

Report on *Crioceris* sp. (Coleoptera: Chrysomelidae) infestation and damage to medicinal plant *Asparagus racemosus* (Liliopsida: Asparagaceae) in New Delhi, India

ABSTRACT

This study reports a species of *Crioceris* infesting *Asparagus racemosus* Willd. The adults exhibit sexual dimorphism, with black males and yellowish-brown females bearing a distinct black spot on their elytra. Morphological and molecular analyses indicate a close relationship (95% genetic identity) with the *Crioceris* genus. Life cycle aspects, including hibernation during winter, spring emergence for mating and egg-laying, and polygynandrous mating behaviour, have been observed. Significant damage caused by *Crioceris* sp. to *A. racemosus*, including broken cladophylls, notched leaves, and defoliation, hindering plant growth, is reported.

Key words: *Asparagus racemosus*, *Crioceris*, chrysomelidae, first report, polygynandrous, Coleoptera, pest, damage, sexual dimorphism, lifecycle.

1. INTRODUCTION

The genus *Asparagus* encompasses over 250 species worldwide, with distribution across India (22 species), Sri Lanka, and the Himalayan regions, as well as certain areas of Australia and Africa. *Asparagus racemosus* Willd, or Shatavari, is a perennial woody climber in the Asparagaceae family, characterized by a highly branched structure reaching 1 to 2 m. Distributed across India, Sri Lanka, the Himalayan regions, Australia, and Africa, it is cultivated in various Indian states. Despite high demand for its applications, destructive practices have led to its endangered status [1]. Shatavari, hailed as the "Queen of Herbs" in Ayurveda, derives its name from the tradition, translating to "Who has a hundred husbands or is agreeable to many." Recognized for its notable efficacy in addressing female reproductive system issues, this herb, found in Ayurvedic texts like the Charak Samhita and the Ashtang

Hridyam, contains steroidal saponins known as Shatavarins I–IV in its roots, exhibiting a spectrum of pharmacological activities, including aphrodisiac, antioxidant, immunostimulant, and antiulcerogenic effects [2-4].

A. racemosus is susceptible to various pests. Aphids are a common pest that feeds on the plant's sap, resulting in stunted growth and distorted leaves. Red spider mites pose another threat, as they cause yellowing and the formation of webbing on the undersides of leaves. Whiteflies gather in clusters on the under surface of leaves and extract the plant's juices, leading to yellowing, stunted growth, and the development of sooty mould. Thrips, slender insects, feed on the plant cells, causing a silvery or bronzing effect on the leaves. Mealybugs, covered in a waxy substance, extract sap from the plant and produce honeydew, which attracts sooty mould [5]. Apart from these pests, the asparagus beetle (*Crioceris asparagi* L.) is a major threat to *A. racemosus*. These beetles are characterised by their distinctive red or orange colour with black spots on elytra. Both adults and larvae feed on the foliage, resulting in considerable damage. The adult beetles emerge in the spring and primarily feed on the tender shoots and foliage. They chew on the leaves, leaving behind characteristic feeding notches and causing skeletonization. The small grubs feed on the foliage and can cause extensive damage and adult beetles lay eggs on the plants [6]. To examine the dynamics of insect pests, including their diversity, dispersal, and invasion within the *A. racemosus*, a periodic survey was conducted in various locations across the National Capital Region (NCR) of New Delhi and the results presented here.

2. MATERIALS AND METHOD

Collection, transportation, preservation of the specimen and identification

In a 100-m² *Crioceris*-infested plot, from ten randomly selected *A. racemosus* plants, 20 adults and 30 larvae of insects were collected. The adult beetles were challenging to collect

due to their active behaviour. However, the larvae were easily collected as they remained attached to the shoots. To collect the adult beetles, a sweep net was used, and they were later transferred to a killing jar. Subsequently, the adults were placed in 30 ml screw-capped vials filled with 70% alcohol and transported to the laboratory [7]. In the lab, the specimens were taken out, and the alcohol was dried using blotting papers. The adult beetles were then pinned at the right elytra using standard insect pins and left to dry completely for three days. After drying, the dried and pinned specimens were stored in standard insect boxes. For species identification, reference specimens and pertinent literature were compared. Further molecular identification, beetle samples were collected and stored in 70% ethanol at -20°C for DNA extraction. DNA extraction was performed using the standard CTAB method [8]. PCR amplification was done for mtCOI gene fragment using the universal primers LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAACTTCAGGGTGACCAAAAAATCA-3') [9]. Dendrograms by the neighbour joining method were generated using MEGA11 software, incorporating the isolate from the current study and reference strain sequences obtained from GenBank [10, 11].

3. RESULT AND DISCUSSION

During the surveys during February 22, 2023, at Amrit Udyan in New Delhi, India (28.6156N, 77.1984E), the report of *Crioceris* sp. on *A. racemosus* was documented. The adult insects displayed colour sexual dimorphism and measured approximately 6.5 ± 0.289 long, with the male being black and the female being yellowish brown with a black spot and a faint black patch on the inner edge of the posterior part of the elytra. Additionally, a faint black patch was observed on the posterior part of the elytra. Both adults had 11 segmented antennae, which were inserted in front of the eyes and widely separated. The front coxa was conical and contiguous, providing a distinctive feature. The prothorax is narrower than the elytra at its base, and a strong middle constriction is typically present. The elytra exhibited

repetitive punctures in several rows, and the tarsal claws were simple and broadly spread out from the base. On the ventral side, only five abdominal segments were visible, with the remaining segments concealed (Fig. 1 and 2). The eggs of *Crioceris* sp. were elongate, conical, and relatively large compared to the adult body size. They displayed a greyish-brown colour. The larvae of the species appeared dark greyish to creamish white, with a black head and three pairs of black legs (Fig. 3). Diagnostic colour characters are commonly used to distinguish between different insect species, and many leaf beetles, including the criocerine, exhibit such colour characters [12]. This species differed from existing ones in terms of its morphological appearance, particularly in its varied coloration and marking patterns compared to those of the available described species (Fig. 4). Furthermore, molecular identification was done by sequencing five insects, and later, using the NCBI BLAST algorithm, it indicated that all the sequences matched with a 95% identity with the *Crioceris* genus, and the obtained sequence was assigned an accession number (OR211845.1). (Fig. 4).

During the winter survey, adult *Crioceris* sp. entered hibernation and emerged in spring, laying eggs on new asparagus shoots. Egg incubation spanned 8.5 ± 3.5 days, and upon hatching, larvae immediately fed on young asparagus for 2.5 ± 0.5 weeks, undergoing four instars. In the final instar, larvae dropped to the soil, forming a pupa in an earthen cell, with adults emerging in 6.5 ± 1.5 days. Mating behavior was observed after hibernation and feeding, revealing polygynandrous tendencies. Males rode on female backs, while females resisted by kicking and moving abdomens away. Post-mating, females laid eggs singly or in groups on leaves or spears, coated with a dark adhesive. The insect displayed high daytime activity, running along plants and employing flight sparingly. When attacked, they occasionally played dead as a defense mechanism.

In terms of plant damage, both the larvae and adults of *Crioceris* sp. fed on *A. racemosus* by chewing. The larvae fed within developing asparagus berries, consuming their way out and

also fed on young spears. The adults fed on young spears and consumed buds, causing damage early in the season. The damage was characterized by broken cladophylls and the removal of photosynthetic tissue (Fig. 5a). The beetles chewed on the leaves, creating distinctive feeding notches along the leaf edges. These notches appeared as irregular, jagged edges. They also fed on the soft tissue between the leaf veins, leaving behind a network of veins and translucent leaf remnants. This gave the affected leaves a skeletal appearance (Fig. 5b). Severe infestations of these beetles could result in extensive defoliation of the plant (Fig. 5c). Their feeding activity weakened the plants, hindering their growth, compared to healthy plants (Fig. 5d). The damage caused by these pests included stunted growth, resulting in smaller and less productive plants. We noticed severe infestation accounting 60-80 grubs per spear and adults were 8 to 10 per spear. This report presents information that is different from previous reports documented across the globe.

In Europe and North America, two species, *Crioceris duodecimpunctata* (Linnaeus, 1758) and *C. asparagi* (Linnaeus, 1758), are recognized as serious pests of *Asparagus officinalis* (L.) [13]. The first record of *C. asparagi* in Lithuania's Vilnius environs was published by Tenenbaum, [14], and *C. asparagi* and *C. duodecimpunctata* were observed in western Massachusetts on *A. officinalis* by Capinera, [15]. Major pests that contributed to the decline of Dutch asparagus production include the asparagus fly (*Platyparea poeciloptera* Schrank) and asparagus beetles (*C. duodecimpunctata* L. and *C. asparagi* L.) [16]. *C. quatuordecimpunctata* (Scopoli), commonly known as the asparagus beetle, has also been reported in Asparagus in China [17]. The asparagus beetle observed in the wild asparagus field differs from the other two chrysomelidae beetles (*C. asparagi* (L.) and *C. duodecimpunctata* (L.)) reported on garden asparagus in California [18]. In 2015, a report of *C. asparagi* (L.) and *C. duodecimpunctata* on *A. racemosus* was documented in Nepal (PQPMC) [19]. In India, there have been previous reports on Chrysomelidae beetles.

Crioceris hampsoni from Niligiri hills of India by Hampson, [20]. And it differs from our specimen by having dark yellow colour with 12 pairs of dark spots on elytra. And another chrysomelidae beetle *Lema downesi* Baly, which was reported on wild asparagus [21]. Even Saravanan and Chaudhary, [22] reported *L. downesi* from *A. racemosus* in India. Additionally, *C. aparagi* has been reported for the first time in India on *Asparagus filicinus* Buch.-Ham. ex D.Don [23] and our specimen clearly differs from this in elytra colour pattern and also absence of three pairs of white spots (Fig. 4).

Conclusion

The present study reported the presence of the *Crioceris* genus on *A. racemosus* in India. Observations covered mating behavior, egg-laying patterns, and feeding habits causing damage to asparagus plants in both larval and adult stages. These findings contribute significantly to our understanding of chrysomelid beetles associated with asparagus crops, underscoring the imperative for additional investigations in this domain.

Fig. 1 Image of new black colour male of *Crioceris* sp. collected from, Amrit Udyan: a: dorsal view adult; b: ventral view of adult; c: lateral view of adult



Fig. 2 Image of new brown colour female of *Crioceris* sp. collected from, Amrit Udyan: a: dorsal view adult; b: ventral view of adult; c: lateral view of adult



Fig. 3 Infestation of new *Crioceris* sp. larva on *Asparagus racemosus*



Fig. 4 Molecular identifications of new *Crioceris* sp.

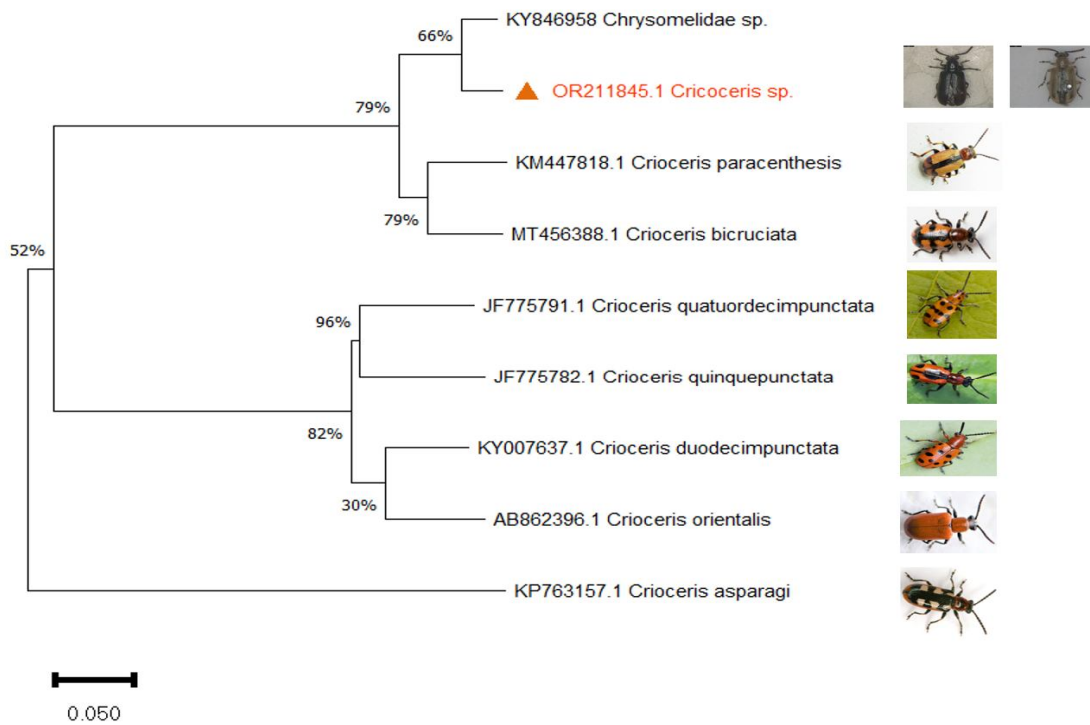


Fig. 5 a) Skeletisation of spears by removal of photosynthetic tissue b) extensive defoliation of the plant c) extensive infestation led to plant death d) healthy plant



a)



b)



c)



d)

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable, as no approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

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