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Nodulation in *Rhynchosia schimperi*: A Rare Legume of Indian Thar Desert

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ABSTRACT: *Rhycnhosia schimperi* Hochst. ex Boiss. is a rare plant of Indian Thar Desert and mostly confined to the Jaisalmer and Barmer district of Rajasthan. There are least information is available on the nodulation in it. In India the comprehensive study on nodulation in native legumes of Thar Desert was done by Gehlot et. al. in 2012. In which they have reported nodulation in more than 30 native legumes (excluding *Rhynchosia schimperi*) with detail study on the morphology & histology of nodules and their associated rhizobia. The present investigation was done to know the nodulation status as well as the morphology & histology of nodules and their associated micro-symbionts in *Rhynchosia schimperi*. The study reveal good amount of nodulation was found in it. The nodules were found perennial and mucunoid type and mainly two types of bacteria viz. fast growing probably *Ensifer* sp. and slow growing *Badirhizobium* sp. were found in it.

KEY WORDS: Nodulation, *Rhynchosia schimperi*, Thar Desert.

INTRODUCTION

India has about 2.34 million km^2 of hot desert called 'Thar' in the western part. It's about 90% part comes under India, and about 10% under Pakistan. [1] It is mainly confined to four district of Rajasthan namely Jodhpur, Jaisalmer, Barmer and Bikaner and covers an area of 19.6 m ha or 61.9% of the total hot arid zone. [2] The extreme temperature, shortness of water and the low nutrients in sandy soil is the major problem in the area. The soil organic carbon is lowest in sandy soils of the arid zone. [3] The legumes are belongs to the third largest family of angiosperm i.e. Leguminosae or Fabaceae [4] and are the major contributor to provide atmospheric nitrogen in compound form for the richness of soil in the desert. Total nitrogen fixation in the world is estimated to be ~175Tg, of which symbiotic nitrogen fixation in legumes counts for ~80 Tg by fixing 20–200 kg N fixed ha^{-1}yr^{-1}.[5] There are 179 Genera and 1152 species of legumes found in India. [6] Much studies had been done in past to know the floral diversity of Thar Desert viz. Blatter & Helburg, [7] Shetty & Singh, [8] and Bhandri M.M. [9] Bhandari explore more than 500 plants sp. in Thar Desert with 38 Genera and 88 Species of legumes including *Rhynchosia schimperi* Hochst. ex Boiss. According to Shetty & Singh it is a rather rare plant of Thar Desert and mostly confined to Jaisalmer and Barmer & few occurrences were reported from Bikaner. [8] Author itself reported it very difficult from Jaisalmer on road side on Lanela road, and from a new site Cheela near Phalodi (Jodhpur). There are least information is available on the nodulation in it. Only the occurrence of nodule was reported by Gehlot in 2007 and was published in Sprent (2009) [10] but lack the detail study. In India the comprehensive study on nodulation in native legumes of Thar Desert was done by Gehlot et. al. [11], Dheeren et. al. [12] and the author's own Ph.D. thesis (unpublished) in which they have reported nodulation in 35 native legumes (excluding *Rhynchosia schimperi*.) with detail study on the morphology & histology of nodules and their associated symbiont. The present investigation was done to know the nodulation status as well as the morphology & histology of nodules and their associated symbionts in *Rhynchosia schimperi* Hochst. ex Boiss. It is a first attempt to know the nodulation in it.

MATERIAL AND METHODS

Taxonomy of plant:

The Regular Field trip was done in monsoon season July-September 2019-22 to explore the plant in Barmer, Jaisalmer and Jodhpur District. The field characters viz. habit and habitat, root type and color, height of plant, flower color, leaf variations ect. were recorded in field note book and the plant twigs containing leaves, flower and fruits were bring to the laboratory in vasculum for detail taxonomic description. The standard taxonomic terminology as describe in Lawrence [13] was used to describe the plant.

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Nodulation Study:

In Field: Regular field trip was done in monsoon season to collect the young seedling of plant. The seedlings were dugout carefully with intact roots and bring to the laboratory for further study. The soil was removed by gentle flush of water jet. Many characters of nodules viz. numbers, positions and attachment on the roots, their color, size and texture was recorded.

In nursery: Several seed were collected from field. The seeds were germinated by acid treatment of analytical grade con. H_2SO_4. The germinated seedlings were planted in 2 kg poly bags (30 replicas) contain the original site soil for detail study of nodule morphology, histology and microsymbiont.

Histology of Nodules:

The fresh and healthy nodules of all stage were selected with a small adjoining root part and were sectioned through hand. Radial-longitudinal and transverse hand sections were made using sharp razor blade. Middle half of nodules were observed under dissecting microscope for presence of leg-hemoglobin. Thin sections of approximately 0.1 to 0.3 mm thickness were selected and partially dehydrated with ethyl alcohol series (30% to 50%). Sections were stained by Safranin-Harris hematoxylin as describe by Johansen [14] and 1% Toluedine Blue. Sections were observed under Olympus dissecting microscope at 10x and under Jainco compound binocular microscope at 25x, 100x, 400x and 1000x. Photographs were taken using Canon EOS 1300D and Samsung Galaxy J5 prime camera. Adobe Photoshop7 were use to edit the photos.

Microbiology of Symbionts:

The micro-symbionts were isolated from the nodules and were cultured on CR-YEMA, and BTB-YEMA for rhizoibial confirmation by standard procedure as describe by Somasegaran and Hoben. [15] The colony characteristics wise size, shape, texture and color were recorded. Gram staining was done to know the bacterial classification.

Host authentication:

Isolated micro-symbionts from the nodules were purified, multiplied and activated in broth before re-inoculation to their natural host then variant of nif TAL tube method (hydroponics system) as well as sterile soil rite, vermiculite and sand mixture moistened with nitrogen free Fahraeus solution were used for authentication.

RESULTS

Taxonomy of Plant:

Rhynchosia schimperi Hochst. ex Boiss. is a perennial under shrub or climbing twiner occurs in Indian desert. Fig. 1 (A-H) shows the external morphology and habitat of plant with characteristics features. Plant has xerophyte characters viz. thick and densely hairy leaves & Stem (Fig. 1 B-D.), leathery thick pod (Fig.1 H), and hard seed coat for desert adaptation. **S**tem terete, densely velvety pubescent with ashy appearance, leaves alternate trifoliate, petiole minute, terminal leaflet obcordate, emarginate, mucronate, slightly leathery, covered with dense hairs both side, yellow dotted shining gland beneath. Inflorescence 2 flowered axillary raceme, flower papilionaceous and yellow (Fig. 1 E), stamen diadelphous 9+1(Fig. 1 G), ovary elliptic oblong and densely hairy (Fig. 1 F), fruit two seeded pod, constricted between seed, apiculate, hairy, brown at maturity (Fig. 1 H), seed globular, black and glabrous.

Morphology and Anatomy of Nodule:

Fresh and healthy nodules with collapse nodules were found on main roots as well as on lateral roots (Fig. 2). Nodules not originate from root axil means infection occurs through root hair. At initial stage all nodules were irregular spherical in shape with little protuberance but later on become elongated or branched, indeterminate Mucunoid type (Fig 3 D & E). Prominent bark was found in all nodules and may arise from the outer cortical. In older nodules deep fissures found between bark which may play role in gaseous exchange like lenticels. Nodules were perennial in pot culture but in field condition after 2-3 months become collapse perhaps due to lack of sufficient moisture. The middle half shows blackish-red color and in few nodules greenish color (not shown here) was found which indicates the occurrence of active N2 fixation (Fig. 3 B). The size ranges from 3 mm (initial stage)

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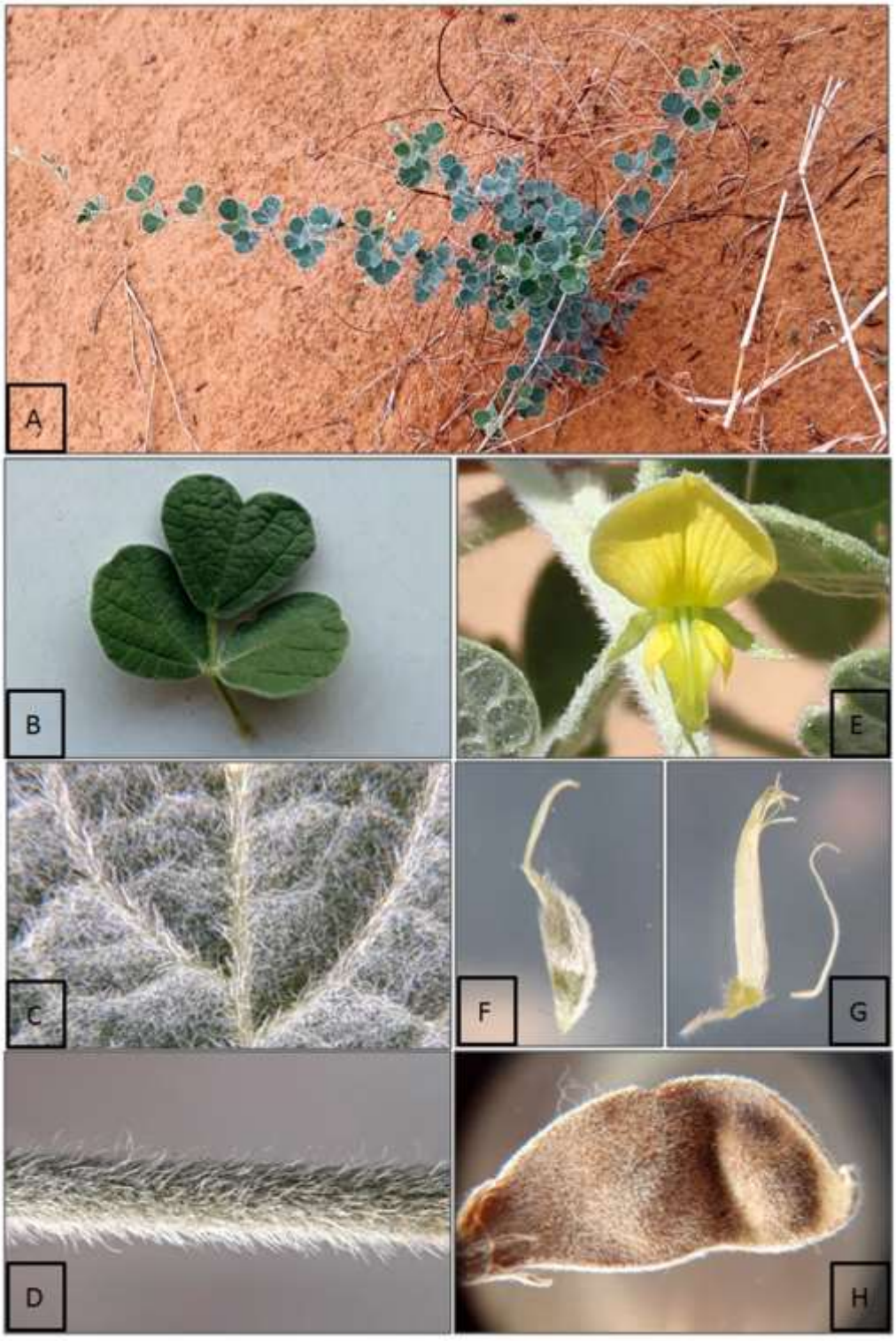

Fig.1. Morphology of *Rhynchosia schimperi* Hochst. ex Boiss. (A) Habit & Habitat: A perennial xerophyte under shrub or Twinner, (B) Leaf, (C) Dorsal leaf surface, (D) Stem surface, (E) Papilionaceous flower, (F) Gynoecium, (G) Androecium, (H) Dried pod.[Note: Thick appearance of leaf in B and dense hairs in C, D, F & H showing xerophytic characters for desert adaptation]. Magnification in C, D, F, G, & H at 10x.

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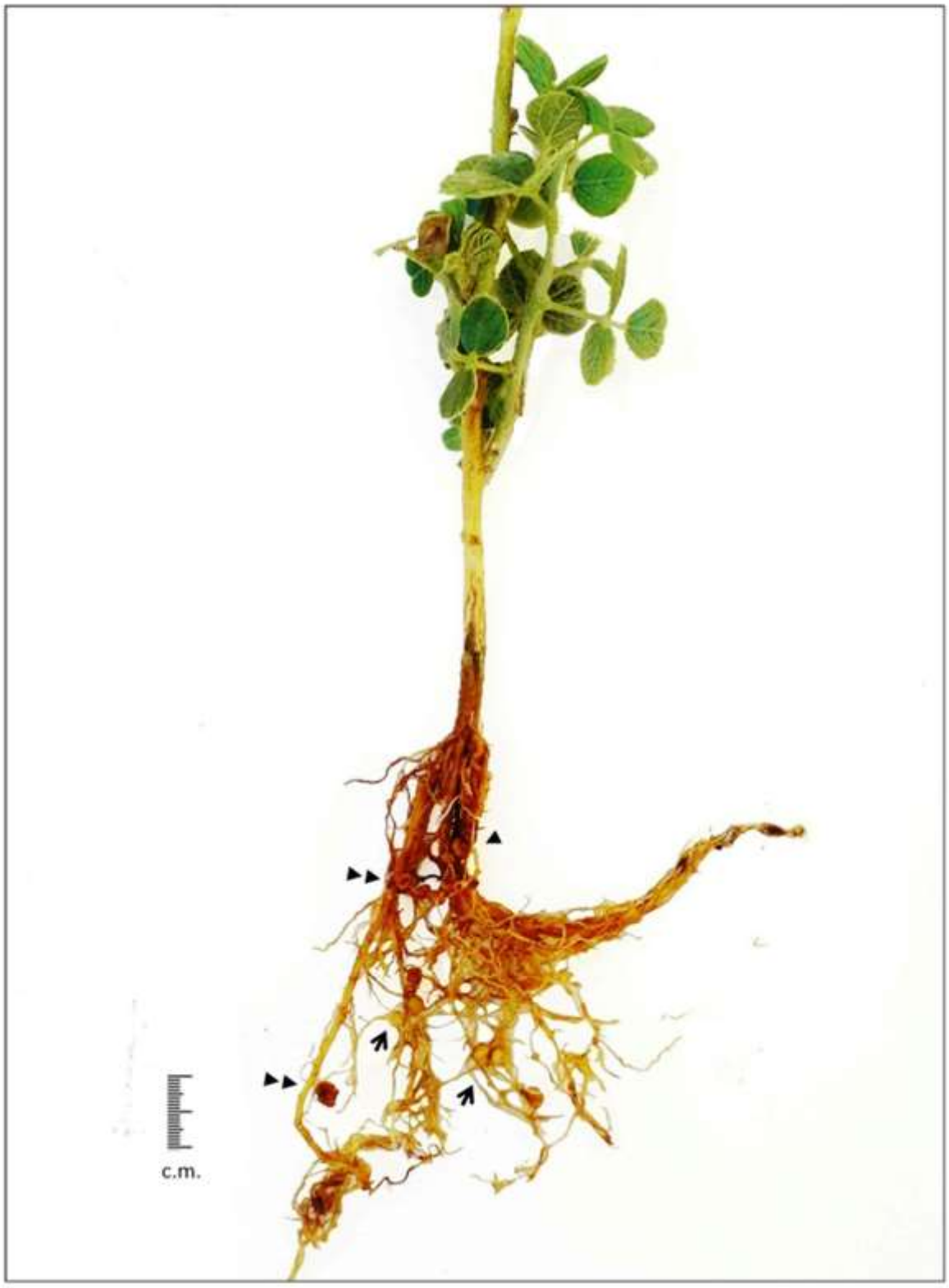

Fig.2. Nodules Arrangement in *Rhynchosia schimperi* Hochst. ex Boiss.: Note nodules on main root (single arrow head) as well as on lateral roots (arrows) and collapse nodules (double arrow head).

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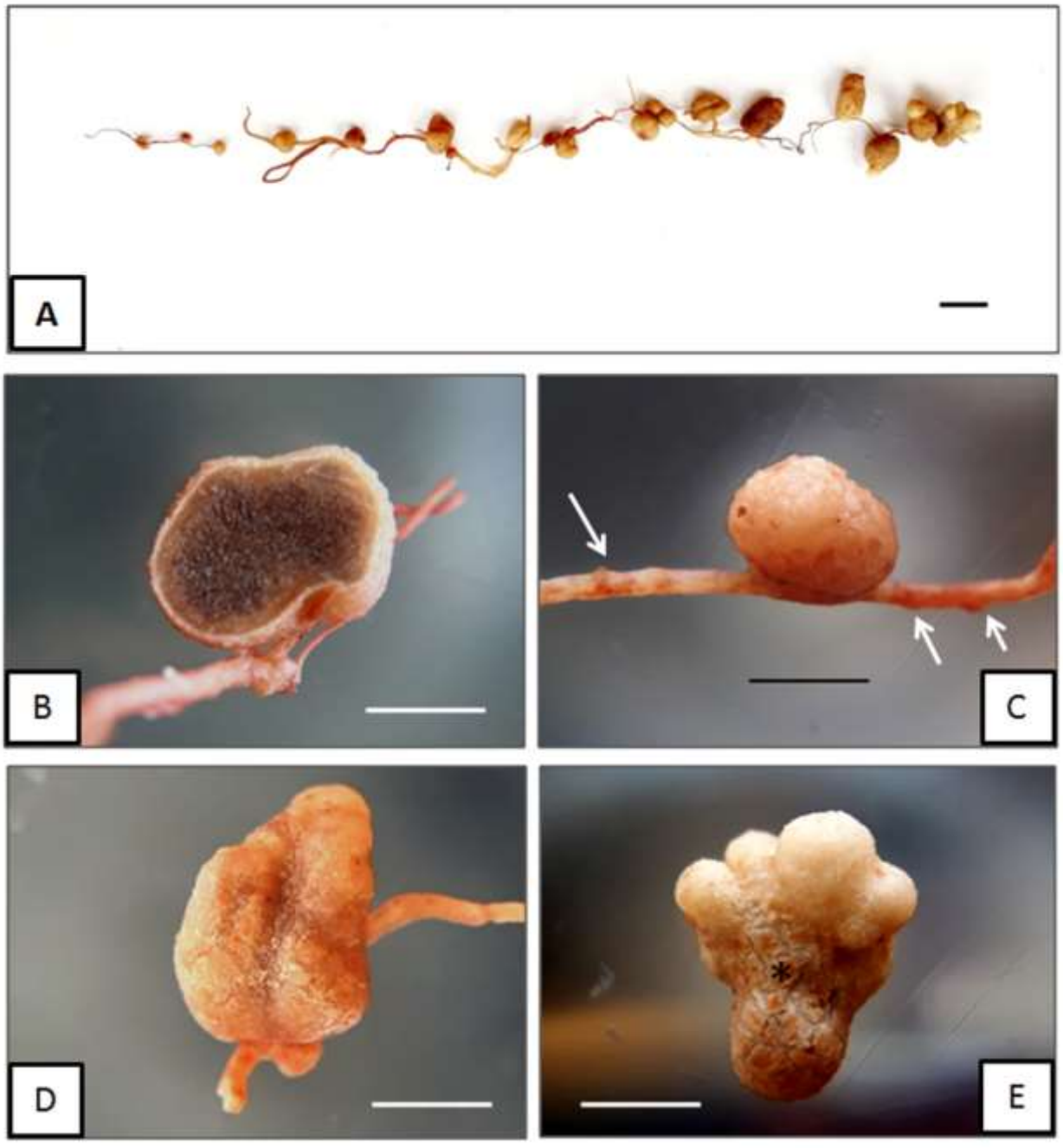

Fig.3. Nodules Morphology: (A) Developmental stages of nodules collected from a single plant (B) Radial Half of nodule [Note dark blackish red pigment the Leghemoglobin] (C) Young Nodule [arrows indicating initiation of new nodules], (D) An un-branched nodule (E) Branched nodule [Note: D & F both are mucunoid type of nodule as describe by Corby (1988), Asterisk (*) indicate bark on surface that has arisen from a cortical phellogen. Magnification in B, C, D, & E at 10x. Bars= 5 mm in A, 2.5 mm in B, C, D & E.

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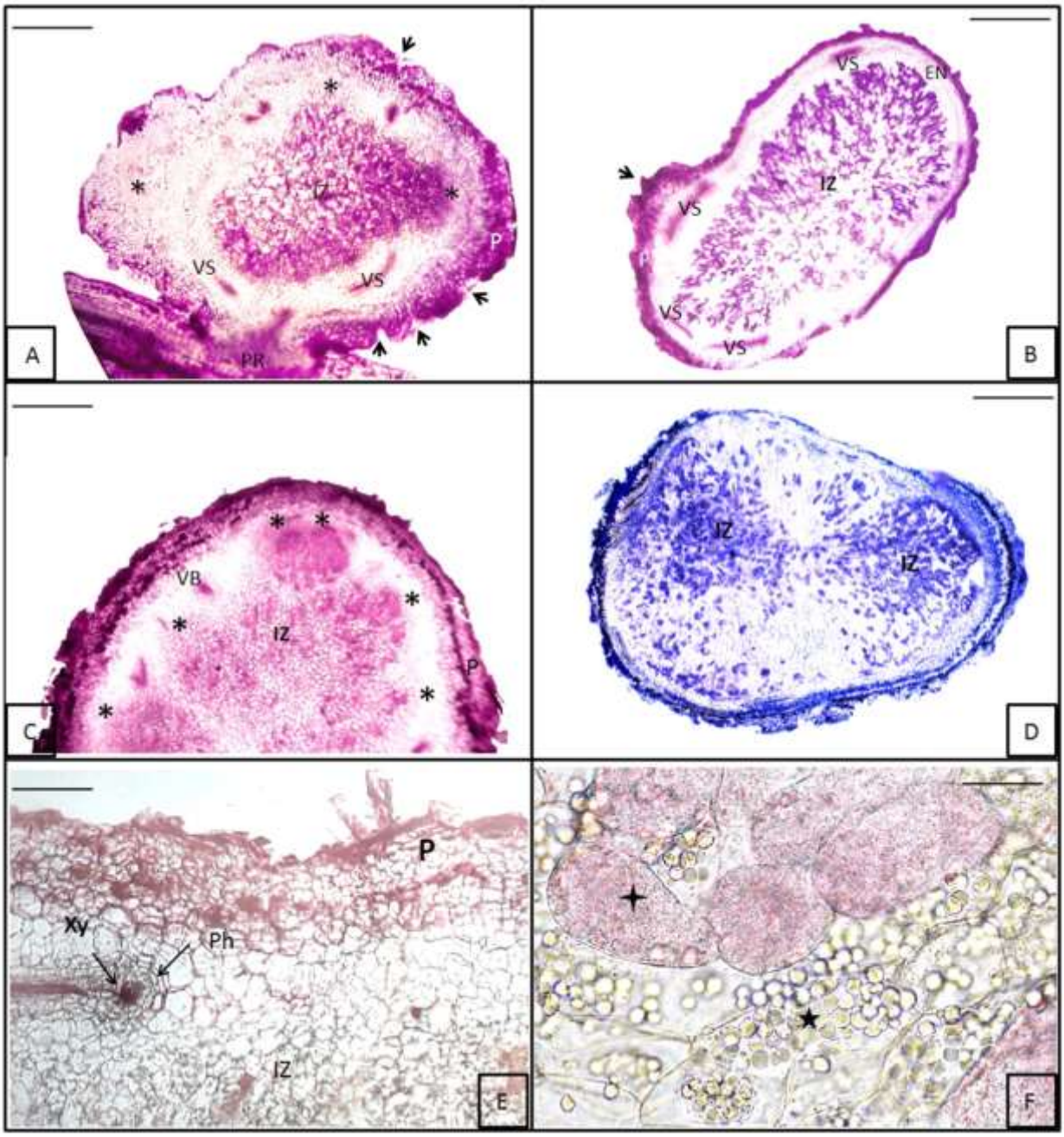

Fig.4. Light Micoscopy of nodules of *Rhynchosia schimperi* Hochst. ex Boiss: (A) Young nodule along with root part and emerging new branch [arrow indicating deep fissures which may play role in gaseous exchange] (B) & (D) Mature nodule: Note-Single infection zone in B and two separate infection zone in D]. (C) Enlarge view of nodule: Note infected [dark purple] & uninfected [blank] cells and multiple provascular meristem [asterisks]. (E) Magnified view of nodule from Fig. B at 100x showing amphicribal xylem and prominent periderm, (F) magnification at 1000x: 4 point star indicating cells filled with bacteroid and 5 point star indicating cells filled with oil droplet a characteristics feature of *Rhynchosia* nodule. Asterisks (*) = meristem, P= periderm, IZ= infection zone, VS= vascular supply, EN= endodermis, R= Root, Xy= xylem, Ph= Phloem. Bars= 500 µm in (A, B, C, D), 200 µm in (E) and 20 µm in (F). Stained by safranin-hematoxylin (A-C & E) and toluidine blue (D).

to 5 (-7) mm (Fig. 3 A). Nodules number and other characters were varying in field condition and in pot culture; data are shown in Table 1. Longitudinal Section of nodules shows vascular supply surrounds the infected region or run parallel outside the

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infected region which is the characteristic feature of rhizobial infection (Fig. 4 A-D). Meristem was apical in position but may split and found more than one or multiple provascular meristem in branched nodule (Fig. 4 A & C). Cells were filed with bacteroid and few uninfected interstitial cells were found (Fig. 4 A-D). Infection zone was found evenly in whole nodule (Fig. 4 B) or in more than one separate zone as in branched nodule (Fig. 4 D). Prominent periderm with deep fissures was present. It is assume that fissures may play role in gaseous exchange. Vascular bundle was amphicribal (Fig. 4 E). The infected cells were fully filled by bacteroid while uninfected interstitial cells were filled with oil droplet (Fig. 4 F). The presence of oliosomes is the characteristics feature of *Rhynchosia* nodule since we have also found these oliosomes in another sp. *Rhynchosia* minima and *Rhynchosia aurea* (unpublished).

Table1. Nodules characteristics in wild and pot culture

Habitat	Number of nodules in 30 days	Number of nodules in 60 days	Number of nodules in 90 days	Shape of nodules	Size of nodules	Lenticels	Leg hemo globin	Nodule color
Field condition	± 10	± 8	± 5	Irregular spherical when young and Mucunoid in older	± 3 mm	Absent	Present	Pinkish dull yellow
Pot Culture	± 20	± 30	± 45	Irregular spherical in young , Elongated Caesalpiniod or Branched Mucunoid in older	± 5mm or may up to 7 mm	Absent	Present	Pinkish in young nodule dull yellow or brown and corky in older

Microbiology:

Rod shape gram negative bacteria were seen from exudates of fresh nodule. Bacteria grow on YEM broth shows motility in hanging drop method. On CR YEMA bacteria remain translucent opaque dull white or white which is the characteristics feature of rhizobia. Pure culture shows two types of bacterial colony from different soil. Translucent dull white fast growing bacteria were isolate from plant grown in soil collected from Cheela (the new site). While fast growing translucent dull white and slow growing white both types of colonies were found from plant grown in soil collected from Jaisalmer. The other characters of fast growing and slow growing bacteria were similar to our publication Gehlot et al (2012). Probably fast growing was *Ensifer sp*. while slow growing was *Bradirhizobium* sp.

DISCUSSION

Corby describes many types of nodules in Leguminosae e.g. Caesalpinioid, Desmodioid, Crotalarioid, Mucunoid ect. [16] According to him in tribe Phaseolae dimorphic nodules were found viz. disomatic mucunoid type and monosomatic Aeschynomenoid, & Desmodioid types. In my study disomatic branched & un-branched Mucunoid and Caesalpiniod both types were found in *Rhynchosia schimperi* Hochst. ex Boiss. The meristem activity was not atypical but interesting as describes by Lotocka et. al. (2012). [17] It was found completely cease in some elongated nodule and no meristem was seen even in fully mature nodules (see Fig. 4 B), while in many nodules apical meristem split into multiple provascular meristem which will allow the elongation of the nodule vascular bundles in accordance with nodule growth properties (see Fig. 4 C). Many author reported that lenticels are uncommon character of an elongated inderminate type of nodule viz. Corby (1988) [16], Sprent (2009) [10], but exceptionally present as in indeterminate nodule of *Indigofera* (see in Gehlot et. al 2012). In my study I have not found typical lenticels on nodules of *Rhynchosia schimperi* Hochst. ex Boiss but in longitudinal section deep fissures were found where endodermis was ruptured hence assume that the gaseous exchange was taken through these fissures. Since I have cut the section by hand therefore it is need of verifying it by thin microtome section. Isolated fast growing and slow growing bacteria may probably *Ensifer* (*Sinorhizobium*) and *Bradirhizobium* species respectively.

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CONCLUSION

It was the first attempt to know the nodulation status with detail study on morphology and histology of nodules in *Rhynchosia schimperi Hochst. ex Boiss*. My study reveals that mainly mucunoid types of nodules were found in it. Two type of meristamatic activities were found viz. no meristem was seen in some mature indeterminate nodule while multiple splitting meristems were observed in almost all branched nodule. The presence of oliosome in interstitial uninfected cells was another interesting character of *Rhychosia* nodule. The bacteria isolated from nodules were both fast and slow growing, but need of further investigation on DNA profiling of these bacteria for species identification.

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