https://doi.org/10.33307/entomon.v48i1.854
ENTOMON 48(1): 123-128 (2023)
Short communication No. ent. 48117

Oviposition behaviour of *Callosobruchus chinensis* (L.) (Coleoptera, Chrysomelidae, Bruchinae) on horse gram seed varieties

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ABSTRACT: Oviposition behaviour of *Callosobruchus chinensis* was tested on six varieties of horse gram seeds (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) to identify the resistance variety. In no choice test, females of *C. chinensis* laid lesser eggs within 24 h of mating on whole seeds of Urmi variety (11.6 ± 0.41) than the other varieties tested. However, females did not lay eggs when seed coat was removed from the above six varieties of horse gram. In choice test, females laid the lowest number of eggs within 24 h of mating on Urmi (1.11 ± 0.23) than the other varieties. The scanning electron micrograph (SEM) study revealed that there were morphological differences on the seed coat texture of the six horse gram varieties. The seed coat texture of Urmi variety is heterobrochate with some nearly bi-reticulate patches and few unevenly depressed muri, which partially inhibited oviposition on Urmi seeds. Urmi is the less preferred variety for oviposition. © 2023 Association for Advancement of Entomology

KEY WORDS: Varietal resistance, choice assay, no choice assay, SEM, seed coat texture

Macrotyloma uniflorum Lam. (Verdc.), commonly known as horse gram, is an important pulse crop in India (Reddy *et al.*, 2008; Prasad and Singh, 2015; Agnihotri and Rana, 2021) besides, Australia, Africa, Malaysia, Burma and the West Indies (Kingwell-Banham and Fuller, 2012). Pulses in tropical and subtropical countries are attacked by pulse beetles, *Callosobruchus* species (Coleoptera, Chrysomelidae, Bruchinae) in the fields and storage. In India, *Callosobruchus chinensis* (L.), *C. maculatus* (F.) and *C. analis* (F.) are reported to cause severe damage of pulse seeds. Among three pulse beetles, *C. chinensis* is the most important bruchid pest in India (Mishra *et al.*, 2013). The larvae of *C. chinensis* complete larval development within horse gram seeds and thus damaged seeds are not suitable for human consumption. Severe attack by this insect pest causes poor germination, low seed weight and no commercial value of horse gram seeds (Divya *et al.*, 2016). Synthetic insecticides are commonly applied to control the insect pests in storage (Jayaram *et al.*, 2022). But applications of synthetic insecticides in stored grains have many harmful effects such as health hazards, toxicity and environmental contamination. Further, insect pests might be resistant to harmful insecticides (Talukder, 2009). So, it will be cost effective and eco-friendly to use resistant horse gram varieties against the attack of *C. chinensis*. The current study was

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undertaken to study the oviposition behaviour of *C. chinensis* on varieties of horse gram seeds to observe the varietal resistance against *C. chinensis*.

Six varieties of uninfested and healthy horse gram seeds (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) were collected from Kota, Rajasthan, India. Adults of *C. chinensis* were collected from local stores containing cowpea seeds at Burdwan (23°16 N and 87° 54 E) during March 2022. Collected adults were also reared on cowpea seeds for three generations. The insects were not reared on horse gram seeds as the insect might be habituated to horse gram seeds. A pair of newly emerged male and female were kept in a glass jar (20 cm H × 10 cm D) for mating. Newly emerged females mate within 2 hr of emergence. Gravid females were used for oviposition experiments.

In no choice test, twenty seeds of each variety of horse gram (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) were placed in a glass jar (20 cm H × 10 cm D) separately. A gravid female was released in each glass jar and observed for 24 hr. The experiment was replicated 35 times for each variety of seeds. In no choice test, seed coats from each variety of horse gram seeds (ANK Black, AK 42, AK 21, BK 1, Urmi and Paiyur 1) were removed keeping the seeds intact. Twenty seeds without seed coat from each variety of horse gram were positioned in a glass jar (20 cm H × 10 cm D) separately. The experiment was replicated 35 times for each variety of seeds.

In choice assays, a rectangular glass chamber (35 cm long × 10 cm breadth × 8 cm height) was used, and emery papers (coarse grade) were put along sides and bottom of each glass chamber to prevent oviposition on the floor and walls of glass chamber. Six Petri dishes (5 cm diam) were placed at equal distance along the length of the rectangular glass chamber. Distance between two Petri dishes is 10 cm along the length of the glass chamber. Each Petri dish contained 20 uninfested seeds of a horse gram variety. A gravid *C. chinensis* female was then released in the centre of the rectangular chamber. For the oviposition assay experiment, 35 gravid females were separately examined eliminating those insects that did not oviposit.

The egg laying behaviour of a gravid female for the above two experiments was monitored for 24 h after releasing in the experimental chamber, and when a female began to lay eggs, she was observed until she stopped ovipositing, at which point the replicate was terminated, the number of eggs counted, and the female was discarded. For scanning electron microscope (SEM) study of seed coat, healthy and uninfested whole seeds were broken cautiously in the laboratory to separate the seed coats. The seed coat surface of each variety of horse gram seed sample was passed through graded alcohol (50, 70, 90 and 100 % alcohol, for each grade 15 min) to dehydrate the seed coats. The dehydrated seed coats for each variety were mounted on aluminium holders (stabs) coated with gold-palladium (2 nm thickness) for SEM study. The data in no-choice and choice tests were subjected to one-way ANOVA followed by Tukey test (Zar, 1999).

In no choice assays, number of eggs laid by a gravid female differed significantly among six varieties of horse gram seeds ($F_{5, 204}$= 14.221, $P < 0.0001$). The number of eggs laid by a female within 24 hr of mating was the lowest on Urmi variety (11.6 ± 0.41) among six horse gram varieties, ANK Black (15.06 ± 0.52), AK 42 (16.57 ± 0.53), AK 21 (15.03 ± 0.51), BK 1 (16.34 ± 0.66) and Paiyur 1 (13.26 ± 0.32) (Fig. 1). Among six varieties, Urmi is the least preferred variety for oviposition by *C. chinensis*. However, females did not lay eggs on seeds when seed coat was removed from the above six horse gram varieties, implicating seed coat morphology and surface waxes influenced the oviposition of *C. chinensis* females. A previous study demonstrated that when seed coat was removed from four varieties (BIO L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1) of *Lathyrus sativ*us L. seeds then *C. maculatus* females did not lay eggs on seed coat removed *L. sativ*us seeds (Adhikary *et al.*, 2016a).

In choice assays, number of eggs laid by a gravid female differed significantly among six horse gram

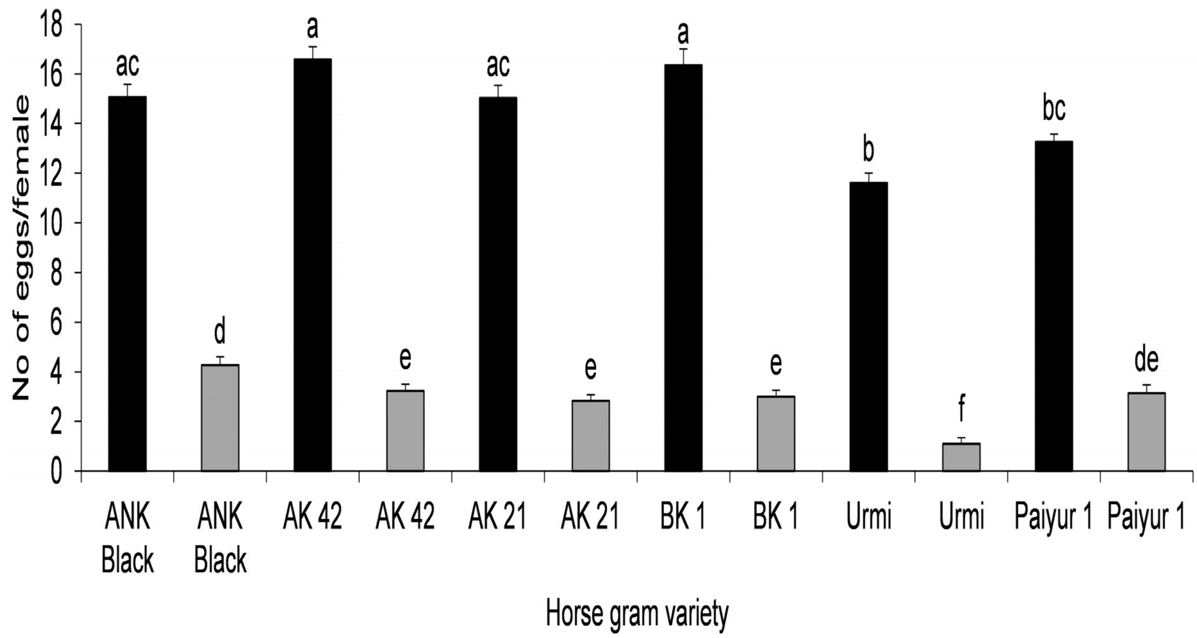

Fig. 1 Oviposition of *Callosobruchus chinensis* within 24 h of mating (n = 35) on different varieties of horse gram seeds in no choice assays (black colour) and choice assays (grey colour). Means followed by different letters are significantly different by Tukey's test at 5% level of significance

varieties ($F_{5, 204}$= 12.919, $P < 0.0001$). The number of eggs laid by a female within 24 hr of mating was the highest on ANK Black (4.26 ± 0.34) followed by AK 42 (3.23 ± 0.27), BK 1 (3.00 ± 0.26), AK 21 (2.83 ± 0.24) and the lowest on Urmi (1.11 ± 0.23). There is no significant difference between ANK Black and Paiyur 1 (3.11 ± 0.34) (Fig. 1). These above observations revealed that females did not prefer to lay eggs on Urmi, implicating that Urmi is the partially resistant variety for ©*C. chinensis* oviposition.

The SEM study revealed that seed coat of ANK Black was homobrochate reticulate with significantly thicker muri and slightly elevated lumen (Fig. 2a), whereas the seed coat of AK 42 rugulate-reticulate (Fig. 2b). In AK 21, the seed coat was rugulate with few patches showing perforate-rugulate-reticulate and perforation elongated (Fig. 2c), whereas the seed coat of BK 1 was faintly rugulate and sometimes ruptured (Fig. 2d). The seed coat of Urmi was heterobrochate with some nearly bi-reticulate patches and few unevenly depressed muri, but some of these muri were incomplete (Fig. 2e). In Paiyur 1, the seed coat was heterobrochate reticulate with thicker muri (Fig. 2f). These above observations indicate that the seed coat of Urmi variety, i.e., heterobrochate with some nearly bi-reticulate patches partially inhibited egg laying of *C. chinensis*. Different studies demonstrated that seed coat texture influenced oviposition in stored grain insect pests (de Sá *et al.*, 2014; Adhikary *et al.*, 2014, 2015, 2016a, b; Szentesi, 2021).

Host resistance is an effective way of pest management in integrated pest management strategy. Resistance against insect pests is manifested through antibiosis, antixenosis (non-preference) and/ or tolerance. Traits contributing to resistance/ susceptibility of legumes to bruchids include seed color, texture, hardness, size and chemical constituents. In the present study, seeds of six horse gram varieties are having the shape of curved beak and more or less similar in size. The texture and colour of seeds of horse gram varieties are as follows – ANK Black is smooth and shiny black, AK 21 has smooth and creamy brown, AK

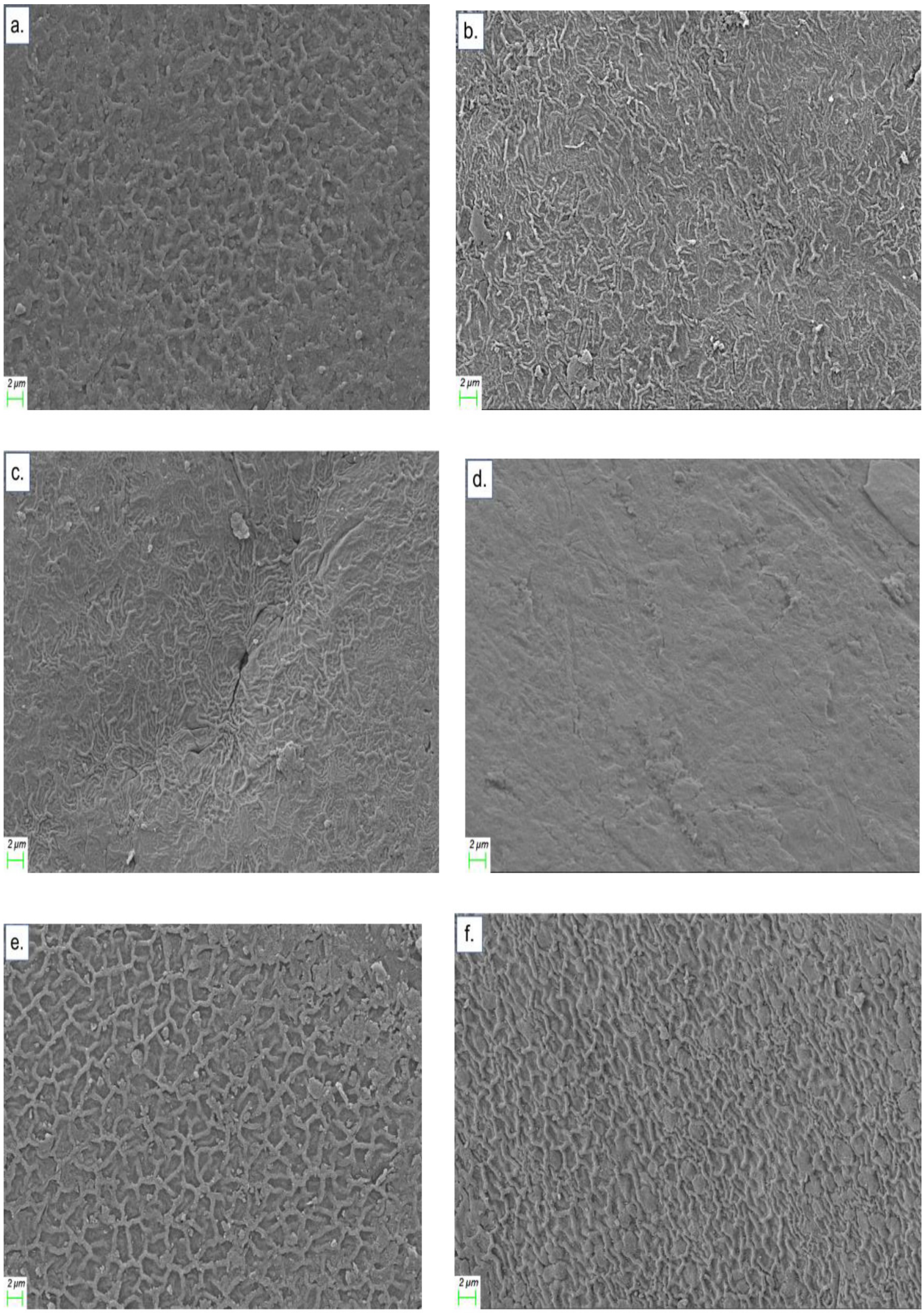

Fig. 2 Scanning electron micrograph of seed coat surface of ANK Black (a), AK 42 (b), AK 21 (c), BK 1 (d), Urmi (e) and Paiyur 1 (f)

42 with smooth and blackish brown, BK1 is smooth and creamy, Paiyur 1 is not so smooth and creamy blackish brown, and Urmi has rough and grey black. The oviposition was low on Urmi variety than the other varieties, suggesting that seed coats with rough texture inhibited egg laying. The uneven and undulating pattern of the rough seed coats of Urmi variety may have provided a stimulus of instability to *C. chinensis* females. Similarly, *C. maculatus* laid more eggs on smooth-coated seeds compared to rough-coated cowpea seeds as smooth-coated seeds allowed firm attachment of the eggs and provided more surface area than rough-coated seeds (Barde *et al.*, 2012). In the present study, ANK Black, AK 21, AK 42, BK 1 and Paiyur 1 were found to be susceptible, while Urmi variety was relatively resistant.

ACKNOWLEDGMENT

The financial assistance from the UGC, New Delhi, Govt. of India to Bhramar Bhattacharyya as a Junior Research Fellow (JRF) [NTA Ref. No. 211610110248] is gratefully acknowledged.

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(Received November18, 2022; revised ms accepted January 25, 2023; published March 31, 2023)