Citrinin is abundantly produced by various species of *Penicillium* preliminary by *Penicillium citrinum* and some species of *Aspergillus* on wheat and other agricultural commodities. Awareness towards the citrinin toxicity originated from the strong implication of their involvement in diseases in living systems. Citrinin is reported to be cytotoxic, genotoxic and nephrotoxic in all species in which it has been tested. Over the last few decades a wide range of control measures (physical, chemical and biological) have been advanced owing to the risks associated with hazardous nature of citrinin, but none of them are fully satisfactory. Undoubtedly, these methods specially the chemical ones came forth with booster results but none of them appeared to be totally satisfactory as they themselves may pose problems related to environmental pollution, mutagenicity, carcinogenicity, development of resistant varieties etc. These methods are also not cost efficient.

As a substitute or supplement, possibilities have been explored in using medicinal plant extracts, essential oils and homoeopathic drugs to control growth of *P. citrinum* and citrinin synthesis as they are reportedly safe and ecofriendly. It therefore, implies that there is a need to revise dogmatic approach towards fundamental approach.

Impressed by such ideas, the author became motivated to use medicinal plant extracts, essential oils and homoeopathic drugs to control the growth of *P. citrinum* and citrinin production. The effects of above mentioned categories of substances were tested under *in vitro* and *in vivo* conditions. Further *in vivo* effects were analysed as pre and post inoculation treatments.

With this view point the experimentation involved the use of 3 categories of unconventional substances containing 10 varieties each. One category belonged to
medicinal plant extracts. It included the leaf extracts of *Azadiracta indica*, *Eucalyptus globusa*, *Datura alba*, *Mentha piperta*, *Osimum sanctum*, *Andrographis paniculata*, *Cajanus cajan* and *Azolla pinnata*, seed extract of *Swietenia mahagoni* and aerial part extract of *Silybum marianum*. Second category belonged to essential oils that include clove oil, eucalyptus oil, garlic oil, ginger oil, spearmint oil, citronella oil, sandalwood oil, jasmine oil, lemon oil and amla oil. The third category involved homoeopathic drugs namely *Arnica montana*, *Arsenicum album*, *Belladona*, *Drosera*, *Iodium*, *Natrum phosphoricum*, *Opium*, *Sulphur*, *Spongia* and *Thuja occidentalis*. 5 different concentrations of each of the categories were used (medicinal plants: 3, 5, 10, 20 & 50mg/ml, essential oils: 10, 50, 100, 250 & 500 ppm and homoeopathic drugs: 3, 6, 12, 30 & 200 potencies) to test their potential as anti *Penicillin*ic and anticitrinic agents under *in vitro* and *in vivo* conditions. The standard toxigenic strain of *P. citrinum* (sopp no. 1910) that produces high amount of citrinin toxin was obtained from Vellore Institute of Technology (VIT), Chennai.

Moving towards the objective, it first involved citrinin isolation from the test fungus, *P. citrinum* using chloroform method and then identification through several parameters such as TLC, IR, H^1^NMR, Spectrophotometry, HPTLC and HPLC. Thereafter standardization of citrinin with respect to incubation time, temperature and pH was achieved. Furthermore efficacy of each of the three categories of substances was tested first under *in vitro* and then under *in vivo* condition.

6.1 MEDICINAL PLANT EXTRACTS

6.1.1 *In vitro* effects

Before testing the efficacy, the plant extracts were proceeded for phytochemical analysis to evaluate the presence of different phytochemicals. The analysis revealed the presence of terpenoids in all the experimental plant extracts. On the other hand
cardioglycosides, alkaloids, flavanoids, tannins, saponins and steroids were found in maximum plant species with few exceptions.

All the test medicinal plant extracts did not possess any striking antifungal activity. Maximum antifungal activity was, however, exhibited by E.globusa (9.070%) followed by S.mahagoni (8.62%) at 50 mg/ml concentration, which is not at all convincing. Contrarily, slight enhancement in fungal growth was recorded with Azadiracta indica and Cajanus cajan plant extracts at 50 mg/ml concentration. The research findings also reveal inhibition of spore formation in a few cases, ie, A.indica, C.cajan and Azolla.

Notwithstanding low antifungal activity, medicinal plant extracts turned out to be an assured anticitrinic agent at 50 mg/ml concentration. A.indica was most promising, inhibiting citrinin upto 72.368% followed by E.globusa (63.674%), A.peniculata (43.126%) and M.piperta (36.493%). Moderate anticitrinic activity (35.74% and 55.14%) was recorded at 20 mg/ml in case of A.indica and E.globusa respectively. E.globusa at 10 mg/ml also showed moderate activity of 37.80%. Rest of the plant extracts proved to show average or poor activity. All the plant extracts in general behaved in a concentration dependent manner.

6.1.2 In vivo effects

In order to test the in vivo efficacy of medicinal plant extracts, only five treatments, ie, A.indica, E.globusa, A.peniculata, M.piperta and D.alba that displayed maximum citrinin inhibition in ‘in vitro’ experiment were carried forward for ‘in vivo’ testing in the form of pre inoculation and post inoculation treatments on the host, ie, wheat grain. Rest of the treatments were not proceeded for ‘in vivo’ experimentation due to lack of promising results.
In case of pre inoculation treatment, plant extracts of *A.indica* again evolved as a potent toxin inhibitor (70.84%) followed by *E.globosa* (56.90%), *A.peniculata* (36.06%), *M.piperta* (34.67%) and *D.alba* (31.90%) at 50 mg/ml. However moderate toxin inhibitory activities were also recorded in case of *A.indica* (38.84%), *E.globosa* (37.45%) and *D.alba* (29.16%). Rest of the plant extracts were poor inhibitors.

In case of post inoculation treatment *A.indica* and *E.globosa* inhibited synthesis of toxin upto the same extent, ie, 36.961% at 50 mg/ml. In both pre and post inoculation treatments dose dependent patterns were observed.

### 6.2 ESSENTIAL OILS

#### 6.2.1 In vitro effects

In general all the test essential oils possessed anti *Penicillinic* activity. Clove oil showed maximum activity inhibiting *P.citrinum* upto 38.78% followed by eucalyptus oil (35.637%), amla oil (28.546%) and jasmine oil (24.820%) at 500ppm. Moderate activity was recorded at 250 ppm concentration with clove oil and eucalyptus oil. Least activity was shown by zinger oil (9.156%).

Not only growth, essential oils subdued citrinin synthesis also upto fairly good extent. The utmost inhibition was achieved in case of clove oil reaching upto 55.853% followed by eucalyptus oil (47.510%), spearmint oil (26.825%) and zinger oil (25.662%).

#### 6.2.2 In vivo effects

Same pattern of experimentation was followed as in case of medicinal plant extracts. Only clove oil, eucalyptus oil, spearmint oil, zinger oil and amla oil were carried forward for their *in vivo* achievement.

In case of pre inoculation treatment maximum inhibition was obtained with clove oil (40.77%). Rest of the oils inhibited citrinin below the considerable limit.
All the oils in case of post inoculation treatment were not at all satisfactory. On the whole essential oils presented dose dependent responses in both *in vitro* and *in vivo* studies.

The inhibitory effects against the test organism or the toxin could be due to the involvement of certain phytochemical components in the categories already mentioned contained in the plant extracts. Similarly, essential oils could not be studied at their component level, mainly phenolic compounds (eg, eugenol, thymol, carvecrol etc.) which are active agents against pathogen and the toxin. Author feels sorry that studies to this level could not be extended on account of lengthy time consuming procedures and insufficient lab facilities.

6.3 HOMOEOPATHIC DRUGS

6.3.1 *In vitro* effects

Unlike medicinal plant extracts and essential oils, sinusoidal patterns of inhibition were recorded with homoeodrugs. There were no co-relations between the potencies of the drugs used and their corresponding effects.

The maximum mycelial inhibition obtained was around 50% which was due to Arnica montana 3, 6; Arsenicum album 30 and Thuja occidentalis 3, 6. Moderate antifungal activities were also noted with Arnica montana 3, 6; Arsenicum album 3, 6; Iodium 6, Natrum phos 30 and Thuja occidentalis 12. Rest of the drugs remained average or poor in this regard.

Homoeodrugs disclosed better anticitrinic properties with Thuja occidentalis 6 standing as the most potent inhibitor of citrinin, inhibiting upto 55.50%. Appreciable toxin inhibition in the range of 35 to 55% could also be attained with Arnica montana 30; Arsenicum album 30; Drosera rotundifolia 200; Iodium 6; Opium 6; Sulphur 3, 30
and Thuja occidentalis 3, 6, 200. Rest of the drug potencies were more or less average or poor.

6.3.2 *In vivo* effects

Unlike the dose dependent effects achieved in case of plant extracts and essential oils, homoeodrugs displayed sinusoidal (dose independent) effects under *in vitro* conditions. Therefore all the 10 homoeodrugs were employed for testing their pre and post inoculation treatments to assess their curative and preventive potentials in controlling the citrinin production.

Investigation revealed the abnormal behaviour of homoeodrugs unlike conventional substances. There was hardly any co-relation between drug potencies and their responses. Drugs acting as good fungicides may or may not be effective in curtailing citrinin synthesis and vice versa. Rather drug potencies behaved in a manner unbecoming of conventional substances in the sense that each potency itself behaved as an independent drug. This deviation is totally puzzling, until one becomes aware of the mystiques of homoeopathic drugs which are class apart from conventional substances.

Here under, in nutshell, the author would undertake at focussing the significant outcomes of the study along with the unconventional features of observation in light of the knowledge of homoeopathy:

(1) Beyond any shade of doubt it was fully demonstrated that citrinin synthesis could be controlled considerably with homoeopathic drugs.

(i) Wheat seeds pretreated with Belladona 200, Drosera rotundifolia 30, Opium 3, 200 and Thuja occidentalis 6 could be protected against citrinin contamination around 75%. This also means that the wheat could be treated with these drugs prior to storage.
(ii) Post inoculation treatments with Arsenicum 12, 200; Natrum 6; Opium 200 and Sulphur 30, 200 were equally effective in bringing about toxin inhibition in the range of 60-75%. This means that these drugs could be used as curatives in case of emergency.

(iii) However in totality it could be concluded that homoeodrugs were more effective when used as preventives rather than curatives.

(2) Surprisingly none of the homoeodrugs used in the study achieved cent percent inhibition neither in citrinin production nor in fungal growth. A few reasons could be brought forward.

(i) It could be due to the fact that limited number of drug potencies were used, whereas more should have been employed to achieve the expected outcome.

(ii) Drugs employed in the study belonged to materia medica developed for treating human sufferings though whatever drugs were used they might have presumably acted homoeopathically. In fact materia medica for treating plant sufferings has not been developed. Homoeopathic law of similar needs be extended to plant world as well using many plant-pathogen-drug systems.

(iii) Another noticeable feature for homoeodrugs performing relatively better under in vivo condition may be due to the reason that the homoeodrugs consider host as main site of action, as the fundamental contradictions of health and disease function optimally at host level, the pathogen playing the subsidiary role.

(iv) As opposed to conventional substances, homoeodrugs were recorded presenting multiple optima reverberating distinguished drug activities related to different phenomena and processes (multiple sites) over an array of potencies corresponding presumably to different physical states achieved during the process of
dynamization. This singularity of homoeodrugs action lies in the unique method of drug preparation, where the substance is not only diluted but also succussed. Due to this reason it would rather be difficult for the pathogens to develop resistance against homoeodrugs since it will not be possible for them to cope up with all the marred loci one and at the same time.

(v) As a natural corollary the homoeodrugs demonstrate dose independent effects so devotedly obeyed by conventional drugs.

(vi) Another unique feature, probably the most striking one is the one related to the paradox of ultramicrodoses presented by homoeodrugs themselves. Homoeodrugs are believed to contain no drug substance beyond 12th potency ($10^{-24}$ dilution) as no substance can exist crossing the theoretical limit imposed by Avogadro’s number ($6.03 \times 10^{23}$ atoms/mole). Despite this, homoeodrugs work. This is a major point of criticism against homoeopathy discrediting it as placebo therapy. On the other hand some of the noble laureates like Dr. Montagnier, Benveniste and Brian Josephson strongly supports homoeopathy and they says that homoeopathy is not pseudoscience, it is not quackery, it is real phenomenon which deserves further study.

But thanks to advancement in molecular chemistry and precision in probes that some perception into the physical nature of homoeopathic substances has become accessible. A few hypothesis are attracting the scientific community. The most popular hypothesis is that the vehicle in which drug is prepared is itself induced to acquire the medicinal dimension of the drug molecules. Thus, the medicinal properties exhibited by higher potencies of homoeodrugs are in fact the attributes of the dynamized “promoted” water-alcohol molecules. This is beneficial. Being composed of water/alcohol these drugs might find it comfortable to permeate into
the interiors of tissues and cell through the cell membranes which otherwise obtrude roadblock for the diffusion of orthodox “macrdoses” of mainstream medicinal substances.

The aforementioned abstraction of literature presented in the thesis indicates that the essential oils employed have not been up to the satisfaction in protecting wheat. Comparatively plant extracts have proved to be better protectants. However, better results have been obtained with homoeopathic drugs, used either as prophylactic or therapeutic agents. Conceding the promising results obtained with homoeodrugs, these might find an important place in plant protection in near future.