INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 16, No. 4, p. 71-82, 2020
http://www.innspub.net

REVIEW PAPER OPEN ACCESS

Efficacy of phytoextracts against fungal diseases of vegetables

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Article published on April 30, 2020

Key words: Phytopathogen, Phenolic compounds, Botanicals, Production, Antifungal

Abstract

Vegetables are the most important source of healthy balanced diet for human beings as they contain essential nutrients, vitamins, antioxidants and dietary fibers. Significant economic losses caused by variety of pathogens including pests, bacteria, virus and fungi. Fungal pathogens majorly responsible for yield losses of vegetable crops. Chemical based synthetic fungicides are noxious and detrimental to environment as they deteriorate the food chain. Now a days ecofriendly managemental strategies are under discussion for healthy safe and sound exploration in scientific era. Jasmonate, glucosinolates, quinones and chitosan containing phytoextracts showed significant antifungal potential thus could be used as an effective alternative to synthetic fungicides. So, phytoextracts could be better choice for pre-harvest and post-harvest management of fungal diseases. Therefore, to avoid the serious consequences of chemicals on human health and environment there is need to focused on the botanical products as well as for betterment of conventional and non-conventional extraction techniques.

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Introduction

According to world vegetable survey more than 53% leafy vegetables, 15% table fruits vegetables and 17% underground plant parts consumed in daily life. There are about 402 vegetable crops belongs to 69 families and 230 genera throughout the world. Nutritional quality, attraction of the crop and availability at moderate prices make it important marketing incentive (Dias, 2012). Plant based food known as vegetable that could also be defined as herbaceous plant parts like fruit, roots, leaves that can be eaten by humans and animals. Nutritionally vitamin K comprised the green vegetables while vitamin A constituent of orange-red vegetables. Mostly vegetables possessed colorful pigments which represents their Phyto-nutritional category like purple for flavonoids, Green for chlorophyll and orange for beta-carotenes. The density level of color indicates the concentrations of phytonutrients pigments present in red food, orange food, yellow food, green food and blue purple food have anti-inflammatory, reproductive health maintenance, gastrointestinal health promoting, cardiovascular health boosting and memory assisting properties respectively (Minich, 2019).

Vegetables possessed higher concentrations of vitamins, minerals, phytochemicals, antioxidants and dietary fibers which are responsible for the healthy food source to human beings as they lower down the chances of like blood pressure, cardiovascular disorder, osteoporosis, cancer and respiratory diseases. The Antioxidants have ability to quench the health detrimental free radicals while dietary fibers of these vegetables enhanced the nutrient absorption in intestine by reducing the passage of intestine. While the flavanol, anthocyanin and procyanidins lowered the risks of cardiovascular diseases. The normal weight of the body can be maintained by the consumption of non-starchy vegetables which have low energy and high in fiber contents (Dhandevi and Jeewon, 2015). Several biotic and abiotic factors affect the production of vegetables crops while fungal pathogens notably cause severe damage. About 18% crop losses occurs due to insects and more than 16% damage caused by microbial pathogens like fungi. Significant qualitative and quantitative crop losses have been noted that reduced quality of ingredients, market quality, storage quality and post-harvest production (Moore *et al.,* 2011).

Crop productivity enhanced through control of fungal pathogen by applications of several fungicides that may have possible side effects as they damage the membranes, nucleic acids, signal transduction, mitosis, respiration, protein synthesis and other non-target microorganisms present in soil (Yang *et al.,* 2011). Chemical control involved synthetic fungicides that bring inhibition by disrupting cell membrane and metabolism. These chemicals become a part of food chain cause deleterious effects on human and biosphere, damaged the soil quality by compaction, declining soil organisms, diminishing resistance to disease. Natural Plant products that do not leave any toxic effect can effectively replace synthetic fungicides (Hada and Sharma, 2004). Natural phytoextracts used as alternative to synthetic fungicides that contain non-toxic acetic acid, jasmonate, glucosinolates, chitosan and essential oils showed antifungal properties (Tripathi and Dubey, 2003). Secondary molecules of plants have antimicrobial, antifungal abilities (Bourgaud *et al.,* 2001). Moreover, pesticides have toxic effects especially organophosphate regularly used pesticides in the world and may lead to death of individual (Binukumar and Gill, 2011).

Plant extracts are natural fungicides effective against fungal diseases of plants, environment friendly as compared to toxic chemical fungicides (Chohan and Parveen 2015). Methanolic extracts and essential oils of medicinal plants such as herbs that contains antimicrobial compounds used as natural preservative for food (Wanchaitanawong *et al.,* 2005). Fungal phytopathogens cause severe economic losses which could be controlled through phytoextracts instead of synthetic fungicides responsible for toxic and hazardous effects to environment. As recent trends shifting towards healthy, safe and sound management of plant diseases phytoextracts have attained a major

attention of researchers (Haider *et al.,* 2020). Extensive pesticides applications have severe effects on human health and ecosystem (Jepson *et al.,* 2014). Scientific research now focusing on naturally plant derived therapeutic, antioxidant and anti-carcinogenic agents as concerned to human health. Synthetic agents used to cure diseases have carcinogenic and toxic properties (Thomas *et al.,* 2017).

The use of ecofriendly plant extracts caused significant fungal inhibition is focusing issue now a days. Plants considered as store house of chemicals and contains more than 10,000 secondary metabolites that defend plants against diseases (Mohana and Raveesha., 2007). Methanolic Crude extracts and Dry powdered material of *Dolichos kilimandscharicus*, *Phytolacca dodecandra* and *Maerua subcordata* significantly reduced the fungal growth invitro and under field conditions, *P.dodecandra* was most effective against sorghum loose and covered kernel smuts (Tegegne and Johan, 2007). Several researchers reported that parts of plants including leaf, flower, fruit and bark possessed antifungal properties. Crude Aqueous and ethanolic extracts *Tithonia diversifolia* and *Byrum coronatum* showed considerable antifungal activities against *Penecillium atrovenetium, Aspergillus niger, Fusarium flocciferum* and *Geotrichum candidium* (Liasu *et al.,* 2008). Antifungal activity of fortynine botanical extracts were tested against *Aspergillus niger* and 86% out of 49 plants represented variable degree of inhibition of mycelial growth. Exceptionally remarkable reductional activity performed by botanical extracts of *Grewia arborea, Melia azedarach, Peltiphorum pterophorus* and *Terminalia chebula* (Bobbarala *et al.,* 2009). Efficacy of plant extracts depends upon PH, temperature and chemical nature of compound present in extracts like citrus peel extract antioxidant activity increased with increase in temperature(Jeong *et al.,* 2004). Antifungal activity of leaf extracts of *Azadirachta indica, Eucalyptus globulus, Artemissia anunua, Ocimum sanctum* and *Rheum emodi* observed against *Fusarium solani* of brinjal (Babu *et al.,* 2008).Stem extracts of 9 plants *Azadirachta indica, Callistemon rigidus, Capsicum annum, Datura inoxia, Lantana camara, Lawsonia enermis, Santalum album, Terminalia theorlii* considerably inhibited pathogenic fungi *Altenaria alternata, Aspergillus niger, Fusarium moniliforme* and, *Trichoderma viride* (Pawar, 2011). Formulations of *Curcuma aromatica, Garcinia indica, Glycyrrhiza glabra* increased the shelf life of vegetables due to their antifungal and antimicrobial activities (Bhagwat and Datar, 2013). This review will focus on the extraction application of phytoextracts for the safe and sound management of fungal diseases of plants.

Techniques for Phytoextraction

Infusion involves sample material placed in a cold or boiled water for short period and readily soluble components of plant material form dilute solutions (Katiyar, 2008). Decoction boiling sample in specified volume of water 1:16 for definite time until volume reduced to one fourth of original volume and this type of extraction suitable for only heat stable compounds. Decoction water base extraction for hard and tough plant material like root and bark that first broken down into small pieces or powdered form then boiled at very low flame (Tandon and Rane., 2008).

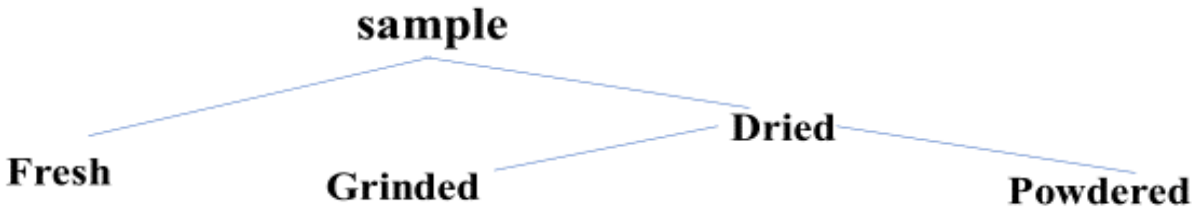

Fig. 1. Describing pre-extraction preparation of plant sample.

Table 1. Chemical profile of solvent extracts.

Solvents			Compounds		References
Water	Lectins	Phenolic acid	Alkaloids	Glycosides	Chikezie *et al.*, 2015
Methanol	Anthocyanin Saponins	Steroids	Flavonoids, polyphenols	Terpenoids, saponins	Jain *et al.*, 2010 Azmir *et al.*, 2013
Ethanol	Alkaloids	Flavonoids	Saponins	Tannins	Azwanida,2015
Acetone	Alkaloids	Terpenes	Saponins	Quinones	Neelamathi and Kannan, 2016
Chloroform	Terpenoids	Flavonoids	Anthocyanin	Tannins	Selvamuthukumaran *et al.*, 2017

Other than conventional methods there are some modern techniques for extraction.

Ultrasonic Assisted Extraction (UAE)

Ultrasounds with frequency greater than 20 kHz to 2000kHz cause cell wall disruption and enhance solvent's penetration ability in cells to acquire better extraction. Ultrasound energy more than 20kHz effect the active components of extract and formulate free radicals. This technique involved Crushed sample with appropriate solvent placed into ultrasonic bath under controlled temperature for extraction (Garcia-Salas *et al.,* 2010).

Microwave Assisted Extraction

Heat of Electromagnetic radiations having frequency between 300 MHz to 300GHz, with wavelength between 1cm to 1m used for extraction. Hexane < Ethyl acetate <Acetone <Methanol <water commonly used as solvent according to their solubility (Altemimi *et al.,* 2017). Microwaves directly transferred the heat to solid plant material without absorption into transparent material and evaporated moisture exert vapor pressure that damaged the cell to release active constituents (Pangarkar, 2008).

Fungal diseases of vegetables and their management

Postharvest disease convicts 10-30% losses of total crop's yields and 19 fungal pathogens found culprit of fruit and vegetable disorder. Aspergillus, Mucor, Rhizopus and penicillium are majorly disease causing agent of fruits and vegetables (Pallavi *et al.,* 2014).

Chilies (Capsicum annum)

Important cash crop of Pakistan. Pakistan is the 5th world biggest exporter of chilies with 0.2 million tons production. Moreover, it is good source of vitamins A, B, C and dietary fibers (Hussain and Abid, 2011). *Capsicum annum* being highly nutritious combating nutrient deficiencies of micronutrients like zinc, iron and bioactive compounds that have anticancer, antiviral, anti-inflammatory activities (Olatunji and afolayan, 2018). In Pakistan 85% of chilies production obtained from Sindh regions and 11% from Punjab (Wahocho *et al.,* 2016). Capsaicin active component of chilies cause body weight loss by shifting oxidation metabolism from carbohydrates to lipids and proteins. It also effective against diabetes and cardiovascular disorders as well as in prevention of Alzheimer's disease (Varghese *et al.,* 2017).

Fusarium wilt of Chilies

Fusarium wilt caused by *Fusarium oxysporum* characterized as yellowing of younger and older leaves, vascular discoloration, stunted growth, Fruit rot and reduced the yield (Bai *et al.,* 2018). Fusarium wilt one of the most devastating fungal disease that cause chlorosis and desiccation of young and older leaves that results in inward rolling of leaves with subsequent wilting of whole plant (Khan *et al.,* 2018). Fusarium wilt under low temperature and high humidity cause maximum crop losses (Kaushal and Kansal, 2019). *F.oxysporum* plant pathogenic and toxigenic fungi that reproduced through a sexual reproduction by formation of uninucleate spores microconidia, multinucleate macroconidia and thick walled spores resistant to desiccation and remain persistent to soil during unfavorable conditions are formed by fungal hyphal modifications known as chlamydospores. Fungal Penetration occurs through roots and colonization in vascular bundles that results

in chlorosis, necrosis and wilting due to toxins like fusaric acid and hydrolytic enzymes secreted by the *F. oxysporum* (Rana *et al.,* 2018).

Antifungal contents of flower extracts of *Tridax procumbens*, fruit and stem extracts of *Capparis decidua* successfully inhibited spore germination of *F. oxysporum* could be used to formulate ecofriendly fungicides (Sharma and Kumar, 2008). Lemon scented *Eucalyptus citriodora* leaf extracts committed 85% disease control in chilies by subduing fungal growth as compared to methanolic extracts of bark and fruits due to caffeic, ferulic,1,8-cineole, coumaric, anthraquinones and phenolic contents of Eucalyptus leaves (Shafique *et al.,* 2015). Maximum Fungal inhibition caused by dry seed extracts of *Azadirachta indica* at all concentrations. Inhibition increased by increasing concentrations of fresh extracts of neem leaves and garlic bulb (Agbenin and Marley, 2006). Considerable antifungal potential presented by aqueous extracts of medicinal plants *Moringa olifera, Cymbopogon citratus* and *Tinospora cordifolia* due to thymol and citral compounds (Dwivedi and Sangeeta, 2015). Different botanicals like neem, garlic and mustard oil at10% concentration exhibit maximum mycelial growth inhibition. Garlic was effective even at 5% concentration (singh *et al.,* 2017). Maximum germination and growth of chilies obtained by plant extract of *Acasia* followed by neem, Giant milk weed and Australian acasia. Phytoextracts of these plants referred as natural fungicides as they reduced disease severity and promote the germination (Khaskheli *et al.,* 2016).

Cercospora leaf spot of chilies

Disease described as dark brown spots or lesions with ashy centers on leaf and fruits that cause reduction in quality and yield of chilies (Islam *et al.,* 2015). Prevalence of disease very high at 92% Relative humidity and temperature less than 28C. *Cercospora* has ability to survive in plant debris and diseases severity maximum under wet weather conditions (Kumar *et al.,* 2017). *C. capsici* form conidia and dormant mycelium for survival up to 195 days in infected plant debris (Swamy *et al.,* 2012).

Maximum conidial germination noted at 25-30C and infection caused through stomata by formation of appressoria from germ tube. Necrotic spots with collapsed mesophyll cells appeared to have conidiophores that gave rise to conidia and encompassed stomatal activity (Meon, 1990).

Extract of Neem, Biskatali, Arjun, Almanda leaves and garlic cloves minimize the disease severity and enhance yield (Uddin *et al.,* 2013). Plant extracts in Combination with plant extracts highly effective against cercospora leaf spot. Applications of Tilt 25%EC @0.05+Euclyptus@ 10%+ *Pseudomonas fluorescens* @ 5g/lt. minimized the infection and maximize the yield under field conditions (Devappa and Thejakumar.,2016).

Okra (Abelmoschus esculentus)

Okra (*Abelmoschus esculentus L.*) important commercial, medicinal vegetable crop source of carbohydrates, fats, proteins, minerals and water soluble mucilage. Rhamnose, galactose, galacturonic acid and amino acids are important components of okra fruit (Benchasri, 2012). Okra fruits used as vegetable with multiple health benefits like mucilage control cholesterol and seeds are rich source of proteins, essential amino acids, Antioxidants and phenolic compounds like flavanol (Habtamu *et al.,* 2015).

Powdery Mildew of Okra

Powdery mildew caused by *Erysipheci choracearum* characterized as powdery minute patches on upper and lower side of leaves older and young leaves results in drying withering and defoliation (Atre *et al.,* 2017). Bulk of White powdery mass appeared over leaves, stem and fruit that spread rapidly and fruiting body known as ascocarp produce ascospores known as sexual spore that spread through rain and air. On the other a-sexual spores conidia also formed in form of long chains (Moyer and Grove, 2012). Powdery mildew caused 17-86% yield losses in Okra (Younes, 2014).

Plant extracts proved effective against powdery mildew under field conditions and remarkable reduction noticed in disease severity.

Phytoextracts of *Allium cepa*, *Datura stromanium, Zingiber officinale, Allium sativum* and *Azadirachta indica* with 3rd spray at 15% concentrations gave considerable reduction in severity of disease incidence (Atiq *et al.,* 2015). Neem leaf extracts and garlic clover extracts at 10% concentration highly reduced spore germination in invitro and extracts of Lantana, Jatropha and Ardusi were least effective for inhibition of spore germination (Jadav and Kadvani, 2019). Oil extracts of *Simmondsia chinensis, Azadirchta indica* seeds and *Rynoutria sachalinensis* provide protection against powdery mildew of Okra, reduced disease severity of potted plants, enhance yield and growth of crop (Moharam and Ali, 2012). Botanicals of neem, lantana, turmeric and Ipomoea effectively inhibited conidial germination due to antifungal contents and their efficacy increased with concentrations (Dinesh *et al.,* 2015).

Cucumber (Cucumis sativus)

Cucumber is a Crop of family Cucurbitaceae and second most important vegetable crop after tomato in Western Europe (Eifediyi and Remison, 2010). *Cucumis sativus* softens the skin, promote the hair growth and reduced the swellings under eyes. Antioxidant flavonoid, tannins, carotenoids, polyphenolic and lycopene contents of cucumber quench the harmful free radicals (Saeed and waheed, 2018). Cucumber extracts possessing p-coumaric acid and syringic acids reduced the glucose level up to 67% and cholesterol to 29% due to their antidiabetic activities which inactivate alpha glucosidase. Cucumber is also nutritionally rich with vitamin C (Kumaraswamy). About 16% annual crop losses occurred due to disease and 10% losses due to seed borne diseases and phytoextracts proved effective to eliminate these fungal diseases (Farrag and Moharam, 2012).

Powdery Mildew of Cucumber

Powdery mildew caused by *Sphaerotheca fuliginea* that reduced the yields of cucmber 20-50% (Velkov, 2007). Leaf, stem and fruits being attacked by fungus white powdery molds formed by fungus (Bettiol *et al.,* 2008). Mycelium produce Conidia which germinate to form cleistothecia fruiting body have ascospores in ascus. Sporulation of fungus produce conidia and ascospores to infect plant tissues (Moyer and grove, 2012).

Extracts of *Euphorbia humifusa* and *Robina Pseudoacacia* could be used as natural fungicides against powdery mildew of cucumber in vivo they had preventive and curative effect due to secondary metabolites (Liu *et al.,* 2010). Methanolic extract of blue gum leaves and thyme gave maximum inhibition of *S. fuliginea* spores (Surhanee, 2013). Botanical products have protective effect against powdery mildew like white mustard oil effective against powdery mildew. Plant oils have greater efficacy with higher concentrations (Masheva *et al.,* 2014). Extracts of Eucalyptus, garlic, onion, ginger and olive (*Olea europaea*) effective against powdery mildew of cucumber (Ahmed, 2005).

Cucumber Anthracnose

Cucumber Anthracnose caused by *Colletotrichum orbiculare* (syn. *Colletotrichum lagenarium*) Chlorotic necrotic lesions formed on leaves and fruits that lead to defoliation, fruit decline and plant death. Moreover, conidia produced by fungi on infected fruit have pinky wet appearance in water soaked sunken lesions on cucumber fruits (Palenchar, 2009).

Plant extract of *Cinnamomum camphora* provide remarkable 95% inhibition of *Colletotrichum* mycelium (Chen and Dai, 2011). Leaf extracts of the weeds of four families *Urticaceae, Onagraceae, Commelinaceae* and *Solanaceae* out 203 species cause highest inhibitions of anthracnose lesions in cucumber as compared to *Papaveraceae* that did not show any inhibition (Inagaki *et al.,* 2008). *Persicaria odorata* due to antifungal, antimicrobial, antioxidant properties inhibit the germination of spore and mycelial growth of the fungi (Yanpirat and vajroaya, 2015).

Alternaria leaf spot of Mustard Plant

Mustard (*Brassica juncea*) important oil seed crop grown during reason (singh *et al.,* 2017). Brassica belongs to family Brassicaceae which rich in nutrients and best source of vitamin C, E and carotenoids

reduces chances of cancer and vascular disorders (Sanlier and Saban, 2018). Mustard acts as heavy metal (Pb and Cd) accumulator and used in phytoremediation meanwhile mustard oil also has medicinal characteristics and used as condiments (Mourato *et al.,* 2015).

Alternaria leaf spot caused by a fungus called *Alternaria brassicae* that form dark brown olivaceous, chlorotic concentric rings that 47% yield losses. *Trichoderma harzianum* highly effective (under laboratory 61.44%, field 23.34%, green and house 38.45%) and *Trichoderma viride* in laboratory 55.42%, field 29.63%, in greenhouse 29.08% growth inhibition *Alternaria brassicae* (Ahmad and Ashraf, 2016). *Solnum nigrum* @10% concentration proved equally effective against *Alternaria brassicae* as the other chemical fungicides. Its due to Saponins found in the plant extract has antipathogenic activity (Sheema and Durai, 2014). *Lantana camera* 80%, *Allium sativum* 54.44%, *Zingiber officinale* 17.78% inhibition of *A. brassicae* (Biswas and Ghosh, 2018). Garlic and Eucalyptus extract at 5% and 10% concentrations gave maximum 100% inhibition while Ashok (90.77%, 100%), Tulsi (87.44%, 100%), Datura (85.09%, 100%) and Neem (82.44%, 100%) inhibition at their 55% and 10% concentrations (Yadav *et al.,* 2019). Extract of Neem leaves @15 concentration enhanced the seed yield of mustard while at 10% concentration of Neem leaves extract and garlic bulb extract also provide better results under field conditions (Mahapatra and Das, 2013).

Gray Mold of Tomato

Gray mold caused by *Botrytis cinerea* that infects all plant parts above ground especially halo white ghosts spots are formed over fruit fluffy gray spores produced by the fungus (Mouekouba *et al.,* 2013).

Invitro Extracts of *Hyssopus officinalis, Satureja hortensis and Allium sativum* with 5,10 and 20% show 80%-100% Anti-botrytis activity invitro (Sesan *et al.,* 2015). Aqueous and organic Plant extracts of *Pulicaria mauritanica*, *Asteriscus imbricatus*, *Lavandula dentata* and *Globularia alypum* were tested against *Botrytis cinerea* under both lab and field conditions. Invitro *Botrytis cinerea* completely inhibited by organic extract of *Asteriscus imbricatus* and *Pulicaria mauritanica* while extracts of *L. dentata* caused moderate inhibition of mycelial growth. The extract *G. alypum* did not show any effectiveness against fungal pathogen. Aqueous extracts of *A. imbricatus* showed highest 90% antifungal activity while 22% of inhibition noted by *P. mauritanica* and remaining two extracts proved ineffective against *B. cinerea*. However under Invivo conditions Aqueous and organic extracts of *A. imbricatus*, *P. mauritanica* with greater concentrations commendably reduced the disease incidence (Senhaji *et al.,* 2014). Phytoextracts of *Capsicum annum* and *Capsicum chinense* at 40% concentrations showed significant decrease in *Botrytis* spore germination and mycelial growth. However, *Capsicum fruitescens* and *Waltheria indica* intermediately reduced the spore germination (Ahmed, 2018). Aqueous and hexanic extracts of *Cystoseira tamariscifolia* and *Bifurcaria bifurcate* showed significant antifungal potential against *B. cinerea* due its bioactive constituents. It may encourage the application of phytoextracts as better substitution of chemical fungicides (Bahammou *et al.,* 2017).

Conclusion

Massive applications of chemical fungicides to control fungal pathogens imposed serious threat to food security and safety standards. Toxins of chemical fungicides accumulate in plant tissues and become part of food chain which lead to serious consequences. But due to public health concerns the utilization of phytoextracts executed permissible antimycotic operations due to bioactive compounds and antimicrobial ingredients in medicinal plants. Exploitation of phytoextracts for crop protection is an ecologically sound and realistic approach for management of phytopathogens which would be a better substitute of chemical pesticides in future. Several researchers focusing on such type of biodegradable strategies however there is need of further exploration about the use of botanicals at commercial scale which would provide cheap, feasible and easily accessible antimicrobial sources to farmers in suitable budget.

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