RADINKA JOURNAL OF SCIENCE AND SYSTEMATIC LITERATURE REVIEW

ISSN 2988-5825

https://www.rjupublisher.com/ojs/index.php/RJSLR

Biosynthesis, Accumulation, and Antimicrobial Potential of Phytoalexin and Natural Product in Plant and Animal Defense System

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Abstract

Most plant potential to resist pathogenic infections requires some sophisticated defense system which basically involves the production of low-molecular-weight antimicrobial metabolites known as phytoalexins. These compounds are known to exhibit toxicity towards a wide spectrum of pathogens, such as fungi, bacteria, nematodes, and other organisms. This review delves into the diverse chemical structures and biosynthetic pathways of phytoalexins across various plant familie and some higher animals human alike. We examine the mechanisms of phytoalexin accumulation, through de novo synthesis, enzymatic biocatalysis activities, and the influence of biotic and abiotic elicitors. Understanding the complex connection between phytoalexin biosynthesis, accumulation, and antimicrobial activity is crucial for developing effective disease management strategies. By employing modern molecular biology tools, researchers can elucidate the genetic pattern of phytoalexin production and identify key genes involved in resistance and susceptibility.

Keywords: Phytoalexins, biosynthesis, accumulation, antimicrobial, plant defense, elicitors, metabolites.

Acknowledgments: Thanks to all parties who have supported the implementation of this research. I hope this research can be useful.

For citation:
Mamman, M. O., Muhammed, H. A., Akowe. I. I., Abdulhakeem, S, J. Iyaji. (2024). Biosynthesis, Accumulation, and Antimicrobial Potential of Phytoalexin and Natural Product in Plant and Animal Defense System. *Radinka Journal of Science and systematic Literature Review*, *2*(3), 442-449.

Introduction

Phytoalexins are group of chemically diverse broad spectrum antimicrobial compounds synthesized by plants in response to pathogen attack .Naturally they are mostly product of shikimic acid(phenylpropanoid) pathway giving rise to the forms of most phytoalexins produced by *leguminosae* i.e.possession of six isoflavanoids structural classes such as isoflavones,isoflavenes,pterocarpans,pterocarpenes ,isoflavans,and coumestans,however a small number of legumes also produce non-iso-flavonoid phytoalexins such as Furanoacetylenes and Stilbenes (Sobolev et al., 2022). Examples of phytoalexins are pisatin from pea,kievitone and phaseolin from bean and glyceollin from soybean .On the other hand product of mevalonic acid pathway giving rise to the forms i .e.phytoalexin with sesquiterpenoid structure such as Gossypol from cotton ,casbene from castor bean and rishitin from the solanaceae as well as Diterpenoid structures such as oryzalexin A in rice varieties. Phytoalexins are not cosmopolitan in nature but they are found in brassicaceae,vitaceae,poaceae (Gramineae) while most of the family of cereals such as Oats,Sorghum,barley, rice and maize are reported to possess Phytoalexins or some other natural products that accumulates to resist inbuilt of disease symptoms (Anjali et al., 2023).Phytoalexins are plant secondary metabolites,small antibiotic molecules synthesized and accumulated in plant tissues under attack from pathogenic microbes.Rapid localized accumulation of phytoalexins restricts pathogen development in many plant -pathogen interactions and contributes an important role in plant defense and as an antifungal agents they are biologically active on most living organisms (Kengne et al., 2021). They accumulate at the sites of infections in concentration sufficient to restrict and inhibit the growth and development of fungi and bacteria and are also reported to be somewhat similar in context between plants within the same family. However a compound known as elicitors have been described as a high molecular substances which induce other plants defence responses due to microbial infections.For example ,an elicitor is found to induce secretion of antimicrobial substances (Meena et al., 2022). Moreover,they accumulate around both resistant and susceptible necrotic tissues but resistance occurs when one or more Phytoalexins reach a concentration sufficient to restrict pathogen development by inhibiting the growth of fungi which are pathogenic to plants while some are also toxic to biotic invasion such as bacteria,nematodes,virus and other organisms as well as abiotic stress e.g. mechanical damages such as heavy metal,UV radiation and mutation. Meanwhile they are also secreted in efficient concentration accumulated enough for microbial protection by inhibiting sporulation,hyphal growth and spore germination of phyto pathogenic fungi while some pathogens are found to be resistant because they have the ability to demethylate or hydroxylated the aromatic rings in the Phytoalexins and render them inactive.

Research Objective: To address the research objective, a combination of Online Research, Surveys and Phonic Conversation was conducted where these methods employed to gather quantitative data and qualitative data insights into specific incidents and their outcomes on the prevalence and characteristics of cyber harassment. I evaluated the credibility and relevance of online sources. This involved assessing the authority of the source, checking for bias, and ensuring that the information is up-to-date. After collecting relevant information, I analyzed the data to draw insights, identify trends, and support their research objectives.I ensured and followed ethical guidelines, including proper citation of online sources and respecting copyright laws. Plants are able to enhance disease resistance and increase food security as well as balances the resource allocation between growth and development if their is manipulation by induction of specific genes encoded to trigger its accumulation among plants whose Phytoalexin is not sufficient enough to resist certain diseases for farmers to have an increasingly yield and productivity (Jeandet et al., 2023)*;* (Osuna-Caballero et al., 2024).. However it is reported that the prevalent defense mechanisms are categorized into three which are ,pathogen -asociated molecule pattern (PAMP) pathogen triggered immunity (PTI),the secondary defense ,effector triggered- immunity (ETI) and the interference of additional defense i.e.the exosome-mediated cross -kingdom RNA (CKRI) system (Alpkvist et al., 2024); (Chen et al., 2014); (Cicchinelli et al., 2024).

Phytoalexins elicitors are generally high molecular weights substance that are constituents of the fungal cell wall such as glucans,chitosan,glycoproteins and polysaccharides and they can also induce other plants defence responses,for example,the synthesis of proteinase inhibitors and the accumulation of hydroxyproline-rich glycoproteins.Phytoalexins are restricted to compounds of low meolecular weight produced from remote precursors through de novo synthesis of enzymes.Their peculiar biosynthesis and regulation mechanism which are very complex includes Phosphorylation'cascades'defense related marker genes,calcium sensors and elicitiors as well as hormone signaling which are important regulators for the modulation of phytoalexin production and pathogen resistance which serves as the knowledge and insight of the control mechanisms of Phytoalexins accumulation as the basis for the genetic manipulation of those compounds in engineered plants for enhanced disease resistance.

Methodology

1. *Online Research:* Online research involves using the internet to gather information and data for your research project. It includes collecting data from websites, databases, online publications, and various digital sources. I used search engines, academic databases, and various online tools. I reviewed existing online sources, academic articles, and relevant websites to gather background information.

Steps:

- Identify research objectives and questions.
- Use search engines and databases to find relevant sources.
- Evaluate the credibility and reliability of online sources.
- Extract and compile data from websites, articles, or digital resources.
- Analyze and synthesize the gathered information for your research.

2. *Surveys:* Surveys are a structured data collection method where researchers design questionnaires or interviews to gather information from a sample of respondents. Surveys can be conducted through various mediums, including paper, online forms, or in-person interviews.

Steps:

- Define research objectives and formulate clear research questions.
- Design a survey instrument, including questions and response options.
- Determine the survey sample and method of distribution (e.g., online, phone, in-person).
- Administer the survey to respondents.
- Collect and analyze the data to draw conclusions and insights.

Designing the Survey: I designed a structured set of questions that are administered to a group of respondents via Google Form. The question was open-ended, closed-ended, and a mix of both.

Sampling: I selected a representative sample of the target population. This was done through random sampling.

Data Collection: The data of this survey was collected from the respondents based on the survey design via Google Form and Phonic Conversation.

Data Analysis: The collected data was analyzed using statistical methods to draw conclusions and insights.

Ethical Considerations: I obtained the information and data through informed consent, ensured confidentiality, and handle data in compliance with relevant ethical and legal standards.

3. *Phonic Conversation:* Phonic conversations, or phone conversations, are verbal interactions with participants conducted over the phone. This method is often used in qualitative research to collect in-depth information through interviews or discussions.

Steps:
- Establish research objectives and design a conversation guide or interview script.
- Identify and select participants (interviewees) who fit the research criteria.
- Schedule and conduct phone interviews, following the guide.
- Record the conversations (with consent) or take detailed notes.
- Transcribe and analyze the data for patterns and insights.

Participant Selection: I identified individuals and groups to engage in phone conversations. These participants was chosen based on specific criteria and as a random sample.
Script or Protocol: I prepared a script or a set of questions to guide the conversation. This can included both open-ended and closed-ended questions.
Conducting the Conversation: I was engaged in phone conversations with participants, asking questions and recording responses.
Data Analysis: The recorded conversations were transcribed, and the data was analyzed to identify patterns, themes, and insights.
Ethical Considerations: I obtained the information and data through informed consent, ensured privacy and confidentiality, and followed ethical guidelines during the conversations.

Results and Discussion

Biosynthesis Forms of Accumulation and Functions of Phytoalexins

An elicitor are molecules that signal plants to begin the process of phytoalexin biosynthesis and accumulation to restrict infection.Naturally on a normal circumstances stimulus is provided by the presence of the micro-organism and it's perception by the host plant may initiate the chain of events leading to phytoalexin synthesis.Elicitors can be categorized into biotic and abiotic elicitors.The biotic elicitors may originate in the invading organism (parasite) and hence usually refer to as endogenous in nature while the abiotic elicitors are having their origin from the plant(host) elicited by the interaction between micro-organism and host plant.

(Eckardt, 2011), Large number of Phytoalexins are products of the shikimic acid (phenylpropanoid) pathway from which many other plant secondary metabolites i.e., flavonoids like lignin and anthocyanins are mostly derived while others are synthesized through the mevalonic acid and Trp pathway.Phytoalexins which are considerably less toxic than chemical fungicides has its fungi toxicity clearly determined by the inhibition of germ-tube elongation ,radial mycelial growth and mycelia dry weight increase ,as illustrated by the action of resveratrol on B.cinerea,the casuals agent for gray mold in grapevine. The Phytoalexins production and accumulation occur in healthy plant or cell cultures surrounding wounded or infected cells and are stimulated by the alarm substances produced and released by the damaged cell and diffusing into the adjacent healthy cells .Accumulation of Phytoalexins after production is regulated by levels of the relevant biosynthesis enzymes i.e.extracellular peroxidases which degraded them oxidatively or by polymerization increased due to an increase in expression of the genes encoding the enzymes while Phytoalexin synthesis is stimulated with elicitors de novo transcription of the genes encoding the biosynthetic reactions.

Phtoalexins are generally regarded as multisite toxicants capable of affecting a variety of physiological and biochemical pathways which tends to produce to produce those with isoflavonoid structures such as pisatin known to cause membrane dysfunction which suggested that their sites of action may be the cell membrane as evident from their collective lipophilicity.Alternatively,it has been proposed that isoflavonoid derivatives uncouple oxidative phosphorylation in the fungal cell,leading to cell death and as such the relative acidity and number of free phenolic groups could determine their potency **(Kruk et al., 2022)**. Asymmetric growth of the germ tube resulting in the production of "curved-germ tubes"has also been observed in

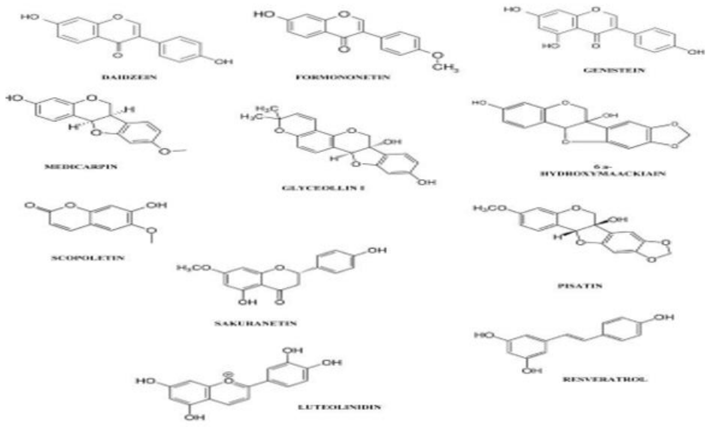

Figure 1. Molecular structures of some plants phytoalexins

B.cinerea conidia treated with sub-lethal doses of resveratrol.Phytoalexins may also affect glucose uptake by fungal cells during the study of the interactions between phaseollin/Kievitone and Rhizoctonia solani conidia of B.cinerea which has showed a complete disorganization of mitochondria and disruption of the plasma membrane upon treatment with the stilbene phytoalexins,resveratrol and pterostilbene (Jian et al., 2016); (Mizuhara et al., 2023).

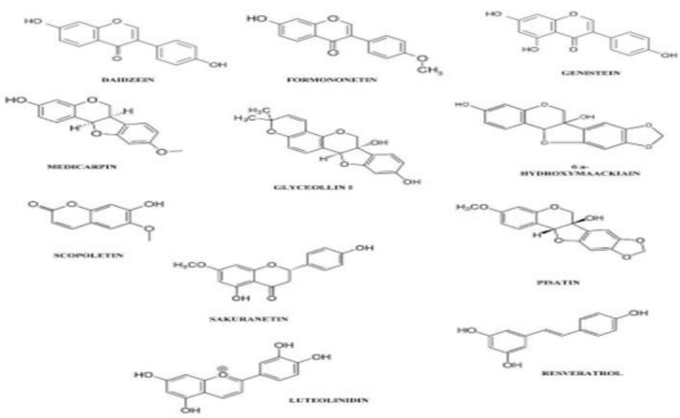

Figure 2. Molecular structures of some plants phytoalexins (culled from (Jeandet et al., 2014))

Most of the elicitors are non-specific while few elicitors, are specific ,as the accumulation of the phytoalexins they caused on certain compatible and incompatible cultivars parallel the phytoalexin accumulation caused by the pathogen races themselves.The formation of phytoal exins in a susceptible (compatible) h ost following infection by a pathogen seems in some cases to be prevented by suppressor molecules produced by the pathogen which seems to be in form of glucans or glycoproteins or one of the toxins produced by the pathogen.Species or races of fungi pathogenic to a particular plant species seem to stimulate the production of generally lower concentrations of Phytoalexins than non-pathogens.For example,in the case of pisatin production by pea pods inoculated with the pathogen Ascochyta pisi a pea varieties which produce concentrations of pisatin that are approximately proportional to the resistance of the variety to the pathogen e.g. in soybean plants infected with the fungus phytophthora megasperma F.sp.glycinea,inoculations of fungal races on incompatible host cultivars resulted in earlier accumulations and higher concentrations of the phytoalexin glyceollin than inoculations of fungal races on compatible cultivars.It has been suggested that the higher concentrations of glyceollin in incompatible host- combinations are the result of reduced bio-degradation rather than increased biosynthesis of the phytoalexin.

However in some host-pathogen systems e.g.,in the bean/colletotrichum lindemuthianum and the potato/ Phytophthora infestans system,with the respective phytoalexins,such as phaseollin and rishitin reach equal or higher concentration in compatible (Susceptible) hosts compared to incompatible (resistant) ones.For examples in the grapevine Vitis vinifera one of the predominant Phytoalexins belongs to a family of compounds called Stilbenes i.e trans-resveratrol (trans-3,5,4¹-trihydroxystilbene(RES).RES is Stilbene polyphenol containing two phenol rings joined by a double styrene bonds (fig.2) and has been shown to possess significant free radical scavenging properties in various cellular models ,reducing elevated malondialdehyde(MDA) and nitrite levels to restore depleted gluthathione (GSH) and is able to reduce Aβ fibril formation in vitro by promoting protease remodellation of this peptide into a non-toxic structure of high molecular weight ,possibly by activation of AMP-activated protein kinase (AMPK) by the calcium /Calmodullin -dependent protein Kinase-beta,which is an important anti-aging pathway (Marin et al., 1999): (Chung et al., 2017).

Benefits of Phytoalexin

Camalexin has recently been involved in the induction of fungal apophotic programmed cell death in *B.cinerea* while resveratrol also exerts some activity against numerous bacteria affecting humans such as Chlamydia,Helicobacter, staphylococcus Enterococcus,pseudomonas and Neisseria as its very clear that phytoalexins exhibit toxicity across much of the biological spectrum, Prokaryotic and eukaryotes alike *Roop and Kuldert.,2017.*The wide spectrum of biological activity potentially poses food safety problems e.g.Xanthotoxin,a furanocoumarin phytoalexins in diseased celery tissue could adversely affect field workers harvesting the celery(*Apium graveolens*)crop while some phytoalexins have beneficial impacts on human health e.g.3-Deoxyanthocyanin phytoalexins from sorghum show activity against gastrointestinal cancers while indole-sulphur phytoalexins from crucifers contribure to the antioxidants, anticarcinogenic and cardiovascular protective function of these vegetable in humans. Resveratrol from peanut and grape show antidiabetic, anticarcinogenic,anti-aging,anti-inflammatory and vasodilator activity in human as well (Zhang et al., 2021); (Gál et al., 2023); (Rudrapal et al., 2022).

Maize (Zea Mays) is commonly used in the control of soil borne disease caused by tomato Fusarium wilt by F.oxysporum,and in the control of brown stem rot of adzuki bean (Vigna angularis) by cepharosporium gregatum.(6R)-7,8-dihydro-3-oxo-$\alpha -$ ionone and (6R,9R)-7,8-dihydro-3-oxo-$\alpha - ionol\ were\ isolated\ from$ the root exudates of maize.They exhibited anti-fungal activity F. and inhibited germination of *C.gregatum*.It is also worth noting that 6-methoxybenzoxazolinone (MBOA) and 6,7-dimethoxybenzoxazolinone. (DMBOA) were isolated from the root extract and are compounds identified as phytoalexins in several gramineous plants.Application of Geranium carolinianum for the control of *Ralstonia solanacearum*,the cause of bacterial wilt of potato and potato scab which is induced by some streptomyces app.,with ethyl gallate as one of the antibacterial constituents.Alliaceous plants release characteristics volatile compounds e.g. allylthiosulfinate which is produced from alliin by alliinase and exhibits strong antibacterial and antifungal activities and for this reason ,Alliaceous plants are use as companion plants of tomato, cucumber, strawberry etc (Cao et al., 2020).

Conclusion

Phytoalexins and other natural products play crucial roles in plant and animal immune responses, offering potent defenses against pathogenic infections. This review has explores their diverse forms of accumulation, biosynthesis pathways, and the wide-ranging benefits they confer in combating microbial threats. Understanding these mechanisms not only enhances agricultural strategies for disease resistance but also illuminates potential therapeutic applications in human medicine.

Conflicts of Interest

The authors declare no conflict of interest.

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