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Screening of legumes against pulse beetle [*Callosobruchus chinensis* (L.), Coleoptera: Bruchidae] under storage condition in Chitwan, Nepal

Janak Pant[1+]
Bhuwan Poudel[2]
Dinesh Phuyal[3]
Prem Pandey[4]
Richa Devkota[5]
Subash Bishwakarma[6]
Suman Khanal[7]

[1,2,4,5,6,7]*Institute of Agriculture and Animal Science, Chitwan, Nepal.*
[1]*Email:* Janakpant500@gmail.com
[2]*Email:* agribhuwan.notes@gmail.com
[4]*Email:* pandeyprem73@gmail.com
[5]*Email:* devkotaricha83@gmail.com
[6]*Email:* agbksubash@gmail.com
[7]*Email:* sumankhanalshow@gmail.com
[3]*Agriculture and Forestry University, Chitwan, Nepal.*
[3]*Email:* phuyaldines@tamu.edu

(+ Corresponding author)

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ABSTRACT

The study was carried out to find out the host preference of pulse beetles against different legumes at the entomology laboratory. It is found that the major cause of heavy loss of grain legumes in the storage condition is the pulse beetles (*Callosobruchus chinensis* L.). Farmers grow large scale of legumes, however, due to the pest especially *Callosobruchus chinensis*, the productivity of grain legumes is low, so the study focused on finding the susceptible and resistant legumes. Study carried out at the entomology laboratory of Institute of Agriculture and Animal Sciences, Rampur, Chitwan. The experiment was carried out in Completely Randomized Design (CRD) with three replications. Seven different legumes were used as treatment in the experiment. After 20 days of treatment chickpea seems most susceptible legumes than other as the average number of beetles were highest in chickpea (35.666) and least number of beetles was found in kidney bean (3.333) which denotes the most resistant legumes against pulse beetle after 20 days after treatment. Later, the average number of pulse beetles increased rapidly in lentils till last recording and the pulse beetle number in kidney beans seems to be null. After 100 DAT (Days After Treatment), the total number of damaged grains per 100 seeds is found maximum in Pea (10.68) and Cowpea (10.68) minimum in Soybean (0.00) and Kidney bean (0.00). Based on host preferences lentil was best host and was most susceptible to pulse beetle and kidney bean as least susceptible to the pulse beetle.

Contribution/Originality: The study focuses on numerous legumes together to identify their resistance level and infestation by the grain pest. The level of pest resistance was different in varying legumes. Research carried for a long period in the laboratory with the application of treatments and reflects the practical challenges of farmers at field level.

1. INTRODUCTION

Grain legumes are the most important type of food after cereals in world, especially southeast Asia. Legumes are ideal for various cropping system, human nutrition, crop diversification, soil nitrogen management, intercropping and adaptability to marginal land. Pulses are a major part of the dietary protein as they are cheaper to animal protein source. Various types of leguminous crop are cultivated throughout the country. They have been playing a significant role in Nepalese Agriculture for meeting the dietary protein need s of the people. Besides these crop residue and byproducts such as fodder, feeds and firewood are valuable [1].

In the world, grain legumes are grown in 69.29 million ha with production of 64 million tons and productivity of 924 kg per ha during 2009 [2]. India is the largest grower with 30 % share in area and 23% share in production. In Nepal grain legumes collectively rank 4th place in terms of area and production after rice, maize, and wheat. They cover 10% of total cultivated area and grown in 319, 472 ha. With production and productivity of 262, 357 ton and 821 kg per ha respectively [2].

Nepal contributes about 0.4% of world pulse area and production. In recent year, the area and productivity has been slowly increasing, which increase to 334323 ha of crop land with production of 319769.8 ton and productivity of 956 kg per ha [3]. In Nepal per capita consumption of pulses is around 10 kg / capita / annum, which is very low compared to the standard of Food and Agriculture Organization (FAO) recommendation of 18 kg / capita / annum (or 50 gm./ capita /day) [4]. Legume is grown in the Terai, Inner Terai, mild hill, high hill after rice and maize. It is traditionally grown either as sole crop or as mixed crop with mustard wheat and / or linseed on marginal land with poor, management [5].

Lower production and productivity of grain legumes is due to the various biotic and abiotic constraints [6, 7]. Among many productions constraints insect pest ranked third based on losses and the storage losses is estimated to be 9% in developed country and 20% or more in developing county [8]. Many species of insect are found in store grains but only few causes damage and loss. The pulse beetles (*Callosobruchus spp*) (Coleoptera: Curculionidae) is a major economically important pest of grain legumes [9, 10]. In Nepal, it was reported that 10.12 % of the total production of pulses destroyed during post-harvest operation [11]. Annual grain loss in storage due to insect is about 15% [12].

For the management of this insect pest various physical, mechanical, and chemical methods have been practice globally. In Nepal, the farmers intensively use chemical pesticides to control pulse beetle in grain legumes [13]. The most used pesticides in storage are Aluminum Phosphide [14]. Nearly 1500 pesticide poisoning related death annually in the county over the last five years [15]. Majority of cases are due to intentional poisoning and a significant number of accidental poisonings is with the use of storage pesticides [16]. Chemical method of insect pest control has several problems, including health hazard to the farmers as well as grain consumers. Therefore, plant product with insecticidal properties can be best alternatives to the chemical pesticides that are extensively being used in developing countries [17, 18]. Present study was conducted to determine pest intensity and their loss in storage pest (*Callosobrochus chinensis*).

1.1. Objectives

1.1.1. Broad Objectives

- Documentation of pest intensity and losses due to storage pest (*Callosobrochus chinensis)* in storage.

1.1.2. Specific Objectives

- To identify the most economical storage pest in legumes.
- To evaluate the intensity of infestation of pulse beetle in different legumes.

2. MATERIALS AND METHODS

The research was conducted in entomology laboratory of Institute of Agriculture and Animal science (IAAS, Rampur Campus, Rampur Chitwan) from September 2015 to January 2016.

2.1. Maintenance of Insect Culture

Insect specimen of *Callosobruchus chinensis L.* was obtained from Rampur Gurung community for the maintenance of insect culture, mass rearing of *Callosobruchus chinensis L.* was performed at room temperature for one generation on bulk of chickpea variety.

2.2. The Seed

The different legumes seeds (lentil, chickpea, cowpea, pea, black gram, kidney bean and soybean) were obtained from nearby shop.

2.3. Major Tools and Equipment

Aluminium sheet bin, Binocular Microscope, magnifying hand lens, muslin cloth, rubber band, aspirators, sieves, net, etc. were the major tools and equipment used during the experiment.

2.4. Experiment on Host Preference of Callosobruchus Chinensis L.

Seven different types of legumes were tested for the host preference of insect. All the legumes were procured from shop before experiment each grain legumes were washed thoroughly with tap water and shade dried, to maintain moisture level of seed. Seeds were made free from dirt, dust, chemicals etc. After that the study was replicated three times with seven treatments. 200gm seed of each legume was kept separately in clean aluminum sheet bin (23cm height and 12.5cm diameter). Adult species of *Callosobruchus chinensis* were taken from the mass culture using aspirator in the glass Petri dish (9cm diameter) and five pairs of pulse beetle was released in the center of bin. Then the mouth of seed bin was capped with fine meshes cotton cloth fastened with rope tightly. Thereafter the experiment was left for observation. Data was recorded in each twenty days intervals.

The experiment was designed on completely Randomized Design (CRD) and consist of seven treatments with single factor with three replications (Table 1).

Table 1. Legumes included in host preference study with moisture content measured by moisture meter.

Treatments	Legumes	Moisture content at experimentation
Trt1	Lentil	12%
Trt2	Pea	13%
Trt3	Cowpea	11%
Trt4	Soybean	10%
Trt5	Chickpea	13.5%
Trt6	Black gram	12%
Trt7	Kidney bean	9%

Note: TRT: Treatment, %: Percentage.

2.5. Design of Experiment

The experiment was design on completely randomized design (CRD), which consists of seven treatments with three replications. The aluminum metal bins were selected as storage structure and cowpea, lentil, chickpea, black gram, soybean, pea, and kidney bean were seven treatments. Thus, there were altogether 21 aluminum bins used and each metal bin was an experimental unit containing 200 gm of each legumes seed (Table 2).

Table 2. Description of treatment and quantity used.

Treatment	Quantity used
T_1 (Lentil)	200g per aluminum sheet bin
T_2 (Pea)	200g per aluminum sheet bin
T3 (Cowpea)	200g per aluminum sheet bin
T_4 (Soybean)	200g per aluminum sheet bin
T_5 (Chickpea)	200g per aluminum sheet bin
T_6 (Black gram)	200g per aluminum sheet bin
T_7 (Kidney bean)	200g per aluminum sheet bin

Note: G: Gram.

2.6. Layout of the Design

Table 3 presents that the layout of the research consists of seven treatments (i.e., lentil, pea, cowpea, soybean, chickpea, black gram, kidney bean), and were replicated 3 times. Each treatment were replicated for three times.

Table 3. Layout of design.

T1R2	T2R3	T1R1
T6R3	T5R2	T3R2
T4R1	T7R3	T2R1
T6R1	T3R1	T7R2
T2R2	T4R3	T7R1
T5R3	T3R3	T5R1
T6R2	T1R3	T4R2

Note: T: Treatment used in the experiment, R: Replication number.

2.7. Observation and Data Recording

The total number of live adult insect after treatment setup was recorded at 20 days after treatment (DAT). At the same time, number of dead insects, number of holes per 100 seed from each experimental unit were also recorded. 100 seeds were randomly selected from each experimental unit. Dead insects counted at each observation were thrown out and live insects were kept in respective metal bins.

2.8. Weather Records

Maximum and minimum temperature, rainfall and humidity were also recorded throughout the experimental period and the daily weather was recorded and presented in Appendix.

2.9. Data Management and Analysis

The recorded data was arranged and analyzed by using M. S. Excel 2013 and R studio.

Table 4. Total no of insects in different legumes at every 20 days interval in lab condition at Rampur, Chitwan, Nepal.

S.no.	Treatment	20DAT	40DAT	60DAT	80DAT	100DAT
1.	Lentil	26.000 ^{bc}	106.00^{a}	167.000^{a}	252.666^{a}	958.333^{a}
2.	Pea	15.000 ^{cd}	52.666 ^{bc}	103.000^{b}	37.333^{b}	73.666^{b}
3.	Cowpea	19.000^{cd}	62.666 ^{ab}	28.333^{c}	24.000^{b}	100.000^{b}
4.	Soybean	16.666^{cd}	12.666 ^{bc}	1.333^{c}	1.333^{b}	0.000^{b}
5.	Chickpea	44.333^{a}	48.333 ^{bc}	82.333^{b}	70.000^{b}	33.333^{b}
6.	Black gram	36.666^{ab}	17.333^{bc}	14.333^{c}	3.666 ^{b}	0.333^{b}
7.	Kidney bean	10.000^{d}	0.000^{c}	0.666^{c}	0.000^{b}	0.000^{b}
8.	Grand mean	23.952	42.809	56.714	55.571	166.523
9.	C.V.	29.008	65.049	40.456	82.058	55.950
10.	LSD	12.360	49.540	40.817	81.124	165.749
11.	P. value	0.0005 ***	0.008**	7.04e-06 ***	0.000198 ***	2.09e-07 ***

Note: a, b, c, d means in a column having same letter(s) do not differ significantly at 5% probability by DMRT, DAT: Days after treatment, C.V: Coefficient of variation, LSD: Least significant difference, P. value: Probability value., *** means they are statistically different at probability values of P 0.001, respectively.

3. RESULTS

3.1. Host Preference of Callsobruchus Chinensis L. to Different Legumes

After 20 days of treatment, the number of pulse beetle is statistically higher in chickpea (44.33) followed by Black gram (36.66), Lentil (26.00), Cowpea (19.00) at par with Soybean (16.66), Pea (15.00) and lowest number of pulse beetle is found in Kidney bean (10.00).

After 40 days of treatment, the number of pulse beetle is statistically higher in Lentil (106.00) followed by cowpea (62.66) at par with pea (52.66), chickpea (48.33), black gram (17.33) and lowest number of pulse beetle is found in kidney bean (0.00).

After 60 days of treatment the number of pulse beetle is significantly higher in lentil (167.00) followed by pea (103.00) as par with chickpea (82.333) followed by cowpea (28.333) which is as par with black gram (14.333), soybean (1.333) and least number of pulse beetle is reported in kidney bean (0.666) (Figure 1).

After of 80 days of treatment, the number of pulse beetle is highest in Lentil (252.666), followed by chickpea (70.000) which is as par with pea (37.333), cowpea (27.000), black gram (3.666), soybean (1.333) and least number of pulse beetle is found in kidney bean (0.000).

After 100 days of treatment, the number of pulse beetle is statistically higher in Lentil (958.333) followed by pea (100.000), cowpea (73.666) as par with chickpea (3.333), black gram (0.333), soybean (0.000) and kidney bean (0.000) (Table 4).

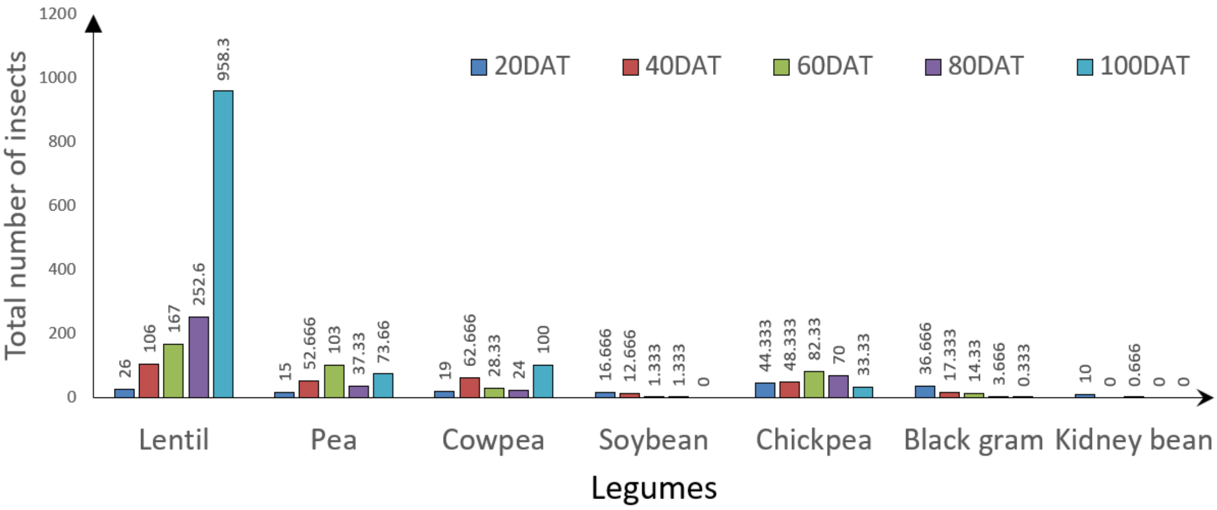

Figure 1. Total no of insects after every 20 days interval in lab condition.

Table 5. Total number of live insects at different legumes in every 20 days interval in lab condition at Rampur, Chitwan, Nepal.

S.no.	Treatment	20DAT	40DAT	60DAT	80DAT	100DAT
1.	Lentil	23.666 ^{bc}	69.000 ^{a}	71.333 ^{a}	217.333 ^{a}	742.000 ^{a}
2.	Pea	13.333 ^{cd}	42.666 ^{abc}	14.666 ^{bc}	24.666 ^{b}	58.666 ^{b}
3.	Cowpea	16.333 ^{bc}	49.000 ^{ab}	6.666 ^{c}	20.000 ^{b}	92.000 ^{b}
4.	Soybean	12.666 ^{cd}	0.000 ^{c}	0.000 ^{c}	0.000 ^{b}	0.000 ^{b}
5.	Chickpea	35.666 ^{a}	36.000 ^{abc}	68.666 ^{ab}	32.333 ^{b}	7.666 ^{b}
6.	Black gram	27.000 ^{ab}	4.666 ^{bc}	0.666 ^{c}	0.333 ^{b}	0.000 ^{b}
7.	Kidney bean	3.333 ^{d}	0.000 ^{c}	0.000	0.000 ^{b}	0.000 ^{b}
8.	Grand mean	18.857	28.761	23.142	42.095	128.619
9.	C.V.	31.212	82.765	131.486	106.198	58.357
10.	LSD	10.470	42.348	54.134	79.528	133.529
11.	P. value	0.001 ‘**’	0.020 *	0.0335 *	0.000643 ***	3.16e-07 ***

Note: a, b, c, d means in a column having same letter(s) do not differ significantly at 5% probability by DMRT, DAT: Days after treatment, C.V: Coefficient of variation, LSD: Least significant difference, P. value: Probability value, *, **, and *** means they are statistically different at probability values of $P \leq 0.05$, ≤ 0.01 and ≤ 0.001, respectively.

After 20 days of treatment, the number of live pulse beetle is statistically higher in chickpea (35.666) followed by black gram (27.000), Lentil (23.666) as par with cowpea (18.333), pea (13.333, soybean (12.666) and least number of pulse beetle is found in kidney bean (3.333).

After 40 days of treatment, the number of live pulse beetle is statistically higher in Lentil (69.00) followed by cowpea (49.00), pea (42.666), chickpea (36.000), black gram (4.666) and least number of pulse beetle is found in soybean (0.000) and kidney bean (0.000). After 60 days of treatment, the number of live pulse beetle is found higher in lentil (71.333) followed by chickpea (68.666), pea (14.666), cowpea (6.666), black gram (0.666) and least number of pulse beetle is found in soybean (0.000) and kidney bean (0.000) (Figure 2).

After 80 days of treatment, the number of live pulse beetle is found higher in Lentil (217.333) followed by chickpea (32.333), pea (24.666), cowpea (20.000), black gram (0.333) and least number of live pulse beetle is found in soybean (0.000) and kidney bean (0.000).

After 100 days of treatment, the number of live pulse beetle is found higher in Lentil (742.000) followed by cowpea (92.00), pea (58.666), chickpea (7.666) and least number of live pulse beetle is reported in soybean (0.000), black gram (0.000) and kidney bean (0.000) (Table 5).

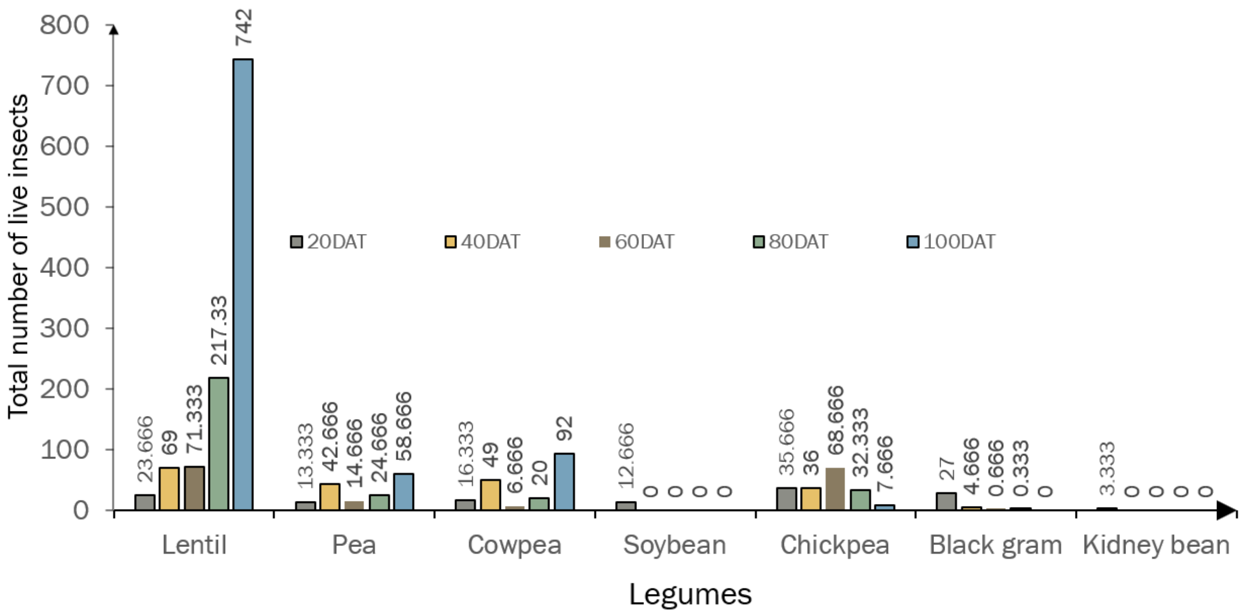

Figure 2. Total no of live insects after every 20 days interval in lab condition.

Table 6. Total no of holes in 100 seeds of the legumes.

S.no.	Treatment	20DAT	40DAT	60DAT	80DAT	100DAT
1.	Lentil	1.666 ab	4.333 ab	4.333 bc	3.666 b	7.3333 ab
2.	Pea	1.333 ab	7.667a	8.666 ab	11.333 a	10.667 a
3.	Cowpea	2.000 a	2.333 bc	4.000 bc	5.666 ab	10.667 a
4.	Soybean	0.666ab	0.000 c	0.000 c	0.000 b	0.000 b
5.	Chickpea	0.333 b	2.333 bc	14.333 a	12.666 a	10.333 a
6.	Black gram	1.333 ab	1.000 bc	0.666 c	0.000 b	0.333 b
7.	Kidney bean	1.000 ab	0.333 bc	4.000 bc	0.000 b	0.000 b
8.	Grand mean	1.1904	2.571	5.142	4.761	5.6190
9.	C.V.	70.199	83.508	62.888	79.372	79.841
10.	LSD	1.486	3.820	5.753	6.723	7.981
11.	P. value	0.288	0.0111 *	0.002 **	0.003 **	0.017 *

Note: a, b, c, d means in a column having same letter(s) do not differ significantly at 5% probability by DMRT, DAT: Days after treatment, C.V: Coefficient of variation, LSD: Least significant difference, P. value: Probability value, * and ** means they are statistically different at probability values of $P \leq 0.05$ and ≤ 0.01 respectively.

3.2. Total Number of Holes In 100 Seeds of Different Legumes at 20 Days Interval

By observing the data above, it is found that after 20 days of treatment the maximum average no of holes in 100 seeds is found in cowpea (2 seeds with holes) at par with lentil (1.66) followed by pea (1.33), black gram (1.33), kidney bean (1.00) and least number of holes in 100 seeds is found in chickpea.

Similarly, after 40 DAT the maximum average number of holes are observed in pea (7.667) at par with lentil (4.33) and followed by cowpea (2.33), chickpea (2.33), black gram (1.00), kidney bean (0.333) and least number of the holes are found in soybean (0).

After 60 DAT the maximum average number of the holes are found in chickpea (14.333) at par with pea (8.666) followed by lentil (4.333), cowpea (4), kidney bean (4) and the least number of holes are found in black gram (0.666), and soybean (0).

After 80 DAT the average no of holes in chickpea is found maximum (12.666), at par with pea 11.333) followed by cowpea (5.666), lentil (3.666) and least number of average holes is found in black gram (0), soybean (0) and kidney bean (0) (Figure 3).

Finally, after 100 DAT the average number of the holes in 100 seeds is maximum in pea (10.667) and cowpea (10.667) at par with chickpea (10.333) and followed by lentil (7.333), black gram (0.333) and average number of holes in 100 seeds is found least in soybean (0) and kidney bean (0) (Table 6).

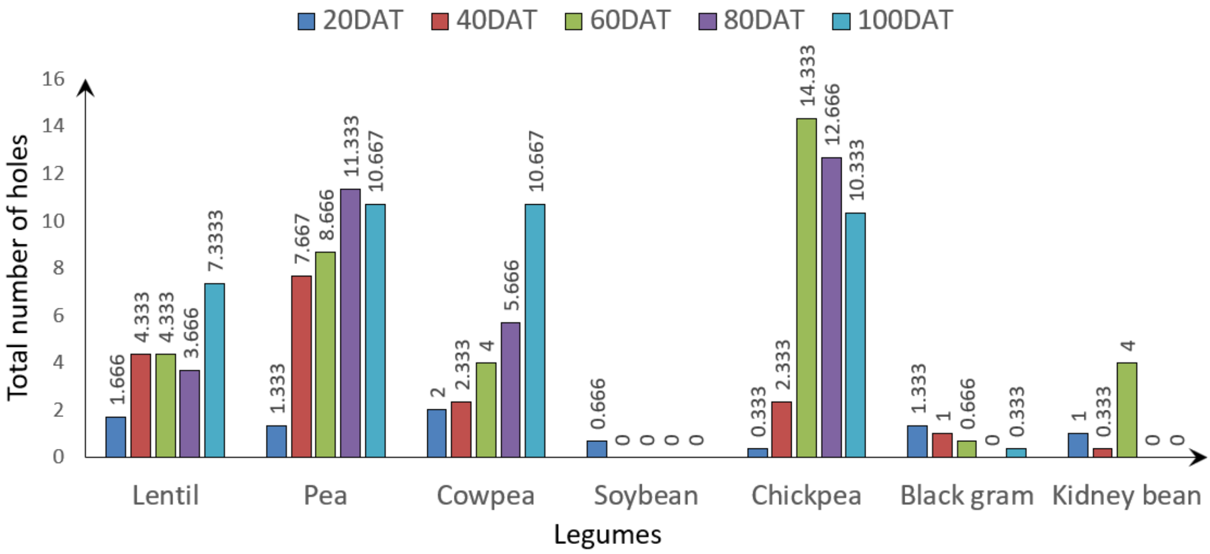

Figure 3. Total number of damaged grains by pulse beetle in 20 days interval in lab condition.

4. DISCUSSIONS

The growth and development of the pulse beetle in different seeds of the legumes depends on the quality, compactness, and size of the seed. Similarly, the factors influencing on oviposition, which consequently delays pulse beetle development [19, 20]. The pulse beetle prefers chickpea, but it was also reported that it feeds several other legumes named lentil (*lens esculenta*), cowpea (*Vigna ungulata*), blackgram (*phaseolus mungo. L.*), soybean (*Glycine max Merr*), kidney bean (*Phaseolus vulgaris L.*) and garden pea (*Pisium sativum*) [21]. Similarly in a study of host preference of *C. chinensis* they found that lentil was the best host substrate followed by gram, pea and cowpea for oviposition and adult emergence. Present study revealed that the response of different legumes against pulse beetle and might be its ovipositional behavior of beetle on legumes. As in our experiment the total number of pulse beetle were recorded maximum in lentil [21].

Although the total number of insects were found maximum in lentil but the number of damaged grains in lentil were comparatively lower than the other legumes because most of the small, seeded legumes are less preferred than the large, seeded legumes by pulse beetle for their oviposition. It was also reported by Pandey, et al. [22]. The maximum number of insects were recorded in lentil followed by chickpea, pea, cowpea, black gram, soybean, and least number of beetles were recorded in kidney bean. Significantly very few numbers of beetle were recorded in kidney bean and soybean due to the poor adult emergence in those legumes whose seed coat is hard and thick.

5. CONCLUSION

The research was conducted to determine potentiality of pest and losses due to striae pest. After 20 days of treatment chickpea seems most susceptible legumes than other as the average number of beetles is highest in chickpea (35.666) and least number of beetles was found in kidney bean (3.333) which denotes the most resistant legumes against pulse beetle after 20 days after treatment. Later, the average number of pulse beetles increases rapidly in lentils till last recording and the pulse beetle number in kidney beans seems to be null. Overall experiment signifies that based on host preferences lentil was best host and is most susceptible to pulse beetle and kidney bean was least susceptible to the pulse beetle.

6. RECOMMENDATION

Farmers are recommended to pay intense attention while storing lentils than other legumes. The findings also proved that other legumes are also highly infested by pulse beetles so special care should be given while storing grains and legumes.

Researchers who are intended to do research in storage pests, especially in pulse beetles are recommended to find the most suitable storing structure for storing grains and legumes, not only this they should recommend the proper management of the pulse beetle.

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Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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