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# *CISTANCHE TUBULOSA* (SCHENK) R. WIGHT AN IMPORTANT MEDICINAL PLANT OCCURRING IN SAND DUNES OF KARAK, N.W.F.P., PAKISTAN

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**Abstract**

*Cistanche tubulosa* (Schenk) R. Wight of the family Orobanchaceae has been found growing as a parasite for the first time on *Capparis decidua* L (Capparidaceae). It is reported for the first time from District Karak (N.-W.F.P.) Pakistan. It was also found growing on *Calligonum polygonoides* L (Polygonaceae), *Calotropis procera* L. (Asclepiadaceae) and *Tamarix indica* Willd (Tamaricaceae). The seed germinates on the host root which is near the soil surface and produces haustoria which then penetrate into the deeper layers of the root i.e. to the centre of metaxylem to tap the nutrition from the host, where it is parenchymatous in nature. The nature and habit of both the host and parasite are described in detail.

## Introduction

Members of the family Orobanchaceae are often fleshy, usually covered with scale leaves. Both the leaves and stem are devoid of chlorophyll and are parasites on roots of a number of plants (Jafri, 1976). It has been reported that the plants are either holo– or hemi-parsitic, annual or perennial herbs or shrubs growing on roots of a host. The plants are succulent and covered with small, membranous, simple and sessile leaves which are arranged in a spiral manner. No well developed stomata, rather a hydathode, are present for the exchange of gases and water vapours. Flowers are aggregated in spikes. The flowers are bracteate and small with each occurring in the axil of a scale. A hypogynous disc is present at the base of flower which is fleshy (Watson & Dallwitz, 1992). The plants are attached to the host by means of haustoria formed at the base of roots which transfer nutrients to the parasite from the host.

The fruit comprises of a dehiscent, non-fleshy, unilocular capsule containing many extremely minute endospermic seeds. The seeds are widely dispersed by wind which increases their chance of inhabiting a new host (Anon., 2008). The embryos are minute and usually undifferentiated i.e., they are immature (Jafri, 1976). Lack of food and immature embryos has led to a parasitic habit. The stem usually remains underground during the early developmental stages and is achlorophyllous. The stem on emergence out of the ground in spring then produces flowers which are arranged in such a way that the inflorescence looks like a pine cone and does not become green. The peduncle is crimson when young which then becomes yellow or whitish yellow as the parasite matures.

The family is economically important because genera like *Orobanche, Aeginetia* etc., cause damage to the host plant. One member of the family viz., *Cistanche tubulosa* alongwith *C. deserticola* Y. C. Ma occurs usually in sandy habitats and inhabit a variety of plants. *C. tubulosa* has been reported from China, Pakistan etc., and is a yellow to yellow-brown herb, with simple, unbranched, erect, glabrous often 40 to 60cm tall stem. It has been reported from various parts of Pakistan (Jafri, 1978; Nasir & Rafiq, 1985) but not from Karak, a district in Kohat Division. Recently it has attracted a lot of interest due to its medicinal properties, especially those related with fertility problems of both the

males and females (Dharmananda, 2004). Various compounds of interest have been isolated from different species of the genus (Song *et al*., *2008*; Xie *et al*., 2008; Leung & Ko. 2008). A decoction of the entire plant is used against jaundice (Palevitch *et al*., 2002). Locally the drug is administered for treatment of whooping cough, stomach aches etc. Either a concoction of the stem is made or the dried stem powdered which is then administered to the patients with sugar. Studies are underway to isolate various active compounds synthesized by the plant along with ethnobotanic importance.

Detailed studies of the species regarding germination, seedling establishment, parasite-host relationship and medicinal importance are not properly understood regarding the local genus *Cistanche tubulosa*. Therefore, the present paper is an effort to report distribution of the plant in the region and understand its nature and habit, while details regarding active compounds isolation and ethnobotanic importance will be reported in a latter communication.

## Materials and Methods

After a thorough survey of the Kohat Division *Cistanche tubulosa (Schenk)* R. Wight was collected from three different localities of district Karak viz., (i) village Bogara *via* Amberri Ada, (ii) Gangai on Indus Highway near Hamadan Chowk and (iii) Thal Wazir on Bannu Link road (Fig. 1). These are indicated as sites 1, 2, and 3 respectively. The soil was mainly sandy with some silt and clay as evident from Table 1. The soil was loose and retained by the host roots. It could be easily eroded by rain/flood water. Samples of both the host and parasite were collected in March-April 2008, dried properly and mounted on herbarium sheets for record. Dried root and stem parts were ground into a powdered form for chemical analysis.

A detailed morphological study of the fresh material was also carried out, especially of the projecting inflorescence. Thin sections of the root of the host (*Capparis decidua L*) (Family Capparidaceae) parasite were cut with the help of a Sledge microtome, stained in saffranine, dehydrated with different grades of alcohol and finally after clearing in clove oil were then mounted in Canada balsam. Thin sections of the parasite stem were cut with the help of a razor. Procedure for staining was the same as described above.

To comprehensively understand the habit of the host and parasite a number of surveys were conducted in early spring, summer and autumn. The stem part of the parasite used was usually dead in summer and autumn. However, the root used to be living with young buds and somewhat elongated stem parts of the future. To determine percentage of parasite, the host plants were counted in a selected area. In case of site 3 an area of 10X10m was selected, while 5X5 at sites 2 and 3 as a unit.

## Results

Map of the Karak district (Fig. 1) indicates the places from where the plant has been collected for the first time. The parasite was found on the host *Capparis decidua L*. (Family Capparidaceae) for the first time in village Bogara (site No. 1). Moreover, *Cistanche tubulosa* (Schenk) R. Wight was found growing on four different types of hosts viz., *Capparis decidua* (Fig. 2) not reported before, *Calligonum polygonoides* L. (Family Polygonaceae*)*, *Calotropis procera* L. (Family Asclepiadaceae) and *Tamarix indica* Willd (Family Tamaricaceae). To ascertain the host-parasite association, the soil was dug to expose the underground parts. As evident from Fig. 3, the *Cistanche* sp., root penetrates into the host root and establishes itself firmly with host root tissue by means of

haustoria. Some thin roots are also formed at the junction of the host and parasite roots which remain thin (slender) and do not develop further. These might help in absorption of water, minerals etc., whenever available from the soil. The anatomical section of the host also reveals that haustoria form a fibrillar structure and then ramify in the vascular tissue of the host root as indicated by arrows. Thread-like structures penetrate into the deeper layers of the host root from the base of the parasite root (Fig. 4).

The host plants prefer sandy soils (desert conditions) exhibiting xerophytic characteristics. Further, these soils are poor in organic matter and other essential elements as evident from Table 1. These soils are coarse and loose in texture with very low water holding capacity. Typical xerophytes and halophytes which form association with parasite grow in the area e.g., *Calotropi*s sp., *Calligonum* sp., *Capparis* sp and *Tamarix* sp., Survey of the area showed that *C. decidua* L plants were sparse and occurred at a reasonable distance from one another and other species growing in the area. However, host plants occurred densely at sites 2 and 3 (Table 1). *Calotropis procera* L., density was not much at site 2, nonetheless it was heavily infested. Although *C. polygonoides* L., occurred densely in the area, it was least attacked by the parasite. During observation it was found that usually numerous parasite *Cistanche tubulosa* plants occurred on the same host viz., *C. procera* L., and *T. indica* Willd. On the contrary, a single well developed, vigorous and healthy parasite occurred on *C. decidua* L. It has been observed that the parasite seed lands on the root branches which are exposed and after germination slowly pushes it to the lower strata. Haustoria are formed at the base of germinating seed which ramify through the vascular tissue of the root and reach the central core of the root xylem where it can be mistaken as pith (Fig. 4), because of parenchymatous–like cells. This firm attachment ensures physical support and nutritional supply of the parasite. The haustoria in the central part of the root then travel along the axis of the root for long distances to tap further sources of nutrition from the host roots. The roots which are not infected do not show the haustorial development (Fig. 5). Here ramification of haustoria is not obvious as seen in Fig. 4. It seems that haustorial ramification occurs at places where the parasite comes out of the host root (as shown by arrows in (Fig. 6). The threads of haustoria then take a $90^0$ turn and form a stem bud when it comes out of root. The haustorial threads are visible in Fig. 6. These haustorial threads are not visible in those portions of the host root which is not infected with the parasite root (Fig. 5). One of the haustorium then forms a slender branch which slowly and gradually swells, forms the succulent stem and remains dormant. This young stem remains underground during late summer and autumn and ultimately comes out to form the inflorescence in late summer or early spring (Fig. 7). The inflorescence/spike, after producing flowers and seeds next spring, withers (Fig. 8). Same is the fate of the underground stem as well. Thus spread of haustoria is local as revealed by Figs. 4 & 6. At these places another seed can land on the same host root, consequently germinating into a new plant, thus making a firm connection at the place of contact with the help of haustoria so produced. This phenomenon is evident from Fig. 3 where at least three parasite roots are visible. These then bear the achlorophyllous stem. In addition to this at a certain distance from the already established contact, haustoria can come out of the host root and help in giving rise to another young plant after the formation of an apical bud. Thus the *Cistanche* sp., plant can be propagated either from the seed or already established haustoria. As noticed during the present studies and evident from various figures, there is a single haustorial connection point which give rise to a single stem.

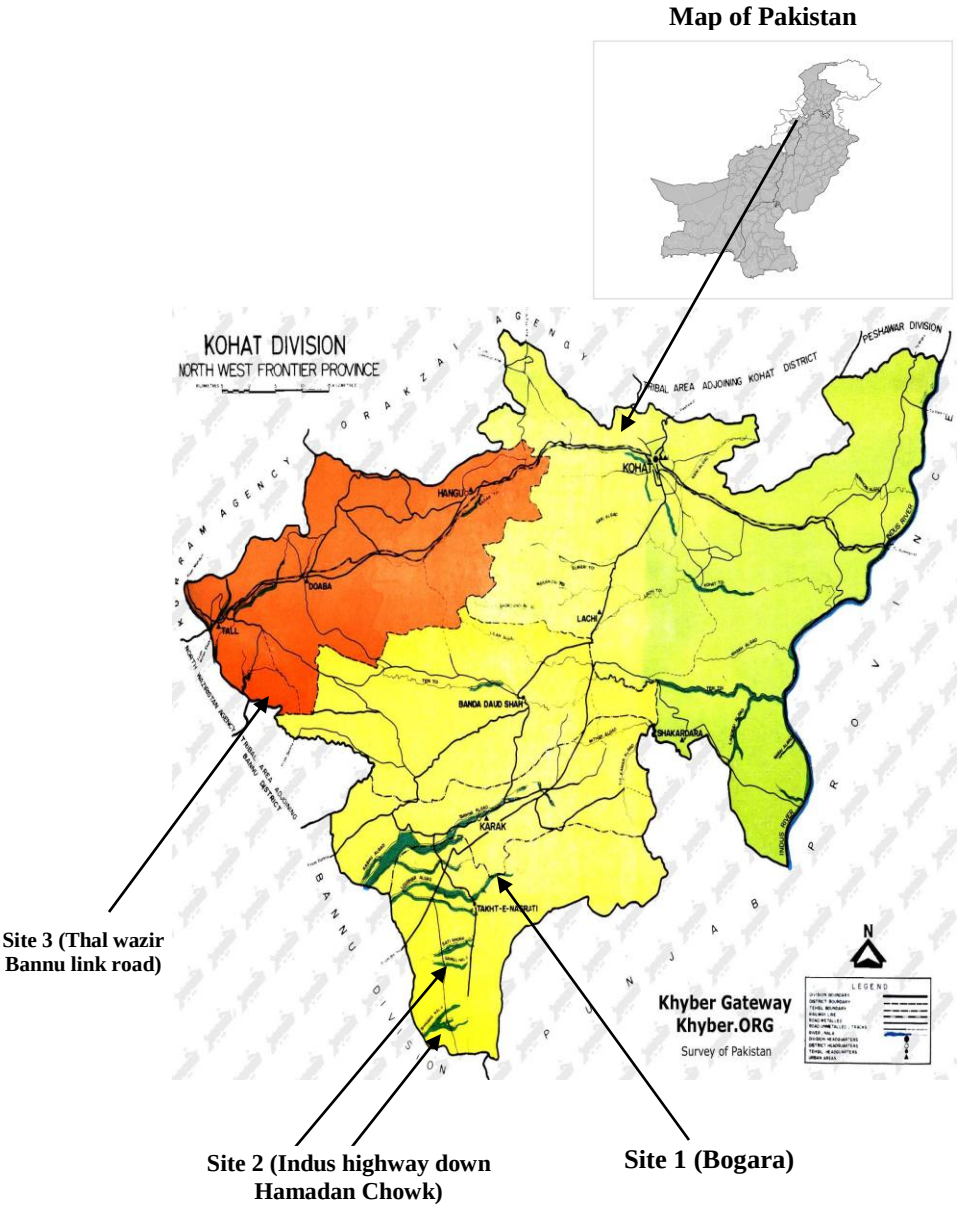


Fig. 1. Map of Kohat Division showing various places (arrows) from where the parasite has been collected.

The root of the parasite is very hard when compared with host root or the succulent stem it gives rise to narrow and slender roots (Figs. 3 & 7). On the tip of the root then a bulbous stem is formed which is fleshy (succulent), erect and swollen at the base and not completely circular due to compression (Figs. 3 & 7). The stem is white and devoid of chlorophyll. The leaves are small (scaly), alternate, arranged in a spiral having an entire lamina. These leaves contain no chlorophyll, thus are non-photosynthetic. Being a

parasite all the nutritional requirements are obtained from the host. The inflorescence, being a spike (almost resembling the pine cone), is a continuation of the underground stem which usually projects above ground in spring. The spike has a bluish or crimson colour when young while the flowers are whitish yellow at maturity. The spike is short lived and dies in 2-3 weeks depending upon the weather after release of the seeds (Fig. 8). The underground stem usually withers up to the root tip and becomes very soft. Next year another stem usually develops below the withered inflorescence and produces a new plant which ultimately gives rise to a fresh inflorescence. This phenomenon is evident from Fig. 7 where two plants are arising near one another from the host root. This phenomenon is clear from Fig. 3 as well where the spike and possibly stem of last year (arrow) have withered but a new stem formed, consequently indicating formation of a new inflorescence at the tip (arrow). The stem usually grows in a serpentine manner and remains underground throughout the year. Only the apical part of the stem comes out where the inflorescence/spike develops which withers after release of semi-mature seeds next spring, thus the stem again goes underground. Hence the plant totally relies on the host for all the nutritional requirements. Following the same pattern, next year a new branch is formed from the stem below the withered inflorescence which grows underground and develops into a spike next spring.


Fig. 2. A close view of *Cistanche tubulosa* parasitizing the host *Capparis decidua* roots. The photograph was taken in early spring. The inflorescence which protrudes from sandy soil has mature flowers at the base of the spike which are senescing and young flowers at the top which have not yet opened. The young flowers are yellow at the base while crimson at the top.


Fig. 3. The picture shows the host root from which at least three parasite roots have originated that support the swollen achlorophyllous stem (The stem is not rounded, but ridged or compressed by the soil). Minute scale leaves are discernable. However, there are no evident nodes and branching. The picture also establishes a single host-parasite connection. The slender roots are also visible.

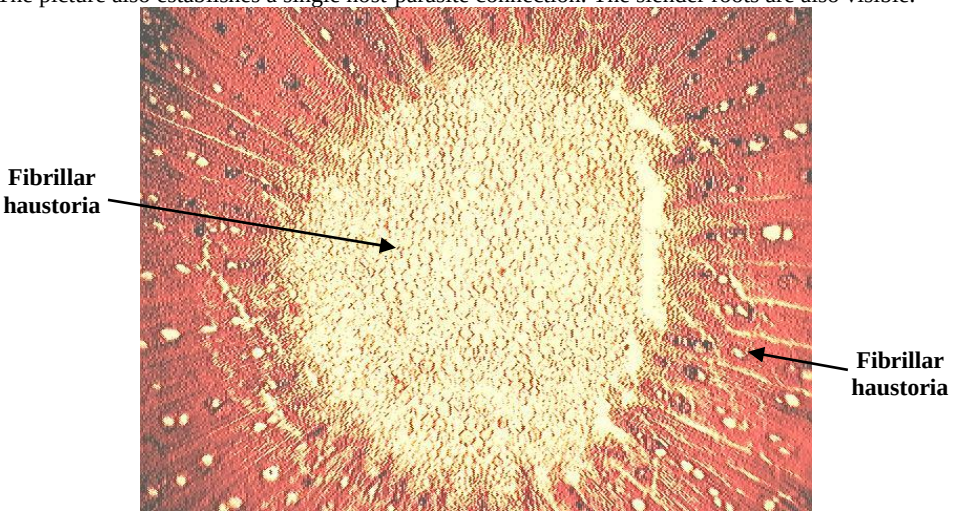


Fig. 4. TS of the host root infected with the parasite. Notice the parenchymatous type cells of the haustoria which give impression of pith.

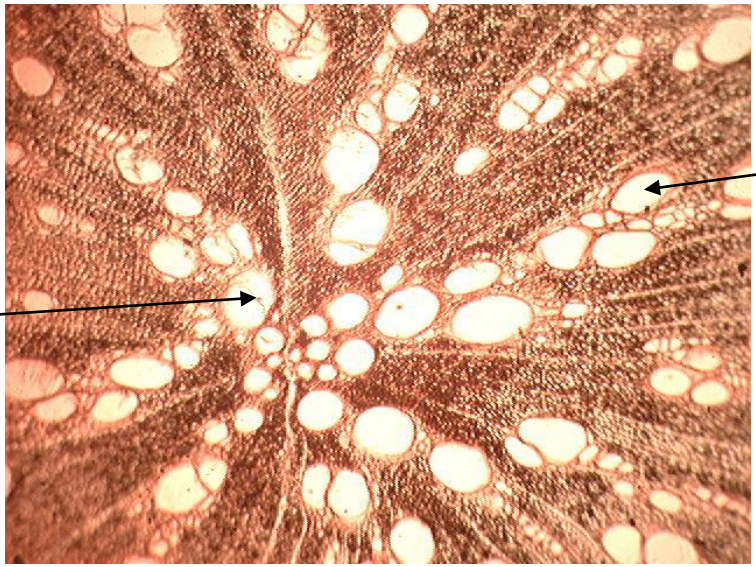


Fig. 5. TS of the host which is not infected with the parasite. Notice the typical central xylem with prominent vessels.

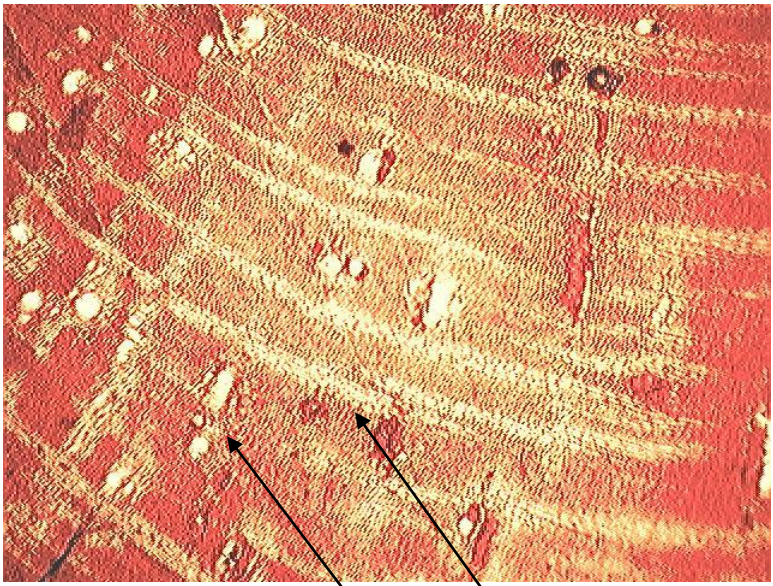


Fig. 6. Further ramification of haustorial threads in mid-central xylem of the host root.


Fig. 7. The bulbous, achlorophyllous underground stem exhibiting a serpentine-like habit.

Fig. 8. A withered stem bearing the flowers.

A close study of TS of the parasite root (Figs. 4 & 5), where the roots of the parasite establish association and at places where there is no such relationship, reveals that the root exhibits a larger proportion of xylem tissue (around 80%), while a lesser portion comprises of phloem. This indicates that there is not much conduction of organic material to the parasite root from the host stem. However, the thin phloem does serve to transport these substances from root to stem which is non-photosynthetic. This aspect needs further studies for confirmation of the findings.

Locally the plant is used for treatment of whopping cough, stomach aches etc. A concoction of the stem is made which is then administrated to the patients. Studies are underway to isolate various active compounds synthesized by the plant and the ethnobotanic importance of the plant. These studies will be helpful for conservation and sustainable marketing of the drug.

**Discussion**

Family *Orobanchaceae* has been described by Jafri, (1976); Nasir & Rafiq, (1995). Although *Cistanche tubulosa* (Schenk) R. Wight has been reported from other parts of Pakistan, there is no such report of it from N.W.F.P. Thus the present finding is the first report regarding its collection from this region. In addition we are the first to report parasitization of *C. tubulosa* (Schenk) R. Wight on *Capparis decidua* L., because it has not been reported occurring as a parasite on this plant either from Pakistan or any other country (Jafri, 1976; Nasir & Rafiq, 1995; Dharmananda, 2004). Nonetheless, it has been reported as a parasite on *Calotropis procera*, *Tamarix indica*, *Calligonum polygonoides L*., *Suaeda nudiflora*, *Salvadora oleoides* etc. (Jafri, 1976). During the present surveys we found *C. tubulosa* growing on a number of host plants, but not *Salvadora* sp., and *Suaeda sp*., as these plants do not grow in places where the parasite usually grows in this part of the country. Our findings showed that *C. decidua* L., *C. procera* L., and *T. indica* Willd were the preferred hosts, because of heavy infestation of theses hosts. *C. polygonoides* L., was the least infected. The parasite grows from a tiny seed which can travel long distances buoyed by winds. The seed attaches itself to the root and then produces a haustorium which penetrates to the deeper (central) layers of the root of host plant where the haustoria ramify in different directions (Figs. 4 & 6). The central xylar portion of the host is then replaced by haustorial cells and can be mistaken with pith because of presence of parenchymatous-type cells. This has been reported by Qasem (2006) for a number of such parasitic species. It has been reported that allelochemicals play a role in seed germination of the parasite on the host (Jorrin *et al*., 1999). A survey of the area revealed that a number of plant species grow in the area but the parasite establishes connection with the specific host and none other, thus supporting the idea of host-parasite relation based on certain chemical interaction. Root of the *Cistanche tubulosa* is hard, smooth and exhibits anatomical characteristics like that of a normal root. The stele of roots is star-shaped with meta-xylem in the centre and protoxylem on the tip of the steler arms. There is tremendous secondary growth, thus the central core is occupied by metaxylem which further pushes the protoxylem outward. The phloem is a few layers thick and occurring on the extreme periphery of the xylem. Contrarily the stem is succulent, swollen and reasonably soft. This is probably to store water and other nutrients obtained from the host which are possibly then further biosynthesized/bioconverted for assimilation into other organic compounds for ultimate utilization by the parasite. Both the soil and parts from the stem were analyzed for detection of minor elements. As evident from Table1. the soil contains aluminium in appreciable quantities. This was absorbed by *Cistanche* stem and might be having added nutritional effects which are under investigation. Apparently it seems that a single root usually attaches to the host root. However, as evident from Fig. 3 other small roots are also formed as reported by Dharmananda (2004). Nonetheless, these roots do not develop further and remain slender. A comparison of host and *Cistanche* plants exhibit differences regarding presence of parenchymatous-like cells in the centre of the metaxylem of host root. Hence there is a peculiar parenchymatous, pith-like central portion which is misleading because roots are devoid of pith. This deserves further investigation.

The economic benefit of the parasite could be its medicinal importance both locally and abroad (Dharmananda, 2004; Song *et al*., 2008; Xie *et al*., 2008; Xue, 2008). The host-parasite relationship could bind the sandy soil, hence possibly reducing erosion etc. Further the spread of the host roots and ramification of the parasite stem within the soil could provide the vegetational cover and thus conserve water resources under desert-like conditions. This characteristic habitat hence affords a medicinal parasitic herb of immense economic importance in the future (Morikawa *et al*., 2008; Xue, 2008). It has been reported that desertification and soil erosion are major causes of food crisis (Khan, 2008), thus increase in vegetational cover of the area with *Cistanche sp*., and host species might be able to control this phenomenon.

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