Chapter 1: Rearing and Host Preference of *Callosobruchus chinensis* (Coleoptera: Bruchidae) in the laboratory conditions

1.1 Introduction

The agricultural industry holds significant importance within the Indian economy, with a contribution of approximately 14% to the country's Gross Domestic Product (GDP) and approximately 11% to its overall export value (Jha et al., 2015; Wagh and Dongre, 2016). It is the primary source of livelihood for almost 2/3rdof India’s population (Manohar, 2016; Dolli and Divya, 2020). India accounts for around 29% of the global area and 19% of the global production of pulses, making it the leading pulses producer and consumer (Shukla and Mishra, 2020; Palai et al., 2019; Bhat et al., 2022). India where a large population follows a vegetarian diet, pulses are the major source of protein for them and that is the reason that they are referred as "poor man's meat" and "rich man's vegetable," (Singh et al., 2015; Tripathi et al., 2019). Pulses play a significant role in the human diet, offering a wide range of nutritional and physiological benefits that positively impact human health (Venkidasamy et al., 2019; Ferreira et al., 2021).

Pulses possess a notable nutritional composition, containing significant amounts of protein, carbs, and dietary fibre. Moreover, they serve as a valuable reservoir of many bioactive constituents. Consequently, the consumption of pulses is prevalent on a global scale (Maphosa and Jideani, 2017). Pulses are a category of legumes that encompass a diverse range of dried seeds, including several types of beans, lentils, peas, green gram, black gram, horse gram, and chickpeas. Pulses, which are rich in protein, are often regarded as a fundamental component of the Indian diet, serving as a primary source of both protein and energy for a significant proportion of the population (Venkidasamy et al., 2019; Hussain et al., 2021). Pulses serve as the primary and economically accessible source of dietary protein in India, a country characterised by a substantial population facing poverty (Minocha et al., 2017; Chakrabarti et al., 2018). Pulses hold a significant role in Indian diets as an affordable and animal-free protein source, with India now holding the top position globally as the producer, user, and importer of pulses (Dizon and Herforth, 2018; Abraham and Pingali, 2021; Singh et al., 2022).

Over the course of recent decades, India has experienced a notable escalation in per capita earnings. This growth may be attributed to the expanding economic prospects stemming from a thriving service sector and enhancements in rural wages (Siddiqui, 2016; Pingali et al., 2019). The combination of demographic change, including population growth, increased life expectancy, and more female workforce involvement, has resulted in a rising need for a variety of dietary categories. (Nagarajan et al., 2016; Agarwal et al., 2020). In the face of dynamic dietary patterns, evolving food preferences, advancements in food processing technologies, shifting cultural norms, and heightened awareness among individuals, there exists a significant obstacle in providing nourishing food options, specifically pulses, to meet the needs of a growing population (Kumar et al., 2014; Changan et al., 2017; Langyan et al., 2022a). The primary inquiry pertains to the means by which the forthcoming years' want for food, which offers a nourishing and wholesome dietary composition, can be fulfilled without jeopardising the finite resources of our planet (Langyan et al., 2022b).

Over the course of the last 25 years, there has been a notable rise in the global population, amounting to an increase of two billion individuals. Projections indicate that this trend will persist, with estimates suggesting that the world's population would reach around 8.5 billion by the year 2030, and further expand to 9.8 billion by 2050, as reported by the United Nations World Population Division in 2017. One of the key components in meeting demand is the increase of pulse production; nevertheless, the task of effectively distributing it to a growing population is a significant problem (Haddad et al., 2016). Food loss and waste is a prevalent phenomenon observed on a global scale. The occurrence of food loss and wastage is more prevalent in the latter stages of the food chain in high-income nations, whereas in low-income countries, a majority of the loss occurs in the earlier stages (FAO, 2013; Dou et al., 2016; Cattaneo et al., 2021; Mokrane et al., 2023). The rates of loss incurred during the various stages of harvesting, threshing, and storage at both farm and processing plants are significantly elevated. The primary factors contributing to the losses include unsuitable and delayed harvesting practises, as well as inadequate post-harvest management techniques (Tibagonzeka et al., 2018; Vishwakarma et al., 2020). The primary cause of significant losses in agricultural activities is attributed to the utilisation of inadequate machinery, particularly during the processes of threshing and harvesting. Additionally, inadequate storage methods employed in families, farms, and warehouses also have a role in the occurrence of these losses (Tibagonzeka et al., 2018).

At the storage level, pulses are vulnerable to degradation due to a range of microbial and abiotic causes. Collectively, these factors contribute to a reduction of 25% of food grain production. The factors encompassing high temperature, wetness, microbes, mites, insects, and rodents are considered to be influential in this context (Singh, 2018; Mundhada et al., 2022; Sharma et al., 2023). The regions experiencing the greatest losses are those characterised by tropical and subtropical climates, as these conditions are particularly favourable for the rapid proliferation and production of organisms that cause harm to food supplies. One example of such species is storage insect pests, which are responsible for approximately 30% of annual losses (Abass et al., 2014; Adu et al., 2014; Kumar and Kalita, 2017; Mohapatra et al., 2017; Delouche et al., 2021).

The economically significant post-harvest insect pests are divided into two main groups: Coleoptera (beetles) and Lepidoptera (moths and butterflies) (Yahia et al., 2019; Demis and Yenewa, 2022; Suleiman and Rosentrater, 2022). Several Coleopteran and Lepidopteran species attack crops both in the field and in store. Crop damage by Lepidoptera is only done by the larvae. However, In Coleopterans, both larvae and adults feed on the crop and these two stages are responsible for the damage (Wielkopolan and Obrępalska-Stęplowska, 2016; Ahmad et al., 2021). Few Coleopteran family which are serious stored grains pest includes Bruchidae, Bostrichidae, Tenebrionidae, Cucujidae, Dermestidae, etc (Kumar, 2017; Banga et al., 2020; Singh et al., 2021).

C. chinensis from family Bruchidae originated in tropical Asia, but is currently distributed all over the tropics and sub-tropics and has become a serious pest of pulses (Kumar, 2017; Ahmad et al., 2021). The tiny, white, legless pest with biting jaws matures inside the grains and emerges from the grain seeds through an emergence window as a fully mature adult (Millsap, 2017). *C. chinensis* is cosmopolitan in nature and a serious pest of green gram, red gram, black gram, cowpea, and chickpea, it has been reported that a loss of 32-64% cow pea is due to *C. chinensis* (Duan et al, 2014;

Jaiswal et al., 2019). Further, Jaiswal et al., (2018) has reported that around 60% weight loss of the pulses by *C. chinensis* and have opined that the seeds became unfit for human consumption and as well as planting.

To combat the menace caused by *C. chinensis* various chemical and biological control measures have been reported (Liu et al., 2019; Dent and Binks, 2020; Takla et al., 2021; Pipariya et al., 2022), however, overuse of pesticides leads to resistance and increases insect pest survival rates (Daglish et al., 2014; Dara, 2017; Fang et al., 2019; Kortbeek et al., 2019). Insects have successfully adapted to most insecticides by becoming physiologically or behaviourally resistant (Nansen et al., 2016; Jallow et al., 2017; Dara, 2017; Ningombam et al., 2017; Onstad et al., 2023). In past few years insecticide resistance has been reported in 504 species of insects (Naqqash et al., 2016; Du et al., 2020), and there is still a steady increase in resistance to specific chemicals, with many species now resistant to several families of molecules like DDT, malathion, pirimiphos-methyl, deltamethrin and permethrin (Karaağaç, 2012; Dara et al., 2013 and 2016; Zhu et al., 2016; Kortbeek et al., 2019).

For scientists to address these challenges, it is imperative to design insecticides that specifically target insects. In order to assess the effectiveness of insect-specific insecticide mass rearing and the use of purebred pests, it is essential to conduct controlled experiments under controlled conditions. This is necessary to minimise random variations and obtain reliable results. Without a comprehensive understanding of the life cycle dynamics of insects, it is not possible to achieve significant progress in pest monitoring and management (Ribeiro et al., 2018; Arai et al., 2022). A tendency for a certain host is a crucial characteristic in the life cycle of every organism. According to Thakkar and Parikh (2018), several organisms not only consume the host grain but also inhabit it as a living environment. This is certainly the case for *C. chinensis* where, the adults meet members of the opposite sex and mate, females oviposit and a larva then feeds. The entire life cycle is therefore dependent on the suitability of the host. Many scientists have delved into the life cycle of *C. chinensis* (Hosamani et al., 2018; Jaiswal et al., 2019; Kumari et al., 2020; Herald et al., 2022), and host preference and host suitability in *C. chinensis* has been explored on chick pea (Chandel and Bhaudaria, 2015; Rana et al., 2020), Green gram (Devi and Devi, 2014; Gopi and Singh, 2020), Black gram (Dalal et al., 2020), Cowpea

(Augustine et al., 2018), moth bean (Meghwal and Singh, 2005) multiple hosts (Patel et al., 2005; Hosamani et al., 2018; Jaiswal et al., 2019; Mehta and Negi, 2020).

Athanassiou et al., (2017) conducted research that demonstrated the occurrence of competition for host preference among coexisting species under controlled laboratory circumstances. Prior research has also documented that the selection of hosts is influenced by factors such as seed size, germ layer composition, moisture content, and nutritional value (Ojo and Omoloye, 2016; Akhter et al., 2017). Based on the existing literature survey, it is evident that there is a substantial amount of information available on the biology and ecology of *Callosobruchus*, approached from various perspectives. However, in the context of the current investigation, the primary emphasis was placed on the culture of *C. chinensis* for the purpose of studying the molecular mechanisms underlying the development of insecticide resistance in this species. So, to have an abundant number of adult *C. chinensis* the host preference was investigated for seven stored pulses (green gram, moth beans, cow pea, black gram, chick pea, pigeon pea, pea) under laboratory conditions.

Hence, the present study was undertaken to investigate a suitable rearing protocol, life cycle as well as screening the preference of different stored pulses by C. chinensis in control laboratory conditions.

1.2 Materials and Methods

Collection of Insect pest

Infested grains were collected from the different granaries of Vadodara. Insect pests were separated from the grains. On keen observation, there were few major insect pests like *Tribolium castaneum, Sitophilus Oryzae, Trogoderma granarium, Callosobruchus maculatus* etc found. Among these pests, *C. chinensis* were found on wide range of host grains and was reported as dominant and hence, *C. chinensis* were selected as insect model for the further study. Morphological identification of *C. chinensis* was done by Raina, 1970.

Animal maintenance

Pulse Beetle, *C. chinensis* host preference on different grains was studied under laboratory conditions at the Zoology Department, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat. After confirming the identification, *C. chinensis* were reared in laboratory conditions for at least three months before starting the final experiment, this was the stock culture. the stock, culture was reared on chick pea sees in plastic jars covered with mesh lids. The cultures were kept under 26-28°C and 60-70% RH, and 12-hour photo period (Thakkar and Parikh 2018). In this way a fresh culture was developed in laboratory and this fresh culture and was used in all further experiments.

Figure: 1.1: Collection and Maintenance of *C. chinensis*

Experimental regime

The grains for the final experiments were purchased from the local market of Vadodara and disinfested by fumigation. The host preference for development by *C. chinensis* on different grains *viz*. green gram, moth bean, chickpea, cowpea, pigeon pea, black gram, pea and kidney beans was carried out under laboratory conditions. 10 pairs of freshly emerged (1-2 days old) *C. chinensis* were released in plastic jars containing 50g each of all hosts respectively. The jars were covered with muslin clothes. These jars were maintained at 26^0-28^0C, 60-70% RH and 12-hour photo period, and they were allowed to mate for ten days, whole set up was replicated three times (Fatima et al., 2016). Statistics of the following parameters was analyzed till the termination of the experiment.

Total number of eggs: To determine the total number of eggs, the eggs laid on different host were counted using magnifying glass and recorded after 10 days and this was considered as the oviposition period of the pulse beetle.

Egg laying percentage: It was calculated by the formula (Giga and Smith, 1987).

$$\text{Egg laying (\%)} = \frac{\text{No. of eggs laid in specific variety}}{\text{Total no. of eggs laid in all varieties}} \times 100$$

Hatching percentage: It was calculated by the formula

$$\text{Hatching (\%)} = \frac{\text{No. of eggs hatched}}{\text{No. of eggs laid}} \times 100$$

Incubation period: The duration from egg laying to emergence pf 1^{st} instar larva was recorded.

Larval + Pupal period: The duration from the 1^{st} instar larva to the emergence of adult was recorded.

Total Development period: The period from the egg laid to adult emergence in each treatment was recorded.

Total adult emergence: Male and Female emerged adults were counted separately and the sum of male and female was calculated as Total adult emergence.

Adult longevity: The number of days that the emerged adult survive were recorded.

Weight loss percentage (%): It was calculated by the formula (Jaiswal et al., 2019).

$$\text{Weight loss (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Figure: 1.2: Depicts the infestation of the host by *C. chinensis* on different hosts (a. cow pea, b. pea, c. pigeon pea, d. moth bean, e. chick pea, f. green gram, f. black gram.

Estimation of nutrition in the grains due to infestation

Estimation of Carbohydrates: The Carbohydrates were determined by DNSA method.

Procedure: Reducing sugars were determined in uninfected and infested grains by employing DNS Method. The underlying principle is that reducing sugars present in wheat reduce 3,5-dinitrosalicylic acid (DNS) to 3amino 5 nitro salicylic acid which absorbs light strongly at 540nm (Patil and Muskan, 2009). The amount of reducing sugar in the samples was calculated using a standard graph prepared from working standard of glucose solution (0 to 500µg/ml).

Estimation of Protein: Protein content was determined by Bradford method.

Procedure: Protein content was determined by Bradford method i.e., a colorimetric protein assay, based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 in which under acidic conditions the red form of the dye is converted into its bluer form at 595nm, to bind to the protein being assayed (Pandey and Budhathoki, 2007). The standard curve was prepared by dissolving 25mg of bovine serum albumin in 0.15 NaCl and the volume was made up to 25ml of 1mg/ml.

Statistical Analysis

This study was in a Completely Randomized Design (CRD) by three replications. Statistical analysis was done by analysis of variance (ANOVA) with GraphPad prism 9.0v followed by multiple comparison test (Tukey's). Results are presented as Mean±SEM. The level of significance was set as *$p<0.05$, ** $p<0.01$.

1.3 Result

Identification of *C. chinensis*

Head brown or black, broader at posterior end; frons carinate, its surface covered with white setae. Eyes bulbous, strongly emarginate; surface of canthus covered with white or golden setae; Maxillary palpi black, protruding. Antennae dark brown, segments 3-11 serrate in female, pectinate and often margined with black in male. Size small; length male 3.23 to 3.36 mm; length female 3.43 to 3.56 mm (Raina, 1970).

Morphological Identification Characters

Morphological identification **(Fig. 1.3)** was done using standard references and the characteristic features of *C. chinensis* are as follow:

- Female antennae are of serrate type; antennae usually with segments 4-11while Male antennae pectinate segments 4-10.
- Female genitalia: median lobe less elongate, apex valve spearhead-shaped, and base with two sclerotized plates;
- Male genitalia: median lobe more elongate, apex with exophallic valve spearhead-shaped, and base with two sclerotized plates.

Life Cycle

The total life span of *C. chinensis* was found to differ with different hosts. However, the average life span of the adult *C. chinensis* was found to be of 23 days to 42 days. After copulation, adult female deposit eggs on the surface of the host. The egg was oval in shape. There was fractional change in the size of the egg of different grains, each egg was found to be tiny, whitish in colour. The egg hatched into larvae in 3-5 days. The tiny, white, fleshy, legless pest with biting jaws develops inside the grains and the fully developed adult emerged out from the seeds of grains through emergence window. The larva is C or bean-shaped, yellowish-white, Head oval, laterally more sclerotized and globous. Ocelli one pair. Length 3.36 mm-3.69 mm. Breadth 1.47 mm-1.89 mm. **(Fig. 1.4)**. The larval and pupal stage lasted for 20-32 days varies according to host. The adults formed after pupation was found to bore its way out of the grains through emergence window.

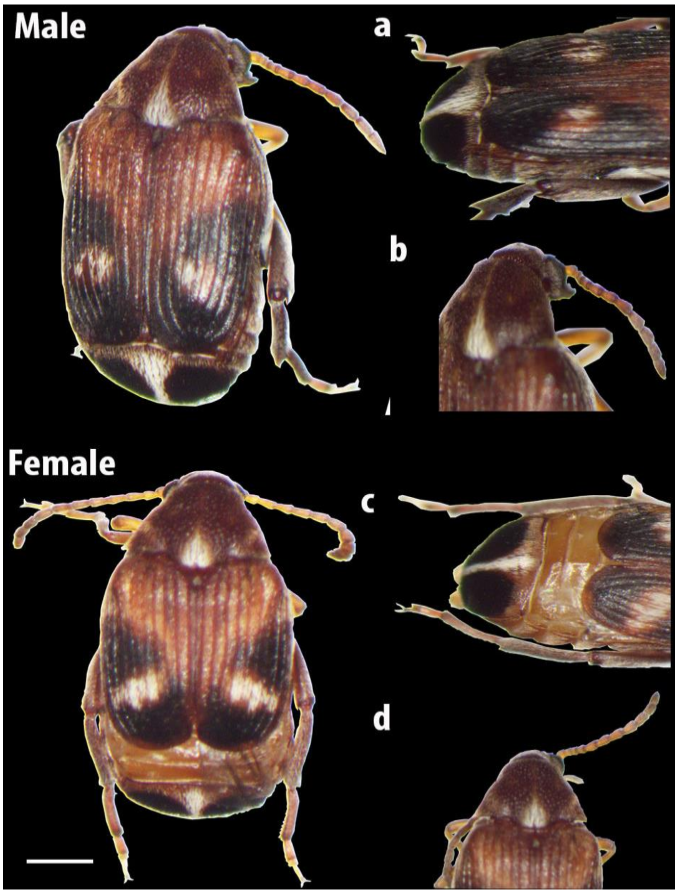

Figure: 1.3: Depicts the morphological characters of *C. chinensis*

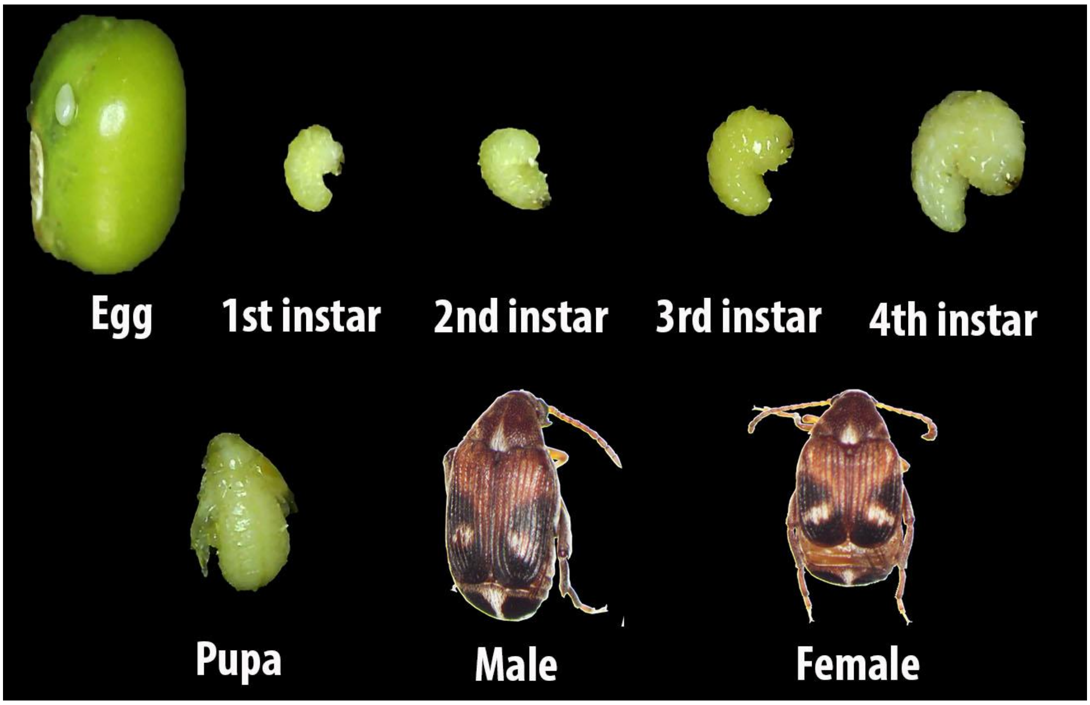

Figure: 1.4 Depicts the life cycle stages of *C. chinensis*

Molecular Identification

To confirm that the desired portion of COI gene has been amplified, gel electrophoresis was conducted. Thermo Fisher GeneRular 100 bp was used as ladder. The gel documentation image obtained by BioDoc Analyzer shows that all the samples selected for gel electrophoresis gave bands between 600 and 700bp of DNA ladder **(Fig. 1.5)**. It reveals that desired COI gene of mtDNA were properly polymerased. The visualized PCR product contained no double bands on agarose gel, thus indicating that sequences obtained were targeted mitochondrial DNA and not nuclear or mitochondrial mistargets.

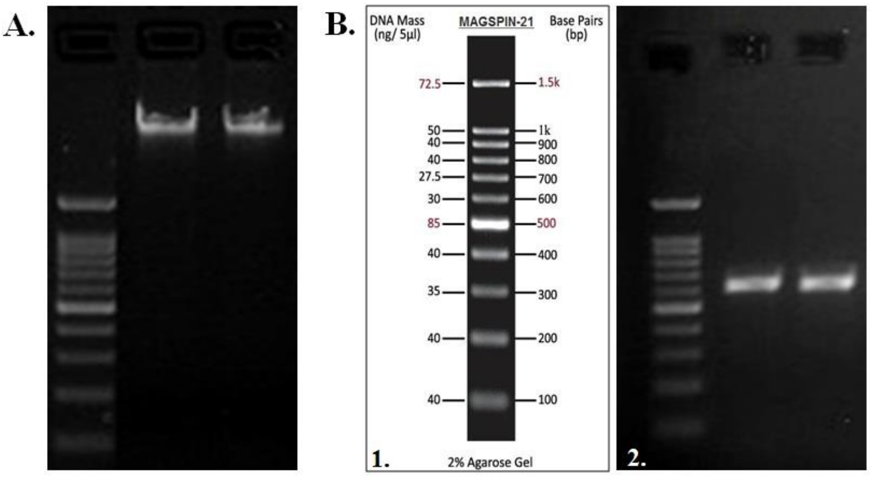

Figure: 1.5: Results of Agarose Gel Electrophoresis. A. Genomic DNA B. PCR Product 1. Base pair size 2. PCR product of COI

Bands of Genomic DNA are shown in Fig. A and that for the COI gene in Fig. B. The COI gene consisted of 85ng/5µl DNA mass with a 500bp length, when run on 2% Agarose gel. Further, the barcode **(Fig. 1.6)** and sequence of amplified COI gene was obtained which on BLAST analysis revealed that the observed sequence shows 99% homology with the sequences in GenBank.

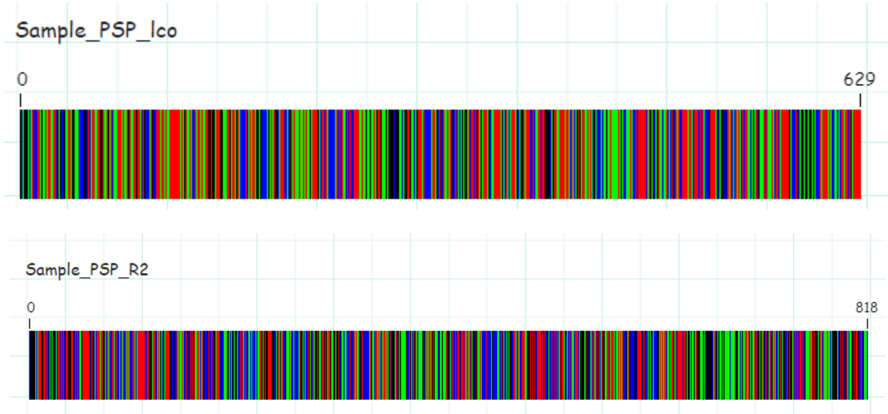

Figure 1.6: Depicts the barcode of the sequence

>***C. Chinensis* contig**

AAAAAATCAGAATAAGTGTTGCGTATAAAATAGCGGATCTCCTCCTCCTGC
ACGGTCAAAAAATGAAGTATTTAGATTTCGATCTGTTAAGAGTATAGAGATA
GCGCCTGCTAAAACGGCTAGAGAAAGTAATAAAAGGATAACAGGGATAGCT
ACTGCTGTTGCATGAAAAAAGAAATAGATGGTCTATTTGTATTATTGTGGGT
CGTATATTAATCACTGTAGTAATCACAGAATTAACTGCCCCAACAGTCCCGG
AAATTGAGGATAATACCTGCTAAATGTATTGTCTAAAAATTACTAAATATCCT
ACAGAAGATCCTCTGTGAGCGATATTAGCAGCTAATGGGGGACAAACTGTT
CAACCCCCTCCCGCCCCTCTTCCTCAACTATTCATCGGATAAAAAGAAAAG
TTAGAAAAGGGCAAAGAAATCATAAATCTTATATTAGTAATAGCCGTGGAAA
TGCCATACCTGGGGCTACGAATATGAGGCAAACTAATCAATCACCAAATCC
ACCAGGTATTATAATAGGAATTACTATAAAAAAAATTATAAGAAAGGCATCG
ACAGTAACAATTACATTATAAATTTGATCATTACCATCATTAGCGACGGGGG
AGCCGAGGTTCTCCTAAATCGAATCAAATTAAGAATCTAACGGATGTCCCT
GCNTCTTGCGGCTCAAGCAAAAAATAAAGAGCATATAGCTTCATTATCTTTA
TGATATGCTGACCAAAAAAGGGCACGCCTGAGCACGACAACAAAATCGAC
AAGGTTATCGTGATATTTCCTGATACCTTGGAGATCAGAA

Host Preference

The total number of eggs exhibits variation across different hosts, as seen in **Table 1.1**. The highest number of eggs was seen on Pea (310±1.15), followed by chickpea (270±0.58), while the lowest number of eggs was recorded on moth beans (180±1.15). There was minimal variation detected among the green gram, black gram, and cow pea, with average values of 190±1.10, 190±0.60, and 210±0.60, respectively **(Fig.1.7)**.

The data indicates that pea (19.49%) had a significantly higher preference for egg laying compared to chick pea (16.98%) and pigeon pea (15.09%). The variation in egg laying preference among Moth Bean (11.32%), green gram (11.94%), black Gram (11.94%), and cow pea (13.20%) exhibited a relatively small range. The findings indicates that pea (60±1.12) had a significantly low hatching compared to green gram (120±1.15) and pigeon pea (100±0.58). The variation in egg laying preference among Moth Bean (90±1.05), chick pea (85±1.10) and black Gram (80±0.55) exhibited a relatively small range of difference **(Fig.1.7)**. The study observed notable differences in the hatching percentage across different host plants. The highest hatching preference was seen on green gram, with a percentage of 63.16%, while the lowest hatching preference was reported on pea, with a percentage of 19.35% **(Fig.1.8)**.

Table:1.1 Total egg count, egg laying %, total hatch and hatching % of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

Host	Total Eggs Count	Egg laying %	Total Hatch Count	Hatching %
Green Gram	190±1.10	11.94	120±1.15	63.16
Cow Pea	210±0.60	13.20	100±0.58	47.62
Moth Bean	180±1.15	11.32	90±1.05	50
Chick pea	270±0.58	16.98	85±1.10	31.48
Black Gram	190±0.60	11.94	80±0.55	50
Pigeon Pea	240±1.73	15.09	75±0.60	31.25
Pea	310±1.15	19.49	60±1.12	19.35

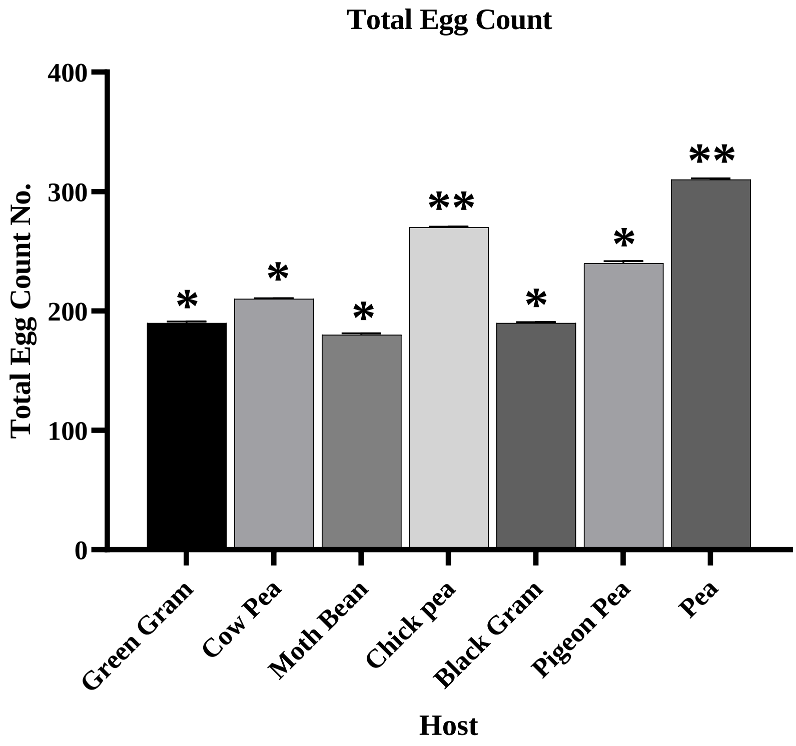

Figure: 1.7: Total egg count of *C. chinensis* on different host. Significant level *(p<0.05); **(p<0.01)

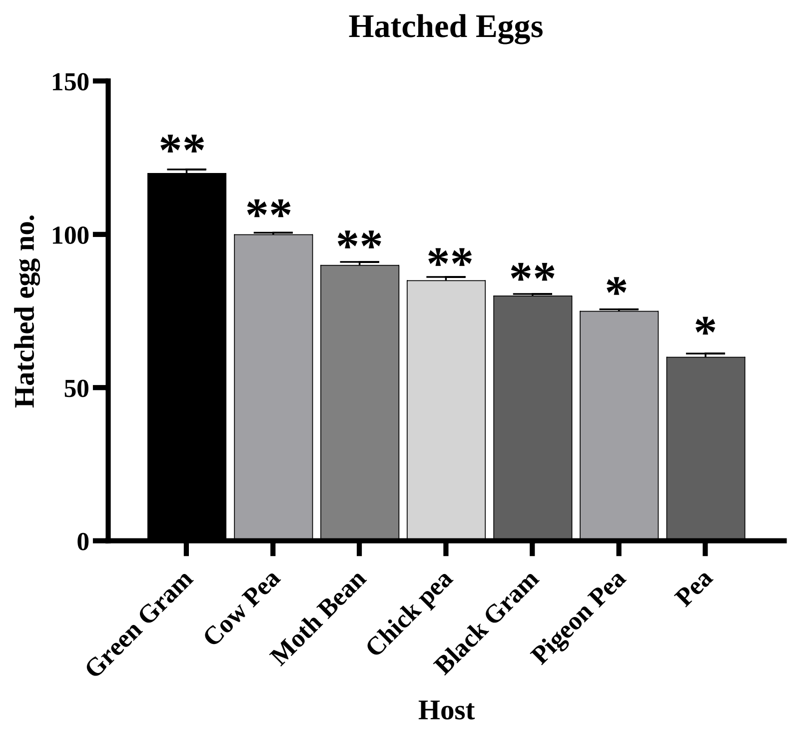

Figure: 1.8 Total hatched eggs of *C. chinensis* on different host. Significant level *(p<0.05); **(p<0.01)

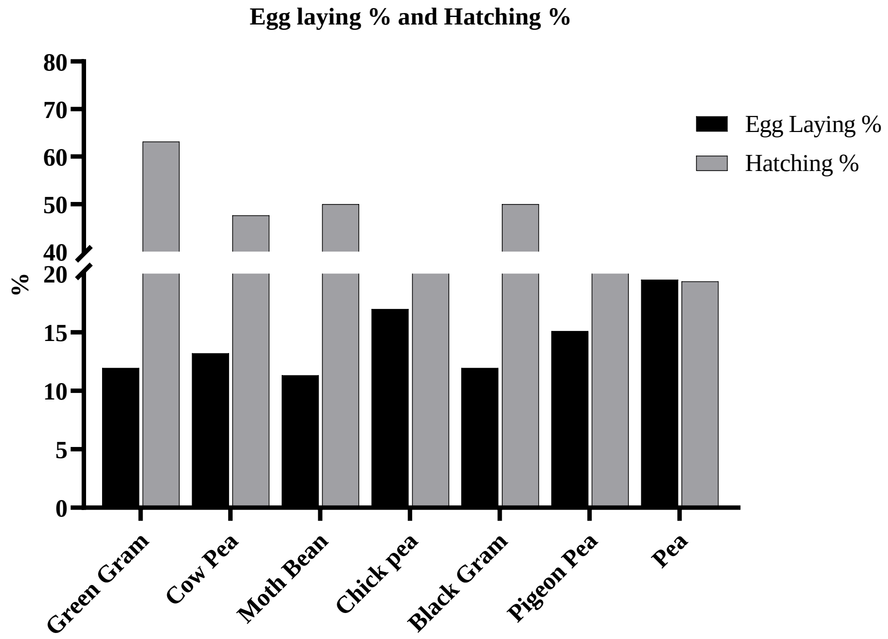

Figure: 1.9 Egg laying % and hatching % of *C. chinensis* on different host.

Table 1.2 presents the recorded highest incubation duration of 10 days for pea, while the minimum period of 3 days was seen for green gram. Moth bean and cow pea exhibited an incubation period of 4 days, but chick pea and black gram displayed an incubation period of 5 days. The data reveals that the longest larval pupal period was seen on pea, lasting for 32 days. This was followed by pigeon pea, with a duration of 26 days. Conversely, the shortest duration was observed on green gram, lasting for 20 days, followed by cow pea, with duration of 21 days. The pea plant exhibited the greatest total development time, lasting around 42 ± 1.15 days. This was followed by the pigeon pea, which had a total development period of around 32 ± 0.54 days. On the other hand, the green gramm plant had the smallest total development period, lasting approximately 22.67 ± 0.33 days. The cow pea plant had a slightly longer total development period of about 25.67 ± 0.54 days. **(Fig 1.9).**

Table: 1.2: Egg incubation, Larval + pupa period and total development period of *C. chinensis* on different host.

Host	Egg Incubation Period (Days)	Larval + Pupa Period (Days)	Total Development Days
Green Gram	3	20	22.67±0.33
Cow Pea	4	21	25.67±0.33
Moth Bean	4	22	26.66±0.34
Chick pea	5	23	28.33±0.35
Black Gram	5	25	30.30±0.35
Pigeon Pea	6	26	32±0.54
Pea	10	32	42±1.15

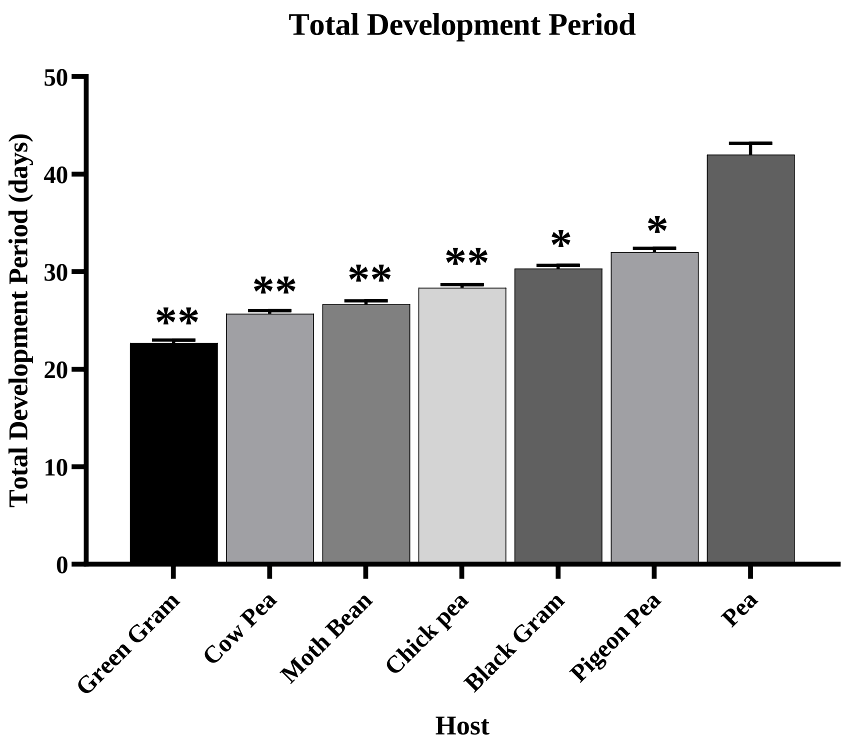

Figure: 1.10: Total development period of *C. chinensis* on different host. Significant level *(p<0.05); **(p<0.01)

The highest recorded adult emergence was observed on green gram (120±1.15), with 58 males and 62 females. Conversely, the lowest recorded emergence was observed on pea (60±1.12), with 32 females and 28 females **(Table 1.3).** The longest recorded adult longevity of emerging adults was seen in green gram, with a span of 16 days. This was followed by cow pea and moth bean, which both exhibited a survival duration of 14 days. Conversely, the shortest adult longevity was observed in pea, with a span of 9 days, followed by pigeon pea with a duration of 11 days **(Fig. 1.10)**.

Table 1.3: Adult longevity of *C. chinensis* on different host.

Host	Male Count	Female Count	Adult longevity
Green Gram	58	62	16±0.25
Cow Pea	46	54	14±0.32
Moth Bean	44	46	14±0.20
Chick pea	40	45	12±0.20
Black Gram	37	43	13±0.15
Pigeon Pea	35	40	11±0.24
Pea	28	32	09±0.12

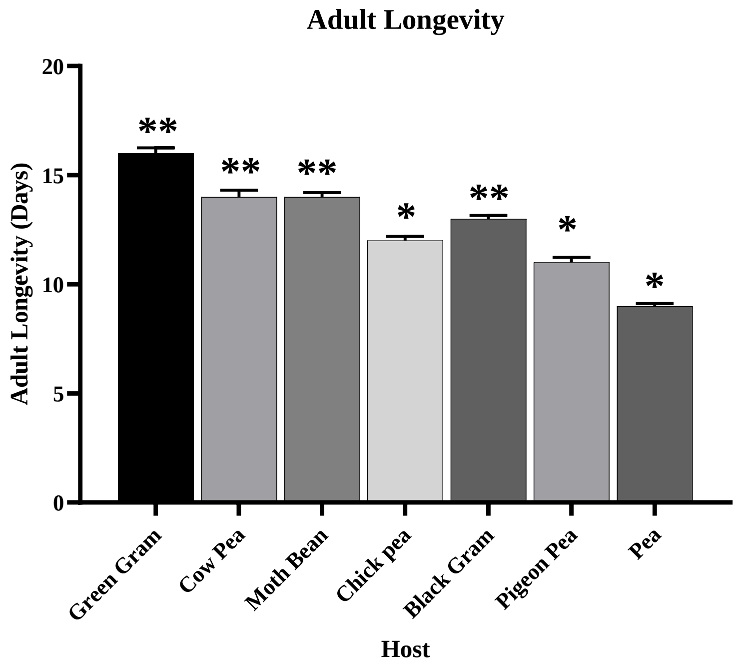

Figure: 1.11 Adult longevity of *C. chinensis* on different host. Significant level *($p<0.05$); **($p<0.01$)

A significant weight loss was recorded among all the hosts. The highest weight loss was reported in green gram (11.4±0.58g), accounting for 27.22% of the initial weight. This was followed by cow pea (9.5±0.46g) with a weight loss of 20.40%. On the other hand, pea exhibited the least weight loss (6.5±0.64g), representing 12.58% of the initial weight. Pigeon pea showed a weight loss of 9.3±0.48g, equivalent to 16.66% of the initial weight. A notable decrease in nutritional composition, namely protein and carbs, was seen across all host samples **(Table 1.4)**. Among the host samples, chickpea exhibited the highest recorded protein loss of 17.34±0.49 mg/dl, followed by green gramme with 10.2±0.47 mg/dl, while pigeon pea had the least protein loss of 5.5±0.44 mg/dl. A same pattern was noted in the reduction of carbohydrates, with chickpea exhibiting the highest loss (26.30±0.51 mg/dl), followed by green gram (21.2±0.45 mg/dl), and pigeon pea displaying the lowest loss (2.8±0.40 mg/dl) **(Fig.1.12)**

Table 1.4: Quantity (weight loss) and Quality losses (protein and carbohydrate loss) by *C. chinensis* on different hosts.

Host	Weight loss (grams)	Weight Loss%	Protein Loss mg/dl	Carbohydrate Loss mg/dl
Green Gram	11.4±0.58	27.22	10.2±0.47	21.2±0.45
Cow Pea	9.5±0.46	20.40	7.7±0.45	3.25±0.48
Moth Bean	9.2±0.50	19.48	8.8±0.46	3.20±0.47
Chick pea	9±0.42	17.12	17.34±0.49	26.30±0.51
Black Gram	9.7±0.51	18.23	5.85±0.40	7.50±0.42
Pigeon Pea	9.3±0.48	16.66	5.5±0.44	2.80±0.40
Pea	6.5±0.64	12.58	7±0.42	6.10±0.43

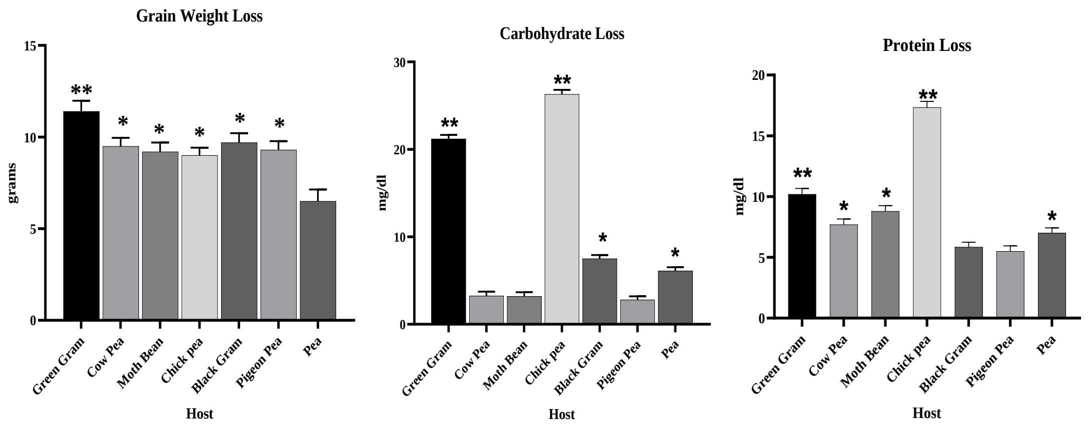

Figure 1.12: Quantity and Quality losses by *C. chinensis* on different host (Grain Weight loss, Carbohydrate loss, Protein loss). Significant level *(p<0.05); **(p<0.01)

1.4 Discussion

Knowledge of host and pest interaction is an important prerequisite when devising a cost-effective pest management package. The type of host plays a very significant role in setting the pattern on life cycle of the pest due to variation in texture, shape, size, hardness or softness and nutritional composition of the seeds (Singh et al., 2013; Mason-D'Croz et al., 2016; Sewsaran et al., 2019). For a polyphagous pest like *C. chinensis,* it is important to know its host range so that storage planning can be made to avoid cross-infestation among susceptible stored grains. In the present study in an attempt to find out the host preference, *C. chinensis* illustrated a definite host preference. According to the observations, the texture of the seed coat and the surface area appeared to play major role in the selection for egg laying. The female preferred seeds with a smooth coat and larger surface area over rough coat and less surface area. Further, throughout the infestation period *C. chinensis* being an internal feeder their entire development cycle occurred within the kernel and it was seen to come out of the grain only after it became adult. Padmasri et al. (2017) in their studies have reported that oviposition depends on softness or hardness of the grain and Adebayo and Ogunleke (2016) reported that increase in the length and width leads to high oviposition activity. In the present study maximum oviposition activity was observed on pea which possess larger surface area as well as hardness. The minimum oviposition activity was recorded on Moth bean followed by green gram, whose seeds have less surface area and more softness compared to pea. Thus, the results of the present study are in congruence with earlier reported work of (Jaiswal et al., 2019; Nisar et al., 2021).

Although the maximum egg was laid on pea due to its large surface area, nevertheless, when it comes to successful hatching it was seen that maximum hatching was on green gram, which was also reported by Sharma et al. (2016) and Nisar et al. (2021) while working on *C. maculatus*. Furthermore, the hatching has been reported to be dependent on the softness and hardness of the grains (Keskin and Ozkaya, 2015). In the present study of all the grains green gram possess soft coat compared to pea which helps easy penetration of the 1st instar larvae into the kernel, *C. chinensis* spend a considerable part of their life inside the grain kernel which decrease the amount of endosperm (Keskin and Ozkaya, 2015; Thakkar and Parikh, 2018).

Based on the obtained results, the incubation period ranged from 3 to 6 days, where minimum incubation was recorded on green gram and maximum on pea. The variation in the range is possibly due to feeding responses which in turn attribute to either the nutritional factors or physical properties of the host (Naseri and Majd-Marani, 2022). The finding of the present work supported by the work of Hosamani et al. (2018), Jaiswal et al. (2019), Dalal et al., (2020), Sathish et al., (2020) and Sekender et al. (2020) who have recorded 3-6 days of incubation by *C. chinensis* on different host. The longest larval and pupal period was recorded in pea followed by pigeon pea and shortest in green gram followed by cow pea. a considerable variation in larval development, pupae formation is also reported to be dependent on the nutritional composition of the host (Fabres et al., 2014), thus the difference is well justified as the nutritional content of the green gram was more favourable than other grains. our results are in agreement of the earlier work where they have reported the shortest developmental period in the green gram compare to multiple hosts on which they have worked (Hosamani et al., 2018; Jaiswal et al., 2019; Nisar et al., 2021).

In the current work maximum adult emergence was recorded in green gram followed by cow pea and least in pea followed by black gram. The drop in adult emergence in pea and black gram could be because of the low hatchability of eggs due to hard seed coat as reported by Padmasri et al. (2017). The chemical composition of seeds plays an important role in the adaptation of host-host relationships (Soumia et al., 2017; Kébé et al., 2020). The diet of the *C. chinensis* is characterized by a degree of specialization because their larvae are found in nature only in the seeds of a small number of host plants (Mishra et al., 2028; Pan et al., 2022). The significant variation in emergence obtained with various host thus confirms the idea of a seed defence system against *C. chinensis*. Seed teguments are also a form of defence against insect pests. The results obtained show that the presence of different integument of host causes a significant reduction in emergence rate. Further, it has also been reported that the leguminous seed coat of legumes, which are rich in tannins and lignin, presents a chemical or mechanical barrier against the penetration of larvae of unsuitable pest. Thus, various factors influence the emergence of the pest on their host, However, the exact mechanism of their low emergence needs to be evaluated in detail. Nevertheless, the maximum emergence on green gram and cow pea are in accordance of earlier work reported on *C. chinensis* of (Chandel and Bhaudaria 2015; Jaiswal et al., 2019; Nisar et al., 2021).

Observations regarding the longevity of adult *C. chinensis* corroborate with the findings of Hosamani, et al., (2018) and Mehta and Negi (2020), who found significant alteration in the longevity of adult of *C. chinensis* in different varieties of stored grain. Host significantly affected the adult longevity of *C. chinensis* where longest adult longevity was recorded on green gram possibly due to easy penetration as well high hatchability. Followed by green gram was cow pea probably due larger surface area and curvature which might have had significance effect over the other hosts. Adult longevity was shortest on the pea and pigeon pea is similar to earlier reported work of Nisar et al., 2021 who have reported that the adult longevity affected by nutrition of adult, adult weight and size, seed availability and fecundity.

Earlier studies have shown that the annual loss of stored grain by *C. chinensis* varies from 15% to 57% with different grains depending on the size and the nutrient content (Nawrocka et al., 2012; Bhandari et al., 2015). The grain weight loss has been correlated with the susceptible and resistant seed, which is more in susceptible inbreeds and less in resistant ones (Dari et al., 2010; Masasa et al., 2013; Derera et al., 2014; Garcia-Lara and Bergvinson, 2014; and Zunjare et al., 2016). In the present inventory, maximum weight loss by *C. chinensis* was observed in green gram and least weight loss was recorded in pea. *C. chinensis* spends its entire immature life in individual seeds, where they cause weight loss of seed, decrease germination potential. Moreover, the size of the green Gram was comparatively smaller than all the other host grains used in the present study. These findings are in accordance with the observation of Gupta and Apte (2016) Bharathi et al. (2017) and Jaiswal et al. (2019) who have reported that the seed loss is due to infestation. Variation of eggs deposition on different host grains can also be attributed to physical (seed size, seed coat texture) and biochemical parameters. Maximum weight loss in green gram also indicates green grams to be more susceptible compared to other host grains for *C. chinensis*.

Insects that attack the stored grains bring about deterioration reducing the moisture content which supplements undesirable metabolites, apart from depletion of calorie and selective nutrilites. (Srivastava and Subramanian, 2016). Depletion of different seed tissues such as germ, bran and endosperm have an individual effect on food quality and calorie supply. Losses in nutritional values are mainly attributed to stored insect pests, which preferentially feed on grain embryos (Taddese et al., 2020), Pulses are great source of proteins and some amount of carbohydrates. In the present study, the nutritional content of the infected grains was also measured, where a significant decrease in protein and carbohydrates were observed. This decrease in protein and carbohydrate was parallel with the severity of infestation, may be a result of the feeding activities of the larvae buried deep in the seeds (Haouel-Hamdi et al., 2017). Further, it was observed that infestation by *C. chinensis* level was negatively correlated with carbohydrate content as the storage insects consumed the carbohydrate over time and the reduced protein can be attributed to the care taken during the experiment to completely remove the eggs, egg cases, excretory products left behind on removal of larval, pupal and adult stages of *C. chinensis* before analysis (Mbah and Silas, 2007). Our observations are in agreement of previous studies by Bamaiyi et al., (2006).

1.5 Conclusion

The present study on deciphering the host preference of *C. chinensis* reports that the hatching percentage, adult emergence, adult longevity, weight loss percentage was highest in green gram, making the pest to develop fastest on green gram with least total developmental period. The study also confirms that the host size, texture (hardness or softness), surface area of the hosts affects survival and oviposition of the offspring. Thus, the knowledge acquired during the present study suggests that the biology of the *C. chinensis* and the host-preference is an obligatory step in the search for alternative methods of protection stored grains. Further, the mass rearing of *C. chinensis* opens an avenue for assessing the mechanism of resistance on exposure of insecticide.