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Ingestion Effect of pathogens on Primary Metabolites of *Tinospora cordifolia*.

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ABSTRACT

Plants play many important roles in the environment. Not only do they provide us with food and useful products, but also they play a main role in maintaining the ecological balance of nature, the food chain and the natural cycles and also have medicinal value. Laboratory evaluations were made to assess the study of primary metabolites of *Tinospora cordifolia*, belongs to the family menispemeaceae. It contains chlorophyll, sugars, starch, protein, ascorbic acid and phenols in, leaves, stem, root and callus of the plant. Levels of plant metabolites are strongly affected via genetic and environmental factors. Different types of pests' are causing changes in plant metabolite production. The results revealed the evidence of different infestation of the plant by common herbivores. In this manuscript we report primary metabolites of the *Tinospora cordifolia* along with the quantification after the pests' effect.

Keywords: *Tinospora cordifolia*, menispemeaceae Chlorophyll, Protein, Ascorbic acid, sugar, starch and callus.

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INTRODUCTION

India is a country known for ancient scripts, the number system, invention of zero and Vedas. Medicines in India are used by about 60 per cent of the world's population. These are used for primary health care not just in rural areas, in developing countries, but in developed countries as well, where modern medicines are predominantly used. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment, of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal (Singh and Ahirwar, 2010)¹.

India is a known mega-diversity centre harbouring a multitude of medicinal plant species, each apparently studded with as yet unidentified genetic and chemical verities of economic importance. Approximately 17,000 higher plant species occurring in India, more than 1000 species are used in the traditional systems of medicine such as- Ayurveda, Siddha and Unani. The villagers and tribal folks extended throughout the country to make use of more than 7000 plant species through oral traditions (Thyagarajan *et al.*, 2002, Shankar and Rawat, 2006, Swapna and Rao, 2013)²⁻⁴. Approximately 75% medicinal products used in the world are available in India. Therefore, the rich and varied plant diversity, especially the genetic diversity of medicinal and aromatic plants, is one of India's important strengths and is the bedrock for all future bio-industrial developments. Unfortunately, the renowned medicinal plant wealth of India has seldom been subjected to genetic scrutiny, keeping in mind the latent and patentable properties and economic utility of the selected plant types. Severe habitat losses and consequent reduction and extinction of well-known to lesser known species of economic value are not uncommon in the Indian subcontinent. It is imperative that heritable variations within the unimproved natural populations of prospective taxa are studied for selection, improvement and development of suitable cultivars (Vaidya and Devasagayam, 2007; Handique, 2009)^{5,6}.

Medicinal plants have several metabolites by which they are using to cure several diseases or use as anti-inflammatory drug (Okigbo *et al.*, 2008; Hassan, 2012)^{7,8}. The use of conventional medicines and medicinal plants in most rising countries as remedial agents is widely observed (Dhawale *et al.*, 2013)⁹. Secondary metabolites are a re-emerging health aid has been fuelled by the rising costs of chemically synthesized drugs in the maintenance of personal health (Lucy and Edgar, 1995)¹⁰. The researches on medicinal plants are increasing day by day due to several reasons, including increasing faith in herbal medicines (Kala, 2006)¹¹. In addition, an increasing dependence on the use of medicinal plants in the developed societies has been traced to the

exploration novel drugs and chemotherapeutics from medicinal plants as well as from traditionally used herbal remedies (Folashade *et al.*, 2012, Lingaraju *et al.*, 2013)^{12,13}.

The studies of primary metabolites have been carried out in some plants in the past such as *Balanitesa egyptiaca*, *Cissusqua drangularis*, *Eclipta alba* and *Nerium indicum* (Vijayvergia and kumar, 2007)¹⁴.

Phytochemicals are naturally occurring biochemical in plants that give plants their color, flavor, smell and texture. Preliminary phytochemical screening of medicinal plants is a useful method for qualitatively determination of different metabolite in crude sample. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs (Vane and Lindsay, 1995; Sanchez and Demain, 2008)^{15,16}. Most of the insects or pests undergo a developmental process known as metamorphosis, which simply means that the insect changes form during its life. Metamorphosis may be complete or incomplete. Complete metamorphosis consists of four stages , these are- egg, larva, pupa, and adult. Plant parts chewed by the insects this processes is known as herbivory. Pests' and insects are also affecting the primary metabolites of plants. Pest of vegetables is also reduces the quality of food. Food quality is largely determined by the availability of these nutrients (protein sugar carbohydrates), and its importance for longevity, size, fecundity, and death rates in herbivorous insects has been recognized early on by Painter (1936). In this manuscript we report primary metabolites of the *Tinospora cordifolia* along with the quantification after the pests effect (Figure 1). *Tinospora cordifolia* also known as Guduchi, Giloy is well known for its immense medicinal properties (Sharma and Batra, 2015), i.e. antimicrobial (Sharma and Batra, 2013),¹⁷ instead of this plant has Berberine alkaloid , which have an anti-diabetic potency (Sharma and Batra, 2013)²⁰, It also has protein content (Sharma and Batra, 2015)¹⁸ and it also have high content of primary metabolites (Sharma and Batra, 2016)¹⁹.



Figure 1: Pathogens on *Tinospora cordifolia*

MATERIALS AND METHOD

Collection of plant material for experiment:

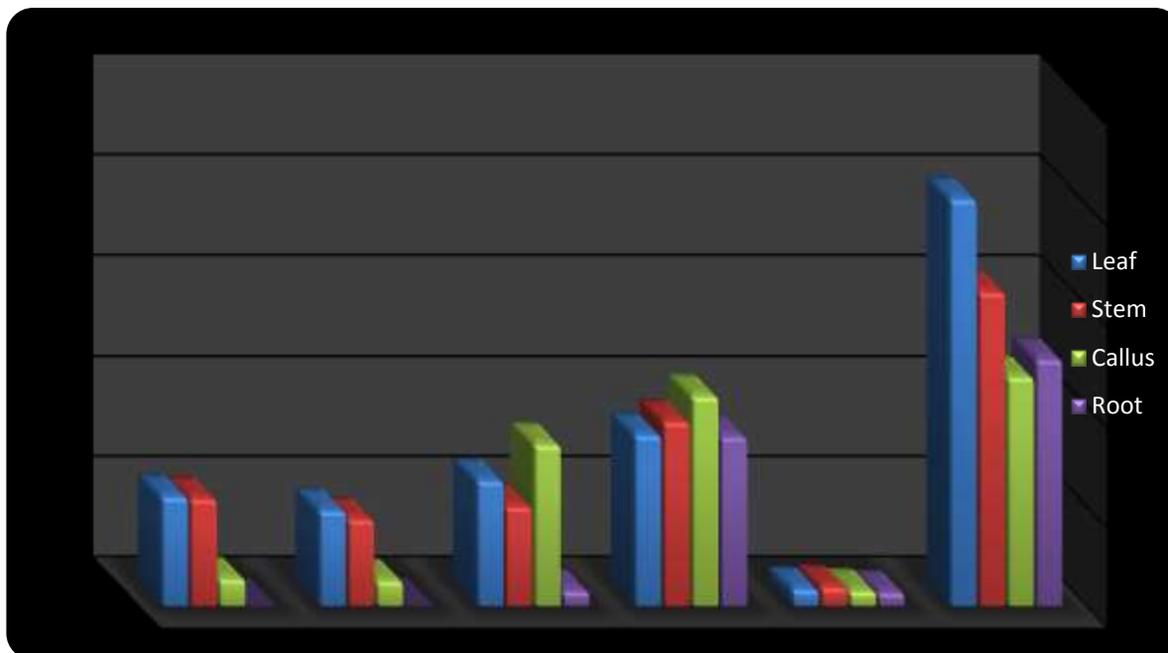
Plant material collected from Department of Botany, University of Rajasthan and authenticated by Herbarium of the University (Rajasthan University).

Preparation of extract for extraction of metabolites:

The stem, leaf and roots of *Tinospora cordifolia* was cut into small pieces, dried and powdered. The resultant was then tried to extract out with benzene, petroleum ether, water, chloroform, and ethanol with soxhlet apparatus. The extracts were then goes in concentration process by using rotary flash evaporator and dehydrated by desiccators. These concentrated extracts were then goes to preliminary phytochemical screening for the detection of various plant ingredients viz, proteins, carbohydrates, starch, phenol, chlorophyll following the established protocols (Kokoshi *et al.*, 1949)²¹, for their extraction of primary metabolites the concentrated extracts dried powder (dried by the help of desiccator) was treated with different types of acids i.e. 1N Hydrochloric acid, Nitric acid, Sulfuric acid etc., and alkaline solutions i.e. 1N Ammonia solution and Sodium Hydroxide solution etc. Callus, Stem, Leaf and Root parts of *Tinospora cordifolia* were evaluated

quantitatively to estimate the total levels of chlorophyll, soluble sugars, starch, proteins, lipids and phenols following the established methods for the lipid (Jayaraman, 1981)²², protein (Lowry, 1951)²³, phenol (Bray and Thorpe, 1954),²⁴ and starch (Dubois *et al.*, 1951)²⁵. All experiments were repeated five times for accuracy.

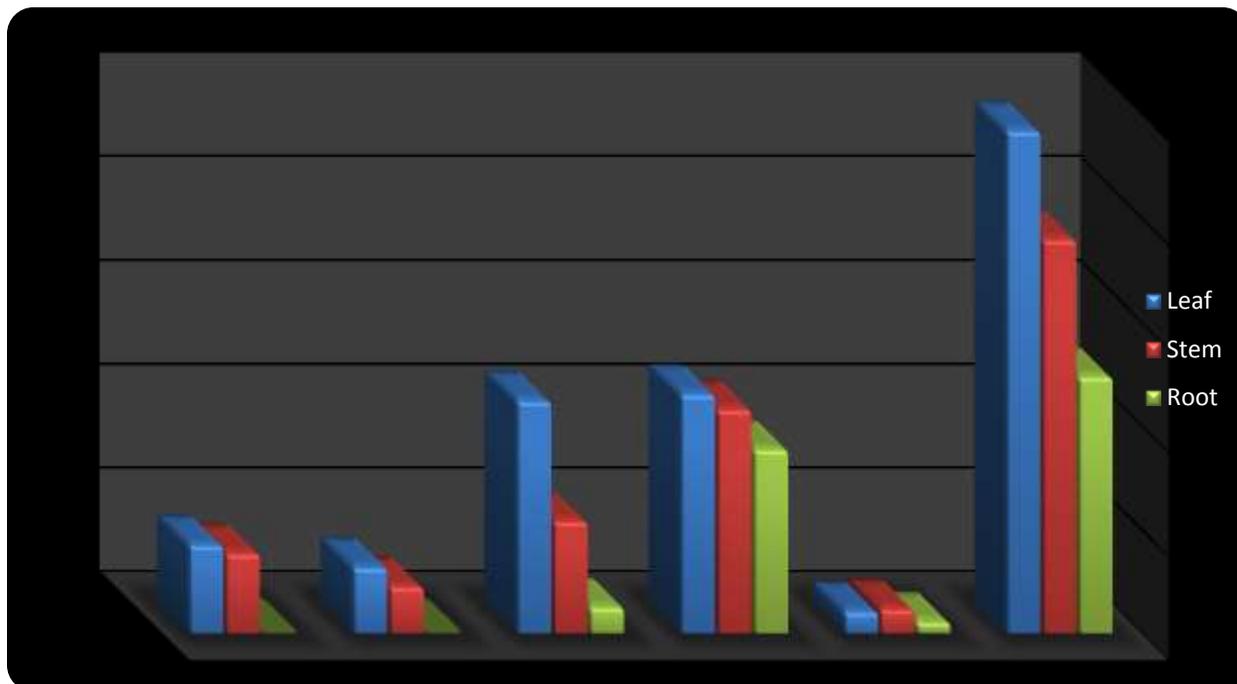
RESULTS AND DISCUSSION



Graph 1: Various primary metabolites in pest free different plant part

Effect of Ingestion of pests on Primary Metabolites-

The inducing factors produced by the pests largely affect the metabolites (Primary and Secondary metabolites) of the plants, It results in the elevated production of certain primary metabolites, viz- Carbohydrates, Phenol and Amino acids. (Muhammad *et al.*, 2009)²⁶. Similar to this *Tinospora cordifolia* ingested via pathogens shows same result (Graph 2).



Graph 2: Various primary metabolites in pest infected plant parts

CONCLUSION

Primary metabolites chlorophyll, proteins, lipid, soluble sugar, starch and total phenol contents are quantified in different plant parts (root, stem, callus and leaves) (in Graph 1). Pest free vegetable part contained total sugar ($7.34 \pm 0.11 \text{ gm/gdw}$) highest in callus, whereas, starch ($10.91 \pm 0.21 \text{ gm/gdw}$) highest in callus and moderate in stem ($9.37 \pm 0.18 \text{ gm/gdw}$), proteins ($91.17 \pm 0.13 \text{ } \mu\text{g/mg}$) highest in stem, chlorophyll a ($5.48 \pm 0.19 \text{ gm/gdw}$) and chlorophyll b ($4.12 \pm 0.15 \text{ gm/gdw}$) highest in leaves, whereas Phenol content highest in leaf ($20.16 \pm 0.17 \text{ gm/gdw}$). Callus show maximum concentration of metabolites as compared to its leaf, stem and roots. Pest infected vegetable parts showed (Graph 2) total sugar highest in leaves ($11.93 \pm 0.17 \text{ gm/gdw}$), starch was also showed highest in leaves ($11.83 \pm 0.14 \text{ gm/gdw}$), proteins showed highest in stem ($112.23 \pm 0.11 \text{ } \mu\text{g/mg}$), chlorophyll a ($4.83 \pm 0.15 \text{ gm/gdw}$) and chlorophyll b ($3.91 \pm 0.16 \text{ gm/gdw}$) both were showed highest in leaves, whereas Phenol showed highest in leaves ($24.10 \pm 12 \text{ gm/gdw}$) and moderate in stem ($18.89 \pm 0.23 \text{ gm/gdw}$). Leaves shows maximum primary metabolites as compared to its stem and roots. Plant synthesizes primary metabolites (lipid, protein, starch, sugars, phenol etc.) for the normal growth and development of itself.

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