CHAPTER 11

Eco-friendly Methods of Management of Seedborne Diseases

Dev (2007) has extensively worked on botanicals, essential oils and aromatic compounds against various seedborne pathogens while working for her Ph.D. thesis. She has very nicely reviewed the green pesticides in the management of seedborne pathogens and I quote "It is estimated that India will need 267 million tons of food-grains by the year 2030 and is expected to produce 222 million tons. Crop losses by pests, diseases and weeds amount to over 20 per cent of total potential productivity and without proper protection, 10-30 per cent of the total crop production would virtually be lost, with peak losses of up to 50 per cent in less developed countries. In U.S. about 37 per cent of all agricultural crop production is lost due to pests (Insects cause an estimated 13% crop loss, plant pathogens 12% and weeds 12%), despite the use of an estimated 320 million kg of pesticides applied in addition to biological control and other non-chemical controls (Pimentel *et. al.*, 1991). A huge amount of chemicals is being used in disease control e.g. European Union Agriculture consumes about 350 million Kg active ingredients per year of fungicides, herbicides, insecticides and nematicides, etc. of which fungicides constitute the major portion, with a 51 per cent of the total (Gorris and Smid, 1995).

Use of chemical fungicides in the control of fungal diseases though effective, is accompanied by plethora of problems such as environmental pollution, health hazards and development of resistance by the target pathogens, and adds to the cost of application. The occurrence of pesticides residues in drinking water has become an issue of public concern; there is also a growing concern all over the world regarding environmental degradation. The fears are not misplaced as there has really been a criminal tampering of the ecology by mankind in order to produce more and more. The increasing toll on environment and human health thus calls for a "back to nature" approach in crop protection.

Over the last decade, an outburst of research activities has been directed towards the development of effective alternative crop protectants. The office of pesticide programmes (OPP) within the United States Environmental Protection Agency (EPA) has initiated several programmes to reduce risks of pesticides associated with their use. Now the pesticides being under attack globally, it is time to go back to traditional approach and adopt eco-friendly pest control meesures. This brings in biocontrol and botanical pesticides in the picture.

11.1 BOTANICALS AS FUNGICIDES

The plant world comprises of a rich storehouse of biochemicals that could be tapped for use as fungicides. Numerous defensive chemicals such as terpenoids, alkaloids, phenols, tannins, etc. which cause physiological effects on pathogens have already been identified in the extracts and exudates of many plants and they are also known for antifungal activities. The total number of plant chemicals exceeds 4,00,000; of these 10,000 are secondary metabolites playing a major role in plant defence and disease resistance. The xanthones from fruit hulls of *Garcinia mangostana* are reported to be active against *Fusarium oxysporum* f.sp.*vasinfectum, Alternaria alternata* and *Drechslera oryzae* (Gopalkrishnan *et al.*, 1997). An isocoumarin, coriandrin with photoactive properties was found active against *Cladosporium cucumerinum* and *Fusarium culmorum* (Delgado *et al.*, 1996). Nine flavonoids obtained from *Ulex jussiaei* and *Ulex minor* (Leguminosae) were active against *C. cucumerinum* (Maximo *et al.*, 2000). From cashew nut shell, Weerasena *et al.* (1993) have isolated a biodegradable quinone having inhibitory effect on *Cladosporium cladosporioides*. 2', 6'-dihydroxy-4-methoxy-acetophenone and related compounds found in the leaves of *Rhododendron daucicum* showed strong activity against *Botrytis cinerea, Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* (Aoyama *et al.*, 1997). As part of a search for botanical pesticides, methanol extracts of a number of plants, including *Ailanthus altissima*, *Acanthospermum hispidum* and *Heritiera littoralis*, and seed cake of *Azadirachta indica* (neem cake), were tested for various biological activities (Kraus *et al.*, 1994). Though higher plants are rich pool of diverse biologically active secondary metabolites (Parmar and Devakumar, 1993) the number of antifungal plants documented is very small.

Garlic juice is reported to exhibit a broad spectrum of activities against pathogenic fungi and many strains of yeast (Murthy *et al.*, 1983; Block, 1985). Seed treatment with garlic extract disinfected the seeds infected with

Peronospora destructor, *Phomopsis vexans*, *Fusarium oxysporum*, *Verticillium albo-atrum*, *Alternana brassicae* and *A. radicina* in various crops and increased the yield (Kuprashvili, 1996). Volatile vapours emanating from crushed garlic bulbs reduced mycelial growth of *Alternaria*, *Curvularia*, *Fusarium* and *Helminthosporium* (Misra and Dixit, 1979; Singh *et al.*, 1979). Qvarnstrom and Ramert (1992) found satisfactory control of black spot (*Marssonina rosae*) on rose cultivars with 5% garlic extract. Inhibitory effect on the mycelial growth, spore germination and pathogenicity of *Drechslera sorokiniana* was also reported (Ashrafuzzaman and Hossain, 1992; Hossain and Ashrafuzzaman, 1994). Effect of cold and hot water extracts of garlic on *Macrophomina phaseolina* was found at par with carbendazim (Raja and Kurucheve, 1998). Efficacy of garlic extract as a foliar spray was found less effective in controlling leaf blight of wheat caused by *D. sorokiniana* (Rahman *et al.*, 2001). Seed treatment with garlic was as effective as vitavax in controlling seed-borne infection of *Colletotrichum corcori*, *Fusarium* spp. and *M. phaseolina* in jute (Khan and Fakir, 1995). Onion wilt caused by *Fusarium solani* and *Erwinia carotovora* pv. *carotovora* aided by *Meloidogyne arenaria* was inhibited by garlic cloves extract (Alice and Sivaprakasam, 1996).

Literature survey on neem has revealed that the various neem products such as, leaves, seed, oil and oil cake can inhibit many soil-borne fungal pathogens, which are detrimental to many field crops. Many workers have carried out an exhaustive study on the utility of neem oil against various fungal pathogens. Its efficacy has been evaluated against *Fusarium moniliforme*, *Aspergillus niger*, *Drechslera rostrata* and *Macrophomina phaseolina* (Dharamvir and Sharma, 1985).

During an antifungal study, neem oil was found to be at par with the fungicide hymexazole in the control of soil pathogens, *Fusarium oxysporum*, *F. ciceri*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *S. sclerotium* (Devakumar, 1997). Four sprays of neem oil (4%) were found to be comparable to the single spray of Baytan (triadimenol 0.1%) in inhibiting the germination of uredospores of leaf rust (*Puccinia recondita*) (Sajid *et al.*, 1995). Muthusamy *et al.* (1988) evaluated the efficacy of neem products against rust disease of groundnut. Neem and pungam (*Pongamia glabra*) oil based EC formulation developed by Tamil Nadu Agricultural University have been found effective against sheath rot disease of rice under field conditions (Narasimhan *et al.*, 1998). The disease incidence of bacterial blight of French bean, the results obtained for the EC formulation were at par with mancozeb and metalaxyl and hence this formulation may be alternated with fungicides. This practice will help to avoid the anticipated environmental pollution due to the sole use of chemicals for disease

management and also will nullify the probable development of fungicide resistant strains in the pathogen if any, arising out of the repeated use of the same chemical (Rajappan *et al.*, 2000). Ilyas *et al.* (1997) found Repelin, neem oil and neem cake to be effective in descending order in inhibiting the *M. phaseolina* the causal organism of charcoal rot of mung bean. Lehman and Benthal (1995) using chromatography identified at least 5 bands with fungicidal potential from neem oil. A polar component of neem reduced the disease intensity of groundnut rust caused by *Puccinia arachidis* (Suresh *et al.*, 1997).

Jeyrajan *et al.* (1987) used neem and other plant products in the management of plant diseases in India and reported that addition of neem cake to soil reduced the pre-emergence and post-emergence mortality of cotton seedlings infected with *Rhizoctonia solani*.

Leaf extract of *Melia* spp. was found to be good inhibitor of *Bipolaris micropus*, *Colletotrichum lindemuthianum* and partially inhibited *Alternaria alternata*, *A. solani*, *Botrytis cinerea* and *Fusarium solani*, while *Trichilia* extract exhibited better activity against *Alternaria alternata*, *A. solani*, *B. cinerea* and *F. solani* (Lovang and Wildt, 1998).

Bhat *et al.* (1994) and Bhat (2000) reported inhibition of *Pythium aphanidermatum* by *Parthenium hysterophorus* (48.3%), *Polyanthia longifolia* (56.6%) and *Caesalpinia pauciflora* (85.5%). Ethanol extract of the aerial parts of *Polygonum chinense* was found to be active against *Alternaria raphani* and *Fusarium* spp. (Joshi *et al.*, 1997). Aqueous extract of *Acalypha ciliata* was found effective at par with benomyl in the control of *Fusarium moniliforme* in maize (Owolade *et al.*, 2000). Mohanty *et al.* (1995) reported control of brinjal blight and fruit rot caused by *Phomopsis vexans* using crude leaf extracts of *Allemande cathertica* (93.75%) and *Aegle marmelos* (85.38%). Soil application of green leaves of *Adhatoda vasica*, *Cymbopogon flexuosus*, *Anisomeles ovata*, *Azadirachta indica*, *Aegle marmelos* and rhizomes of *Curcuma* and resins of *Ferula fostida* at 2 and 5 per cent concentration reduced pre- and post-emergence collar rot of chickpea caused by *Sclerotinia rolfsii* (Daya Ram and Tewari, 1994). Leaf extracts (1:5) of *Pongamia pinnata* were highly fungitoxic to *Fusarium pallidoroseum* and *F. moniliforme* var. *intermedium* inhibiting mycelium by 78.2 and 84.3%, respectively, whereas *Calotropis gigantea* and *Azadirachta indica* were most effective against *F. oxysporum* inhibiting mycelial growth by 78.5% and 73.2%, respectively, (Gupta *et al.*, 1996).

Leaf extract of *Datura stramonium* and the xanthones from fruit hulls of *Garcinia mangostana* and their synthetic derivatives are reported to be active against *Fusarium oxysporum* f.sp.*vasinfectum*, *Alternaria alternata* and

Drechslera oryzae (Gopalkrishnan *et al.*, 1997). Leaf extract of *Datura stramonium* was found to inhibit germination of uredospores of *Puccinia* rust after 18 hrs of incubation. In a pot experiment, foliar sprays reduced the number of pustules on plants (Intizarul-Hassan *et al.*, 1992). Aqueous extracts of shoot and roots of clammy inula (*Inula viscosa*) inhibited leaf spot disease of wheat caused by *Drechslera sorokiniana* (Qasim *et al.*, 1995). Dichloromethane extract of aerial parts of *Chenopodium procerum* inhibited the growth of *Cladosporium* spp. (Bergeron *et al.*, 1995). *Oxalis, Melia* and *Ageratum* were found as effective as Dithane-M 45 against hill bunt of wheat (Pant *et al.*, 1997). Extracts of *Oxyspora paniculata* and *Macaanga denticulata* gave 94.4% and 100% inhibition, respectively, in *Pythium aphanidermatum*.

A dichloromethane extract from roots of *Eriosema tuberosum* (leguminosae) inhibited the growth of *Cladosporium cucumerinum* and *Candida albicans* on silica gel TLC plates and the flavones and the isoflavones isolated showed antifungal activities (Ma *et al.*, 1995).

Several workers reported that the plant extracts retained the inhibitory effect even after autoclaving. This might be due to the thermo stable nature of the active principles present in the extracts. Alcohol preparations were generally more active than Aqueous and acetone extracts (Sas-Potrowska *et al.*, 1996).

Latex from *Jatropha gossypiifolia* and *J. tanjorensis* was reported to be highly toxic to *Alternaria brassicicola* and *Drechslera miyabeanus* and some toxicity retained even after 25-fold dilutions (Pandey *et al.*, 1996). Flavonoids isolated from roots of *Clerodendron infortunatum* exhibited good inhibition of spores of *A. carthami* and *D. oryzae* (Roy *et al.*, 1996).

Extracts of *Ipomoea cornea* and *Prosophis julifera* were found to be as active as bavistin in controlling rice sheath rot (Eswaramurthy *et al.*, 1996). Aqueous and methanolic extracts of *Calycophyllum multifolium*, *Geophila repens* and *Tabebuia avellanedae* showed a high degree of inhibition in *Aspergillus niger*, *A. fumigatus* and *Cladosporium cladosporioides* (Portilo *et al.*, 2001). Anti-fungal activities were observed in many plants like bark of Cinnamomum (Kim *et al.*, 1996). The poisonous fruits of the shrub *Diplolophium maritima* are used as a pesticide in the Ryukyu Islands of Okinawa, Japan. From fresh fruits of *D. maritima* were isolated 3 new naphthoquinones (3-bromoplumbagin, ethylidene-

inhibitory activity, and antifungal activity. The naphthoquinone derivatives from the fruits of *D. maritima* showed various biological activities; plumbagin showed strong activity in all 3 bioassays. Three phenylpropanoids (myristicin, elemicin, and trans-isoelemicin), and 2 furanocoumarins (oxypeucedanin and oxypeucedanin hydrate), were isolated from the leaves of *Diplolophium buchanani* (collected from Malawi) by a separation strategy involving the almost exclusive use of centrifugal partition chromatography. All 5 compounds exhibited activity against *Cladosporium cucumerinum* (MIC values of 1-20 mg/plate). A new melampolide, sonchifolin isolated from leaves of *Smallanthus sonchifolius* exhibited good activity against *Pyricularia oryzae*, this being the first report of melampolide as fungicidal compound (Inoue *et al.*, 1995). Aqueous extracts from *Aegle marmelos* (bael) and *Ocimum sanctum* (tulsi) leaves are reported to be very effective in controlling blast disease of rice (*Pyricularia grisea*). Prophylactic sprays of these products are reported to be comparable with the application of carbendazim in the field (Mathur, 1998). *Piper betle* is reported to have antimycotic activity against *Pyricularia oryzae*, *Cochliobolus miyabeanus*, *Rhizoctonia solani* and *Botryodiplodia theobromae* (Mohamed *et al.*, 1996).

Yokoto *et al.* (1978a&b) have isolated anti-fungal compounds from the bark of *Hibiscus syriacus* and *Betulae* cortex. Singh and Pandey (1966) reported the inhibitory effect of green and mature plant residues and compost on the population of *Pythium aphanidermatum* in soil. Aqueous extracts of *Impatiens balsamia* and *Lawsonia inermis* gave complete inhibition in *Fusarium oxysporum* causing wilt disease in lentil (Singh and Tripathi, 1995). Antifungal activity of hot water extract of *Vernonia amygdalina* was exhibited against *Curvularia lunata* and *Fusarium semitectum* (Ekpo, 1995). Rhizome extract and the powder of *Curcuma longa* were found to be effective against *Alternaria alternata* and *Aspergillus flavus* and the oils were found to be as effective as benlate.

11.1.1 Essential Oils

Essential oils of some higher plants being volatile have recently been proved successful in providing effective control of storage losses by several fungi (Dixit *et al.*, 1983; Kala *et al.*, 1984; Asthana *et al.*, 1989; Dixit *et al.*, 1995). Hasan

deterioration during storage (Kishore *et al.*, 1993). Essential oils of *Tagetes erecta* (Kishore and Dwivedi, 1991); *T. minuta* and *T. filifolia* (Zygadlo and Guzman, 1994); *Artemisia scoparia* (Mohammad *et al.*, 2000); *A. annua* (Mohammad and Siddiqui, 2000) and *Curcuma longa* (Saju *et al.*, 1998) were also reported to have antimicrobial activity. Control of post harvest fungal diseases is also achieved by using essential oils of plant origin (Gorris *et al.*, 1994).

The essential oil hydrodistilled from the flowering shoots of *Tanacetum annuum* is reported to completely inhibit the mycelial growth of *Helminthosporium oryzae*, *Alternaria solani*, *Pyricularia oryzae* and *Botrytis cinerea* by using GC and GCMS. Sabinene (22.3%) and camphor (13.2%) were detected (Greche *et al.*, 2000). Essential oils of *Salvia officinalis*, *Mentha sylvestris*, *M. piperita*, *M. arvensis and Casearia sylvestris* were shown to be active against *Candida albicans* and *Aspergillus oryzae* (Carvalho *et al.*, 1997). *Ocimum* and *Citrus* oils gave significant control for pathogens during storage (Asthana *et al.*, 1989; Dixit *et al.*, 1983).

All the cultivars of *Mentha spicata* yielded oil rich in carvone. The carvone content was the highest in the oil of cultivars Arka and MSS-5. The Neer-Kalka cultivar of *Mentha spicata* had menthone and neo-menthol in its oil (Bahl *et al.*, 2000). The essential oils of some higher plants viz. *Ocimum gratissimum*, *Zingiber cassumunar*, *Cymbopogon citratus* and *Caesulia axillaris* have shown strong antifungal activity against *Aspergillus flavus*. The minimum inhibitory concentration (MIC) of the essential oils varied between 500 and 1300 ppm. The oils of *Cymbopogon citratus* and *Caesulia axillaris* were altogether fungistatic in nature. Fumigation of wheat samples with *Caesulia* and *Cymbopogon* oils at their MICs indicated their *in vivo* applicability as herbal fumigants (Dubey *et al.*, 2000). Handa and Kaul (1997) gave a detailed information about essential oils. The essential oils of citronella Java (*Cymbopogon winterianus*) and rose geranium (*Pelargonium graveolens*) were partitioned into different fractions under high vacuum in a packed fractionating column for their separation into pure constituents such as citronellal, d-citronellol [(+)-citronellol], l-citronellol [(+)-citronellol] and geraniol. The formate derivatives of geraniol, l- and d-citronellol could be prepared with optimum yield and confirmed through GC. The spectra of the antimicrobial activities of the essential oils and their constituents in relation to optical isomers and their derivatives were analysed. Differential antimicrobial activities were studied through in vitro bioassays against 12 bacterial (*Bacillus subtilis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus mutans*, *Yersinia enterocolitica*, *Salmonella typhi*, *Escherichia coli* and

Mycobacterium smegmatis) and 7 fungal (*Microsporum gypseum, Aspergillus niger, A. flavus, Trichophyton rubrum, Sporothrix schenckii, Candida albican* and *C. albicans*) strains. The distinct activity patterns indicated structure-function relationships for the optical isomers (Aggarwal *et al.*, 2000). The essential oils from lemon grass [*Cymbopogon citratus*], palmarosa grass [*C. martinii* var. *motia*], geranium, citronella [*C. nardus*], *Eucalyptus globulifera* and *E. citriodora* were tested for their antifungal activity at conc. of 1, 5, 10, 15 and 20% against *Beauveria bassiana*. Geranium oil showed the best antifungal activity, inhibiting fungal growth for 26.3 mm and spore germination by 79.7%. The antifungal properties of all essential oils increased with an increase in conc. (Raghavaiah and Jayaramaiah , 1987). Essential oils from 4 types of *C. winterianus* (Java-I, Java-II, Guatemala and Burma) exhibited antifungal activity against *Alternaria solani, Drechslera oryzae* [*Cochliobolus miyabeanus*], *Rhynchosporium sativum, R. oryzae* f.sp. *sativum, Sclerotium* [*Corticium*] *rolfsii* and *Fusarium oxysporum. F. oxysporum* growth was completely inhibited by oils from all 4 types whilst the other fungi were inhibited to varying degrees. It is suggested that these results provide evidence of genetic variation in fungitoxicity within the 4 types of this oil-bearing species (Kole *et al.*, 1993). Essential oil from *P. graveolens* exhibited *in vitro* antifungal activity against *C. gloeosporioides* [*Glomerella cingulata*] the cause of anthracnose in fruits such as mango, citrus and pawpaws. The major constituents of this oil, geraniol and citronellal, were more active (100% inhibition) than geranium oil (60% inhibition) at a conc. of 250 ppm. Linalool (an isomer of geraniol), showed considerably less activity, causing only 8% inhibition of *G. cingulata* (Chandravadana and Nidiary, 1994). Aegle, ageratum, citronella, eucalyptus, geranium, lemongrass, orange, palmarosa, patchouli and peppermint essential oils were tested for antibacterial activity against 22 bacterial strains, including Gram-positive cocci and rods and Gram-negative rods, and 12 fungi (*Alternaria citri, Aspergillus fumigatus, A. oryzae, Candida albicans, Cryptococcus neoformans, Fusarium oxysporum, F. solani, Helminthosporium compactum, Macrophomina phaseolina, Sclerotium rolfsii* [*Corticium rolfsii*], *Sporothrix schenckii* and *Trichophyton mentagrophytes*) by the disc diffusion method. Lemongrass, eucalyptus, peppermint and orange oils were effective against all the 22 bacterial strains. Aegle and palmarosa oils inhibited 21 bacteria; patchouli and ageratum oils inhibited 20, and citronella and geranium oils were inhibitory to 15 and 12 strs. respectively. The oils of aegle, citronella, geranium, lemongrass, orange, palmarosa and patchouli inhibited all the 12 fungal spp. Eucalyptus and peppermint oils were effective against 11 fungi. Ageratum oil was inhibitory to only 4 of the fungi tested. The minimum inhibitory concentration of eucalyptus, lemongrass, palmarosa and peppermint oils ranged from 0.16 to

minimum lethal concentration (MLC) of this oil and citral (a component of the oil) against 35 clinical isolates of 4 dermatophytes (*Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum and Microsporum gypseum*) were determined by agar dilution method. The MIC and MLC values of lemon grass oil were higher than those of citral. The most resistant strain was *M. gypseum* followed by *T. rubrum, T. mentagrophytes* and *E. floccosum*, respectively. The mode of action of lemon grass oil and citral were shown to be fungicidal. A comparative study of the efficacy of cream containing 4 concentrations (1 %, 2, 2.5 or 3%) of lemon grass oil was performed *in vitro* by hole-diffusion assay. The minimum concentration of lemon grass oil for the preparation of an antifungal cream for subsequent clinical study was 2.5% (Wannissorn *et al.*, 1996). A total of 52 plant essential oils and extracts were investigated for activity against *Acinetobacter baumanii, Aeromonas veronii* biogroup *sobria, Candida albicans, Enterococcus faecalis* [*Streptococcus faecalis*], *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* sub sp. *enterica* serotype *typhimurium, Serratia marcescens* and *Staphylococcus aureus*, using an agar dilution method. Lemon grass (*Cymbopogon citratus*), oregano (*Origanum vulgare*) and bay (*Laurus nobilis*) inhibited all organisms at concentrations of 2% (v/v). Six essential oils did not inhibit any organisms at the highest concentration tested (2% v/v). Twenty of the essential oils and extracts were investigated for activity against *C. albicans, Staphylococcus aureus* and *Escherichia coli* using a broth micro dilution method. The lowest minimum inhibitory concentrations were 0.03% (v/v) for thyme (*Thymus vulgaris*) essential oil against *C. albicans* and *Escherichia coli*, and 0.008% (v/v) for vetive (*Vetiveria zizanioides*) essential oil against *Staphylococcus aureus* (Hammer e *al.*, 1999).

11.1.2 Aroma Compounds

Noorman (1968), Fries (1973), Rytych and Zyska (1977) and Mallik and Nar (1982) have recommended the use of volatile compounds in the control mould infection during storage. Volatile compounds move faster as gases physiological temperature and biologically more active at extremely l concentrations.

11.1.3 Use of traditional medicines

The plants which are used in traditional medicine to treat cold, bronch

>20 µl/ml for 18 bacteria and from 0.25 to 10 µl/ml for 12 fungi (Pattnaik *et al.*, 1986). The antifungal activity of the essential oils of palmarosa (*C. martini*) cv. CIMAP/PRC-1, lemon grass (*C. flexuosus*) cv. Pragati and citronella (*C. winterianus*) cv. BIO-13, as well as some essential oil components, viz. citral, geraniol, citronellol and citronellal, were tested against 4 human pathogenic fungi (*Microsporum gypseum, Candida albicans, Sporothrix schenckii* and *Aspergillus niger*) to identify plant substances for future antifungal formulations. Among the essential oils and components tested, lemon grass oil and geraniol, respectively, recorded the highest antifungal activity. *M. gypseum* was highly sensitive to all the essential oils and tested components, with inhibition zones 1.5- to 2-fold larger than the other fungal pathogens and generally low minimum inhibitory dilutions (MID). Citral produced the smallest inhibition zones but demonstrated the highest activity in terms of MID and minimum fungicidal concentration (MFC) values, which were comparable to lemon grass oil. Lemon grass, palmarosa oil and geraniol recorded the highest inhibition of *C. albicans, A. niger* and *S. schenckii*, respectively. However, the highest antifungal activity in terms of MFC was recorded by lemon grass for all organisms tested. The antifungal activity of lemon grass oil (LGO), the essential oil of *Cymbopogon citratus*, was evaluated using fungistatic (min. inhibitory conc. and agar diffusion tests) and fungicidal (spore germination) studies. Appreciable activity was observed against various isolates of *Candida* (*C. albicans* and *C. pseudotropicalis* [*C. kefyr*]) and clinical isolates of *Aspergillus fumigatus, Microsporum gypseum and Trichophyton mentagrophytes*. The most resistant organism was *A. fumigatus*, while *M. gypseum* and the *Candida* spp. were the most susceptible of the isolates. Comparative studies with pure samples of citral and citronellal, constituents of LGO, showed good activity against the test fungi while dipentene and myrcene showed no activity. Exposure of the spores of *A. fumigatus* to 0.1% LGO for 5 min resulted in 93% of spores not germinating, while lower conc. (0.08 and 0.05%) caused 80 and 60% reduction in spore germination, respectively. Challenge tests on a formulated aqueous cream containing LGO indicated the 0.25% LGO effectively preserved the cream against fungal contamination (Onawunmi , 1989). The essential oil of leaves of *C. maritinii* exhibited fungitoxicity against 3 species of Aspergillus (namely *A. flavus, A. fumigatus* and *A. parasiticus*) at 3000, 2000 and 900 ppm, respectively. The oil was not phototropic to the seeds of groundnut. Upon chemical investigations geraniol was identified as the active constituent and it was fungitoxic for all the test species (Misra *et al.*, 1988).

Lemon grass oil was steam-distilled from wilted leaves of *Cymbopogon citratus* (cultivated in Thailand). The minimum inhibitory concentration (MIC) and

minimum lethal concentration (MLC) of this oil and citral (a component of the oil) against 35 clinical isolates of 4 dermatophytes (*Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum and Microsporum gypseum*) were determined by agar dilution method. The MIC and MLC values of lemon grass oil were higher than those of citral. The most resistant strain was *M. gypseum* followed by *T. rubrum, T. mentagrophytes* and *E. floccosum*, respectively. The mode of action of lemon grass oil and citral were shown to be fungicidal. A comparative study of the efficacy of cream containing 4 concentrations (1.5, 2, 2.5 or 3%) of lemon grass oil was performed *in vitro* by hole-diffusion assay. The minimum concentration of lemon grass oil for the preparation of an antifungal cream for subsequent clinical study was 2.5% (Wannissorn *et al.*, 1996). A total of 52 plant essential oils and extracts were investigated for activity against *Acinetobacter baumanii, Aeromonas veronii biogroup sobria, Candida albicans, Enterococcus faecalis* [*Streptococcus faecalis*], *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* sub sp. enterica serotype *typhimurium, Serratia marcescens* and *Staphylococcus aureus*, using an agar dilution method. Lemon grass (*Cymbopogon citratus*), oregano (*Origanum vulgare*) and bay (*Laurus nobilis*) inhibited all organisms at concentrations of 2% (v/v). Six essential oils did not inhibit any organisms at the highest concentration tested (2% v/v). Twenty of the essential oils and extracts were investigated for activity against *C. albicans, Staphylococcus aureus* and *Escherichia coli* using a broth micro dilution method. The lowest minimum inhibitory concentrations were 0.03% (v/v) for thyme (*Thymus vulgaris*) essential oil against *C. albicans* and *Escherichia coli*, and 0.008% (v/v) for vetiver (*Vetiveria zizanioides*) essential oil against *Staphylococcus aureus* (Hammer *et al.*, 1999).

11.1.2 Aroma Compounds

Noorman (1968), Fries (1973), Rytych and Zyska (1977) and Mallik and Nandi (1982) have recommended the use of volatile compounds in the control of mould infection during storage. Volatile compounds move faster as gases at physiological temperature and biologically more active at extremely low concentrations.

11.1.3 Use of traditional medicines

The plants, which are used in traditional medicine to treat cold, bronchitis, rheumatism and pneumonia, also have been found to be active against plant pathogenic fungi (Tan *et al.*, 1996). Sesquiterpene lactones from *Rabtibida*

mexicana that is used in Mexico to treat rheumatism, headache and colds are found active against *Helminthosporium* spp. and *Pythium* spp. (Calera *et al.*, 1995).

11.1.4 Use of weeds

Fungicidal property was also reported in some common weeds. Aqueous extract of *Rananculus asiaticus* was found to be effective against *Alternaria solani*, *Rhizoctonia solani* and *Drechslera sativum* (Qasem and Abu-Blan, 1996) and the extracts of *R. asiaticus*, *Erodium cruciatum*, *Galium tricornutum* and *Sisymbrium* were found to be toxic and reduced the growth of *Verticillium dahliae* significantly (Qasem and Abu-Blan, 1996).

11.1.5 Bacterial control

Csizinszky *et al.* (1993) reported the inhibition of strains of *Xanthomonas campestris* pv. *citri* (bacterial canker of citrus) with *Matricaria reticulata*. Seed treatment with root and leaf extracts of *Adhatoda zeylanica* completely suppressed the development of bacterial blight caused by *Xanthomonas campestris* pv. *vignicola* in cowpea and the root extract was found to be more effective (Thamaiah *et al.*, 1995). The effect could be attributed to the presence of water-soluble alkaloids in plant extracts. Antibacterial properties in the floral petals of many higher plants belonging to families Mimosaceae, Myrtaceae, Amaranthaceae, Solanaceae, Cucurbitaceae, Rubiaceae, Convolvulaceae, Oleaceae and Labiatae have been reported (Darokar *et al.*, 1998). Garlic, eucalyptus and *Thymus vulgaris* were found effective against tomato pith necrosis pathogens *Pseudomonas viridiflora*, *P. corrugata*, *P. cichori*, *Erwinia carotovora* and *E. chrysanthemi* though autoclaved extracts were not found effective (Aysan and Yildiz, 2000). The leaf essential oils of *Syzygium cumini* and *S. travancoricum* when tested against *Bacillus* spp. former was found to be good while the latter gave moderate inhibition (Shafi *et al.*, 2002). *Morinda tinctoria*, *Mussaenda frondosa*, *Psychotria gardneri* and *Psychotria stenophylla* displayed a wide spectrum of antibacterial activity (Jayasinghe *et al.*, 2002). The fatty acids from *Schotia brachypetala* exhibited high degree of antibacterial activity against gram-positive bacteria (McGaw, *et al.* 2002).

11.1.6 Viral control

Essential oils from geranium (*Pelargonium graveolens*), lemon grass (*Cymbopogon citratus*), peppermint (*Mentha piperita*) and spearmint (*Mentha*

spicata) were effective against potato virus Y (PVY) on *Datura metel* (Ismail, 1994). Extracts of *Basella alba*, *Phyllanthus fraternus* were found effective against pumpkin mosaic virus (Louis and Balakrishanan, 1996) and the extract of *pithecolobium dulce* was found to be promising when sprayed on tobacco against *Tobacco mosaic virus* (Murty and Nagarajan, 1986). Water and acetone extracts of ginger at dilution 1:10 were reported to inactivate tobacco mosaic tobamovirus (TMV) 67-87% and 75-85% respectively (Sindelarova *et al.*, 1996). Antiviral effect of strawberry leaves extract was shown against tomato mosaic virus and cucumber mosaic virus (Stoimenova and Angelov, 1995). Antiviral properties were observed in extracts of *Crotolaria juncea* (Velazhahan *et al.*, 1994) and *Bougainvillea spectabilis* (Vairamani and Sekar, 1994).

11.1.7 Seed-treatment

In soybean seeds ginger, garlic and neem extracts gave excellent control of seed-borne *Colletotrichum dematium* when seeds were dipped for 30 min. *Macrophomina phaseolina* and *Cercospora kikuchi* were completely inhibited by dipping seeds for 5 min. (Hossain *et al.*, 1999). Maize seeds when treated with the crude extract of *Acalypha ciliata*, the effect on control of *Fusarium moniliforme* was comparable with benomyl (Owolade *et al.*, 2000). *Calotropis procera* seed treatment was found to be effective against fungal pathogens in chickpea . The paddy seeds treated with rhizome powder of *Acorus calamus* (1 & 2%) have inhibitory effect on fungi and bacteria but did not have any adverse effect on taste, smell or colour of cooked rice (Rao and Ratnasudhakar, 1992). Seed treatment with *Clerodendron* extract completely checked the radial growth of *Curvularia lunata* in rice seeds and the treatment gave greater seed germination and root and shoot length (Pari and Francis, 1999). Shetty and Shetty (1987) and Shetty *et al.*(1989) found plant extracts effective in controlling seed-borne pathogens in rice.

11.1.8 Effect on seed germination and viability

Kumar and Vijayan (1999) found no adverse effect of extracts of leaves of *Calotropis gigantea*, *Ocimum sanctum*, *Tagetes patula*, onion bulb and ginger

mycoflora of *Colletotrichum dematium*, *Fusarium oxysporum* and *Macrophomina phaseolina* in chickpea.

11.1.9 Seed-storage

Many workers have shown control of *Penicillium* spp. the fungus responsible for major losses in seeds during storage, using extracts from various plants e.g. *Dioscorea alata* (Adetiye *et al.*, 1996) and *Vernonia scorpioides* (Freire *et al.*, 1996). Methanol extracts of *Cocos japonica* and *Anemarrhena asphodeloides* effectively inhibited the mycelial growth of *Botryosphaeria* sp., *Glomerella cingulata* and *Penicillium expansum*, which cause the storage disease of apples (Paik and Chung, 1997). Methanol and hexane extracts of neem seed kernel and methanolic extract of neem seed coat were found to be effective in controlling associated mycoflora in soybean during storage (Nakka *et al.*, 1998)

11.1.10 Commercial products

Some extracts have been commercialized. *Reynoutria sachalinensis* is used as a plant health enhancer named *Milsana*. *R. sachalinensis* is also effective against *Phytophthora infestans* (Schmitt, 1996). SPIC Science Foundation has developed a fungistatic product "*Wanis®*" which has a single monoterpene as an ingredient and it is reportedly very effective in controlling more than 30 different types of phytopathogenic fungi. It is non-toxic to human beings and livestock. Recently an antifungal agent by name *TALENT®* containing carvone as the active ingredient derived from essential oil of *Carum carvii has been developed*" unquote.

11.2 THERMOTHERAPY

The term thermotherapy was coined by Baker (1962) to include various types of heat to control the seed infection. In thermotherapy one can use dry heat or wet heat. The dry heat can be used in the form of solar heat or increased temperatures while wet heat can be used in the form of hot water or aerated steam. Since water is good conductor of heat, the wet heat is more effective than dry heat in controlling seedborne pathogens.

11.2.1 Hot water treatment

Hot water treatments of seed and plant material are classical thermo-physical methods of plant protection. As early as at the end of the 19th century the method was applied to control loose smut (*Ustilago nuda*) in cereals by Jensen

(1888). In the 1920s hot water treatment was used to treat the cabbage seeds to control *Phoma lingam* causing black leg disease in the USA (Walker, 1923). Hot water treatment has been recommended against the fungus *Ciborie batschiana* during storage of oak seed (Natzke, 1997). Further examples for application of hot water treatment are shown by Baker (1962), Gabrielson (1983), Hoffmann et al. (1994) and Jahn *et al.* (2000). In the second half of the 20th century hot water treatment was displaced by the application of more effective chemicals. The method fell into oblivion and due to this the method was not extended to other fields and crops. In the light of current knowledge, practical application on a broad spectrum of crops is not possible.

Hot water treatment gets more and more importance for organic farming and for the production of spices and medicinal plants (Trueman and Wick, 1996). It could also become an alternative method for conventional farming especially in case of failure of chemicals permitted for seed treatment.

The method was used for the first time by Jensen (1888) when he gave hot water treatment to potato tubers for the control of potato rot caused by *Phytophthora infestans*. The hot water treatment has an edge over the aerated steam treatment as this treatment is twice as effective as steam treatment. Many seedborne pathogen can be controlled by hot water treatment, e.g. *Ustilago segetum* var. *tritici* causing loose smut of wheat and barley can be controlled by steeping the seeds in hot water (49°C) for 90-120 minutes or 5-6 hours at 41°C (Doling,1965). However, Walker (1969) suggested that seeds be pre-soaked for 5 hours at 21°C followed by one minute at 59°C and 11 minute at 52°C to eradicate the pathogen. Similarly, *Sclerospora graminicola* causing downy mildew of *bajra* was controlled by steeping the seeds for 10 minutes at 55°C (Thakur and Kanwar,1977). *Xanthomonas campestris* var. *campestris* causing black rot in crucifers can be effectively controlled if the seeds are soaked in hot water at a temperature of 52°C for 20 minutes (Gaur *et al*, 1984). Grondeau *et al* (1992) found the control of *Pseudomonas syringae* pv. *pisi*, the incitant of blight in pea, if the seeds are exposed at 55°C for 15 minutes. In hot water treatment, majority of seeds are exposed to a temperature close to 50°C for a maximum period of 30 minutes (Baker, 1972). Though the treatment is quite effective and economical , yet it has the disadvantage of treatment damage. If the temperature and duration are not precisely controlled, the treatment may result either in total loss of germination or in stunted and weak seedlings. Some of the hot water treatments against fungal and bacterial pathogens have been given below in table 11.1.

		brassicicola		
Brassica spp	Black rot	*Xanthomonas campestris pv.campestris*	30min. at 50°C	Walker,1923
Brassica spp		*Xanthomonas campestris pv.campestris*	20min. at 40°C in ACA	Schaad,1980
Brassica spp		*Xanthomonas campestris pv.campestris*	20min. at 35-40°C in ACA	Lin,1981
Brassica spp		*Xanthomonas campestris pv.campestris*	20min. at 38-40°C in AZS	Huang and Lee,1988
Brinjal	Fruit rot	*Phomopsis vexans*	30min. at 50°C	—
Celery	Leaf spot	*Septoria apicola*	30min. at 48-49°C	Krout,1921
Chilli		*Xanthomonas campestris pv.campestris*	10min.at 50°C	—
Cotton		*Xanthomonas campestris pv.campestris*	10min.at 60°C	—
owpea		*Xanthomonas campestris pv.campestris*	30min. at 50°C	—
ucumis spp.	Foot rot	*Fusarium solani*	15min. at 56°C	—

Crop	Disease	Causal organism	Treatment	Reference
Mung bean	Black rot	*Xanthomonas axonopodis* pv. *phaseoli*	45min. at 52°C	Gaur et al, 1992
Pea	Blight	*Pseudomonas syringae* pv. *pisi*	15min. at 52-55°C	Chitrampalam et al, 1994
Potato		*Erwinia carotovora* sub sp. *atroseptica*	10min. at 50°C	—
Potato		*Erwinia carotovora* sub sp. *caratovora*	20min.at 50°C	—
Rice	Bacterial Leaf blight	*Xanthomonas oryzae* pv. *oryzae*	Soaking in normal water for 12hrs. and then at 53°C for 30min.	—
Tomato	—	*Xanthomonas campestris* pv. *vesicatoria*	50°C for 60min. or 55°C for 30 min. or 60°C for 15min.	—
Wheat	Loose smut	*Ustilago segetum* var. *tritici*	90-120min. at 49°C or 5-6hrs. at 41°C	Doling,1965

11.2.2 Solar heat treatment

The solar heat treatment was developed in India for controlling loose smut of wheat caused by *Ustilago segetum* var. *tritici* by Luthra and Sattar (1934). The wheat seeds were soaked in water for 4 hours from 6am to 10am in the morning on a sunny day followed by drying them for 6 hours, from 10am to 4pm, when the temperature is above 35°C. The soaked seeds should be spread in a single layer on threshing floor or *pucca* floor. This method is quite effective in North India where afternoon temperatures during the months of May and June are above 35°C.

11.2.3 Hot air treatment

The dry heat seed treatment is quite effective in some cases, *e.g. Pseudomonas syringae* pv. *phaseolicola* in beans can be reduced if the seeds are exposed to 50°C for 3days, without adversely affecting seed germination (Tamiethi and Garabaldi,1984). Similarly, the incidence of *P. syringae* pv. *pisi* in pea was reduced when seeds were exposed to 56°C for a day without affecting germination (Groundeau *et al.*, 1992). Incidence of *Xanthomonas campestris* pv. *malvacearum* can be reduced if the seeds are kept at 95°C for 5hrs. or 85°C for 48-72hrs. Seed infection of *Pseudomonas syringae* pv. *pisi* can be reduced if the seeds are held at 65°C for 72hrs. Similarly, the seed infection of *Pseudomonas avenae, P.fuscovaginae* and *P.glumae* in rice can be reduced if the seeds are kept at 65°C for 2 days. Some other examples of hot air treatment are given in the table 11.2:

Table 11.2 Hot air treatment for some bacterial pathogens

Crop	*Disease*	*Causal organism*	*Treatment*	*Reference*
Barley	—	*Xanthomonas campestris* pv.*translucens*	4days at 72°C	Fourest *et al*,1990
Bean	—	*Pseudomonas syringae* pv. *phaseolicola*	One day at 60°C or 3days at 50°C	Naumann and Karl,1988
Bean	—	*Pseudomonas syringae* pv. *phaseolicola*	120min. at 70°C	Belletti and Tamietti, 1982
Cucumber	—	*Pseudomonas syringae* pv. lachrymas	3days at 70°C	Umekawa,1987
Pea	—	*Pseudomonas syringae* pv. pisi	One day at 65°C	Grondeau *et al*,1992
Rice	—	*Pseudomonas glumae*	2days at 65°C	Ziegler and Alvarez,1989
Tomato	—	*Clavibacter michiganense* sub.sp. *michiganense*	60min. at 80°C	Marinescu,1975

However, this method is least effective as compared to other two methods of hermotherapy. Since in dry heat treatments normally ovens are used which ncrease the probability of fire hazards. Thus, this method is not very much in use articularly on commercial scale.

11.2.4 Aerated steam treatment

According to Baker (1969) the efficacy of aerated steam is about 50 per cent less than that of the hot water, thus requiring a longer exposure to achieve similar level of control of seedborne pathogens. It is possible to eradicate or significantly control the seedborne pathogens, e.g. *Drechslera maydis* in maize can be controlled if the seeds are treated with aerated steam at 54°C for 17 minutes (Pritchard, 1974). Similarly, *Septoria nodorum* in wheat requires an exposure of 30 minutes to the temperature ranging from 52°-62°C (Navratnam *et al*, 1980). *Pseudomonas syringae* pv. *phaseolicola* can be controlled by subjecting the seeds to a temperature of 55-60°C for a duration lasting from30-60 minutes (Ralph, 1974).