REVIEW OF LITERATURE
Over the years, organic and synthetic pesticides have played a vital role in pest management programs and also have enormously benefited mankind. However, excessive and non-judicious use of these pesticides lead to risks like resurgence and outbreak of new pests, development of resistance by pests, toxicity to non-target organisms and perilous effects on the environment endangering the sustainability of ecosystems necessitating the urge for new and alternative pest control methods. The natural chemical defense systems that plants have evolved through the ages by producing and exuding constituents of the secondary metabolism to protect themselves, may lead us to natural products that might be used to control insects. Earlier studies by several scientists, as reviewed in this chapter, provides evidences enough to think and work in this line of pest control. As a prelude, to plan the current research work; as well as, to lead in a right direction during the course of work, an extensive literature survey was carried out. Review of the literature thus surveyed is presented as follows:


### 2.1. Botanicals as pesticides

An interesting way of searching for biorational pesticides involves screening naturally occurring compounds in plants (Isman, 1997; 2000). Plants, over their lifetime, always have resisted attackers by production and exudation of constituents playing a key role in their defense mechanisms. Phytochemicals can be subdivided into two major groups viz., primary and secondary metabolites. The substances that are produced by the plant cells that are directly involved in its growth, development and reproduction (sugars, proteins, amino acids, and nucleic acids) are primary metabolites while components that are not directly involved in growth or reproduction but they are often involved with plant defense (terpenoids, phenolics, and alkaloids) are categorized as secondary metabolites (Harborne, 1973).
Pest management using these secondary metabolites involves either their direct application as pesticides or can be used as model compound for the development of chemically synthesized derivatives (Isman, 2000). Extensive literature is available concerning the use of plants crude or refined extracts for crop protection against various pests such as insects, fungi, nematodes, bacteria etc. The efficacy of botanicals can be attributed to specific constituent or compound(s) that bring about specific bioactivity (Akhtar & Mahmood, 1994).

2.1.1. Role of phytochemicals in plant defense against herbivore attack
Most healthy plants may produce a broad range of chemical barriers that hardly have any direct roles in the physiological processes like photosynthesis, respiration, translocation, solute transport, nutrient assimilation and differentiation (Harborne, 1973). Prohibitins or phytoanticipins are constitutively expressed, high energy, carbon consuming, fitness cost exhibiting defense metabolites involved in first line of defense while phytoalexins are induced metabolites formed within two or three days, in response to an infection comprising de novo synthesis (Van Etten et al., 1994; Grayer and Harborne, 1994; Jianqiang and Baldwin, 2010). The production of these defense metabolites is thought to be costly and is believed to reduce the plant growth and reproduction (Karban and Baldwin, 1997; Harvell and Tollrian, 1999).

A systematic evaluation of the kinds of plants fed upon by larvae leads to the conclusion that secondary plant metabolites play a leading role in determining patterns of utilization for all phytophagous insects (Harvell and Tollrian, 1999). Insects select plants for both food and as sites for oviposition with the help of a range of chemoreceptors (gustatory and olfactory chemoreceptive systems) located mainly on their antennae and mouthparts. These chemoreceptors enable them to discriminate a wide range of chemical compounds even at very low concentrations
and help to encode this information by the decoding command centers localized in the central nervous system (Dethier, 1960).

Plant constituents that make unpalatable to an insect are secondary metabolites in sufficient concentration to exert an undesirable physiological effect in them. Such a plant is protected from the attacks of phytophagous insects; therefore, plant secondary compounds have received much attention as proximate and ultimate determinants of host-plant range in phytophagous insects.

The main groups of secondary metabolites that contribute for plant defense can be broadly classified as alkaloids, nitrogen compounds (cyanide and cyanogenic glycosides), glucosinolates, cardenolides, iridoid glycosides, lectins, tannins and lignins, phenolics and phenylpropanoids, and terpenoids and steroids (Harborne, 1973).

Each of the group of phytoconstituents affects one or the other physiology of the invading insects in order to provide plant protection. They may have an individual effect or work in synergy. They exhibit antifeedancy, toxicity, enzyme inhibition, growth inhibition, oviposition deterrence or ovicidal effect on the herbivores. An array of botanicals with bioactive constituents and their mode of action against insects have been listed by Gahukar (2010) (Table 1).

### 2.1.2. Botanicals as antifeedants

Antifeedants are also called ‘feeding inhibitors’ or ‘feeding deterrents’ (Dethier et al., 1960). They deter feeding through a direct action on peripheral sensilla (taste organs) in insects (Isman, 2002).

Antifeedants are generally considered to play an important role in insect-plant interactions, especially in host plant recognition and host plant specialization of
herbivorous insects (Isman, 2002). Recognition of host plants by herbivorous insects is largely directed by chemical information. When insects encounter plants while searching for food, chemical information obtained from the plant is decisive in either accepting or rejecting it as food plant (Koul, 2004).

The mode-of-action of most antifeedants is directed at the taste cells. A gustatory sensillum in an insect contains receptors selective for deterrents and others for stimulants (such as sugars and amino acids). Most of the antifeedants act likely by stimulating a deterrent receptor, which further sends a signal to the feeding center in the insect’s central nervous system while some antifeedants are believed to block or interfere with the perception of feeding stimulants, while others may cause erratic bursts of electrical impulses in the nervous system preventing the insect from acquiring appropriate taste information on which it may choose an appropriate feeding behaviour (Isman, 2002). Insect antifeedants isolated from terrestrial plants.

Antifeedants can be found amongst all the major classes of secondary metabolites – terpenoids, alkaloids, and phenolics (Harborne, 1973). Isman (2002) has provided a list of insect antifeedants belonging to various classes of secondary metabolites isolated from various terrestrial plants (Table 2). Terpenoids are the largest and most diverse class of phytoconstituents that exhibit enormous chemical variety and complexity with respect to structure as well as their biological activities (Gershenzon and Croteau, 1991).

The monoterpenoids esters like pyrethroids from the leaves and flowers of Chrysanthemum species are best known insect toxins (Casida, 1973). The toxic effect of monoterpenes from Pseudotsuga menziesii against western spruce budworm Christoneura occidentalis affecting the larval growth and survival have been well documented by Cates et al., (1987). Monoterpenes like pulegone from Mentha pulegium and resins from conifers have a toxic and repellent effect against bark
Table 2. Insect antifeedants isolated from terrestrial plants (Isman, 2002)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Chemical type</th>
<th>Compound</th>
<th>Plant source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Monoterpene</td>
<td>Thymol</td>
<td>Thymus vulgaris (Lamiaceae)</td>
</tr>
<tr>
<td>2</td>
<td>Sesquiterpene lactone (germacarnolide type)</td>
<td>Glaucolide A</td>
<td>Vernonia species (Asteraceae)</td>
</tr>
<tr>
<td>3</td>
<td>Sesquiterpene (drimane type)</td>
<td>Polygodial</td>
<td>Polygonum hydropiper (Polygonaceae)</td>
</tr>
<tr>
<td>4</td>
<td>Diterpene (abietane type)</td>
<td>Abietic acid</td>
<td>Pinus species (Pinaceae)</td>
</tr>
<tr>
<td>5</td>
<td>Diterpene (clerodane type)</td>
<td>Ajugarin I</td>
<td>Ajuga remotas (Lamiaceae)</td>
</tr>
<tr>
<td>6</td>
<td>Triterpene (limonoid type)</td>
<td>Azadirachtin</td>
<td>Azadirachta indica (Meliaceae)</td>
</tr>
<tr>
<td>7</td>
<td>Triterpene(cardenolide type)</td>
<td>Digitoxin</td>
<td>Digitalis purpurea (Scrophulariaceae)</td>
</tr>
<tr>
<td>8</td>
<td>Triterpene(ergostane type)</td>
<td>Withanolide E</td>
<td>Withania somnifera (Solanaceae)</td>
</tr>
<tr>
<td>9</td>
<td>Triterpene (spirostane type)</td>
<td>Aginosid</td>
<td>Allium porrum (Liliaceae)</td>
</tr>
<tr>
<td>10</td>
<td>Alkaloid (indole type)</td>
<td>Strychnine</td>
<td>Strychnos nuxvomica (Loganiaceae)</td>
</tr>
<tr>
<td>11</td>
<td>Alkaloid (steroidal glycoside)</td>
<td>Tomatine</td>
<td>Lycopersicon esculentum (Solanaceae)</td>
</tr>
<tr>
<td>12</td>
<td>Phenolic (furanocou arin)</td>
<td>Xanthotoxin (=8-methoxy psoralen)</td>
<td>Pastinaca sativa (Apiaceae)</td>
</tr>
<tr>
<td>13</td>
<td>Phenolic (lignan)</td>
<td>Podophyllotoxin</td>
<td>Podophyllum peltatum (Berberidaceae)</td>
</tr>
<tr>
<td>14</td>
<td>Phenolic (benzoate ester)</td>
<td>methyl salicylate</td>
<td>Gaultheria procumbens (Ericaceae)</td>
</tr>
</tbody>
</table>
beetles as well serving as insect feeding deterrents (Rosenthal, 1991). Several types of diterpenes (based on a 20-carbon skeleton) including the clerodanes from *Teucrium tomentosum*, (Soundaryadevi et al., 2003), isoryanodane diterpenes from *Persea indica* (Braulio Fraga et al., 1997) are well known antifeedants against *Spodoptera litura* along with grayanoid diterpenes from *Rhododendron molle* against *Leptinotarsa decemlineata* and *Spodoptera frugiperda* (Klocke et al., 1991). Sesquiterpenes (15-carbon skeleton) with potent antifeedant action include the drimanes, and the sesquiterpene lactones from Asteraceae. One particularly well-studied example is the drimane polygodial extracted from the foliage of *Polygonum hydropiper* (Luis Moreno-Osorio et al., 2007; Adeyemi, 2010) and stem bark of *Drimys winteri* (Zapata et al., 2009) against lepidopteran larvae. The deterrent effect of polygodial and other drimane dialdehydes may be as a result of blocking of the stimulatory effects of glucose and sucrose on chemosensory taste receptor cells found on the mouthparts (Jansen and de Groot, 1991).

The sesquiterpene lactones from *Viguiera tucumanensis* (Clarisa Vaccarini, 2002), *Centaurea maculosa* (Landau et al., 1994) belonging to asteraceae have also been reported to impart antifeedancy in generalist herbivores like *Spodoptera littoralis* and *Leptinotarsa decemlineata* (Azucena Gonzá’lez-Colomaa, 2005) and specialist herbivorous insects like *Strenodes straminea* (Landau et al., 1994; Adekenov, 1995).

Among the triterpenoids, well studied ones include limonoids from the neem (*Azadirachta indica*) against Lepidopterans like *Helicoverpa armigera* (Murugan et al., 2011; Koul et al., 2004), *Cnaphalocrocis medinalis* (Guene’e) (Senthinathan et al., 2005) and Coleopterans like *Epilachna paenulata* Germ. (Maria Carpinella et al., 2003). The antifeedant effects of limonoids from chinaberry trees (*Melia azedarach*) on *Agrotis ipsilon* (Mekhli, 2009) as well as azadirachtin, toosendanin, and limonin from Citrus species against several Hemipterans like *Aphis glycines* Matsumura (Kraiss & Cullen, 2008), Lepidopteran *Spodoptera litura* (Isman,
2002), and Coleopteran pests like Harmonia axyridis Pallas (Kraiss & Cullen, 2008) are also reported. Other antifeedant triterpenoids include steroidal saponins, cardenolides, and withanolides extracted from solanaceous plants belonging to the genera Withania, Acnistus, Physalis, Jaborosol and Datura (Isman, 2002; Adeyemi, 2010; Gebreyesus and Chapya, 1983).

The best known example of simple phenolics being feeding barriers to insect herbivores include the coumarins from Clausena anisata (Rutaceae) against Spodoptera exempta (Gebreyesus and Chapya, 1983) and neolignans represented by salicylates in Salix leaves that affect the feeding and growth of the polyphagous larvae of Operophtera brumata (Havill and Raffa, 2000). Alkaloids with well documented antifeedant effects on insects include certain indoles on Agrotis ipsilon, (Jeyasanker, 2012) and the solanaceous glycoalkaloids on Leptinotarsa decemlineata (Melville et al., 1985).

2.1.3. Botanicals as growth inhibitors
Plants offer a rich source of novel compounds with insect growth regulatory (IGR) properties. The ample potential in using phytoecdysones as IGRs cannot be overlooked. It has been reported that there are more than 1,000 species of plants belonging to more than 200 families, 800 genera containing these bioactive substances (Varma and Dubey, 1998).

The chemistry and mechanism of toxic action of these plant products to insects mainly focuses on insect growth hormones like juvenile hormone and ecdysones. If insects undergoing metamorphosis are treated with juvenile hormone (JH), they moult into intermediates, which are half immature and half adult, which die without gaining reproductive competence. The insect growth regulators (IGRs) are generally selective in their toxicity, slow in their killing action, and safe to both the environment and the natural enemies of insect pests. JH and JH analogues have
been found in various plants that interfere with the control of several species of insects. Phytojuvenoids such as juvabione, farnesol, juvocimene I and II, sesamin and sesamolin, thujic acid, sterculic acid, tagetone, ostruthin, echinolone, baku-chiol, juvadecene have been isolated from plants (Varma and Dubey, 1998).

Antijuvenile hormones such as precocenes isolated from *Ageratum haustorianum* (Bowers *et al.*, 1976) show anti allatotropic activity (prevent JH synthesis) and accelerated the development of insects, producing dwarf sterile animals, which were unable to survive. Analogues of precocone, b-asarone which is a constituent of the rhizome of sweet flag (*Acorus calamus*) showed antagonadal activity in insects. The vapours of the oils have exhibited complete inhibition of ovarian development when given to a number of stored grain insects (Mathur and Saxena, 1975; Koul *et al.*, 1977a; Schmidt and Strelko, 1994).

Most plants contain an array of flavonoids that are distributed across different families, genera and species. Plant flavonoids affect the behaviour, development and growth of a number of insects. In comparison to many other secondary metabolites, flavonoids are apparently not very toxic and have a low physiological activity in most insects. Isoflavonoids like maackiain and judaicin decreased the weight gain of early stadia larvae of *Helicoverpa armigera*. Renwick *et al.* (1987) found that flavones glycoside, isovitexin-6"-D-β-glucopyranoside (XXI), retarded the growth of Native American butterfly, *Pieris napi oleracea*. Similarly, Hypercalin A, a phenolic from *Hypericum calycinum* flowers was deterrent and toxic to *Utetheisa ornatrix* larvae (Gronquist *et al.*, 2001).

### 2.1.4. Botanicals as oviposition deterrents

Among the phytophagous insects, host selection by ovipositing adults is crucial for the survival and development of their offspring. The oviposition stimulants and deterrents may often co-exist in plants and chemoreceptors sensitive to either
stimulants or deterrents may play a key role in determining the final decision by an insect (Renwick and Radke, 1990). Specialist herbivores would lay their eggs only on the presence of characteristic stimulants, whereas generalists would oviposit based on the absence of deterrents (Renwick, 1983). Cardenolides mediate discriminatory behavior of ovipositing butterflies. Recent studies by Oyeyele and Zalucki, (1990) and Zalucki et al., (1990) have shown a relationship between cardenolide content of milkweeds and oviposition by the monarch butterfly, Danaus plexippus wherein the females oviposited in plants that had significantly lower cardenolide levels compared to the ones with higher levels and control plants. Similar oviposition deterrent activity by cardenolides of crucifer, E. cheiranthoides was studied in cabbage butterfly, P. rapae, (Dimock and Renwick, 1991) and the active compounds were identified as strophanthidin glycosides (Sachdev-Gupta et al., 1990).

Oviposition deterrents for one insect may affect the behavior of associated insects in different ways. It was found that a fraction containing cardenolides from E. cheiranthoides that would deter P. rapae oviposition were actually stimulatory to Plutella xylostella (Renwick and Radke 1990). More studies are needed to better understand the host selection mechanisms of phytophagous insects and in particular to evaluate the involvement of deterrents in insect-plant interactions.

In nature host recognition by phytophagous insects involves multiple sensory modalities, involving visual, olfactory and gustatory cues. It is observed that the volatile fatty acids, detected by the antennae of females in Ostrinia furnacalis (Lei Guo, 2009); in Helicoverpa armigera (Xu et al., 2006), deterred females from oviposition. Similarly, the contact chemoreceptors, located on the fore tarsi in some of the female insects, are responsible for detection of both host and non-host allelochemicals at oviposition site (Roessingh et al., 1991; Nishida, 1995).
The lipid components normally found on the surfaces of the aerial parts of the plant contribute for the plant defense mechanisms. Texture of the leaf is influenced by these epicuticular lipid components hence indirectly they play a role in insect attachment and, locomotion also. The non polar extracts of plants parts i.e., lipid materials may mask the polar stimulants present in the leaf, thus preventing the insect's tarsal receptors from making the necessary contact with the substratum (Koon and Njoya, 2004; Dethier et al., 1960). In some insects for example gravid cabbage butterflies polar as well as non polar extracts of non host crucifers were deterrent (Renwick and Chew, 1994). Ayyangar and Rao (1989), Srinivasan and Sundarababu (1999) have reported that the methanol and hexane extracts of neem seed kernel are not only larval repellents but also oviposition deterents to the adults of Spodoptera litura and Leucinodes orbanalis respectively. Oviposition deterrence by neem under no-choice and choice conditions have been reported in Cnaphalocrosis medinalis (Saxena et al., 1981), Earis vittella (Sohtra ad Patel, 1992; Gajmer et al., 2001) and Crocidolomia pavonana (Fagoonee, 1981). The main chemical components of oviposition deterrents that were observed in the larval frass or on the egg masses of Ostrinia species are myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids (Li and Ishikawa 2004). These results indicate that the oviposition-deterring fatty acids in the larval frass or on the surface of eggs are volatile and are probably detected by female antennae.

In addition to toxicity, feeding deterrent effect and oviposition deterrent effect has been exhibited by Pieris rapae when fed upon cardenolides from Erysimum cheiranthoides (Sachdev-Gupta et al., 1993). However, several insect species sequester cardenolides for their own defense against vertebrate predators (Zalucki, 1990).
2.1.5. Botanicals as ovicidal agents

Coumarins are a class of natural compounds comprising of a 2H-1-benzopyran-2 one nucleus and more than 800 coumarins that are widespread across 70 plant families have been identified and characterized so far. These coumarins have a varied range of effects on the herbivores. A simple coumarin bergamottin has ovicidal effect against *Leptinotarsa decemlineata* (Carrasco *et al*., 1981), *Drosophila melanogaster* (Kogan *et al*., 1983). Furanocoumarins also exhibit deterrent and toxic to a range of insects like *Battus philenor*, *Depressaria pastinacella*, *Eurytides marcellus* and *Heliothis zea* (Berenbaum and Neal, 1985).

2.1.6. Botanicals as enzyme inhibitors

Many plants and seeds contain proteins that specifically inhibit pathogen and pest enzymes by forming complexes that block active sites or alter enzyme conformation, ultimately reducing enzyme function. These proteins are generally small and rich in the amino acid cysteine. They include defensins, amylase inhibitors, lectins, and proteinase inhibitors. Unlike simple chemicals such as terpenoids, phenolics, and alkaloids, proteins require a great deal of plant resources and energy to produce; consequently, many defensive proteins are only made in significant quantities after a pathogen or pest has attacked the plant.

Defensins are small cysteine-rich proteins that inhibit digestive proteins in herbivores. The precise mechanisms employed by plant defensins act upon molecular targets in the plasma membrane of pathogens. These defensins may inhibit pre-existing ion channels or form new membrane pores that disrupt cellular ion balance.

Digestive enzyme inhibitors are proteins that block the normal digestion and absorption of nutrients by vertebrate and invertebrate herbivores. Alpha-amylase inhibitors are proteins commonly found in legumes that bind to amylase enzymes
and inhibit starch digestion. Mehrabadi et al., (2011) have documented the amylase-inhibitory activities of the extracts of *Punica granatum* L. (Punicaceae), *Rheum officinale* B.(Polygonaceae), *Rhus coriaria* L. (Anacardiaceae), *Artemisia sieberi* B. (Compositae), *Peganum harmala* L. (Nitrariaceae), *Datura stramonium* L. (Solanaceae) and *Thymus vulgaris* L. (Lamiaceae) against stored grain pest *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), *Sitophilus granarius* L. (Coleoptera: Curculionidae), and *Trogoderma granarium* E. (Coleoptera : Dermestidae).

Lectins are non-enzymatic proteins and glycoproteins that bind to carbohydrates and exhibit a wide range of functions including disruption of digestion in insects, disruption of protein synthesis and agglutination of blood cells in vertebrates. The harmful effects of lectins on biological parameters of insects are larval weight decrease, mortality, feeding inhibition, delays in total developmental duration, adult emergence and fecundity on the first and second generation (Powell et al., 1993; Habibi et al., 1993). Various lectins from different sources have already been found to be toxic towards important members of insect orders, including Lepidoptera (Czapla & Lang, 1990), Coleoptera (Gatehouse et al., 1984; Czapla & Lang, 1990) and Homoptera (Powell et al., 1993).

Protease inhibitors are typically produced in response to herbivore attack and inhibit digestive enzymes including trypsin and chymotrypsin. They occur widely in nature but have been well studied in legumes, solanaceous plants, and grasses. Trypsin inhibitors present in soybean were shown to be toxic to the larvae of flour beetle, *Tribolium confusum* (Lipke et al., 1954) and *Callosobruchus maculatus* (Gatehouse and Boulter, 1983) and *Manduca sexta* (Shulke and Murdock, 1983). Serine proteinase inhibitors have anti-nutritional effects against several lepidopteran insect species (Shulke and Murdock, 1983). Broadway and Duffey (1986a) demonstrated the
growth inhibitory effects of purified SBTI and potato inhibitor II (an inhibitor of both trypsin and chymotrypsin) on larvae of Heliothis zea and Spodoptera exigua.

2.2. Calendula officinalis-a probable pesticide

Calendula officinalis Linn. belongs to the family, Asteraceae, and is commonly known as Zergul (Hindi), African marigold, Calendula, Common Marigold, Garden Marigold, Marigold, Pot Marigold (English), Butterblume (German), Chin Chan Ts’ao (Chinese), Galbinele (Romanian) and Ringblomma (Swedish).

2.2.1. Habitat

Calendula is native to the Mediterranean area i.e., Central and Southern Europe, Western Asia and also the US. However, some believe it comes from Egypt although it is widely spread throughout the world as an ornamental plant. Though it prefers clay soil, it can grow in every kind of soil and hence has been usually planted in urban flower beds and gardens.

2.2.2. Botanical description of the plant (flower, leaves, root and fruit)

Calendula officinalis L. is an aromatic annual plant that belongs to the Asteraceae (Compositae) family. The stems are straight, ramified. The leaves are oblong-lanceolate, hairy on both sides, 5 to 15 cm long, with toothed margins. The inflorescences are thick capitula or flower-heads (3-8 cm) surrounded by two rows of hairy bracts. The tubular, hermaphrodite, central flowers are generally, of a more intense orange-yellow colour than tridentate, peripheral flowers. The flower-heads appear all year long. The fruit is a thorny curved achene. On the tip of each stem, there is a 5 to 7 cm composite flower head, consisting of an epicalyx of numerous narrow-lanceolate sepals, which are densely covered on both sides with glandular hairs. The inner section of the flower head is made up of orange-yellow tubular florets. The disc florets are pseudohermaphrodites but the female is sterile. The
Calendula officinalis L. Plants

Diagramatic Representation of Calendula plant parts
zygomorphic ray florets at the edge are female, their stamens are completely absent, and their inferior ovaries are much more developed than those of the tubular florets. The fruit forms only in the female ray flowers. The heterocarp achenes are sickle-shaped, curved and ringed. The plant is an annual, seldom biennial. It grows to between 30 and 50 cm high, and has about 20 cm long tap root and numerous thin, secondary roots. The stem is erect, angular and downy and branched from the base up or higher. The alternate leaves are almost spatulate at the base, oblong to lanceolate above and are all tomentosae.

2.2.3. Traditional uses

In Europe, the leaves are considered resolvent and diaphoretic while the flowers are used as a stimulant, and antispasmodic. In England, the decoction of the flowers was used as a posset drink for the treatment of measles and smallpox, and the fresh juice as a remedy for jaundice, costiveness (constipation) and suppression of menstrual flow. In India, the florets are used in ointments for treating wounds, herpes, ulcers, frostbite, skin damage, scars and blood purification. The leaves, in infusion, are used for treating varicose veins externally. The botanical name comes from kalendulae, the Latin word for the first day of the month in the Roman calendar, in reference to the plant’s tendency to bloom at the beginning of almost every month (Gracie Lynn, 2011).
2.2.4. Taxonomic position

Kingdom : Plantae
Subkingdom : Tracheobionta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Asteridae
Order : Asterales
Family : Asteraceae
Tribe : Calenduleae
Genus : Calendula
Species : officinalis

2.2.5. Medicinal uses

Even when its origins are still uncertain, the uses of calendula as a medicine and as a
colouring agent were known to the old herbalists, especially Arabs and Hindus. Calendula has long been used in topical applications, to treat skin ulcers, infected
wounds, diaper rash, eczema, varicose veins, hemorrhoids, periodontitis and
conjunctivitis (www.lotioncrafter.com/calendula-extract-certified-organic.html).

2.2.6. Chemical composition

Essential oils: Calendula flower-heads have variable essential oil concentrations in
the ligulate flowers and in the inflorescence receptacle. The flower-heads are rich in
oxygenated monoterprenes and sesquiterpenes: carvone, geranylacetone,
cariophyllene ketone, menthone, isomenthone, γ-terpinene, γ and δ-cadinene,
cariophyllene, α and β-ionone, 5,6-β-ionone epoxide, pedunculatine, dihydro-
actinidiolide, α-muurolene, etc (Latra Valdés H & Piquet García R, 1999).

Flavonoids: Calendula inflorescences harbor flavonoids like isorhamnetin
3-O-glycoside, isorhamnetin neohesperidoside, quercetin glucoside, calendoflaside,
calendoflavoside, calendoflavobioside, narcassin, isoquercetin, quercetin, rutoside and kaempferol (Lastra Valdés H & Piquet García R, 1999).

**Triterpene alcohols:** Pentacyclic triterpene alcohols like arnadiol, faradiol, α & β-amyrin, faradiol-3-myristic acid, lupeol, taraxasterol, faradiol-3-palmitic acid, calenduladiol, etc are found in the capitula of Calendula species (Alonso J, 2004).

**Phenol acids:** Calendula is known to have the following phenolic acids; coumaric, gentísic, vanillic, caffeic, syringic, o-hydroxyphenylacetic, protocatechnic, ferulic, p-hydroxybenzoic, salicylic, chlorogenic, veratric, o-coumaric and quinic acids (Lastra Valdés H & Piquet García R, 1999).

**Tannins:** Several researchers have observed the presence of catechol- and pyrogallol like tannins in Calendula plants (Lastra Valdés H & Piquet García R, 1999).

**Carotenoids:** The ligulate flowers and receptacles of Calendula have been found to contain carotenoids. The identified compounds are α-, β- and γ-carotene, violaxanthin, rubixanthin, citroxanthin, flavochrome, galenin, lutein, lycopene, valenciaxanthin, auroxanthin, microxanthin, 5,6 epoxycarotene, β-zeacarotene, mutatoxanthin and lutein epoxyde (Lastra Valdés H & Piquet García R, 1999).

**Saponosides:** Oleanic acid derivatives like calendulosides A, B, C, D, D2, F, G, and H are also present in Calendula.

**Other active principles:** Calendula flowers also contain coumarins – such as scopoletin, umbelliferone and esculetin – sterols, sugar and paraffin. Researchers identified water soluble polysaccharides, pectin-like substances and hemicellulose on a dry basis in the aerial parts of Calendula (Lastra Valdés H & Piquet García R, 1999).
2.2.7. Uses in pest management

The members of Asteraceae are also known to contain characteristic secondary plant compounds with known anti-herbivore effects (Landau et al., 1994). Several plants belonging to Asteraceae such as *Chrysanthemum* sps., *Artemisia* sps., *Tagetes* sps., *Synedrella* sps., have been reported to be effective against the control of many pests like *Ageratum conyzoides* (Hussain, et al., 1990) and *Rudbeckia hirta*. The extracts from *Chromolaena christieana* (stem and bark), *Achyrocline satureoides* (leaves and flowers) and *Mikania cordifolia* (root and stem) have moulting inhibition activity against a hematophagous insect (Rojas Arias, et al., 1995). The leaf extracts of *Chromolaena odorata* were proved to be fatal to coconut rhinoceros beetle, *Oryctes rhinoceros* L. (Leena et al., 2008).

In addition to other Asteraceae plants, *Calendula officinalis* L. has been given a lot of credit as a pest deterrent. The pungent odor of the marigold has been used as an effective pesticide and also as an insect repellant to deter white flies. Intercropping with Pot Marigold was the most effective pest control on cabbage as they lowered the number of cabbage aphid *Brevicoryne brassicae* L. and flea beetles *Phyllotreta* on the cabbage plants. The lower number of eggs of the small white butterfly *Pieris rapae* L., large white butterfly *P. brassicae* L., cabbage moth *Mamestra brassicae* L. and larvae and pupae of the diamondback moth *Plutella xylostella* L. were observed on plots intercropped with Calendula (Beata Jankowska, 2009).

2.3. *Spodoptera litura*- the pest.

*S. litura* (Fabricius) is the scientific name of the pest under current investigation. English armyworm, cluster caterpillar, common cutworm, cotton leafworm, cotton worm, egyptian cotton leafworm, rice cutworm, tobacco budworm, tobacco caterpillar, tobacco cutworm, and tobacco leaf caterpillar are some of the common names for *Spodoptera litura*.
2.3.1. Taxonomic position

Kingdom : Animalia
Phylum : Arthropoda
Class : Insecta
Order : Lepidoptera
Superfamily : Noctuoidea
Family : Noctuidae
Subfamily : Acronictinae/Amphipyrinae
Genus : Spodoptera
Species : litura
Author : Fabricius, 1775

2.3.2. Geographic distribution

The tobacco caterpillar, S. litura, is one of the most important insect pests of agricultural crops in the Asian tropics. It is widely distributed throughout tropical and temperate Asia, Australasia, and the Pacific Islands (Feakin, 1973; Kranz et al., 1977). The pest under consideration is widely distributed in different countries across the globe as shown in (Fig 1 and 2).

2.3.3. Host plants and host preferences

The pest is polyphagous in nature (Brown & Dewhurst, 1975) and the host range covers over 40 families (Salama et al., 1972). Among the main crop species attacked in the tropics are Colocasia esculenta, cotton, flax, groundnuts, jute, lucerne, maize, rice, soyabean, tea, tobacco, vegetables (aubergines, Brassica, Capsicum, cucurbit vegetables, Phaseolus, potatoes, sweet potatoes, Vigna etc.). Other hosts include ornamentals, wild plants, weeds, and shade trees (e.g. Leucaena leucocephala, the shade tree of cocoa plantations in Indonesia). This pest is a defoliater of more than 120 species of host plants, many of which are commonly produced in the US.
Figure. 1. The geographical distribution map of *Spodoptera litura* Fab.

(Source EPPO, 2006)
Figure. 2. The distribution map of *Spodoptera litura* Fab. in the Indian subcontinent

(Source: http://www.nabg-nbaii.res.in)
2.3.4. Pest status and economic impact

Larvae is the most harmful insect pest of crops, which remains in the crop up to maturity and exhibits maximum damage during the vegetative phase of the plant where enough chlorophyll is present. Normally the damage caused by small larvae is easily missed out, but for careful inspection. Damage may not be noticed until the larvae are almost full-grown. Large larvae may invade from adjacent cereal crops or grasslands and eat out the entire area. Infestations are indicated by the eaten-out margins of leaves due to feeding of the older larvae and also by the faecal pellets around the base of the plant. The larvae often feed on the leaf blades leaving the midrib and so leaving the plant with a tattered appearance. Leaves up to 45cm from ground level are stripped (Elder, 2007). The caterpillars cause severe defoliation resulting in huge loss. The larvae feed voraciously on almost all plant organs. Generally, young leaves are preferred leading to skeletonization of the leaves, but when they consume leaves, they attack other parts (e.g. stems, buds or pods). An infestation frequently leads to complete defoliation. Besides devouring the leaves, the caterpillars interfere with plant development by destroying growth points and flowers. They bore into buds and capsules and feed inside them, soiling them with frass. On light soil, they can continue feeding during the daytime, when they hide underground (www.nabg-nbaii.res.in).

2.3.5. Resistance to insecticides

Pesticide resistance is now an immense problem associated with the chemical control of any pests. More than 500 species of arthropods have evolved resistance to pesticides (Georghiou and Lagunes-Tejeda, 1991). *Spodoptera litura* is no exception for this. They are reported to be resistant or highly resistant in some areas, to all classes of insecticide viz., organochlorines, organophosphate, and synthetic pyrethroid (Issa et al., 1984), thus occupying new habitats and substituting other pest species. Undoubtedly they have spread worldwide because of the market distribution of plants or plant parts, but resistance developed to most pesticides
applied frequently in protected and open field crops has been of great importance. Furthermore, mismanagement like applying broad spectrum insecticides at high dosages and using repeatedly the same active ingredients, etc., can lead to the development of resistance in any insect with in very few years. Again *S. litura* is not an exception for this rule hence; pesticide selective pressure must be reduced by way of finding alternative methods to control this insect.

2.4. **Integrated Pest Management**

These days, crop failures have been addressed by integrated pest management approach based on combination of all methods of control like chemical and biological control methods along with the use of pheromones (JA Wightman, ICRISAT, Andhra Pradesh, India, personal communication, 1996).

Various natural based synthetic insecticides such as cypermethrin, a synthetic pyrethroid have been deployed to control *S. litura*. However, criteria like short generation time; excessive usage contributing to rapid development of resistance and from an environment standpoint, the use of various synthetic insecticides is certainly not an advisable control measure.

At different life stages, *S. litura* is attacked by as many as 131 species natural enemies reported from different parts of the world. These include egg parasitoids like *Telenomus chilonis, Cotesia sp.*, *Meteorus sp.*, *Orius sp.*, *Geocoris sp.*, *Nabis sp.*, *Nomurea rileyi*. (Joshi et al., 1979), larval parasitoids like *Chelonus formosanus* (Rai 1974), *Peribaea orbata* (Jayanth and Nagarkatti, 1984), *Zele chlorophthalma* (Rao and Satyanarayana, 1984), pupal parasitoids like *Ichneumon sp.* and *Chelonus sp.* and predators like ants, earwigs, pentatomid bugs and predaceous beetles. Several fungal pathogens viz., *Aspergillus flavus, Beauveria bassiana, Nomuraea rileyi* and *Metarhizium anisopliae* (Zaz and Kishwaha (1983), some strains of *Bacillus*
thuringiensis (Salama et al., 1989) and viruses like Nuclear Polyhedrosis Viruses and Granulosis virus (Narayanan 1985) are also involved in the control of this pest.

2.5. Methodology for isolation of biofactor from plants

Plant harbors innumerable number of secondary metabolites that have a distinct function against herbivory and if a detailed study about their chemical structures, biosynthesis, turnover, metabolism, natural distribution, and biological functions have to be carried out, one has to extract, analyze, separate, purify and identify these phytococonstituents in an organized way (Harborne, 1973).

2.5.1. Extraction of plant material

In order to isolate the bioactive factor, the plant material (fresh or shade dried) has to be subjected to extraction which depends upon the texture, water content of the plant material being extracted as well as the type of compound that has to be isolated. The classical chemical procedure to procure organic constituents from dried plant material is to continuously extract powdered material in a Soxhlet apparatus with an array of solvents in the increasing order of their polarity (petroleum ether and chloroform to isolate terpenoids and lipids while alcohol and ethyl acetate for more polar compounds). The extracts thus obtained can be further subjected to preliminary screening/qualitative analysis for the detection of various groups of phytococonstituents present in them. The qualitative tests include screening for alkaloids, terpenoids, steroids, saponins, flavonoids, glycosides, proteins, carbohydrates etc (Prashanth Tiwari et al., 2011). Subsequent to screening, the extracts can be used for conducting bioassays to establish their biological activity. Once their biological activity is studied, the extracts can be separated into fractions, to isolate individual components responsible for the bioactivity. Soxhlet extraction of the plant materials followed by their phytochemical screening have been followed
by many of the groups of researchers like Carpinella et al. (2003), Martin Rathi et al.,(2006).

2.5.2. Separation and Purification of bioactive principle

The separation and purification of bioactive principle can be achieved by using different chromatographic techniques like column chromatography (CC), paper chromatography (PC), thin layer chromatography (TLC), gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC) either singly or in combinations (Harborne, 1973; Citoglu & Acikara, 2012). The solubility and volatilities of the compounds to be separated forms the baseline for the use of each of these techniques. TLC finds its application for separation of hydrophobic components like lipids, steroids, carotenoids and chlorophylls as practiced by different group of workers like Cetkovic et al., 2003 and Chakraborthy et al., 2010 in isolating various phytocostituents in Calendula officinalis. GLC, a method of choice to separate volatile compounds like fatty acids, mono- and sesquiterpenes, hydrocarbons and sulphur compounds from Calendula flower extracts for researchers Okoh et al., 2007; Stela Georgieva et al., 2008. While less volatile compounds from Calendula was separated through HPLC by Eszter Bako et al., 2002; Cecilia Veronica Nuñez et al., 2004; Petrovi et al., 2007. All the aforesaid techniques can be used both on a macro- and a microscale level. Various reports about the preparative TLC of Calendula extracts carried out using thick layers (upto 1 mm) of adsorbent is available wherein the separated constituents are recovered by scrapping off the adsorbent at the appropriate places on the developed plate, eluting the powder with a solvent such as ether or acetone and finally centrifuging to remove the adsorbant (Ostad et al., 2004; Braga et al., 2009).
2.5.3. Identification, Molecular weight determination and Structural elucidation of compound

Once the plant constituent has been isolated and purified, firstly it is important to determine the class of the compound as well as its homogeneity i.e., it must move as a single spot on a TLC plate. Also, complete identification within the class depends upon other properties like melting point, boiling point, optical rotation, Rf. spectral characteristics like ultra violet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectral (MS) measurements (Harborne, 1973). A known compound can be identified on the above basis. Later on final confirmation can be done by directly comparing with the standard compound. If the standard compound is not available, careful comparison with the literature data may help for identification. Suppose, a new compound is obtained, all the above data can come in hand for characterization.

The mass spectroscopy technique can be employed to determine the molecular weight of the compound, yielding a complex fragmentation pattern, which is often characteristic of and may identify any specific compound. If MS helps in determining the mass, proton NMR spectroscopy essentially helps to determine the structure of an organic compound by measuring the magnetic moments of its hydrogen atoms that are attached to different functional groups as –CH2, -CH3, -CHO, -NH2 etc and provides a record of the number of hydrogen atoms present. The nature of the carbon skeleton of the molecule can be determined by 13Carbon NMR spectroscopy in a similar fashion.

The combination of all the spectral data were used to predict the probable compounds in the plant extracts by a number of researchers like Ferreria et al. (2009), Li He et al. (2010), Neri & Tringali (2001). The probable compounds in the flower extracts of Calendula was determined by using these