

Fluorescence emission based spectral signatures from endogenous flurophores from *Lantana camara*.

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Introduction

Under natural conditions, plants are exposed to a number of abiotic stresses. To counteract these stresses, eukaryotic and prokaryotic cells accumulate a number of auto-fluorescent molecules that produce fluorescence when excited with UV light, yielding information on their accumulation and function inside the cells [1]. Most important endogenous flurophores are molecules widely distributed in cells and tissues, like secondary metabolites like: pterins, some phenols, alkaloids, flavins, proteins containing aromatic amino acids and lipopigments [2]. Many enzymatic cofactors, such as FMN, FAD, NAD and porphyrins, which are also intrinsically fluorescent, add to the protein fluorescence play important roles in the cellular energy metabolism. Plants produce a vast variety

Abstract

Lantana camara is a noxious and invasive weed from family *Verbenaceae*, native to tropical America, but now widespread in many countries. However, there has been little research on its auto-fluorescence producing flurophores that play very crucial biological role inside the cells. In the present study, the fluorescence based spectral fingerprint analysis of leave extract was studied in *Lantana camara*. A peculiar pattern of fingerprints were observed, indicating accumulation of metabolites and enzymes in cells. Further, as per the excitation λ , different fingerprints were detected. Based on these findings possible role of spectral fingerprints in correlation with accumulation pattern of metabolites was discussed.

Keywords: Endogenous flurophores, Fluorescence, invasive plants, *Lantana camara*

of secondary metabolites in response to interaction with environment which confer resistance to them. Hence it was postulated by many authors that plant resistance to abiotic stress can be monitored by the accumulation pattern/ fingerprint of endogenous.

Lantana camara, belongs to *Verbenaceae*, world's 10 worst weeds, is an ornamental and noxious weed as it harms other plants, crop productivity, animals, organisms, now widespread in North America, many parts of Australia, Africa and India [3]. *Lantana* can grow in a wide range of warm environment like tropical, sub-tropical, temperate areas, forestlands, along roadsides, etc. It can also grow in various soil types and has high reproductive capability and adaptability to extreme stress conditions. There is very little knowledge



about fluorescence based fingerprint analysis and related mechanisms involved in the tolerance of *Lantana camara*. We hypothesised that fluorescence based

methods is the key technique to study fluorescence producing secondary metabolites in *Lantana camara*.

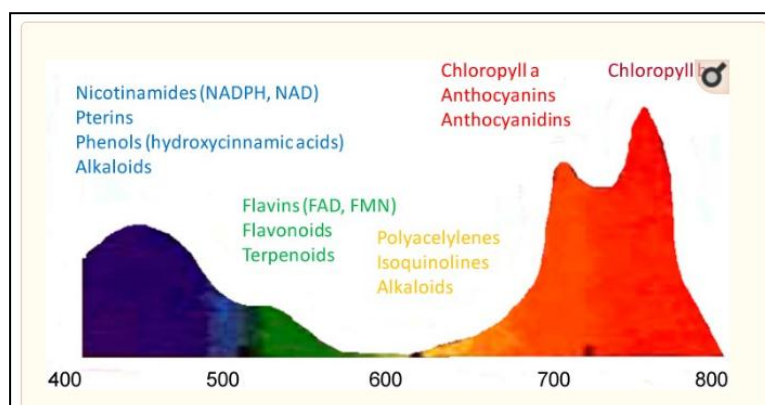


Fig. 1: Fluorescence emission spectrum of a typical green leaf under UV-radiation ($\lambda_{exc.} = 355 \text{ nm}$). The fluorescence bands in blue (430–450 nm), green (520–530 nm), red (680), and far-red (740 nm) regions, adapted from, adapted from [1]

Material and methods

Plant material

Lantana camara growing under natural conditions, under vicinity of Jalandhar located at 71°31 east latitude and 30°33' north longitude, were collected. Sample selection was random to avoid any bias. Leaves were collected and then pooled and used together. The experiments were designed in a complete randomized way with 3 replicates.

Fluorescence spectroscopy analysis (FSA)

FSA of plant extract was carried out as described in Sharma et al [4]. Briefly, tissue (leaves) was sun dried and ground to powder. Extract were prepared by grinding 1 g of the tissue in 5 ml of Tris buffer (1 mM, pH 8.0). The final volume taken for FSA analysis was 1.0 ml. The fluorescence spectrum of each sample was then measured on Perkin Elmer Spectrophotometer

(FL6500). All experiments were done at room temperature ($\sim 30^\circ\text{C}$).

Results and Discussion

Auto-fluorescent molecules are abundant in plant cells and their spectral images often used to analyze their spectra which give information about their accumulation and function under environmental conditions. Secondary metabolites and proteins are exclusively modulated in plants in response to interaction with environment [5]. However, yielding information about their fluorescent based analysis in *Lantana camara* is still not known. In the present study, laser induced fluorescence emission spectrum of extract from leaves of *Lantana camara* was studied. It was postulated that metabolites and enzymes involved in the biosynthesis have been localized in a variety of plant cell [6]. Endogenous fluorophores are particularly abundant in plant tissue and

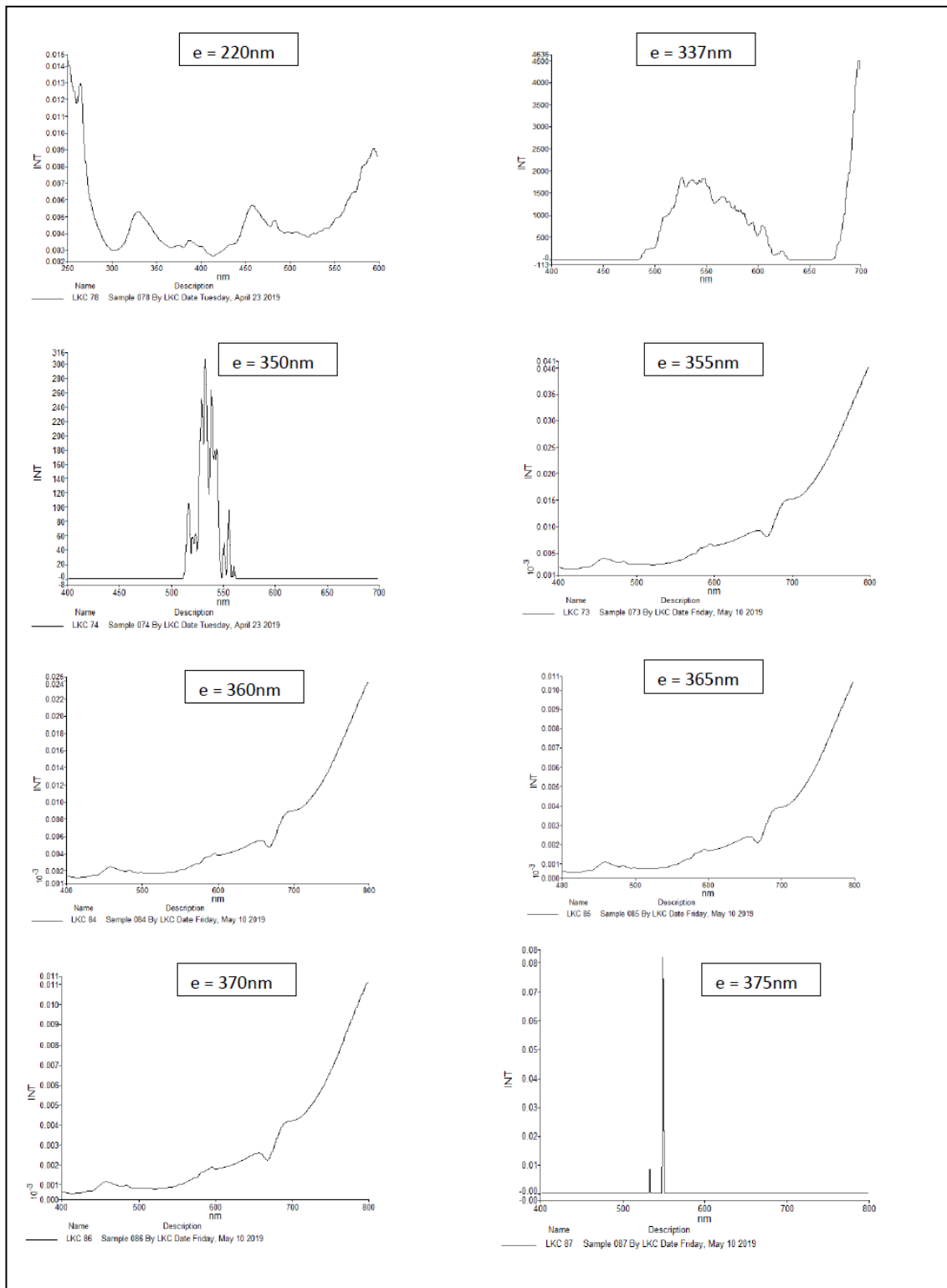


Fig: 2: Fluorescence emission spectral fingerprint from *Lantana camara*



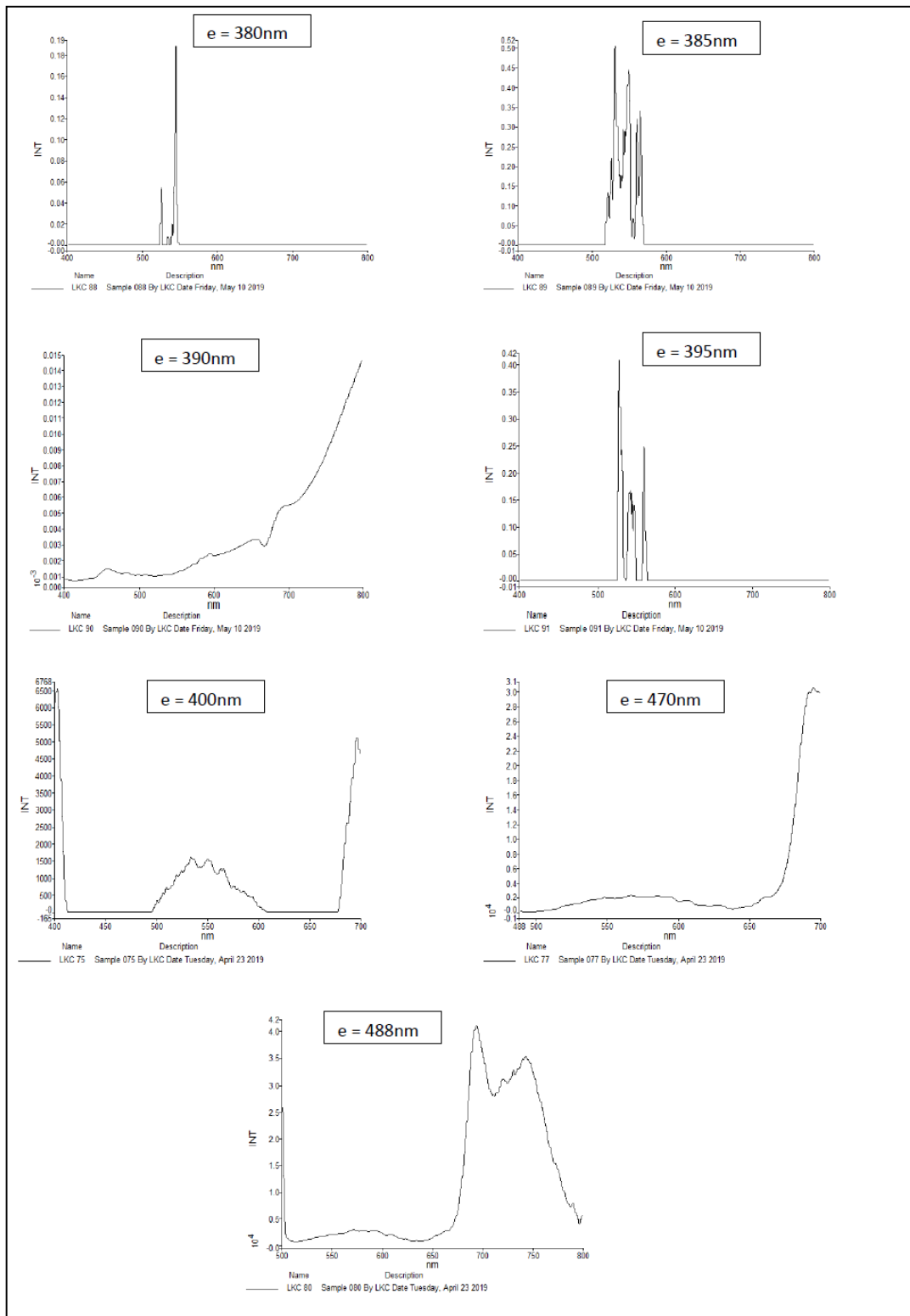


Fig: 1: Continued...



are involved in structural and metabolic functions at cell and tissue level. [23]. As shown in Fig 1, different spectral signatures were detected as per the excitation maxima. Earlier studies have shown that different metabolites when excited with radiation of suitable wavelength, molecules widely distributed in cell and tissue, like protein and enzymes, behave as endogenous fluorophores [7]. The relative pattern of this fluorescence is highly sensitive and species-specific and depends on environmental factors. For instance: when Lantana leave extract was excited at wavelength λ : 400; 488, a peculiar spectral fingerprint was detected in blue fluorescent (BF) region. It was reported that candidates for BF emission (λ near 450 nm) are phenolic substances such as NADPH, NAD, Pterins, chorogenic acid, caffeic acid, coumarins (aesculetin, scopoletin) and stilbenes (t-stilbene, rhaponticin) and alkaloids[8]. Based on these findings it can postulated that secondary metabolites could be responsible for invasiveness of this plant under abiotic conditions. Further studies are underway to analyze laser induced spectral emission under natural conditions at different developmental stages.

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Conflict of interest

Authors declares no conflict of interest

Compliance with Ethical Standards

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or

human participants performed by any of the authors

Author contributions

ADS: designed the study and prepared manuscript

PN: performed expts

NS: helped in performing expts

References

1. Talamond, P. Verdeil JL, Conejero G. 2015. Secondary metabolite localization by auto-fluorescence in living plant cells. *Molecules*, 20, 5024-5037.
2. Sander RW. 2006. Taxonomy of Lantana sect. Lantana (Verbenaceae). I. Correct application of Lantana camara and associated names, SIDA, *Contrib. Bot.* 22:381-421
3. Mylle E, Codreanu MC, Boruc J, Russinova E. 2013. Emission spectra profiling of fluorescent proteins in living plant cells. *Plant Methods*. 9:1-8.
4. Sharma AD, Nischal P, Singh N, Nanda JS. 2019. Fluorescence spectroscopy based folding-unfolding of Protein [Bovine Serum Albumin (BSA)]: an UG/PG experiment in Biotechnology. *Res Rev. Biotechnol. Biosci.*, 1: 1-4.
5. Hartmann T. 2007. From waste products to ecochemicals: Fifty years research of plant secondary metabolism. *Phytochemistry*. 68:2831-2846.
6. Littlejohn GR, Meckel T, Scharzländer M, Costa A. 2014. Functional imaging in living plants—cell biology meets physiology. *Front. Plant Sci.* 5:1-3.
7. Conejero G, Talamond P, Verdeil JL. 2010. A new approach to visualize secondary metabolites in plants; Proceedings of the FESPB, XVII Congress of Federation of European Societies of Plant Biology; Valencia, Spain. 4-9 July 2010.
8. Lang M, Stober F, Lichtenthaler HK. 1991. Fluorescence emission spectra of plant leaves and plant constituents *Radiat. Environ. Biophys.*, 30: 333-47