Indian Journal of Agricultural Sciences **92** (3): 393–6, March 2022/Article
https://doi.org/10.56093/ijas.v92i3.122703

Morphometric, molecular characterization and management of *Callosobruchus chinensis*

D SINGH[1] and T BOOPATHI[2]*

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110 012, India

Received: 24 September 2021; Accepted: 01 November 2021

ABSTRACT

Pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) is the most economic insect pest of pulse and can cause huge quantitative losses and also decreases the nutritional value of stored products. The morphological and molecular characterization of pulse beetles was determined and different non-edible oils against *C. chinensis* were assessed at ICAR-National Bureau of Plant Genetic Resources, New Delhi during 2019–20. Eggs of *C. chinensis* were 264.74±3.716 µm in width and 452.33±4.531 µm in length. The final instar larva of *C. chinensis* was 1703.12±4.692 µm in width and 3062.19±33.119 µm in length. The width and length of the pupae was 1696.09±5.589 µm and 3281.60±73.641 µm, respectively. The length of the adult body was 2520.85±23.278 µm for females and 2469.70±29.570 µm for males with a width of 1426.78±41.334 µm for females and 1456.54±23.606 µm for males. Both *C. maculatus* and *C. chinensis* got amplified by *COI* primer. A band of approximately 710 bp was obtained from both pulse beetles (*C. maculatus* and *C. chinensis*). DNA barcode helps in identification of pests at all life stages. Hundred per cent of egg mortality, larval mortality and adult mortality were reported in all non-edible oils such as *Pongamia glabra* L., *Hydnocarpus wightiana* Blume, *Madhuca longifolia* Konig, *Callophyllum inophyllum* L. and *Azadirachta indica* A. Juss. Similarly, all non-edible oils had ovipositional deterrence. To summarize, these oils can be used for the management of pulse beetles during storage.

Keywords: DNA barcode, Morphology, Mortality, Non-edible oils, Pulses

Pulses are a major source of food and nutrients for millions of people worldwide, especially for low-income countries or underdeveloped nations. The Food and Agriculture Organization (FAO) of the United Nations proclaimed 2016 the international year of pulses with the goal of enhancing public understanding of the nutritional benefits of pulses in sustainable food production. This was intended for global nutrition and food security (Singh 2017). Pulses have a higher protein, zinc, iron, magnesium, folate and potassium (Dahl *et al*. 2012).

One of the main constrains of pulse production are the insect pests that cause huge losses in both field and storage. Insect pests account for about 30% of pulse losses in India, which amount to about $815 million in monetary losses. Among all insect pests attacking the pulses during storage, the pulse beetle (Coleoptera: Chrysomelidae) is a notorious pest for chickpea, cowpea, green gram, garden pea, lentil, black gram and pigeonpea. Three species of pulse beetle, mainly *Callosobruchus chinensis* (L.), *C. maculatus* (F.) and *C. analis* (F.), are significant pests of green gram, cowpea, lentil, black gram and pigeonpea. Among the genus of *Callosobruchus*, *C. chinensis* is the most significant pulse pest and is considered to be abundant and fast in reproduction. This insect is cosmopolitan in nature but is more common in all tropical and subtropical regions. The newly hatched larvae bore into the seed and keeps feeding on its content until the entire endosperm is eaten. However, *C. chinensis* is not only found to be the most damaging insect, but also causes serious losses in various pulse crops up to a maximum of 93.33%. The present study was carried out to determine morphological characterization of *C. chinensis* and molecular characterization of pulse beetles (*C. chinensis* and *C. maculatus*) and to evaluate the non-edible oils against *C. chinensis* in green gram.

MATERIALS AND METHODS

Callosobruchus chinensis *culture*: *Callosobruchus chinensis* was mass reared on green gram under laboratory conditions (27±1°C temperature with a 70±1% RH) at ICAR-National Bureau of Plant Genetic Resources, New Delhi during 2019–20. To start with, adult beetles collected from infested stored grains were introduced into open mouthed plastic containers containing 100 g of disinfested green gram. The plastic containers (10 cm × 5 cm) were kept in cages made of wire mesh and glass. Three to five days after the release, the beetles were removed after they

[1]Chaudhary Charan Singh University, Meerut, Uttar Pradesh; [2]ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, Telangana. *Corresponding author email: boopathiars@gmail.com

laid the eggs. When the adults emerged from the infested grains, they were collected and released into fresh green gram as done earlier. They were allowed to remain in the grains for mating and oviposition and removed from the containers. In the similar way, cultures were initiated at 15 days interval for the uninterrupted supply of all stages of the insects for conducting different studies.

Morphology of Callosobruchus chinensis : The biometric studies were made on various life stages of *C. chinensis* cultured on green gram. Measurements of eggs, final larval instar, pupae and adults (female and male) were made using stage and occular micrometers on fifty samples for each stage. The effect of different pulses, green gram, lentil and cowpea were also studied on characters of egg, larva and pupa.

Molecular characterization of Callosobruchus chinensis and *C. maculatus*: The DNA was extracted with a slight modification using the cetyl trimethylammonium bromide (CTAB) method (Gawel and Jarret 1991). In a sterile microcentrifuge, the female of *C. chinensis* and *C. maculatus* were grounded by adding 200 µL of preheated CTAB buffer. The samples were kept for 45 min at 65°C. The tubes were centrifuged at 4°C for 15 min at 16,000 g after adding an equal volume of chloroform: isoamyl alcohol mixture (24:1). A one-third volume of ice-cold isopropanol was added in the clear aqueous phase and the tubes were incubated at -20°C overnight. The tubes were then centrifuged at 16,000 g at 4°C for 20 min. It was dissolved in 20 µL of 1X TE buffer (*p*H 8.0) after washing the DNA pellet with 70% ethanol.

A set of COI primers [(F: C1-J-2183): CAA CAT TTT TTT TTT TTT GG and R (TL-2-N-3014-ANT): TGA AGT TTA AGT TCA ATG CAC] was used to amplify the DNA samples (Simon *et al*. 2006). In a thermocycler (BIORAD DNA Engine, PTC-0200, Mexico) polymerase chain reactions (PCR) was performed. PCR reactions were conducted with a volume of 25.0 µL containing a buffer (10 mM Tris–HCl *p*H 9, 50 mM KCl), 200 µM dNTPs, 1.5 mM $MgCl_2$, 10 pmol of both forward and reverse primers, 1 unit Taq DNA polymerase, and 4 ng of the extracted DNA. The PCR cycle included pre-denaturation at 94°C for 2 min, 40 amplification cycles (denaturation at 94°C for 1 min, annealing at 55 for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min). Following amplification, a 4 µL aliquot of PCR amplified product was separated from a 2% agarose gel containing 0.5 µg/mL ethidium bromide at 90 V for 45 min and viewed under UV light and data acquired from an image documentation system (Gel Doc XR System, Model: 1708170EDU, Bio-Rad Laboratories, Hercules, California, USA). A co-migrating 100 bp DNA ladder (MBI Fermentas) was compared to the size of the DNA fragment.

Assessment of non-edible oils against Callosobruchus chinensis: The non-edible oils, viz. Pungam (*Pongamia glabra* L.), Maravetty (*Hydnocarpus wightiana* Blume), Mahua (*Madhuca longifolia* Konig), Pinnai (*Callophyllum inophyllum* L.) and Neem (*Azadirachta indica* A. Juss.) were assessed for their efficacy in inhibiting the eggs hatchability, oviposition deterrence, larval mortality, and adult mortality. Treatments with four replicates were arranged in a complete randomized design (CRD). A quantity of 100 g of green gram was thoroughly treated with all non-edible oils at 1.0% (1.0 ml/100 g of seeds) and transferred to separate plastic containers. For egg mortality, 10 freshly laid eggs were introduced in each container. The observations were made on the number of eggs hatched 7 days after the release. In case of ovipositional deterrence, a pair of freshly emerged adults was released in each container. The number of eggs laid by the female was recorded daily up to 10 days. For larval mortality, 10 second instar larvae were released into each container. The mortality of the larvae was recorded on first, third and seventh day after treatment. In case of adult mortality, 25 freshly emerged adults were released into each container. The mortality of adults was recorded on first, third and seventh day after treatment.

Statistical analyses: Data were analyzed using SAS Software Version 9.3 (SAS 2011) for morphology and effect of oils on the mortality of *C. chinensis*. Data were analyzed using analysis of variance (ANOVA) and the mean separated using Tukey's HSD test at $P \leq 0.001$.

RESULTS AND DISCUSSION

Morphology of Callosobruchus chinensis: The eggs were dome-shaped structures with flat oval bases. They were small, translucent grey and unnoticeable when newly laid. Eggs were 452.33±4.531 µm length and 264.74±3.716 µm width (Table 1). The eggs occurred individually and had a yellow colour which became opaque when hatched. Larva was yellowish-white in colour with reduced legs and brown head. The final instar larvae were 3062.19±33.119 µm length and 1703.12±4.692 µm width. They were found mostly inside the seed. The pupae were dark brown in colour. The length and width of pupae were 3281.60±73.641 µm and 1696.09±5.589 µm, respectively, were found mostly inside the seed. The adults emerged from the grain through windows, leaving the main evidence of round holes. It was also observed that females were bigger than males. Body length of adults was 2469.70±29.570 µm in male and 2520.85±23.278 µm in female with breadth of 1456.54±23.606 µm in male and 1426.78±41.334 µm

Table 1 Morphometric parameters (mean ± standard error) of different life stages of *Callosobruchus chinensis*

Stage	Length (µm)* (Mean ± SE)	Width (µm)* (Mean ± SE)
Egg	452.33±4.531	264.74±3.716
Final instar larva	3062.19±33.119	1703.12±4.692
Pupa	3281.60±73.641	1696.09±5.589
Male adult	2469.70±29.570	1456.54±23.606
Female adult	2520.85±23.278	1426.78±41.334

#The mean ±SEM was determined by Tukey's post-doc test at $P<0.01$ (n=50). SEM, standard error of the mean.

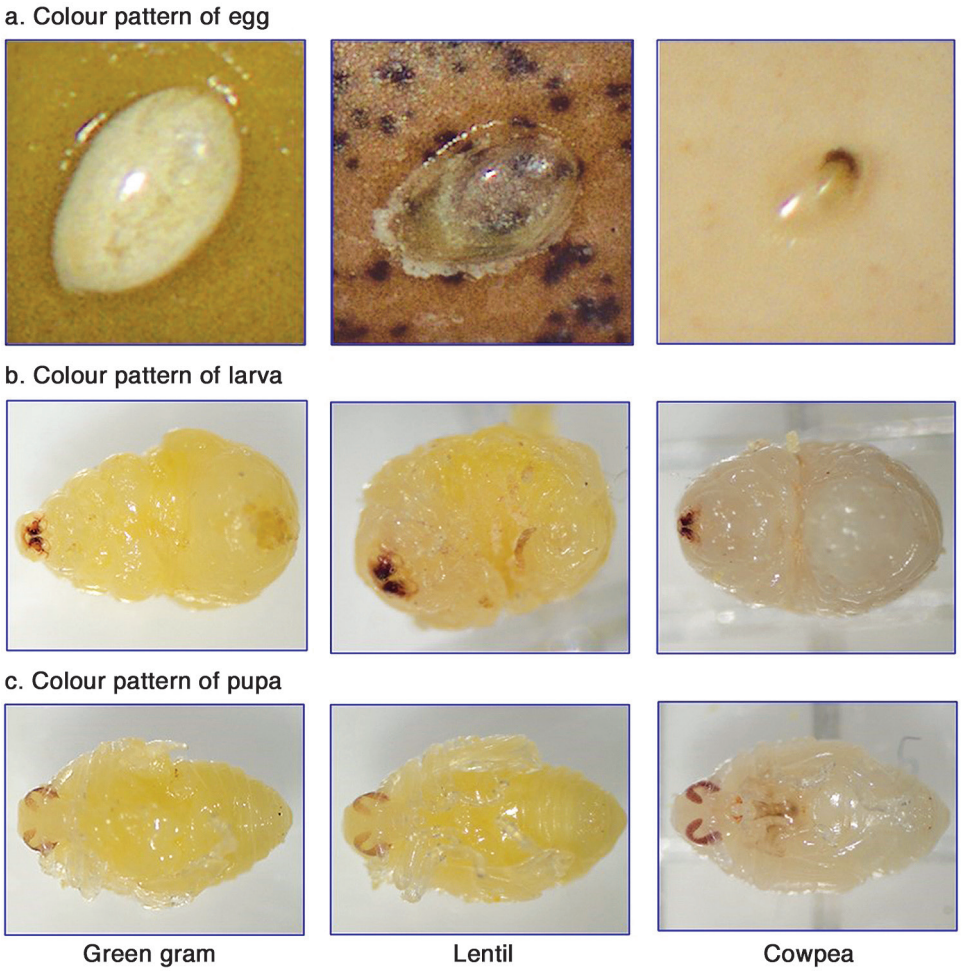

Fig 1 Effect of different pulses, green gram, lentil and cowpea on colour pattern of (a) egg; (b) larva; and (c) pupa of *Callosobruchus chinensis*.

in female. The antennae were pectinate in males while in females, the antennae are serrate. The prothorax and female and male pygidium covered with silver or white setae of *C. chinensis* were the main characters for identification. When *C. chinensis* fed on different pulses, different color pattern of egg, larva, and pupa were noticed (Fig 1).

Determination of non-edible oils against Callosobruchus chinensis: Hundred per cent egg mortality was noticed in all non-edible oils, *P. glabra*, *H. wightiana*, *M. longifolia*, *C. inophyllum*, and *A. indica* compared to control (Table 2, F = 6003.95; df = 5,18; P< 0.001). Similarly, all non-edible oils had ovipositional deterrence as compared to control (F = 126.140; df = 5,18; P<0.001). All non-edible oils caused hundred per cent larval mortality than control (*F* = 6241.00; df = 5,18; P <0.001). Similarly, all non-edible oils produced hundred percent adult mortality as compared to control (F = 5525.82; df = 5,18; P<0.001).

Amplification of cytochrome oxidase I gene from Callosobruchus chinensis *and* C. maculatus: *Callosobruchus chinensis* and *C. maculatus* got amplified by *COI* primer. A band of approximately 710bp was obtained from them. DNA barcode for *C. chinensis* and *C. maculatus* were developed.

Cowpea was observed to be an appropriate host for *C. chinensis* rearing and to culture in laboratory conditions throughout the year. When newly laid, the eggs are small, translucent grey and unnoticeable and are dome-shaped structures with flat oval bases. Individually, the eggs occured and had a yellow color that became opaque when hatched. When *C. chinensis* fed on different pulses, it noticed different pattern of egg color. Larvae and pupae were mostly found within seed. Larva had a brown head in yellowish-white, reduced legs and the pupae were dark brown in colour. The adults emerged

Table 2 Effect of non-edible oils on the per cent egg hatchability larval and adult modalities of *Callosobruchus chinensis*

Treatment	Percent egg hatchability[#]	Number of eggs laid/female[#]	Larval mortality (%)[#]	Adult mortality (%)[#]
Pongamia glabra oil	0.00	0.00	100.00	100.00
Hydnocarpus wightiana oil	0.00	0.00	100.00	100.00
Madhuca longifolia oil	0.00	0.00	100.00	100.00
Callophyllum inophyllum oil	0.00	0.00	100.00	100.00
Azadirachta indica oil	0.00	0.00	100.00	100.00
Control	96.67	49.67	1.75	1.25
df	5,18	5,18	5,18	5,18
F-value	6003.95	126.140	6241.00	5525.82
P-value	<0.001	<0.001	<0.001	<0.001

[#]The mean ±SEM was determined by Tukey's post-doc test at P<0.01 (n=4). SEM, standard error of the mean.

from the grain through windows, leaving the primary signs of round holes. Compatibility with the host could also be expected to differ comparatively. Indeed, a recognized host of *C. chinensis*, garden peas, show preferential resistance to the Japanese strain. Speculation about the significance of the fact that the origin of both garden peas and *C. chinensis* is in the Far East (which remained the main world producer of this legume seeds) raises the question of whether the propinquity of the beetle to this host might have contributed to selection for resistance. The potential to overcome such resistance, present in Israeli strain genetic material, can have economic implications to some extent. With regard to soybeans, there were slight differences between the beetle strains, and their strict carbohydrate requirements as an essential ingredient of their diet minimize the possibility of susceptible soybean varieties. For all practical purposes, *C. chinensis* does not appear to be capable of presenting any hazard to soybean production.

All non-edible oils caused larval and adult mortality to 100%, and also had ovipositional deterrence. The bruchids are controlled by oils extracted from some plant materials (Aliyu and Ahmed 2006). Aliyu and Ahmed (2006) revealed that groundnut oil and *Mentha arvensis* (L), *M. spicata* (L), *M. piperata* (L) and *Cymbopogon nardus* (L) have an effect on bruchid. Essential plant oils should be used as effective pesticides worldwide, since they are environmental friendly and safe for non-target organisms than chemical pesticides (Dubey *et al*. 2008). These are a complex mixture of primarily terpenoids, especially monoterpenes and sesquiterpenes, and a variety of aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones that decide the characteristic aroma and smell of the donor plant (Batish *et al*. 2008). Several plant extracts have been well documented by researchers for both their insect repellent and insecticide effects (Batish *et al*. 2008, Nerio *et al*. 2010). A study by Koona *et al*. (2007) showed that the use of impregnated jute bags with *Chenopodium ambrosioides* and aqueous extracts from *Lantana camara* significantly reduced damage to stored pulses of *Acanthoscelides obtectus* (Say) and *C. maculatus*. Those locally available plant extracts can be used as an inexpensive source of insecticide.

In this investigation, *C. chinensis* and *C. maculatus* got amplified by *COI* primer with a band of ≈710bp. The mitochondrial genes and the rDNA gene 16S were used to examine the insect relations between the level of the genus and the level of the species. Within the topology, the mitochondrial genes provide support below the genus level and this will help in confirming the current positions of the species in bruchid evolution. These patterns may be controlled by the gene function, and may be caused by mitochondrial genes as well. The 16S rDNA was used to study higher taxonomic levels of groups Anisoptera and Odonata (Hasegawa and Kasuya 2006). The incorporation of morphological characteristics, ecological characteristics and DNA sequence are the most important to know about the entire phylogeny of bruchid beetles.

The DNA barcoding has emerged as a molecular method for identification of species over the last decade. Although the major food insect pests are widespread globally, only a few studies have been done on DNA barcodes for these species (Cho *et al*. 2013). This work is therefore carried out to attempt to build a DNA reference dataset using the mitochondrial *COI* gene from store species of food-associated insects. This dataset can be used effectively to classify food-associated insect pests that are relevant in commercial food markets at the moment. DNA barcoding can aid in identifying pests at any stage of life, making it easier to control them saving farmers from pest damage costing billions of dollars (Kaur 2015, Sarvananda 2018). To summarize, these non-edible oils (*P. glabra*, *H. wightiana*, *M. longifolia*, *C. inophyllum*, and *A. indica*) can be used for the management of pulse beetles during storage.

REFERENCES

Aliyu M and Ahmed B I. 2006. Comparative efficacy of different rates of groundnut oil for the control of cowpea weevils *Callosobruchus maculatus* (F.) in stored cowpea (*Vigna unguiculata* (L) Walp). *Global Journal of Agricultural Sciences* **5**(2): 123–26.

Batish D R, Singh H P, Kohli R K and Kaur S. 2008. Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management* **256**(12): 2166–74.

Cho S Y, Suh K I and Bae Y J. 2013. DNA barcode library and its efficacy for identifying food□associated insect pests in Korea. *Entomological Research* **43**(5): 253–61.

Dahl W J, Foster L M and Tyler R T. 2012. Review of the health benefits of peas (*Pisum sativum* L.). *British Journal of Nutrition* **108**(S1): S3–S10.

Dubey N K, Srivastava B and Kumar A. 2008. Current status of plant products as botanical pesticides in storage pest management. *Journal of Biopesticides* **1**(2): 182–86.

Gawel N J and Jarret R L. 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Plant Molecular Biology Reporter* **9**(3): 262–66.

Hasegawa E and Kasuya E. 2006. Phylogenetic analysis of the insect order Odonata using 28S and 16S rDNA sequences: a comparison between data sets with different evolutionary rates. *Entomological Science* **9**(1): 55–66.

Kaur S. 2015. DNA barcoding and its applications. *International Journal of Engineering Research and General Science* **3**(2): 602–04.

Koona P, Tatchago V and Malaa D. 2007. Impregnated bags for safer storage of legume grains in West and Central Africa. *Journal of Stored Products Research* **43**(3): 248–51.

Nerio L S, Olivero-Verbel J and Stashenko E. 2010. Repellent activity of essential oils: a review. *Bioresource Technology* **101**(1): 372–78.

Sarvananda L. 2018. Short introduction of DNA barcoding. *International Journal of Research* **5**(4): 673–85.

SAS Institute Inc. 2011. *SAS® 9.3 System Options: Reference,* 2nd Edn. SAS Institute Inc., SAS Campus Drive, Cary, NC.

Simon C, Buckley T R, Frati F, Stewart J B and Beckenbach A T. 2006. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systems* **37**: 545–79.

Singh N. 2017. Pulses: an overview. *Journal of Food Science and Technology* **54**(4): 853–57.