CHAPTER 1
General Introduction
1.1 Introduction

Modern chemical technology has produced many organic compounds, which exhibit action as insecticides and pesticides in order to control the insect population. These compounds are mainly used because of their chemical stability and toxic properties. Though these chemical compounds are believed to be stable and safe, their continuous use can interfere with various life processes of living things. Herbal folklore has long included the use of aromatic herbs and oils as insect repellents. Certain oils like use of citronella oil (Cymbopogon nardus) in candles and in products designed to be used on the skin are well known. Other aromatic herbs used for centuries for their insect repellent qualities include Pennyroyal (Mentha pulegium), Elderflower leaf (Sambucus nigra) which also repels mice, and Mugwort (Artemesia vulgaris) and Rosemary (Rosmarinus officinalis) both are known for repelling moths. Natural insecticides such as Tansy (Tanacetum vulgare), Pyrethrum flower (Anacyclus pyrethrum), Anise (Pimpinella anisum) and Birch (Betula alleghaniensis) have also been used since ancient times. Some of the prominent essential oils used are oils of Cajeput (Melaleuca leucadendron L.), Citronella, Eucalyptus (Eucalyptus globulus), Geranium (Pelargonium roseum), Lavender (Lavandula angustifolia), Peppermint (Mentha x piperita), Red Cedar wood (Juniperus virginiana) and Rosemary (Rosmarinus officinalis). These oils are used either singly or in combinations diluted into hydrosol or water and sprayed on the skin.

There is an abundance of plants that can be used for their natural insecticide properties. Over 1500 are presently being used for control of pests. This provides a variety of methods to get rid of unwanted insects. Many factors will determine which kind of natural insecticide can be used. Some are more inexpensive. Some are more easily obtained than others. Some are safer to humans and pets.

Synthetic chemical insecticides often contain ingredients that kill beneficial insects. The use of synthetic chemical insecticides has long been associated with a variety of chronic health conditions. The advantage of using a natural insecticide is that these conditions rarely occur with their use. Therefore, an objective of this work is to provide a natural insecticide /pesticide exhibiting broad-spectrum insecticidal activity towards a variety of insects and pests harmful to mankind thereby preventing toxic chemicals to affect the environment.

The present endeavor is to study the plant extract of seeds of Cassia tora for identifying the potential insecticidal activity on the pests, which are menace to
households, agriculture and public health. This piece of work thus would be able to contribute towards an Integrated Pest Management approach of treating the pests wherein a synergistic effect of insecticides can play a vital role in saving the environment as well as provide a safe and effective way of eradication of pests.

The study plan covers an in depth literature survey on the plant extract under experiment to ensure no study on the insecticidal activity of this extract of *Cassia tora* was undertaken however some references were obtained to give the direction to the study on various aspects of the extracts and their potential activity on other types of studies such as antimicrobial, antihelminthic etc.

The objective of the invention was to study the broad-spectrum pesticidal activity of the extract by employing various methods of evaluations of bioefficacy properties.

The **Chapter one** of introduction of the thesis gives a detail account on literature review pertaining to the plant, and different extracts studied for establishing various properties the plant exhibits. An extensive screening of chemical abstracts and entomological abstracts was carried out.

A detail description of plant material, preparation of different types of extracts by Soxhlet distillation method is provided. All the extracts were prepared by the same methodology by using Soxhlet distillation method and the extracted material was used to carry out different studies by preparation of a standard stock solution.

The main objective of the study being entomological evaluation of the extract on different insects, no tear down of the extract was carried out.

**Chapter two** gives an account of experiments carried out on a household pest *Musca domestica*. The testing methods adopted are given in detail to establish the bioefficacy activity of the extract.

**Chapter three** covers an in-depth study of a mosquito larvicidal activity of the extract on larvae of *Aedes aegyptii*. The actual methods carried out from rearing of the larvae to the bioefficacy tests are explained. The histopathological effects of the extract on the larvae were also studied.

**Chapter four** emphasizes on the Antifeedent activity on *Spodoptera littoris*, an insect which is of prime importance to agriculture. The study was directed towards an
Antifeedent activity of the larvae of *Spodoptera littoris* thereby preventing damage to the crop.

The promising results obtained for a broad-spectrum activity of the extract prompted to carry out the toxicological studies on non targets to study the harmful effects if any of the plant extracts under investigation. **Chapter Five** presents acute oral, acute dermal toxicity findings and skin irritation studies on both rabbit and mice.

**Chapter Six** covered an allelopathy activity, which is a peculiar property of very few plants. A study was conducted on *Parthenium histrophorous*, another harmful pest for the mankind.

The **Chapter Seven** on Summary provides an overview of the observations, discussions and inferences resulted during a course of this study and the promising outcome has prompted to carry out further research in order to commercialize the applications.

1.2 Literature Review:

**Fig. No.1 Cassia tora Plant**

*Cassia Tora L.*, (*Cassia obtusifoliais* L.) of family, Caesalpinaceae, is a wild herb that grows in most parts of India as a weed. According to Ayurveda (Anonymous), the leaves and seeds are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiotonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders. (Yun-Choi H.S., 1990) Surveys on different chemical components of *Cassia tora* reveal that the plant has constituents such as anthraquinones (chrysophanol, emodin, obtusifolin, obtusin, chryso-obtusin, aurantio-obtusin, and their glycosides naphthopyrones. Kitanaka and Takido (1986) carried out studies on the constituents in the roots of
Cassia obtusifolia L. and the antimicrobial activities of the roots and the seeds. They were found to contain rubrofusarin, norrubrofusarin, rubrofusaringentiobioside, toralactone, torachrysone (Yakugaku, 1986)^ ^ Roots contain 1, 3, 5-trihydroxy-6-7-dimethoxy-2-methylanthraquinone and betasitosterol. While seeds contain Naptho-alpha-pyrone-toralactune, chrysophanol, phycision, emodin, rubrofusarin, cchrysophonic acid-9-anthrone. Emodin, tricontan-1-01, stigmasterol, -sitosterol,-D-glucoside, freindlen, palmthic, stearic, succinic and d-tartaric acids uridine, quercitin and isoquercitin are isolated from leaves. Antibacterial (Kitanaka, et al 1988), anti-platelet aggregation, hepatoprotective (Wong et al, 1989) cAMP-phosphodiesterase inhibitory activities (Shibata et al, 1969) antifungal, antiyeast, antiinflammatory (Maity et al, 1998) estrogenic and antiestrogenic (El-Halawany et al, 2007), Hypolimpidemic (Umesh et al, 2004), antimutagenic (Young-Mi Kim et al, 2004) antioxidant (Yen Gow-Chin and Chuang Da-Yon, 2000) activities in seeds have also been evaluated. Seeds are hard, 1 cm long, 3-4 mm thick, oblong or rhombohedral, both ends appear as if cut off obliquely, greenish-brown to brownish-black, smooth and shiny; odorless; taste, bitter. Seeds of Cassia tora show seed coat consisting of longitudinally elongated cells, covered with thick, smooth cuticle, followed by palisade layer composed of closely packed, radially arranged, non-lignified, thickened columnar cells, and by a single layer of dumb-bell shaped, thick-walled, parenchymatous cells. A wide zone of thick-walled, parenchymatous cells forming inner layer of testa is differentiated into outer 8 – 10 layers of tangentially elongated, parenchymatous cells and a single layer of broad cells which are squarish in shape. A few vascular bundles scattered in this zone are also seen. Embryo consists of radicle, plumule and two cotyledons. Epidermis of cotyledon consists of a single layer, externally covered with cuticle, followed by two layers of palisade-like cells of mesophyll. Mesophyll of ventral side composed of rectangular to polygonal cells filled with round to oval starch grain, measuring 8-12 µ in diameter, a few vascular bundles and a few rosette crystals of calciihrollalate upto 49 µ in diameter are seen scattered in this region. The seed powder is light brown in color, and shows fragments of testa, small parenchymatous cells, simple, round to oval, starch grains measuring 8-12 µ in dia. (Singh, 2009). The seeds are used as coffee substitutes, health drinks and for curing many human ailments. (Patil et al, 2004). A seed of Cassia tora was found to contain anthraquinones some of which (emodin and rhein) are still debated as carcinogenic or antitumor agents (Gopal et al, 2007).
Emodin was isolated and identified from the 90% Methanol extract (1% w/v) of the dried leaves of *Cassia tora*. This was tested on albino mice for its purgative action. At 50 mg/kg it was more effective than a sennoside cathartic at 2.5 mg/kg (Pal et al, 1977).

Seeds of *Cassia tora* were found to contain 10.38% total ash, containing Ca (0.35%), Na (0.19%), K (1.0%), and P (2.90%), 10.5% oil (sp. gr. 0.9251 and refractive index 1.4708 at 29°, acid value 7.4, saponification no. 198.4, and I no. 101.1), and the sugars glucose, galactose, xylose, and raffinose. The seed glycerides contain a minor component (2.5%) characterized as 9-hydroxy-cis-12-octadecenoic acid on the basis of spectral analysis and chemical transformations. The other usual fatty acids present have mainly C-16 and C-18 chain lengths. (Singh et al, 1981). From the seeds of *Cassia tora*, a new naphthalene glycoside was isolated and characterized as 2-acetyl-3-O-b-D-apiofuranosyloxy-8-O-b-D-glucopyranosyloxy-1,6-dimethoxynaphthalene (cassitoroside) (Choi et al, 1995).

Seed extract of *Cassia tora* and herb extracts of *Alternanthera sessilis* were tested against some human and plant pathogenic bacteria. Seed extracts of *Cassia tora* in alcohol had a good inhibitory effect against all the tested organisms. Moreover, the herb extracts of *Alternanthera Sessilis* in petroleum ether and benzene inhibited the growth of all the bacteria (Sahu et al, 1994).

To explore the brassinosteroid-active component in *Cassia tora*, a methanol extract (1% w/v) of immature seeds was purified by a sequence of solvent fractionation, silica gel adsorption chromatogram, Sephadex LH-20 chromatogram, charcoal adsorption chromatogram, and Bondesil chromatogram. The activity of brassinosteroid was monitored by the rice inclination test and its presence could be confirmed in each purification step. The purified active components were separated by silica gel adsorption chromatogram. Brassinosteroid substances in separated active fractions were identified as teasterone, castasterone, and brassinolide by TLC and HPLC. This is probably the first report of endogenous brassinosteroid in *Cassia tora*. The content of brassinosteroid in *Cassia tora* as converted into brassinolide was 3.5~5.5 ng/g fresh weight. The order of brassinosteroid contents was teasterone > castasterone > brassinolide (Park et al, 1993).

Thirteen phenolic glycosides including six new compounds were isolated from seeds of *Cassia tora* (Leguminosae). The structures of the new compounds, rubrofusarin...
triglucoside, nor-rubrofusarin gentiobioside, demethylflavasperone gentiobioside, torachrysone gentiobioside, torachrysone tetraglucoside and torachrysone apioglucoside, were elucidated on the basis of spectroscopic and chemical evidence. The effects of the phenolic glycosides, their aglycons and several other compounds structurally related to them on *Escherichia coli* K12, *Pseudomonas aeruginosa* PAO1 and some strains of *Staphylococcus aureus* were then examined. Among them, torachrysone, toralactone, aloe-emodin, rhein and emodin showed noticeable antibacterial effects on four strains of methicillin-resistant *Staphylococcus aureus* with a minimum inhibitory concentration of 2-64 mg/mL. On the other hand, the phenolic compounds tested did not show strong antibacterial effects on *E. coli* and *P. aeruginosa* (Hatano et al, 1999).

The protein content of *Cassia* seed (*Cassia obtusifolia* L) is 18.69%, in which there are all kinds of amino acids that are indispensable to human body. SDS-PAGE analysis showed that protein of *Cassia* seed mainly consists of three classes of protein, the molecular weight of which are 42 kDa, 40 kDa and 38 kDa respectively. When mercaptoethanol was added to the samples, they decomposed into two classes of peptide. Their molecular weights are 24 kDa and 16.5 kDa respectively. The protein of *Cassia* seed was extracted by ammonium sulfate fractionation and the high quantity of protein was obtained at 40% ~ 55% saturation of ammonium sulfate. The sequence of N-terminal 20 amino acids of Ps, one of the components of the peptides, was determined. Results showed that, to a certain degree, there were homologues between the Ps and apolipoprotein B in the sequence. As a result, it was possible for protein of *Cassia* seed to perform the lipid-lowering action through its participation of the lipid metabolism. (Li et al, 2001).

One recent study showed lack of genotoxicity of *Cassia tora* seeds in Allium cepa model (Solanke et al, 2007). Similar study revealed *Cassia tora* seed decoction (water boiled extract) enhances or reduces genotoxicity of a potent mutagen sodium azide and unprescribed analgesic-antipyretic drug acetaminophen in many part of world (Insel, 1996) because both are known genotoxicants in Allium cepa model (Rank et al, 1997).

*Cassia tora* Linn. root/ leaf paste is used against ring worm and eczema. (Sikdar and Dutta, 2008). Decoction of leaves and flowers is externally used for bronchitis and
asthma. Roots work as antidote against snakebite (Deore et al, 2009). Alcohol and aqueous extracts from the seeds of *Cassia tora* were investigated for their anthelmintic activity against Pheretima posthuma and Ascardia galli. Three concentrations (25, 50 and 100 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Both the extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/ml. Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control. The anthelmintic activity of alcohol and aqueous extracts of *Cassia tora* has therefore been demonstrated for the first time. The ointment containing benzene extract of *Cassia tora* showed activity in 23 psoriatic patients. The significant point was reduction in number of papulo-squamous lesions in majority of the patients (Malhotra et al, 2005).

Chrysophanic acid has significant anti-psoriatic activity. Three naphthopyrone glucosides, cassiaside, rubrofusarin-6-O-β-D-gentiobioside, and toralactone-9-O-β-D-gentiobioside, were isolated from the Butyl alcohol, soluble extract of the seeds of *Cassia tora* as active constituents, using an, *in vitro* bioassay based on the inhibition of advanced glycation end products (AGEs) to monitor chromatographic fractionation. (Lee et al, 2006). The structures of 1–3 were determined by spectroscopic data interpretation, particularly by extensive 1D and 2D NMR studies. All the isolates (1–3) were evaluated for the inhibitory activity on AGEs formation *in vitro*. (Zhang et al, 2007) The seed of *Cassia tora* L. has been used as a traditional Chinese medicine for a long time and it is also an herbal tea in China.

Chrysophanic acid [CAS No.481-74-3] was present in *Cassia tora* seed oil at 1.07%. 28.3 % of it was removed by soaking the seeds for 24 hours in cold water and 37.5% removed by soaking the seeds in boiling water, respectively (Desai and Shukla, 1978).

Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an Antifungal Property against Phytopathogenic Fungi. The fungicidal activities of *Cassia tora* extracts and their active principles were determined against *Botrytis cineria*, *Erysiphe graminis*, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia grisea*, and *Rhizoctonia solani* using a whole plant method *in vivo* and were compared with synthetic fungicides and three commercially available anthraquinones. The responses varied with the plant pathogen tested. The chloroform fraction of *Cassia tora* showed
a strong fungicidal activity against *B. cinerea, E. graminis, P. infestans, and R. solani.* Emomin, physcion, and rhein were isolated from the chloroform fraction using chromatographic techniques and showed strong and moderate fungicidal activities against *B. cinerea, E. graminis, P. infestans, and R. solani.* Furthermore, aloe-emodin showed strong and moderate fungicidal activities against *B. cinerea and R. solani,* respectively, but did not inhibit the growth of *E. graminis, P. infestans, P. recondita,* and *Py. grisea.* Chlorothalonil and dichlofluanid as synthetic fungicides were active against *P. infestans and B. cinerea* at 0.05 g/L, respectively. Results demonstrate the fungicidal actions of emodin, physcion, and rhein present in *Cassia tora.*

Dried seeds contain protein (up to 24 percent) and is given as a protein rich feed for livestock and birds. Roasted seeds are substituted for coffee like Tephrosia seeds. Seeds yield tannins and dyes (yellow, blue and red) and a gum (7.50%), which is good agent for suspending and binding. The aqueous extracts of whole plant and leaves produces inhibitory allelopathic effects on common weeds specially on *Parthenium hysterophorus.* Recommended to grow in *Parthenium* infested areas as smoother crop. Stimulatory allelopathic effects on rice and wheat have been reported. Seeds used in preparation of sweet dishes. Leaves are popular potherb. In organic farms of India, *Cassia tora* is used as natural pesticide. Fungicidal activity of chrysophanic acid-9-anthrone from *Cassia tora* have been reported.

Ethers and esters of galactomannan from *Cassia tora* are useful as thickeners, especially for textile printing pastes. Thus, 162 parts *Cassia tora* galactomannan was treated with 58 parts propylene oxide in aqueous alkali at 60° to give the hydroxypropyl ether derivative with degree of substitution of 0.65, and viscosity (3% aq. soln. at 20°) u 20000 mPa-s. (Bayerlein et al, 1985).

This brief review of literature cited in chemical abstracts gave an account of the studies conducted on the plant under study.

A very less literature is known of the insecticidal activity of medicinal plants, herbs and seeds on mosquitoes at different stages i.e. larva and adults. Only one reference of use of emodin from *Cassia obtusifolia* (Young-Cheol Yang, et al 2003) is reported against *Aedes aegyptii* and no other work on *Cassia tora* is reported. Emomin isolated from *Cassia obtusifolia* (Leguminosae) seed showed larvicidal activity against three mosquito species. Mosquito larvicidal activity of *Cassia obtusifolia* (Leguminosae) seed-derived materials against the fourth-instar larvae of *Aedes aegypti, Aedes togoi,*
and Culex pipiens pallens was examined. The chloroform fraction of C. obtusifolia ext. showed a strong larvicidal activity of 100% mortality at 25 mg/L.

The biological active component of Cassia obtusifolia seeds was characterized as emodin by spectroscopic analyses. The LC50 values of emodin were 1.4, 1.9, and 2.2 mg/L against C. pallens, A. aegypti and A. togoi, respectively. Pirimiphos-Methyl acts as a positive control directly compared to emodin. Pirimiphos-Methyl was a much more potent mosquito larvicide than emodin. Nonetheless, emodin may be useful as a lead compound and new agent for a naturally occurring mosquito larvicidal agent. In tests with hydroxyanthraquinones, no activity was observed for alizarin, danthron, and quinizarin, but purpurin has an apparent LC50 value of ~19.6 mg/L against A. aegypti.

No reports have been found on activity of Cassia tora against house fly. Present work aims at establishing activity of Cassia tora against different household pests like houseflies (Musca domestica) public health pests like mosquitoes (Aedes aegyptii), crop pests like Spodoptera litura and agricultural menace like Parthenium hysterophorus.

1.3 Preparation of Materials
1.3.1 Collection of Plant of Cassia tora:

Cassia tora plant was collected from University of Pune during July to September, 2005.

Description of the plant

English Name: Foetid cassia, The Sickle Senna, Wild Senna

Common (Indian) names:
Hindi: Charota, Chakvad, Chakavat.
Bengali & Oriya: Chakunda
Gujrati: Kawaria
Canarese: Gandutogache
Malyalam: Chakramandakam, takara
Marathi: Takala
Sanskrit : Chakramarda,Dadmari,Dadrughra,Taga
Tamil: Tagarai
Telugu: Chinnakasinda

**Family:** Leguminosae

**Habitat:** In India it occurs as wasteland rainy season weed.

**Botany:** It is an annual foetid herb, 30–90 cm high.

**Roots** contain chemical constituents such as 1, 3, 5-trihydroxy-6-7-dimethoxy-2-methylanthraquinone and beta-sitosterol.

**Leaves:** Pinnate, up to 10 cm long rachis grooved, conical gland between each of two lowest pairs of leaflet, leaflets in 3 pairs, opposite, obovate, oblong and base oblique. The chemical constituents include Emodin, tricontan-1-ol, stigmasterol, -sitosteral-D-glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin and isoquercitrin

**Flowers:** In pair in axils of leaves, petals five, pale yellow. The flowering time is after the monsoon rains (in Indian conditions)

**Fruit:** The pod of the fruits is obliquely separate.

**Seed:** 30-50 rhombhedral, seeds contain chemical constituents such as Naptho-alpha-pyrone-toralactune, chrysophanol, physcion, emodin, rubrofusarin, chrysophonic acid-9-anthrone. The seeds of *Cassia tora* were rhombohedral and brown in color, about 30 to 50 in number. The plant bears flowers in the rainy season and fruits in the winter.

The pods of the *Cassia tora* plant were collected from campus of University of Pune and were peeled to get the seeds. Seeds specimen was confirmed by Agharkar Research Institute, Pune. The seeds were also procured from Shree Vinayak Ingredients of Ahmedabad.
1.3.2 Preparation of extracts from the seeds

*Chemicals and Reagents:* De-mineralized water, Distilled water. AR grade Methanol, Acetone, Petroleum Ether from Rankem.

*Apparatus:* Soxhlet apparatus (Borosil), Rotary flash evaporator buchi type manufactured by Positive Infotech, Vacuum oven manufactured S. D (India corporation), Copper sieve of mesh size of #10,20.

The seeds were preserved and dried in the oven at 45 deg. c. Then they were ground to make a fine powder which was sieved through a mesh size of #20 and stored in cool and dry place.

50 gm of the seed powder was subjected to extraction with 500 ml of both polar (Water, Methanol, Acetone) and non-polar solvents (Petroleum Ether) by using a Soxhlet apparatus.

A *Soxhlet extractor* is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids.
The seed powder was placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a flask containing the extraction solvent such as Methanol, Acetone, Water and Petroleum Ether. The Soxhlet was then equipped with a condenser.

The solvent was heated to reflux. The solvent vapor traveled up a distillation arm and flooded into the chamber housing the thimble of solid. The condenser ensured that any solvent vapor that cools, drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly filled with warm solvent. The compound then got dissolved in the warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat many times, over 8 hours. During each cycle, a portion of the non-volatile compound dissolved in the solvent. The reflux was then continued till 8 hours.

The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is used and which can be recycled.
The distillate was then filtered through a Millipore filter paper. The extract was then concentrated in a rotary flash evaporator. A sticky brown colored mass was obtained which was dried in a vacuum oven at 27 deg.c., percentage yield of each extract was calculated and the dried extract was stored in air tight containers for further studies.

A stock solution of 1% w/v (10,000 ppm) was prepared in methanol and water.

Percentage yield of various extracts of seeds of *Cassia tora* obtained was as follows.

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Average Percentage yield*</th>
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</thead>
<tbody>
<tr>
<td><em>Cassia tora</em> (%)</td>
<td></td>
</tr>
<tr>
<td>Pet ether</td>
<td>22.84±0.66</td>
</tr>
<tr>
<td>Methanol</td>
<td>16.58±0.85</td>
</tr>
<tr>
<td>Water</td>
<td>29.09±0.46</td>
</tr>
<tr>
<td>Acetone</td>
<td>20.32±0.45</td>
</tr>
</tbody>
</table>

These extracts were used to carry out different tests on insects. The detailed methodologies, protocols of studies employed are given in each chapter respectively.

Out of these extracts, methanol extract (1%w/v) which showed broad spectrum pesticidal activity was selected to carry out the insecticidal tests.

Water extract was considered for evaluation in studies related to agricultural application.