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Effect of Diet Containing Genetically Modified Maize on *Drosophila melanogaster*

Olusegun Emmanuel Ogundele

Department of Biological Sciences,
Tai Solarin University of Education,
Ijagun,
Ogun State.

Email: ogundeleoe@tasued.edu.ng

Abstract
The widespread use of genetically modified (GM) crops has raised concerns about their potential effects on non-target organisms. Drosophila melanogaster, commonly called fruit fly, serves as an ideal model for assessing toxicological impacts due to its short life cycle and sensitivity to environmental changes. This study was designed to investigate the effect of GM maize on the reproductive capacity and developmental outcomes of D. melanogaster, focusing on fecundity, pupation rate, and adult emergence. Flies were reared under controlled laboratory conditions and divided into four groups: a control group fed with non-GM maize and four experimental groups fed with diets containing 0.5g, 1g, 2.5g and 5g of GM maize, respectively. Observations were made on the number of eggs laid, the progression of larvae to pupae, and the emergence of adult flies. Results show significant decline in all measured parameters with increasing concentrations of GM maize. The control group had the highest adult emergence rate of 34.1%, while the 5g GM maize group showed a reduced emergence rate of 24.3%. Fecundity and pupation rates also declined progressively with higher GM maize exposure. These findings suggest that high intake of GM maize may adversely affect the reproductive success and development of fruit flies. The results raise important questions about the long-term effects of GM crops on insect populations and highlight the need for further ecological and toxicological studies.

Keywords: *Drosophila melanogaster*, Genetically Modified, Diet, Maize

INTRODUCTION

The advent of genetically modified organisms (GMOs) has revolutionised agricultural practices, particularly in the cultivation of maize, which is one of the most widely grown crops globally (Hamdan *et al*., 2022). Genetically modified (GM) maize, particularly those engineered to express insecticidal proteins from *Bacillus thuringiensis* (*Bt*), has been developed to enhance pest resistance and reduce reliance on chemical pesticides (Zhang *et al*., 2019). While the benefits of GM maize in agricultural productivity are well-documented, concerns regarding its ecological impact and potential effects on non-target organisms remain a significant area of research. Among the various model organisms used to study the effects of GMOs, *Drosophila melanogaster* has emerged as a prominent choice due to its genetic tractability, short life cycle, and well-characterised biology (Adekoya & Ogundele, 2013; Miller *et al*., 2023). The use of *Drosophila melanogaster* as a model organism allows researchers to investigate the physiological and genetic responses to dietary components, including those derived from GM maize. Studies have shown that dietary composition can significantly influence various biological processes, including metabolism, reproduction, and behaviour in *Drosophila* (Kumari *et al*., 2023). The introduction of GM maize into the diet of *Drosophila*

**Author for Correspondence*

provides a unique opportunity to assess the potential impacts of transgenic crops on insect physiology and ecology, particularly in terms of metabolic health and reproductive success.

Research has indicated that the consumption of GM maize can lead to alterations in metabolic pathways in *Drosophila* (Mesnage *et al.*, 2016). For instance, a study demonstrated that diets containing GM maize can affect the expression of genes involved in lipid metabolism and energy homeostasis (Hu *et al.*, 2020). These metabolic changes are critical, as they can influence the overall fitness of the organism, including its ability to survive and reproduce in varying environmental conditions (Koyama *et al.*, 2020; Rodrigues *et al.*, 2024). Furthermore, the interaction between diet and genetic background in *Drosophila* has been shown to modulate responses to dietary components, highlighting the importance of genetic diversity in understanding the effects of GM diets (Rohde *et al.*, 2021).

In addition to metabolic effects, the consumption of GM maize has been linked to changes in the gut microbiota of organisms, for example, in broiler chicken (Czerwiński *et al*. (2017), and *Drosophila's* gut microbiota have been shown to be sensitive to dietary changes, including the ones induced by GM maize consumption (Singh *et al*., 2024). The gut microbiome plays a crucial role in nutrient absorption, immune function, and overall health (Gale *et al.*, 2024). A recent study has shown that dietary components, including those from GM sources, can significantly alter the composition and diversity of gut microbiota in *Drosophila*, which in turn can affect host physiology and behaviour (Massey *et al.*, 2024). This interaction between diet and microbiota is particularly relevant in the context of GM maize, as the potential for altered microbial communities may have downstream effects on the host's health and fitness. Moreover, the ecological implications of GM maize consumption by *Drosophila* extend beyond individual health. The potential for transgenic traits to be transferred to wild populations through horizontal gene transfer raises concerns about the long-term ecological consequences of GM crops (Xu *et al*., 2021). Understanding how *Drosophila* responds to diets containing GM maize is essential for assessing the risks associated with the cultivation and consumption of GM crops in natural ecosystems.

Studies have explored the effects of GM maize on *Drosophila*, focusing on various aspects such as growth, reproduction, and behaviour (Belcher *et al.*, 2024; Sahu & Armstrong, 2024). For example, research by Sahu and Armstrong (2024), demonstrated that *Drosophila* fed a diet containing GM maize exhibited altered reproductive parameters compared to those on a non-GM diet. These findings suggest that the introduction of GM maize into the diet can have significant implications for the reproductive success of *Drosophila*, potentially affecting population dynamics in natural settings. In addition to reproductive effects, the impact of GM maize on locomotor activity and stress responses in *Drosophila* has also been investigated (Elias *et al*., 2021). Studies have shown that dietary components can influence locomotor behaviour, which is critical for foraging and mating success (Belcher *et al.*, 2024; Meunier *et al*., 2007; Shu *et al*., 2021). The ability of *Drosophila* to adapt to dietary changes is essential for its survival, and understanding how GM maize affects these behaviours can provide insights into the ecological consequences of GM crop cultivation.

Furthermore, the potential for GM maize to induce metabolic disorders in *Drosophila* has been a topic of interest. Research has indicated that diets high in sugar and fat can lead to insulin resistance and other metabolic syndromes in *Drosophila*, mirroring conditions observed in humans (Dhar *et al.*, 2018). The introduction of GM maize, particularly those with altered nutritional profiles, may exacerbate these conditions, leading to further health implications for the organism. The effect of diets containing genetically modified maize on *Drosophila melanogaster* encompass a wide range of physiological, ecological, and genetic factors. As research continues to elucidate the complex interactions between diet, genetics, and health in *Drosophila*, it is crucial to consider the broader implications of GM crop consumption on non-target organisms and ecosystems. This study aimed to contribute to the growing body of literature on the effect of GM maize on *Drosophila*, providing insights that may inform future agricultural practices and regulatory policies regarding GM crops.

MATERIALS AND METHODS

Fly collection

Drosophila melanogaster flies were collected from selected areas near the departmental laboratory at Tai Solarin University of Education, Ijebu-Ode, Ogun State. Sterile culture bottles containing fermenting bananas were placed overnight at strategic collection sites. By the following morning, the bottles, having trapped fruit flies, were sealed with foam plugs and transported to the laboratory for identification and sorting.

Fly separation

Insects in the sterile culture bottles were exposed to fumes of carbon (IV) oxide to anesthetise them and facilitate the separation of male and female *Drosophila melanogaster* according to the protocol of Ashburner (1989). The *D. melanogaster* flies were sexed based on morphological characteristics as previously described by Giwa and Adekoya (2017), and were introduced into vial bottles containing a banana-*Garri* medium prepared according to the method described by previous researchers (Williams & Akpabio, 1993).

Test substance

Tela maize (*SAMMAZ 75 T*) was obtained from the International Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Kaduna State. The grains were dried and ground into a fine powder, which was then incorporated into the Banana-*Garri* medium at various concentrations (weight/ volume): 0% (control), 0.5%, 1%, 2.5% and 5% by adding 1000 mL of distilled water to it. A 10 mL sample of each concentration was added to the banana-*Garri* medium following the method of previous researchers (Giwa & Adekoya, 2017; Williams & Akpabio, 1993).

Medium preparation

The medium was prepared by following previously described protocols (Giwa & Adekoya, 2017; Williams & Akpabio, 1993). Briefly, 1000 mL of distilled water, 250 g of banana, 20 g of agar, 5.5 mL of propionic acid and 55 g of *Garri* were used. A pot containing 1000 mL of distilled water was placed on a hot plate and brought to a boil. Once boiling, 20 g of agar was added and stirred until fully dissolved. The weighed banana was mashed using a glass mortar and pestle, added to the boiling mixture, and stirred continuously. After 13 minutes, *Garri* was added, and stirring continued for another 15 minutes. The pot was then removed from the hot plate, and 5 mL of propionic acid was immediately added to prevent mould formation.

Subsequently, 50 mL of the prepared medium was measured and added to 10 mL of each test substance concentration in separate jars. The resulting mixtures were then poured into pre-labelled vials using laboratory markers. In this medium, banana served as the nutrient source for the flies, while *Garri* and agar functioned as binding agents. To prevent contamination, the vials were placed in trays, covered with cheesecloth to prevent external flies from laying eggs, and left to cool. Once cooled, they were stored in a refrigerator pending the introduction of *Drosophila melanogaster*.

Experimentation

Both larval and adult feeding methods were employed. *Drosophila melanogaster* flies were crossed in a 1:3 male-to-female ratios and reared on media containing varying concentrations of the test substances (Giwa & Adekoya, 2017; Holsopple *et al.*, 2023). The flies were allowed to mate and lay eggs over a 6–8-day period, after which they were released into the wild. Developmental progression from larval to adult stages was observed and documented across all concentrations tested. The flies were reared at 25°C, while the control group was maintained on a medium without exposure to GM maize (Linford *et al.*, 2013).

Progeny analysis

The first filial generation (F1) progeny flies were anesthetised using carbon (IV) oxide fumes for the purpose of sexing, counting, and conducting morphological observations (Ashburner, 1989). This process continued until no further adult emergence (eclosion) was observed from the medium. Similar method was used for subsequent generations.

Statistical analysis

Data collected were recorded in a Microsoft Excel spreadsheet. Descriptive statistics, including frequencies, percentages, and means were conducted, while inferential statistics involving analysis of variance (ANOVA) was performed using the Statistical Package for Social Sciences (SPSS), version 22.

RESULTS

Survival of parent flies and mortality rate within treatment groups

Table 1 presents the activities of the parent flies in the vials after 7 days. There is an increase in the mortality rate as GM maize concentration increases. A total number of 14 flies died after 7 days. The control group had the lowest mortality, suggesting that non-GM maize supports optimum living conditions in *Drosophila* flies. In contrast, higher GM maize levels might have negatively impacted the lives of *Drosophila* flies. This reduction could be attributed to stressors induced by the GM maize, such as anti-nutritional factors or metabolic imbalances. This suggests that even moderate levels of GM maize in the diet may affect the physiological ability of *Drosophila* flies to lay eggs.

Table 1: Survival of parent flies and mortality rate within treatment groups

Treatment (GM maize)	Parent flies	Total dead	Dead males	Dead females
5g	31	5	3	2
2.5g	39	3	2	1
1g	54	2	1	1
0.5g	60	3	2	1
Control (0g)	71	1	1	0

Survival of the F1 generation flies

After 12 days, the parent flies had given rise to F1 generation as depicted in Table 2. Notable differences were observed between the GM diet treatment groups and the control group. While there was an increase in the number of flies seen in the control group, the GM diet groups declined. The control group increased by 128.17%; marginal increase was also observed in the flies exposed to 0.5 g GM maize treatment (113.33%) and those exposed to 2.5 g GM maize treatment (105.13%). Notably, parent flies exposed to the highest level of GM maize treatment (5 g) suffered significant decrease (70.97%) in number of F1 flies. Parent flies fed with 1 g of GM maize diet also suffered a decrease of 96.30%.

Table 2: Survival of the first filial generation (F1) flies

Treatment (GM maize)	Number of flies			Number of dead F1 flies		
	Parent	F1	F1 % of parent	Total	Female	Male
5 g	31	22	70.97	8	5	3
2.5 g	39	41	105.13	6	3	3
1 g	54	52	96.30	7	4	3
0.5 g	60	68	113.33	10	4	6
Control	71	91	128.17	11	8	3

Survival of the F2 generation flies

For the second filial generation of files, the control group increased by 162.64% after 23 days as shown in Table 3. Increase was also observed in the flies exposed to 0.5 g GM maize treatment (135.29%) and those exposed to 2.5 g GM maize treatment (129.27%). F1 flies exposed to the highest level of GM maize treatment (5 g) also increased, though the least, by 127.27%). F1 flies that were fed with 1 g of GM maize diet also increased by 130.77%).

Table 3: Survival of the second filial generation (F2) flies

Treatment (GM maize)	Number of flies			Number of dead F2 flies		
	F1	F2	F2 % of F1	Total	Female	Male
5 g	22	28	127.27%	10	7	3
2.5 g	41	53	129.27%	15	9	6
1 g	52	68	130.77%	11	7	4
0.5 g	68	92	135.29%	18	12	6
Control	91	130	162.64%	26	12	14

Table 4 shows the observations from the flies in the F1 and F2 generations in response to various treatments. Expectedly, the control group had high reproduction rate and the flies were very active with no visible sign of abnormal behaviour among the flies. The reproduction rate, larva count and level of activity were observed to decrease with increasing concentration of GM maize treatment in the diets.

Table 4: Observations in the first filial generation flies

Treatment groups (GM maize)	Observations in F1 and F2
5 g	Low reproduction, slow hatching of egg into larvae, slow development of larva into fly and less activity amongst flies.
2.5 g	Flies found at the food level, low larva count, sluggish and inactive flies.
1 g	Male flies seen at the top of the vial, low larva count, adult flies crawl at the food level and slow larva movement.
0.5 g	Flies were active within the vial, moderate larva count, more female flies and eggs found at the food level.
Control	High reproduction level, very active flies, larva moves towards the food level, no abnormal behaviour noticed

Effect of GM diet treatments on *Drosophila* development

Table 5 reveals a descending trend in the number of eggs laid as GM maize concentration increased. The control group had the highest fecundity, suggesting that non-GM maize supported optimal reproductive conditions. In contrast, higher GM maize levels impaired egg production. It is also noted that there was a high recorded increase in the fecundity level of the control group compared to the experimental groups. The significant ($p=0.041$) increase in total number of eggs laid, in the control group compared to the 0.5 g GM treatment group suggests that even moderate levels of GM maize in the diet may affect the physiological ability of *Drosophila* flies to lay eggs.

There was a high decline in the pupation rate of flies fed with GM maize. This suggests that the larvae might be experiencing stress and reduced viability, preventing them from reaching the pupal stage. Lower pupation rates are indicative of developmental interference. As shown in Table 6, the highest pupation rates were observed in the control diet group (80.5) while higher concentrations, 5 g (48.3), 2.5 g (55.2), 1 g (68.8) and 0.5 g (71.6) GM diet groups led to sharp decline.

The control group maintained a high adult emergence rate, while flies exposed to 5 g GM maize had notably low emergence percentages. The flies in 5 g had an emergence rate of 24.3% while the control groups had an emergence rate of 34.1%. In the highest GM group, the emergence was drastically reduced, indicating high lethality or sub-lethal impacts that prevent completion of development. This may reflect cumulative developmental toxicity over the larval and pupal stages, incomplete metamorphosis and emergence of weak adults may all contribute to this pattern.

Table 5: Effect of GM diet treatments on *Drosophila* development

Treatment with GM maize (g)	No. of F2 flies	Total eggs [a]	Avg. eggs [a]	Days to laying eggs	No. of larvae [a]	Pupation rate from larvae [a]	Adult emergence (%)
5.0	28	168	42	7	64	48.3	24.3
2.5	53	334	84	7	139	55.2	28.8
1.0	68	434	109	6	201	68.8	31.6
0.5	92	600	150	6	292	71.6	30.2
Control (0)	130	876	219	5	447	80.5	34.1

GM, genetically modified; No., number; Avg., average; [a] Significantly different by analysis of variance ($p<0.05$)

DISCUSSION

The study investigated the effect of diet containing genetically modified (GM) maize on the survival, reproduction, and development of *Drosophila melanogaster*. The results indicate that increasing concentrations of GM maize in the diet significantly impacted the survival rates of both parent flies and their progeny, with notable declines in reproductive success and developmental milestones. Specifically, the control group exhibited the highest survival and fecundity rates, while the highest concentration of GM maize resulted in the lowest survival and reproductive output. These findings suggest that GM maize may introduce stressors that adversely affect the physiological and ecological dynamics of *Drosophila* populations.

This present study observed increased mortality rate among parent flies exposed to higher concentrations of GM maize. The control group exhibited the lowest mortality, while the highest GM maize treatment group showed a marked increase in mortality. This aligns with previous research indicating that GM crops can introduce anti-nutritional factors or metabolic imbalances that may compromise the health of non-target organisms (Meissle *et al.*, 2022). The observed mortality rates suggest that even moderate levels of GM maize can impose physiological stress on *Drosophila*, potentially leading to reduced fitness and survival. Yaqoob *et al.* (2016), in their review of the ecological risks of *Bt* transgenic crops on non-target, plant-associated insects and soil organisms. consistently found no significant harmful effects associated with commercially approved *Bt* events on a wide range of non-target organisms, including pollinators, beneficial predators, rhizobacteria, soil fauna, and other ecological components. This broader evidence suggests that direct toxicity from *Bt* proteins is unlikely to account for the increased mortality observed in *Drosophila*. Instead, the present results may reflect subtle physiological stress or nutritional imbalances introduced by the GM maize diet, effects that lie outside the direct scope of *Bt*-specific risk assessments. Together, these insights underscore the importance of extending risk assessments beyond the direct action of *Bt* toxins to encompass the holistic nutritional and metabolic impacts of GM diets. The elevated mortality in the GM maize treatments may, therefore, stem not from the insect-resistant proteins themselves, but from altered nutritional profiles, digestion, or xenobiotic stress, none of which are typically captured in standard *Bt*-centric investigations.

The results of this study revealed that the control group produced significantly more offspring than those fed GM maize, with the F_1 generation from the control group showing the highest increase, while reproductive output in the highest GM maize group declined sharply. This aligns with findings by Sahu and Armstrong (2024), who reported that GM maize diets negatively affect reproductive parameters in Drosophila, likely due to physiological stress impairing egg-laying capacity. The observed decline in fecundity is further supported by Ng'oma *et al.* (2019), who demonstrated that Drosophila lifespan and fecundity are highly sensitive to dietary quality and genetic background, with significant genotype × diet interactions, suggesting that GM maize may impose both direct nutritional stress and gene-diet interactions that reduce reproductive success.

Complementing this, Semaniuk *et al.* (2018) showed that, within a fixed diet, variation in food consumption and reproduction did not trade off against lifespan, implying that the reduced fecundity and lower survival in our GM maize groups stem from between-diet imbalances or stressors inherent to the GM feed rather than energy allocation conflicts. Furthermore, the survival patterns in the F_2 generation reinforce the detrimental impact of GM maize, as control groups exhibited substantial increases while the highest GM maize group showed poor survival, echoing Kumari *et al.* (2023), who reported that dietary stress in parents can compromise offspring viability. Collectively, these findings indicate that GM maize diets impair reproductive success and offspring survival in Drosophila, with potential transgenerational consequences that raise concerns about the long-term ecological impacts of GM crop consumption.

Additionally, the study observed a significant decrease in the number of eggs laid as the concentration of GM maize increased. The control group had the highest fecundity, while the 5g treatment group exhibited the lowest egg production. This observation aligns with findings from previous studies that have indicated that dietary components can significantly influence reproductive output in *Drosophila* (Dhar *et al.*, 2018). The reduction in egg-laying capacity may be linked to the physiological stress induced by GM maize, which could disrupt hormonal pathways essential for reproduction. Accordingly, Nunes and Drummond-Barbosa (2023), demonstrated that a diet with high sugar content impaired female *Drosophila* fertility primarily by causing more death of premature germline cysts and vitellogenic egg chambers, rather than as a consequence of obesity. They further showed that supplementation with water could rescue fertility despite persistent obesity, implicating elevated glucose levels, rather than obesity, as a key mediator of impaired oogenesis.

The pupation rates detected in the present study also revealed a concerning trend, with the control group maintaining the highest rates while the GM maize groups experienced sharp declines. The highest concentration of GM maize resulted in a pupation rate of less than average, indicating that larvae may be experiencing stress that prevents them from reaching the pupal stage. This finding is consistent with research indicating that dietary stress can lead to developmental interference in *Drosophila* (Hu *et al.*, 2020). The reduced pupation rates observed in this study suggest that GM maize may negatively impact larval viability and development, potentially leading to decreased adult emergence rates. The adult emergence rates were significantly lower in the GM maize treatment groups compared to the control group. The emergence rate for the highest GM-diet group was only the least, indicating high lethality or sub-lethal impacts that prevent successful development. This observation aligns with findings from studies that have reported developmental toxicity associated with GM diets (Massey *et al.*, 2024). The reduced emergence rates may reflect the cumulative effects of dietary stress experienced during the larval and pupal stages, highlighting the potential for GM maize to disrupt normal developmental processes in *Drosophila*.

CONCLUSION

The findings of this study underscore the potential negative impact of diet containing genetically modified maize on the survival, reproduction, and development of *Drosophila melanogaster*. The observed trends in mortality, fecundity, and developmental success suggest that GM maize may introduce stressors that compromise the physiological health of *Drosophila* populations. These results contribute to the growing body of literature on the ecological implications of GM crops and highlight the need for further research to assess the long-term effects of GM maize on non-target organisms and ecosystems.

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