



Research Article

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FIRST RECORD OF A TORYMIDAE INFESTATION IN THE SEEDS OF *CORDIA MACLEODII* HOOK. F. & THOMSON: INHIBITING SEED GERMINATION

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ABSTRACT

Background: The seed germination percentage was found to be very low; hence, the investigation was carried out to find out the seed infestation. Aim and Objective: To discover the cause of the low percentage of seed germination. To report the Torymidae infestation in *Cordia macleodii* Hook. F. & Thomson. Material and methods: Mature fruits and seeds were collected from 10 plants of two selected localities. Longitudinal sections were taken to determine the infection in buds, immature, mature fruits and seeds. Documentation of different stages was recorded. Identification and authentication were performed based on characters observed and reports from ICAR- National Bureau of Agricultural Insect Resources, Bangalore. Discussion: The insect species was identified as a species belonging to the Family Torymidae (Hymenoptera: Chalcidoidea). The fruits of *Cordia macleodii* Hook. F. & Thomson showed internal as well as external infestation. Conclusion: The investigation's findings confirmed that the low seed germination is due to the infestation of the Torymidae insect, which was reported in this communication for the first time.

Keywords: *Cordia macleodii*, Dahipalasha, infestation, seed germination, *Torymidae*

INTRODUCTION

The genus *Cordia* belongs to the family Boraginaceae, harbouring 300+ species, most of them being trees or shrubs showing their existence across Asia, Africa, and the American continents. In India, there are 21 species of *Cordia*, of which five species of *Cordia*, namely *C. gharaf* (Forssk.) Ehrenb & Asch., *C. macleodii* Hook. F. & Thomson, *C. domestica* Roth fulvosa Wight, *C. wallichii* G. Don and *C. dichotoma* Forst. f. are predominant in Maharashtra.¹⁻⁵

Among these, *C. macleodii* is distributed across India, Bangladesh and Pakistan. It is a rare and endangered plant known as Dahiman, Dahipalash.⁶ It is a deciduous, 8-10 m high tree, showing flowering and fruiting between March – June.⁷ The flowers are white with yellow throat; drupes are 1.2-1.9 cm long, acuminate, and ovoid seated on persistent densely tomentose calyx. The branchlets and young shoots show a woolly appearance, and the leaves are heart-shaped ovate with glabrous/ rough lamina and pubescent adaxial side with 2-3.5 cm long leaf stalks.^{2,8,9}

The plants of *Cordia macleodii* exhibit varied pharmacological activities; some of the remarkable ones are antibacterial, antifungal, anti-inflammatory, antinociceptive, antsnake bite, immune-modulatory, antioxidant, insecticidal, antifertility, antihyperlipidemic, antibiotic. It has also been reported for various ethnomedicinal uses, such as wound healing, skin disease, jaundice, and aphrodisiac.¹

A study carried out in the Vindhyan region of India (Covering Bihar, Gujarat, Chhattisgarh, Madhya Pradesh, and Southern parts of Uttar Pradesh), following the IUCN guidelines, reported *C. macleodii* to be Critically Endangered/Rare (CR/R). Whereas, it is mentioned as Endangered in Orissa.¹⁰ Another study concluded *C. macleodii* is Critically Endangered in Eastern India based on IUCN (2001) Red List Criteria B1, B2, C, C2, and D; however, its IUCN categorization is not available, and there is a need for setting up conservation measures for this plant.^{7,11}

This therapeutically important plant is on the verge of extinction; hence, conserving and cultivating this important ayurvedic medicinal plant is essential. Efforts to preserve the plant have been reported.¹² However, a maximum of 6-8 % seed germination was observed in this study. After a thorough investigation, it was observed that insects infested almost all *Dahipalasha- C. macleodii* fruits. Thus, it was found necessary to identify the root cause of infestation. Hence, this study aimed to report the occurrence of species responsible for the infestation in fruits and seeds of *C. macleodii* and, eventually, the germination rate.

In this communication, the occurrence of *Torymidae* infestation (Hymenoptera: Chalcidoidea) in *C. macleodii* seeds leads to the main cause of seed damage and affects germination, which has been reported for the first time.

MATERIALS AND METHODS

Collection of plant material

C. macleodii plant located in Katrajghat (N 18°24'36.0"; E 073°51'35.4"), Mangdari area (N 18°16'77.6"; E 073°46'90.2") of Pune, Maharashtra, India was selected for study. Locations were recorded with the help of a GPS tracker device (Garmin-Oregon 750). Plants were identified by botanist Dr Arun Gurav (Research Officer-Botany), Regional Ayurveda Research Institute, Pune, and authenticated with the help of Flora. The herbarium of the collected sample was prepared and deposited in the herbarium section of the institute with Accession No. 14974 for further reference.¹³ Five plants from each, Katraj and Mangdari, were identified and selected for further experiments.

Traps to collect the insect

To collect stages of insects, including larvae and adults, twigs with buds, flowers, and fruiting were tied with Nylon nets during the blooming season, i.e., March-April (Figure 1a). In subsequent weeks twigs were cut down with nylon bags to collect intact floral parts and fruits. Mature fruits observed during the visit were collected from five selected plants twice a month in May and June during the experiment.

Fruits collected from each tree were kept separately in transparent polybags, and holes were made for aeration. The 15 fruits of each tree/collection were selected randomly from these collected fruits. Thus, a total of 150 fruits were chosen for further observation.

Incubation of eggs

To maintain moisture and temperature suitable for the incubation of eggs located in seeds, all seeds were kept in polybags at room temperature at 28 °C±5, placed in a closed box, and observed daily for up to 2 months or till the eggs were incubated.

Hatching of larvae

Larvae hatching from incubated seeds were separated and kept in glass bottles. The mouth of the bottle was covered with muslin cloth for air circulation.

Identification of insects

Different stages of larvae and adult flies hatched from seed were collected, packed in an Eppendorf container, and sent to ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, for identification and authentication.

Confirmation of infestation

All the fruits were observed under a zoom lens for any morphological characters, holes, traces of insects, and damage. Longitudinal sections of fruits were cut with a scalpel and observed under a stereo zoom microscope (Laica- S9D), and digital images were captured with the help of a Leica EC4 microscope camera. Documentation of records was maintained for identification and authentication of insects.

RESULT AND DISCUSSION

During the seed germination experiments, it was observed that a maximum of 6-8 % of seeds germinated. However, seeds were subjected to various treatments such as conc. H₂SO₄, soaking in

water, multiple concentrations of GA₃ and different combinations of acid, water and GA₃ to facilitate seed germination. After a thorough investigation, it was observed that almost all Dahipalasha fruits were infested by insects. It was also observed that at the maturity stage of fruit and in bud condition, larvae ate all the essential whorl of flower, cotyledons, entire embryo, and endosperm except the seed coat. The seeds of *C. macleodii* contain high amounts of nutrients like carbohydrates, proteins, and lipids, which can favour the larval development of phytophagous insects within them.¹⁴ The different stages of larvae of certain species were found in the seeds of *C. macleodii*. The infestation was confirmed by observations recorded during the longitudinal section (LS) of bud, flower, and mature seeds (Figure 1).

The reports received from ICAR- National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, confirmed that the different stages of development of larvae (Figure 2) are of Family Torymidae. The insect species was identified as a species belonging to the Family- Torymidae (Hymenoptera: Chalcidoidea) with accession number – NIM/NBAIR/TORY/23721.

Chalcidoidea is a highly diverse superfamily within Hymenoptera. 375,000 to 500,000 species were estimated as diverse; more than 22,500 species have been described.¹⁵ Chalcidoidea are mostly parasitoids, and thus, they are important natural enemies of insects. Torymidae is one of the recognized, mid-sized families of Chalcidoidea that comprises 69 genera and up to 1,100 described species with a wide range of hosts of the largest and most influential groups from biological and morphological views. Phylogenetic analysis of Torymidae is based on 24 morphological characters and 46 taxa.¹⁶

As per the nomenclature, the classification of family is as given below¹⁷

Domain: Eukaryota
Kingdom: Metazoa / Animalia
Phylum: Arthropoda
Class: Hexapoda/ Insecta
Order: Hymenoptera
Superfamily: Chalcidoidea
Family: Torymidae

Literature survey and reports from ICAR- National Bureau of Agricultural Insect Resources (NBAIR), Bangalore showed that the infestation of *C. macleodii* in the current study area can be concluded to be of two types: external (Figure 1b,c,h,i,k) and internal infestation (Figure 1d,e,f,g,j).

The external infestation was localized and limited to the fruits of *C. macleodii*, which were semi-ripened and fully ripened. The infestation was observed to be caused chiefly by wingless ants (Family- Hymenoptera) and fruit worms. These organisms were noticed to be specifically feeding on the fruit pulp and did not cause any internal damage to the seed.

Meanwhile, the larvae of Torymidae insects explicitly caused internal infestation, leading to seed damage at different stages of their growth. Torymidae is a family of wasps under the superfamily- Chalcidoidea exhibiting metallic colours and a peculiar feature shown by females, i.e. a long ovipositor (Figure 2f). Also, most of these Torymidae insects survive as parasitoids on other insects that show gall formation in plants. Here, in *C. macleodii*, the presence of beetles or other parasitic insects has not been observed in the interior as well as the exterior of the fruits, which delimits the possibility of the Torymidae insect

entering the fruit along with any parasitic insect which is, otherwise the most probable way of infestation of Hymenopteran parasitoids. Hence, it confirms the direct infestation of Torymidae insects without the involvement of any parasitic insect or beetle.

Elaborating further, many fruits of *C. macleodii* showed the presence of entry and escape holes on the fruits, which indicated the external infestation.¹⁸ However, the seeds selected for this study were completely intact and healthy in their external appearance and did not have any entry/escape holes (Figure 2), confirming the only possible mode of entry of the Torymidae insect through the flowers. The flower morphology of *C. macleodii* shows a merged corolla forming a tube-like structure. On the other hand, the ovipositor of the Torymidae insect is complementary to this flower structure, creating a perfect host and parasite interaction.

Hence, it is concluded that the infestation must be caused at the flowering stage as the Torymidae insect lays the eggs into the flower, and further, the different developmental stages of the

Torymidae insect are nurtured by the food material from the developing seeds, as it was observed that the insect completes its life cycle inside the seeds. Infestation of *Paradecatomia bannensis* Masi belongs to Hymenoptera order as a seed parasite has been reported in seeds of *Cordia africana*.¹⁹ In other species, i.e. *Cordia trichotoma* seeds are also reportedly damaged by *Amblycerus long esuturalis* and *A. profaunar*, impairing seed germination.²⁰

The presence of any disease agents or pests in the seeds decreases the yield and quality. Also, the morphological features of the flower and fruit are essential factors that affect the dispersal seed infestation.²¹ *Cordia macleodii*, the infestation of insects is the main problem, and due to the infestation of *Torymidae* insects, the plant is becoming Critically Endangered/Rare (CR/R).

However, expertise in the subject is highly required to identify the species.



Figure 1: Internal and external infestation in the buds and fruits of *Cordiamacleodii* Hook. f. & Thomson
 (a- traps set to catch the insects; b,c- bud, fruit showing external infestation; d,e- dorsal and ventral view of fruit showing no entry holes, f,g- seeds damaged internally by larvae; h, i- LS of fruit and bud showing damage; j, k- exit /entry holes)



Figure 2: Stages and body parts of *Torymidæ* insect.

(a- larval stages; b- intermediate or pupae of female; c- intermediate or pupae of male; d-adult fly ventral view; e- adult fly dorsal view; f- female fly; g- wing; h-abdomen; i- antenna; j- head and compound eyes.)

CONCLUSION

The fruits of *Cordia macleodii* collected from the Pune region are observed to have external and internal infestations. It is concluded that the *Torymidæ* infestation in the seeds of *Cordia macleodii* leads to degradation/damage of all essential whorl of flower, cotyledons, entire embryo, and endosperm. *Torymidæ* species infestation decreases seed germination. It is one of the most prominent reasons for the endangered status of *C. macleodii* in the studied area. Therefore, this is the first report of *Torymidæ* infection in *Cordia macleodii* from Pune, Maharashtra, India. To consider the findings of this research, further study must be conducted to control insect manifestation. So, it is a need of the hour to find suitable insecticides to prevent insect manifestation.

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