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Plant Extracts as an Alternative to Synthetic Chemicals: A Review

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ABSTRACT: Earth is blessed with large numbers of plant species which is approximately 30,000–50,000. This diverse group of plants contains a range of unique organic compounds like steroid, saponin, alkaloids, flavonoids, glycosides, phenols and terpenoids etc which are having antimicrobial properties. These antimicrobial properties of plant extracts like *Allium sativum, Ocimum sanctum, Allium cepa, Allamanda cathartica* and *Azadirachta indica* etc were evaluated by a number of workers and they found them effective at different levels in both *in vitro* as well as *in vivo* conditions. Agricultural crops are attacked by several fungal and bacterial plant pathogens resulting considerable economic losses which are controlled by using synthetic chemicals that lead to damage to natural flora and fauna. These synthetic pesticides may enter the food chain and ultimately reach human and animal body causing dangerous abnormalities like hypersensitivity, cancer, asthma, hormonal disturbances and skin diseases etc. Many plants contain alkaloid, phenolic and other organic compounds which can be alternative to chemical pesticides to some extent and provide opportunities for organic agriculture. Many techniques are available to evaluate different plant extracts against microorganisms, but due to some limitations, they are less competitive with commercial synthetic chemicals.

Keywords: Chemicals, pathogens, antimicrobial, plant extract.

INTRODUCTION

Almost all types of crops are subject to numerous pests and diseases attack. According to FAO plant diseases reduce production levels considerably which costs the global economy around $220 billion. These diseases are caused by a variety of biotic agents including bacteria, fungi, viruses, nematodes etc. Generally, synthetic chemicals are used to manage these diseases which have a large impact on both humans and the environment. Inappropriate use of chemical pesticides resulted chemical residues in food as well as in the soil and water, which has encouraged researchers to search for suitable substitutes. Additionally, this has resulted in the emergence of resistant plant disease strains as well as many risks to the environment and public health (Bhagat *et al*., 2014). There were several reports of natural products and botanicals that have antimicrobial properties and can be the best alternatives to chemical pesticides for the management of plant diseases. Plant products have the potential to be used in pest management because of their non-phytotoxicity, systemic character to some extent, simple biodegradability and stimulatory nature of the host metabolism. For the control of various plant diseases, secondary metabolites present in plants such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, terpenoids and coumarins can be utilized (Cowan, 1999). Some isolated plant extracts may have more antifungal activity than some commercially available synthetic fungicides (Tamuli *et al*., 2014). In order to assist the researchers in choosing botanicals for management of fungal and bacterial plant diseases under both *in vitro* and *in vivo* circumstances, we will describe the major botanicals that have been demonstrated to be effective against a variety of plant pathogens in this review paper.

Efficacy of botanicals against fungal plant pathogens. The ongoing use of synthetic fungicides has resulted in the emergence of fungicide-resistant plant pathogen strains, destruction of natural enemies and food chain contamination etc. Considering the negative effects of artificially synthesized pesticides, numerous studies were conducted in the past by researchers to assess the plant extracts against various plant pathogens. Many studies have documented the antifungal properties of different indigenous local herbs and plant products. A hot water leaf extract of *Eucalyptus microtheca* inhibit damping off (*Pythium aphanidermatum*) of brinjal by 90 percent *in vitro* (Narayana *et al.*, 1994). Likewise, Allamanda water extract was found to have inhibitory effect against *Phomopsis vexans, Phytophthora capsici, Fusarium*

oxysporum, *Rhizoctonia solani*and *Sclerotium rolfsii* (Panda *et al.*, 1996; Khan, 1999; Jannat, 2006; Tania, 2007; Masuduzzaman *et al*., 2008). The antimicrobial activity of different plant extracts depends on the extract and solvents used. Aqueous extracts of *AIlium cepa*, *Allium sativum*, eucalyptus, neem leaves and *Lawsonia inermis* inhibit the growth of *Pythium aphanidermatumin in vitro* and among all *A. sativum* bulbs at 10% had higher fungitoxicity and minimum radial mycelia growth followed by *L. inermis* extract (Kurucheve and Padmavathim 1997).

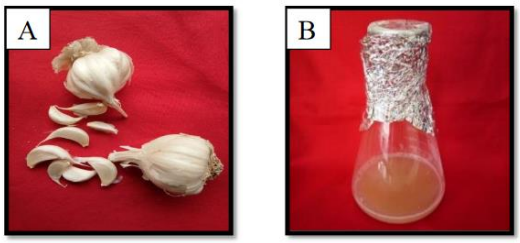

Fig. 1. Garlic (*Allium sativum*) A. Bulb B. Water extract.

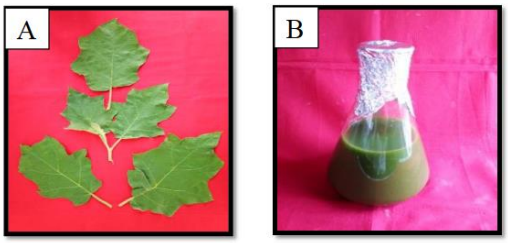

Fig. 2. Dhatura (*Datura wrigtii*) A. Leaf B. Water extract.

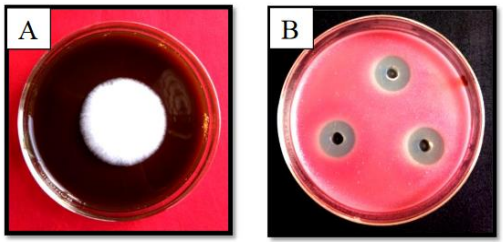

Fig. 3. A. Poison food technique B. Agar well diffusion technique.

Organic solvents are the best medium to extract the metabolites from the samples and help in the estimation of antimicrobial compounds present in any plant parts. Shivpuri *et al.* (1997) evaluated ethanol extracts of *Allium sativum, Allium cepa, Ocimum sanctum* and *Catharanthus roseus* against five pathogenic fungi *viz., Alternaria brassicicola, Colletotrichum capsici, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* under *in vitro* at two concentrations (500 and 1000 micro g/ml) and found leaf extracts of *O. sanctum* and *C. roseus* were the most fungitoxic against all the test fungi at 1000 micro g/ml. Similarly, Singh *et al.* (1993) evaluated Tulsi against the post-harvest disease of banana (*Fusarium oxysporum*) and they found a reduced amount of symptoms on banana. There are several reports of botanicals used against different post-harvest diseases. Adaskaveg and Forste (2000) reported chilli (*Capsicum frutescence*) for post-harvest diseases of citrus fruit (*Fusarium* spp.). Jadhav *et al.* (2015) observed Hena (*Lawsonia inermis*) against storage rot of ginger (*Pythium aphanidermatum*).Mconie (1964) and Serrano *et al*. (2005) recorded Curry leaf (*Murraya koenigii*) and Lemon grass (*Cymbopogon citrullus*) against Green mold of citrus (*Penicillium digitatum*). Plant extracts of some weeds *viz., Datura alba, Asplenifolia, Tridax procumbens* and *Calototropris procera* are effective at 10 percent concentration and inhibit 60 percent mycelial growth of *Fusarium oxysporum* f.sp. *zingiberi* (Siddiqui and Kausal 2002). Dutta *et al*. (2004) reported that a 10% concentration of crude *Allium sativum* extract completely inhibited the sclerotial production and 20% concentration showed excellent mycelial inhibition of *Rhizoctonia solani* causing sheath blight of rice *in vitro*. Neem (*Azadirachta indica*) leaf and garlic (*Alium sativum*) showed retardation of mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* causing chickpea wilt and decreased mycelia growth with increasing concentrations ranging from 5% to 30% (Agbenin and Marley 2006). Okigbo and Ogbonnaya (2006) tested the leaf extracts of *Ocimum gratissimum* and *Aframomum melegueta* on spore germination and mycelial reduction of fungal pathogens causing soft rot of yam tuber and found them effective against *Aspergillus niger, A. flavus, Fusarium oxysporum, Rhizopus stolonifer, Botryodiplodia theobromae* and *Penicillium chrysogenum*. Phyto-extracts of *Azadirachta indica, Lantana camera*, Ardusa, *Ocimum sanctum, Ricinus communis* and bulb extracts of *Allium sativum, Allium cepa, Datura stramonium* and *Parthenium hysterophorus* at (10%), tested for their antifungal activity through poisoned food techniques against *Fusarium oxysporum* f.sp. *lycopersici in vitro*. Plant extracts inhibited mycelial growth by 67-70% over control. The lowest effect (15.3%) on mycelial growth was observed with *R. communis* (Singh and Lekhram, 2007). Leaf extracts of *Azadirachta indica, Catharanthus roseus, Eucalyptus globulus, Lantana camara* and *Withania somnifera* were able to reduce mycelial growth of *Fusarium oxysporum* and the highest inhibition of mycelial growth was observed in *E. Globules* followed by *C. roseus, L. camara, A. indica* and *W. somnifera* on the third day of incubation (Kanherkar*et al.*, 2007).Sagar *et al.* (2007) studied the efficacy of fourteen plant extracts at 5% and 10% concentrations against rhizome rot of ginger caused by *Pythium aphanidermatum* and *Fusarium solani in vitro*. Among all the extracts evaluated against *F. solani*, maximum inhibition of mycelial growth was observed in *Ferulafeotida* powder extract (68.51%) followed by *Ocimum* leaf extract (60.16%). AquSeous leaf extracts (2.0%) of 15 plants against *Alternatalini*, the causal organism of leaf and bud blight in linseed, showed the maximum inhibition by *Azadirachta indica* (67.7%) followed by *Lawsonia inermis* (63.0%), *Datura metel* (39.2%), *Calotropis procera* (37.8), *Lantana camara* (36.6%) and *Citrus medica* (28.1%) under *in vitro* condition (Singh and Singh 2007). Vijaya *et al.* (2007) conducted an experiment to test the fungitoxicity of 12 plant extracts at 5 and 10 % concentrations against *Ceratocystis paradoxa* (causing sett rot of sugarcane)

under *in vitro* and found that the extracts at 10% were significantly superior over 5%. Among them mycelial growth inhibition (53.13%) in Garlic extract at 10% was the best and significantly superior over all other plant extracts.

An effort was made by Ram and Thakore (2009) to evaluate the efficacy of 19 plant extracts against *Fusarium solani* and *Pythium aphanidermatum* and they found that *Allium sativum* and *Lantana camera* were most effective *in vitro*. In pit storage ginger treated with *A. sativum* and *Azadirachta indica* combination and *A. sativum* alone reduces the loss of weight, disease incidence and highest rhizome recovery during storage. Shinde and Gawai (2011) studied the aqueous and alcoholic extracts of *Azadirachta indica, Ocimum sanctum, Tridex procumbens, Clerodendron inermis, Cathranthus roseus, Ricinus communis* and *Citrus limon* to determine their effect against *Colletotrichum capsici*. Out of these medicinal plants tested at 15% concentration, alcoholic extracts of *O. santum* and *C. inermis* were found to be inhibitory on the growth of *C. gloeosporioides*. Similarly, Gupta and Dikshit (2010) found lime (*Citrus limon*) controlling post harvest anthracnose of mango caused by *Colletotrichum gloeosporiodes*.

An investigation was conducted by Siddiquee *et al.* (2011) to check the efficacy of a foliar spray with seven fungicides and a botanical to control the scab and die-back of citrus. The result showed that fungicides and *Allamanda* leaf extract are effective in achieving a significant reduction in severity of the scab and dieback disease and increasing the fruit yield of lemons. Botanicals have been tested by different workers against various post-harvest diseases which they found effective. Okigbo and Nmeka (2005) found black pepper (*Piper nigrum*) reduced the rot of yam caused by *Fusarium oxysporum*. Similarly, the effectiveness of Dawn Redwood (*Metasequoia glyptostroboides*) against anthracnose disease of Chilli (*Phytophthora capsici, Colletotrichum capsici*) and Papaya (*Carica papaya*) against post-harvest rot of fruit was reported by Bajpai *et al.* (2007); Bautista-Banos *et al*. (2003) respectively. Antifungal activity of 26 plant extracts has been tested against *Phytophthora infestans* and out of which, *Xanthium strumarium, Laurus nobilis, Salvia officinalis* and *Styrax officinalis* were found to be most effective and totally inhibited the mycelial growth of *P. infestans* while other plant extracts exhibited moderate activity. The observed average radial growth of the fungus was only 0.8 to 5.0 mm/day significantly lower than the control (Yanar *et al.,* 2011).

In vitro tests revealed that the extracts of onion, garlic and agave were highly efficacious in limiting the mycelial growth of *Pythium aphanidermatum* (Dohroo *et al.,* 2012). Ahmed *et al.* (2012) evaluated *in vitro* efficacy of the acetate extract of *Allamanda* at 0.2% and 0.3% concentrations which resulted in 100% inhibition of the mycelial growth of *Phomopsis vexans* while the methanol extract was not effective in suppressing growth but rather in arresting the growth of the fungus. The medicinal plants i.e. neem, safedaak, begunia, bael, clove of garlic and onion bulb possess antimicrobial properties. Bal and Behera (2012) studied the efficacy of some plant extracts of neem, safedaak, begunia, bael, clove of garlic and bulb of onion against *Colletotrichum capsici*, causing anthracnose or ripe fruit rot of chilli. The maximum growth inhibition (81.04%) was recorded with neem, followed by garlic (80.54%) and onion (80.17%), each at 5% concentration. Haron *et al.* (2013) reported that leaf extracts of *Allamanda blanchetti*, *A. cathartica*, *A. cathartica* 'Alba', *A. cathartica* 'Jamaican Sunset' and *A. oenotheraefolia* inhibit the growth of *Colletotrichum gloeosporioidein vitro*, a causal agent of anthracnose in papaya. A significantly lower disease incidence, severity, and index were observed in Allamanda treated papaya fruits. Gholve *et al*. (2014) tested the bio efficacy of ten plant extracts *viz.*, Mehandi (*Lawsonia inermis*), Ginger (*Zingiber afficinale*), Datura (*Datura metel*), Tulsi (*Ocimum sanctum*), Parthenium (*Parthenium hysterophorus*), Neem (*Azardirachta indica*), Garlic (*Allium sativum*), Turmeric (*Curcuma longa*), Satawari (*Asparagus racemosus*) at 10, 15, and 20% *in vitro* against *Pythium ultimum* by poisoned food technique and found Adulsa (75.53 %) and Datura (60.65 %) most effective. Tripathi and Dubey (2004) observed inhibition of *Botrytis cinerea* causal agent of grey mould of grapes by Ginger (*Zingiber officinale*) rhizome extract.

Chaudhary *et al.* (2017) evaluated the extract of Neem leaf, Garlic bulb, Datura, Turmeric, Onion bulb and Ginger rhizome extract at 10 per cent concentration *in vitro* and pot culture conditions against *Pythium ultimum* and they observed minimum mycelia growth in Neem leaf followed by Garlic in both *in vitro* and pot culture conditions. Cruz (2003) observed that an extract of Turmeric (*Curcuma longa*) was effective against *Colletotrichum musae* the causal organism of crown rot in banana. Kumar *et al*. (2019ab) evaluated water extract of ten botanical extracts against *Pythium aphanidermatum* and *Fusarium oxysprum*. They found *Allium sativum* highly effective followed by *Allamanada cathertica* and *Laurus nobilis* using the poison food technique.

Efficacy of botanicals against bacterial pathogens. Khan (1974) tested Garlic (*Allium sativum* L.) and Onion (*Allium cepa*) extracts against *Ralstonia solanacearum* and found that Garlic had an inhibitory effect on the growth of *Ralstonia solanacearum* under *in vitro* condition, whereas the Onion extract had no such effect. Dedtyareva *et al*. (1970) evaluated the transparent greenish brown fluid prepared from *Myrtus communis* L. leaves against Gram positive bacteria and they found considerable antibacterial effects against tested the bacteria.

Nigam and Rao (1978) tested the effect of essential oil from the leaves of plants such as *Cinnamomum zeylanicum, Cymbopogaon flexuosus* Wats, *Eucalyptus citridora* Hook, *Skimmia laureola HKf, Mentha arvensis* L., *Mentha spicata* on *Bacillus fumilis, Erwinia caratovora, Micrococcus* spp., *Peudomonas mangiferae, R. solanacearum, Sarcina lutea, Staphylococcus albus, Staphylococcus* spp., *Shigella* spp., and *Xanthomonas campestris.* They found that *Cinnamomum zeylanicum* oil showed the highest activity while *S. laureola* oil showed the least activity.

Ahmed and El-Shazlez (1987) studied the biological activity of aqueous extracts of *Medicago hispida* Gertn., *Melilotus* spp., *Caromopus squamatus, Anagallis arvensis* L. and *Ammi majus* L. against *R. solanacearum*, *Bacillus subtilis, Erwinia carotovora and Erwinia* sub sp. *atroseptica*. The extracts were moderately toxic against the tested bacteria except *E. carotovora* subsp. *atroseptica*. Extracts of *Melilotus* sp. Juss. and *Ammi majus* L were most toxic and extracts of *Anagallis arvensis* L. was found to be least toxic. Among all the bacteria, *R. solanacearum* was found to be the most sensitive bacterium for the tested extract.

Antibacterial activity of some plant extracts controlling the incidence of bacterial wilt on tomato was shown by extracts of garlic, shallot and *Tagetus erecta* L. which suppressed the disease in inoculated plants (Hannudin, 1987). Prasad and Alankara Rao (1987) evaluated the antimicrobial effects of essential oils of the five species of *Ocimum*, all the samples showed antimicrobial activity against Gram +ve and Gram –ve bacteria. Garlic bulbs have been reported to have antimicrobial property against incidence of bacterial wilt on tomatoes. It was found that 10 ml suspension of 34 ground Garlic bulb 77 ml sterile water suppressed the disease (Hutagalung, 1988). Hannudin and Djantnika (1989) evaluated the effect of some plant extracts on *R. solanacearum* growth *in vitro.* Extracts from Onion and Garlic bulbs, roots and stems of *Crotalaria* sp. L., *Tagetus* sp. and Pawpaw leaves were evaluated for suppression of *R. solanacearum.* They found that extracts from Garlic bulbs inhibited bacterial growth. According to Karuna and Khan (1993) plant extracts obtained from Ocimum, Eucalyptus, Citronella, Neem, inhibited the growth of *R. solanacearum* under *in vitro* condition. Maximum inhibition zone of 43.9 mm diameter was obtained in extract of Eucalyptus followed by Citronella (40.4 mm) and Ocimum (37.2 mm) respectively.

Meena *et al*. (2007) evaluated the partially purified plant extracts against *R. solanacearum*, *Xanthomonas campestris* pv. *campestris, X. auxonopodis* pv. *citri*, and *X. axonopodis* pv. *Cyamopsidis* by the disc diffusion technique. Out of seven partially purified plant extracts tested, only Mahua and Satyanashi were found effective at 1000 ppm. Sukanya *et al.* (2009) reported that methanol extracts of *Chromolaena odorata* showed inhibition zones against *Escherichia coli, Staphylococcus aureus, X. vesicatoria* and *R. solanaccearum* respectively followed by chloroform extract of the same plant leaf. Moussa *et al*. (2010) investigated activity of 4 different extracts of 25 local plant species belonging to 17 families against two phytopathogenic bacteria, *Erwinia carotovora* and *R. solanacearum,* the pathogens of soft rot and brown rot diseases of many important cultivated plants. The biological evaluation results indicated that *Myrtus communis* had the most potential to combat these pathogens.

Kumar *et al*. (2023) evaluated aqueous extracts of ten plants i.e. *Acorus calamus, Allamanda cathertica, Allium cepa, Allium sativum, Curcuma longa, Datura wrightii, Lasiaspinosa, Laurus nobilis, Ocimum sanctum,* and *Piper betle* against *R. solanacearum* in Assam. Out of ten botanicals only *Allium cepa* was able to inhibit the bacterial growth *in vitro*.

Limitations of plant extracts in disease management

— Optimal Extraction method for botanicals is not standard
— Rapid degradation of the active ingredient in extract
— Formulations are not standardized
— Mostly effective in lab conditions (*in vitro*)
— Some chemicals are harmful to humans as well as animals
— Less effective
— Not available in different formulations

Methods of testing botanicals against microorganisms

Food Poison Technique. This is one of the simplest techniques to evaluate botanicals against target fungal pathogens. It can be employed for plants as well as clinical pathogens. In laboratories, the antifungal activity of any extract is determined by the poison food technique (Grover and Moore 1962; Mishra and Tiwari 1992; Nene and Thapliyal 2000). Under this technique liquid extracts of botanicals are mixed with molten agar media followed by inoculation with fungal disc made by cork borer. After that, the inoculated plates are incubated at 25°C for interpretation of inhibition.

Spore germination assay. Chemicals present in botanicals inhibit the multiplication of fungal spores, so this principle is used in testing the antifungal activity of plant extracts using the slide technique (Nair *et al*., 1991). Plant extract of desired concentration and volume are added to the cavity slides containing fungal spores along with one containing water. These slides are kept for incubation for spore germination.

Agar disk diffusion assay. Agar disk diffusion method was developed to test the antimicrobial activity of penicillin in 1940 (Heatley, 1944). The technique which was accepted by NCCLS and used by a large number of researchers these days. This is an easy way to check the antibacterial compound present in any extract and for that an agar disc containing the antimicrobial compound is placed on the bacterial lawn which is further incubated at 28°C. If zone is formed around the disc then the extract is considered as effective against the target bacteria. MIC (Minimum Inhibitory Concentration is determined by using different concentrations of extract.

Agar well diffusion assay. The concept of the agar well diffusion method is similar to agar disc diffusion, in the first method hole is used for putting extract where as in the later method disc is used. In this method a standardized concentration of inoculum is spread on a agar gelled agar medium and a hole with a diameter ranging from 5 to 7 mm is punched with the help of sterile cork borer in the center of the Petriplate and the hole is filled with 50 – 70 µl of extract (Perez *et al*., 1990). An inhibition zone will be formed if the extract is effective against inoculums.

CONCLUSIONS

Plants are valuable sources of novel, physiologically active compounds with antimicrobial properties. Because of their bioactive components, plant extracts

can directly or indirectly inhibit pathogen growth. Extracts can therefore be thought of as a management tool for microbial infections since the changes brought about by the extracts are reflected in reduction of pathogen infection in plants. To assess the effectiveness of phytochemicals that have inhibitory effects on all sorts of microorganisms, *in vitro* and *in vivo* research should be conducted.

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Conflict of Interest. None.

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