



## Oviposition behavior of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) on four varieties of *Lathyrus sativus* L. seeds

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**ABSTRACT:** *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) is an important stored grain pest of *Lathyrus sativus* L. (Leguminosae). Olfactometer assay using the surface waxes of the four varieties, Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds @ 1, 1, 2 and 2  $\mu\text{g ml}^{-1}$  showed that the surface waxes of all the varieties attracted *C. maculatus* females and the least attraction was to WBK-13-1. Oviposition by *C. maculatus* was significant on all the varieties with surface waxes in no choice assay. The highest preference was to Bio L 212 Ratan and it was followed by Nirmal B-1, WBK-14-7 and WBK-13-1. The insect did not prefer wax removed seeds for egg laying. The study suggests that WBK-13-1 and WBK-14-7 are the less preferred varieties of *L. sativus* by *C. maculatus* for oviposition, and these varieties might be promoted for cultivation.  
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**KEY WORDS:** *Callosobruchus maculatus*, *Lathyrus sativus*, surface waxes, olfactometer assay, ovipositional preference.

### INTRODUCTION

In recent years, consumption of *Lathyrus sativus* L. (Leguminosae), commonly known as khesari, has been growing worldwide, because it is now perceived as part of a healthy diet. The crop is cultivated in India, Bangladesh, China, Nepal, Pakistan and Ethiopia (Gaur and Maloo, 2011; Girma and Korbu, 2012). Farmers grow this pulse crop due to low-cost cultivation, and resistance to drought, salinity and stress (Gaur and Maloo, 2011). Earlier khesari seeds are considered as a staple food because of neurotoxin ( $\beta$ -ODAP) which is making a comeback because of new plant varieties (Rao, 2011; Singh and Rao, 2013). Further, the seeds contain both homoarginine and  $\beta$ -ODAP, which are important to human health, in areas of

cardiovascular physiology, hypoxia and nutrition (Singh and Rao, 2013).

*Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) is a polyphagous pest of stored legumes in tropics and subtropics (Utida, 1972; Fox *et al.*, 2010). A cursory review of literature indicate the existence of the 'active' and 'normal' morphs of *C. maculatus* on different stored legumes, the two forms that are thought to represent adaptations to the two very different environments of field and seed store, respectively (Utida, 1972; Huignard *et al.*, 1985; Messina and Renwick, 1985; Thanthianga and Mitchell, 1990; Fox, 1993; Appleby and Credland, 2001; Zannou *et al.*, 2003; Fox *et al.*, 2010; Arnold *et al.*, 2012; Adhikary *et al.*, 2015; 2016). The active form of

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this insect has an important role in dispersal of populations from stores due to its increased flight activities than normal form (Utida, 1972; Appleby and Credland, 2001; Arnold *et al.*, 2012). However, normal females are the primary target of growers for the management of infestations of legume seeds because they are the principal egg layers (Zannou *et al.*, 2003). Infestation by this insect to cowpea, in three months of storage results in damage up to 30 % (Ouedraogo *et al.*, 1996), and total loss of this seed had been shown to occur within six months of storage (Caswell, 1961). The adults lay eggs on khesari seed surface and the larvae of *C. maculatus* feed for four instars in the seeds and complete their development within 12–16 days (Adhikary and Barik, 2012). Infestation by this insect reduces nutritional quality and viability of the seeds.

It is well established that successful use of varieties with good resistance has a number of merits over control by chemical insecticides as these insecticides are not safe to the users as well as to the environment. Further, application of chemical insecticides need periodic repetition and consequently it is not cost effective. Methyl bromide which is used in the developing countries for disinfections of the stored grain will be restricted worldwide by 2015 under the terms of Montreal Protocol (United Nations Environment programme, 1998). Further, resistance to treatments with insecticides *viz.* dimethoate, permethrin, carbosulfan and to the fumigant phosphine has been reported in *C. maculatus* (Bogamuwa *et al.*, 2002). Therefore, it is necessary to find suitable varieties which are resistant to the bruchid attack. The first physical contact between the insect and seed occurs on the seed coat surface. Hence, surface wax plays an important role in egg laying by *C. maculatus*. So, an attempt has been made to find whether *C. maculatus* display any differences on egg laying behavior on four varieties of khesari seeds, two cultivars, Bio L 212 Ratan and Nirmal B-1 currently grown by farmers and another two cultivars, WBK-14-7 and WBK-13-1 that are being considered for commercialization. The purpose of the investigation was to study variations in the surface waxes of four varieties of khesari seeds, analyse the role of

surface waxes in short range attraction of *C. maculatus* and oviposition by the bruchid on the four varieties of khesari seeds.

## MATERIALS AND METHODS

### Seed

Four varieties (Bio L 212 Rattan, Nirmal B-1, WBK-14-7 and WBK-13-1) of khesari seeds were collected from Pulses and Oilseeds Research Station, Behrampore, West Bengal, India.

### SEM study of seed coat

Uninfested whole seeds were broken cautiously in the laboratory to separate the seed coats for scanning electron microscope (SEM) study. The seed coat surface of each variety of khesari seed sample was mounted on aluminium holders (stabs) coated with gold-palladium (2 nm thickness) using Hitachi made Scanning Electron Microscope (Model: S 530 with IB 2 ion cotter, Japan). The SEM study was replicated thrice.

### Extraction of waxes from seed coat

Hundred grams of uninfested seeds of Bio L 212 Ratan (number =  $1226 \pm 6.5$ ), Nirmal B-1 (number =  $1332 \pm 8.3$ ), WBK-14-7 (number =  $1242 \pm 5.9$ ) and WBK-13-1 (number =  $1314 \pm 3.4$ ) (mean  $\pm$  SE) were separately immersed in 1 L *n*-hexane for 5 min under room temperature (27°C). The extract was filtered through Whatman No. 41 filter paper, and the solvent was removed under reduced pressure. The dried crude extract was used for olfactory assay. Five replications for each variety were maintained. Wax removed seeds were used for ovipositional assays to find any role of wax.

### Test insects

*C. maculatus* infesting chickpea seeds (variety: Radhey) were collected from local stores at Burdwan, West Bengal, India during June 2014. They were maintained in 1 L glass jars containing seeds of Radhey for one generation, and were covered with fine-mesh nylon nets at 14 L: 10 D photoperiod,  $27 \pm 1^\circ\text{C}$  and  $70 \pm 3\%$  relative humidity

in a BOD incubator (ADS instruments and Tech., Calcutta). Active/inactive male and female forms were determined by flight activity, elytra size and intensity of pigmentation on elytra. Newly emerged  $F_2$  inactive males and females (male: antennae long and deeply serrated, and pygidium uniformly covered with golden setae; female: antennae short and subserrated, and pygidium with a pair of black postero-lateral spots) were separated morphologically from the stock cultures everyday at 8 AM and 8 PM and were kept in separate glass jars without chickpea seeds. For mating, virgin inactive females collected within 12 h of adult emergence were provided with a single inactive male in a 60 mm Petri dish. After a single copulation, inactive females were transferred to a 15 × 8 cm glass jar containing a small Petri dish (2 × 1 cm) with water (Howe and Currie, 1964; Fox, 1993). The behavioral of 4–6 day-old mated inactive females was observed in olfactory assays; whereas mated females were used for ovipositional assays.

### Olfactory assay

Surface waxes (2 mg) from each variety of khesari seed were dissolved in petroleum ether to prepare four concentrations of 0.5, 1, 2 and 4  $\mu\text{g/ml}$  for olfactory assays. The dose of the waxes was lowered until the insect did not produce any response. Further, dose of the wax was tested until the insect produced highest significant ( $P < 0.0001$ ) attraction. The insect displayed highest attraction ( $P < 0.0001$ ) to 2  $\mu\text{g ml}^{-1}$  of surface waxes from Bio L 212 Ratan and Nirmal B-1 seeds, 4  $\mu\text{g ml}^{-1}$  of surface waxes were not tested for these varieties. Further, response of the insect to 2  $\mu\text{g ml}^{-1}$  surface waxes was tested as following combinations: Bio L 212 Ratan vs. Nirmal B-1, Bio L 212 Ratan vs. WBK-14-7, Bio L 212 Ratan vs. WBK-13-1, Nirmal B-1 vs. WBK-14-7, Nirmal B-1 vs. WBK-13-1 and WBK-14-7 vs. WBK-13-1 to find which variety of seed surface wax was most attractive.

The behavioral responses of adult *C. maculatus* females were observed in a short glass Y-tube olfactometer (5 cm long stem and arms; 0.6 cm radius, 60° Y-angle) as the wax compounds are

semivolatile (Mukherjee *et al.*, 2014; Sarkar and Barik, 2014; Malik and Barik, 2015). Each arm of the olfactometer was connected to glass-made micro kit adapter fitted into a glass vial (1 × 3 cm). One glass vial contained a 2 cm<sup>2</sup> Whatman No. 41 filter paper moistened with 1 ml of the tested concentration of wax, whilst the other glass vial contained a filter paper of the same size moistened with 1 ml of the control solvent (petroleum ether) which did not attract the test insect. Preliminary assays with 90 naïve insects were tested with petroleum ether solvent loaded air flow against clean air flow, and it was observed that the test insects did not indicate any positive response to the solvent control (petroleum ether) ( $\chi^2=0.18$ ;  $df=1$ ;  $P>0.05$ ). Charcoal filtered air was pushed into the system at 150 ml  $\text{min}^{-1}$ . The stem of the olfactometer was connected to a porous glass vial (1 × 3 cm) in which test insect was released. The experiment was conducted at  $27 \pm 1^\circ\text{C}$ ,  $70 \pm 3\%$  R.H. and 150 lux. One milliliter of tested wax concentrations / petroleum ether were applied to the filter paper pieces and allowed to evaporate and these filter papers were introduced into the glass vials before the first insect was released into the olfactometer, for each experiment. One adult female *C. maculatus* was introduced into the porous glass vial, which was then attached with the stem of the olfactometer. The behavior of each female was observed for 2 min and was considered to have made a choice if it reached at the end of either arm. The insect was removed from the Y-tube, and the choice made was recorded as a positive response or negative response by one unit, respectively. In contrast, a female was considered to have not made a choice if it remained in the common arm of the Y-tube during the observation period (Mukherjee *et al.*, 2013 and 2015; Adhikary *et al.*, 2015). Each dose was conducted until a total of 90 naïve female insects had responded (each insect was used once throughout olfactory bioassays). After testing five insects the olfactometer set-up was cleaned with petroleum ether followed by acetone, and the position of the two arms was changed in order to avoid positional bias.

## Ovipositional assay

After a single copulation of the newly emerged male/ female, the female was placed in a sterilized glass jar (15 × 8 cm) containing 25 khesari seeds of the tested variety on a Petri dish (8 cm) in no choice test. The glass jars were lined with coarse grade emery paper to prevent oviposition on the wall of glass jars and bottom of the glass jars were covered by Whatman No. 41 filter paper. Twenty five inactive females were evaluated for each variety of khesari seed. The number of eggs per seed was counted daily and the seeds in each glass jar were replaced with uninfested seeds until death of the ovipositing female. Similar tests were conducted with wax removed khesari seeds of each variety to find out the role of surface waxes on egg laying.

In choice test, a specially designed square glass chamber (25 cm<sup>2</sup>) was used. Inside the jars, emery paper/ Whatman No. 41 filter paper were used. Twenty five khesari seeds of each variety were placed separately in Petri dishes (8 cm) at the four corners of the square glass chamber. Newly emerged female, mated once was placed centrally in the glass chamber and covered with a glass lid to prevent outward movement of the insect from the glass chamber. The number of insects taken for the study and the observations on oviposition were similar to that mentioned in no choice test.

## Statistical Analyses

The data obtained on responses of *C. maculatus* to surface waxes of khesari seeds were analyzed by Chi-square test (Sarkar and Barik, 2014 and 2015; Mukherjee *et al.*, 2015; Malik and Barik, 2015; Sarkar *et al.*, 2015). Insects that did not respond to any selection offered in the olfactometer were excluded from the analyses. The data on number of eggs laid by *C. maculatus* in no choice tests were subjected to *t*-test; whereas one-way ANOVA followed by Tukey test were adopted in choice tests (Zar, 1999).

## RESULTS AND DISCUSSION

### SEM study

The surface of Bio L 212 Ratan seed showed an obscure framework of cuticular hydrocarbon depositions surmounted by multiplied, flat, disc like asteroid configurations, and asteroids with a central flat disc and 7-12 radiating ribs which was diffused over seed surface (Fig. 1a). In Nirmal B-1 seed surface, a series of tent like (a framework of trabecular) configurations with fimbriated margin emanating from spermoderm was observed with numerous tertiary granular and a few large globular depositions, and the fimbria appear to be interlinked by trabecular strands (Fig. 1b). The surface of WBK-14-7 seed indicated a subtending trabecular framework of hydrocarbon depositions with asteroids both tent like as well as flat discoid, and tertiary depositions of different shapes (Fig. 1c); whereas in WBK-13-1, a trabecular framework with asteroids (disc part of asteroids very irregular and radiating area variable) and granular depositions were observed (Fig. 1d).

### Surface waxes and olfactory assay

The *n*-hexane extracts from 100 g of Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds yielded  $15.1 \pm 0.6$ ,  $14.1 \pm 0.9$ ,  $13.0 \pm 1.0$  and  $10.6 \pm 0.6$  mg of surface waxes, respectively. Total amount of seed surface waxes differed significantly among the khesari seed varieties ( $F = 5.68$ ;  $df = 3, 16$ ;  $P < 0.05$ ). The Tukey test revealed that total amount of surface waxes was lower in WBK-13-1 than Bio L 212 Ratan and Nirmal B-1, but the amount of surface waxes in WBK-14-7 seeds did not differ significantly between Bio L 212 Ratan or Nirmal B-1 or WBK-13-1 seeds.

The data on olfactory assay are presented in Table 1. Waxes from Bio L 212 Ratan seeds were attractive at  $1 \mu\text{g ml}^{-1}$  ( $\chi^2 = 7.51$ ;  $df = 1$ ;  $P < 0.01$ ) and  $2 \mu\text{g ml}^{-1}$  ( $\chi^2 = 23.51$ ;  $df = 1$ ;  $P < 0.0001$ ). The surface waxes from Nirmal B-1 seeds elicited

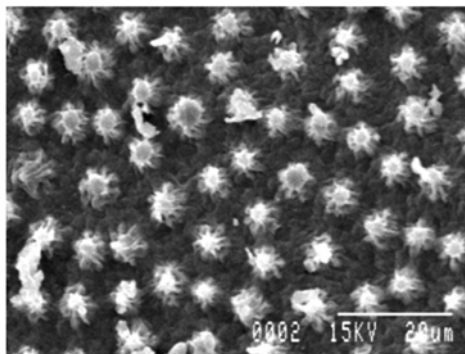


Fig. 1a.

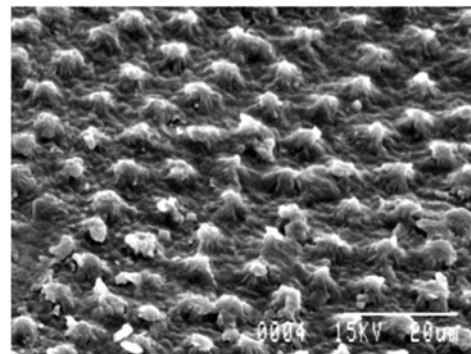


Fig. 1b.

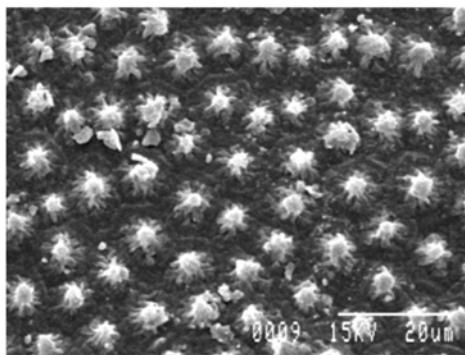


Fig. 1c.

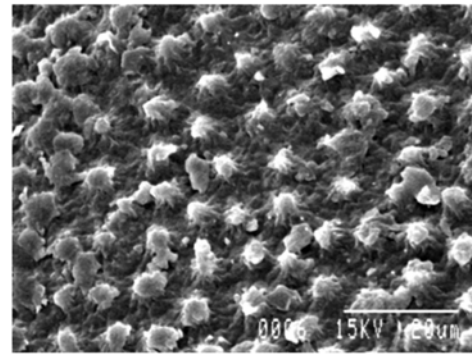
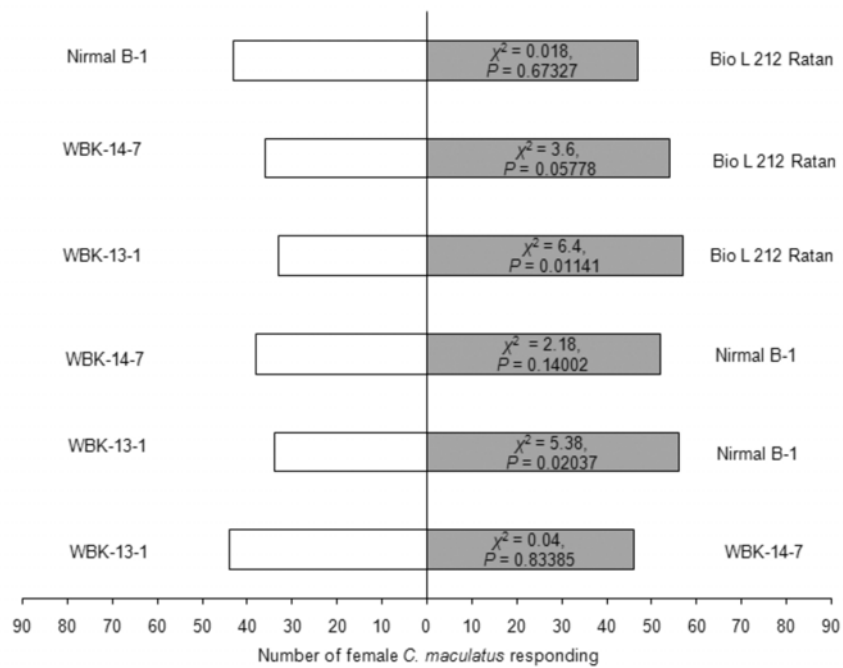
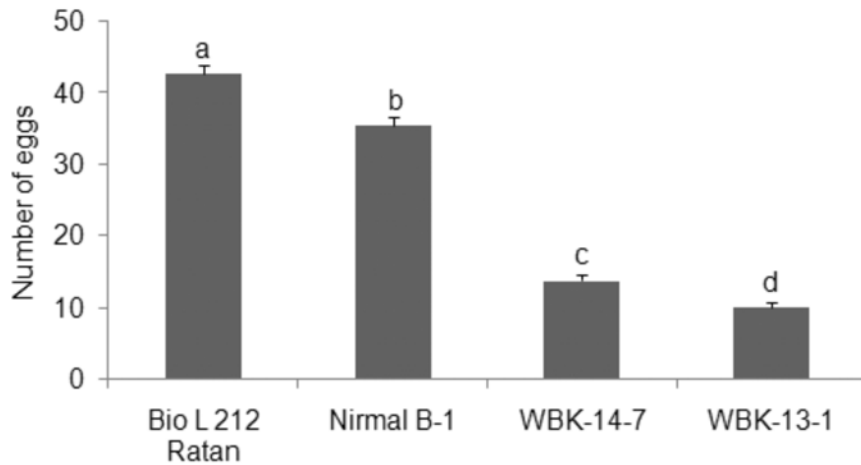


Fig. 1d

**Fig. 1.** Scanning electron micrograph of seed coat surface of Bio L 212 Ratan (a), Nirmal B-1 (b), WBK-14-7 (c), and WBK-13-1 (d) khesari seeds



**Fig. 2.** Response of female *C. maculatus* to  $2 \mu\text{g ml}^{-1}$  surface waxes from four varieties of khesari seeds tested against each other in olfactometer bioassay



**Fig. 3.** Mean number of eggs laid by *C. maculatus* on different varieties of *Lathyrus sativus* seeds in choice test

**Table 1.** Response of female *C. maculatus* to surface waxes of different varieties of *Lathyrus sativus* seeds in olfactometer assay

T1	T2	Insects responded		$\chi^2$ (df = 1)	P Values
		T1	T2		
Waxes	Control				
Bio L 212 Ratan (0.5 $\mu\text{g/ml}$ )	Solvent	52	38	2.18	0.67327
Bio L 212 Ratan (1 $\mu\text{g/ml}$ )	Solvent	58	32	7.51	0.00613
Bio L 212 Ratan (2 $\mu\text{g/ml}$ )	Solvent	68	22	23.51	<0.0001
Nirmal B-1 (0.5 $\mu\text{g/ml}$ )	Solvent	50	40	1.11	0.29184
Nirmal B-1 (1 $\mu\text{g/ml}$ )	Solvent	55	35	4.44	0.03502
Nirmal B-1 (2 $\mu\text{g/ml}$ )	Solvent	67	23	21.51	<0.0001
WBK-14-7 (1 $\mu\text{g/ml}$ )	Solvent	54	36	3.6	0.05778
WBK-14-7 (2 $\mu\text{g/ml}$ )	Solvent	59	31	8.71	0.00316
WBK-14-7 (4 $\mu\text{g/ml}$ )	Solvent	70	20	27.78	<0.0001
WBK-13-1 (1 $\mu\text{g/ml}$ )	Solvent	52	38	2.18	0.14002
WBK-13-1 (2 $\mu\text{g/ml}$ )	Solvent	58	32	7.51	0.00613
WBK-13-1 (4 $\mu\text{g/ml}$ )	Solvent	68	22	23.51	<0.0001

solvent – petroleum ether, N = 90

attraction at 1  $\mu\text{g ml}^{-1}$  ( $\chi^2= 4.44$ ;  $df = 1$ ;  $P < 0.05$ ) and 2  $\mu\text{g ml}^{-1}$  ( $\chi^2= 21.51$ ;  $df = 1$ ;  $P < 0.0001$ ). *C. maculatus* responded to the waxes from WBK-14-7 khesari seeds at 2  $\mu\text{g ml}^{-1}$  ( $\chi^2= 8.71$ ;  $df = 1$ ;  $P < 0.05$ ) and 4  $\mu\text{g ml}^{-1}$  ( $\chi^2= 27.78$ ;  $df = 1$ ;  $P < 0.0001$ ); whereas no clear positive or negative responses was observed at 1  $\mu\text{g ml}^{-1}$  ( $\chi^2= 3.6$ ;  $df = 1$ ;  $P > 0.05$ ). Waxes from WBK-13-1 seeds were attractive at 2  $\mu\text{g ml}^{-1}$  ( $\chi^2= 7.51$ ;  $df = 1$ ;  $P < 0.05$ ) and 4  $\mu\text{g ml}^{-1}$  ( $\chi^2= 23.51$ ;  $df = 1$ ;  $P < 0.0001$ ), but the insect did not elicit clear positive responses ( $\chi^2=$

2.18;  $df = 1$ ;  $P > 0.05$ ) at 1  $\mu\text{g ml}^{-1}$ . The insect showed preferences to 2  $\mu\text{g ml}^{-1}$  concentration of surface waxes from Bio L 212 Ratan seeds ( $\chi^2 = 6.4$ ,  $df = 1$ ,  $P < 0.05$ ) vs. WBK-13-1 seeds, and Nirmal B-1 seeds ( $\chi^2 = 5.38$ ,  $df = 1$ ,  $P < 0.05$ ) vs. WBK-13-1 seeds; whereas the insect did not indicate clear positive or negative responses to surface waxes from Bio L 212 Ratan ( $\chi^2= 0.18$ ;  $df = 1$ ;  $P > 0.05$ ) vs. Nirmal B-1, Bio L 212 Ratan ( $\chi^2= 3.6$ ;  $df = 1$ ;  $P > 0.05$ ) vs. WBK-14-7, Nirmal B-1 ( $\chi^2= 2.18$ ;  $df = 1$ ;  $P > 0.05$ ) vs. WBK-14-7

**Table 2. Oviposition by *C. maculatus* on different varieties of *Lathyrus sativus* seeds with waxes and without waxes in no choice assay**

Variety	Experiment 1		Experiment 2	
	Eggs laid on seeds with waxes	Eggs laid on filter paper	Eggs laid on seeds without waxes	Eggs laid on filter paper
Bio L 212 Ratan	105.24 ± 2.08	2.04 ± 0.23	54.6 ± 0.83	53.88 ± 0.67
Nirmal B-1	101.2 ± 3.02	6.96 ± 1.23	55.6 ± 0.52	54.84 ± 0.71
WBK-14-7	94.7 ± 2.9	11.8 ± 1.5	55.28 ± 0.62	53.64 ± 0.62
WBK-13-1	91.2 ± 3.01	15.1 ± 2.5	54.36 ± 0.49	53.56 ± 0.68

N = 25

and WBK-14-7 ( $\chi^2 = 0.04$ ;  $df = 1$ ;  $P > 0.05$ ) vs. WBK-13-1 (Fig. 2). This observation indicated that the test insect showed less attraction to surface waxes from WBK-13-1 khesari seeds than the other varieties of khesari seeds used in this study.

### Ovipositional assay

*Callosobruchus maculatus* females showed significant oviposition in khesari seeds with waxes on Bio L 212 Ratan ( $t = 32.9$ ,  $df = 48$ ,  $P < 0.0001$ ), Nirmal B-1 ( $t = 27.9$ ,  $df = 48$ ,  $P < 0.0001$ ), WBK-14-7 ( $t = 25.7$ ,  $df = 48$ ,  $P < 0.0001$ ) and WBK-13-1 ( $t = 19.6$ ,  $df = 48$ ,  $P < 0.0001$ ) seeds in no choice assay (Table 2); whereas the insect did not indicate significant oviposition on khesari seeds without waxes on Bio L 212 Ratan ( $t = 0.678$ ,  $df = 48$ ,  $P > 0.05$ ), Nirmal B-1 ( $t = 0.859$ ,  $df = 48$ ,  $P > 0.05$ ), WBK-14-7 ( $t = 1.896$ ,  $df = 48$ ,  $P > 0.05$ ) and WBK-13-1 ( $t = 0.959$ ,  $df = 48$ ,  $P > 0.05$ ) seeds in no choice assay (Table 2). From this study, it is clear that waxes from four varieties of khesari seeds influenced oviposition of *C. maculatus*.

In choice tests, when *C. maculatus* were provided with four varieties of khesari seeds, and it was observed that *C. maculatus* displayed significant difference in egg laying through one-way ANOVA ( $F = 261.69$ ;  $df = 3, 96$ ;  $P < 0.0001$ ), and the Tukey multiple pair wise comparisons test revealed that total number of eggs laid by the insects were significantly higher on Bio L 212 Ratan followed by

Nirmal B-1, WBK-13-1 and WBK-14-7 (Fig. 3).

The cuticular wax serves many physiological functions. It also play an important role in seed-insect interaction (Schoonhoven *et al.*, 2005) and act as attractants for oviposition (Phelan *et al.*, 1991; Parr *et al.*, 1998; Nietupski *et al.*, 2005) and for feeding (Manosalva *et al.*, 2011; Mukherjee *et al.*, 2014; Mukherjee and Barik, 2014; Sarkar and Barik, 2014 and 2015; Malik and Barik, 2015). The amount of surface waxes was lower in WBK-13-1 seeds than Bio L 212 Ratan and Nirmal B-1 seeds, and the SEM study indicated considerable variations in their micro morphology, ranging from amorphous films to mixed arrays of wax tubes, rods, granules and plates (Sharma *et al.*, 1977; Murthy and Sanjappa, 2002; Mallick and Sawhney, 2003; Gohary and Mohammed, 2007; Al-Ghamdi and Al-Zahrani, 2010; Gandhi *et al.*, 2011; Tabaripour *et al.*, 2013). The results of the olfactory assay revealed clear olfactory responses to surface wax compounds, which are low-volatile substances that might act as close range allelochemicals after arrival of the insect to the seed. *C. maculatus* could detect wax compounds from Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds at 1, 1, 2 and 2  $\mu\text{g ml}^{-1}$  respectively, and among the four varieties, the insect showed less attraction to surface waxes from WBK-13-1 than the rest three varieties indicating that surface wax compounds from WBK-13-1 are less attractive to *C. maculatus*.

In the present study, *C. maculatus* laid higher number of eggs on Bio L 212 Ratan followed by Nirmal B-1, WBK-14-7 and WBK-13-1 seeds with surface waxes in no choice assay; whereas the insect did not indicate significant oviposition preference on wax removed seeds of any variety in no choice assay, implicating that surface wax compounds played an important role in oviposition of *C. maculatus*. Further, the insect preferred to lay higher number of eggs on Bio L 212 Ratan followed by Nirmal B-1, WBK-14-7 and WBK-13-1 seeds in choice assay, indicating that surface wax compounds from Bio L 212 Ratan and Nirmal B-1 might have influenced the insect for laying higher number of eggs on these two varieties which result in higher losses by the feeding *C. maculatus* larvae in these two varieties than in WBK-14-7 and WBK-13-1. Our previous study revealed that the amount of volatiles were highest in Bio L 212 Ratan, followed by Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds, and the insect showed lowest attraction to the WBK-14-7 and WBK-13-1 (Adhikary *et al.*, 2015), and lower amounts of carbohydrates, proteins, lipids, nitrogen, water content and higher trypsin inhibitor activity of WBK-14-7 and WBK-13-1 caused higher developmental time and lower fecundity of *C. maculatus* on these varieties than Bio L 212 Ratan and Nirmal B-1. Further, WBK-14-7 and WBK-13-1 khesari seeds indicated lower amount of  $\beta$ -ODAP content than Bio L 212 Ratan and Nirmal B-1 (Adhikary *et al.*, 2016). It is well established that variation in seed size plays an important role in the oviposition of *C. maculatus* (Mitchell, 1983; Cope and Fox, 2003), but in this study since almost the same size for the different varieties khesari seeds were used it is seen that seed sizes did not play any role in the oviposition. On the basis of the present study and also on the basis of the previous investigations, it is suggested to promote the production of WBK-14-7 and WBK-13-1 khesari seeds than Bio L 212 Ratan and Nirmal B-1 seeds to reduce the loss caused by *C. maculatus*.

Long chain (>C16) free fatty acids constitute a large proportion of the surface waxes and these compounds have infrequently been shown to affect insect oviposition (Parr *et al.*, 1998). Therefore, it

remains to be seen whether ovipositing female bruchids can similarly perceive differences between fatty acids in the four varieties of khesari seeds.

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## REFERENCES

- Adhikary P. and Barik A. (2012) Effect of temperature on biology of *Callosobruchus maculatus* (F.). Indian Journal of Entomology 74(3): 261–266.
- Adhikary P., Malik U. and Barik A. (2016) Effects of four varieties of *Lathyrus sativus* L. seeds on the bionomics of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). The Canadian Entomologist 148(1): 102–111.
- Adhikary P., Mukherjee A. and Barik A. (2015) Attraction of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) to four varieties of *Lathyrus sativus* L. seed volatiles. Bulletin of Entomological Research 105(2): 187–201.
- Al-Ghamdi F. A. and Al-Zahrani R. M. (2010) Seed morphology of some species of *Tephrosia* PERS. (Fabaceae) from Saudi Arabia. Identification of species and systematic significance. Feddes Repertorium 121(1-2): 59–65.
- Appleby J. H. and Credland P. F. (2001) Bionomics and polymorphism in *Callosobruchus subinnotatus*. Bulletin of Entomological Research 91(4): 235–244.
- Arnold S. E. J., Stevenson P. C. and Belmain S. R. (2012) Odour-mediated orientation of beetles is influenced by age, sex and morph. PLoS ONE 7(11): e49071.
- Bogamuwa M. M.S., Weerakoon K. C. and Karunaratne S. H. P. P. (2002) Insecticide resistance in the bruchid *Callosobruchus maculatus*, a storage pest of legumes. Ceylon Journal of Science (Biological Science) 30(1): 55–66.
- Caswell G. H. (1961) The infestation of cowpea in western region of Nigeria. Tropical Science 3(4): 154–158.
- Cope J. M. and Fox C. W. (2003) Oviposition decisions in the seed beetle, *Callosobruchus maculatus*



- (Coleoptera: Bruchidae): effects of seed size on superparasitism. *Journal of Stored Products Research* 39(4): 355–365.
- Fox C. W., Bush M. L. and Messina F. J. (2010) Biotypes of the seed beetle *Callosobruchus maculatus* have differing effects on the germination and growth of their legume hosts. *Agricultural and Forest Entomology* 12(4): 353–362.
- Fox C. W. (1993) The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia* 96(1): 139–146.
- Gandhi D., Albert S. and Pandya N. (2011) Morphological and micromorphological characterization of some legume seeds from Gujarat, India. *Environmental and Experimental Biology* 9(3-4): 105–113.
- Gaur V. and Maloo S. R. (2011) Genetic divergence in grass pea (*Lathyrus sativus*). *Indian Journal of Agricultural Science* 81(2): 172–173.
- Girma D. and Korbu L. (2012) Genetic improvement of grass pea (*Lathyrus sativus*) in Ethiopia: an unfulfilled promise. *Plant Breeding* 131(2): 231–236.
- Gohary I. and Mohammed A. H. (2007) Seed morphology of *Acacia* in Egypt and its taxonomic significance. *International Journal of Agriculture and Biology* 9(3): 435–438.
- Howe R. W. and Currie J. E. (1964) Some laboratory observations on the rates of development, mortality, and oviposition of several species of Bruchidae breeding in stored pulses. *Bulletin of Entomological Research* 55(3): 437–477.
- Huignard J., Leroi B., Alzouma I. and Germain J. F. (1985) Oviposition and development of *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) in *Vigna unguiculata* (Walp) in cultures in Niger. *Insect Science and its Application* 6(6): 691–699.
- Malik U. and Barik A. (2015) Free fatty acids from the weed, *Polygonum orientale* leaves for attraction of the potential biocontrol agent, *Galerucella placida* (Coleoptera: Chrysomelidae). *Biocontrol Science & Technology* 25(5): 593–607.
- Mallick D. K. and Sawhney S. (2003) Seed coat ornamentation in wild and cultivated lentil taxa. *Phytomorphology* 53(2): 187–195.
- Manosalva L., Pardo F., Perich F., Mutis A., Parra L., Ortega F., Isaacs R. and Quiroz A. (2011) Behavioural responses of clover root borer to long-chain fatty acids from young red clover (*Trifolium pratense*) roots. *Environmental Entomology* 40(2): 399–404.
- Messina F. J. and Renwick J. A. A. (1985) Dispersal polymorphism of *Callosobruchus maculatus* (Coleoptera: Bruchidae): variation among populations in response to crowding. *Annals of the Entomological Society of America* 78(2): 201–206.
- Mitchell R. (1983) Effects of host-plant variability on the fitness of sedentary herbivorous insects. In: *Variable plants and herbivores in natural and managed systems* (Eds. Dunno R. F. and McClure M. S.), Academic Press, New York, 343–369 pp.
- Mukherjee A. and Barik A. (2014) Long-chain free fatty acids from *Momordica cochinchinensis* Spreng flowers as allelochemical influencing the attraction of *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Allelopathy Journal* 33(2): 255–266.
- Mukherjee A., Sarkar N. and Barik A. (2013) Alkanes in flower surface waxes of *Momordica cochinchinensis* influence attraction to *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Neotropical Entomology* 42(4): 366–371.
- Mukherjee A., Sarkar N. and Barik A. (2014) Long-chain free fatty acids from *Momordica cochinchinensis* leaves as attractants to its insect pest, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Journal of Asia Pacific Entomology* 17(3): 229–234.
- Mukherjee A., Sarkar N. and Barik A. (2015) *Momordica cochinchinensis* (Cucurbitaceae) leaf volatiles: semiochemicals for host location by the insect pest, *Aulacophora foveicollis* (Coleoptera: Chrysomelidae). *Chemoecology* 25(2): 93–104.
- Murthy G. V. S. and Sanjappa M. (2002) SEM studies on seed morphology of *Indigofera* L. (Fabaceae) and its taxonomic utility. *Rheedea* 12(1): 21–51.
- Nietupski M., Szafranek B., Ciepiewska D., Synak E., Fornal Ł. and Szafranek J. (2005) Correlation between bean seed surface lipids and *Acanthoscelides obtectus* Say development. *Journal of Plant Protection Research* 45(2): 125–133.
- Ouedraogo A.P., Sou S., Sanon A., Monge J.P., Huignard J., Tran B. and Credland P. F. (1996) Influence of temperature and humidity on populations of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinarmus basalis* (Pteromalidae) in two climatic zones of Burkina Faso. *Bulletin of Entomological Research* 86(6): 695–702.

- Parr M. J., Tran B. M. D., Simmonds M. S. J., Kite G. C. and Credland P. F. (1998) Influence of some fatty acids on oviposition by the bruchid beetle, *Callosobruchus maculatus*. *Journal of Chemical Ecology* 24(10): 1577–1593.
- Phelan P. L., Roelofs C. J., Youngman R. R. and Baker T. C. (1991) Characterization of chemicals mediating ovipositional host-plant finding by *Amyelois transitella* females. *Journal of Chemical Ecology* 17(3): 599–613.
- Rao S. L. N. (2011) A look at the brighter facets of  $\beta$ -N-oxalyl-L- $\alpha$ ,  $\beta$ -diaminopropionic acid, homoarginine and the grass pea. *Food and Chemical Toxicology* 49(3): 620–622.
- Sarkar N. and Barik A. (2014) Alkanes from bitter gourd as allelochemicals in olfactory responses of *Epilachna dodecastigma* (Wied.). *Allelopathy Journal* 33(1): 43–52.
- Sarkar N. and Barik A. (2015) Free fatty acids from *Momordica charantia* L. flower surface waxes influencing attraction of *Epilachna dodecastigma* (Wied.) (Coleoptera: Coccinellidae). *International Journal of Pest Management* 61(1): 47–53.
- Sarkar N., Mukherjee A. and Barik A. (2015) Attraction of *Epilachna dodecastigma* (Coleoptera: Coccinellidae) to *Momordica charantia* (Cucurbitaceae) leaf volatiles. *The Canadian Entomologist* 147(2): 169–180.
- Schoonhoven L. M., Van Loon J. J. A. and Dicke M. (2005) *Insect-Plant biology*. Oxford University Press, Oxford, 421 pp.
- Sharma S. K., Babu C. R., Johri B. M. and Hepworth A. (1977) SEM studies on the seed-coat pattern in *Phaseolous mungo-radiatus-sublobatus* Complex. *Phytomorphology* 27(2): 106–111.
- Singh S. S. and Rao S. L. N. (2013) Lessons from neurolathyrism: A disease of the past & the future of *Lathyrus sativus* (Khesari dal). *Indian Journal of Medical Research* 138(1): 32–37.
- Tabaripour R., Koohdar F., Sheidai M. and Gholipour A. (2013) Intra-specific variations in *Silene*: Morphometry and micromorphometry analyses. *African Journal of Biotechnology* 12(33): 5208–5217.
- Thanthianga C. and Mitchell R. (1990) The fecundity and oviposition behaviour of a south Indian strain of *Callosobruchus maculatus*. *Entomologia Experimentalis et Applicata* 57(2): 133–142.
- United Nations Environment Programme (1998) *Montreal Protocol on Substances that Deplete the Ozone Layer, 1998: Assessment of Alternatives to Methyl Bromide*. Methyl Bromide Alternatives Committee, Nairobi, Kenya.
- Utida S. (1972) Density dependent polymorphism in the adult of *Callosobruchus maculatus*. *Journal of Stored Products Research* 8(2): 111–125.
- Zannou E. T., Glitho I. A., Huignard J. and Monge J. P. (2003) Life history of flight morph females of *Callosobruchus maculatus* F.: evidence of a reproductive diapause. *Journal of Insect Physiology* 49(6): 575–582.
- Zar J. H. (1999) *Biostatistical analysis*. Prentice Hall, New Jersey, 661 pp.

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