

Review Article

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A Review on Eco-Friendly Natural Plant and Animal Products for Plant Diseases Management

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ABSTRACT

The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Pyrethroids, and neem products are well established commercially as botanical pesticides and recently some essential oils of higher plants and animals products have also been used as antimicrobials against diseases. In the context of agricultural disease management, botanical pesticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post-harvest protection of food products in developing countries. To achieve these multiple functionalities, sustainable plant disease management should place emphases on rational adaptation of resistance, avoidance, elimination and remediation strategies individually and collectively, guided by traits of specific host-pathogen associations using evolutionary ecology principles to create environmental (biotic and abiotic) conditions favourable for host growth and development while adverse to pathogen reproduction and evolution.

Keywords

Botanical, Plant extract, Plant diseases and Antimicrobial compounds

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Introduction

India is the second most populous country in the world. With the increasing population, the cultivable land resource is shrinking day to day. To meet the food, fibre, fuel, fodder and other needs of the growing population, the productivity of agricultural land and soil

health needs to be improved. Green Revolution in the post-independence era has shown path to developing countries for self-sufficiency in food but sustaining agricultural production against the finite natural resource base demands has shifted from the “resource degrading” chemical agriculture to a “resource protective” biological or sustainable

agriculture. The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides.

Chemical control of most of the diseases of plants may be available and could extensively reduce the impact of plant diseases, but field application of synthetic fungicides may not always be desirable. Increasing awareness of environmental impact of conventional high input intensive farming system has led to a move towards alternatives. Use of natural and conventional resources for the management of plant diseases is currently being advocated. Furthermore, these compounds are natural in origin, have minimum adverse effects on the physiological processes of plants and are easily convertible into common eco-friendly organic materials (Gnanamanickam, 2002).

In Indian agriculture, the yield gap in various crops still remains large even after following the best management practices. Also the agricultural lands continue to shrink and there is a greater threat to global environment and soil resources in the form of erosion of biodiversity and climate change marching towards desertification and environmental pollution. Hence, there is now a great concern to maintain soil health and protect environment by popularizing eco-friendly and cost effective organics. Rapid increase in population and demand of food materials has initiated the large scale use of chemicals resulting in harmful biomagnifications and infertility of soils. Indiscriminate use of these chemicals has led to resistance, killing of beneficial microorganisms and imbalance in

the ecosystem. To overcome these problems, plant and animal based substances and certain indigenous practices offer safe alternatives in disease management.

With this background a review has been made to study the effect of various plant and animal based substances and certain indigenous practices in plant disease management.

Plant extracts

Plant extracts, essential oils, gums, resins etc. have been shown to exert biological activity against plant fungal pathogens *in vitro* and *in vivo* and can be used as bio-fungicidal products (Fawzi *et al.*, 2009; Jalili *et al.*, 2010; Romanazzi *et al.*, 2012). These products are generally assumed to be more acceptable and less hazardous for the ecosystems and could be used as alternative remedies for treatment of plant diseases (Chuang *et al.*, 2007).

Natural plant products have a narrow target range with specific mode of action, therefore are suitable for a specific target, mostly nontoxic for antagonistic microorganisms, show limited field persistence and have a shorter shelf life and no residual threats. They often constitute a part of integrated pest management (IPM) programs, generally safe to humans and environment in comparison to conventional synthetic chemical pesticides. They can easily be adopted by farmers in developing countries who traditionally use plant extracts for the control of diseases (Nuzhat and Vidyasagar, 2013). Many reports approve the efficacy of natural products of plants in controlling fungal growth and mycotoxin production, e.g. cinnamon, clove, oregano, palmarosa and lemongrass oils (Marin *et al.*, 2004), tea tree oil (Burgiel and Smagłowski, 2008), common thyme, cinnamon leaf and aniseed oils (Cosic *et al.*, 2010), sweetbasil, neem, eucalyptus, datura, garlic and oleander extracts (Nashwa and

Abo-Elyousr, 2012) (Table 1). Thymol and carvacrol are definitely the most effective active constituents against most fungal species tested (Numpaque *et al.*, 2011; Shin *et al.*, 2014; Villanueva Bermejo *et al.*, 2015; Gavaric *et al.*, 2015). The mechanism of action of these compounds against fungi is not completely understood but it is supposed to be in relation to their general ability to dissolve or otherwise disrupt the integrity of fungal cell walls and cell membranes (Isman and Machial, 2006). Some more examples regarding antifungal potential of plant products are listed in table 2. Chemical composition and mode of action of plant products the most commonly plant products used for plant disease management are essential oils and extracts. These two types of plant based products have many similarities but also differ for some characteristics. Essential oils are oily liquids obtained from plants through fermentation and steam distillation (Burt, 2004), whereas plant extracts, in contrast are obtained from dried plant products by filtration and evaporation using various solvents (Wang *et al.*, 2004). The major compounds that have been investigated to date include phenols, flavonoids, quinones, terpenens, tannins, alkaloids, lectins, polypeptides, saponins and sterols (Halama and Van Haluwin, 2004). These products may have fungicidal or fungistatic activity on plant pathogens or they can create conditions unfavourable for establishment and multiplication of pathogenic microorganisms on host plants (Scheuerell and Mahaffee, 2002). Simple phenols and phenolic acids are bioactive phyto-chemicals consisting a single substituted phenolic ring. Phenolic toxicity to microorganisms is due to the site (s) and number of hydroxyl groups present in the phenolic compound. Quinones are characteristically highly reactive, colored compounds with two ketone substitutions in aromatic ring. Flavones, flavonoids and flavonols are phenolic structures with one

carbonyl group. They are synthesized by plants in response to microbial infection and are often found effective *in vitro* as antimicrobial substance against a wide array of microorganisms. Tannins are polymeric phenolic substances possessing the astringent property (Figure 1). These compounds are soluble in water, alcohol and acetone (Gurjar *et al.*, 2012). The mechanisms of some important natural compounds on plant pathogenic fungi are given in table 3.

Species of some plant families such as *Solanaceae* for their high alkaloid contents, *Mimosaceae* for their high tannins contents and *Lamiaceae* and *Meliaceae* for their wide diversity of terpenoids may be more feasible for investigations regarding their biofungicidal compounds. For production of active ingredients, there are some factors that determine variability in quality and quantity of metabolites. The concentration of a chemical in different parts of a plant such as roots, leaves, flowers and fruits may differ. It may even be absent in one or more parts, therefore it is convenient to collect integral samples (Montes-Belmont and Carvajal, 1998).

Some plant contains components that are toxic to pathogens. When extracted from the plant and applied on infested crops, these components are called botanicals. Commonly used botanicals: Plant extracts: Neem (*Azadirachta indica*, A. Juss), Garlic (*Allium sativum*, Linn.), Eucalyptus (*Eucalyptus globulus*, Labill., Turmeric (*Curcuma Longa*, Linn., Tobacco (*Nicotiana tabacum*, Linn., Ginger (*Zingiber officinale*, Rosc. Essential oils: Nettle oil (*Urtica* spp.), Thyme oil (*Thymus vulgaris*, Linn.), Eucalyptus oil (*Eucalyptus globulus*, Labill. Rue oil (*Ruta graveolens*, Linn.), Lemon grass oil (*Cymbopogon flexuosus* (Steud.) Wats. and Tea tree oil (*Melaleuca alternifolia*). Gel and latex: *Aloe vera* (*Tourn. Ex Linn.*) (Gurjar *et al.*, 2012).

Why consider botanicals?

Sustainable solutions in agriculture
Reduce crop losses
Cheaper and
Eco-friendly
Organic farming and
Easily bio-degradable
Integrated Diseases Management.

Plant extracts have assumed special significance in the present day strategy of developing ecologically safe method of plant disease management (Ranjana Sarma *et al.*, 1999). The plants and their constituents are less phytotoxic, more systemic, easily bio degradable and induced host metabolism (Malik, 1987; Gupta, 1997) to resist pathogen infection.

Antimicrobial activity of plant extracts have also been reported by many workers (Pandey and Pant, 1997). Among the higher plants, medicinal plants and their products (Gohil and Vala, 1996) have been shown to possess good efficacy against fungi, bacteria and viruses (Ushiki *et al.*, 1996; Hussain *et al.*, 2000; Parveen and Kumar, 2000). The root extracts of medicinal plants like *Geranium pretense* L., *Eupatorium fortunei* L. and *Sanguisorba officinalis* L. were affective against *P. aphanidermatum*, *R. solani*, *Verticillium dahliae* and *Fusarium oxysporum* (Ushiki *et al.*, 1996). Parveen and Kumar (2000) reported that the extracts from *Azadirachta indica* A. Juss, *Polyalthia longifolia* Benth and Hook, F. *Ocimum tenuiflorum* Benth and Hook, *Catharanthus roseus* L., and rhizome of *Zingiber officinale* L. inhibited *Alternaria triticina*. Antifungal activity of some of the plants is presented in table 4.

Tewari and Mandakini (1991) reported that, leaf extracts of four plant species, *Piper betel*, *Ocimum sanctum*, *Nyctanthesarbor-tristis* and *Citrus limon* were effective in reducing the

radial growth of *Pyricularia oryzae*, *Cochliobolus miyabeanus* and *Rhizoctonia solani*. *Piper betel* was found to be the best, followed by *O. sanctum*, in reducing the growth of the pathogens completely under *in vitro* and in checking the spread of blast, brown spot and sheath blight diseases of rice under *in vivo*.

Ansari (1995) tested antifungal activity of *Trachispermum ammi*, *Cymbopogon citrates*, *Ocimum* spp., *Mentha* spp., *Rauwolfia* spp., *Lawsonia inermis* and *Vitex trifolia*, against *Rhizoctonia solani* and found *T. Ammi* (seed), and *Ocimum* spp. (leaves) extracts were fungicidal whereas the other extracts had fungistatic activity. Spray of *T. ammi* and *Ocimum* spp. Extracts at 1:20 dilutions reduced the disease by 72.25 and 69.58 per cent respectively.

Tewari (1995) reported that leaf extract of *Ocimum sanctum* significantly inhibited both conidial germination and mycelial growth of *Pyricularia oryzae in vitro* and also observed that the effect of leaf extract was comparable to that of ediphenphos and carbendazim in controlling the blast disease.

The plant extracts (10%) prepared from *Acacia concinna* L. and *Basia latifolia* (Roxb.) effectively controlled *A. solani* (Babu *et al.*, 2000). The extracts of all parts of *Cleome isocandra* L. were reported to inhibit the mycelial growth and spore germination of *A. solani*. The extracts of *Allium sativum* L. bulb, *Aegle marmelos* L. leaf and *C. roseus* flower inhibited spore germination and mycelial growth of *A. solani* (Vijayan, 1989). Sumathi (1996) reported that the extracts of *Prosopis chilensis* (Molina) Stundz, *Abutilon indicum* Mill. *Sorghum vulgare* L. and *Cocos nucifera* L. were more effective in inhibiting the mycelial growth and spore germination of *A. solani* by 90 per cent. The rhizome extract of *Curcuma longa* L. and *Valeriana officinalis* L.

reduced the mycelial growth of *Alternaria alternata* (Kazami *et al.*, 1993).

The flower extracts of *Cassia fistula* L., *Rhododendron arboreum* J.E. Smith and *Thevetia peruviana* (Pers.) Merr. Inhibited the growth of *A. solani* (Sundriyal, 1991). Shivapuri *et al.*, (1997) reported that the ethanol extract of *A. indica*, *Datura stramonium* L., *O. sanctum* L., *P. longifolia* and *Vinca rosea* G. Don. Were more fungitoxic to *Alternaria brassicicola*, *R. solani*, *Colletotrichum capsici* and *F. oxysporum*. The inhibitory effect of leaf extract of *Adenocalymna alliaceum* Mart., *A. indica*, *Murraya koenigii* Spreng. on *A. tenuissima* under *in vitro* as well as in field condition was observed by Singh *et al.*, (2001). Joy *et al.*, (2004) stated that leaf, fruit and shell extract of *Anacardium occidentale* L. suppressed the growth of *A. solani*, *F. solani*, *Phytophthora palmivora* and *Sclerotium rolfsii*.

Field evaluation of garlic bulb extract and neem oil cake reduced disease incidence of leaf blight in onion caused by *A. alternata* (Kannan and Subbaraja, 1999). Kuruchev and Padmavathi (1998) reported that soil drenching and seed treatment of 10 per cent leaf extracts of *Lawsonia inermis* L., *Eucalyptus globulus* Labill., *A. indica* and bulb extract of *A. sativum* reduced the per cent disease incidence of damping off in chilli. Leaf extract of Piper betle (Alice, 1984) and *E. microthecia* (Narayana Bhat *et al.*, 1990) was reported to control *Pythium* species when applied as soil drench. The juice obtained from shade dried *Eupatorium cannabinum* L. exhibited 100 per cent inhibition of mycelial growth of *P. debaryanum*, *F. oxysporum*, *R. solani* and *S. rolfsii* at 1:1 dilution (Kumar and Tirpathi, 1991). Shivapuri *et al.*, (1997) reported that ethanol extracts of 10 plant species (*A. cepa*, *A. sativum*, *A. indica*, *O. sanctum*, *P. longifolia* F., *Tagetes erecta* L.,

Vinca rosea G. Don., *D. stramonium*, *Calotropis procera* L. R.Br. and *Withania somnifera* L.) have showed antimicrobial properties against five pathogens (*R. solani*, *A. brassicicola*, *F. oxysporum*, *C. capsici* and *S. sclerotiorum*). Meena and Muthusamy (1999) found that application of neem cake (150 kg/ha) in combination with palmarosa oil (0.1%) caused 73.6 per cent reduction in sheath blight disease incidence over control. Sindhan *et al.*, (1999) evaluated the efficacy of water extracts of *A. indica*, *Mentha arvensis* L., *E. globules*, *O. sanctum*, *D. alba*, *Bougainvillae spectabilis* Willd., *Z. officinalis* and *A. cepa* against mycelial growth of *R. solani* and *R. bataticola* at 5 per cent concentration. The methanolic extract of *Agrimonia eupatoria*, *Hypericum scabrum*, *Mentha longifolia*, *Onobrychis tournefortii*, *Peganum harmala*, *Phlomis sieheana*, *Pimpinella anisum* and *Tanacetum vulgare* were found to possess the antimicrobial activity against several pathogenic fungi and bacteria (Sokmen *et al.*, 2000).

Shivapuri *et al.*, (1997) reported that ethanol extracts of ten plant species (*Allium cepa* L., *Datura stramonium* L., *Ocimum sanctum* L., *Polyanthea longifolia*, *Tegetes erecta* L., *Vinca rosea* and *Withania somnifera* L.) showed fungistatic properties against five pathogens (*Rhizoctonia solani*, *Alternaria brassicicola*, *Fusarium oxysporum*, *Colletotrichum capsici* and *Sclerotinia sclerotianum*). Concentrations of 500µg/ml and 1000 µg/ml were tested. Higher dose of the plant extracts was more efficient.

Ranjana *et al.*, (1999) tested fifteen common weed species found in crop fields for their efficacy in suppressing sheath blight. They found that aqueous weed extracts of bracken fern (*Diplazium esculentum*), goat weed (*Ageratum houstonianum*), sickle pod senna (*Cassia tora*), black night shade (*Solanum nigrum*), and polygonum (*Polygonum*

plebium) exhibited more than 90 per cent inhibition at 20 per cent concentration.

Kamalakannan *et al.*, (2001) studied the efficacy of different plant extracts against blast disease under pot culture conditions and reported that, pre and post inoculation spray of *Prosopis julifera*, *Zizyphus jujuba* and *Azadirachta indica* exhibited greater reduction in disease incidence. However, pre-inoculation spray was comparatively more effective than post inoculation spray in reducing disease incidence. Reddy *et al.*, (2002) revealed that aspirin was highly effective (100 %) in inhibiting mycelia growth and sclerotial production of *Rhizoctonia solani* followed by extracts of *Allium sativum* (61.8 %). *Azadirachta indica* (24.9%) and *Allium cepa* (19.3 %).

Muralidharan *et al.*, (2003) reported that among the different plant derived products most of them reduced disease incidence and also increased grain yield harvests. The degree of disease reduction and yield increase was more pronounced when these products were applied to control blast and sheath blight diseases. Biswas and Roychoudhury (2003) evaluated the relative efficacy of six botanical formulations against the development of sheath blight disease of rice and compared with the standard check fungicide (Propiconazole 25 EC). All the botanicals significantly reduced the disease severity over control. Among the botanicals, spectaf followed by neemazal and ahook performed best in reducing the disease severity and also improved yield.

Systemic resistance inducing protein (CA-SRIP) was identified from *Clerodendrum aculeatum* against PRSV infection in papaya by Srivastava *et al.*, (2006). No virus symptoms were observed up to 6 months after inoculation, following challenge inoculation with PRSV, to the CA-SRIP treated papaya

plants. There was also a significant difference in the vegetative growth of the treated plants as compared to control set of plants.

The plant extracts from *Plectranthus tenuiflorus*, *Azadirachta indica*, *Clerodendrum inerme*, *Schinus terebinthifolius* and *Mirabilis jalapa* as antiviral materials were evaluated against *Bean common mosaic virus* (BCMV) in bean plants (Elsharkawy and El-Sawy, 2015). All the tested plant extracts were effective in reducing the number of local lesions formed by BCMV. The mixing of *P. tenuiflorus* extracts with BCMV inoculum achieved the highest reduction in BCMV infection (92%), while the mixing of *S. terebinthifolius* extracts with BCMV inoculum recorded the lowest reduction in BCMV infection (68%).

Medicinal plant extracts

Plant extracts used to control of the phytopathogens have been obtained mainly from tree species such as eucalyptus and neem (24% of the studies with extracts) and herbaceous species like garlic, citronella, mint, rue, yarrow, ginger, basil, camphor, turmeric and *ocimum* (54%). Besides these there are other 237 plants from the Brazilian flora whose antimicrobial potential was tested by Brazilian researchers. With respect to groups of pathogens, the majority of the work is with those that cause disease in the plant canopy (30% of the works with extracts), like the genus *Alternaria*, *Bipolaris*, *Crinipellis*, *Corynespora* and *Colletotrichum*, which respond alone for 15% of the works. The soil-borne pathogens represent 20% of the researches, especially *Rhizoctonia*, *Sclerotium*, *Sclerotinia*, *Fusarium* and *Phytophthora*. Post-harvest pathogens like *Penicillium*, *Aspergillus* and *Rhizopus* are in 9% of the works and *Meloidogyne* nematode in 9.5%. For the host plants, 30% of the work are with crops like beans, soybeans, coffee, wheat,

cotton and cassava 20% with vegetables like cucumber and tomato, this later representing alone 15% of all the researches with extract; and 10% with the fruits like papaya, strawberry and cocoa. Details about these data can be found in the review of Stangarlin and colleagues (1994). From these presented numbers we can have an overview of the researches on alternative control of disease in plants in Brazil using natural extracts. Besides that, other researches have been made to identify plants whose extracts have compounds with biological action against phytopathogens or that induce plant resistance.

To evaluate the post-harvest control of anthracnose in banana, an *in vitro* assay was conducted to measure the mycelial growth of *Colletotrichum musae* in solid and liquid media containing 1, 5, 10, 15, 20 and 25% of camphor (fresh weigh/volume). To *in vivo* assay was evaluated the post-harvest control by immersing banana fruits for 3 min in the camphor extracts, using as control treatments Benomyl (0.25 g/L) and distilled water. The results showed that camphor inhibited 41% the mycelial growth and 86% the sporulation, in solid medium, although had stimulated the mycelial growth in liquid medium. The severity of *Anthracnose* was reduced to 67% and 56% for camphor and fungicide treatments, respectively (Carré *et al.*, 2007).

Aqueous extract (AE) of eucalyptus in concentrations of 0.1, 1, 5, 10, 15, 20 and 25% was used in the following experiments: induction of phytoalexins in sorghum etiolated mesocotyls and soybean cotyledons; *in vitro* fungitoxicity against *Colletotrichum lagenarium*; and local or systemic induction of resistance in cucumber plants against anthracnose. Water and acibenzolar-S-methyl were used as control treatments. The eucalyptus extract induced phytoalexin synthesis in sorghum starting from the concentration of 1%. In soybean, glyceollin

synthesis took place from 10% of aqueous extract. The phytoalexin synthesis in different incubation periods showed that greater inductions take place from 48 hours on. There was total inhibition in the spores germination and apressoria formation in concentrations of 20%. Based on the parameter size of lesion, the aqueous extract of *E. citriodora* was capable to induce local resistance in cucumber plants against *C. lagenarium* (Bonaldo *et al.*, 2004).

Aqueous extract from *E. citriodora* fresh leaves, in concentrations up 20%, were efficient to inhibited in 100% the mycelial growth of *Colletotrichum sublineolum*, *Phytophthora* sp. and *Sclerotium rolfsii*, in 75% to *Rhizoctonia solani* and in 45% to *Alternaria alternate*, under *in vitro* assays (Bonaldo *et al.*, 2007).

Zingiber officinale (ginger) the effect of aqueous extract of ginger was evaluated at concentrations of 1, 5, 10, 15, 20 and 25% on *Sclerotinia sclerotiorum* mycelial growth and sclerotia production, *in vitro*. The efficiency of protection of ginger was also verified in lettuce plants growth organically and inoculated with the pathogen. Besides the disease incidence, the crop yield and the peroxidase induction were analysed in the tissue plants. Water and the resistance inductor acibenzolar-S-methyl were used as control treatments. Additionally, the elicitor ability of ginger extract in inducing accumulation of the phytoalexins 3-deoxyanthocyanidin and glyceollin was evaluated in sorghum and soybean bioassays, respectively. The results showed the antimicrobial activity of ginger with mycelial growth and sclerotia production inhibition. In the lettuce crop, it was observed that the application of ginger mass on the soil near to plants basis caused a bigger activity of peroxidase enzyme and the reduction of disease incidence. The presence of elicitor

compounds in the ginger extract was detected by the production of phytoalexins in sorghum and soybean, with dose-dependent response (Rodrigues *et al.*, 2007).

Ocimum gratissimum (wild basil) the *in vitro* effect of wild basil crude extract (CE) (autoclaved or sterilized by filtration) as well as the iprodione + thiram fungicide on the mycelial growth and sporulation of *Bipolaris sorokiniana* was evaluated. Besides, wheat seeds naturally infected with *B. sorokiniana* were submitted to the following treatments: 1) fumigation with essential oil; 2) immersion with autoclaved CE and; 3) non autoclaved CE at different concentrations (1, 5, 10, 15, 20, 25, and 50%; w/v); 4) sprinkling with dry leaves of the medicinal plant and with the fungicide. The autoclaved CE showed, even partially, higher inhibition of mycelial growth in all the tested concentrations than CE sterilized by filtration. In greenhouse, the fumigated seeds (7 days) showed the same emergency speed (EV) concerning to the control (water). There were no statistics differences among the analyzed parameters (EV, emergency percent, height, dry mass, fresh mass, and transmission rate (TR)) when the treatment by immersion was compared with the no treated control. Seeds treated with the dry powder of medicinal leaves did not differ statistically from the chemical control, when the same parameters were evaluated, but presented higher TR of the pathogen. In the laboratory, seeds submitted to the fumigation process presented a higher number of health seeds than the non-treated control, while the treatments by immersion in the CE and sprinkling with dry leaves did not differ concerning to the same parameter (Rodrigues *et al.*, 2006). Aqueous extracts of the leaves of *Ocimum gratissimum* at 10, 25, 40 and 50% (w/v) concentrations induced the production of phytoalexins in soybean cotyledons and sorghum mesocotyls. The aqueous extracts also induced systemic resistance in cucumber

against *Colletotrichum lagenarium*, reflected by reduction in disease incidence and an increase in chitinase production (Colpas *et al.*, 2009). The essential oil and crude extract of wild basil, harvested in different seasons, were tested *in vitro* on the mycelial growth of *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora* sp. and *Alternaria alternata*. To evaluate the effect of the essential oil, sterilized oil aliquots of 20 μ L, 40 μ L and 60 μ L were distributed on the surface of potato-dextroseagar (PDA). The aqueous extracts were filtrated and incorporated in PDA at 1%, 5%, 10%, 15%, 20%, 25% and 50%. The results showed total inhibition of mycelial growth in the different aliquots of essential oil. The extract collected from plants in autumn had better results; in this season the aqueous extract at 5% was enough to provide inhibition of 100% on mycelial growth of *A. alternata* and *S. rolfsii* (Benini *et al.*, 2010). *Tagetes patula* (marigold) Aqueous extract of flowers, leaves and roots of *Tagetes patula* were obtained by infusion in the proportion of 50 grams of dehydrated material in 1,000 ml of distilled water and tested *in vitro* on eggs and second-stage juveniles (J2) of *Meloidogyne incognita*, without dilution and diluted 1:1, 1:2, 1:3 and 1:4 (extract: water, v:v), and in plants of tomato cv. Santa Cruz Kadá cultivated in pots, without dilution and diluted 1:1. For *in vivo* assays, the extract was sprayed on the leaves, soil or both by four ways: weekly during eight weeks; at the transplanting (one week before inoculation); and together or one week after inoculation. The extract of flowers, leaves and roots inhibited the hatching, the mobility, and caused mortality of J2 *in vitro*.

Greater nematicide effect was obtained with roots extract, which promoted mortality up to 68% of J2. In tomato plants, results were not satisfactory with only one application of *Tagetes* extract. Weekly applications promoted plant development and decreased

galling and the nematode population. Greater effect was obtained by flower extract application, followed by leaves extract. Slight or non-effect was observed by root aqueous extract. Flower extract without dilution inhibited galling up to 62.2% and decreased 61.5 and 52.8% the number of J2 in the soil and eggs in the roots, respectively. Applications on the soil or leaves presented similar results. These results demonstrated the potential of *T. patula* extract, mainly of flowers, to protect tomato plants against *M. incognita*. Furthermore, it was hypothesized that induced resistance was involved in the responses observed (Franzener *et al.*, 2007).

In Kharif season *Sphaerotheca fuliginea* was dominantly present on *Cucurbita maxima*, *Lagenaria siceraria* and *Cucurbita pepo*, where as *Erysiphe cichoracearum* noted on *Citrullus lanatus* was not reported in 2005 and 2006. *Cucurbita maxima*, *Lagenaria siceraria*, *Luffa acutangula* and *Luffa cylindrica* showed maximum occurrence of *Sphaerotheca fuliginea* in the rabbi season. Incidence of powdery mildew is more at post flowering and fruiting stage.

However powdery mildew also reported at seedling stage of *Cucurbita pepo* and *Cucurbita maxima*. Neem leaf extract at 15% concentration, *Parthenium* leaf at 10% concentrations. *Ocimum* leaf at 20 % concentration, Citrus leaf at 20% concentration, *Annona squamosa* leaf at 10% concentration, Ipomea at 15% and Jowar leaf at 20% control the powdery mildew of cucurbits (Vasant and Ashok, 2010). Cow urine at 15 % concentration proved to be effective to control powdery mildew on cucurbit. 20 % to 25% of Butter milk spray successfully controlled the powdery mildew of cucurbit. Similarly 20% ash spray also found to be successful to control the growth of powdery mildew of cucurbits. Spray of Dashparni ark, a bi-product of plant and

animal at 10 % concentration inhibited the growth of *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*

Current aspects of commercially available natural products

Although considerable research efforts throughout the world have been conducted and an ever-increasing volume of scientific literature on the pesticides properties of plant products and their constituents are available, but some pest control products based on plant essential oils/extracts have been appearing in the market which seems not enough for substituting with current synthetic pesticides. In the United States, commercial development of insecticides based on plant essential oils/extracts has been greatly facilitated by exemption from registration for certain oils commonly used in processed foods (Quarles, 1996). This opportunity has encouraged the development of some essential oil-based pesticides using rosemary oil, clove oil, and thyme oil as active ingredients for agricultural and industrial applications. Application of these products has been interesting, particularly for control of greenhouse pests and diseases. The natural plant product Milsana ®, extracted from the giant knotweed (*Reynoutria sachalinensis*) is probably the best known natural fungicide. This product has been reported to control powdery mildew, caused by *Sphaerotheca fuliginea*, in the long English cucumber under greenhouse conditions, and also showed broad spectrum activity against powdery mildew of tomato, apple and begonia as well as downy mildew of grapevine and rust of bean (Daayf *et al.*, 1995). A product based on rosemary oil is available fungicide sold under the trade name 'Sporan TM '. A volatile natural product, Carvone™, derived from dill and caraway seed, has been developed to inhibit the growth of storage pathogens (Moezelaar *et al.*, 1999).

Table.1 Botanicals

Plant	Part used	Preparations	Diseases/pathogen	References
Datura/thorn apple (<i>D.stamonium</i>) <i>Calotropis procera</i> (Ait.) R. Br. <i>Oscimum</i> spp.	Root, stem, Leaf, flowers	Crude extract	<i>Curvularia lunata</i>	Manoharachary and Gourinath (1988)
Turmeric (<i>Curcuma longa</i> Linn.), Ginger (<i>Zingiber officinale</i> Rosc.)	Rhizome	Crude extract	<i>Phytophthora infestans</i> , <i>Fusarium solani</i> , <i>Pyricularia oryzae</i>	Bandara <i>et al.</i> , (1989)
Neem/Margosa (<i>Azadirachta indica</i> A.Juss.), Sugar apple (<i>Annona squamosa</i> Linn.), Holy basil (<i>Oscimum sanctum</i>Linn.)	Leaf, Stem, Bark, root	Crude extract	Anthracnose of pepper	Nduagu <i>et al.</i> , (2008)
Neem/Margosa (<i>Azadirachta indica</i> A.Juss.),	Seed kernel	Oil	<i>A. alternata</i>	Dharam and Sharma (1985)
<i>Ambrosioides</i> Linn., <i>Oscimum</i> spp.	Leaf	Essential oils	<i>Aspergillus flavus</i>	Mishra <i>et al.</i> , (1989)
Garlic (<i>Allium sativum</i> Linn.), Datura (<i>D. stramonium</i> Linn.)	Bulb, Leaf	Ethanol extracts	<i>Curvularia lunata</i>	Upadhyaya and Gupta (1990)
Spearmint (<i>Mentha spicata</i> Linn.), Greek Sage (<i>Salvia fruticosa</i> Mill.), <i>Thymbra</i> spp.	Leaf	Essential oils	<i>Rhizoctonia solani</i> , <i>Sclerotium sclerotiorum</i>	Yegen <i>et al.</i> , (1992)
Neem/Margosa (<i>Azadirachta indica</i> A.Juss.),	Seed, Leaf	Crude Extracts	Early blight of tomato	Patil <i>et al.</i> , (2001)
Madar (<i>Calotropis procera</i> (Ait.) R.Br.	Leaf	Crude Extracts	Tikka leaf spot disease of groundnut	Srinivas <i>et al.</i> , (1997)
Neem/Margosa (<i>Azadirachta indica</i> A.Juss.),	Seed	NSKE	Powdery mildew of pea	Surwase <i>et al.</i> , (2009)
Neem/Margosa (<i>Azadirachta indica</i> A.Juss.),	Leaf	Achook formulations (azadirachtina)	Sheath blight of rice	Kandhari <i>et al.</i> , (2007)
Neem/Margosa (<i>Azadirachta indica</i> A.Juss.),	Seed kernel	Neem oil	Rice tungro virus	Muthamilan and Revathy (2007)
Neem/Margosa (<i>Azadirachta indica</i> A.Juss.),	Leaf, Seed	Achook, Neemazal,	Bacterial blight of rice	Sunder <i>et al.</i> , (2005)
Black pepper (<i>Piper nigrum</i> Linn., Clove (<i>Syzygium aromaticum</i>(Linn.) Merr. & Perry, Geranium (<i>Pelargonium graveolens</i> L'Herit), Nutmeg (<i>Myristica fragrans</i> Houtt.), (<i>Origanum vulgare</i> spp. <i>hirtum</i> (Link) Letsw. and thyme [<i>Thymus vulgaris</i> Linn.	Leaf	Volatile oil	Anti bacterial (gram positive and gram negative)	Dorman <i>et al.</i> , (2000)

Table.2 Botanicals produced by plants having antimicrobial activity

Common name	Scientific name	Compound	Class	Activity
Apple	<i>Malus pumila</i> Mill.	Phloretin	Flavonoid derivative	General
Aswagandha	<i>Withania somnifera</i> Dunal.	Withafarin A	Lactone	Bacteria, fungi
Bael tree	<i>Aegle marmelos</i> Linn.	Essential oil	Terpenoid	Fungi
Blue germ tree	<i>Eucalyptus globulus</i> Labill.	Tannin	Polyphenol	Fungi, Bacteria, Viruses
Onion	<i>Allium cepa</i> Linn.	Allicin	Sulfoxide	Fungi, Bacteria
Thyme	<i>Thymus vulgaris</i> Linn	Caffeic acid	Terpenoid	Fungi, Bacteria, viruses
Turmeric	<i>Curcuma longa</i> Linn.	Curcumin	Terpenoids	Fungi, Bacteria, protozoa
Thome apple	<i>Datura stramonium</i> Linn.	Hyoscyamine	Scopolamine	Alkaloids Fungi
Black pepper	<i>Piper nigrum</i> Linn.	Piperine	Alkaloid	Fungi
Castor	<i>Ricinus communis</i> Linn.	Ricinine, Ricininoleic	Alkaloids	Fungi
Neem/ Margosa tree	<i>Azadirachta indica</i> A.Juss.	Azadirachtin	Terpenoides	Fungi, Bacteria
Garlic	<i>Allium sativum</i> Linn.	Allicin	Solfoxide	Fungi, Bacteria

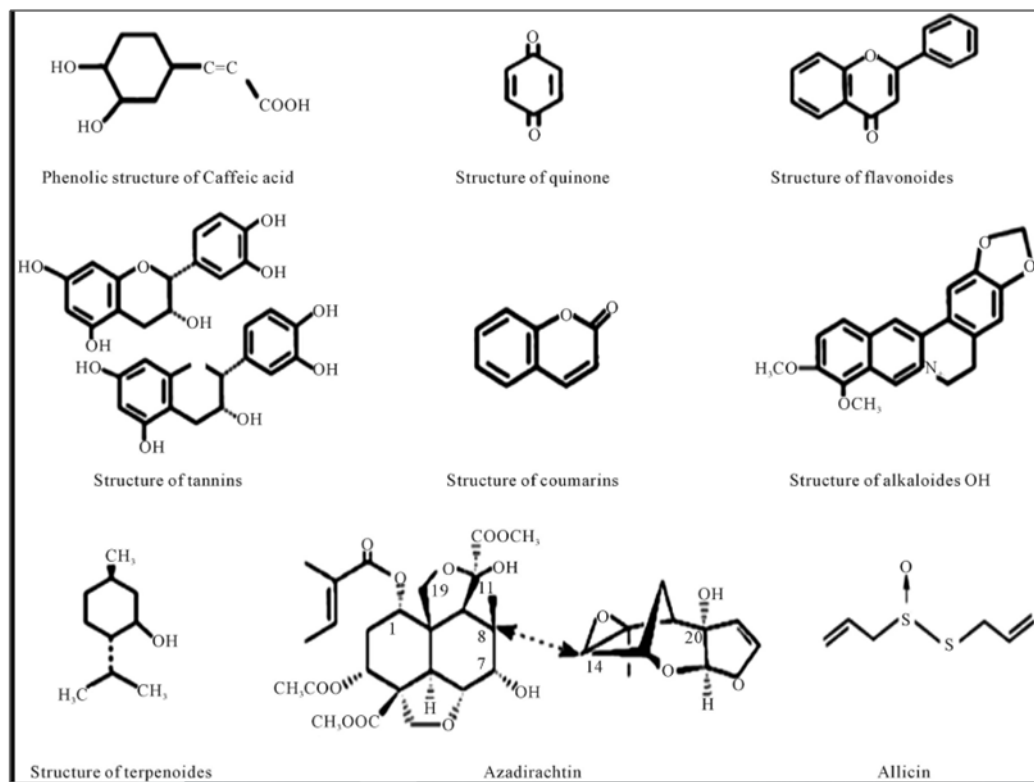
Table.3 Mechanisms of action of phytochemicals (Cowan, 1999)

Name of compound	Mode of action
Simple Phenols	Membrane disruption, substrate deprivation
Phenolic acids	Bind to adhesins, complex with cell wall, inactivate enzymes
Terpenoids	Membrane disruption
Essential oils	Membrane disruption
Alkaloids	Intercalate into cell wall
Tannins	Bind to proteins, enzyme inhibition, substrate deprivation
Flavonoids	Bind to adhesins, complex with cell wall, Inactivate enzymes
Coumarins	Interaction with eukaryotic DNA
Lectins	Form disulfide bridges
Polypeptides	Form disulfide bridges

Table.4 Fungicidal properties of some of plant products

Name of plant product /compound	Controlled plant pathogen	Reference
Acacia, sapodilla, datura, eucalyptus, pomegranate and black plum extracts	<i>Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. ochraceus</i>	Satish <i>et al.</i> , (2007)
Eucalyptus and lavender extracts	<i>Alternaria alternata</i>	Zaker and Mosallanejad (2010)
Clove bud, cinnamon, ginger, black pepper, garlic and onion extracts	<i>Aspergillus niger</i>	Avasthi <i>et al.</i> , (2010)
Neem, chinaberry, garlic and turmeric extracts	<i>Fusarium oxysporum, Rhizoctonia solani</i>	Hadian (2012)
Artemisia, thyme and eucalyptus extracts	<i>Fusarium solani</i>	Zaker (2014)
Indian beech, milk weed, oleander and turmeric extracts	<i>Aspergillus fumigatus, Alternaria solani, Helminthosporium spp</i>	Masih <i>et al.</i> , (2014)
Kokum, wild turmeric and jasmine extracts	<i>Rhizopus stolonifer, Colletotrichum coccodes</i>	Bhagwat and Datar (2014)
Grape seed, thyme, rosemary, mint, basil and sage essential oils	<i>Botrytis cinerea</i>	Mermer-Doğu and Zobar (2014)
Anise, cumin, caraway, ammin, pennyroyal, thyme and cinnamon essential oils	<i>Aspergillus flavus, Phoma sorghina, Alternaria alternata, Botrytis cinerea</i>	Behdani <i>et al.</i> , (2012)
Pepper and cassia extracts, neem, mustard and cinnamon essential oils	<i>Phytophthora nicotianae</i>	Bowers and Locke (2004)
Citral, eugenol and geraniol	<i>Fusarium moniliforme, Curvularia lunata</i>	Krishna Kishore <i>et al.</i> , (2007)
Carvacrol, eugenol, citronellol, geraniol, citral, perillyl and menthol	<i>Monilinia fructicola, Botrytis cinerea</i>	Tsao and Zhou (2000)
Thymol, carvacrol, 1,8 cineole,Y-terpinene, p-cymene and anethole	<i>Fusarium moniliforme, Rhizoctonia solani, Phytophthora capsici</i>	Mueller-Riebau <i>et al.</i> , (1995)

Fig.1 Structure of antimicrobial compounds



Carvone™ is currently marketed as Talent ® in the Netherlands. Additionally, Fungastop™ and Armorex™ II, are two natural products developed in the USA by Soil Technologies Corp., and are commercially available for the control of various plant diseases in agriculture. TimorexGold ® manufactured by Stockton group (Switzerland) is the new generation of bio-fungicides based on a plant extract of *Melaleuca alternifolia* for the control of powdery mildews, downy mildews, rust and early and late blight diseases in vegetables, grapevines and orchards and is harmless to beneficial insects and bees. For organic farming also some plant essential oils are marketed as fungicides. These include E-Rase™ from jojoba (*Simmondsia californica*) oil, Sporan™ from rosemary (*Rosemarinus officianalis*) oil, Promax™ from thyme (*Thymus vulgaris*) oil, Trilogy™ from neem (*Azadirakhta indica*) oil and GC-3™ being a mixture of cottonseed (*Gossypium hirsutum*)

oil and garlic (*Allium sativum*) extract. Bla-S™ is used against rice blast disease in eastern Asia, Kasugamin™, against rice blast and other crop diseases in Japan, Mildiomycin™, for controlling powdery mildews mainly in Japan, Delvolan™, against fungal diseases of ornamental plants and Validacin™, for controlling *Rhizoctonia* root rot of a variety of crops (Dayan *et al.*, 2009; Zaker, 2014).

Animal based products

From the ancient period in India, cow urine has been used for several medicinal purposes and the description on its use has been in several classical Ayurveda texts like Charaka samhita and Shushruta samhita. Cow is believed to be a sacred animal in India its urine is known to cure several diseases. In Veda, cow urine is compared with the nectar (Krishnamurthi *et al.*, 2004; Gururaja *et al.*,

2011). Gaw-mutra (cow's urine) has been described as a liquid with innumerable therapeutic values, capable of curing several incurable diseases in human beings and plants. Cow urine is rich source of macro, micronutrients and has disinfectant and prophylactic properties. It purifies the atmosphere and improves the soil fertility. Cow urine has got applications in agriculture. It has been found that cow urine has potential to control *Meloidogyne incognita* in *Lycopersicon esculentus* (Abubakar *et al.*, 2004) and aphids and pickleworms in watermelon cultivation (Burubai and Eribo, 2012). It is observed that cow urine has inhibitory effect against several plant pathogens such as *Sclerotinia sclerotiorum* (Basak *et al.*, 2002a), *Fusarium solani* f.sp. *cucurbitae* (Basak *et al.*, 2002b), *Bipolaris sorokiniana* (Akhter *et al.*, 2006) and *Xanthomonas oryzae* pv. *oryzae* (Murugan *et al.*, 2012). It has been shown that cow urine extract of certain plants as well as cow urine in combination with certain plant extracts are found to possess marked inhibitory effect on plant pathogens (Akhter *et al.*, 2006; Yadav *et al.*, 2008; Rajapandiyn *et al.*, 2011; Tiwari & Das, 2011)

Inhibitory efficacy of fresh and stored cow urine was tested against two phytopathogenic fungi of ginger viz., *Pythium apahidermatum* and *Fusarium oxysporum* f.sp. *zingiberi* isolated from soft rot specimen of ginger. Urine was collected from an indigenous cow variety 'Malnad gidda'. The effect of various concentrations of fresh and stored cow urine (3 months) viz., 5, 10, 20 and 40% was tested against the mycelial growth of test fungi by poison food technique. The extent of growth of test fungi in poisoned plates was recorded and compared with the control. Both fresh and stored cow urine displayed concentration dependent inhibition of test fungi. Inhibitory efficacy was recorded higher for stored cow urine than fresh cow urine. The inhibition of

test fungi was >50% at 20% and higher concentrations of cow urine. *P. apahidermatum* was inhibited to higher extent than *F. oxysporum* f. sp. *zingiberi*. It can be concluded that cow urine has got potential to inhibit pathogenic fungi causing rhizome rot of ginger in vitro. Field experiments are to be carried out to justify the possible utilization of cow urine against rhizome rot of ginger (Rakesh *et al.*, 2013).

Antifungal efficacy of cow urine against *Colletotrichum capsici* isolated from anthracnose of chilli (*Capsicum annum* L.). Poisoned food technique was employed to determine antifungal activity of different concentrations of cow urine (5, 10 and 15%). Cow urine was found to display concentration dependent inhibitory activity against fungal growth. An inhibition of >50% was observed at 5% concentration indicating, the use of cow urine can be the cost effective and eco-friendly approach for controlling anthracnose in chilli (Yashoda Kambar *et al.*, 2013).

Cow urine extract, hexane, chloroform, ethyl acetate, alcohol, methanol and aqueous fractions of *Pongamia pinnata* Linn seed were tested against *X. oryzae* pv. *oryzae* for its antibacterial activity. Streptomycin sulphate (30 µg) and dimethyl sulfoxide (DMSO, 15 µL) are used as positive and negative control. All the extracts and fractions were effective and showed 10 to 13 mm zone of inhibition. Phytochemical analysis also showed the presence of terpenoids, quinine, coumarin, tannin and phenol, with flavonoid available in higher quantity (1.56 mg.kg⁻¹) (Murugan *et al.*, 2012).

Gotora *et al.*, (2014) was carried out an in vitro assay to establish if cow urine at different concentrations (500µL.mL⁻¹, 300µL.mL⁻¹, 200µL.mL⁻¹, and 100µL.mL⁻¹) can be used to control the growth of *Fusarium lateritium*, the fungal agent causing Fusarium

bark disease in coffee. The growth characteristics selected were conidial germination, germ tube length, mycelial growth rate, and sporulation. Copperoxychloride 50% W.P. was the standard, distilled water was the negative control, and undiluted cow urine was the positive control. The undiluted cow urine was most effective in inhibiting fungal growth with the rest of the cow urine concentrations showing dose dependent efficacy compared to the negative control ($P < 0.01$). Copperoxychloride had the highest efficacy of all treatments with the exception of the inhibition of mycelia growth where undiluted cow urine had higher efficacy and sporulation where efficacy was comparable to undiluted cow urine. There is potential for the use of cow urine as a means of controlling Fusarium bark disease with other advantages being availability, low cost and limited environmental damage.

Studies on use of jeevamrutha in management of leaf spot diseases in pomegranate were conducted in farmer's field at two locations in karnataka during 2006. The effect of jeevamrutha on groundnut registered low leaf spot incidence (2.5%) compared to the control (8.5%). It is attributed to the phenolic compounds and other elements present in the cow urine and dung led to the inhibition and multiplication of the pathogen and further restricts the spread of pathogen. This imparts the disease resistant once and reduced incidence (Upperi *et al.*, 2009.).

Due to hazardous impact of most of synthetic fungicides, in the near future the use of such chemicals must be strictly regulated by governments which may lead to a growing demand for biologically plant protection materials such as plant origin products. Based on approved data and scientific publications it seems evident that plant essential oils/extracts are biodegradable and do not cause similar

environmental risks like widely used synthetic chemicals. The option of replacing fossil oil based chemicals with plant product formulations fits well with food and agriculture policies directed to the future. Sustainable agriculture and food security cannot rely on the use of fossil oil as has been the case for a long time in the developing countries. Local resources should be utilized and thus production of more biopesticides should become a common practice.

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