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VARIETAL RESPONSE OF CHICKPEA GENOTYPES TO PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* L. (BRUCHIDAE: COLEOPTERA)

Farid Asif Shaheen[1]*****, Muhammad Farooq Nasir[1], Anila Irshad[1], Rubab Aslam[1], Talha Javed[1]

[1]*Department of Entomology, PMAS Arid Agriculture University, 46300- Rawalpindi*

**Corresponding Author: shaheen@uaar.edu. Pk*

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Abstract-Chickpea (*Cicer arietinum*) is third most imperative pulse crop followed by peas and soybean. Fifteen percent of the total pulse production in the world is contributed from chickpea. Chickpea grains are severely damaged in storages. *Callosobruchus chinensis* (Bruchidae: Coleoptera) is most severe pest in stored grains of chickpea. Seed weight loss 55 to 60 percent and protein content loss 46 to 66 percent is caused by infestation of *C. chinensis* in pulses. The present research was carried out to check the varietal response of chickpea genotypes to *C. chinensis* in year 2015-16 at Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. Ten chickpea genotypes including; Five DESI (CM-98, 09-AG006, CH-10/8, PB-2008 and BHAKR-2011) and five KABULI (TG-12K-05, NOOR-2013, K-09015, CH-6808 and 09012) were obtained from the Pulses Research Program (PRP), National Agricultural Research Centre (NARC), Islamabad. Antixenosis and antibiosis tests were carried out. The present results of antibiosis test showed that CM-98 proved to be resistant showing minimum (0.84) eggs per grain compared to the maximum (4.58) eggs per grain observed in CH-6808 genotype. Similarly number of holes also showed that CM-98 having minimum (0.047) holes per grain seemed to be resistant and chickpea genotype CH-6808 (susceptible) showing maximum (0.66) holes per grain. The maximum F_1 adults of *C. chinensis* were observed in NOOR-2013 (37.67) proved to be susceptible genotype. NOOR-2013 was taken as the control genotype to calculate the % inhibition rate. However, the genotype, K-09015 (10.35 %) was seemed to be susceptible chickpea genotypes. Maximum inhibition rate (96.1%) was observed in CM-98 chickpea genotype. The results of percent weight loss and till 100% mortality of PB adults showed that all KABULI chickpea genotypes were showing susceptibility while all DESI were seemed to be resistant. In preference and non-preference test, attracted PB adults were counted minimum in DESI chickpea genotypes while dead PB adults were observed maximum in these varieties proved to be tolerant, when compared to all KABULI genotypes (susceptible) against *C. chinensis*. On the basis of morphological characteristics chickpea genotypes, CM-98, 09-AG006, CH-10/8, PB-2008 and BHAKR-2011 were statistically similar with each other and classified as resistant genotypes while chickpea genotypes, TG-12K-05, NOOR-2013, K-09015, CH-6808 and 09012 were statistically different with above five genotypes and these were observed susceptible varieties.

Keywords-

I. INTRODUCTION

Chickpea (*Cicer arietinum*) ranks the third most important pulse crop followed by peas and soybean and it contributes 15 percent of the total pulse production in the world (FAO, 2010). In Pakistan, chickpea production has increased to 484 thousand tonnes in 2015 as compared to 399 thousand tonnes in 2014, showing an increase of 21 percent (GOP, 2015). It is mainly grown in rainfed conditions in Thal areas of Punjab and Kyber Pakhtunkhwa provinces. In Sindh and Baluchistan, the crop is grown on residual moisture after rice harvesting. The Punjab province contributes about 80% of chickpea production in the country where the 90% area of chickpea is grown under rainfed conditions (Hussain *et al.*, 2015). In Pakistan three chickpea genotypes TG1203, TG1221 and TG1219 are cultivated in stress environment, which followed best drought resistance, good harvest index, least drought susceptibility index and minimum loss in seed yield (Hussain *et al*., 2015). The grains of chickpea are severely damaged in storages. The main constraint for production of chickpea is post-harvest loss during storage. Due to heavy infestation the chickpea grains lose their germination capacity and become unfit for human consumption (Farukh *et al*., 2007). The pulse beetle and other species of bruchidae have been seemed to be mainly imperative species in storage grains of chickpea (Sarwar *et a1*., 2005). Once damaged chickpea grains by bruchids are not suitable for planting due to poor germination and not acceptable for food or feed due to spoilage, bad smell and toxin is produced (Adugeena, 2006). *Callosobruchus chinensis* (Bruchidae: Coleoptera) is the most severe pest in stored grains of chickpea. It is commonly known as pulse beetle (PB). Pulses have been seriously damaged by C. *chinensis* in India, Bangladesh and in many other countries. *C. chinensis* has the ability to infest plants in the field and grains in storages (Fahad, 2011). It is reported that in pulses, seed weight loss 55 to 60 percent and protein content loss 46 to 66 percent is caused by infestation of *C. chinensis* (Faruk *et al*., 2011).

Different measures are used to protect the pulses from infestation of pulse beetle. Synthetic pesticides and fumigants are commonly used to manage this pest. But chemical method has several problems, which include health hazards to the users and grain consumers. It causes residual toxicity, environmental pollution and development of pesticide resistance against bruchids (Khan *et al*., 2015). Serious harms due to inherited resistance by insect species, lasting toxicity, pest resurgence, vertebrate toxicity, photo toxicity, ecological hazards and increasing price of have emerged the need for efficient use of eco-friendly pesticides (Elhag, 2000).

Chickpeas cultivars in storages and in the field have different characteristic of resistance and susceptibility against insects infestation (Sarwar *et al*., 2006). The most environmental pleasant and consistent method to manage the storage pest is the use of resistant cultivars (Sarwar *et al*., 2009). The resistance source in the chickpea cultivars is the best option for the management of pulse beetle (Siddiqa *et al*., 2013). For the management of this beetle resistant chickpea genotypes are concluded by the evaluation of different chickpea genotypes (Erle *et al*., 2009). The resistant chickpea cultivars against PB could be used for hybridization to minimize the chemicals use (Shaheen *et al*., 2006). Resistant or less susceptible varieties are important for developing as well as the developed countries, which are exporting grains. The cultivars having good yields and acceptable storage characteristics are the best to grain protection against pulse beetle (Shafique and Maqbool, 2005). For reducing the pest damage in chickpea genotypes during storages to pulse beetle, seed morphological characteristics were evaluated with a relationship to seed resistance or susceptibility (Sarwar, 2012). The present study was carried out to screen chickpea genotypes for their genetic resistance to manage pulse beetle in stored chickpea grains.

II. MATERIALS AND METHODS

Maintenance of pulse beetle culture: The pulse beetle (PB), *Callosobruchus chinensis* culture was maintained followed by Shaheen *et al.*, (2006) in the "Stored Product Entomology" laboratory of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, to execute insect bioassays.

Experimental chickpea genotypes: Ten chickpea genotypes including Five DESI (CM-98, 09-AG006, CH-10/8, PB-2008 and BHAKR-2011) and five KABULI (TG-12K-05, NOOR-2013, K-09015, CH-6808 and 09012) were obtained from the Pulses Research Program (PRP), National Agricultural Research Centre (NARC), Islamabad. Before execution of insect bioassays, chickpea grains were made un-infested, followed by Shaheen *et al.*, (2006). Grain morphological characteristics were observed on visual basis in consultation with experts at PRP, NARC and are mentioned in Table 2.

Antibiosis test: In this experiment, plastic jars of 250 g capacity were used as experimental units. Fifty grams of each genotype was placed in separate jars and twelve pairs of one day old beetles were released in each jar. Each genotype was replicated thrice. The jars were then being placed in incubator at temperature of 30±2 ^oC and 70±5% relative humidity. For antibiosis, following parameters were studied:

Number of eggs per grain: Eggs laid by PB were calculated to check the outcome of treatment on fecundity. To count the number of eggs, ten grains were selected randomly**.** The data was recorded on weekly basis.

Number of holes per grain: To count the holes per grain, ten grains were randomly selected in each replication. The data was recorded on weekly basis.

Number of F_1 adults emerged: To see the inhibition of pulse beetle emergence, in each jar number of F_1 adults were counted.

Percentage inhibition rate: Percentage inhibition in F_1 adults emergence was calculated by following formula:

$$\text{Percent IR} = \{(C_n - T_n) / C_n\} \times 100$$

Where

T_n = Number of F_1 adults in treated jar

C_n = number of F_1 adults in untreated jar

Weight loss (%) of grains: At the end of experiment, the percent weight loss was calculated by using the formula;

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

Adult Mortality: In this experiment, ten adults of PB were released in each jar instead of twelve pairs. The mortality of beetles was recorded at 24 hours interval until 100% mortality of them.

Antixenosis test: In this test, preference and non-preference of PB to the exposed genotypes was observed. Fifteen grains of each genotype were put in seperate petri plates and kept in insect rearing cabins. In every cabin forty adults of PB were kept in a small round tray which was placed in the centre of cabin in such a way that every petri dish is equally distanced. These cabins were placed in the laboratory and temperature and humidity was recorded daily. The number of beetles attached to each genotype was recorded up to seven days at interval of 24 hours.

Statistical analysis: The software SSPS 21.0, MS Excel and DMRT were used for statistical analysis.

III. RESULTS

Antibiosis test:

Eggs per grain laid by *C. chinensis*: Table 1 showed that the minimum eggs (0.84) per grain were showed in the genotype, CM-98 compared to the maximum eggs per grain (4.58) observed in CH-6808 genotype. CM-98 was statistically alike with 09-AG006, CH-10/8, PB-2008 and BHAKAR-2011 and all these were seemed to be resistant against pulse beetle compared to the remaining ones. On the other hand, CH-6808 genotype was significantly similar to K-09015, 09012, TG-12 K-05 and Noor-2013 and proved to be susceptible genotypes. The number of eggs ranged from 0.84 to 1.42 per grain in resistant genotypes while in susceptible ones, this range was 3.71 to 4.58 eggs per grain.

Holes per grain made by *C. chinensis*: The highest numbers of holes (0.66) per grain was seemed in the genotype, CH-6808 that was susceptible and the least holes (0.047) per grain were found in CM-98 and proved to be resistant (Table 1). CM-98 genotype was statistically non-significant with BHAKAR-2011. Chickpea genotype, 09012 was not significantly different with K-09015, NOOR-2013 and TG-12K-05 showing a range of 0.33 to 0.58 holes per grain; however, all these were classified as partially susceptible genotypes. The genotype CH-10/8 was statistically alike with chickpea genotypes namely PB-2008 and 09-AG006 where holes per grain ranged from 0.10 to 0.18 and proved to be partially resistant genotypes (Table 1).

F_1 adults of *C. chinensis* emerged: It was seen in table 1 that BHAKAR-2011 was seemed neither resistant nor susceptible because it was significantly similar to all the genotypes. Chickpea genotypes 09-AG006, CM-98 and PB-2008 were found resistant against PB emerging 1.8, 1.47 and 1.73 adults, respectively. However, CH-10/8 (4.33) was observed as partially resistant but it was significantly not different with all the resistant genotypes. The maximum adults of *C. chinensis* (37.67) were observed in NOOR-2013 proved to be susceptible genotype. Chickpea genotypes, K-09015, CH-6808 and 09012 showing 30.00, 30.67 and 29.67 emerged F_1 adults respectively were significantly not different with one another and proved to be partially susceptible.

Percentage inhibition rate: The genotype NOOR-2013 was the most susceptible showed the maximum number of F1 adults emerged (37.67) and was also cultivated in arid areas particularly of Pothowar tract. Hence it was taken as the control to calculate the % inhibition rate. The genotype, K-09015 (10.35 %) was statistically related to TG-12K-05, CH-6808 and 09012 showing % inhibition rate 17.51, 14.60 and 20.16 percent inhibition rate respectively seemed to be susceptible chickpea genotypes. BHAKR-2011(65.47%) was proved neither susceptible nor resistant against *C. chinensis* because that was statistically different with all discussed genotypes. Maximum inhibition rate (96.1%) was observed in CM-98 chickpea genotype and that was statistically non-significant with PB-2008, 09-AG006 and CH-10/8 showing 88.49 percent to 96.1 percent inhibition rate seemed to be resistant (Fig.1).

Percent Weight loss: The genotype CH-10/8 proved as resistant against adults of pulse beetle showing the minimum weight loss (18.2%). The maximum weight loss (48.46%) of chickpea grains was calculated in TG-12K-05 chickpea genotype and that genotype was observed susceptible genotype (Fig. 2). Which was statistically non-significant with NOOR-2013 (45.206 % weight loss) and 09012 (41.513 % weight loss). The genotypes, 09-AG006, CM-98 and CH-6808 were statistically alike having 24.72, 24.11 and 26.33 % weight loss respectively proved to be partially resistant chickpea genotypes. PB-2008, the chickpea genotype was significantly different with BHAKAR-2011 and K-09015 showing 30.56, 29.79 and 31.23 % weight loss respectively seemed to be partially susceptible chickpea genotypes.

Mortality of *C. chinensis* adults: The 100% mortality of adults of *C. chinensis* in minimum time (48 hours) was observed in CM-98 and 09- AG006 and both genotypes were seemed to be resistant. Chickpea genotypes, CH-10/8, BHAKAR-2011 and PB-2008 (72, 72, and 96 hours) were proved to be partially resistant against adults of *C. chinensis*. NOOR-2013 and CH-6808 chickpea genotypes were observed susceptible to pulse beetle showing maximum longitivity (168 hours). TG-12K-05, 09012 and K-09015 chickpea genotypes were found to be partially susceptible showing 120, 144 and 144 hours longitivity (Fig. 3).

Antixenosis test: Figure 4 showed that the attracted number of (0.61) PB adults were minimum towards grains of chickpea genotype 09-AG006 which was resistant to pulse beetle and statistically similar with CM-98, CH-10/8, PB-2008 and BHAKAR-2011. The number of dead adults was observed maximum ranged from 0.86 to 1.28 adults per petri plate in resistant genotypes. Maximum number of (2.04) adults were calculated in TG-12K-05 grains which were found susceptible to pulse beetle. TG-12K-05 was non-significantly different with NOOR-2013, CH-6808, K-09015 and 09012.

Table 1: Number of eggs laid, average number of holes and F1 adults emerged of C. chinensis in chickpea genotypes

Chickpea genotypes	**Mean Number of eggs ± SE**	**Mean Number of holes ± SE**	**Mean Number of adults ± SE**
09-AG006	1.21±0.206a	0.18±0.132ab	1.80±0.626a
CM-98	0.84±0.147a	0.047±0.336a	1.47±0.524a
CH-10/8	1.42±0.305 a	0.10±0.047ab	4.33±1.848ab
PB-2008	1.20±0.206 a	0.140±0.532ab	1.73±0.784a
BHAKAR-2011	1.32±0.244 a	0.087±0.363a	13.00±5.205abc
TG-12 K-O5	4.25±1.053 b	0.48±0.206abc	30.67±1.144bc
NOOR-2013	4.09±1.091 b	0.46±0.187abc	37.67±1.443c
K-09015	3.82±0.945 b	0.58±0.232bc	30.00±1.091bc
CH-6808	4.58±1.165 b	0.66±0.227c	30.67±1.128bc
09012	3.71±0.984 b	0.33±0.134abc	29.67±1.197bc

Table 2: Seed of chickpea genotypes with morphological characteristics

Chickpea genotypes	**Morphological characters of seed (texture testa, color and shape)**
09-AG006	Rough, light brown, angular (R L_b A)
CM-98	Rough, dark brown, angular (R D_b A)
CH-10/8	Rough, dark brown, angular (R D_b A)
PB-2008	Rough, dark brown, angular (R D_b A)
BHAKAR-2011	Rough, dark brown, angular (R D_b A)
TG-12K-05	Smooth, beige, Pea shaped (S B P_s)
NOOR-2013	Smooth, beige, angular (S B A)
K-09015	Smooth, beige, Pea shaped (S B P_s)
CH-6808	Smooth, beige, angular (S B A)
09012	Smooth, beige, angular (S B A)

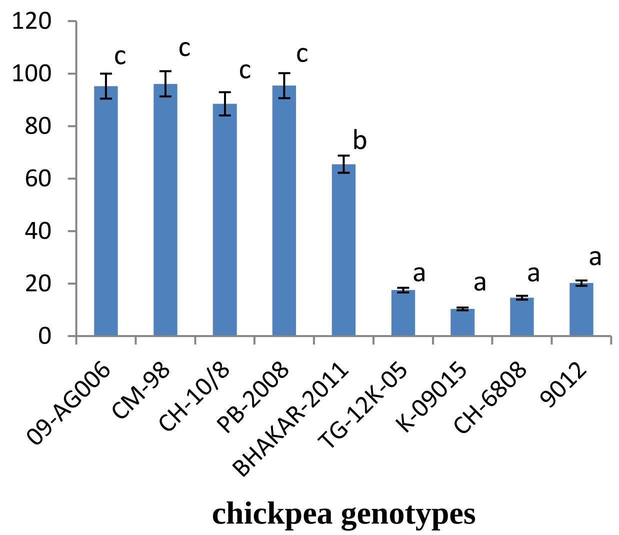

Figure 1: Inhibition rate of F_1 adults of *C. chinensis* emerged in different genotypes of chickpe

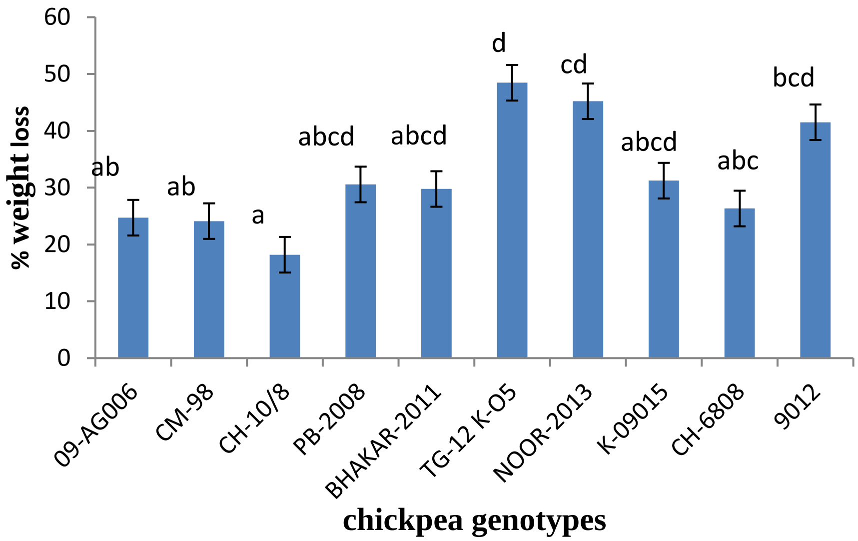

Figure 2: Percent weight loss in different chickpea genotypes by *C. chinensis*

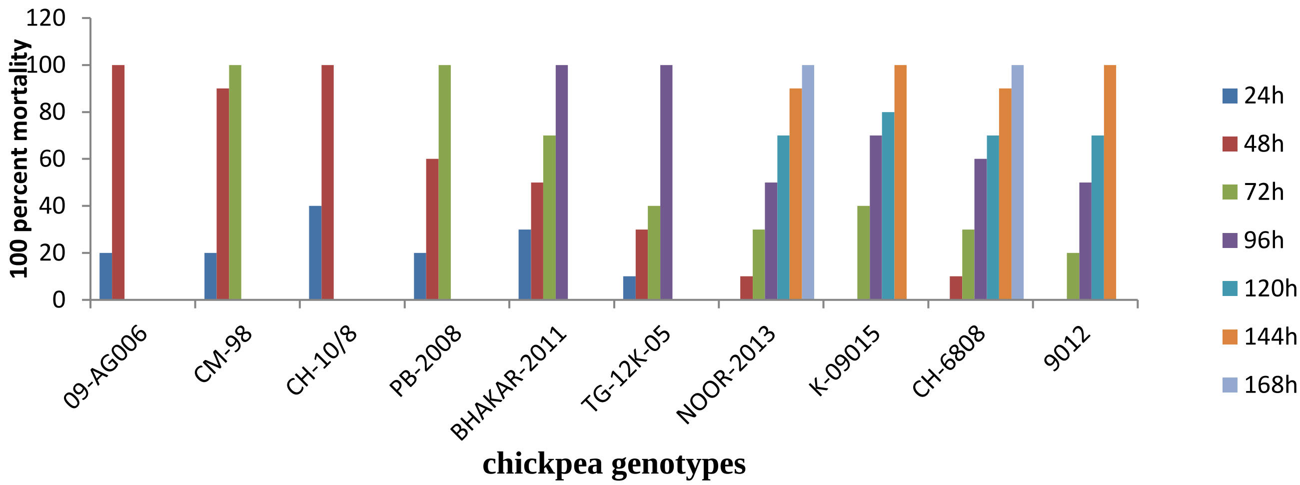

Figure 3: 100 percent mortality of adults of *C. chinensis* in different chickpea genotypes

Minimum numbers of dead adults ranged from 0.48 to 0.8 were seen in susceptible varieties. The range of attracted adults towards resistant genotypes was recorded from 0.61 to 1.24 while in susceptible cultivars ranged from 1.76 to 2.04 adults were observed (Fig. 4).

IV. DISCUSSION

The present results showed that the response of chickpea genotypes to pulse beetle was different in all cultivars. Five DESI (CM-98, 09-AG006, CH-10/8, PB-2008 and BHAKR-2011) and five KABULI (TG-12K-05, NOOR-2013, K-09015, CH-6808 and 09012) genotypes were tested against *C. chinensis* , however all five DESI cultivars were statistically similar classified as resistant genotypes while KABULI genotypes were statistically different with DESI genotypes and these were observed susceptible varieties. It could be seen in Table 2 that morphological appearance of DESI cultivars (dark brown, rough and angular) was different with KABULI cultivars (beige, smooth and angular/pea shaped). Siddiqa *et al.*, (2015) studied that on the basis of morphology of chickpea varieties in free choice test, the response of two *Callosobruchus* species for oviposition and percent damage was different. Sarwar (2012) revealed that the resistant genotypes have thick seed coat, dark brown colour and small grain size while susceptible chickpea genotypes have thin and soft seed coat, whitish seed colour and larger grain size. In the studies of Panzarino *et al*., (2011) the no. of eggs, adult emergence, development time and pre-adult mortality were assessed under antixenosis and antibiosis bioassays on three local genotypes and three commercial varieties. In assayed genotypes the local one Grumo Appula Black was least susceptible to the pulse beetle. Erle *et al.*, (2009) showed that in both antixenosis and antibiosis tests, the genotype, ICC 4969 out of the total eleven tested genotypes observed resistant against pulse beetle. However, they proved that resistance was observed in Desi chickpea genotypes while Kabuli chickpea genotypes were susceptible against PB. Parameshwarappa *et al.*, (2007) conducted twelve varieties of chickpea for level of seed quality, damage, susceptibility and varietal resistance to pulse beetle. It was found that complete resistance was not observed in any one genotype out of the 12 cultivars of chickpea against *C .chinensis*.

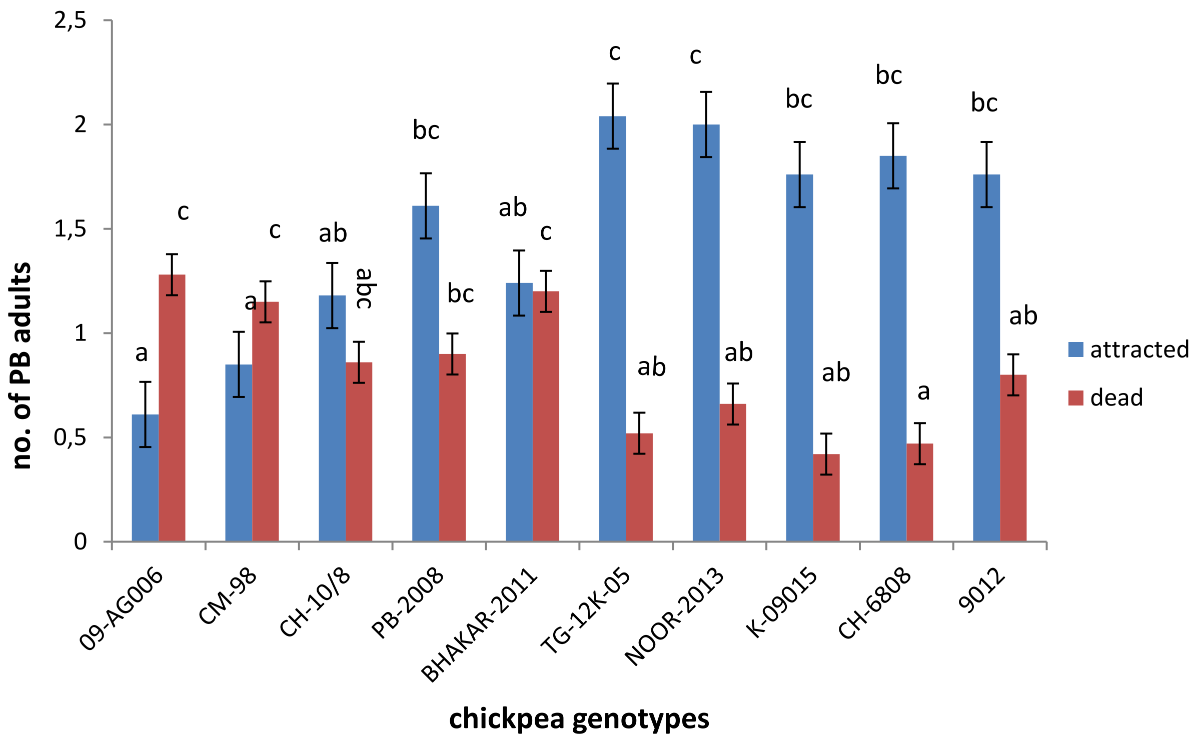

Figure 4: Number of attracted and dead *C.chinensis* adults in chickpea genotypes (preference and non-preference)

But susceptibility difference was observed in different genotypes to pulse beetle attack. Aslam *et al.*, (2006) reported six genotypes of stored chickpea against pulse beetle. Results were showed that Parbat seemed to be most susceptible to *C. chinensis*. The genotype CM-2000 observed to be susceptible. The cultivar, Punjab-91 and Pb-2000 showed to be partially resistant while Bittle-98 noted to be highly resistant to pulse beetle. Shaheen *et al*., (2006) showed that genotypes with hard, thick and rough seed coat were highly resistant but thin seed coat, smooth and soft were not found resistant against pulse beetle. Chickpea cultivars of Punjab-91, Dasht, Bittle-98 and Parbat were resistant against *C. chinensis* while Paidar-91 and Flip 97-192C were found susceptible.

According to Shafique and Ahmad (2005), results showed that in antixenosis test oviposition, development of adult progeny, seed damage and weight losses were significantly different in all chickpea cultivars. The genotypes with thick seed coat and black in color were proved to be resistant because eggs were laid in lesser number and pulse beetle preference for host selection was proved to be sensory to higher level.

Chickpea genotypes of CM-98, CH-10/8, PB-2008, 09-AG006 and BHAKAR-2011(DESI) were found tolerant to *C. chinensis*, however recommended for long time storage. In future, the results of this work can support to screen the genetic resistance in chickpea genotypes to manage pulse beetle in stored chickpea grains.

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