucleic acids in living systems (Chinny, 1969a).

The binding of ascorbic acid to DNA molecule will disrupt electronic configuration of DNA and as a result the H-bond break down and hence DNA will unbind itself.
Price (1966) demonstrated that ascorbic acid increased RNA synthesis by about 125% in isolated wheat nuclei. This RNA was found to be m-RNA. Schopffer (1966) reported that ascorbic acid removes histones from inducible chromatin thus paving the way for increased RNA synthesis. Schopffer (1967) has also shown an appreciable increase in RNA synthesis in isolated nuclei by ascorbic acid treatment. According to Follenberg (1969) ascorbic acid intensified DNA activity by binding the excess histones. It also reduced the binding capacity of histones to DNA in vitro. This view is supported from the finding of Levine (1974). According to him ascorbate ion can link with cationic sections of histones thereby unmasking the relevant portion of DNA template. Gifford and Tepper (1962) also attributed the increased RNA production to altered DNA-histone relation during induction. Chinoy and Saxena (1972) were able to increase RNA synthesis by supplying exogenous ascorbic acid during germination. Addition of macromolecules like RNA and DNA to ascorbic acid solutions containing a small amount of hydrogen peroxide not only shifted the peak of ESR spectra but also a new peak in the U.V. absorption spectra appeared which was not given by ascorbic acid or RNA alone. Further at different concentrations of the macromolecules (RNA and DNA) the
intensity of the formation of free radicals was increased and its rate of decay was accelerated considerably. Parallel to this, the complexing of AA with RNA or DNA also showed a rising trend (Chinoy et al., 1972). Bogatyreva and Znamenskaya (1962) have observed changes in the properties of DNA following its reaction with ascorbic acid.

According to Chinoy (1970), ascorbic acid complexes with the DNA to produce charge transfer complexes. When the excited charge transfer complexes break up they give rise to nucleotide triplets as well as free radicals of nucleotides constituting an energized pool of these constituents. The excitation energy of these nucleotides is utilized for replication of RNA which in its turn gives rise to m-RNA and various grades of t-RNA. On the basis of the above findings it is postulated that ascorbic acid acts as a derepressor on the DNA by removing the complexed inhibitory histone from it. This paves the way for the creation of additional templates in the DNA molecule which produce m-RNA and finally specific structural and enzymic proteins.

Stern and Timonen (1954) have shown a close association between ascorbic acid concentration and mitotic processes in the lily anthers. Sharma and Datta (1956) were able to induce mitotic divisions as well as mitotic
reduction in adult polyploid root cells of *Allium cepa* by treatment with ascorbic acid solutions of high and low concentrations. Ascorbic acid was shown to increase cell division activity (Garg, 1956, 1960).

The rate of electron transport plays an important role in determining the rates of biosynthesis of metabolites including DNA as well as rates of enzymatic reactions on which depend to a great extent, rates of cell division, enlargement and maturation of cells and ultimately growth, differentiation and development (Chinoy, 1970). The involvement of ascorbic acid in the plant metabolism is shown in figure 20.

It is well known that any treatment or factor which significantly influences metabolism, germination and growth of a plant during its juvenile phase leaves an inexorable impression upon its growth and development during the adult phase. Presowing treatment leads to an increase in tissue hydration, respiratory activity, redistribution of nutrient reserves and enhancement of seedling growth (Henckel, 1961; Dawson, 1965).

Dawson (1965), Chinoy (1968), Abraham (1969) and Dave (1969) have reported the beneficial effect of ascorbic acid and sucrose pretreatment on various growth and development characters as well as yield, in a number of
wheat varieties. Pretreated seeds show a protoplasmic colloid with increased hydrophilic viscosity and protoplasm elasticity. All these contribute to the water holding capacity of plants.

Presowing treatment with ascorbic acid and sucrose affected inductive stages of plant growth by changing the course of metabolism. An upsurge in ascorbic acid and ascorbigan during the period of reproductive differentiation suggests that ascorbic acid turnover provides energy for physiological processes leading to flowering in wheat. The beneficial effect of pretreatment with ascorbic acid was not carried over to the next generation as far as growth, ear emergence, flowering and harvest data are concerned (Saxena, 1969).

The results of Chinoy and his co-workers (Chinoy, 1967a, 1967b, Chinoy et al., 1969a) revealed that the concentration of ascorbic acid free radical increased as shown by enhanced activity of ascorbic acid free radical peroxidase during the reproductive phase, as well as by the fact that ESR signals were more intense in the reproductive apex, fertilized carpel and in the corresponding leaf, at all the stages of differentiation as compared to those obtained from vegetative shoot apex. Free radicals of ascorbic acid were also reported in the endosperm during the early stages of
germination suggesting that they were involved in energy transfer mechanism required for the 'active' transport of solubilized nutrients to the embryo axis (Chinoy et al., 1969b). Commoner and his associates (1954, 1957, 1958) have established the participation of free radicals in one electron transfer of electrons in enzymatic reactions by measuring the electron spin resonance of biological materials. Synchronization of enhanced activity of a number of enzymes both during germination and reproductive phases (Chinoy et al., 1969b) with increased ascorbic acid content is suggestive of the role of ascorbic acid as an enzyme mobilizing hormone (Saxena, et al., 1969).

It may be considered that enzyme mobilizing function in plants is done by the electron energy supplied by ascorbic acid turnover involving the creation of suitable redox environment, stimulation of biosynthesis of macromolecules including enzyme proteins and formation of charge transfer complex. The electron energy from redox systems and charge transfer complex also powers the transport of sugar to embryo axis from endosperm for growth (Chinoy, et al., 1969c).

A number of workers have shown that ascorbic acid turnover increased with advancement of germination. Ascorbic acid stimulated germination in June (Mitra and Datta, 1951) and tomato and lupine (Lee, 1955). Exoge-
nous application of ascorbic acid increased induction as well as accelerated the rate of floral differentiation (Chinoy et al., 1957; Chilakhyan, 1956). Ascorbic acid has profound effect on germination as well as on early seedling growth. It acted as a triggering agent and also as germination factor (Michnievicz, 1961; Chinoy et al., 1961). Koch et al. (1967) reported that ascorbic acid increased molecular nitrogen fixation considerably when supplied to nodular bries and cell free bacteroidal extracts from nodules.

Ascorbic acid pretreated plants showed significantly higher mean relative growth ratios for the period of reproductive differentiation and during the ripening period. The pretreated plants registered higher dry weights in stem, leaf and main spike and the number of spikes was also more. The tiller production and total number of leaves was also reported to be high in ascorbic acid treated plants (Chinoy, J.J., 1969b). Ascorbic acid treatment accelerated flower formation and delayed ripening of grain, thereby increasing the yield (Chinoy et al., 1969b). Ascorbic acid treatment resulted in earliest branch initiation in the cotton plants. Combined treatment with 100 mg./1. ascorbic acid and 20 mg./1. gibberellic acid showed a synergistic effect (Gurbaksh Singh and Garg, 1969). The exogenous application of ascorbic acid has
been found to induce amylase activity in wheat and barley and the effect was more pronounced than gibberellic acid (Saxena et al., 1969). Ascorbic acid as well as gibberellic acid enhanced the initiation of nodules, their number and weight and nitrogen content of the plants, the former being more effective than gibberellic acid in all respects (Swaraj and Gary, 1969). Low concentrations of ascorbic acid increased the rate of water uptake in potato slices (Yodava, 1970). Pretreatment of cumin seeds with ascorbic acid showed some beneficial effects on crop yield (Chinoy et al., 1975). Pretreatment with ascorbic acid resulted in stimulated germination, more efficient water absorbing capacity, ability to better withstand against the atmospheric drought and more vigorous growth than the untreated seeds in a number of cultivars of wheat, oat, barley, sesame, ragi, maize and other crop plants (Chinoy and Saxena, 1978).

Seedlings of *Brassica juncea* from seeds pretreated with ascorbic acid showed advanced growth stage when compared to control during the course of germination. Concomitant with this, weight, per cent moisture content, per cent ascorbic acid utilized and ascorbic acid free radical peroxidase were recorded significantly at a higher level (Patel et al., 1980).
Recently, a notable effect of ascorbic acid was also reported on the secondary metabolites of some medicinal plants. 500 mg./l. and 1000 mg./l. solutions of ascorbic acid in the culture medium enhanced the growth, free ascorbic acid, and pyrethrine content in the callus grown from *Tagetes erecta* (Khanna and Khanna, 1976). Ascorbic acid promoted the synthesis of trypine alkaloids in *Atropa belladonna* tissue cultures (Sharma, 1977), and opium alkaloids in tissue cultures of *Papaver somniferum* (Khanna, 1978). When fenugreek seeds were germinated in 40 ppm. ascorbic acid, an increase of 16.48% in the diosgenin content was observed (Bhavnar et al., 1980). But Khanna et al. (1982) found a decrease in diosgenin content in the tissue cultures of various diosgenin yielding plants when ascorbic acid was incorporated in to the medium.