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Host range, Anatomy, Biochemistry and Impacts of *Cuscuta reflexa* Roxb. (wonderful parasitic plant) on Flora: A Case Study from the Betla National Park, Jharkhand, India

Preeti Kumari[1], S. K. Tiwari[2] and A. K. Choudhary[1]

[1]Department of Botany, Ranchi University, Ranchi, India, 834002.
[2]BRSM College of Agricultural Engineering and Technology, IGAU, Raipur (C.G.), India

Abstract

The *Cuscuta reflexa* Roxb. is an angiospermic leaf less parasitic plant belonging to the family Convolvulaceae and it directly attaches to the host plants through the haustoria. During this investigation we found that the *Cuscuta reflexa* Roxb. is a hemi-and holoparasite, living on wood, fruit yielding and medicinally important plants species. In the present study systematic survey and identification of the host plants has been undertaken. Surveys were conducted to find out the host plants of *Cuscuta reflexa* Roxb. from different localities of Betla National Park areas of Jharkhand, India. In a survey 32 species, representing 30 genera belonging to 23 families were recorded as host plants of *Cuscuta*. Host plants were also examined for anatomical and biochemical studies. *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 reaching up to the secondary xylem. Poly-phenol oxidase activity and protein content have also been studied in healthy and infected stems of *Zizyphus mauritiana*, *Cajanus cajan* and *Ficus glomerata* by *Cuscuta reflexa*. The common trend of enzyme activity is stimulatory in infected host plants. It is interesting to note that the protein content is markedly stimulated in all infected host plants. The maximum stimulation occurs in *Zizyphus mauritiana* compared to all host plants. We have also studied impact of *Cuscuta reflexa* and found that it has major impacts on host growth, allometry and reproduction, which lead to changes in competitive balances between host and non host species and therefore affect community structure, vegetation and population dynamics. Impacts on hosts may further affect herbivores, pollinators and seed vectors, and the behaviour and diversity of these is often closely linked to the presence and abundance of parasitic plants.

Author for Correspondence: Dr. Shashi Kant Tiwari, Email: shashikant.au@gmail.com,

Cuscuta is an obligate angiosperm parasitic climber found commonly throughout India. It has about 100-170 species which attach to various trees, shrubs, herbs and affect commercially valuable crops[9]. 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[13]. *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[24]. Fungi, nematodes, bacteria, and viruses are probably the first things that come to mind when thinking of plant pathogens. These organisms certainly do cause damage to plants of economic importance, but it may surprise you to know that parasitic flowering plants are also important pathogens. The purpose of this chapter is to provide an overview of the life cycles and evolutionary relationships of these fascinating and unusual plants and also to focus upon those that negatively affect food and fiber crops. Most plants are autotrophs and produce their own carbon sources through photosynthesis. Although some plants such as Indian pipe (*Monotropa*) lack chlorophyll and appear to be parasitic, they are myco heterotrophs (parasites of mycorrhizal fungi) and, hence, only indirectly parasitize the trees on which the mycorrhizal fungi are found. Here we define a parasitic plant as an angiosperm (flowering plant) that directly attaches to another plant via a haustorium. A haustorium is a modified root that forms a morphological and physiological link between the parasite and host[12]. It is useful to make a distinction between the terms "parasite" and "pathogen." Parasite is from the Greek para (beside) and sitos (grain or food) which literally means "beside the food". If a plant also induces disease symptoms in a host, then it is a pathogen as well as parasite. A general term that refers to both parasites and mycotrophs that derive carbon from sources other than their own photosynthesis is heterotrophic, which simply means "different feeding." *Cuscuta*, dodder (Convolvulaceae-Morning Glory Family) Species of *Cuscuta*, commonly known as dodder, are among the best known of all parasitic plants. The biology and control of dodders was reviewed[2]. Dodders have a broad host range, although monocots are less preferred. The genus *Cuscuta* contains three subgenera. Members of the subgenus *Monogyna* are robust vines that may attack and kill fruit trees, while species in the subgenus *Cuscuta* are more delicate in structure and favor herbaceous hosts, as do species of the entirely New World subgenus, *Grammica*. Many researchers have been reported about *cuscuta* but no anybody has been studied and work carried out related to cuscuta in BNP.

The present investigation is emphasizing on the host plants of *Cuscuta reflexa* Roxb. from different localities of Betla National Park areas of Jharkhand, India. Anatomy of *Cuscuta* and its host plants has been studied. We have also studied the biochemical attributes like

enzyme poly-phenol oxidase and protein content from healthy and infected host plants, as well as impacts of *Cuscuta reflexa* has been also reported.

Study Area: Betla National Park (BNP) situated between latitude 23°25' N to 23°55' N and longitude 83°50' to 84°36' E, was notified in 1973 as one of India's first nine tiger reserves established under Project Tiger. It is located in the western part of the Chhotanagpur Plateau and spans an area of 1129.93 square km comprising the Palamau Wildlife Sanctuary and Betla National Park is spread over Latehar, Palamau and Garhwa District in Jharkhand. It is also part of the Central India Landscape and extends into the Sanjay-Dubri Tiger reserve and Achanakmar-Kanha tiger landscape through the Jashpur and Mahan forest of Chhattisgarh. The vegetation types mainly categorized as dry moist forest, dry Sal forest, moist Sal forest, high level plateau Sal forest and moist forest. BNP is also becoming home to many unwanted non native plants.

Data collection :

The diversified host species of *Cuscut reflexa* Roxb. were studied in Betla National Park of Jharkhand, India. Extensive field survey was undertaken during the year *i.e.* from 2015-2016 at different areas including forest villages and out skirts, higher plant parasites occurring on the host species. These are photographed and collected the host species and parasites for identification and confirmed with the help of existing literature.

The host plants of *Cuscuta* were collected from different localities of Betla National Park of Jharkhand, India and identified by using recent standard books and current literature. 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 poly-phenol oxidase and protein content were studied from healthy and infected host plants of *Cuscuta* using the following methods.

Poly-phenol oxidase analysis :

The Poly-phenol oxidase (PPO) activity has been analyzed as per the procedure of Mahadevan and Sridhar[15]. The reaction mixture consisted 1.5 ml of 0.1M Sodium phosphate buffer (pH 6.5) and 200µl of the enzyme extract. 0.01ml Catechol has added to the reaction mixture to start the reaction. Poly-phenol oxidase activity has expressed as change in absorbance at 412 nm per minute/g fresh weight of tissue.

Protein analysis:

Fresh steam sample 100 mg has extracted in 0.1M Sodium phosphate buffer (pH 7.0) at 0-4°C. The homogenate has centrifuged for 20 minutes at 12000rpm. The protein content of the sample has been determined by the method of Lowry *et al.*[14]. Protein (100µg) from different tubes has taken and mixed with 10µl of sample buffer in eppendorf tube, boiled for 3-4 minutes and incubated at 4°C for 30 minutes. The samples

containing equal amount of protein have loaded into wells of 12% polyacrylamide gel. Electrophoresis was carried out at constant voltage of 75 volts for two hours. The gels have stained with 0.2% coomassie brilliant blue (R-250) solution and distained with acetic acid/ water. The hierarchical cluster analysis has performed on gel documentation system using NTSYS-pc software and the dendrogram has prepared using average linkage between groups based on presence/absence of protein bands in different lanes of the gel.

Study of the host plant of Cuscuta reflexa:

In the present investigation, surveys have made during 2015 to 2016 to locate the host plants of *Cuscuta* in the Plamu Tiger reserve areas of Betla National Park in Jharkhand, India. In a survey 33 species, representing 30 genera belong to 23 families have been recorded as a host plants of *Cuscuta* (Shown in Table 1). The hosts include ephemeral, annual, biennial and perennial life span; herb, shrub, climber, liana and tree habits; and agricultural, horticultural, medicinal, weeds, and economically important plants. The present results clearly indicate that, dodder ranges in severity based on the species of host. The very common plants viz. *Ziziphus mauritiana, Cajanus cajan and Ficus glomerata* have very favorable hosts of *Cuscuta*, (Figures 1-3) and when other suitable hosts were nearby *Cuscuta* shoots, *Cuscuta* spread from host plant to host plant often forming a dense mat of intertwined 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. Sandip S. Nikam *et al.*,[17] reported *Vitex negundo* and *Duranta plumier* plat is a most favorable host plant in Baramati area of Pune district of Maharashtra and Reddy *et al.*,[20] reported *Vitex negundo Linn.*, hedge plant as a new host for *Cuscuta reflexa* in Bidar, Karnataka. 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[23]. Zerman and Saghir[26] 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.*[22] in Sibiu, Romania. Maiti and Chauhan[13] 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.*[7] *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.*[18] presented tabulated data of 48 host plants parasitized by *Cuscuta* species in North Gujarat, India. From thedifferent experimental studies Schoolmaster[21] 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[11] *i.e.* in greenhouse experiments *C. europaea* accept (coil) host of high nutritional status and grow away from (reject) hosts of poor quality.

Table-1. List of host plants of *Cuscuta reflexa Roxb*, collected from Betla National Park, Plamu Tiger reserve areas in Jharkhand.

S. No.	Botanical names of *Cuscuta* host plants	Vernacular names of *Cuscuta* host plants	Family
1	*Carisa spinarum*	Karonda	Apocynaceae
2	*Eugenia heyneana*	kathjamun	Myrtaceae
3	*A. catechu*	khair	Mimosaceae
4	*A. nilotica*	Babul / Kikar	Mimosaceae
5	*Achyranthes aspera*	chirchiri	Amaranthaceae
6	*Adhatoda zeylanica*	Adosa	Acanthaceae
7	*Arotocarpus integrifolia*	Jack fruit / Kathal	Moraceae
8	*Aegle marmelos(Linn.)*	Bel	Rutaceae
9	*Aerva lanata*	Gorakhbuti	Amaranhaceae
10	*Spondias mangifera*	Amra / Amda	Anacardiaceae
11	*Ageratum conyzoides*	Jangli pudina	Asteraceae
12	*Zizyphus xylopyrus*	kathber	Rhamnaceae
13	*Azadirachta indica* A. Juss.	Neem	Meliaceae
14	*Bombax ceiba*	semal	Bombacaceae
15	*Butea monosperma*	Palas	Fabaceae
16	*Albizia lebbeck* (Linn.)	Sirish	Mimosaecae
17	*Emblica officinalis* Gaertn.	Amla.	Euphorbiaceae
18	*Lawsonia inermis* Linn.	Mehendi,	Lythraceae
19	*Moringa oleifera* Lam.	Sahjan,	Moringaceae
20	*Tamarindus indica* Linn.	Imali	Caesalpiniaceae
21	*Terminilia. bellirica*	Bahera	Combretaceae
22	*Terminalia chebula* Retz.	Harra	Combretaceae
23	*Madhuca longifolia*	Mahua	Sapotaceae
24	*Vitex negundo* L.	Nirgundi	Verbenaceae
25	*Cajanus cajan*	Arhar	Fabaceae
26	*Zizyphus mauritiana*	Ber / plum	Rhamnaceae
27	*Shorea robusta*	Sal / Sakhua	Dipterocarpaceae
28	*Annona squamosa*	Sitaphal	Annonaceae
29	*Pyrus communis*	Naspati	Rosaceae
30	*Dalbergia sissoo* Roxb.	Shisam	Fabaceae
31	*Nerium oleander* L.	Kaner	Apocynaceae
32	*Ficus glomerata*	Gular	Moraceae

Figure 1: *Zizyphus mauritiana* plant affected by *Cuscuta reflexa* Roxb, present in Betla National Park areas in Jharkhand.

Figure 2: *Cajanus cajan* plant affected by *Cuscuta reflexa* Roxb. present in Betla National Park areas in Jharkhand.

Figure 3: *Ficus glomerata* plant affected by *Cuscuta reflexa* Roxb. present in Betla National Park areas in Jharkhand.

Anatomical study of host plant of Cuscuta reflexa:

The light microscopic anatomical observations of *Cuscuta reflexa* Roxb. and its host stem showed tremendous diversity (Figures 4-6). 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 things 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[27]. The anatomical studies of *Cuscuta* made by Ihl and Wiese[6] 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 sub apical 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.*[1] 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[13], 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.

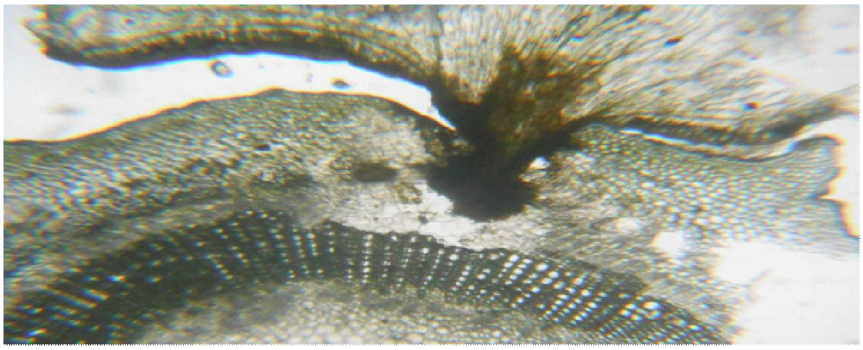

Figure 4: Anatomical structure of *Zizyphus mauritiana* host plant stems infected by *Cuscuta reflexa* Roxb., present in Betla National Park areas in Jharkhand.

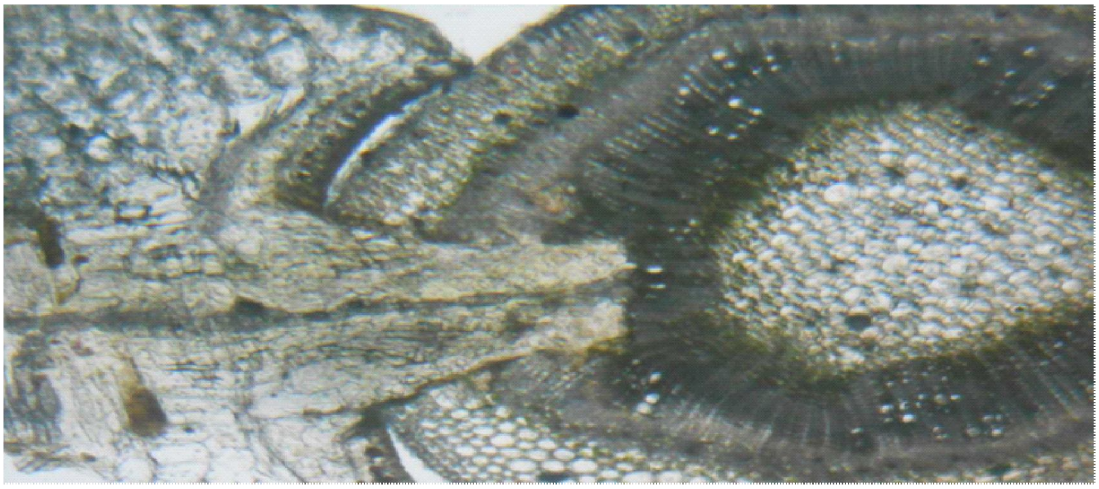

Figure 5: Anatomical structure of *Cajanus cajan* host plant stems infected by *Cuscuta reflexa* present in Betla National Park areas in Jharkhand.

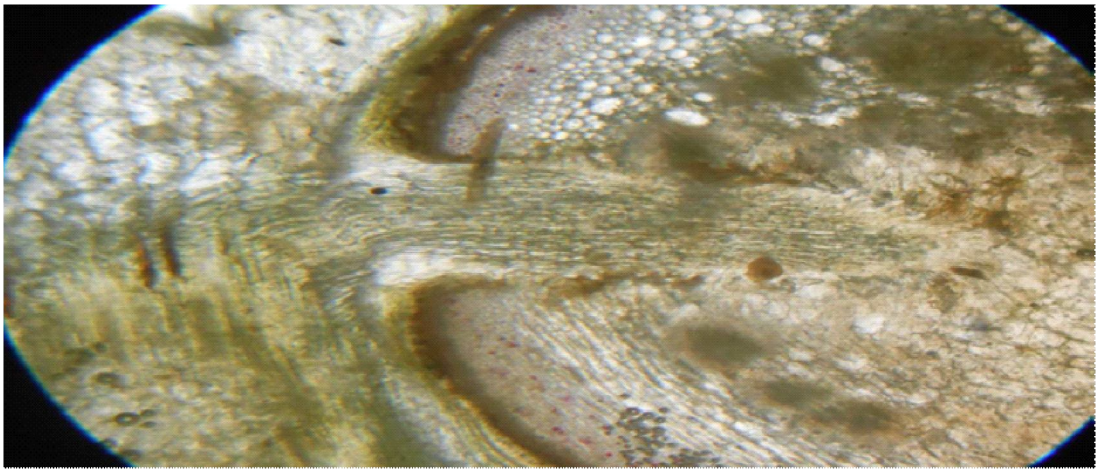

Figure 6: Anatomical structure of *Ficus glomerata* host plant stems infected by *Cuscuta reflexa* present in Betla National Park areas in Jharkhand.

Study the Biochemistry of Cuscuta reflexa:

Phenolic compounds are believed to impart resistance to disease in plants and poly-phenol oxidase (Catecholase and Cresolase) enzyme has been reported to be responsible for *in vivo* synthesis and accumulation of these compounds[25]. In many cases, a close correlation has been found between the enhanced activity of poly-phenol oxidase and per-oxidase and the concentration of Phenolic substances on one hand and between plant resistances on the other[4]. In the present investigation poly-phenol oxidase activity studied in healthy and infected stem of *Ziziphus mauritiana*,

Cajanus cajan, Ficus glomerata, Emblica officinalis Gaertn, and *Arotocarpus integrifolia* (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 (Figure 7). Present results clearly indicates the role of poly-phenol oxidase activity in plant diseases, so here it may concluded that increasing activity of poly-phenol oxidase enzyme markedly involve in physiological defense mechanism of host plants. The similar results are also proposed by many workers. The increase in poly-phenol oxidase activity in a number of diseases has been linked with resistance and with increase in respiration, which usually accompanies resistance. Jite and Tressa[8] found an increase in polyphenol oxidase activity in infected *Jasminum* plants with *Uromyces hobsoni*. Gawande *et al.*,[5] concluded that enzymes polyphenol oxidase and peroxidase are responsible for resistance or susceptibility of host plants against pathogen.

Protein content studied in healthy and infected host plants steams of *Ziziphus mauritiana, Cajanus cajan, Ficus glomerata, Emblica officinalis Gaertn,* and *Arotocarpus integrifolia* by *Cuscuta reflexa* .has 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 *Ziziphus mauritiana,* compared to another plants. Again increasing protein content proves its role in plants defense mechanism (Figure 8).

Table-2. Poly-phenol oxidase activity in healthy and infected host plant steam by *Cuscuta reflexa* present in Betla National Park areas.

S. No.	Plant material (Stem material)	Poly-phenol oxidase activity (ΔOD/min/g fresh wt)	
		Healthy	Infected
1	*Zizyphus mauritiana*	5.20	12.78
2	*Cajanus cajan*	6.31	11.45
3	*Ficus glomerata*	5.63	9.12
4	*Emblica officinalis* Gaertn	6.63	7.78
5	*Arotocarpus integrifolia*	9.34	9.92

Table-3. Protein content in healthy and infected host plant steam by *Cuscuta reflexa* Roxb. present in Betla National Park areas.

S. No.	Plant material (Stem material)	Protein content (µg/100 gm dry tissue)	
		Healthy	Infected
1	*Zizyphus mauritiana*	60	190
2	*Cajanus cajan*	85	205
3	*Ficus glomerata*	72	139
4	*Emblica officinalis* Gaertn.	68	120
5	*Arotocarpus integrifolia*	167	210

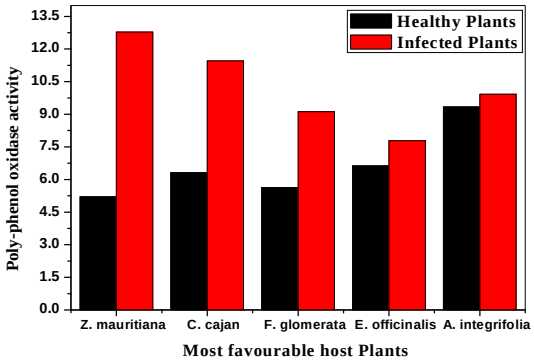

Figure 7. Poly-phenol oxidase activity of most favorable host plant of *Cuscuta reflexa* Roxb. present in Betla National Park area, Jharkhand.

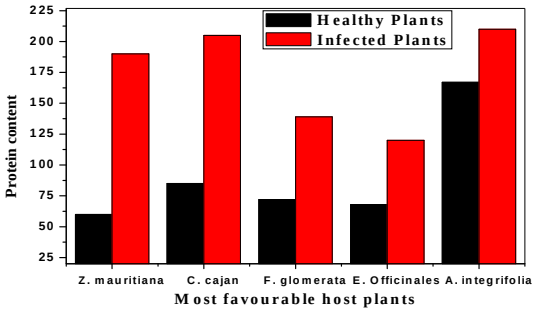

Figure 8. Protein content of most favorable host plant of *Cuscuta reflexa* Roxb. present in Betla National Park area, Jharkhand.

Impact of Cuscuta reflexa Roxb. (Dodder plant) :

Dodder is parasitic on a very wide variety of plants, including a number of agricultural and horticultural crop species such as *Ziziphus mauritiana, cajanus cajan*, etc. Others dodder ranges in severity based on its species and the species of the host, the time of attack. Parasitism has major impacts on host growth, allometry and reproduction. Impacts on hosts may further affect herbivores, pollinators and seed vectors, and the behaviour and diversity of these is often closely linked to the presence and abundance of parasitic plants.

Ecological and economic impact of dodder species:

The presence of a holoparasitic anthophyte, namely *Cuscuta* spp., which has a deep impact on vegetation due to the fact that it determines modifications in the structure of the vegetal carpet, it decreases the arable surface, results in quantitative and qualitative crop losses, represents a vector concerning the transmission of such diseases as viroses and microplasmoses to the host plant, and its impact on the biodiversity determines the degradation of the landscapes' decorative aspect.

Impacts of parasitic plants on the plant community :

Over one season, for instance, a single *Cuscuta* plant may form thousands of connections with many host species and may cover an area greater than 100 m [10], resulting in considerable impacts on the plant community despite its being perhaps less than 5% of vegetation biomass[19].

Impacts of the parasite on other tropic levels:

It is not only plants within communities that can be heavily affected by parasitic plants. Many other organisms, including birds and insect herbivores, other parasites and mycorrhizal fungi can be affected, either directly or

indirectly. Parasite also affects the trophic levels because it is part of our biodiversity. Crop damages are particularly significant. Monitoring *cuscuta* species and their spreading tendencies, as well as their prevention and therapy generates positive and immediate economic and social effects, by means of creating an integrated protection system of cultures. Moreover, it might also determine a qualitative and quantitative increase of agricultural production, which benefits farmers on the short, medium and long run. Furthermore, they will be mirrored in the quantity and quality of fodders, animal health and welfare of farmers; since it is universally acknowledged that the decrease in risk of diseases, parasites and weeds within agricultural ecosystems would influence public health and environmental protection in a very positive way.

Impacts of the parasite on the abiotic environment:

The abiotic environment is also affected by parasitic plants and most significant types of ecosystems affected by cuscuta species are the pratologic ecosystems. However, there is a huge number of species which represent host plants for dodders; this fact affecting the biodiversity of ecosystems at a process level, as well as with regard to human society and animal health.

In present study systematic survey and identification of the host's plants has been conducted to find out the host plants of *Cuscuta reflexa* Roxb. from different localities of Betla National Park areas of Jharkhand in India. In a survey total 32 species, representing 30 genera belong to 23 families have been observed as host plants of *Cuscuta* and *Ziziphus mauritiana, Cajanus cajan,* and *Ficus glomerata* are most favorable host of cuscuta. Host plants have also examined for anatomical and biochemical studies. *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 things 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 has observed that, the *Cuscuta* haustorium penetration in the host stem has affected on the cortex tissue and this tissue shows markedly elongation towards the *Cuscuta* stem and host stem structure has completely changed. The common trend of enzyme activity is stimulatory in infected host plants. Here it may concluded that increasing activity of poly-phenol oxidase enzyme markedly involve in physiological defense mechanism of host plants. Protein content is markedly stimulated in all infected host plants. The maximum stimulation occurs in *Ziziphus mauritiana,* compared to another plants. Parasitism has major impacts on host growth, allometry and reproduction. Impacts on hosts may further affect herbivores, pollinators and seed vectors, and the behavior and diversity of these is often closely linked to the presence and abundance of parasitic plants.

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