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 silvering 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 -20º 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.

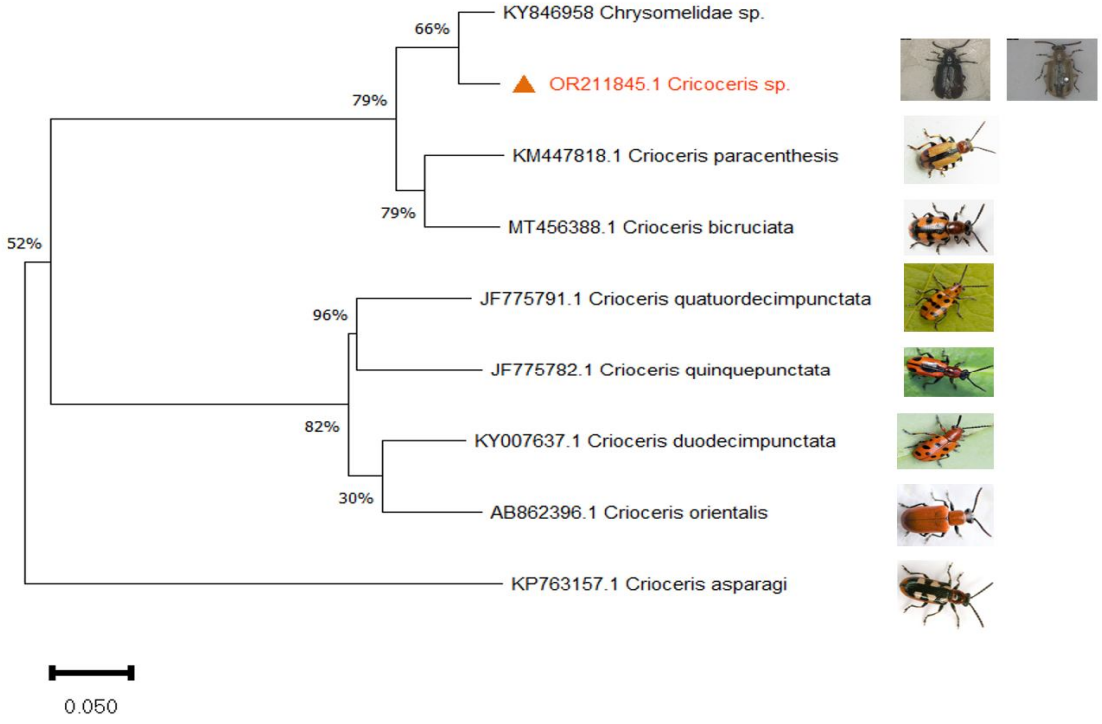

Fig. 5 a) Skeletisation of spears by removal of photosynthetic tissue b) extensive defoliation of the plant c) extensive infestation led to plant death d) healthy plant

a)

b)

c)

d)

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version,

model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

References:

1. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M (2013) Plant profile, phytochemistry and pharmacology of *Asparagus racemosus* (Shatavari): A review. Asian Pacific J Trop Dis 3(3):242-251

2. Karunarathne YAUD, Amarasinghe APG, Weerasooriya TR, Samarasinghe UKA, Arawwawala LDAM (2020) *Asparagus racemosus* (Willd) of Indian Origin: in Terms of Physico-Chemical, Phyto-Chemical and Nutritional Profiles. Sch Int J Tradit Complement Med 3(7):140-143
3. Gogte VM (2000) Medicinal plants. Part III in Ayurvedic Pharmacology and Therapeutic Uses of Medicinal Plants (Dravyagunavignyan), Mumbai Bhartiya Vidya Bhavan, 325-327
4. Bopana N, Saxena S (2007) *Asparagus racemosus*—Ethnopharmacological evaluation and conservation needs. J Ethnopharmacol 110(1):1-15
5. Tripathi AK (2018) Pests of Medicinal and Aromatic Plants. Pests and Their Management 719-822.
6. Morrison WR, Linderman S, Hausbeck MK, Werling BP, Szendrei Z (2014) Disease and insect pests of asparagus. Extension Bulletin E 3219.
7. Gouda MR, Pavan JS, Nazrin MR, Sharath R (2024) Emerging Threat of *Oxyrachis tarandus* Fabricus Infestation on Banyan Trees (*Ficus benghalensis* Linnaeus). International Journal of Economic Plants 11(2): 100-103

8. Gouda MR, Pavan JS, Yogananda M, Rajarushi CN (2024) A New Host Record for Leptocentrus taurus and Extended Host Records for *Oxycarachis tarandus*. Indian Journal of Entomology 1-3
9. Gouda MR, Rajna S, Gambhir S, Subramanian S (2024) Unravelling genetic diversity of whitefly species on different host plants from New Delhi. Journal of Environmental Biology 45: 259-267
10. Gouda MR, Subramanian S (2024) Variations in the expression of odorant binding and chemosensory proteins in the developmental stages of whitefly *Bemisia tabaci* Asia II-1. Scientific Reports 14(1):15046
11. Gouda MR, Kumaranag KM, Ramakrishnan B, Subramanian S (2024) Deciphering the complex interplay between gut microbiota and crop residue breakdown in forager and hive bees (*Apis mellifera* L.). Current Research in Microbial Sciences 6:100233
12. White RE (1993) A revision of the subfamily Criocerinae (Chrysomelidae) of North America north of Mexico. Technical Bulletin-United States Department of Agriculture 1805
13. Morrison WR, Szendrey Z (2014) The Common Asparagus beetle and Spotted Asparagus beetle (Coleoptera: Chrysomelidae): identification, ecology, and management. J Integr Pest Manag 5(3):1–6.
14. Tenenbaum S (1931) Nowe dla Polski gatunki i odmiany chrząszczy, oraz nowe stanowiska gatunków dawniej podawanych [New species and varieties of beetles for Poland and new records of known species]. Fragmenta Faunistica Musei Zoologici Polonici 1(1):329–359.
15. Capinera JL (1974) Biology of the asparagus beetle, Crioceris asparagi and Crioceris duodecimpunctata, in western Massachusetts [Master's thesis]. University of Massachusetts

16. Blok WJ (1997) Early decline of asparagus in the Netherlands: Etiology, epidemiology and management [Doctoral dissertation]. Wageningen Agricultural University
17. Jia HM, Zhao JY, Jia HT, Chen D (2013) Biological characteristics of asparagus beetle: Crioceris quatuordecimpunctata (Scopoli). In: XIII International Asparagus Symposium 1301 pp. 169-172
18. Hexamer FM (2010) Asparagus, its culture for home use and for market: A practical treatise on the planting, cultivation, harvesting, marketing, and preserving of asparagus, with notes on its history. Retrieved from http://www.gutenberg.net.
19. Pest list of five highly traded medicinal and aromatic plants of Nepal (2015) Department of Plant Resources, National Plant Quarantine Program, Nepal Herbs and Herbal Products Association. https://nepalindata.com/resource/PEST-OF-FIVE-HIGHLY-TRADED-MEDICINAL-AND-AROMATIC-PLANTS-OF-NEPAL/. Accessed 18 July 2023
20. Hampson GF (1908) The Fauna of British India: Including Ceylon and Burma. Taylor & Francis Vol 11
21. ICAR (2009) A new insect infesting satavari. Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat. ICAR Newsletter 10(1):ISSN 0975-3958.
22. Saravanan L, Chaudhary V (2012) Life history and seasonal incidence of Asparagus beetle, Lema downsei Baly on Asparagus racemosus in India. Ann Plant Prot Sci 20(1):83-87.
23. Razak N, Ahmad I (2020) Diversity of insects infesting medicinal and aromatic plants in the Kashmir Valley. In: Biodiversity of the Himalaya: Jammu and Kashmir State pp. 801-820.