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Resistance Mechanism Exhibited by Selected Maize Varieties to Asian Corn Borer *Ostrinia furnacalis* Guenee (Lepidoptera: Crambidae), Philippines

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ABSTRACT

The resistance mechanisms exhibited by the five selected maize varieties against *Ostrinia furnacalis* [Gueene] or the Asian corn borer (ACB) were identified and evaluated in the study in terms of the insects' percent mortality and development. The detached leaf-feeding assay was used in the experiment and laid out in Complete Randomized Design (CRD) with 5 treatments and replicated three times. Findings among the five selected maize varieties tested against ACB, Bt corn had exhibited a high degree of antibiosis mechanism. This was expected because of the Bt toxin from the maize variety conferring toxicity to Lepidopterous insect pests like ACB. A similar result with Variety 4 (Yellow Dent corn) was observed which shows a high degree of antibiosis mechanism that is innate within the maize leaves of the variety. Variety 1 (UPLB Lagkitan), Variety 2 (Yellow corn), and Variety 3 (White corn) show high susceptibility to ACB. Tolerance of the corn varieties to ACB needs to be further investigated because it is unknown to date whether the varieties possess or lack plant tolerance characteristics. Leaf whorl feeding assays used in the study only measure the antibiosis and antixenosis mechanisms in maize. Furthermore, a test to measure tolerance mechanisms is suggested among the maize varieties.

INTRODUCTION

Plants have ways of defenses to combat insect attacks (Chuang et al., 2014). These defenses are classified into two, direct or indirect defenses. Direct defenses involve toxic compounds or called antifeedant, which inhibits the insect pest from feeding on the plant, while the indirect defense involves excretion of herbivore-induced plant volatiles (HIPVs) that attracts the natural enemies of the pests' (Howe & Jander, 2008; Turlings & Erb, 2018). There are three general mechanisms of plant resistance against insects classified as antixenosis, antibiosis, and tolerance (Stout, 2013). Plant resistance to insect pests can be attributed to several factors, such as genetic, morphology, and chemical as well as its ecology. The genetic factor is resistance that is managed by inherited genetic traits. Morphological and chemical factors are resistance caused by the formation and presence of certain substances in plants that harm pests. Then, the ecological factor is the resistance influenced by the environmental factor (War et al., 2019).

Maize is one of the important staple cereal crops in the world. Countries in the continents of Africa, Latin America, and South Asia primarily depend on maize as a source of nourishment (Kaul, Jain, & Olakh, 2019). In the Philippines, maize is considered a staple crop along with rice (*Oryza sativa*). Generally, there are two color types of maize purposely planted in the Philippines. The white maize is for human consumption and 15% of the total country's population considers it as their staple food commonly from the Visayas and Mindanao regions. On the other hand, Yellow maize is cultivated mainly as a source of raw material for animal feed (Labios & Malayang, 2015). Based on the standing crops, the January to March maize production was estimated to be 2.40 million metric tons which are reduced by 0.9 percent in 2019 with 2.43 million metric tons (PSA, 2020). Different factors contribute to the decrease in the production of maize. One of these is the *Ostrinia*

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furnacalis, a maize insect pest infesting all stages of maize development, damaging the stem, cobs, and male flowers.

The selected test entries identified were open-pollinated varieties acquired from the Institute of Plant Breeding (IPB) through the National Plant Genetic Resources Laboratory (NPGRL). The accessions were coded as Variety 1 (UPLB Lagkitan), Variety 2 (Yellow corn), Variety 3 (White corn), Variety 4 (Yellow Dent corn), and Variety 5 (BT Corn). Variety 3 (White corn) is a white flint type dubbed as Quality Protein Maize (QPM) bred purposely for corn grits as a substitute for rice with higher lysine content than the ordinary corn. Variety 2 (The Yellow corn) is a single cross yellow hybrid with tolerance to ACB and resistant to downy mildew. Variety 4 (Dent corn) is a yellow dent type bred for animal feed, while Variety 1 (Lagkitan) is an opaque white glutinous variety bred for green corn. Variety 1 is known to be highly susceptible to the corn borer, while Variety 4 (Yellow Dent corn) and Variety 3 (White corn) were resistant to lodging and moderately resistant to ACB. GM corn with innate insect resistance and herbicide tolerance traits was used as the resistant control check in the experiment. The GM corn hybrid is commercially available and purchased from a local agricultural farm supply. The effect GM corn hybrid to ACB larvae is when the larvae ingest leaves of the Bt corn, the Cry toxin is activated due to proteolytic enzymes that are present in the alkaline environment of the insect's digestive tract. The activated toxin binds to specific receptors that are only present on susceptible insects such as ACB. The binding of the toxin causes swelling of the cells that continues until the cells lyse or burst. Then alkaline gut juices of the insect leak to the hemocoel that causes the pH of hemolymph to increase. This increased pH results in paralysis of the insect leading to its death (Soberón et al., 2010).

In the Philippines, the damage of *O. furnacalis (*ACB) varies from 20 to 80% (Nurkomar & Santoso, 2014), and the yield loss may reach 100% during the wet season (Caasi-Lit et al., 2018). The length of the *O. furnacalis (*ACB) life cycle depends on the host, diet, and weather conditions as the number of instars varies due to the host season and its overall growth condition (Nafus & Schreiner, 1991). Generally, *O. furnacalis* ACB has 5 to 6 instars larval stage lasting for 14-20 days. The larval infestation usually begins at four weeks of corn after planting. The first instar larvae feed on the leaves, causing pin-head size holes, and that the second to the early fourth instar causes match head-size holes. The third instar larval stage causes the greatest damage due to feeding on the silk of the developing corn ear and later boring into the developing cobs. Currently, there are methods identified for the control of *O. furnacalis*. Chemical systemic insecticides were used over localized insecticides due to the *O. furnacalis* damage and behavior. However, the reliance and improper application of these pesticides lead to safety issues and the development of insect pest resistance. Other methods of control such as biological, cultural, and plant breeding techniques for resistant corn cultivars offer safer and more reliable *Ostrinia furnacalis* management. Javier & Morallo-Rejesus (2004) used *Trichogramma spp.* a parasitoid, and found it effective as a biological control for *O. furnacali* in the Mindanao region.

Meanwhile, through selective breeding, the utilization of resistant varieties is considered an alternative action in combating insect pests that is more economical and ecological in minimizing pest damage. The reported occurrence of pest resistance in some insect pests had greatly influenced the breeding and utilization of this technology. According to Nafus & Schreiner (1991), the use of insect-resistance cultivars may slow down the rate of the development of resistance in the insect population. With the great importance of corn in the Philippines, high damage and yield loss inflicted by *O. furnacalis* and, at the same time, the availability of control tactics in corn that farmers can choose to overcome these damages by the pest is a crucial concern. There are limitations to the use of other control methods in corn and the urging use of resistant varieties as an alternative method in minimizing pest damage in crops is sensible. Here, we specifically aimed to identify the resistance mechanism exhibited by the five local maize varieties against *Ostrinia furnacalis* [Gueene] through laboratory assay and evaluated ACB performance in terms of mortality and development. This endeavor allows us to get more insight into the possible molecular and biochemical defenses against *O. furnacalis.*

MATERIALS AND METHODS

Time and Place of the Study

The study was conducted at the D110 Insect Physiology Laboratory of the Institute of Weed Science, Entomology and Plant Pathology (IWEP), College of Agriculture and Food Science, University of the Philippines Los Baños, College Laguna 4031 from August to December 2019.

Test Entries

The test entries used were open-pollinated varieties acquired from the Institute of Plant Breeding (IPB), University of the Philippines Los Baños, through the National Plant Genetic Resources Laboratory (NPGRL). The accessions were coded as follows: Variety 1 (UPLB Lagkitan), Variety 2 (Yellow corn), Variety 3 (White corn), Variety 4 (Yellow Dent corn), and BT Corn (*delta endotoxin)*.

Planting of Test Entries and Experimental Design

The test entries were planted at IWEP UPLB experimental station following the recommended rates of fertilizer and other agronomic practices for corn. Two seeds were planted per hill to ensure complete germination and thinning was done 14 days after planting (DAP). Regular irrigation and application of fertilizer were also done. The detached leaf-feeding assay was used and laid out in Complete Randomized Design (CRD), five (5) treatments were used and replicated three times.

Insect Collection and Rearing

The feral population of *O. furnacalis* was collected in the Field Experiment Station of IPB. The egg masses were collected in the cornfield 21 days after transplanting, mass rearing was done following the methods of Caasi-Lit et al. (2012) and with the recommended artificial diet. The F1 progeny of the feral population was used throughout the experiment to ensure a standardized test insect for the bioassay and artificial infestation.

Leaf Whorl Feeding Bioassay

Leaf Whorl feeding resistance to ACB was tested for the different varieties of maize under laboratory conditions at the Entomology Laboratory (Fig. 1). Leaves were collected from 30-45 DAP maize plants and brought to the laboratory. These were cut into leaf discs with an area of 3 cm. In every assay cup containing leaf sample were seeded one (1) newly 2^{nd} instar *O. furnacalis*. After seeding, it was moistened with distilled water, and placed in the Bioassay Room. Leaf samples were replaced every two days. A total of fifty (50) *O. furnacalis* was placed in every replicate. Larval survival was recorded for seven (7) consecutive days and computed as a percentage of larvae that survived over the total number of fifty larvae seeded.

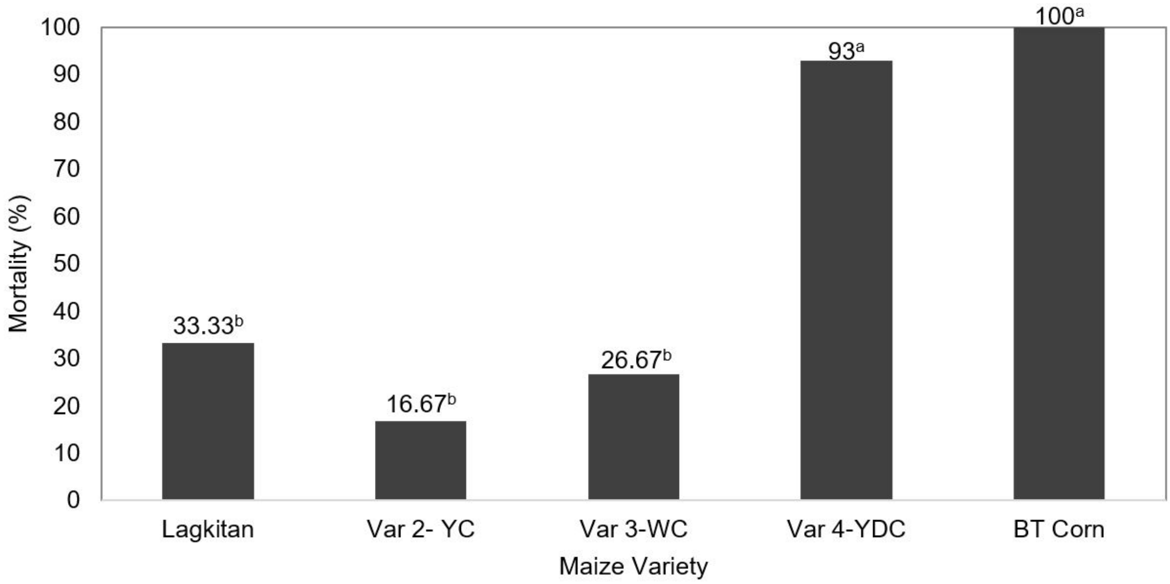
Development of *Ostrinia furnacalis* (Asian Corn borer)

The surviving larvae in the leaf whorl feeding bioassay were continuously reared using the same varieties of corn to reach the pupal stage by leaf replacement. Observations on the number of *O. furnacalis* adults and the total percentage of the successful pupa were recorded.

Percentage Mortality

The larval mortality was recorded for seven consecutive days and computed as a percentage. There were 10 larvae per treatment and replicated three times. A total population of 150 larvae were used for all the treatments. The larvae mortality was computed by dividing the number of dead larvae in every treatment by number of larvae that survived over the total number (30) of larvae seeded.

Fig. 1. (a) Detached leaves of Variety 1 (Lagkitan, Variety 2 (Yellow corn), Variety 3 (White Corn), Variety 4 (Yellow dent Corn), and BT Corn, (b) Leaf whorl placed in an assay cup for bioassay against Asian Corn Borer (ACB).



Remarks: Means with a column having similar letters are not significantly different at the 5% level by Least Significant Different (LSD).

Fig. 2. Percentage mortality of *Ostrinia furnacalis* on the different corn varieties after seven days of observation.

Initial Weight (g) and Final Weight (g)

A total of 150 2nd instar ACB were weighted as initial weight in analytical balance prior to the conduct of the Leaf Whorl Feeding Bioassay. All samples were seeded into each leaf sample inside the assay cup moistened with distilled water and observed for 7 consecutive days**.**

All larvae survived in the assay after 7 days was recorded and weighted using analytical balance and continuous to feed for three to four weeks until pupal stage.

Pupation and Adult Viability

Three to four weeks of observation was done in the study to obtain the total number of successful larvae that developed into pupal stage and emerged as adult per maize variety. Total number of pupa and adult were recorded after seven to nine days before the moth emerges.

Statistical Tools

The data were analyzed using a one-way analysis of variance (ANOVA) or Microsoft Excel using the analysis of variance (ANOVA) with a 5% level of significance. The Least Significance at 5% (LSD 0.05%) was used to compare treatments.

RESULTS AND DISCUSSION

Percent Mortality of Asian Corn Borer (ACB)

Antibiosis includes mechanisms which reduce life history traits such as mortality or survivability. Fig. 2 shows the comparison among treatment tested and indicated Bt Corn and Variety 4 (Yellow dent corn) to be highly resistant to the Asian corn borer with mortalities of 100% and 93%, respectively. Mortality of ACB for the Bt corn was expected to be 100% because of the Cry1Ab toxin trait that is toxic to Lepidopterous pests like Asian corn borer. On the other hand, Variety 4 (Yellow dent corn) is a yellow open-pollinated variety that displays resistance to lodging, has long ear length, and remains green during maturity. The assay recorded 93% ACB larval mortality after 7 days. There are currently no available studies tackling the resistance of Variety 4 (Yellow dent corn) to ACB. However, based on possible sources of resistance in corn to ACB, secondary metabolites may have a role in the plant defense from insect pests. The dominant benzoxazinoid hydroxamic acid in maize (*Zea mays* L.) is a novel class of alkaloids that possess 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one or DIMBOA. Secondary metabolites serve as important factors of resistance against insects, microbial diseases, and allelochemicals used in competition with others (Li et al., 2013). These alkaloids possessed strongly biological activity against various organisms such as European, Asian, and Southwestern corn borer. Plant age affects the levels of DIMBOA, with younger plants having high concentrations of DIMBOA compared to older plants.

In maize, benzoxazinoids toxicity and deterrence toward European corn borer, *O. nubilalis* has been extensively studied. The degradation of hydroxamic acids and N-O-methylated hydroxamic acids into toxic benzoxazolinones is facilitated by the alkalinity of the guts of lepidopteran insect pest (War et al., 2019). In a study conducted by Houseman et al. (1992), an artificial diet containing DIMBOA tested on the larvae of *O. nubilalis* showed high mortality rate and had shown influence on the developmental time of the insect during pupation.

Mean Initial and Final Weight (g)

To measure antibiosis as a mechanism of resistance in the different corn varieties, we measured the initial and final weight gain of ACB after seven days of observation through leaf-feeding assay. Result shows that the Lagkitan variety obtained the highest ACB mean weight gain (g), followed by Variety 3 (White corn) and Variety 2 (Yellow corn). Weight gain for ACB larvae feeding on Variety 4 Yellow Dent Corn, obtained the lowest percent weight gain of 18.06% (Fig. 3), suggesting the effects of plant metabolites such as DIMBOA in Variety 4 Yellow Dent corn in the larval nutritional status. This may be linked to benzoxazinoids toxicity because of the degradation of products such as benzoxazolinones that abbreviates insect digestibility by impeding the activities of chymotrypsin and trypsin. These two main enzymes are responsible for breaking down protein into smaller peptides that will eventually be absorbed through the lining of the small intestine. Maag et al. (2014) state that less consumption of maize leaves by the *O. nubilalis* was reported on account of the higher concentration of these compounds. Benzoxazinoids had also been reported as plant defensive traits against aphids which are piercing and sucking insect pests. Moreover, Lagkitan (33.33%), Variety 3 (White corn) (26.67%), and Variety 2 (Yellow corn) (16.67%) have shown high susceptibility reactions to Asian Corn Borer (ACB). It coincides with the report of the National Plant Genetic Resources Laboratory (NPGRL) that UPLB Lagkitan was highly susceptible to corn borer feeding damage. Variety 2 (Yellow corn) flint full-season single cross corn hybrid that is suitable for animal feed and other corn-based products. Variety 2 (Yellow corn) was the most susceptible variety based on the leaf-feeding assay.

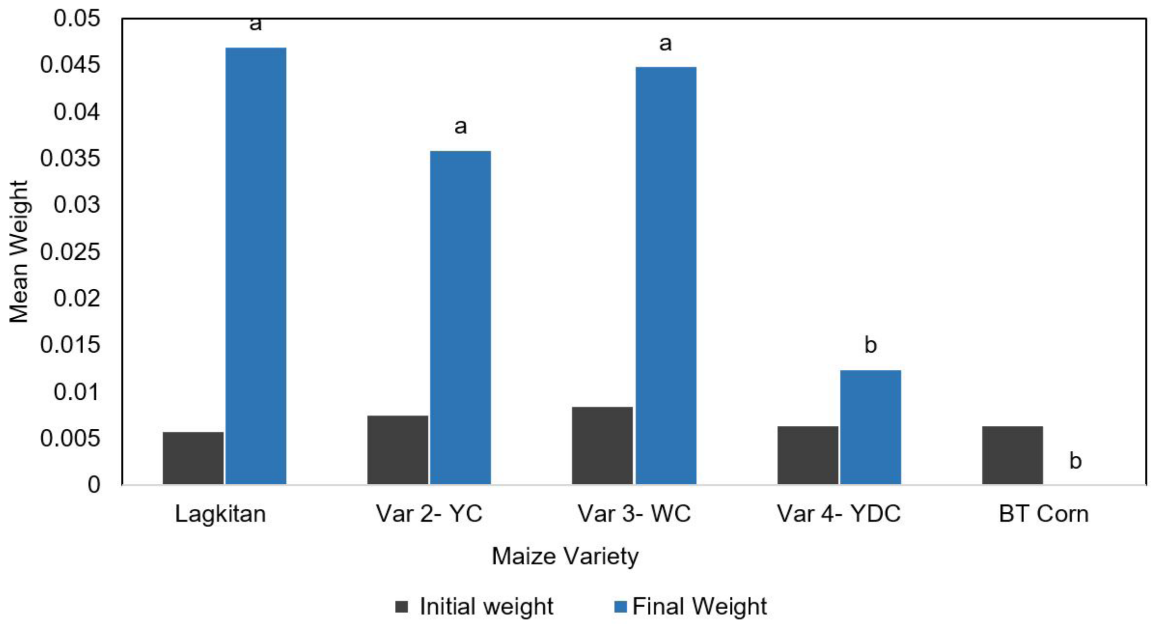


Fig. 3. Data on the Initial weight (g), Final weight (g) and Weight gain of *Ostrinia furnacalis* after seven days of leaf feeding assay using different maize varieties.

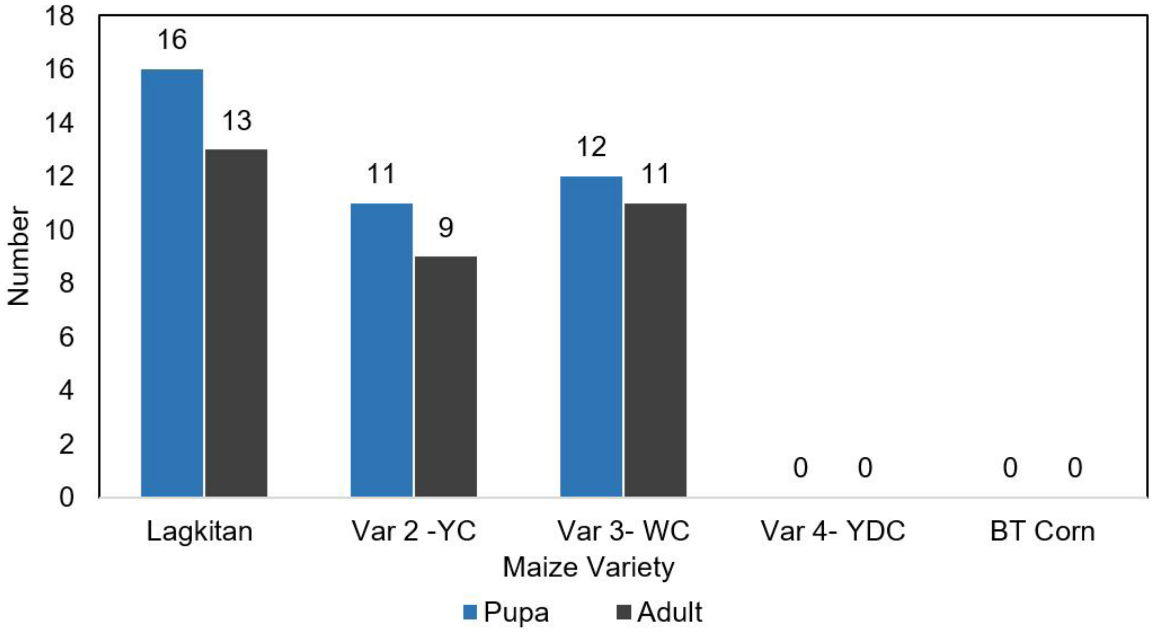


Fig. 4. Total number of *O. furnacalis* larvae that reached pupal stage and adult from the total 20 surviving larvae.

Based on literature, Variety 2 Yellow corn is a moderately tolerant variety that may not be detected based on mortality-based methods (Caasi-Lit & Fernandez, 2006). Tolerance refers to the ability of a host plant to resist or tolerate insect damage such that, under equivalent insect injury, economic traits (agronomic yield or quality) of tolerant plants were affected to a lesser extent than of plants lacking the ability to tolerate damage (Sandhu & Kang, 2017). There are currently no studies explaining the tolerance mechanism of Variety 2 (Yellow corn) to insect pest feeding specifically, on ACB feeding. Though, it is crucial to note the possible tolerance mechanism exhibited by Variety 2 (Yellow corn). Statistical analysis showed that Variety 4 (Yellow dent corn) and Bt corn were significantly different from Variety 2 (Yellow corn), Variety 3 (White corn), and Variety 1 (Lagkitan).

Lagkitan variety was a white glutinous open-pollinated corn variety that is known to be highly susceptible to ACB damage. This is commonly used in bioassay studies as their susceptible control. Variety 3 (White corn), a tall variety, confirms the trait for lodging resistance but this particular variety also needs to be checked for tolerance characteristics as lodging tolerance lends a certain degree of tolerance to Asian corn borer feeding in particularly on the stalk boring stages of the insect. Tensile strength of the stalk may provide antixenosis properties to this variety by providing a barrier to the stalk during feeding as exhibited by the lower number of larvae surviving to pupae and adult stages. According to Caasi-Lit et al. (2018), variability in tunnel length measurements is a useful character in determining ACB resistance, and this may be related to stalk toughness measured in tensile strength and fiber content. Variety 2 (Yellow corn) is a moderately tolerant variety that may not be detected based on mortality-based methods (Caasi-Lit & Fernandez, 2006). As statistical analysis shows that the Lagkitan, Variety 3 (White corn) and Variety 2 (Yellow corn) are comparable to Variety 4 (Yellow dent corn) and Bt corn based on the weight gained during the duration of the study. To verify the existence of tolerance mechanism occurring in Variety 2 (Yellow corn), against ACB feeding further experiments could be considered such as photosynthetic activity measurement, chlorophyll content in leaves, and phenology. The increase in photosynthetic activity of the plant can be considered as a tolerant mechanism when insects feed. Also, the association of ACB feeding in increasing photosynthetic activity in Variety 2 Yellow corn must be established. There was minimal weight gain in Variety 4 (Yellow dent corn), while no weight gain was recorded for Bt corn since there were no surviving individuals.

Number of Pupa and ACB Adult

We looked on the pupal development and exclusion to adult as additional parameters in checking the effect of the different maize varieties on ACB viability. Among the varieties, Variety 1 (Lagkitan) obtained the highest number of larvae that reach the pupal stage and adult stage with a total number of 16 and 13, respectively (Fig. 4).

These were followed by Variety 2 (yellow corn) (12 pupae; 11 adults), Variety 3 (White corn) recorded the lowest number of pupal, 11 and 9 on the adult stage. Moreover, Variety 4 (Yellow dent corn) and BT corn showed zero results because of the 100% mortality during the leaf whorl feeding bioassay after seven days of observation. The quality of the food source is a crucial factor for the growth and development of any insect. On the study done by Shi, Lu, Shi, & Yang (2011) on the effect of insect resistant transgenic maize on *O. furnacalis* growth and development, they have shown that ACB neonates fed transgenic maize were not able to complete their life cycle due to a high percentage of mortality. However, ACB that was able to pupate shows smaller in size and weight for about 10 to 14 mg lower than the control. The adult that successfully emerged had manifested low fecundity. As viewed by the authors, Wang et al. (2009) reported that transgenic Bt cotton had a significant effect on the growth of cotton bollworm by inhibiting the nutrient utilization of the insect.

CONCLUSION

Among the Maize varieties tested, Bt corn and Variety 4 (Yellow Dent Corn) exhibited a high degree of antibiosis mechanism of resistance based on the mortality data. We suspect that BT corn efficacy was based on the presence of Bt toxin (delta endotoxin). Variety 4 expresses its innate antibiosis mechanisms found within its leaves. Variety 1 (Lagkitan), Variety 2 (Yellow corn), and Variety 3 (White corn) were highly susceptible to ACB. The leaf-disc feeding assay was limited only in measuring the antibiosis recommend mechanisms from the maize varieties. The study recommends further experiments to measure the antixenosis, and tolerance mechanisms specifically for Variety 2 (Yellow corn). Measuring the amount of DIMBOA from the different corn varieties can be also be performed and to correlate its effect on the development of *O. furnacalisi*.

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