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**Central European Journal of Experimental Biology, 2014, 3 (2):6-12**
*(http://scholarsresearchlibrary.com/archive.html)*

Central European Journal of Experimental Biology
Scholars Research Library
**ISSN: 2278–7364**

# Study of *Cuscuta reflexa* Roxb. with reference to host diversity, anatomy and biochemistry

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**ABSTRACT**

*Surveys were conducted to find out the host plants of Cuscuta reflexa Roxb. from different localities of Baramati area of Pune District of Maharashtra, India. Host plants were examined for anatomical and biochemical studies. In a survey 29 species, representing 23 genera belong to 15 families were recorded as host plants of Cuscuta. Cuscuta haustorium penetration in host stem and size of the haustorium was specific to host and Cuscuta species. Each transverse section of host stem shows Cuscuta haustorium reached up to the secondary xylem. Polyphenol oxidase activity studied in healthy and infected stem of Bougainvilliea spectabilis, Ficus glomerata, Vitex negundo, Santalum album and Acalypha hispida. The common trend of enzyme activity is stimulatory in infected host plants. Protein content studied in healthy and infected host plants of Bougainvilliea spectabilis, Ficus glomerata, Vitex negundo, Santalum album and Acalypha hispida by C. reflexa Roxb. It is interesting to note that the protein content is markedly stimulated in all infected host plants. The maximum stimulation occurs in Bougainvilliea spectabilis, Vitex negundo, Santalum album and Acalypha hispida compared to Ficus glomerata.*

**Key words :** *Cuscuta* and host plants, anatomy, polyphenol oxidase, protein

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## INTRODUCTION

*Cuscuta* (Family - Convolvulaceae) is an obligate angiosperm parasitic climber found commonly throughout India. It has about 100-170 species which attach various trees, shrubs, herbs and affect commercially valuable crops [1]. It is generally accepted that water and inorganic nutrients are absorbed through the xylem connections between host and parasite, while organic substances are transported from the phloem of the host to that of the parasite via the phloem connections. *Cuscuta* ranges in severity based on its species and the species of host, the time of attach and whether any viruses are also present in the host plants [2].

*Cuscuta reflexa* Roxb. has been used from ancient times, for various purposes *viz*. as a purgative, in the treatment of liver disorders, cough and itching and for its carminative and anthelmintic actions. The *Cuscuta* is known to contain several antibacterial, antiviral and antiproliferative substances. It is known to contain compound like phenolics and flavonoids and since flavonoids exhibit anti-inflammatory and anticancer activities [3].

The present investigation is emphasizing on the host plants of *Cuscuta reflexa* Roxb. from different localities of Baramati area of Pune District, Maharashtra, India, anatomy of *Cuscuta* and its host plants and study of biochemical attributes like enzyme polyphenol oxidase and protein from healthy and infected host plants.

## MATERIALS AND METHODS

The host plants of *Cuscuta* were collected from different localities of Baramati area of Pune District of Maharashtra and identified by using recent standard books and Flora of the Presidency of Bombay. The hosts were categorized in herbs, shrubs, climbers, lianas, trees; angiosperms, gymnosperms, and their families; medicinal, insecticidal, antimicrobial, herbicidal and economically important plants. The transverse sections of *Cuscuta* host stem were taken from affected area of the host (where *Cuscuta* shoots made firm attachment with host stem). Then sections stained with dilute safranin and dilute light green using double staining technique and made it permanent using Canada balsam. The ready slides were observed under light microscope to study the anatomical details in host stem and *Cuscuta* shoot association. The activity of an oxidative enzyme polyphenol oxidase and protein content were studied from healthy and infected host plants of *Cuscuta* using the methods of Mahadevan and Sridhar [4] and Lowry *et al*. [5] respectively.

## RESULTS AND DISCUSSION

In the present investigation, surveys were made during 2013 to 2014 to locate the host plants of *Cuscuta* in the Baramati area of Pune district of Maharashtra, India. In a survey 29 species, representing 23 genera belong to 15 families were recorded as host plants of *Cuscuta* (Table 1). The hosts include ephemeral, annual, biennial and perennial life span; herb, shrub, climber, liana and tree habits; and agricultural, horticultural, medicinal, weeds, forest and economically important plants (Plate 1).

The present results clearly indicate that, dodder ranges in severity based on the species of host. The very common hedge plants viz. *Vitex negundo* and *Duranta plumieri* were very favourable hosts of *Cuscuta*, and when other suitable hosts were nearby *Cuscuta* shoots, *Cuscuta* spread from host plant to host plant often forming a dense mat of interwined stems. Hence, it is very clear that, *Cuscuta* infection or multiplication is mostly caused by vegetative method via stems or shoots. It grows on each and every type of plants. In shaded areas, twining and attachment were greatly reduced. Reddy *et al.* [6] reported *Vitex negundo* Linn. hedge plant as a new host for *Cuscuta reflexa* in Bidar, Karnataka. Liu [7] reported tobacco as a new host for *C. japonica* in China. Extensive parasitic infestation on *Pueraria phaseoloides* by *C. campestris* and its sporadic infestation of *Hevea brasiliensis* (Rubber) and *Mucuna bracteata* in India were reported by Thankamma and Marattukalam [8]. Zerman and Saghir [9] conducted field surveys in Algeria during 1981, 1987 and 1994 for different species of *Cuscuta*, which parasitized field crops, vegetables, fruit trees and weeds and they reported 12 *Cuscuta* species. Approximately 26 new host plants contain some rarely found hosts reported by Tanase *et al.* [10] in Sibiu, Romania. Maiti and Chauhan [11] made survey of hosts of *C. reflexa* in Gangtok, Sikkim, India and identified the 53 hosts, from 27 families. They include both herbaceous species (42%), shrubs (26%), climbers (21%) and trees (11%) and concluded that, tree species are parasitized in their early stages of growth only. According to Jayasinghe *et al.* [12] *Cuscuta* is widely distributed in Sri Lanka. They searched 161 host plant species including rice, belonging to 59 families and 139 genera. Patel *et al.* [13] presented tabulated data of 48 host plants parasitized by *Cuscuta* species in North Gujarat, India. From the different experimental studies Schoolmaster [14] concluded that, *Impatiens capensis* Meerb. (Balsaminaceae) was a necessary nurse host for the parasitic plant *Cuscuta grovonii* in Schultes in Southeastern Michigan wetlands. One very interesting thing was revealed by Kelly [15] i.e. in greenhouse experiments *C. europaea* accept (coil) host of high nutritional status and grow away from (reject) hosts of poor quality.

The light microscopic anatomical observations of *C. reflexa* Roxb. and its host stem shoed tremendous diversity. The Photoplate 2 contains entire transverse structures of *Cuscuta* and their hosts stem anatomy. The present result clearly indicates that, *Cuscuta* haustorium penetration in host stem and size of the haustorium is specific to host and *Cuscuta* species. Each transverse section of host stem shows *Cuscuta* haustorium reached up to the secondary xylem. And here one of the interesting thing is that, if food material is available from phloem tissue to *Cuscuta* haustorium then what is the necessity of insertion of these haustoria next to phloem tissue of host stem. But these haustoria insertion was not up to the pith and shows limited specific growth. The another common character was observed that, the *Cuscuta* haustorium penetration in the host stem was affected on the cortex tissue and this tissue shows markedly elongation towards the *Cuscuta* stem and host stem structure was completely changed. Haustoria in *Cuscuta* never have apical meristems and root caps and develop from cortical parenchyma cells without any involvement of the pericycle. In addition, during the formation of the haustoria, cell elongation predominates over cell division, and therefore the number of cells of the parasite endophytic system in the host is determined by the number of *Cuscuta* cortical parenchyma cells undergoing transformation. Furthermore, the haustoria have limited growth capacity [16].

The anatomical studies of *Cuscuta* made by Ihl and Wiese [17] concluded that, the induction of haustoria formation in *C. reflexa* proved to be independent of the presence of a suitable potential host. Haustoria formation was

restricted to a subapical region of *C. reflexa* stem, which is the area where the most intensive elongation of the stem takes place. During haustorial development, the growth rate of *C. reflexa* is retarded. According to Arnaud *et al.* [18] while the *Cuscuta* easily attached itself to its hosts, the first difficulty was to establish connection between xylem vessels and sieve-tubes. As per the studies of Day and Pati [19], transverse sections of the affected area of the stem of *Digitaria ciliaris* showed that the haustoria penetrate the host by rupturing the bulliform cells or epidermal pores. Information about many aspects of the parasitism of *Cuscuta* is still in its elementary stage. The mechanism of haustoria penetration is not clearly understood and there are very few works carried out on the anatomical studies of *Cuscuta* and its host association, hence it wants further investigation.

Phenolic compounds are believed to impart resistance to disease in plants and polyphenol oxidase (Catecholase and Cresolase) enzyme has been reported to be responsible for *in vivo* synthesis and accumulation of these compounds [20]. In many cases, a close correlation has been found between the enhanced activity of polyphenol oxidase and peroxidase and the concentration of Phenolic substances on one hand and between plant resistance on the other [21].

In the present investigation polyphenol oxidase activity studied in healthy and infected stem of *Bougainvilliea spectabilis, Ficus glomerata, Vitex negundo, Santalum album* and *Acalypha hispida* is depicted in Table 2. The common trend of enzyme activity is stimulatory in infected host plants. None of the infected host shows decreasing trend of polyphenol oxidase activity. Present results clearly indicates the role of polyphenol oxidase activity in plant diseases, so here it may concluded that increasing activity of polyphenol oxidase enzyme markedly involve in physiological defence mechanism of host plants. The similar results are also proposed by many workers.

The increase in polyphenol oxidase activity in a number of diseases has been linked with resistance and with increase in respiration, which usually accompanies resistance. Jite and Tressa [22] found an increase in polyphenol oxidase activity in infected *Jasminum* plants with *Uromyces hobsoni*. Gawande *et al*. [23] concluded that enzymes polyphenol oxidase and peroxidase are responsible for resistance or susceptibility of host plants against pathogen.

The activity of polyphenol oxidase enzyme generally higher in infected tissue of resistant varieties than in the infected tissue of susceptible genotype [24]. The oxidative enzymes especially polyphenol oxidase is involved in necrotic browing that results into environment unfavourbale for the growth of potential pathogen [25]. Gogoi *et al.* [26] concluded that the oxidative enzyme converts phenolic compounds of plants into polyphenols and quinines, the toxic substances for the extra cellular enzymes produced by the pathogen. Increased activity of polyphenol oxidase has been seen by Nicholson and Hammerschmidt [27] in wheat leaves infected by *Neovossia indica* (Karnal bunt).

Inducible defense-related proteins have been described in many plant species upon infection with oomycetes, fungi, bacteria, or viruses, or insect attack. Several types of proteins are common and have been classified into 17 families of pathogenesis-related proteins (PRs). Others have so far been found to occur more specifically in some plant species. Most PRs and related proteins are induced through the action of the signaling compounds salicylic acid, jasmonic acid, or ethylene, and possess antimicrobial activities in vitro through hydrolytic activities on cell walls, contact toxicity, and perhaps an involvement in defense signaling. However, when expressed in transgenic plants, they reduce only a limited number of diseases, depending on the nature of the protein, plant species, and pathogen involved. Several defense-related proteins are induced during senescence, wounding or cold stress, and some possess antifreeze activity. Many defense-related proteins are present constitutively in floral tissues and a substantial number of PR-like proteins in pollen, fruits, and vegetables can provoke allergy in humans [28].

Protein content studied in healthy and infected host plants of *Bougainvilliea spectabilis, Ficus glomerata, Vitex negundo, Santalum album* and *Acalypha hispida* by *C. reflexa* Roxb. is recorded in Table 3. It is interesting to note that the protein content is markedly stimulated in all infected host plants. The maximum stimulation occurs in *Bougainvilliea spectabilis, Vitex negundo, Santalum album* and *Acalypha hispida* compared to *Ficus glomerata.* Again increasing protein content proves its role in plans defence mechanism. Similar results also reported by many workers.

Protein content increased moderately in mango leaves infected with *Colletotrichum gloesporioides* reported by Hossain *et al*., [29]. Rahman *et al*., [30] recorded increased total protein content in infected *Moringa* fruits by *Rhizopus stolonifer*. A remarkable increase in total soluble protein content was observed by Srivastava and Alok [31] in the cultivars of black gram, T-9 and IPU 94-1 'Uttara' at 10, 20 and 30 days after inoculation. Among the two cultivars, the susceptible one i.e.T-9 showed a relatively higher increase in protein level as compared to the resistant variety IPU 94-1 'Uttara'. Ashfaq *et al*., [32] noticed urdbean leaf crinkle virus (ULCV) infected blackgram plants, both susceptible and resistant, appeared to have increased total soluble protein contents at 15 and 30 days after inoculation. Leaves from Mash-88 (susceptible genotype) had slightly higher protein content than the CM-2002 (resistant one). According to Charitha Devi and Radha [33] there was a significant increase in the total protein

content in cucurbit plants treated with cucurbit mosaic virus (CVM). In Healthy plants the protein content was 34 µg g-1 while in treated plants it varied from 36 to5 9 µg g-1.

On the other hand many workers noticed the decreased protein content in infected plants. Panda [34] reported decreased protein contents in inoculated leaves of *Solanum melongena* L. by little leaf disease compared to healthy plants. 62.74% crude protein decreased on the 6$^{th}$ day infection of chilli leaves inoculated by *Alternaria sonali* was noticed by Veeramohan and Ramaswamy [35]. Soluble proteins were studied by Herclito *et al.*, [36] from infected leaves of *Theobroma grandiflorum* leaves infected by *Crinipellis perniciosa* fungi and reported decreased trend.

**Table 1 : List of host plants of *Cuscuta* collected from Baramati area of Pune district of Maharashtra.**

| Sr. No. | Botanical names of *Cuscuta* host plants | Vernacular names of *Cuscuta* host plants | *Family* |
|---|---|---|---|
| 1. | *Acalypha hispida* | --- | Euphorbiaceae |
| 2. | *Adhatoda vasica* Nees. | Adulsa | Acanthaceae |
| 3. | *Alstonia scholaris* R.Br. | Saptaparni | Apocynaceae |
| 4. | *Annona reticulata* Linn. | Ramphal | Annonaceae |
| 5. | *Annona squamosa* L. | Sitaphal / Custard apple | Annonacea |
| 6. | *Azadirachta indica* A. Juss. | Neem | Meliaceae |
| 7. | *Bougainvillaea spectabilis Willd.* | Bogenvel | Nyctaginaceae |
| 8. | *Calotropis giganta* (L.) R.Br. | Ruee | Asclepiadaceae |
| 9. | *Catharanthus roseus* Don. | Sadafuli | Apocynaceae |
| 10. | *Citrus medica* Linn. | Limbu | Rutaceae |
| 11. | *Dalbergia sissoo* Roxb. | Shisam | Fabaceae |
| 12. | *Duranta plumieri* Jacq. | Duranta | Verbenaceae |
| 13. | *Euphorbia geniculata* Orteg. | Dudhani | Euphorbiaceae |
| 14. | *Euphorbia hirta* | Dudhani | Euphorbiaceae |
| 15. | *Euphorbia tirucalli* L. | Sher | Euphorbiaceae |
| 16. | *Ficus bengalensis* L. | Wad | Moraceae |
| 17. | *Ficus benjamina* L. | Weeping fig | Moraceae |
| 18. | *Ficus glomerata* Roxb. | Umber | Moraceae |
| 19. | *Ficus religiosa* L. | Pimpal | Moraceae |
| 20. | *Hamelia erecta* Jacq. | --- | Rubiaceae |
| 21. | *Hibiscua rosa-sinensis* L. | Jaswant | Malvaceae |
| 22. | *Ixora coccinea* L. | --- | Rubiaceae |
| 23. | *Lantana camera* Roxb. | Ghaneri | Verbenaceae |
| 24. | *Nerium oleander* L. | Kaner | Apocynaceae |
| 25. | *Phyllanthus niruri* L. | Bhuiavala | Euphorbiaceae |
| 26. | *Pithecellobium dulce* (Roxb.) Benth. | Vilayti chinch | Fabaceae |
| 27. | *Punica granatum* L. | Anar / Dalimb | Myrtaceae |
| 28. | *Santalum album* L. | Chandan | Santalaceae |
| 29. | *Vitex negundo* Linn. | Nirgudi | Verbenaceae |

**Table 2 : Polyphenol oxidase activity in healthy and infected host plants of *C. reflexa* Roxb.**

| Sr. No | Plant material (Stem material) | Polyphenol oxidase activity (ΔOD/min/g fresh wt) | |
|---|---|---|---|
| | | Healthy | Infected |
| 1 | *Bougainvilliea spectabilis* | 7.78 | 9.42 |
| 2 | *Ficus glomerata* | 5.63 | 9.12 |
| 3 | *Vitex negundo* | 9.47 | 11.99 |
| 4 | *Santalum album* | 9.34 | 10.17 |
| 5 | *Acalypha hispida* | 6.73 | 9.21 |

**Table 3 : Protein content in healthy and infected host plants of *C. reflexa* Roxb.**

| Sr. No | Plant material (Stem material) | Proline content (µg $100^{-1}$ gm dry tissue) | |
|---|---|---|---|
| | | Healthy | Infected |
| 1 | *Bougainvilliea spectabilis* | 56 | 156 |
| 2 | *Ficus glomerata* | 72 | 102 |
| 3 | *Vitex negundo* | 130 | 138 |
| 4 | *Santalum album* | 88 | 182 |
| 5 | *Acalypha hispida* | 138 | 306 |

*Alstonia scholaris* R.Br.

*Ficus benjamina* L.

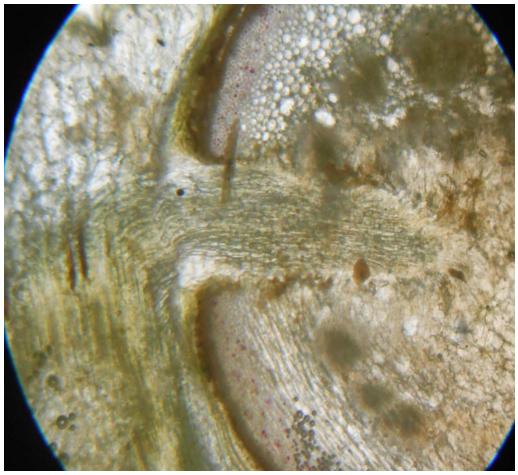
*Bougainvillaea spectabilis Willd.*

*Duranta plumieri* Jacq.

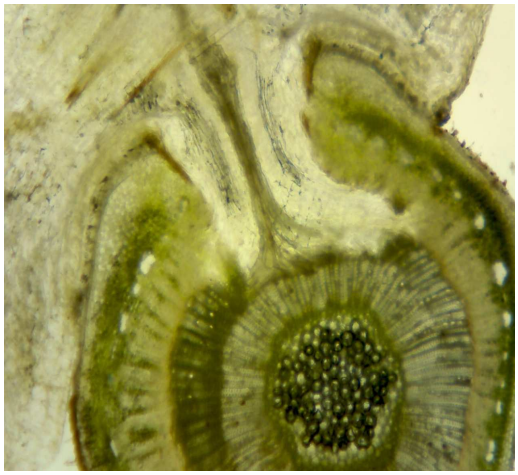
*Euphorbia tirucalli* L.

*Vitex negundo* Linn.

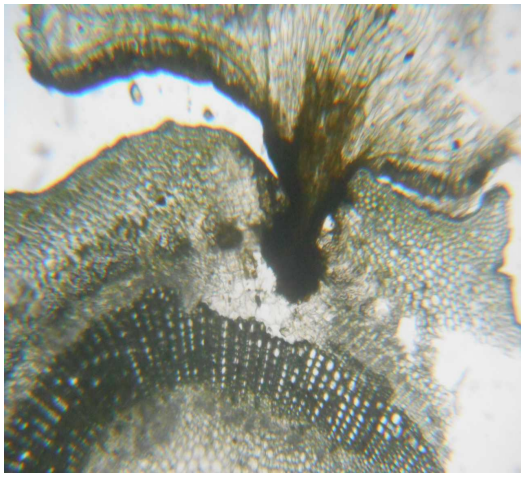
**Plate 1 : Hosts of *C. reflexa* Roxb. in Baramati area**



*Ficus glomerata* Roxb.

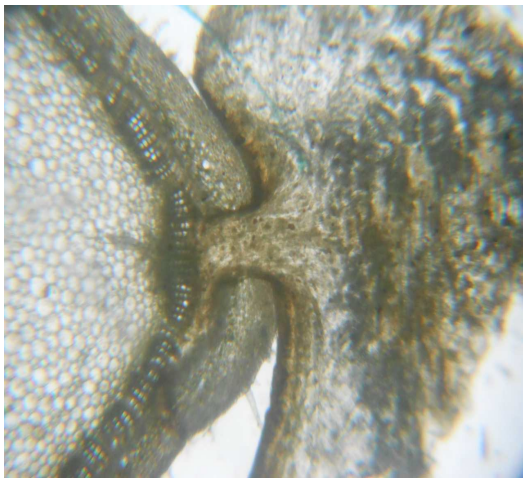


*Duranta plumieri* Jacq.

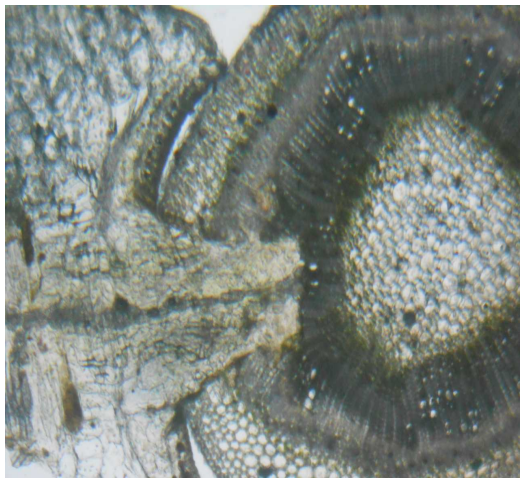


*Catharanthus roseus* Don.

*Euphorbia tirucalli* L.



*Hamelia erecta* Jacq.



*Ixora coccinea* L.

**Plate 2 : Anatomy of infected host plant stems of *C. reflexa* Roxb.**

**Acknowledgements**

Authors are sincerely thankful to Dr. Chandrashekhar V. Murumkar, Principal, Tuljaram Chaturchand College, Baramati and Dr. S. J. Chavan, Co-ordinator M. Sc. Botany and all Teachers of Botany Department for constant encouragement and guidance in research activities.

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