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Role of medicinal plants as green pesticides against *Alternaria blight*

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Abstract

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Medicinal plants have been playing significant role in management of several plant diseases. Numerous medicinal plants contain several components and pigments that show toxicity against various microbes including fungi that play role in spreading plant diseases. Phytochemicals isolated from medicinal plants are key ingredients of green pesticides. *Alternaria* blight is one such prevalent plant disease caused by *Alternaria* species worldwide that causes damage to agricultural crops including vegetables, fruits and cereals. It is also a major concern for nutritious and economically important oil yielding crops of the world. Innumerable chemicals are applied to combat diseases caused by species of this devastating fungi. The frequent use of chemical fungicides results in various environmental problems which directly interfere with the sustainability factor. Therefore, current attention has been paid towards exploiting the extracts of medicinal and aromatic plant sp. for plant protection. The secondary metabolites secreted by medicinal plants used throughout the world are accountable for their biological characteristics. The microbial growth in varied situation is controlled by these plant derived products. Eucalyptus, moringa and neem products are now used commercially as green pesticide and also investigations are carried out to make use of essential oils of higher plants to test their efficacy against Alternaria sp. Azardirachitin a plant pigment isolated from seed kernel has major contribution in the development of several green pesticide formulations like *Margosan-O, Bio-neem, Azatin, Neemies, Safer's ENI, RD-Repelin, Neemguard, Nimbin* etc used successfully worldwide.

Keywords: green pesticides; plant protection; medicinal plants; *Alternaria* blight

Introduction

Alternaria blight disease caused by *Alterneria* species like *A. alternata, A. brassicae, A. solani Alternaria brassicicola and Alternaria raphani* etc results in large scale destruction of vegetables and various economically important crops. The fungus Alterneria belongs to the Pleosporaceae family (Mamgain et al., 2013).They comprises of pigmented multicellular beak shaped spores present in chains or branching fashions. The various spp. of fungus *Alternaria* that comes under family Deuteromycetes are saprophytic as well as endophytic in nature which consists of highly destructive members of plant pathogen. They have reported to have destructive action against plants belonging to families *Brassicaceae, Solanaceae* and *Cucurbitaceae* etc. which are highly nutritious and economically important as well. *Alternaria alternate* was found to be responsible for causing early blight of potato, leaf spot disease in *Withania somnifera* (Pati et al., 2008), *Alternaria brassicae* and *Alternaria brassicicola* (infests many vegetables and roses), *Alternaria brunsii* leads to cumin blossom blight, *Alternaria carotiincultae* results in leaf blight on carrot, *Alternaria solani* (causing early blight of tomato) are few of the examples. The Cruciferous crops such as broccoli, cauliflower, turnip and field mustard are most probable host of pathovars belonging to the genus *Alternaria* (*Brassica oleracea* L. var. *botrytis* L.). When the host plant is attacked by *Alternaria* series of concentric rings appear on place where the infection has taken place. This provides a "target spot" for early blight. Appearance of small black concentric spots on lower leaves is the initial symptom of the disease. These spots further enhance into larger and prominent ones affecting the rest of the parts of the plant. The spores of *A. brassicae* reside in both soil and atmosphere (Verma & Verma, 2010).

Application of fungicides as a control of *Alternaria* is the simplest and most common method we used today. But keeping in mind their adverse effects on human health, new alternatives of disease control such as growing disease resistant varieties, use of plants and parts thereof, introducing bio-control agents, alterations in agronomic practices and many more should be implemented as they are eco-friendly and safe. Moreover, these are natural product that's why show minimum or negligible adverse effects in various physiological processes of plants and can be broken down into simpler organic substances with great ease. Much work is being done for the investigation of antimicrobial properties of plant products like plant extracts, essential oils and volatile oils. Numerous medicinal plants release phytochemicals which have inhibitory effect on plant pathogens. The use of herbal products and secondary metabolites secretes by medicinal plants is in high demand all over the world (Nalawade et al., 2003; Cole et al., 2007). Plant extracts, essential oils, gums, resins etc. as bio-fungicidal products due to their resistant activity against phytopathogens in both *in vitro* and *in vivo* conditions (Fawzi et al., 2009; Jalili et al., 2010; Romanazzi et al., 2012).The different secondary metabolites can be separated by various isolation techniques and then purified to get pure components and bioactive properties of those components can be used against the phytopathogens in diverse manner for human welfare. Plant extracts are gaining high popularity as green pesticides because they are safe, highly efficient against the disease and are available at much cheaper cost (Yedida & Singh, 2018). These products serves as good alternative for synthetic fungicides as they are eco-friendly and widely acceptable and comprises great potential to overcome the problem of plant pathogens (Chuang et al., 2007).

Medicinal Plants as Green Pesticides

Plants comprehend some natural bioactive compounds toxic to phytopathogens. These plants have narrow target with explicit mechanism of action. They are safe to humans and environment, have short shell life and do not have residual threat. This property favours them to be a part of Integrated Pest Management (IPM). They can be used by farmers of developing nations where plants extracts are already being used in pharmaceutical sector (Nuzhat & Vidyasagar, 2013). The methanolic extract was assessed against spore germination of plant pathogenic fungi, *Alternaria brassicae, A. melongenae* (Pandey & Singh, 2019). More than 250 000 plants species have been reported to offer reservoir of natural bioactive compound that have potential application in agrochemicals (Cowan, 1999). The bioactive compounds that are extracted from different medicinal plants had contributed a lot in development of the phytochemicals for agriculture industry in different ways as follows:

- By acting as natural pesticides in raw form for e.g.- crude extracts
- By providing simpler substances that can be accordingly converted into complex substances
- Available as better and new alternative to various scientific products for prevention of various plant pathogenic fungi and bacteria (Cox, 1990).

These extracted plant products when applied on the infected crop are called Bio pesticides or Green pesticide. Several workers have reported the efficiency of natural phytochemicals in controlling mycotoxin production of *Alternaria* blight. Some commonly used plants are various parts of cinnamon, clove, oregano, palmarosa and essential oil of lemon grass oils, neem extracts, tea tree oil, common thyme, and eucalyptus (Marin et al., 2004; Burgiel & Smaglowski, 2008; Ćosić et al., 2010; Nashwa & Abo-Elyousr, 2012).

Common Medicinal Plants Used against *Alternaria*

The evaluation of crude and boiled forms of various medicinal plants namely Neem, Eucalyptus, Datura, Pudina, Tulsi, Lantana was carried out in vitro condition against *A. brassicae* (Sasode et al., 2012). The management of blight disease caused by *Alternaria* species via bulb extract of *Allium sativum* in Indian mustard was reported by Meena et al. (2004). The gradual decrease in radial growth, spore formation and germination of *A. brassicae* was observed when treated with leaf extract of *Eucalyptus globules* (Patni & Kolte, 2006). Kadam et al. (2018) reported the efficacy of *Allium sativum, A. indica* and *Z. officinale* in reducing *A. alterneta* causing blight disease of pomegranate. The ethanol extract (n-butanol fraction) when applied in specific concentration say 25 mg/L show great efficacy against fungal pathogens and Bu-11-13, derived sub fraction completely prohibits spore germination (Lin et al., 2011). The methanol extract of *Polygonum perfoliatum* (speed weed) was reported to restrict the germination of conidia in *A. brassicicola* that causes leaf spot of spoon cabbage (Ching, 2007). Spore germination of *A.brassicae* collected from cauliflower leaves has been totally inhibited by extracts of plants like *Cenchrus catharticus*, *Canna indica*, *A. sativum,* Convolvulus arvensis, *Menthapiperita, Prosopsiss-*

picigera, Allium cepa, Lawsonia inermis, Ipomoea palmata, Argemone mexicana, Datura stramonium and Clerodendro ninerme (Sheikh & Agnihotri, 1972). The infestation of *A. solani* and its radial growth was prohibited by leaf extract of neem when applied in specific concentration say 43.3 and 26.7% at 0.1% and 0.01%, respectively (Sharma et al., 2007). The mycelial growth of brinjal leaf spot disease was reported to be effectively cured by inhibitory effect of garlic bulb extract caused by *A. tenuis* Datar (1996). In a study, *Alternaria alterneta* causing blight disease of brinjal was controlled significantly by application of rhizome extract of turmeric, garlic and neem (Rajput & Chaudhari, 2018).

Mode of Action

Medicinal plants have infinite ability to produce aromatic secondary metabolites like phenols, phenolic acids, flavones, flavonoids, quinones, terpenens, tannins, alkaloids, lectins (Halama & Van Haluwin, 2004). These secondary metabolites have antimicrobial activity against phytopathogens as they create unfavourable conditions for the pathogenic fungi growth (Scheuerell & Mahaffee, 2002). The various plant extracts and their essential oil are most commonly used for management of diseases. Phenols and phenolic acids present in plants are important bioactive phytochemical and the efficacy of phenolic toxicity depends on concentration of hydroxyl groups and their location in the compound. Quinones were found to be very effective and serve as an important fungicide to control the disease. After microbial infestation phenolic compounds like flavonoids and flavonols synthesized in plants are observed to be very effective against wide array of microorganisms. Tannins are also one of the major phenolic compounds, polymeric in nature, show astringent property and are easily soluble in acetone and water (Gurjar et al., 2012). Environmental stress like air temperature and air humidity also play a key role in influencing the health quality of essential oils, carotenoids and polyphenols (Manukyan, 2019). Allicin is volatile compound comprising antimicrobial properties and is synthesized in garlic by passing through a no. of cascades. It controls the infection of seed-borne *Alternaria* spp. in car-

Table 1. 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 eucaryotic DNA
Lectins	Form disulfide bridges
Polypeptides	Form disulfide bridges

Table 2. Antifungal actions of Products secreted by Plants

Name of the Plant	Part Used	Controlled Pathogen/Disease	References
Eucalyptus and lavender	Leaf	*Alternaria alternata*	Zaker & Mosallanejad (2010)
Indian beech, milk weed, oleander and turmeric	Leaf, Seed	*Alternaria solani*	Masih et al. (2014)
Essential oils, cumin	Leaf, Root	*Alternaria alternata,*	Behdani et al. (2012)
Adhatoda vasica (Nees), *Azadirachta indica* (A Juss). *Ocimum sanctum* (L), *Allium sativum* (L), *Datura metal* (Linn) and *Zingiber officinale* (Rose)	Leaf, Flowers,Root	*A. solani,*	Chapol Roy et al., 2019
Spanish flag (L. camara Linn), Pongam (Pongamia pinnata L.Pierre)	Leaf	Leaf light of Onion	Bhosale et al.,2008
Thyme(*Thymus vulgaris*),Eucalyptus sp. and *Achilleamille folium* L.	Leaf	*Alterneria alternata*	Hadizadeh et al.,2009
Garlic*(Allium sativum),*Datura, Tulsi, Lantana	Bulb, Leaf	*Alternaria brassicae*	Meena et al., 2010
A.indica, Michelia champaca, Adhatoda vasica and Eryngium *foetidum*	Aqueous leaf extract	*Alternaria brassicae*	Yengkhom Premlata Devi., 2018
Azadirachta indica and *Eucalyptus, Allium cepa, Zingiber officinalis, Parthenium, Datura.* and *Lantana camara*	Leaf (crude), rhizome, seeds and bulb	*Alternaria cyamopsidis* of clusterbean	Manasa & Reeti, 2018

rot, mustard and Phytophthora leaf blight of tomato and potato (Slusarenko et al., 2008) (Table 1 and Table 2).

Methods for Extraction and Evaluation of Plant Extracts

Extraction of medicinally active secondary metabolites of plant tissues from inactive components is done by using selective solvents like methanol, ethanol, water, chloroform, ether, acetone and dichloro-methanol. These solvents separate the active metabolites by diffusing into solid plant tissues and hence solubilize the compounds of similar polarity. The quality of the plant extracts depend upon the choice of solvent used and the plant material. The various techniques are described as follows:

Ultrasound assisted extraction (UAE)

The technique is based on the size of various particles present in the extracts of the plant, solvent used and moisture concentration. In this technique ultrasound waves are used for extraction as they help in penetration of solvent liquid into the tissues by breaking cell wall and enhance diffusion of solutes into the solvent by increasing the surface area in contact (Tang-Bin, 2011). Xing-Yu (2019) extracted essential oil from seeds of *Iberis amara* using ultrasound assisted hydro-distillation (UAHD).

Microwave-assisted extraction (MAE)

The technique involves extraction of secondary metabolites by means of microwaves. The principle behind using these rays is generation of heat inside the tissues that ultimately leads to dipole rotation and conduction of ions within the tissues (Jain, 2009). The moisture present in the tissues absorbs the heat energy produced by microwaves results in cell disruption and diffusion of bioactive compounds from tissues (Takeuchi et al., 2009). The mechanism involves separation of secondary metabolites from plant tissues at specific temperature and pressure, introduction of solvent into tissues and diffusion of these metabolites into solvent liquid (Alupului, 2012).

Supercritical fluid extraction (SFE)

SFE was reported to be a good alternative to other extraction techniques using solvent as this technique involves application of carbon dioxide for the extraction process (Yepez et al., 2002). Carbon dioxide itself is non-selective but by using certain co- factors or modifiers its selectivity and its capacity of extraction can be increased and these cofactors can be easily separated after achieving the goal (Ankit, 2012).

Identification, Characterization and Purification Techniques

Secondary metabolites are generally present in the complex matrices of plant extracts in very less amount and hence purification is necessary for identification of the extracts. In order to separate the pure compounds there are various chromatographic and non- chromatographic techniques that are described as follows:

Chromatographic techniques

The various chromatographic techniques are available in the market for the purpose of separation or identification of specific components from a mixture. The separation is based on the different adsorbent tendencies of different components in a mixture. These techniques are widely used in industries for purification of intermediates and products in a no. of processes.

Gas chromatography (GC)

This technique is generally used for identification purpose and analysis of non- polar and volatile compounds. Moreover, gas chromatography mass spectrometry i.e. GC-MS is used for the profiling of various metabolites and for the identification of new compounds and metabolites (Krone et al., 2010). It is not suitable for the identification of highly polar compounds as they are less volatile in nature (Yusuke, 2012).

High performance liquid chromatography (HPLC)

This technique is most probably used for the separation of specific products from a mixture of compounds and is best suitable for the identification, purification and quantification of various components in a mixture (Boligon & Athayde, 2014). It is best suited for the active processing of samples for analysis and preparative scale due its high resolving power (Marin et al., 2004). It is based on the principle that different compounds have particular rates of migration through the column and the stationary phase can be adjusted according to the type of constituent which has to be separated or for the removal of the contaminants. The appropriate conditions namely mobile phase, sensible detectors, flow rate etc. can be adjusted for analysis of particles by characteristic peak that is obtained under chromatographic condition (Sasidharan et al., 2011).

Thin-layer chromatography (TLC)

The chromatic technique is most probably used for the herbal analysis as it is simple, rapid and cheap. Fluorescent images are formed as compared to chromatograms in other techniques that are more visual and provide profiling at dif-

Table 3. IDM combinations used against Alternaria blight

IDM Combinations	References
Eucalyptus leaves (250 kg/ha)+ *Trichoderma harzinum* (2.5 kg/ha)	Patni & Kolte, 2006
Seed treatment with *Trichoderma viride* (5 g/kg seed)+*P. fluorescens*+Soil application of *T. viride* +Soil application of *P. fluorescens* @2.5 kg/ha	Shiv Shakti et al., 2013

ferent levels and integral data that can undergo processing by digital means. But the major disadvantage of the technique is inability of detection of trace components due to low resolution and less sensitivity (Zhang et al., 2011).

Over Pressured Layer Chromatography (OPLC)

It is recently generated technique and is used in separation of essential oils and several types of extracts from plant tissues in highly efficient manner (Pothier et al., 2001; Botz et al., 2001).The system involves programmable pump that deliver the sample into column with high force that results in rapid and specific separation of material. It is much more efficient than TLC (Nyiredy, 2001). It serves as bridge between the TLC and HPLC as it is rapid and efficient than TLC for identification of complex extracts (Nyiredy, 2001). It is very helpful in rapid identification of potential plant active principles (Tabanca et al., 2007; Cheel et al., 2007)

Non-Chromatographic Techniques

Phytochemical screening assay

Secondary metabolites are generally phytochemicals that are present in plant extracts. This techniques is comprises different chemical tests and is usually called as Phytochemical screening. It is cheap, easy to handle and provide instant results.

Medicinal Plants in Integrated Pest Management

The concept of integrated disease management involves the combination of Bio control agents with plant extracts. It is very beneficial as it uses efficient naturally existing products and makes their best possible combinations. They are eco-friendly and are also cost effective. Various strains of *Trichoderma* fungus and *Psuedomonas* bacteria was found to be effective against a no. of pathogens thus are widely used as an antagonist (Biswas et al., 2002) particularly as seed treatment followed by neem extracts spray in managing many fungal diseases in various host pathogen combinations. Patni et al. (2005) reported that application of plant extracts in combination with *Psuedomonas fluorosensces* significantly reduced *Alternaria* blight intensity and also increased the test weight and yield. The application of *Trichoderma harzanium* as seed treatment and foliar spray was found superior over non treated control to reduce disease severity by 25.2% and 23.3% at the leaf and pod stage, respectively (Shiv et al., 2013).Leaf extracts of *Camellia sinensis* and root extracts of *Asparagus racemosus*p *Aloe vera, Acacia nilotica* and *Anthocephalus cadamba* were found to be most effective ones when antifungal assay of approximate 20 plants were examined by Bhardwaj & Laura (2007) for leaf spot disease of Brassicaceae and also for brown rot of cauliflower whereas *Astercantha longifolia* showed moderate inhibition. *Aloe-vera, Acacia nilotica* and *Anthocephalus cadamba* whereas *Astercantha longifolia* showed moderate inhibition. Importance of IDM is increasing tremendously among scientists because this practice just not reduce the crop from fungal disease but also reduces its chances of attack by other plant pathogens like nematodes, bacteria and viruses (Anwar et al., 2017) (Table 3).

Conclusion

The recurrent use of chemicals against plant pathogens deteriorates the environment. The present concern of the scientists is to find a sustainable solution to the above problem. Therefore, emphasis has been laid on eco-friendly alternative to dreadful fungicides for crop protection. Traditional medicinal plants have great potential to overcome plant pathogens as they comprise of biologically active compounds which limit the disease. There is a huge gap between lab research and availability of the medicinal plants at land. Further research is required for making medicinal plants available to the farms either individually or integrated with bio control agents.

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