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### Research Article

## IN VITRO EVALUATION OF MAIZE VARIETIES ON THE DEVELOPMENT AND REPRODUCTION OF *CHILO PARTELLUS* (LEPIDOPTERA: CRAMBIDAE)

<sup>a</sup>Khalid Hussain Dhilloo, <sup>a</sup>Kainat Shahid, <sup>b</sup>Jamal-U-Ddin Hajano, <sup>a</sup>Ali Ahmed, <sup>c</sup>Waqar Ali Chandio, <sup>a</sup>Kaneez Fatima, <sup>a</sup>Aneeta Lashari, <sup>a</sup>Din Muhammad Soomro

<sup>a</sup> Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University Tandojam, 70050, Sindh, Pakistan.

<sup>b</sup> Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University Tandojam, 70050, Sindh, Pakistan.

<sup>c</sup> College of Forestry, Sichuan Agricultural University, Chengdu 611130, China.

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### ABSTRACT

*In vitro* experiments were conducted to evaluate the developmental and reproductive responses of *Chilo partellus* on different maize varieties in the Pesticide Toxicology and Application Technology Laboratory, Sindh Agriculture University, Tandojam. The maize stem borer, *C. partellus*, is a highly destructive insect pest that severely affects both fodder and cultivated crops worldwide. Although pesticides provide limited control of early infestations, they are ineffective against internal feeders and pose environmental risks. In contrast, utilizing resistant maize cultivars offers a sustainable, ecologically safe, and socially acceptable pest management strategy. The study revealed significant differences in the survival rates and developmental durations of *C. partellus* between hybrid and conventional maize under laboratory conditions. On conventional maize, the total developmental period ranged from 46.22 to 69.34 days (mean: 57.78 ± 11.56 days), whereas on hybrid maize, it ranged from 26.87 to 40.99 days (mean: 33.93 ± 7.06 days). Each developmental stage was shorter on hybrid maize. Females survived longer than males on both maize varieties, but their lifespan was notably shorter on hybrid maize. Regarding reproductive traits, *C. partellus* showed higher mean fecundity on conventional maize (604 ± 104.67 eggs, range: 500.11-709.44) compared to hybrid maize (302 ± 95.06 eggs, range: 207.44-397.56). Egg fertility was also significantly higher on conventional maize (mean: 55.44 ± 1.81%) compared to hybrid maize (mean: 24.61 ± 1.77%). Statistical analysis confirmed significant differences in developmental durations and reproductive traits between the two maize varieties. These findings highlight the potential of hybrid maize as a resistant cultivar against *C. partellus*.

Corresponding Author: Khalid Hussain Dhilloo

Email: khdhiloo@sau.edu.pk

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### INTRODUCTION

After rice and wheat, maize (*Zea mays* L.) ranks as the third most important crop in Pakistan. It is a vital

edible grain and serves as a raw material for the production of various industrial products (Afzal et al., 2009). Belonging to the Poaceae family, *Z. mays* was

first domesticated in Central America and Mexico (Hussain et al., 2016). Globally, it is recognized as a highly productive cereal and fodder crop (Shiferaw et al., 2011).

However, maize crops face significant threats from insect pests, largely due to their high susceptibility throughout the growing season. Among these, the maize stem borer (*Chilo partellus*; Lepidoptera: Pyralidae) is the most prevalent and destructive pest (Andow and Hilbeck, 2004; Groote et al., 2011). Originating in the India-Pakistan region, *C. partellus* has emerged as a major pest in many Asian and African countries, causing production losses ranging from 15% to 75% (Jafari and Jalali, 2007; Kumar and Ashwani, 2017; Nabeel et al., 2018). The damage inflicted by this pest includes dead hearts, impaired translocation, ear damage, lodging, premature leaf senescence, and, in severe cases, complete crop failure (Naz et al., 2003; Gupta et al., 2010).

The larva of *C. partellus* attacks all aerial parts of the plant, including ears, tassels, stems, and stalks, by boring into them. Although various management strategies are employed to combat this pest, chemical control remains particularly effective due to its rapid knockdown effect (Tende et al., 2010).

Insecticide spraying offers limited protection against stem borers, as these pests often continue feeding inside the stems and ears despite the effectiveness of insecticides against early infestations (Jotwani, 1983). However, for smallholder farmers, the use of insecticides is often not economically viable and poses significant health and environmental risks. Consequently, many farmers either resort to limited pesticide use or neglect stem borer management entirely. Although traditional stem borer-resistant maize germplasm exists and host plant resistance is considered a safer and more sustainable approach, no stem borer-resistant maize variety has been officially released or commercialized due to the high costs and complexities associated with breeding and registration (CIMMYT, 1993).

Plants have evolved a variety of resistance mechanisms to combat insect pests, which are essential for their survival and adaptation. These mechanisms can be broadly classified into physical defenses, such as trichomes, tough leaf structures, and waxy cuticles, and chemical defenses, including the production of secondary metabolites like alkaloids, phenolics, and terpenoids. For example, some plants release exudates

that deter herbivorous insects or attract natural enemies of the pests. Furthermore, systemic acquired resistance (SAR) enables plants to enhance their defense mechanisms following an initial pest attack, resulting in a faster and stronger response to subsequent infestations.

Understanding these resistance mechanisms not only deepens our knowledge of plant ecology but also informs the development of sustainable pest management strategies that reduce dependence on chemical pesticides (Jansen et al., 2020).

Utilizing plant-resistant cultivars is a socially acceptable, commercially viable, and ecologically sustainable approach to pest management. In host plant resistance research and breeding programs, resistant plant materials can be directly employed during variety testing before their recommendation or release. However, previous studies have predominantly focused on general pest resistance, leaving a significant gap in understanding how specific maize genotypes influence the life cycle and dynamics of particular pests.

Traditional and hybrid maize varieties differ in their genetic backgrounds, often resulting in varying levels of resistance to pests and diseases. Research has highlighted that traditional maize varieties frequently exhibit unique traits that contribute to host plant resistance, such as the increased expression of defensive compounds and structural barriers that deter herbivores (Fritten and Fischer, 2021). In contrast, hybrid maize varieties, though developed for enhanced yield potential, may exhibit higher susceptibility to certain biotic stresses. This dichotomy underscores the need to evaluate how traditional and hybrid maize types can be strategically integrated into agricultural practices to improve resilience against biotic stressors.

The central hypothesis of this research posits that traditional maize varieties demonstrate greater host plant resistance compared to hybrid varieties. Testing this hypothesis will offer valuable insights into sustainable pest management strategies. Consequently, this study investigates the interactions between maize genotypes and the maize stem borer under laboratory conditions. By assessing the impact of these genotypes on the growth and reproduction of the pest, this research aims to contribute to more effective agricultural strategies that enhance crop resilience while reducing pest populations, ultimately supporting sustainable maize production systems.

## MATERIALS AND METHODS

### Study area

The present study was conducted in the Laboratory of Pesticide Toxicology and Application Technology at the Department of Entomology, Faculty of Crop Protection, Sindh Agricultural University, Tandojam. The research aimed to assess the effects of different maize varieties on the development and reproduction of the maize stem borer, *C. partellus*, under laboratory conditions.

### Experimental design

The experiment was designed as a Completely Randomized Design (CRD) with three replications, carried out under laboratory conditions. The treatments were as follows:

**T1:** Conventional Maize (*Azam*)

**T2:** Hybrid Maize (*Pioneer-3025*)

### Insect culture and rearing in a controlled environment

A laboratory colony of *C. partellus* was established by collecting eggs from maize fields at Latif Farm, Sindh Agricultural University, Tandojam, during the summer of 2024. The specimens were reared in the Laboratory of Pesticide Toxicology and Application Technology under controlled conditions ( $24 \pm 2^\circ\text{C}$ ,  $75 \pm 5\%$  relative humidity, and a photoperiod of 16:8 L h).

### Development of *C. partellus* culture on maize varieties

Plastic containers (20 × 8 × 8 cm) with aerated lids covered with muslin fabric were used for rearing the neonate larvae. Freshly hatched larvae were carefully transferred to fresh maize cobs, leaves, and stubbles using a fine, wet camel hairbrush. These materials were replaced regularly.

Pupae were transferred to Petri dishes lined with tissue paper and later moved to plastic containers (15 × 10 cm) with butter paper lining. Adult *C. partellus* used the butter paper as a substrate for egg deposition. A 15% honey solution, soaked into a cotton swab placed in a 5 ml penicillin bottle, was provided as an adult food source. To simulate natural photosensitivity, the containers were kept in the dark, covered with a black cloth. Butter paper was regularly checked for deposited eggs, which were then collected and maintained under controlled laboratory conditions.

The newly emerged neonate larvae were separately transferred to conventional and hybrid maize cobs and leaves to initiate the F1 generation.

### Developmental observations

Following the establishment of the first-generation culture, the life stages of *C. partellus* were studied on both conventional and hybrid maize. Observations were recorded for egg incubation period, larval duration across all instars, pupal duration, adult male and female lifespans and total life span of *C. partellus*.

### Egg observation

The number of eggs laid by each female was recorded until their death. The hatching rate of eggs was calculated using the following formula:

$$\text{Egg hatching percent} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

### Larvae

The larval development of *C. partellus* was observed across six distinct instars. The duration of each instar was recorded, reflecting the number of days required for the larvae to develop to the pupal stage.

### Pupae

A total of 60 pupae were used to study the pupal period in both maize varieties. The overall mean duration of the pupal stage was calculated.

### Adults

Five pairs of male and female moths were housed in plastic containers, each provided with a 15% honey solution, to study adult longevity and behavior.

### Total life cycle

The total lifespan of *C. partellus* was recorded by summing the durations of each developmental stage. The mean duration for the entire life cycle was also calculated.

### Effects of conventional and hybrid maize on fecundity and fertility of *C. partellus*

To evaluate fecundity and fertility, a minimum of five pairs of adults were provided with a 15% honey solution. Fecundity was assessed by counting the total number of eggs laid by each female during the first 10 days after oviposition. Fertility was determined by the hatch rate of the eggs, measured as the proportion of eggs that hatched following the initial oviposition.

### Statistical analysis

The proportion of eggs that hatched was used to determine female fertility. Replication data for each parameter were pooled, and the maximum and minimum values were calculated. The results for conventional and hybrid maize varieties were compared. Finally, the data were analyzed using a paired t-test, and the means were compared via correlation using Statistix 8.1 statistical software.

## RESULTS

### Duration of developmental stages of *C. partellus* reared on conventional maize

The results indicated that all developmental stages of *C. partellus*, from egg to adult, performed poorly when reared on a hybrid maize variety compared to conventional maize under laboratory conditions. The duration of the life stages of *C. partellus* reared on conventional maize is presented in Table 1.

The findings showed that the total developmental period of *C. partellus* on conventional maize ranged from 46.22 to 69.34 days, with a mean duration of  $57.78 \pm 11.56$  days. The incubation period varied between 6.11 and 8.33 days, with an overall mean of  $7.22 \pm 1.11$  days. The

study recorded six larval instars in total, with the larval duration ranging from 2.00 to 6.33 days. Among these, the shortest larval duration was observed in the 2<sup>nd</sup> instar ( $2.39 \pm 0.39$  days), while the longest duration was recorded in the 5<sup>th</sup> instar ( $5.56 \pm 0.56$  days).

The total larval period spanned 20.78 to 30.78 days, with an overall mean duration of  $25.78 \pm 5.00$  days. The pupal period ranged from 6.33 to 9.56 days, with an average duration of  $7.95 \pm 1.62$  days. Furthermore, the results revealed that female adult longevity was greater than that of males. Male adult longevity ranged from 5.89 to 9.78 days, with a mean of  $7.84 \pm 1.95$  days, while female adult longevity ranged from 7.11 to 10.89 days, with an average of  $9.00 \pm 1.89$  days.

Table 1. Duration of life stages of *C. partellus* reared on conventional maize in laboratory conditions.

Sr. No.	Developmental stage(s)	Minimum* (Days)	Maximum* (Days)	Mean $\pm$ S.E
1	Egg incubation period	6.11	8.33	$7.22 \pm 1.11$
2	Possible 1 <sup>st</sup> Instar Larva	3.78	4.44	$4.11 \pm 0.33$
3	Larval 2 <sup>nd</sup> Instar Larva	2.00	2.78	$2.39 \pm 0.39$
4	Stages 3 <sup>rd</sup> Instar Larva	3.67	5.45	$4.56 \pm 0.89$
5	period 4 <sup>th</sup> Instar Larva	4.11	6.33	$5.22 \pm 1.11$
6	5 <sup>th</sup> Instar Larva	5.00	6.11	$5.56 \pm 0.56$
7	6 <sup>th</sup> Instar Larva	2.22	5.67	$3.95 \pm 1.73$
8	Total larval period	20.78	30.78	$25.78 \pm 5.00$
9	Pupal Period	6.33	9.56	$7.95 \pm 1.62$
10	Adult Male Longevity	5.89	9.78	$7.84 \pm 1.95$
11	Adult Female Longevity	7.11	10.89	$9.00 \pm 1.89$
12	Total Life span	46.22	69.34	$57.78 \pm 11.56$

\* Represents the average mean of the three replications.

### Duration of developmental stages of *C. partellus* reared on hybrid maize

The results presented in Table 2 highlight the minimum and maximum durations (in days) for the developmental stages of *C. partellus* from egg to adult, along with the overall mean values. The total developmental period of *C. partellus* on hybrid maize ranged from 26.87 to 40.99 days, with an overall mean of  $33.93 \pm 7.06$  days. The incubation period varied between 3.22 and 5.22 days, with an average of  $4.22 \pm 1.00$  days.

For the larval stages, the duration of individual instars ranged from 1.22 to 3.89 days. The second larval instar recorded the shortest duration of  $1.50 \pm 0.28$  days, while the fifth instar had the longest duration of  $3.45 \pm 0.44$  days. The total larval period spanned 12.55 to 17.89 days, with an average duration of  $15.22 \pm 2.67$  days.

The pupal period ranged from 3.33 to 5.00 days, with an overall mean of  $4.17 \pm 0.84$  days. Female adult longevity was observed to be longer than that of males. Male adult

longevity ranged from 3.33 to 5.33 days, with a mean of  $4.33 \pm 1.00$  days, whereas female longevity ranged from 4.44 to 7.55 days, with an average of  $6.00 \pm 1.89$  days (Table 2).

### Comparison of the biology of *C. partellus* reared on conventional and hybrid maize

Figure 1 presents a comparison of the developmental duration of *C. partellus* reared on stubbles, leaves, and cobs of conventional and hybrid maize under laboratory conditions. The graph indicates that the duration of each life stage, from egg to adult, was significantly shorter when *C. partellus* specimens were fed on hybrid maize compared to conventional maize. Furthermore, the results show that females emerging from caterpillars reared on conventional maize had a longer lifespan compared to males. A similar trend was observed in cultures fed on hybrid maize; however, the lifespan of both sexes was notably shorter than those reared on conventional maize.

Table 2. Duration of different developmental stages of *Chilo partellus* reared on Hybrid maize under laboratory conditions.

Sr. No.	Developmental Stage (s)	Minimum* (Days)	Maximum* (Days)	Mean±S.E	
1	Egg incubation period	3.22	5.22	4.22±1.00	
2	Possible Larval Stages period	First Instar Larva	1.89	2.78	2.34±0.45
3		Second Instar Larva	1.22	1.78	1.50±0.28
4		Third Instar Larva	2.33	3.33	2.83±0.50
5		Fourth Instar Larva	2.67	3.67	3.17±0.50
6		Fifth Instar Larva	3.00	3.89	3.45±0.44
7		Sixth Instar Larva	1.44	2.44	1.94±0.50
8		Total larval period	12.55	17.89	15.22±2.67
9	Pupal Period	3.33	5.00	4.17±0.84	
10	Adult Male Longevity	3.33	5.33	4.33±1.00	
11	Adult Female Longevity	4.44	7.55	6.00±1.56	
12	Total Life span	26.87	40.99	33.93±7.06	

\* Represents the average mean of the three replications.

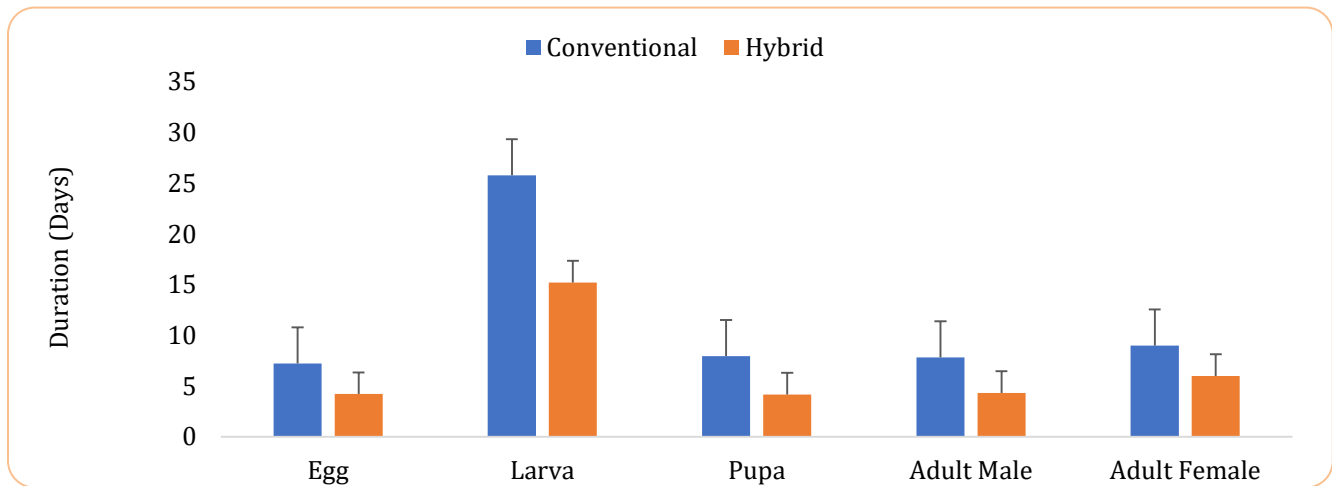


Figure 1. Comparison of the life cycles of *Chilo partellus* reared on conventional and hybrid maize under laboratory conditions.

**Reproductive traits (fecundity and fertility) of *C. partellus* reared on conventional maize**

The reproductive data presented in Table 3 indicated that the highest fecundity was observed when *C. partellus* was reared on conventional maize plant parts. In contrast, the lowest fecundity was recorded in females reared on hybrid maize plant parts. Specifically, Table 3 showed that the overall mean fecundity in the conventional maize trial was 604 ± 104.67 eggs, with a range of 500.11 to 709.44 eggs per female. On the other hand, the mean fecundity of *C. partellus* reared on hybrid maize ranged between

207.44 and 397.56 eggs, resulting in an overall mean of 302 ± 95.06 eggs, which was significantly lower than that observed on conventional maize.

Furthermore, the reproductive performance results indicated that egg fertility was significantly higher when *C. partellus* was reared on conventional maize plant parts compared to hybrid maize plant parts. The highest overall mean fertility percentage (55.44 ± 1.81) was recorded on conventional maize, whereas the lowest (24.61 ± 1.77) was observed when *C. partellus* was fed on hybrid maize plant parts (Figure 2).

Table 3. Mean Fecundity of *C. partellus* reared on conventional and hybrid maize varieties under laboratory conditions.

Treatments	Minimum*	Maximum*	Mean ± S.E
Conventional	500.11	709.44	604 ± 104.67
Hybrid	207.44	397.56	302 ± 95.06

\* Represents the average mean of the three replications.

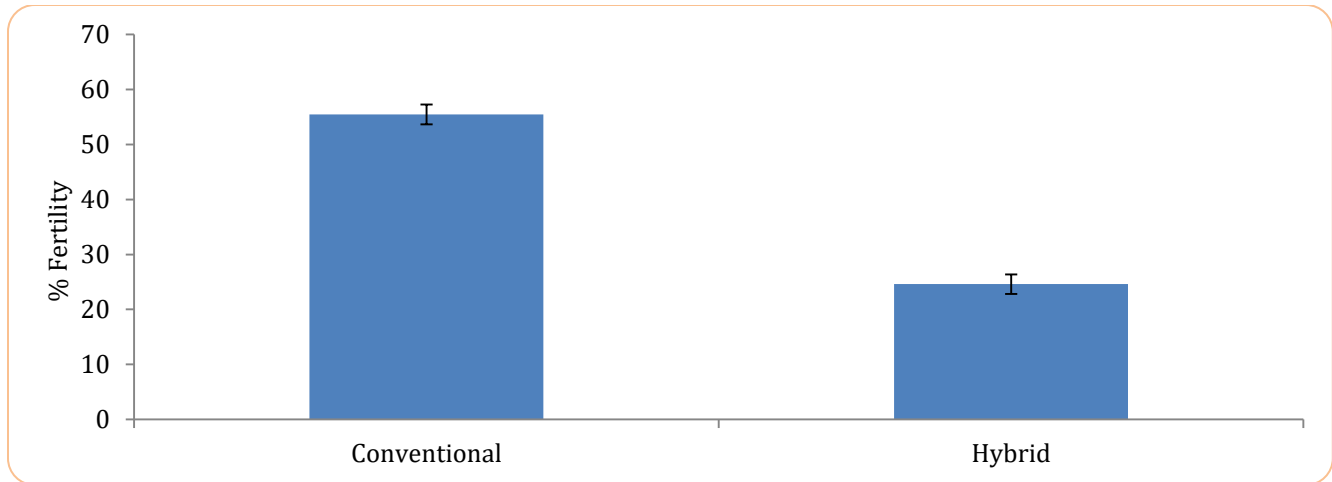


Figure 2. Comparison of egg fertility percentage in *C. partellus* reared on hybrid and Conventional maize under laboratory conditions.

## DISCUSSION

After wheat and rice, maize is one of the most important food and fodder crops globally (Kumar and Ashwani, 2017). It serves as a primary staple in many tropical and subtropical nations. However, maize production is significantly impacted by the attack of various insect pests, particularly the stem borer *C. partellus*. Despite steady annual increases in maize yield, productivity remains suboptimal due to pest infestations (Prakash et al., 2017). Yield losses of up to 75% have been attributed to *C. partellus* alone (Shahid et al., 2018). The infestation typically begins when adult females lay eggs on the maize leaves (Ajala and Saxena, 1994). Given the importance of maize and the substantial damage caused by the maize stem borer, this study aimed to examine the biological cycle of *C. partellus*, encompassing its egg, larval instars, pupal, and adult stages, along with reproductive traits such as fecundity and egg fertility.

Hybrid and conventional maize varieties exhibit notable differences in growth potential and yield performance. Hybrid maize often outperforms conventional varieties due to its enhanced adaptability to varying environmental conditions and its selection for desirable traits, such as improved resistance to pests and diseases, as well as superior drought tolerance (Chen et al., 2020). In contrast, conventional maize varieties offer greater genetic diversity and natural resilience to local pests and diseases, making them well-suited for specific agroecological zones and organic farming systems (Groote and Kanampiu, 2018). However, hybrid maize typically demands higher inputs and intensive

management practices, while conventional varieties are more suitable for low-input agricultural systems. Ultimately, the choice between hybrid and conventional maize depends on factors such as environmental conditions, farming practices, and market demands.

The current study was conducted under laboratory conditions, focusing on both conventional and hybrid maize varieties. Similar research has been undertaken to enhance understanding of *C. partellus* biology. Siddalingappa et al. (2010) conducted extensive investigations into the lifecycle and behavior of the pest, providing a critical foundation for pest management. Furthermore, Saranya and Samiayyan (2017) explored the biological characteristics and control measures for *C. partellus*, contributing significantly to existing knowledge. Khokhar et al. (2019) further examined various aspects of *C. partellus* biology and its interactions with maize crops, emphasizing the urgent need for effective management strategies.

The present study revealed that the total developmental period of *C. partellus* on conventional maize ranged from 46.22 to 69.34 days, with an overall mean of  $57.78 \pm 11.56$  days. Female adult longevity was greater than male adult longevity, with males living between 5.89 and 9.78 days (mean:  $7.84 \pm 1.95$  days) and females living between 7.11 and 10.89 days (mean:  $9.00 \pm 1.89$  days). In contrast, the total developmental period of *C. partellus* on hybrid maize ranged from 26.87 to 40.99 days, with an overall mean of  $33.93 \pm 7.06$  days. Similarly, female adult longevity on hybrid maize exceeded male adult longevity, with males living between 3.33 and 5.33 days (mean:  $4.33 \pm 1.00$  days) and females living between

4.44 and 7.55 days (mean:  $6.00 \pm 1.89$  days).

These results align with the findings of Panchal and Kachole (2013), who reported that *C. partellus* could complete its life cycle in 35 to 42 days when fed on a maize-based diet, including an incubation period of 5 to 6 days, a larval period of 28 to 35 days, a pupal period of 7 to 10 days, and adult longevity ranging from 3 to 8 days for males and 3 to 7 days for females.

The comparative analysis of *C. partellus* biology showed that each developmental stage of the pest was shorter on hybrid maize than on conventional maize. Females from caterpillars reared on conventional maize lived longer than males, a trend also observed on hybrid maize, although both sexes had shorter lifespans on hybrid maize. These findings corroborate those of Siddalingappa et al. (2010), who reported similar developmental durations for *C. partellus*.

The results of the study for reproductive traits, such as fecundity and egg fertility, revealed that the highest fecundity was observed when *C. partellus* was reared on conventional maize plant parts. Conversely, the lowest fecundity was recorded for females reared on hybrid maize plant parts. The highest overall mean fecundity of 604 eggs per female was recorded on conventional maize, ranging from 500.11 to 709.44 eggs. In contrast, mean fecundity on hybrid maize ranged from 207.44 to 397.56 eggs, with an overall mean of  $302 \pm 95.06$  eggs, which was significantly lower than on conventional maize. These results partially align with those of Kega et al. (2008), who reported a mean fecundity of 278.6 eggs per female for *C. partellus* reared on an artificial maize leaf powder-based diet.

Furthermore, the results indicated that egg fertility was significantly higher when *C. partellus* was reared on conventional maize plant parts compared to hybrid maize. The highest overall mean fertility rate of 55.4% was recorded on conventional maize, while the lowest overall mean fertility rate of 24.6% was observed on hybrid maize. These findings are partially consistent with those of Songa et al. (2013), who reported that *C. partellus* adults reared experimentally exhibited a fecundity of 150 eggs per female, an incubation period of 4 to 5 days, a larval stage of 30 to 32 days, and a pupal stage of 8 days.

## CONCLUSIONS

The study concluded that the hybrid maize variety "Pioneer-3025" was effective in reducing the developmental duration of *C. partellus*. Females of this

pest laid fewer eggs, and a lower percentage of egg fertility was observed compared to the conventional maize variety "Azam". The results also indicated that the overall lifespan of *C. partellus* was significantly longer on conventional maize than on hybrid maize. These findings suggest that the shortened developmental period and reduced pest biology on hybrid maize could be attributed to its genetic and chemical properties, which influenced *C. partellus* development under laboratory conditions.

Based on these results, it is recommended that the hybrid variety "Pioneer-3025" be cultivated in field conditions to help suppress the population of *C. partellus*, as it exhibits genetic traits that hinder the development and reproductive capacity of the pest. In addition to summarizing the key findings, field trials should be conducted to confirm the laboratory results and further investigations into the specific biochemical and molecular mechanisms underlying the resistance of the hybrid maize variety would deepen our understanding.

## AUTHORS' CONTRIBUTIONS

KHD and KS highlighted the problem and designed the *in vitro* studies; KHD, KS, KF and AL conducted research trials, and arranged data; DMS and AA fed the diet to *C. partellus*, KHD and WAC wrote the manuscript; KHD and AL analyzed the data; JH proofread the manuscript.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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