TECHNIQUES FOR THE DETECTION AND IDENTIFICATION OF PHYTOTOXINS: MINI-REVIEW

Abstract

Phytotoxins may result from different sources such as plants (rosary pea, castor bean, tobacco, white snakeroot), microorganisms (fungi, bacteria, viruses, protozoa), human activity (herbicides), or natural chemical reactions. These phytotoxinsimpose danger not only to the plants but also to animals and humans that are exposed to it. Furthermore, the study of phytotoxins results in the development of new products having different biological properties. Complex processes require very costly and specialized equipment to identify, extract and detect phytotoxins. However, simple, low cost and effective bioassay methods are also available for the detection of plant toxins. Various methods have been adopted for the isolation and identification of phytotoxins. However, to be able to isolate and identify phytotoxin successfully, specific techniques must be employed. Herein, the study highlighted some of these techniques that may lead to the isolation and elucidation of a pure phytotoxin. Chromatographic techniques (thin layer chromatography, column chromatography, ion exchange chromatography, high performance liquid chromatography) are mostly used. Whereas, for elucidation, techniques like nuclear magnetic resonance spectroscopy, mass spectrometry, infrared spectroscopy, and X-ray diffraction are used. In addition, ELISA and PCR are highly utilized. In conclusion, for a phytotoxin to be isolated from a pool of organic compounds, selection of good and sensitive chromatographic techniques needs to be applied carefully. Additionally, the development of new advanced techniques which are based on biosensors, biochemistry, nanotechnology, and robotics

Authors

Ho Soonmin
Faculty of Health and Life Sciences
INTI International University
Putra Nilai, Negeri Sembilan, MALAYSIA
soonmin.ho@newinti.edu.my

Muhammad Akram
Department of Eastern Medicine
Government College University
Faisalabad, Allam Iqbal Road,
Faisalabad, Pakistan

Mohammad Ali Shariati
Kazakh Research Institute of Processing and Food Industry
(Semey Branch), Semey, Kazakhstan

Muhammad Riaz
Department of Allied Health Sciences
University of Sargodha,
University Road, Sargodha, Pakistan

are needed in the future for the better understanding of phytotoxins and their functions.

Keywords: Phytotoxin; mass spectrometry; isolation; X-ray diffraction; identification; elucidation; organic compounds; chromatography; microorganism.

I. INTRODUCTION

Toxin is a substance poisonous for life and may have originated from a natural source such as plants, animals, or microorganisms. Cytotoxin showed a direct toxic effect on cells. For example, neurotoxins and nephrotoxins could damage the nervous system and kidney systems, respectively. Phytotoxins are poisonous substances originating from plants [1]. Plants may appear harmless at first but some of them can cause harm to human or animal life through the production of chemical compounds generally referred to as phytotoxins [2]. Plants may produce phytotoxins as a part of their defense mechanisms, but they may also in one way or the other become a victim of toxins produced by certain fungi and bacteria using plants as their hosts. These toxins produced by bacteria and fungi diffuse into the plants and cause various abnormalities in the physiological and biochemical functions of the plant [3]. Fungi and bacteria are the most common plant pathogens that produce phytotoxins which aid in the progression of a disease [4].

After decomposition, organic matter of plants becomes an important component of soil. Phytotoxins may spread through soil to water stores and pollute them with toxins that are dangerous to life [5]. Over the years, several efforts have been made in research to isolate and identify the phytotoxins that impose danger to not only plant life but also that of animals and humans that consume them. It is therefore necessary to isolate and identify these phytotoxins so that they can be effectively countered using specific herbicides [6].

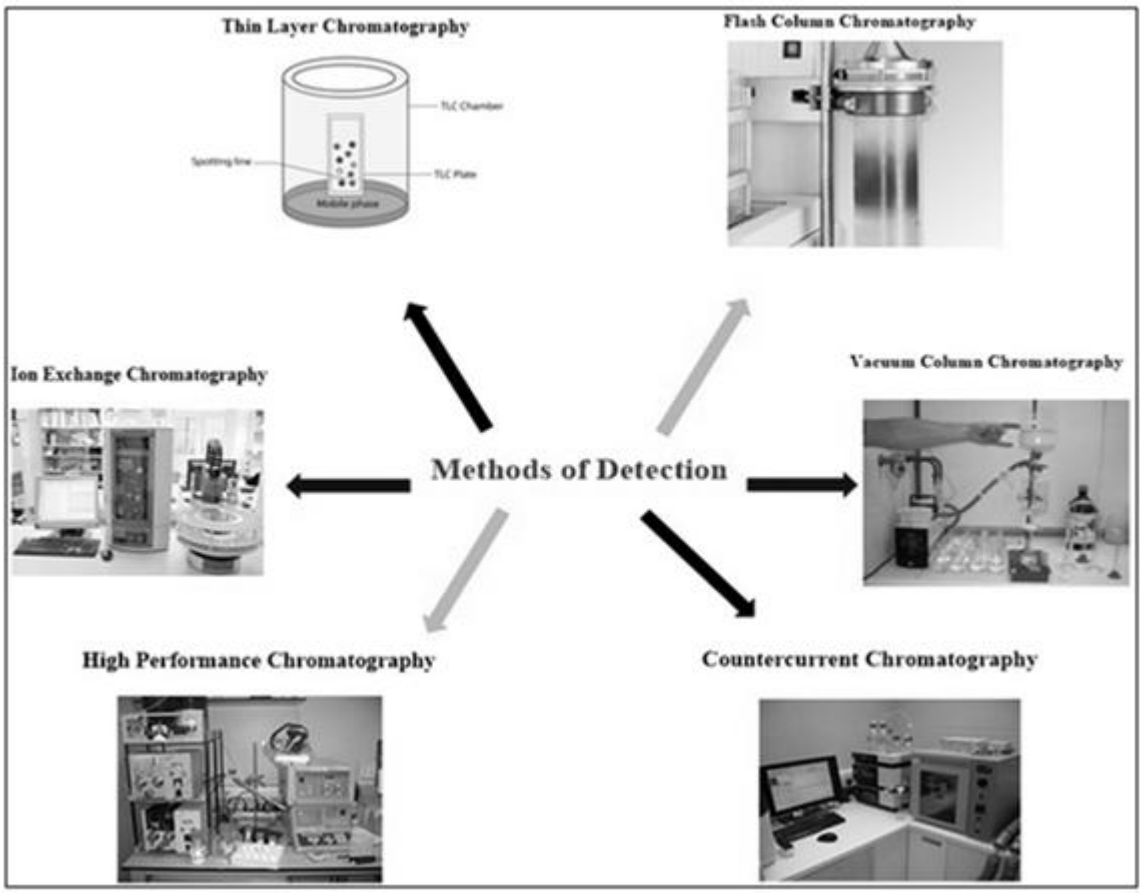


Figure 1: Different Techniques for Identification of Phytotoxins

Various methods have been adopted to achieve the goal of isolation and identification of phytotoxins [7,8]. These methods include in-vitro cultivation of the phytopathogen to detect its phytotoxicity followed by the identification of its structure and chemistry. To isolate the pure phytotoxin, organic extracts of the culture media are fractionated using chromatography techniques such as vacuum column chromatography, thin layer chromatography, flash column chromatography, direct and reverse phase open column chromatography, gel filtration chromatography, ion exchange chromatography, high performance liquid chromatography and counter-current chromatography (figure 1).The advantages and limitations of these techniques have been highlighted in table 1. However, based on the quantity and chemical nature of a phytotoxin, different chromatographic techniques could be used for its isolation [9]. After a phytotoxin is isolated into pure form, the next step is to elucidate its structure. This could be done via several physical methods including mass spectrometry (MS), infrared spectroscopy (IR), ultraviolet-visible spectroscopy (UV-vis), X-ray diffraction or nuclear magnetic resonance spectroscopy [10].

Table 1: Advantages and disadvantages of chromatography methods

Technique	Advantages	Disadvantages
Thin layer chromatography	• The separation process could be carried out in a short time. • Several samples could be analyzed simultaneously. • Low solvent usage (per sample). • High accuracy and precision.	• The findings were hard to reproduce. • Not an automatic process, and this technique belongs to qualitative analysis. • The obtained results were strongly depending on temperature and humidity.
High performance chromatography	• High resolution. • Reproducible. • Automated analysis. • Reusable columns.	• Costly. • Complexity. • Low sensitivity.
Counter current chromatography	• No on-column absorption. • The process was rapid. • The obtained results are versatile. • No expensive stationary phase is needed. • No solid phase disposal complications.	• Flow rates were very low. • Poor mixing was observed.
Flash column chromatography	• Economical and fast process. • Large amounts	• Irreversible adsorption of solute happened. • Time consuming.

	(samples) could be separated. • A wide range of mobile phases could be employed.	• Costly.
Ion exchange chromatography	• High flow rate could be observed. • The buffer was non-denatured. • High yield could be seen.	• Binding profile could be affected by pH value. • The resolution was influenced by particle size. • All the samples should be loaded at low ionic strength.
Vacuum column chromatography	• Efficient and rapid technique.	

In this book chapter, several techniques have been used to detect phytotoxins. The chromatography method biomass essay has been employed for the characterization of the obtained samples. Results will be briefly discussed based on the published papers.

II. LITERATURE SURVEY

Phytotoxic fungi can be identified and then isolated by direct and indirect means. Literature survey showed that, the direct means involves the transfer of the pathogenic element such as hyphae, spores, sclerotia, rhizomorphs from the host plant tissues infected after surface sterilization [11-15] while the indirect means involves selecting parts of the infected plant tissues to form colonies after surface sterilization. To achieve this, fungi are relocated to petri dishes (consisted of solid media) for the isolation and production of metabolites. It is cultured at a certain time for mycelial and spore biomass to be formed then the solid culture fragments are transferred to a growth medium to subculture the fungi [16-18] or a suspension of spores and mycelium can be transferred [19]. For this process to be cultivated properly, a liquid or solid culture medium could be used [20]. Improper use of culture media composition may result difficulty in isolating the phytotoxins. In this regard, it is preferred to use a medium which is synthetic or semi-synthetic. Also, some factors can be considered such as culturing the fungus under different conditions such as using various culture media.

Several studies have reported that the production of phytotoxins can be affected by various factors such as the composition of the media, temperature, type of cultivation (static or shaken) as well as the time of cultivation. It is noticed that a fungus cultured in a malnourished media did not form as many phytotoxins as compared to when in a properly nourished media. Rao and co-workers [21] observed that the synthesis of fumonisin B1 from (Fusarium moniliforme) was affected by composition of the culturing media.

Before embarking on extracting a phytotoxin, it is wise to screen for the plant part that is rich with phytotoxins. Plant parts such as seeds, leaves, fruits are the richest with phytotoxins. When plant pathogens are being studied for their phytotoxins, the host plant can

be assayed for their toxicity. To extract phytotoxin compound, it should first be subjected to an aqueous medium which can be distributed in liquid and solid phases as reported by Barrow [22]. Then filter, by subjecting to filtration membranes such as nylon membrane or cotton cloth or gauze. Filter papers are used for further filtering the media [23, 24]. Centrifugation can also be used to separate the solid and liquid phases. The filtrate is then subjected to extraction techniques such as liquid-liquid extraction or liquid-solid extraction. Organic solvents are used for liquid-liquid extraction while polymeric adsorbent resins are used for liquid-solid extraction [25-28] Alternatively, the filtrate can be loaded onto the chromatographic column for separation and identification of phytotoxins using polymeric adsorbent resin [29, 30].

When performing the extraction with organic solvents, it is advisable to reduce the volume of culture filtrate to enhance the efficiency of the extractions. To achieve this, the filtrate is centrifuged in a vacuum. This can be done until a required volume is arrived and obtained [31]. Salts like sodium chloride or other inorganic salts can be added to the culture filtrates to enhance the efficiency of extracts and to help in preventing the emulsion formations between the aqueous phase and the organic solvent [32, 33]. Organic extracts have been found to contain some number of other organic compounds which may probably be a constituent of the fungi or the culture medium. To separate the phytotoxins, chromatography techniques are mostly deployed.

To extract phytotoxin using chromatographic techniques, the filtrate obtained from the extraction is fractionated in a column chromatography where the eluents are then analyzed using analytical thin layer chromatography or ultraviolet-visible spectroscopy. At this point, the fractions obtained through these techniques may still contain a mixture of compounds and may interfere in the complete identification of phytotoxins. These fractions are then further subjected to fractionation until phytotoxins are completely isolated. On the other hand, another significant process is the use of ion exchange resins to determine the ionic nature of the phytotoxin [34]. Nonpolar solvents and organic solvents (methanolare) have been used to remove non-polar compounds and proteins, respectively [35, 36].

After partial isolation of phytotoxin, it is then subjected to bioassay technique to obtain a pure form. It is good to use a bioassay that is fast, inexpensive, simple, and reproducible [37]. This is because, if the concentration of phytotoxin is low in the culture media the bioassay is sensitive enough to detect the toxicity [38, 39]. For instance, a study was conducted to ascertain the causative agent of the leaf blight of garlic (Stemphyliumsolani).After determining its phytotoxicity, the toxin was extracted using ethyl-acetate where its isolation was done via bioassay guided thin layer chromatography then purified using preparative liquid chromatography [40].

After successful isolation of a phytotoxin, it is necessary to get its full identity. According to researcher [41-43], this could be done by some physical methods including X-ray diffraction technique, mass spectrometry (MS), infrared spectroscopy (IR), ultraviolet-visible spectroscopy (UV-vis), and nuclear magnetic resonance spectroscopy (Figure 2**)**. Other advanced techniques include ELISA and PCR. A successful study by Bultreysand co-workers [44] used PCR to detect strains of pseudomonas syringae which produce toxic lipodepsipeptide in plants. PCR can be used alongside Restriction Fragment Length Polymorphism [45].

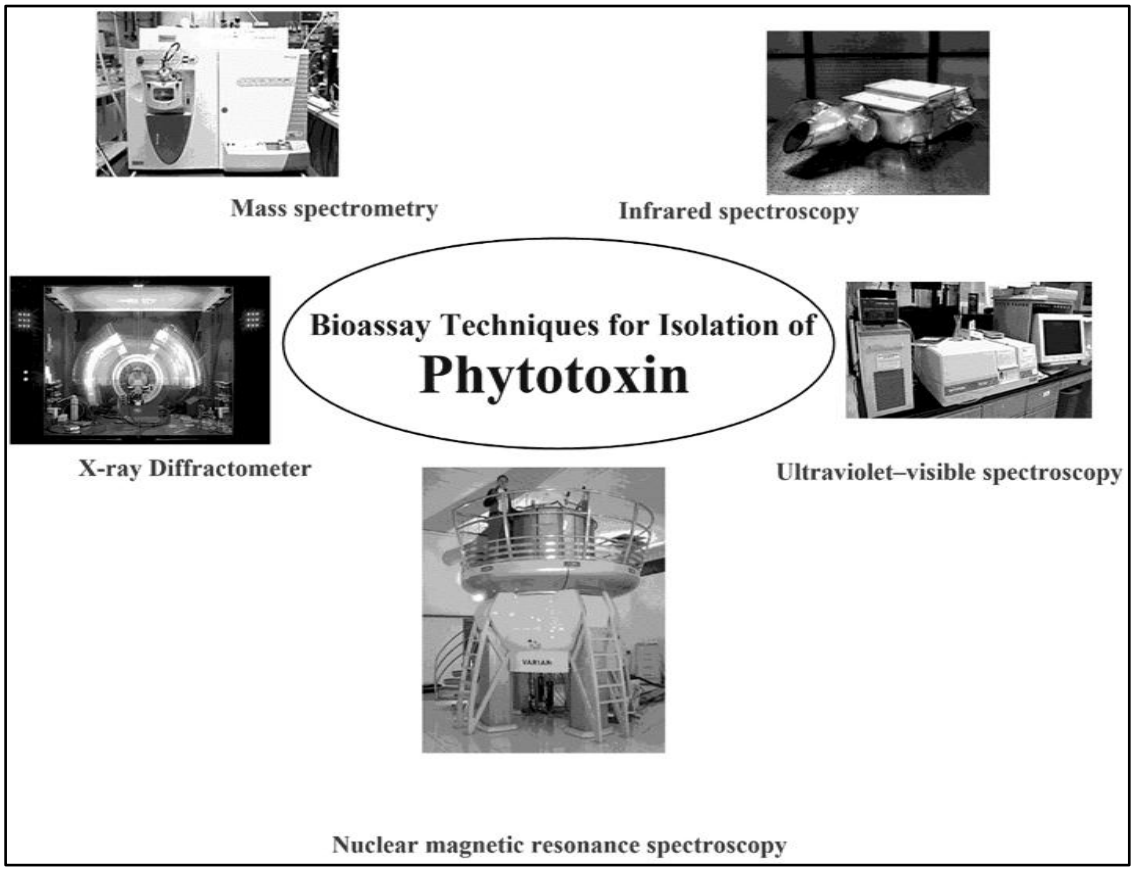
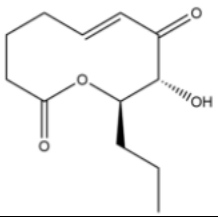
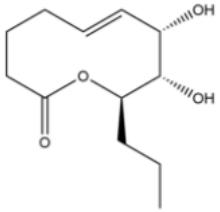
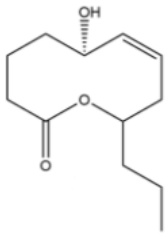
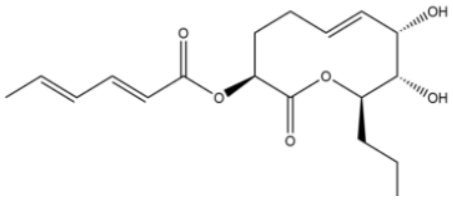
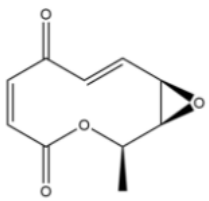
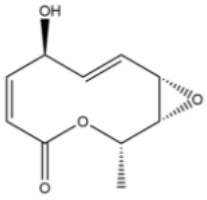
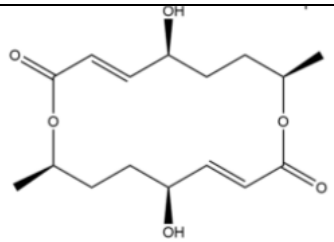
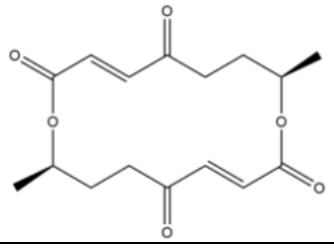
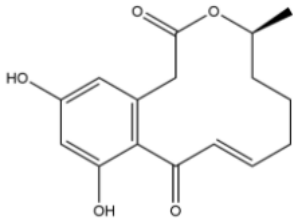
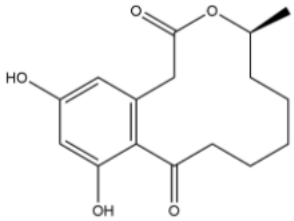
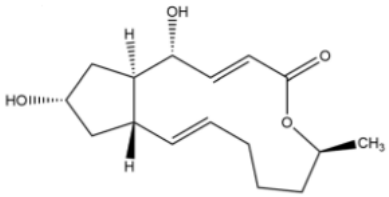


Figure 2: Bioassay Techniques for Isolation of Phytotoxins

Tyutereva and co-workers [46] reported that stagonolide A and herbarumin I have different mechanisms of action. HBI could inhibit carotenoid biosynthesis, while stagonolide A could inhibit intracellular vesicular traffic (could be observed from endoplasmic reticulum to Golgi). Table 2 shows several examples of phytotoxic 10-membered lactones. Morphological changes could be seen in the root cells (Arabidopsis seedlings). Experimental results (figure 3) confirmed that significant destructive effect in the presence of stagonolide A, while herbarumin I resulted in few cells of elongation zone (partially plasmolysis). Mathieu and co-workers [47] studied three phytopathogenic fungi (Table 3) in vitro anti-cancer activity. They observed that radicinin has potential to be used as bioherbicides and anticancer agents if compared to other fungal phytotoxins (figure 4). Samperna and co-workers [48] pointed out that SeiridiumCupressi could form two major toxins, namely cyclopaldic acid (figure 5) and seiricuprolide. Cyclopaldic acid was synthesized using fungal (Seiridium), indicated herbicidal, insecticidal, and fungicidal properties. The biochemical assays indicated this metabolite inhibited root proton extrusion in vivo, induced ion leakage, leaf chlorosis and hydrogen peroxide production. The secondary metabolites (Fusarium oxysporum) were isolated from the plant (Aglaonema hookerianum Schott) as described by Hoque and co-workers [49]. Several compounds (compound 1,2, 5) were found to be cytotoxic agents (figure 6), while other compounds (compound 3, 4) were acted as highly mutagenic based on silico analysis. The obtained results showed that compound 1 could protect kidney cells. Compound 2 has hydroxyl moiety, resulting in great impact on the absorption and solubility profiles. Compound 5 was identified as lipophilic, active against pancreatic carcinoma, breast adenocarcinoma and glioblastoma cells.

Table 2: Structure, Formula and Molecular Weight of Phytotoxic

	Structure	Molecular formula	Molecular weight
stagonolide A		$C_{12}H_{18}O_4$	226.27 g/mol
herbarumin I		$C_{12}H_{20}O_4$	228.28 g/mol
putaminoxin		$C_{12}H_{20}O_3$	212.28 g/mol
pinolidoxin		$C_{18}H_{26}O_6$	338.4 g/mol
Pyrenolide A		$C_{10}H_{10}O_4$	194.18 g/mol
stagonolide H		$C_{10}H_{12}O_4$	196.2 g/mol

pyrenophorol		$C_{16}H_{24}O_6$	312.36 g/mol
pyrenophorin		$C_{16}H_{20}O_6$	308.33 g/mol
10,11-dehydrocurvularin		$C_{16}H_{18}O_5$	290.3 g/mol
curvularin		$C_{16}H_{20}O_5$	292.33 g/mol
brefeldin A		$C_{16}H_{24}O_4$	280.36 g/mol

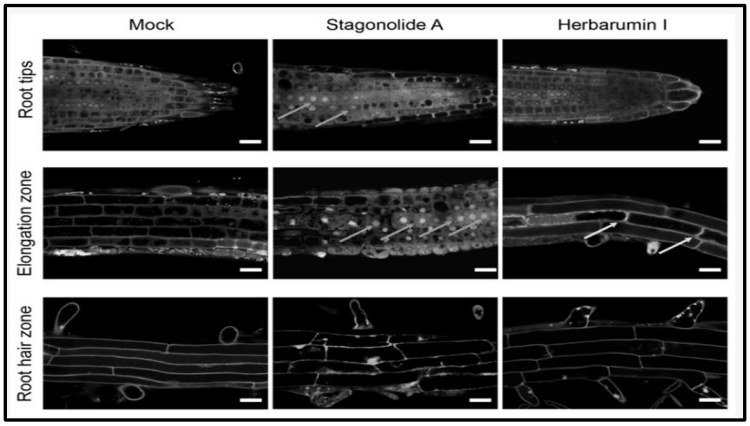


Figure 3: Morphological changes of Arabidopsis seedlings (in the root cells), in the presence of stagonolide A or herbarumin I. [46]

Table 3: Metabolites Isolated From Three Phytopathogenic Fungi, Namely Kalmusiavariispora, Cochliobolusaustraliensis, and Hymenoscyphusfraxineus [47].

Fungus	Host Plant	Disease	Metabolite
Cochliobolus australiensis	Buffelgrass (*Cenchrus ciliaris* L.)	Leaf Spots	Radicinin
			Radicinol
			3-*epi*-Radicinin
			Cochliotoxin
			Chloromonilinic acid B
			Chloromonilinic acid C
			Chloromonilinic acid D
Hymenoscyphus fraxineus	Ash (*Fraxinus excelsior* L.)	Dieback	Viridiol
			1-Deoxyviridiol
			Demethoxyviridiol
			Nodulisporiviridin M
			Hyfraxinic acid
Kalmusia variispora	Grapevine (*Vitis vinifera* L.)	Trunk disease	Massarilactone D
			Massarilactone H

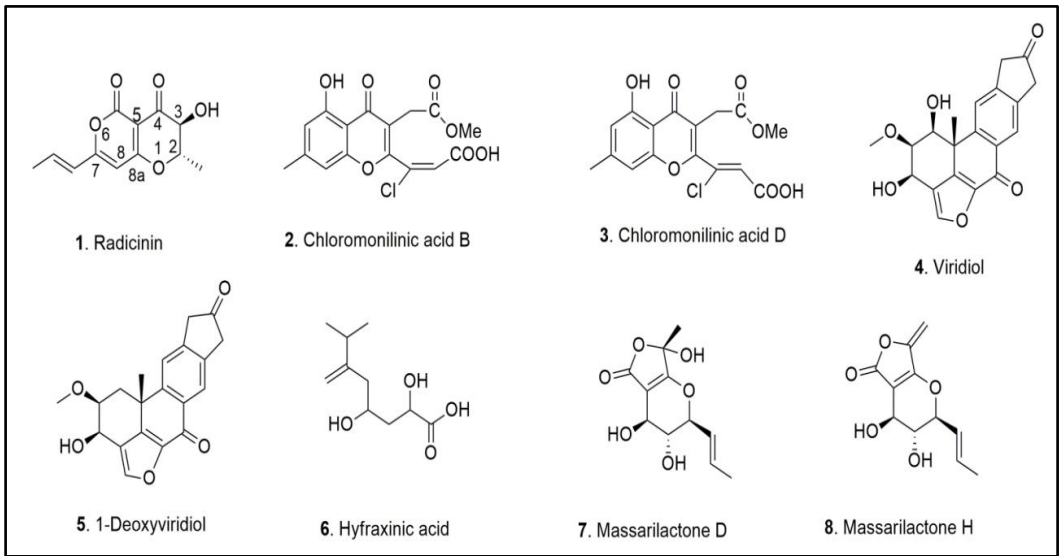


Figure 4: Several types of fungal phytotoxins with different carbon skeletons [47].

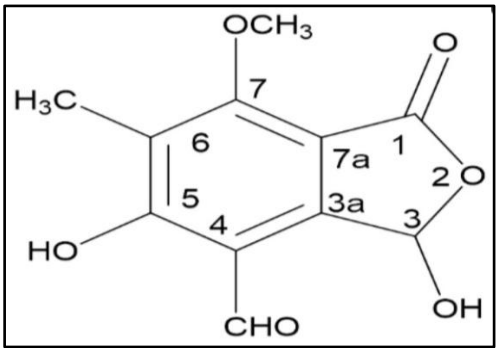


Figure 5: Structure of cyclopaldic acid

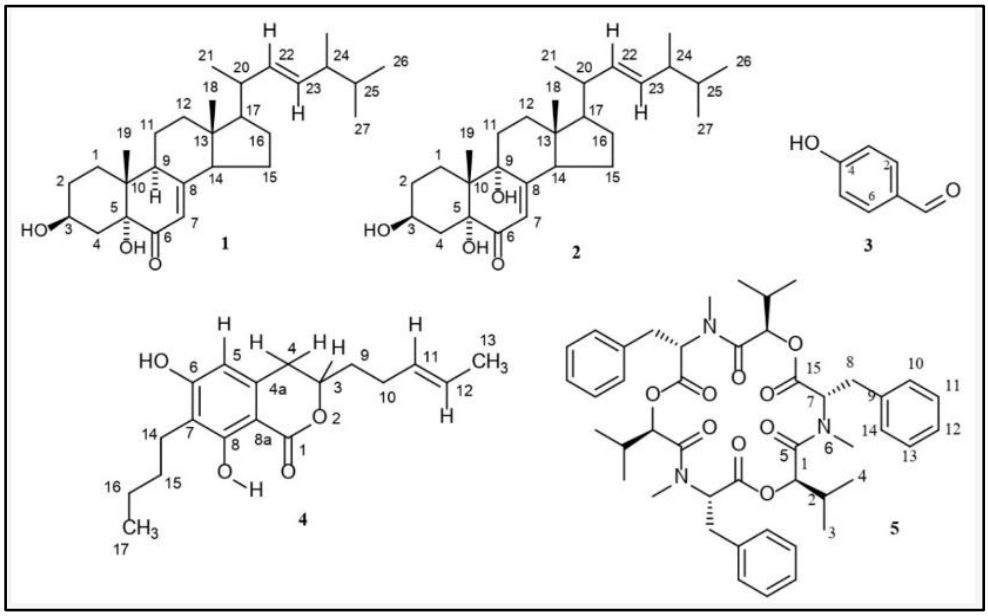


Figure 6: Several compounds could be isolated from Fusarium Oxysporum [49]

III. CONCLUSIONS

Phytotoxins are chemical compounds secreted by some microorganisms hosted by plants or by the plants as their defense mechanism. These chemical compounds on the other hand may impose danger on the plants or to animals and humans that are exposed to it. Herein, techniques for both identification and isolation of phytotoxins are highlighted. Chromatographic techniques or ultraviolet-visible spectroscopy are most used for the identification and isolation of phytotoxins while ultraviolet-visible spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry, infrared spectroscopy, X-ray diffraction among other are highly used for the elucidation. Careful and diligent applications of these techniques may lead to successful identification, isolation, and elucidation of phytotoxin from the pool of other compounds for effective utilization.

IV. ACKNOWLEDGEMENT

This research work [Ho SM] was supported by INTI INTERNATIONAL UNIVERSITY.

REFERENCES

[1] H. Kato, “Phytotoxic Substances Involved in Teak Allelopathy and Agroforestry,” Applied Sciences, https:// doi.org/10.3390/app11083314, 2021.
[2] P. Bora, J. Saikia, P. Saikia, and H. Saikat, “Evaluation of Postharvest Drying, Key Odorants, and Phytotoxins in Plai (Zingiber montanum) Essential Oil,” Journal of Agricultural and Food Chemistry, 69, 5500-5509, 2201.
[3] J. Tang, H. Ling, L. Yu, and H. Shao, “Two Phytotoxins Isolated from the Pathogenic Fungus of the Invasive Weed Xanthium italicum,” Chemistry & Biodiversity, https://doi.org/10.1002/cbdv.202000043, 2020.
[4] G.A. Strobel, “Phytotoxins,” Annual Review of Biochemistry, 51, 309-333, 1982.

[5] X. Liang, H. Jan, and N. Juul, "Source-Supported Suspect Screening (4S) of Phytotoxins in Terrestrial and Aquatic Environments: A Field Study of Lupinus angustifolius L. (Blue Lupin),"Environmental Science & Technology, 57, 2333-2340, 2023.
[6] P. Bhaskar, M. Lal, and H. Saikat, "Phytotoxic essential oil from black turmeric (Curcuma caesia Roxb.) rhizome: Screening, efficacy, chemical basis, uptake and mode of transport," Industrial Crops and Products, https://doi.org/10.1016/j.indcrop.2022.114788, 2022.
[7] M. Macías, and Y. Garrido, "Phytotoxic compounds from endophytic fungi," Applied Microbiology and Biotechnology, 106, 931–950, 2022.
[8] N. Chaves, T. Sosa, and J. Alías, "Identification and Effects of Interaction Phytotoxic Compounds from Exudate of Cistus ladanifer Leaves," Journal of Chemical Ecology, 27, 611–621, 2001.
[9] E. Varejão, J. Demuner, A. Barbosa, and R. Barreto, "The search for new natural herbicides–Strategic approaches for discovering fungal phytotoxins," Crop Protection, 48, 41–50, 2013.
[10] L. Pavia, M. Lampman, J. Kriz, and R. Vyvyan, "Introduction to Spectroscopy, fourth ed. Brooks/Cole Cengage Learning, Belmont.
[11] J. Robeson, and A. Strobel, "Monocerin, a phytotoxin from Exserohilumturcicum," Agricultural Biology and Chemistry, 46, 2681-2683, 1982.
[12] D. Smith, "Culturing, preservation and maintenance of fungi. In: Waller, J.M., Lenné, J.M., Waller, S.J. (Eds.), Plant Pathologists' Pocketbook. CABI Bioscience, Egham, pp. 384-409, 2001.
[13] S. El-Sayed, "Production of thaxtomin A by two species of Streptomyces causing potato scab," Folia Microbiologica, 45, 415-422, 2000.
[14] M. Zerroug, Z. Bouznad, L. Larous, and R. Strange, R.N., "Production of solanapyrone A by Algerian isolates of Ascochytarabiei as the cause of the toxicity of culture filtrates to chickpea (Cicer arietinum) seeds and seedlings," Journal of Plant Pathology, 89, 227-232, 2007.
[15] W. Chen, and W. Swart, "The in vitro phytotoxicity of culture filtrates of Fusariumoxysporum to five genotypes of Amaranthushybridus," Euphytica, 127, 61-67, 2002.
[16] H. Löffler, and R. Mouris, "Fusaric acid: phytotoxicity and in vitro production by Fusariumoxysporum f. sp. lilii, the causal agent of basal rot in lilies," Netherland Journal of Plant Pathology, 98, 107-115, 1992.
[17] B. Andersen, A. Dongo, andM. Pryor, "Secondary metabolite profiling of Alternariadauci, A. porri, A. solani, and A. tomatophila," Mycological Research, 112, 241-250, 2008.
[18] O. Berestetskiy, O. Yuzikhin, S. Katkova, and A. Dobrodumov, "Isolation, identification, and characteristics of the phytotoxin produced by the fungus Alternariacirsinoxia," Applied Biochemistry and Microbiology, 46, 75-79, 2010.
[19] A.Cimmino, A. Andolfi, A. Beretetskiy, and A. Evidente, "Production of phytotoxins by Phomaexigua var. exigua, a potential mycoherbicide against perennial thistles," Journal of Agricultural and Food Chemistry, 56, 6304-6309, 2008.
[20] E. Li, R. Tian, S. Liu, and X. Chen, "PestalotheolsAeD, bioactive metabolites from the plant endophytic fungus Pestalotiopsistheae," Journal of Natural Products, 71, 664-668, 2008.
[21] N. Rao, V. Reddy, S. Girisham, and S. Reddy, "Factors influencing fumonisins (B1) production by Fusariummoniliforme," Indian Journal of Science and Technology, 3, 213-215, 2010.
[22] Barrow, R.A., 2006. Isolation of microbial natural products. In: Sarker, S.D., Latif, Z., Gray, A.I. (Eds.), Natural Products Isolation, second ed. Humana Press, New Jersey, pp. 27-46.
[23] A.Evidente, A. Andolfi, and M. Vurro, "Drazepinone, a trisubstitutedtetrahydronaphthofuroazepinone with herbicidal activity produced by Drechslerasiccans," Phytochemistry, 66, 715-721, 2005.
[24] C. Cruz-Cruz, K. García, andF. Escalante, "Production of hydrophilic phytotoxins by Mycosphaerellafijiensis," Journal of General Plant Pathology, 75, 191-195, 2009.
[25] L. Buchwaldt, and H. Green, "Phytotoxicity of destruxin B and its possible role in the pathogenesis of Alternariaalternata," Plant Pathology, 41, 55-63, 1992.
[26] R.Suemitsu, K. Ohnishi, and M. Horiuchi, "Isolation and identification of 6-(3',3'-dimethylallyloxy)-4-methoxy-5-methylphthalide from Alternariaporri," Bioscience, Biotechnology, and Biochemistry, 56, 986, 1992.
[27] P. Nussbaum, W. Günther, and S. Heize, "New tricycloalternarenes produced by the phytopathogenic fungus Alternaria alternate," Phytochemistry, 52, 593-599, 1999.
[28] M. Horiuchi, K. Ohnishi, and N. Iwase, "A novel isoindoline, porritoxin sulfonic acid, from Alternariaporri and the structure-phytotoxicity correlation of its related compounds," Bioscience Biotechnology Biochemistry, 67, 1580-1583, 2003.
[29] M. Gardner, Y. Kono, H. Tatum, and Y. Suzuki, "Structure of the major component of ACRL-toxins, host-specific pathotoxiccompounds produced by Alternariacitri," Agricultural Biology and Chemistry, 49, 1235-1238, 1985.

[30] S. Chen, X. Xu, and X. Dai, "Identification of tenuazonic acid as a novel type of natural photosystem II inhibitor binding in QB-site of Chlamydomonasreinhardtii," Biochimica et BiophysicaActa Bioenergetics, 1767, 306-318, 2007.
[31] A.Andolfi, A. Boari, A. Evidente, and M. Vurro, "Metabolites inhibiting germination of Orobancheramosa seeds produced by Myrothecium verrucaria and Fusarium compactum," Journal of Agricultural and Food Chemistry, 53, 1598-1603, 2005.
[32] M. Peña, A. Armingeon, andS. Chilton, "Toxins from weed pathogens, I. Phytotoxins from a Bipolaris pathogen of Johnson grass," Journal of Natural Products, 51, 821-828, 1988.
[33] A.Evidente, A. Cimmino, A. Berestetskiy, and G. Mitina, "Stagonolides B-F, nonenolides produced by Stagonosporacirsii, a potential mycoherbicide of Cirsiumarvense," Journal of Natural Products, 71, 31-34, 2008.
[34] Sarker, S.D., Latif, Z., Gray, A.I., 2006. Natural Products Isolation, second ed. Humana Press, New Jersey.
[35] B.Bashan, S. Levy, andM. Cojocaru, "Purification and structural determination of a phytotoxic substance from Exserohilumturcicum," Physiological and Molecular Plant Pathology, 47, 225-232, 1995.
[36] A.Graniti, L. Sparapano, and A. Evidente, "Cyclopaldic acid, a major phytotoxic metabolite of Seiridiumcupressi, the pathogen of a canker disease of cypress," Plant Pathology, 41, 563-568, 1992.
[37] Hoagland, R.E., Williams, R.D., 2003. Bioassays - useful tools for the study of allelopathy. In: Macías, F.A., Galindo, J.C.G., Molinillo, J.M.G., Culter, H.G. (Eds.), Alellopathy e Chemistry and Mode of Action of Allelochemicals. CRC Press, Boca Raton, pp. 315-351.
[38] M. Vurro, A. Evidente, A. Andolfi, and C. Zonno, "Brefeldin A and a,b-dehydrocurvularin, two phytotoxins from Alternariazinniae, a biocontrol agent of Xanthium occidentale," Plant Science, 138, 67-79, 1998
[39] O.Yuzikhin, G. Mitina, and A. Berestetskiy, "Herbicidal potential of stagonolide, a new phytotoxic nonenolide from Stagonosporacirsii," Journal of Agricultural and Food Chemistry, 55, 7707-7711, 2007.
[40] L. Zheng, R. Lv, and J. Huang, "Isolation, Purification, and Biological Activity of a Phytotoxin Produced by Stemphyliumsolani," Plant Disease, 94, 1231–1237, 2010.
[41] M. Islam, F. Zaman, and A. Iwasaki, "Isolation and identification of three potential phytotoxic compounds from Chrysopogon aciculatus (Retz.) Trin," Acta Physiologiae Plantarum, https://doi.org/10.1007/s11738-021-03221-5, 2021.
[42] C. Hartung, G. Nair, and R. Putnam, "Isolation and characterization of phytotoxic compounds from asparagus (Asparagus officinalis L.) roots," Journal of Chemical Ecology, https://doi.org/10.1007/BF01014102, 1990.
[43] D. Bouknana, S. Jodeh, and M. Sbaa, "A phytotoxic impact of phenolic compounds in olive oil mill wastewater on fenugreek "Trigonella foenum-graecum," Environmental Monitoring and Assessment, https://doi.org/10.1007/s10661-019-7541-x, 2019.
[44] A.Bultreys, and I. Gheysen, "Biological and molecular detection of toxic lipodepsipeptide-producing Pseudomonas syringae strains and PCR identification in plants," Appliedand Environmental Microbiology, 65,1904-1909, 1999.
[45] H. Phan, R. Ford, and T. Bretag, "A rapid and sensitive polymerase chain reaction (PCR) assay for detection of Ascochyta rabiei, the cause of ascochyta blight of chickpea," Australasian Plant Pathology, 31, 31–39, 2002.
[46] E. Tyutereva, A. Dalinova, and K. Demchenko, "Effects of Phytotoxic Nonenolides, Stagonolide A and Herbarumin I, on Physiological and Biochemical Processes in Leaves and Roots of Sensitive Plants," Toxins, https://doi.org/10.3390/ toxins15040234, 2023.
[47] V. Mathieu, S. Superchi, and M. Masi, "In Vitro Effects of Fungal Phytotoxins on Cancer Cell Viability: First Insight into Structure Activity Relationship of a Potent Metabolite of Cochliobolus australiensis Radicinin," Toxins, https://doi.org/ 10.3390/toxins14080517, 2022.
[48] S.Samperna, M. Masi, and M. Vurro, "Cyclopaldic Acid, the Main Phytotoxic Metabolite of Diplodia cupressi, Induces Programmed Cell Death and Autophagy in Arabidopsis thaliana," Toxins, 2022, 14, 474. https:// doi.org/10.3390/toxins14070474, 2022.
[49] N.Hoque, F. Afroz, and F. Khatun, "Physicochemical, Pharmacokinetic and Cytotoxicity of the Compounds Isolated from an Endophyte Fusarium oxysporum: In Vitro and In Silico Approaches," Toxins, https://doi.org/10.3390/ toxins14030159, 2022.

