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PLANT SECONDARY METABOLITES IN PLANT DISEASE MANAGEMENT: NATURAL EXTRACTS, REGULATORY FUNCTIONS AND INTERACTION WITH PRIMARY METABOLISM

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ABSTRACT

The plant kingdom is known to produce several organic compounds of low molecular weight which has been broadly classified as Primary metabolites, secondary metabolites and hormones based on their functions in plants. While primary metabolites are required for the plant growth and hormones are required for various metabolic processes, the secondary metabolites play a very important role in the survival of plants by protecting them against various pathogens, environmental stresses and herbivores, in addition to providing a variety of valuable natural products. It is an evolved mechanism in plants which provides defensive functions in plants and regulates defense signaling pathways. Plants are sessile in nature and need to accumulate such compounds to combat various biotic and abiotic stresses. Recent advances in genetic and chemical studies have also shown that secondary metabolites are multifunctional and it can help in plant defenses, regulate plant growth and may also function as potent primary metabolites. Extracts of plants have wide range of activity against plant pathogens and have been reported to contain many phytochemicals such as alkaloids, flavonoids, tannins, etc, which help in preventing disease development. Understanding the roles and functions of secondary metabolites in plant defense can provide valuable insights for developing sustainable agricultural practices, improving crop resistance, and discovering new bioactive compounds with potential applications in medicine and other industry.

Keywords : Secondary metabolites, defenses, stress, extracts

Introduction

Plants produce several low molecular weight organic compounds which can be grouped into Primary metabolites, Secondary metabolites and Hormones on the basis on their functions. The primary metabolites are conserved and directly take part in plant growth and development, eg. carbohydrates, proteins, lipids, etc. (Erb and Kliebenstein, 2020). The secondary metabolites help the plants to mediate the biotic and abiotic interactions, eg. phenolics, terpenes. The hormones are small compounds which regulate metabolic processes in plants, eg. auxin, cytokinins etc. (Davies, 2004). Recently various studies have shown that secondary metabolites can help in plant defenses, help in regulating plant growth and may also involve in

primary metabolism (Erb and Kliebenstein, 2020). Also, extracts of higher plants contain many secondary metabolites such as flavonoids, tannins, etc. that can act against plant pathogens (Enyiukwu *et al.*, 2014). Therefore, how extracts of higher plants can be used in plant disease management is discussed here, also with an emphasis on how in various instances plant secondary metabolites can regulate some plant metabolic functions or involve in primary metabolism.

What are Plant Secondary Metabolites?

In the environment, plants are surrounded by a wide range of natural enemies like herbivores, pathogens such as bacteria, fungi, viruses and pests including human beings. To protect themselves, they

produce various compounds which are known as secondary metabolites, which have no direct function in plant growth and development but defend them against such potential enemies and various biotic and abiotic stress. It can be described an evolved mechanism in plants which takes part in defense signaling pathways (Divekar *et al.*, 2022). Unlike primary metabolites, absence of a secondary metabolite does not result in the immediate death, but the organism's survivability, fecundity, or aesthetics is impaired in the long term or there may be no significant change at all (Tiwari and Rana, 2015).

Role of plant secondary metabolites

1. Protect the plants from invading pathogens.
2. Helps to improve the shelf life of plant
3. Decreases infection by some pests and pathogens.
4. Attracts pollinators and seed dispersing animals.
5. Improves the immunity of plant.

Classification of Plant Secondary Metabolites

Based on the pathways by which they are synthesized, plant secondary metabolites can be classified into four classes (Divekar *et al.*, 2022), as shown below:

1. Terpenes, such as, Monoterpenes (eg. Citronellol), Sesquiterpenes (eg. Beta-Caryophyllene) and Triterpenes (eg. Azadirachtin)
2. Phenolics, such as Coumarin (eg. Hydroxycoumarins), Furano-coumarin (eg. Bergamottin), Flavonoids (eg. Quercetin) and Lignin (eg. Resveratrol).
3. Nitrogen-containing compounds such as Alkaloids (eg. Nicotine), Cyanogenic glycosides, (eg. Dhurrin) and non-protein amino acids (eg. Azetidine).
4. Sulphur-containing compounds such as Glutathione, Glucosinolates, Phytoalexins and Defensin

Mode of Action of Plant secondary Metabolites

Phenols behave as antioxidants which aid in scavenging reactive oxygen species produced during pathogen attack. Glucosinolates when hydrolysed, produce compounds like isothiocyanates which exhibit toxic effect on microbes. Terpenoids show antimicrobial properties by disrupting pathogen cell membranes. Alkaloids interfere with DNA replication, protein synthesis and enzyme activity, leading to cytotoxic effect on microbes. Tannins make complexes with macromolecules in pathogens leading to precipitation and inhibition of their activity. Flavonoids inhibit enzymes essential for pathogen growth.

Role of Plant Secondary Metabolite Extracts in Plant disease management

Why Plant Secondary metabolites should be used against pathogens?

- Eco-friendly and sustainable solution
- Reduce crop losses
- Easily bio-degradable
- Cheaper alternative to synthetic chemicals
- Organic farming and Integrated Disease Management

Sources of Plant Secondary metabolites

Plant extracts are obtained by extracting from different parts of the plants. For example, tulsi extract, garlic extract, etc.

Essential oils are volatile compounds that are extracted from plants through steam distillation or cold pressing. e.g. tea tree oil, lavender oil, etc.

Extracts of higher plants have been reported to contain many phytochemicals such as alkaloids, flavonoids, tannins, etc, which can help in preventing disease (Enyiukwi, 2014).

Techniques for testing of inhibition efficiency of plant extracts

Various techniques can be used to test the antimicrobial activity of plant extracts as discussed below (Balouiri *et al.*, 2016).

1. Agar well diffusion method

In this method, the pathogen is spread evenly over the agar medium plate with the help of a L rod. The compounds that are to be investigated are put in the holes made with a 5mm cork borer in the agar medium. It is left for observation for a period of 7 to 10 days based on the time of growth of the pathogen. The pathogen growth was observed every 24 hrs against the control.

2. Agar disk diffusion method

In this method, instead of wells, certain small disks dabbed with plant extract is placed over the agar medium plate and are left for observations against the control.

3. Poison food technique

It is mostly used to evaluate the antifungal effect. The antifungal agent or the extract is incorporated into the molten agar at a desired final concentration and mixed well. The medium is poured into Petri dishes and then the inoculation of pathogen can be done by a mycelial disc ranging from 2 to 5 mm, which is deposited in the center of the plate with the help of an

inoculating loop. After further incubation under suitable conditions for the fungal strain tested, the diameters of fungal growth in control and sample plates are measured, and then antifungal effect is estimated.

Methods of extraction of Plant secondary metabolites

Extraction is the crucial step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization (Fotsing *et al.*, 2022). Some of the commonly employed extraction methods are given below.

1. Maceration method

Simplest and widely used. Plant materials (coarse or powdered) are soaked in a closed stoppered container in a solvent and left at room temperature for 2–3 days with frequent stirring. The mixture is then pressed or strained by filtration or decantation after a specific time.

2. Decoction method:

It involves boiling the plant material in water to obtain plant extracts. The sample is boiled in a specified volume of water for a defined time (15 to 60 minutes). It is then cooled, strained, filtered, and added enough water through the drug to obtain the

desired volume. The choice of solvents will determine the type of compound extracted from the plant material

3. Soxhlet extraction or Hot continuous extraction:

The first Soxhlet apparatus was developed in 1879 by Franz von Soxhlet. Finely ground sample is placed in a porous bag or “thimble” made from a strong filter paper or cellulose. Extraction solvents are heated in a round bottom flask, vaporized into the sample thimble, condensed in the condenser, and dripped back. When the liquid content reaches the siphon arm, the liquid content is emptied into the bottom flask again, and the process is continued.

Fractionation and Identification

Plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals. For separation, a number of different separation techniques are employed such as Fractional distillation, TL Chromatography, HPL chromatography and Gas chromatography.

Identification is done on the basis of functional group, presence of multiple bonds and rings, hydrogen and carbon arrangement as well as full structural elucidation. The techniques used include mass spectroscopy (MS), ultraviolet spectroscopy (UV) and infrared spectroscopy (IR).

Effect of different compounds in inhibiting different pathogenic microbes

Table 1 shows some compounds found in plants and their effectiveness against various pathogens.

Table 1 : Plant Compounds and their effectiveness against various pathogens.

Compound	Plant	Pathogen	Reference
Psoralen and Furano-coumarin	Cauliflower	<i>Alternaria brassicola</i>	Al-Barwani and Eltayeb, 2004
Zealexin	Maize	<i>F. graminearum</i> , <i>Aspergillus flavus</i> and <i>R. microsporus</i>	Huffaker <i>et al.</i> , 2011
Flavonoids	Bark and stem of Cassia fistula plant	<i>Tobacco mosaic virus</i>	Zhao <i>et al.</i> , 2013
Coumarins	Tobacco	<i>Tobacco mosaic virus</i>	Liu <i>et al.</i> , 2016`
Limonene (terpene)	Rice	<i>Xanthomonas oryzae</i>	Lee <i>et al.</i> , 2016

Important plant diseases controlled with extracts of higher plants

Table 2 shows some extracts derived from various plant parts and their effectiveness against various diseases of plants.

Table 2 : Plant extracts and their effectiveness against plant diseases

Extracts used	Disease	Crop	Pathogen	Reference
<i>P. guineense</i> , <i>Allium</i> sp.	Bacterial Blight	Egg plant	<i>Xanthomonas campestris</i> pv. <i>vesicatoris</i>	Opara and wokocha, 2008
<i>A. meleguata</i> seed, <i>M. myristica</i> seed	Stem rot	cowpea	<i>S. rolfsii</i>	Okwu and Njoku, 2009
<i>A. indica</i> seed oil	Blast	Rice	<i>Pyricularia grisea</i>	Amadioha, 2000
<i>A. indica</i> (ethanolic extract)	Brown spot	Rice	<i>Drechslera oryzae</i>	Amadioha, 2002
<i>P. guineense</i> seed, <i>C. papaya</i> root, seed	Antracnose	Cowpea	<i>Colletotrichum destructivum</i>	Enyiukwu and Awurum, 2013

Role of Plant Secondary Metabolites as regulators of plant growth and development

So far, it has been discussed how plant secondary metabolites from plant extracts are effective in inhibiting various plant diseases. Now the next aspect is, how plant secondary metabolites can be involved in the growth and development of plants. By dynamically regulating their growth, plants very often decrease their investment in growth and development in response to attacks by pathogens or herbivores. Growth-defense patterns are further regulated by plant secondary metabolites and their breakdown products, which are emerging as plant growth modulators. A study has been discussed below to show how glucosinolate can affect growth and development in plants.

Katz *et al.* (2015) reported that exogenous indole-3-carbinol (I3C) treatment inhibits root elongation in *Arabidopsis thaliana*. The indole-3-carbinol is a Glucosinolate breakdown product which are produced in the Cruciferae family and *Arabidopsis thaliana* and it protects them against various insects. When these plants undergo damage in the tissue by herbivores or any other means, hydrolysis of glucosinolates occurs by beta-thioglucosidases produced in the plant. Ultimately, further breakdown produces wide variety of products, out of which one of them is I3C.

They analyzed primary root lengths of 14 day old *Arabidopsis* seedlings germinated on increasing concentrations of I3C and observed that I3C inhibited root elongation in a dose-dependent manner. Extrapolation of growth rates revealed 50% inhibition of root growth (IC₅₀) at 125 μ M I3C, with 95% inhibition (IC₉₅) at approximately 400 M.

The study shows one example of how plant secondary metabolites can regulate growth and development in plants. Similarly, other workers have reported that breakdown products of glucosinolate can regulate stomatal opening in *Arabidopsis* and *Vicia faba* (Khokon *et al.*, 2011). Also, some reports suggest that they may regulate the circadian clock and flowering time of plants (Kliebenstein *et al.*, 2001).

Can a Plant Secondary Metabolite act as a primary metabolite?

One of the major difference between primary metabolite and secondary metabolite is that primary metabolites are highly conserved while secondary metabolites can evolve over time and vary in structure, production, etc. (Wink, 2008). While some of the secondary metabolites are very ancient and conserved like flavonoids and terpenes, some have evolved very recently such as glucosinolates and benzoxazinoids (Erb and Kliebenstein, 2020). Various workers have

reported that secondary metabolites may not directly act like primary metabolite but some secondary metabolites may be involved in primary metabolism.

Soubeyrand *et al.* (2018) reported that in *Arabidopsis*, mutations in the flavonoid biosynthesis pathway, particularly upstream of the enzyme FLAVANONE-3-HYDROXYLASE (F3H), result in reduced levels of ubiquinone. The reduced ubiquinone levels in these mutants can be restored by the addition of dihydrokaempferol or kaempferol which indicates that these compounds, which are flavonoids, can compensate for the disrupted pathway and help in the biosynthesis of ubiquinone. Labeling experiments have shown that the aromatic ring structure of kaempferol is integrated into ubiquinone. This provides direct evidence that kaempferol or its derivatives are utilized in the formation of ubiquinone. They reported that heme-dependent peroxidases likely use kaempferol to produce 4-hydroxybenzoate. 4-hydroxybenzoate is a precursor for the synthesis of ubiquinone, indicating that kaempferol undergoes a transformation facilitated by peroxidases to contribute to ubiquinone biosynthesis.

Conclusion and Future prospects

Plant secondary metabolites play a vital role in the plants defense mechanism. These compounds aid in the plant's ability to protect against pathogens, deter herbivores and withstand environmental stresses. Also these metabolites may help in plant growth regulation and take part in primary metabolism. If the roles and functions of phytochemicals in plant defense is properly understood, it can provide valuable information in developing sustainable agricultural practices, improving crop resistance, and discovering new bioactive compounds which have potential applications in medicine and other fields. After a compound has been found to be effective against certain pathogen in *in vitro* testing, it should be taken to the field trials so that commercialization of the product can be done. Only when more and more such products become available in the market, farmers will have a wide range of ecofriendly options to choose from, instead of the conventional pesticides.

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