Proceedings of the Pakistan Academy of Sciences: B Life and Environmental Sciences 59(3): 9-21 (2022)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-3)716

Pakistan Academy of Sciences

Research Article

Distribution and Damage Potential of Pulse Beetles, *Callosobruchus* spp. (Coleoptera: Bruchidae) in Sindh, Pakistan

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Abstract: *Callosobruchus* spp. are global serious pests of pulses. Therefore, two studies were carried out to determine their distribution in three ecological zones of Sindh, Pakistan along with their damage potential to major pulses. A survey was conducted in Zone one (Ghotki, Sukkur, and Larkana districts), Zone two (Hyderabad, Mirpur Khas, and Shaheed Benazirabad districts), and Zone three (Karachi district) from March – April 2020. Grain samples of main pulses i.e., chickpea, field pea, cowpea, green lentil, and yellow lentil were collected from three locations in each district and brought to Stored Grain Research Laboratory, Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam for further examination. Three pulse beetle species i.e., *C. maculatus, C. chinensis* and *C. analis* were identified from the collected pulse samples as the highest populations of adults, eggs, grubs, and pupae were recorded from green lentil, followed by cowpea and chickpea. No population of pulse beetle was recorded from yellow lentil and field pea. *Callosobruchus maculatus* was the most dominant species in all sampling as higher beetle populations were recorded from Mirpur Khas and Sukkur districts. Therefore, both these strains were further evaluated for their feeding preference and weight loss assessment on various pulses i.e., chickpea, cowpea, green lentil, and yellow lentil in no-choice under laboratory conditions. *C. maculatus* showed a significant feeding preference among pulses with cowpea and chickpea being the most preferred, whereas yellow lentil was the least preferred, hence suffering the highest and lowest weight losses, respectively. Therefore, proper storage and quarantine measures should be taken in the transportation and storage of pulses to restrict the spread and damage of pulse beetles.

Keywords: Beetles, *Callosobruchus*, Damage, Distribution, Population, Stored grains

1. INTRODUCTION

The cultivation of pulses i.e., peas, beans, and lentils can be traced back to ancient civilizations as back as 8,000-10,000 BC [1]. The world's largest producer and consumer of pulses is India, whereas Pakistan, Canada, Myanmar, Australia, and United States are the other major exporters of pulses [2, 3]. The major pulses grown in the world are dry beans, dry peas, chickpeas, and lentils [3, 4]. Pulses are excellent sources of proteins (20-40 %), carbohydrates (50-60 %), minerals, and vitamins i.e., thiamin, niacin, calcium, and iron [4].

During 2018-19, the total sowing area of pulses in Pakistan was 1,174 thousand hectares with a production of 167.4 thousand tons. Chickpea accounts for about 71 % of the total pulse production, whereas green lentil, red lentil, black lentil, and field pea are the other pulses grown in the country [5]. In recent years, a decline has been observed in the area and production of pulses in Pakistan as pulses cover only 7 % of the total cropping land of Pakistan [6]. Moreover, the grain losses due to storage pests ranged from about 5-15 % of the total grain production and may increase up to 50 % in case of severe infestation [7, 8].

More than 150 insect pests attack pulses during storage. Among them, the most important pests are

Received: April 2022; Accepted: September 2022
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bruchids (*Callosobruchus* spp.) i.e., *Callosobruchus maculatus* (Fabricius), *C. chinensis* (Linnaeus), *C. analis* (Fabricius), and *C. phaseoli* (Gyllenhal) [9, 10]. *Callosobruchus* spp. are considered major pests of legumes that originated from tropical and subtropical regions of the world [9, 11]. Mainly, larvae cause damage by feeding inside the grains and in case of severe infestation, can consume entire contents of grains [1]. Moreover, the presence of larvae and pupae inside the pulse seeds deteriorates the quality and marketability of the pulse [12]. Pest attacking grain storage products has become a major concern for all the stakeholders worldwide, thus, needs effective management practices to reduce storage losses by insect pests. Therefore, effective management necessitates assessing the pest status effectively along with the level of losses that might have occurred or likely to occur during the storage [13]. As the infestation and damage of *C. maculatus* is continuously increasing in warehouses in Pakistan and particularly in Sindh province, no considerable research has been done in this regard. Therefore, the study aimed to determine the distribution of *Callosobruchus* spp. at various areas of Sindh along with their damage status to major pulses, so adequate strategies should be devised to manage their spread and losses.

2. MATERIALS AND METHODS

2.1 Distribution of *Callosobruchus* spp.

2.1.1 Study locations

Sampling surveys were carried out in three ecological zones of Sindh i.e., Zone one (Ghotki, Sukkur, and Larkana districts), zone two (Hyderabad, Mirpur Khas, and Shaheed Benazirabad districts), and zone three (Karachi district) (Table 1; Figure 1). The selection of the districts and the particular locations was done on the geography of the Sindh province along with the availability of pulse processing units, and a large warehouse of the pulses. The sampling at various locations was carried out only once during March and April 2020.

2.1.2 Data collection and experimental design

Grain samples of major pulses i.e., chickpea, field pea, cowpea, green lentil, and green lentil were collected from various storehouses. Three sampling points (warehouses), treated as replications, were selected from each zone. A 500 g sample of each pulse was collected from the individual storehouse based on their availability in the particular sampling location. Samples were collected from different points of warehouses and placed in plastic jars immediately with the lids of the jars kept tight. The collected samples were then brought to the Stored Grain Research Laboratory (temperature 30±2 °C and relative humidity 60±5 %) Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam to examine and count both adults as well as immature stages of beetles. The data on the population of larvae and pupae were taken using destructive sampling by breaking the grains with a sharp-edged needle. Species identification was based on the available guidelines from scientific literature [14-16] and taxonomists of the Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam. A sampling of various pulses was done using a completely randomized design as samples were collected randomly on their availability, whereas three samples of each pulse were used as replications.

2.2 Damage Potential of *Callosobruchus maculatus* on Major Pulses

Two population strains of *C. maculatus* i.e., Sukkur and Mirpur Khas were selected for the damage potential studies because of their large-scale presence in all survey locations (results of section 2.1). Fresh 100 g grains of chickpea, cowpea, green lentil, and green lentil were obtained from the local supermarket and placed in separate plastic jars. Five virgins (0-24 h old) pairs (male plus female) of adult *C. maculatus* obtained from the laboratory-reared culture were released in each replicated jar on June 1, 2020. Observations on the population fluctuation of *C. maculatus* were taken fortnightly for three months to record the number of alive and dead beetles. At the end of the experiment, the percent weight loss of pulse grains was calculated using the formula:

% weight loss =
(Final weight of grains/ Initial weight of grains) * 100

Experiments were conducted in the Stored Grain Research Laboratory, Department of Entomology, Sindh Agriculture University, Tandojam, Pakistan (temperature 30±2 °C and relative humidity

Table 1. GPS coordinates of sampling locations

City	Sampling point	Address	GPS	
			Latitude	Longitude
Sukkur	1	Al-Noor Daal Mill, Golimar Sukkur	N 27°42'42.866"	E 68°51'15.466"
	2	Jamal Sons (Ajmeri Daal waala) Liaqat Bazar Sukkur	N 27°41'34.300"	E 68°51'55.911"
	3	Shaikh whole seller, Khudadaad Khan road old Sukkur	N 27°42'14.832"	E 68°52'53.257"
Larkana	4	Super Matni Anaaj Dealer, Jelaus Bazar, Old Anaaj Mandi Larkana	N 27°33'30.255"	E 68°13'12.139"
	5	New Dero road, near Dr. Khalid Mahmood Madarsa, Ayoub Colony Larkana	N 27°33'48.166"	E 68°13'21.617"
	6	Warehouse, near Khosa goth, Rato dero road Larkana	N 27°35'34.184"	E 68°13'24.880"
Ghotki	7	Warehouse, near new bus stand Ghotki	N 27°58'50.464"	E 69°17'16.774"
	8	Laal Dealer, New market Ghotki	N 28°00'31.560"	E 69°18'52.132"
	9	Chander Bhaan Seed store, near noor masjid, new market, Ghotki	N 28°00'31.670"	E 69°18'52.314"
Shaheed Benazira bad	10	Hamdard pansaar and daal centre, chakra bazar Nawabshah	N 26°14'37.676"	E 68°24'37.105"
	11	Haji gareeb shah jadoon daal and rice marchant, markeet road 1. Nawabshah	N 26°14'36.716"	E 68°24'37.347"
	12	Ab Majeed daal, rice and garam masala merchant, market road 1. Nawabshah	N 26°14'36.513"	E 68°24'37.564"
Hyderabad	13	Sagar pulse and rise industry, site area, Hyderabad	N 25°21'23.059"	E 68°23'57.736"
	14	Sun shine Daal Mills, E-29/A site area, Hyderabad	N 25°21'24.119"	E 68°23'52.243"
	15	Sooraj Daal mill, site area, Hyderabad	N 25°21'37.525"	E 68°23'46.161"
Mirpur Khas	16	Zafar Nayab, daal and rice centre, Anaaj market, market chowk Mirpur Khas	N 25°31'40.762"	E 69°00'50.156"
	17	Farooq Nayab, daal centre, Anaaj market Mirpur Khas	N 25°31'40.466"	E 69°00'50.615"
	18	Qalandari daal dealer, near Mehran Cotton factory, Paak coloney Mirpur Khas	N 25°31'41.184"	E 69°00'52.516"
Karachi	19	Haji Kareem (Importer and Exporter) M.R 1/57, Joria Bazaar Karachi	N 24°51'17.668"	E 67°00'14.387"
	20	Zafar Traders and Co. (Importer and Exporter), Joria Bazaar Karachi	N 24°51'18.846"	E 67°00'11.366"
	21	Tariq hole sale, G-13, Batulhina, Gulistan e johar, block-18, Karachi	N 24°54'31.237"	E 67°07'49.160"

60±5 %) in a completely randomized design as each treatment was replicated five times. The humidity of various grains used was standardized at 12 % before the release of insects by treating them in a hot air oven.

2.3 Data Analysis

Collected data for both the studies were analyzed using Analysis of Variance (ANOVA) with the Least Square Difference (LSD) used to separate means with significant differences. The Statistix 8.1 computer software was used for the analysis.

3. RESULTS

3.1 Identification of Collected Pulse Beetles

In seven surveyed districts of Sindh province, three species of pulse beetle i.e., *C. maculatus*,

Fig. 1. GPS co-ordinates (landmarks/legends) of various sampling districts (locations) of Sindh, Pakistan

C. chinensis, and *C. analis* were identified based on their morphological characteristics as suggested by [14-16]. *Callosobruchus maculatus* can easily be identified by its general appearance like coloration on the plate covering the end of the abdomen, *C. chinensis* have a distinguished inner tooth on the hind femur having sides parallel, converging near the apex, whereas the pronotum of *C. analis* has uniformed cuticle (reddish-brown) along with sparse golden setae.

Among the identified species, relatively higher populations of *C. maculatus* were recorded in all the survey districts. Moreover, the population of *Callosobruchus* spp. was observed only on chickpea, cowpea, and green lentil, whereas no population was recorded from green lentil and field peas (Table 2).

3.2 Population of *Callosobruchus* spp. Eggs at Various Survey Locations

The highest mean population of pulse beetle eggs at Sukkur were recorded on green lentil (659.33±168.34 eggs), followed by cowpea (409.33±215.64 eggs) and chickpea (8.67±4.91 eggs), whereas eggs on cowpea (376.33±215.94 eggs), chickpea (17.67±17.67 eggs) and green lentil (1.00±1.00 eggs) were recorded at Larkana. At Ghotki, egg population was recorded only on chickpea (43.67±37.83 eggs), whereas chickpea (89.33±80.02 eggs) and green lentil (6.00±6.00 eggs) showed the population of eggs at Shaheed Benazirabad. The number of *Callosobruchus* spp. eggs at Hyderabad were recorded from green lentil (764±233.89 eggs), chickpea (275.67±151.88 eggs), and cowpea (29.00±29.00 eggs), whereas the population of eggs on the same pulses at Mirpur Khas was 692.67±281.29, 193.67±170.23 and 22.00±11.53 eggs, respectively. Furthermore, in Karachi, commodities that suffered from the population of eggs were chickpea (10.00±6.08 eggs), green lentil (12.00±12.00 eggs), and cowpea (12.00±12.00 eggs). Among sampling districts, Hyderabad and Mirpur Khas showed a relatively higher population of eggs on green lentil (Table 2).

3.3 Population of *Callosobruchus* spp. Grubs at Various Survey Locations

The population of *Callosobruchus* grubs was recorded only in green lentil (56.33±17.07 grubs) and cowpea (43.33±33.12 grubs) at Sukkur, whereas chickpea (56.33±17.07 grubs) and cowpea

(20.00±16.33 grub) showed the presence of grubs in Larkana. In Ghotki district, 6.67±6.67 grubs, and in Shaheed Benazirabad district, 24.33±23.84 grubs were recorded from chickpea only. In Hyderabad, the population of grubs was recorded on chickpea (64.67±32.46 grubs), green lentil (57.00±24.85 grubs), and cowpea (2.00±2.00 grubs), whereas the population of grubs on same pulses in Karachi was 4.67±2.6, 44.00±29.1 and 1.67±1.67 grubs, respectively. At Mirpur Khas, grubs were observed only in chickpea (17.00±11.53 grubs) and green lentil (141.67±95.83 grubs). Among sampling districts, a relatively higher population of grubs was observed at Mirpur Khas in green lentil commodity (Table 2).

3.4 Population of *Callosobruchus* spp. Pupae at Various Survey Locations

Results indicated that the population of pulse beetle pupae was recorded only in cowpea (26.67±14.77 pupae) and green lentil (18.00±5.51 pupae) at Sukkur, whereas cowpea (32.00±16.17 pupae) and chickpea (11.00±11.00 pupae) were the only commodities showing pupae population at Larkana. Chickpea was the only commodity where pupae were recorded at Shaheed Benazirabad (6.67±5.7 pupae) and Ghotki (2.33±2.33 pupae) districts. In Hyderabad, the population of pupae was recorded in chickpea (32.00±23.58 pupae), green lentil (31.33±9.61 pupae), and cowpea (6.00±6.00 pupae), respectively, whereas the population of pupae on same pulses at Mirpur Khas was 6.00±3.46, 86.33±52.7 and 6.00±4.58 pupae, respectively. Furthermore, in Karachi, a population of pupae was observed in green lentil (41.33±32.75 pupae), chickpea (6.00±3.46 pupae), and cowpea (0.67±0.67 pupae). Among sampling districts, a relatively higher population of pupae was observed at Mirpur Khas followed by Karachi (Table 2).

3.5 Population of Adult *C. maculatus* at Various Survey Locations

A great variation was recorded in the population of *C. maculatus* adults from various pulses at different sampling locations (Table 2). Thus, a significant difference (F =6.82, P =0.0045) was recorded in the population of adult *C. maculatus* on various commodities, whereas overall populations at sampling districts were not significantly different (F =1.50, P =0.2425) from each other. In the Sukkur district, the population of *C. maculatus* was recorded only from green lentil (51.33±8.57 beetles) and cowpea (21±10.12 beetles), whereas cowpea (34.33±18.62 beetles) and chickpea (12.00±11.50 beetles) suffered from *C. maculatus* infestation in Larkana. In Ghotki, the *C. maculatus* population was only recorded from chickpea (2.00±1.15 beetles); in Shaheed Benazirabad, chickpea (0.33±0.33 beetles) and green lentil (0.67±0.67 beetles) suffered the beetle attack. The green lentil (18.33±11.67 beetles) and chickpea (6.00±4.58 beetles) showed the population of *C. maculatus* in Karachi. Among sampling districts, Mirpur Khas and Hyderabad showed a relatively higher population of *C. maculatus* on three pulses i.e., chickpea, cowpea, and green lentil. At Hyderabad, the overall population of *C. maculatus* recorded on chickpea, cowpea and green lentil were 22.00±11.06 beetles, 3.00±3.00 beetles, and 43.00±13.01 beetles, respectively, whereas the population of adult beetles on same pulses in Mirpur Khas was 4.00±4.00 beetles, 2.00±2.00 beetles, and 93.00±46.51 beetles, respectively.

3.6 Population of Adult *C. chinensis* at Various Survey Locations

Results also indicated that at Sukkur and Shaheed Benazirabad, population of *C. chinensis* (0.33±0.33 beetles and 16.67±16.67 adults, respectively) was only recorded from chickpea. However, at Mirpur Khas, adult C. chinensis population was recorded from cowpea (2.67±2.67 beetles) and green lentil (4.00±4.00 beetles), only. Furthermore, at Karachi, C. chinensis adults (5.00±5.00 beetles) were only recorded from green lentil (Table 2).

3.7 Population of Adult *C. analis* at Various Survey Locations

It has been also observed that population of adult *C. analis* was recorded only on cowpea (1.67±1.67 beetles) and chickpea (0.67±0.67 beetles) at Sukkur. However, C. analis population in Larkana (1.67±1.67), Ghotki (0.67±0.67), Shaheed Benazirabad (1.67±1.67) and Mirpur Khas (5.00±5.00) was recorded on chickpea, only. Furthermore, at Karachi, population of *C. analis* adults was recorded only on cowpea (2.00±2.00 beetles) (Table 2).

Table 2. Overall mean population of *Callosobruchus* spp. in different pulses at various sampling districts of Sindh, Pakistan.

City	Commodity	Eggs	Grubs	Pupa	*C. maculatus*	*C. chinensis*	*C. analis*
Sukkur	Chickpea	8.67±4.91e	0.00	0.00	0.00	0.33±0.33b	0.67±0.67b
	Cowpea	409.33±215.64a-d	43.33±33.12b	26.67±14.77b	21±10.12b-d	0.00	1.67±1.67ab
	Green lentil	659.33±168.34ab	56.33±17.07b	18.00±5.51b	51.33±8.57b	0.00	0.00
	Yellow lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Field pea	0.00	0.00	0.00	0.00	0.00	0.00
Larkana	Chickpea	17.67±17.67de	56.33±17.07b	11.00±11.00b	12.00±11.5cd	0.00	1.67±1.67ab
	Cowpea	376.33±215.94a-e	20.00±16.33b	32.00±16.17b	34.33±18.62b-d	0.00	0.00
	Green lentil	1.00±1.00e	0.00	0.00	0.00	0.00	0.00
	Yellow lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Field pea	0.00	0.00	0.00	0.00	0.00	0.00
Ghotki	Chickpea	43.67±37.83c-e	6.67±6.67b	2.33±2.33b	2.00±1.15d	0.00	0.67±0.67b
	Cowpea	0.00	0.00	0.00	0.00	0.00	0.00
	Green lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Yellow lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Field pea	0.00	0.00	0.00	0.00	0.00	0.00
Shaheed Benazirabad	Chickpea	89.33±80.02cde	24.33±23.84b	6.67±5.7b	0.33±0.33d	16.67±16.67a	1.67±1.67ab
	Cowpea	0.00	0.00	0.00	0.00	0.00	0.00
	Green lentil	6.00±6.00e	0.00	0.00	0.67±0.67d	0.00	0.00
	Yellow lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Field pea	0.00	0.00	0.00	0.00	0.00	0.00
Hyderabad	Chickpea	275.67±151.88b-e	64.67±32.46ab	32.00±23.58b	22.00±11.06bcd	0.00	0.00
	Cowpea	29.00±29.00de	2.00±2.00b	6.00±6.00b	3.00±3.00d	0.00	0.00
	Green lentil	764±233.89a	57.00±24.85b	31.33±9.61b	43.00±13.01bc	0.00	0.00
	Yellow lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Matar	0.00	0.00	0.00	0.00	0.00	0.00
Mirpurkhas	Chickpea	193.67±170.23c-e	17.00±11.53b	6.00±3.46b	4.00±4.00d	0.00	5.00±5.00a
	Cowpea	22.00±11.53de	0.00	6.00±4.58b	2.00±2.00d	2.67±2.67b	0.00
	Green lentil	692.67±281.29a	141.67±95.83a	86.33±52.7a	93.00±46.51a	4.00±4.00b	0.00
	Yellow lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Field pea	0.00	0.00	0.00	0.00	0.00	0.00
Karachi	Chickpea	10.00±6.08e	4.67±2.6b	6.00±3.46b	6.00±4.58cd	0.00	0.00
	Cowpea	12.00±12.00de	1.67±1.67b	0.67±0.67b	0.00	0.00	2.00±2.00ab
	Green lentil	439.33±300.83a-c	44.00±29.1b	41.33±32.75ab	18.33±11.67b-d	5.00±5.00b	0.00
	Yellow lentil	0.00	0.00	0.00	0.00	0.00	0.00
	Field pea	0.00	0.00	0.00	0.00	0.00	0.00

3.8 Overall Population of *Callosobruchus* spp. at Various Locations and Pulses

It has been also observed that overall, the highest population of *Callosobruchus* spp. adults (110.67) was recorded in Mirpur Khas district, whereas, the highest population of eggs (1077.33), grubs (158.67), and pupae (98.33) were recorded in Sukkur and Mirpur Khas districts, respectively. Moreover, among pulses, green lentils suffered the highest population of various stages of *Callosobruchus* spp. followed by cowpea (adults and eggs and pupae) and chickpea (grubs) (Table 3).

3.9 Population Fluctuation of Alive *C. maculatus* (Sukkur and Mirpur Khas strains) on Various Pulses

Population fluctuation of both *C. maculatus* strains on four major pulses over the entire study duration exhibited a highly significant variation ($F= 14.15$, $P < 0.001$). According to Table 4, during the first week after the release of *C. maculatus,* the maximum population of alive beetles was observed in cowpea (38.00±3.78 beetles) for the Mirpur Khas strain, followed by green lentil (31.00±3.61 beetles) for the same strain. Afterward, a great variation was recorded in a number of alive and dead beetles in various pulses for both Sukkur and Mirpur Khas strains because of their short adult longevity. Thus, fortnightly the highest mean *C. maculatus* population was recorded in cowpea (800.40±57.55 beetles) for the Mirpur Khas strain, followed by 784.40±36.50 beetles recorded in green lentil for the same strain. The maximum fortnightly mean population for the Sukkur strain i.e., 736.80±57.51 beetles was recorded in green lentil, followed by 676.20±120.94 beetles in cowpea, both observed during the fifth fortnight of the study.

Overall mean population results indicated that among pulses, cowpea suffered significantly ($F = 6.00$, $P = 0.0007$) the highest population of both Sukkur (268±67.04 beetles) and Mirpur Khas (327.64±74.50 beetles) strains of alive *C. maculatus,* followed by the population of Mirpur Khas strain observed in chickpea (263.08±59.29 beetles) and green lentil (252.72±62.33 beetles) (Figure 2). Overall, the lowest alive *C. maculatus* population was recorded in green lentils (139.96±32.32 beetles) for the Mirpur Khas strain, followed by 202.80±60.06 beetles recorded in the same pulse for the Sukkur strain.

3.10 Population Fluctuation of Dead *C. maculatus* (Sukkur and Mirpur Khas strains) on Various Pulses

Like population fluctuation of alive *C. maculatus* in various pulses throughout the study duration

Table 3. Overall mean population of various stages of *Callosobruchus* spp. recorded at various locations (A) and pulses (B)

A.

District	Adults	Eggs	Grubs	Pupae
Sukkur	75	1077.33	99.66	44.67
Larkana	48	395	76.33	43
Ghotki	2.67	43.67	6.67	2.33
Shaheed Benazirabad	19.34	95.33	24.33	6.67
Hyderabad	68	1068.67	123.67	69.33
Mirpur Khas	110.67	908.34	158.67	98.33
Karachi	31.33	461.33	50.34	48

B.

Pulse	Adults	Eggs	Grubs	Pupae
Chickpea	73.01	638.68	173.67	64
Cowpea	66.67	848.66	67	71.34
Green lentil	215.33	2562.33	299	176.99

Table 4. Population fluctuation of alive *C. maculatus* strains (Sukkur & Mirpur Khas) on different pulses under lab conditions

Observation interval (Fortnight)	Chickpea		Cowpea		Green lentil		Yellow lentil	
	Sukkur	Mirpur Khas	Sukkur	Mirpur Khas	Sukkur	Mirpur Khas	Sukkur	Mirpur Khas
One	8.00±1.34i	10.60±1.21i	8.00±1.84i	38.00±3.78i	9.60±3.09i	31.00±3.61i	9.60±4.30i	7.00±2.43i
Two	18.20±6.48i	28.80±4.27i	21.40±10.45i	22.60±3.96i	8.40±6.16i	18.80±3.41i	38.40±15.18i	25.00±4.28i
Three	486.40±53.15ef	622.00±47.68c	605.40±63.83cd	731.00±50.64ab	246.80±72.08h	393.40±27.72fg	297.00±48.83gh	234.20±22.64h
Four	28.40±4.21i	48.80±6.30i	33.40±7.06i	46.20±2.99i	12.40±6.07i	26.00±2.92i	53.20±20.23i	29.60±3.50i
Five	513.20±75.13de	605.20±22.48cd	676.20±120.94bc	800.40±57.55a	736.80±57.51ab	784.40±36.50a	611.00±66.99cd	404.00±19.32f

*Means followed by the same letters are not significantly different from each other (LSD = 103.87, P < 0.05)

Table 5. Population fluctuation of dead *C. maculatus* strains (Sukkur & Mirpur Khas) on different pulses under lab conditions

Observation interval (Fortnight)	Chickpea		Cowpea		Green lentil		Yellow lentil	
	Sukkur	Mirpur Khas	Sukkur	Mirpur Khas	Sukkur	Mirpur Khas	Sukkur	Mirpur Khas
One	0.00±0.00n	0.80±0.37n	0.00±0.00n	1.20±0.49n	9.20±2.73mn	20.80±4.04l-n	8.20±3.58mn	8.00±1.14mn
Two	36.40±11.95j-n	46.40±7.50j-n	33.40±12.97j-n	46.20±3.90	21.80±7.72l-n	31.00±3.45j-n	12.80±5.61	10.60±1.36
Three	32.20±3.97j-n	62.20±5.91i-m	33.60±8.31j-n	112.20±5.94hi	34.40±7.68j-n	48.00±4.74j-n	32.80±6.74j-n	22.80±1.91k-n
Four	600.40±33.02c	703.20±21.92ab	653.40±38.47bc	749.00±16.58a	346.40±57.99e	424.00±38.63d	399.20±71.18de	215.40±20.97f
Five	77.20±9.20ijk	192.80±11.78fg	80.20±13.09ij	165.00±12.47f-h	82.40±14.54ij	145.00±15.39gh	70.20±8.95i-l	28.40±3.08

*Means followed by the same letters are not significantly different from each other (LSD = 55.130, P < 0.05)

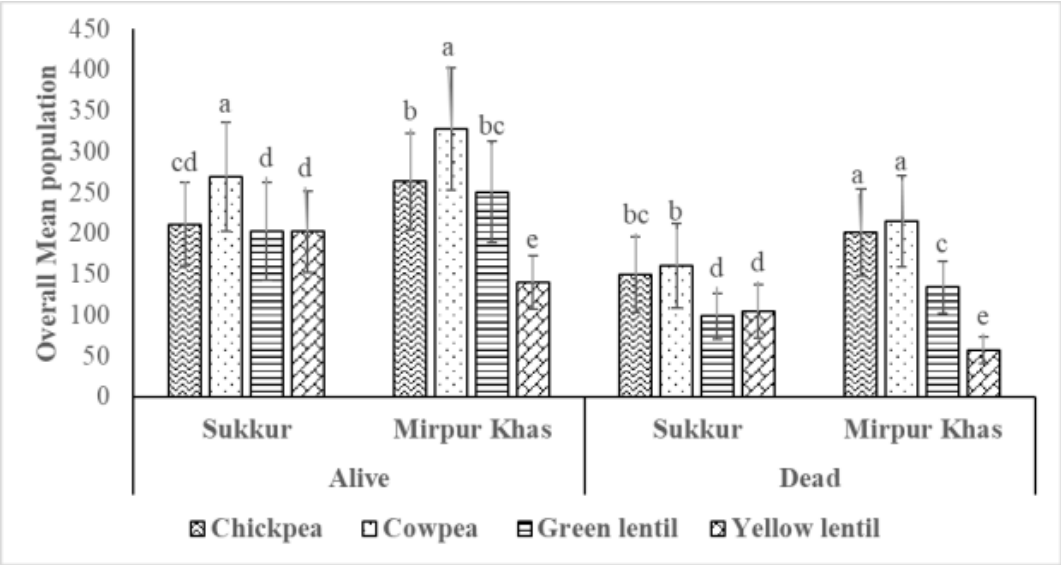

Fig. 2. Overall mean population of *C. maculatus* (alive and dead) on various pulses for Sukkur and Mirpur Khas strains

*Means followed by same letters individual for Alive (LSD = 46.451) and Dead (LSD = 24.655) are not significantly different from each other

(Table 5), a great variation was also observed in the number of dead beetles in different pulses with a highly significant difference (F= 4.39, P < 0.001). Accordingly, the highest number of dead *C. maculatus* (749.00±16.58 beetles) was observed from cowpea for the Mirpur Khas strain, followed by chickpea (703.20±21.92 beetles) and cowpea (653.40±38.47 beetles) for Mirpur Khas and Sukkur strains, respectively.

Overall, a highly significant difference (F = 14.88, P < 0.001) for the *C. maculatus* population was recorded in various pulses for both strains. According to the results, the highest overall mean population of beetles (214.72±55.85 beetles) was observed from cowpea for Mirpur Khas strain, followed by chickpea (201.08±53.10 beetles) and cowpea (160.12±51.23 beetles) for Mirpur Khas and Sukkur strains, respectively. Overall, the lowest population of dead *C. maculatus* (57.04±16.70 beetles) was recorded in green lentils for the Mirpur Khas strain and 98.84±28.06 beetles in the same pulse for the Sukkur strain.

3.11 Cumulative Mean population of *C. maculatus* Strains on Different Pulses

Figure 3 described the cumulative overall mean population of alive and dead *C. maculatus* of both strains on various pulses. According to the results, a significantly higher population of alive (F = 4.26, P = 0.0407) and dead (F = 14.10, P = 0.0002), *C. maculatus* on different pulses was recorded for Mirpur Khas strain i.e., 245.35±29.90 and 151.65±21.87 beetles, respectively than Sukkur strain having an overall cumulative mean population of alive (221.09±28.36) and dead (128.21±20.33) beetles.

3.12 Percentage Damage of Various Pulse Grains due to Feeding of *C. maculatus*

The percentage damage in the shape of weight loss of various pulses due to the feeding of

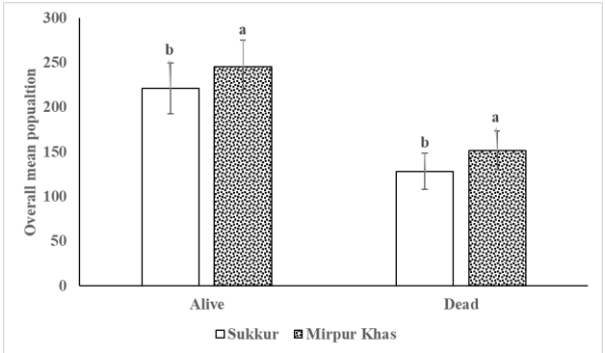

Fig. 3. Collective overall mean population of *C. maculatus* strains (alive and dead) on various pulses

*Means followed by the same letters individually for Alive (LSD =23.225) and Dead (LSD = 12.327) are not significantly different from each other

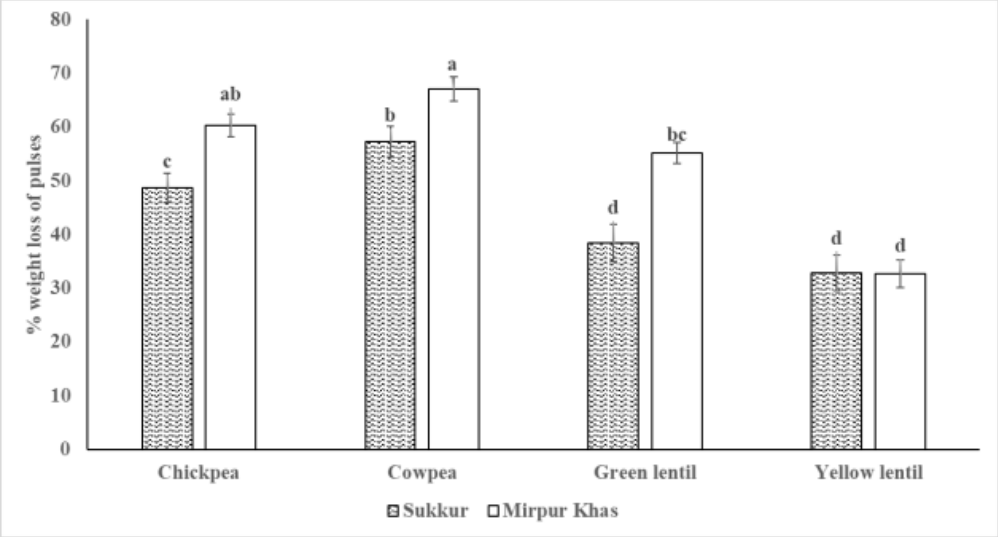

Fig. 4. Percentage damage of various pulse grains due to feeding of *Callosobruchus maculatus*
*Means followed by same letters are not significantly different from each other (LSD = 7.8296, P < 0.05)

C. maculatus is given in Figure 4 which confirmed a significant difference among various pulses (F = 3.39, P = 0.0298). Mirpur Khas strain of *C. maculatus* significantly damage more grains of cowpea (67.07±2.29 %), followed by chickpea (60.24±2.14 %), whereas the highest damage of Sukkur strain was also recorded in cowpea (57.20±2.87%), followed by chickpea (48.64±2.71%). The lowest percentage damage of both the strains was recorded in green lentils i.e., 32.82±3.35 and 32.60±2.56 % for Sukkur and Mirpur Khas strains, respectively.

4. DISCUSSION

Three pulse beetle species i.e., *C. maculatus*, *C. chinensis*, and *C. analis* were recorded from seven sampling locations in Sindh, Pakistan. Moreover, populations of these species were recorded only from chickpea, cowpea, and green lentil, whereas no beetle population was observed from green lentils and field peas. Among insect pests, *Callosobruchus* spp. have been reported as the primary pests of stored grains that cause damage by feeding inside the grains [17, 18]. Moreover, among *Callosobruchus* spp., *C. maculatus* is a more devastating and widely distributed pest of pulses in the tropical and subtropical areas of the world [19]. The results of this study support the findings of our study as at all the sampling locations, *C. maculatus* was the most abundant and widely distributed species on various stored pulses. Many other research studies also suggested that *C. maculatus* is a comparatively more abundant and destructive pest of pulses, where grubs are the most destructive stage that can consume entire contents of the grains in case of severe infestation [18]. The relatively higher and continuous infestation of *C. maculatus* to a wide variety of pulses has also been reported in various geographical regions of the world [10, 20, 21]. Similarly, the infestation of pulse beetles especially *C. maculatus* has not only been found in warehouses but their population is also recorded from the pulses in field conditions [22, 23].

Thus, results of survey and damage potential studies conducted also confirmed significant differences in the presence and damage percentage of *Callosobruchus* spp., especially *C. maculatus* on various pulses. Relatively more population and damage of *C. maculatus* was observed in the cowpea, followed by chickpea and green lentils than green lentils in this study. Such differences in population fluctuations and damage of *C. maculatus* on different pulses may be due to the physical texture, seed coat, and size along with nutrient contents of the seeds. It has been mentioned in previous studies that pulses with smooth seed coats and greater seed weight along with surface area are more preferred by the Callosobruchus spp. for feeding and oviposition [24, 25]. Tripathi et al. (2020) also found a significant impact of various

physical features i.e., colour, shape, testa texture, length, width, and seed hardness of 103 cowpea accessions on oviposition, damage, and percentage weight loss caused by *C. maculatus* [26]. The comparative preference study of *C. chinensis* on lentil, black gram, mungbean, and chickpea showed that it prefers to lay a relatively higher number of eggs on chickpea, whereas black grams were least preferred for oviposition. Accordingly, chickpea and black gram suffered the highest and lowest weight loss due to feeding of the beetle, whereas mungbean and lentil were moderately affected pulses [27, 28]. Due to variable preference of *C. maculatus* on different pulses, they also affect its growth and reproductive parameters as the latest studies by Bidar et al. (2021) found that among chickpea, cowpea, lentil, and green gram, lentil supported the lowest reproduction parameters and higher developmental time, whereas chickpea was the more preferred pulse to support growth and reproductive parameters of *C. maculatus* [29].

In our study, *C. maculatus* showed more feeding preference for cowpea, followed by chickpea, whereas green lentil was the least preferred, hence most of the above-mentioned studies partially supported our findings as most of them found chickpea as the most susceptible pulse against *C. maculatus*.

5. CONCLUSION

Three species of pulse beetles i.e., *Callosobruchus C. maculatus*, C. *chinensis*, and C. *analis* were collected and identified from all sampling locations of Sindh, Pakistan with *C. maculatus* being more abundant and widely distributed. Among sampled pulses, the population of *Callosobruchus* spp. was recorded from green lentil, cowpea, and chickpea, whereas no population was recorded from green lentil and field pea. Overall, the maximum pulse beetle population was recorded on green lentils, with Mirpur Khas and Sukkur districts being the most affected. The feeding preference of Mirpur Khas and Sukkur strains of *C. maculatus* also exhibited significant variation in their feeding towards various pulses with cowpea being the most preferred, followed by chickpea, whereas green lentil was the least preferred, accordingly, suffered the highest and lowest damage and weight loss. Therefore, it is suggested that proper storage and quarantine measures should be taken in the transportation and storage of pulses to restrict the spread and damage of pulse beetles from more susceptible areas and pulses to new areas and relatively resistant pulses.

6. CONFLICT OF INTEREST

The authors declare no conflict of Interest.

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