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Artificial Seed Bioassay for Assessing Genotypic Resistance of Pigeon Pea to *Callosobruchus chinensis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pulse beetle, *Callosobruchus chinensis* is a significant pest of stored legumes, particularly pigeon pea (*Cajanus cajan),* causing substantial damage and economic losses. This study investigates the resistance of various pigeonpea genotypes to *C. chinensis* using artificial seeds in a controlled single-choice bioassay. Artificial seeds in bioassays help provide consistent conditions for studying pigeon pea resistance to pests like *C. chinensis*. Unlike natural seeds, which can vary in traits like size, texture, and composition, artificial seeds ensure that any observed resistance is due to the plant's genetics, not differences in the seeds themselves. Despite using seeds with different proportions of resistant and susceptible genotypes, no significant differences were observed in the beetle oviposition behavior. The findings suggest that factors other than nutritional content may

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influence bruchid resistance, emphasize the need for further research into specific phytochemicals responsible for resistance. This work has important implications for the development of more resistant pigeon pea cultivars.

Keywords: Pulse beetle; pigeon pea; artificial seeds; oviposition.

1. INTRODUCTION

Callosobruchus chinensis commonly known as the cowpea weevil or bruchid beetle. It is a notorious pest of stored legumes, with pigeon pea (*Cajanus cajan*) being one of its primary hosts [1,2]. *C. chinensis* has a widespread distribution particularly in tropical and subtropical regions, where it inflicts significant damage to stored legumes, including pigeonpea, chickpea, and cowpea [3]. *C. cajan* is a vital staple crop in many regions particularly in Asia and Africa, where it is a critical crop due to its high nutritional value, being rich in proteins, essential amino acids and micronutrients, which makes it a key dietary component. Economically, it is important for smallholder farmers as a source of income and sustenance [4]. Its resilience to harsh environmental conditions makes it crucial for food security in these areas. The lifecycle of *C. chinensis* is closely tied to its host, as the female lays eggs on the surface of the seeds. Upon hatching the larvae burrow into the seeds, where they feed, develop, and eventually emerge as adults [5]. This process causes substantial damage to the seeds, reducing their viability, nutritional value, and marketability [6]. Pulse beetle is a ‘niche generalist’ in that it can infest seeds within pods. Its initial infestation occurs in the field before harvest and from there beetles are carried to store houses where the population can increase rapidly [7]. *C. chinensis* causes substantial quantitative and qualitative loss, manifested by seed perforation, reduction in weight, market value, and seed germination. About 10-40% of the total annual production valued as billions of US dollars are lost annually alone to *Callosobruchus* spp in tropical countries [8]. Traditional breeding methods for developing resistant cultivars have focused on general resistance traits, but these approaches often lack precision and may not be effective against specific pests like bruchids [9]. This limitation emphasizes the need for more targeted studies and advanced techniques to develop pigeonpea varieties with robust resistance, ensuring the sustainability and productivity of this essential crop. The use of artificial seeds in bioassay studies offers a controlled and reproducible method to investigate resistance mechanisms against pests like *C. chinensis*. By mimicking natural seeds while eliminating variables such as genetic and environmental factors, researchers can focus on specific traits influencing oviposition behaviour and pest resistance. The objective of this study is to evaluate the resistance of different pigeonpea genotypes to bruchid infestation, specifically focusing on *C. chinensis* using a single-choice bioassay with artificial seeds. This study holds significant implications for sustainable pest management, as it aims to identify key resistance factors that can be utilized in breeding programs, ultimately contributing to the development of pigeonpea varieties that are better equipped to withstand bruchid infestations in stored grains.

2. MATERIALS AND METHODS

2.1 Rearing of *Callosobruchus chinensis*

The *C. chinensis* was available in the storage laboratory, Division of Entomology, ICAR-IARI, New Delhi. The insect was reared in glass jars (15 x 10 cm) on pigeonpea seeds under controlled conditions of 27±2^{0}C and 68±5% RH to maintain uniform culture. The freshly emerged adults were used for bioassay.

2.2 Preparation of Artificial Seeds

Preparation of artificial seeds (AS) by soaking the pigeon pea seeds namely, IPAB 18-21, ICPP171581, Pusa Arhar -16 and ICPP171112 in distilled water for about 1 hr. Seed coat was removed manually and the seeds were dried at 27^{0}C for 48 h. The de-coated seeds were then ground into fine powder using pestle and mortar. Susceptible genotype ICPP171112 was mixed in different proportion with resistant cultivars to create different percentage of artificial seed. In the present study, above mentioned flour of resistant genotypes were thoroughly mixed with susceptible genotype ICPP171112 in 25%, 50%, 75% and 100% combinations (Table 1). One gram of de-coated pigeon pea flour was mixed with 0.40–0.44 ml of 1% Tween 80 to form a fine paste. Later, the paste was converted into similar sized balls as that of pigeon pea seeds and kept in deep freezer for 12hrs. Further, AS was dried at 27ºC temperature and 35 % RH for 1 week to equilibrate moisture content of AS with atmospheric levels. The average weight of the Artificial seed was 1.35 g (n = 15) in comparison with pigeonpea that weighed 1.46 g (n = 15) (Plate 1) [10,11,12].

Table 1. Proportion of pigeon pea genotypes used for single choice test screening against pulse beetle, *C. chinensis*

Genotypes
25% IPAB 18-21 + 75% ICPP171112
50% IPAB 18-21 + 50% ICPP171112
75% IPAB 18-21 + 25% ICPP171112
100% IPAB 18-21
25% ICPP171581 + 75% ICPP171112
50% ICPP171581 + 50% ICPP171112
75% ICPP171581 + 25% ICPP171112
100% ICPP171581
25% Pusa Arhar – 16 + 75% ICPP171112
50% Pusa Arhar – 16 + 50% ICPP171112
75% Pusa Arhar – 16 + 25% ICPP171112
100% Pusa Arhar – 16
100% ICPP171112

2.3 Single Choice Test

Fifteen AS from each of the four genotypes were taken in separate plastic vials (6 x 4 cm) and three pairs of newly emerged adult *C. chinensis* were transferred into each vial and allowed for oviposition. The insects had no opportunity to select a preferred host and were compelled to exclusively feed on the seeds of a single genotype. The oviposition rate was recorded for every 24 hours until three days. To validate the susceptible or resistance reaction of each genotype, the experiment was repeated thrice.

2.4 Statistical Analysis

One way analysis of variance (ANOVA) was carried out by Tukey's Honest Significant Difference (HSD) test at P= 0.05 using SPSS 16.0 software. The means were compared based on the least significant difference (LSD) test at P=0.01.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Bruchid Resistance Using Artificial Seeds under 'Single Choice' Test

Artificial seeds prepared with different proportion of de-coated flour from susceptible (ICPP171112) and resistant (IPAB 18-21, ICPP171581, Pusa Arhar-16) genotypes were exposed to freshly emerged adults of *C. chinensis* to determine their oviposition preference. It was observed that artificial seed does not influence on egg laying behaviour of bruchid, as there was no significant difference in number of eggs laid on these seeds except that higher number of eggs (21.67±1.53) was recorded on susceptible genotype ICPP171112 (Table 2). We tried to validate the resistance mechanisms observed in resistant genotypes by preparing synthetic seeds but surprisingly we could not observed any significant difference in oviposition. This may be because the non-nutritional parameters could be

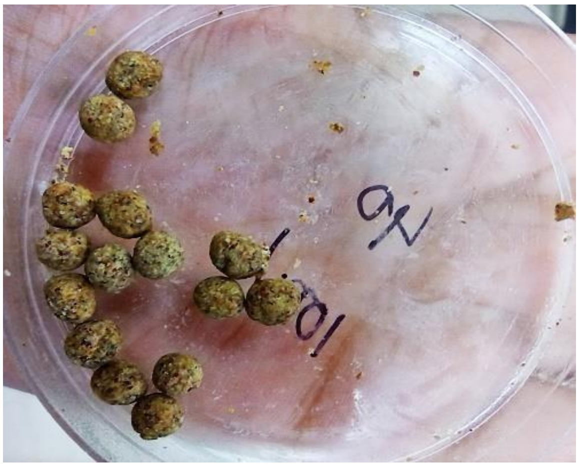

Plate 1. Bioassay with Artificial seeds

Table 2. Oviposition preference of *C. chinensis* from various combinations of artificial seeds made from resistant and susceptible pigeon pea genotypes

Genotype	No. of eggs/15 seeds
25% IPAB 18-21 + 75% ICPP171112	18 ± 3.00 [ab]
50% IPAB 18-21 + 50% ICPP171112	15.67 ± 2.31[ab]
75% IPAB 18-21 + 25% ICPP171112	13 ± 3.61[ab]
100% IPAB 18-21	16.33 ± 1.53[ab]
25% ICPP171581 + 75% ICPP171112	15 ± 4.36[ab]
50% ICPP171581 + 50% ICPP171112	12.33 ± 2.08[ab]
75% ICPP171581 + 25% ICPP171112	11 ± 2.00[ab]
100% ICPP171581	10.67 ± 3.06[b]
25% Pusa Arhar-16 + 75% ICPP171112	16.67 ± 2.08[ab]
50% Pusa Arhar-16 + 50% ICPP171112	13.67 ± 1.53[ab]
75% Pusa Arhar-16 + 25% ICPP171112	10.33 ± 0.58[b]
100% Pusa Arhar-16	15.67 ± 2.31[ab]
100% ICPP171112	21.67 ± 1.53[a]
F value	3.157
P value	<0.01

Note: All values represent mean of three replications. Means in a column followed by different letter(s) are significantly different in all cases. Mean were separated by Tukey's Honest Significant Difference at p<0.01

influencing larval development rather than oviposition choice. This approach has also been demonstrated to identify resistance governing factors [12,13]. Alkaloids and non-protein amino acids to be highly toxic to larval development [13]. Vicillins-like 7S storage proteins have been found in the testa of various legumes and linked to seed resistance to herbivory [14,15,16] as have lectins, lectin-like α-amylase inhibitors and arcelin [17]. It is also evident that several secondary metabolites are responsible for governing resistance against that may be working simultaneously against seed infesting insects [18]. For full understanding of resistance factors more in-depth studies are required on phytochemicals constituents of resistance and susceptible seeds which can be then exploited for developing resistant pigeonpea cultivars.

4. CONCLUSION

There was no significant difference in the oviposition behavior of *C. chinensis* on artificial seeds, regardless of the resistant or susceptible genotype. This suggests that factors other than nutritional content may influence the oviposition preference of the bruchid beetle. The findings highlight the need for further research into the specific phytochemical constituents that govern resistance in pigeonpea seeds. Ultimately, understanding these factors could lead to the development of more resistant pigeonpea cultivars, contributing to sustainable pest management strategies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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