

Research Article

# Groundnut Host Plant and Vector Aphid (*Aphis craccivora*) Interaction in the Transmission of Groundnut Rosette Disease as a Basis for Physiological Studies

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## Abstract

Groundnut (*Arachis hypogaea* L.) is an important food crop in sub-Saharan Africa. Among the major causes for low yields is the susceptibility of cultivated varieties to the Groundnut Rosette Disease (GRD). The Groundnut Rosette is a viral disease, the most destructive where groundnut is grown, that can lead to 100% yield loss. The objectives of the study were to; 1) investigate the effect of leaf colour and plant architecture on aphid colonization; 2) determine the relationship between aphid colonization and disease development. An experiment was carried out in the glasshouse during winter of the 2018/19 and 2019/2020 growing seasons. Sixteen (16) test genotypes with known field reaction to the groundnut rosette disease were used. The results for disease severity concur with field ratings as all genotypes rated resistant had severity score of <1.39 and all genotypes rated moderate resistant, had scores <1 while susceptible genotypes recorded scores > 2.8. All susceptible genotypes had high aphid population (40 aphids on average per plant) whereby CG 7 had the highest. The results clearly show that, genotypes with dark green colour attracted more aphids (52.6) than the light green. Plant architecture may play a role in the migration of aphids within plants but does not influence plant preference by aphids. Genotypes ICGV-SM 01514, ICGV-SM 06637 and ICGV-SM 07544 attracted minimal number of aphids and were resistant to the rosette disease, a similar behaviour to ICG 12991, that is known to be aphid resistant. It can be concluded that these 3 genotypes are resistant to aphids. Genotypes ICGV-SM 01709, ICGV-SM 03710, ICGV-SM 08503 and ICGV-SM 01731 had considerable infestation by aphids (>40) but did not show any signs of the rosette disease, a trait that is common with ICGV-SM 90704, a variety resistant to groundnut rosette virus (GRV strain), an implication that they are resistant to the virus. The valuable results about these genotypes forms a basis for further characterization of these genotypes using molecular markers to understand the physiological basis of the varied reaction to vector and disease incidence. Sequencing the genome of the aphid species on groundnut is crucial to inform the diversity of the vector and give insights on how microbial effector proteins, host targets and plant immune receptors co-evolve.

## Keywords

Aphids, GRV, Genotypes, Characterization, Physiological

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## 1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil, food forage crop grown in many countries of sub-Saharan Africa. The average yields of 800 kg/ha is below potential and one of the reasons for low yields is the susceptibility of currently grown cultivars to the groundnut rosette disease (GRD) [8]. The Rosette disease is known to be endemic to sub-Saharan Africa and its off-shore islands, including Madagascar. There are two main forms of the disease; chlorotic rosette and green rosette described based on symptoms. The disease is caused by a complex of three agents: Groundnut Rosette Virus (GRV), and its satellite RNA (sat RNA), and Groundnut Rosette Assistant Virus (GRAV). The three agents of the disease must be present in the host plant for successful transmission by the vector [6, 7]. Disease symptoms are largely due to sat RNA and variants of sat RNA are responsible for the different forms of rosette. The symptoms occur in two predominant forms, chlorotic and green rosette although other symptomatic forms have been reported. The groundnut aphid, *Aphis craccivora*, is the principal vector of the disease. Rosette disease has been and continues to be responsible for devastating losses to groundnut production in Africa [11].

According to reviews by [12], there is evidence for behavioural response of aphids to colours. This was proved from an experiment where aphids were exposed to a number of illuminated papers. It was found out that there was a considerable number of probing made by aphids on differently coloured and illuminated paper with the highest in orange, yellow and green, and low on red, grey and blue. This was classified as evidence for colour vision in aphids. With an extensive series of experiments, it was further demonstrated the effect of coloured stimuli on aphid landing in the field. Pure yellow without UV showed the strongest attraction for winged aphids, with orange, yellow-green and green following, whereas the aphids responded with low landing rates to red, blue, purple, white, grey and black. Current knowledge on epidemiology with respect to the predominant colours for groundnut; green, dark green and light green as well the general plant architecture is very scanty and yet these may be important factor for attracting or promoting migration of aphids. Such information may help shape groundnut breeding programs or to form the basis designing comprehensive disease management strategies.

The study was premised on the fact that field observations showed that most resistant genotypes are Virginia which are

generally green to dark green in colour with bunched stem structures. The varieties that are light green in colour with an open stem architecture are Spanish or Valencia and often susceptible to groundnut rosette disease. However, whether these traits contribute to aphid attraction and eventually the observed disease reactions is not known. Further investigation is important to elucidate the doubts existing on host plant aphid relationship. Therefore, an experiment was carried out to; 1) investigate the effect of leaf colour and plant architecture on aphid colonization and 2) investigate whether aphid colonization translate into high disease incidence.

## 2. Materials and Methods

### 2.1. Test Lines

The experiment was established at ICRISAT Malawi glasshouses during winter of the 2018/2019 and 2019/2020 growing season. Sixteen (16) test genotypes with known field reaction to rosette of the three botanical groups of groundnuts; Virginia, Spanish and Valencia were obtained from the groundnut breeding program. These test lines included; CG 7, Chalimbana, JL 24, ICG 12991, ICGV-SM 01514, ICGV-SM 01709, ICGV-SM 01731, ICGV-SM 01739, ICGV-SM 03710, ICGV-SM 06637, ICGV-SM 06711, ICGV-SM 07544, ICGV-SM 07599, ICGV-SM 08503, ICGV-SM 90704 and ICGV-SM 99568. These varieties had also been evaluated under the Regional Groundnut Variety Trials (RGVT) with high rosette pressure using an infector row technique. Among them, six (6) of these materials had already been released in Malawi and often used as resistant (ICG 12991 (aphid resistant), ICGV-SM 90704 (GRV resistant) and ICGV-SM 99568 (moderate resistant)) and susceptible (JL 24, CG 7 and Chalimbana) checks. Each botanical group had genotypes classified as resistant, moderate resistant and susceptible using a 0-5 scoring scale where; 0-1.5 was resistant, 1.6-3.9 moderate resistant and 4-5 susceptible. These groups were further divided into two categories; 1) three groups based on the prevalent leaf colour for the groundnut varieties; light green, green and dark green and 2) the structural arrangement of the stems grouping them into bunch and open types (Table 1).

**Table 1.** Genotype characterization based on colour and architecture.

Colour			Stem structure	
Light green	Green	Dark green	Bunch	Open
ICGV-SM 01514	ICGV-SM 01739	ICGV-SM 07599	ICGV-SM 01739	ICGV-SM 01514
ICG 12991	ICGV-SM 90704	ICGV-SM 08503	ICGV-SM 90704	ICG 12991

Colour			Stem structure	
Light green	Green	Dark green	Bunch	Open
ICGV-SM 99568	ICGV-SM 01731	CG 7	ICGV-SM 01731	ICGV-SM 99568
JL 24	ICGV-SM 06637	ICGV-SM 03710	ICGV-SM 03710	JL 24
	ICGV-SM 07544	ICGV-SM 6711	ICGV-SM 06637	Chalimbana
	ICGV-SM 01709		ICGV-SM 01709	ICGV-SM 6711
	Chalimbana		CG 7	ICGV-SM 07544
			ICGV-SM 08503	ICGV-SM 07599

## 2.2. Experimental Layout and Aphid Inoculation

The experiments planted in a glasshouse were arranged in a Complete Randomized Design (CRD). There were three replications for each experiment. Each genotype had three pots per replication, three plants were planted in each pot, and eventually thinned to two (Figure 1). All test plants were flanked on one side with a row of JL 24, a rosette susceptible variety from the front of the bench to serve as the infector row technique used to transfer viruliferous aphids under field conditions. The infector row technique involves planting a test row of uninfected plants flanked on either side by a row of plants infested with aphids reared in glass house [3, 4]. This infector row technique is the only known method for rapid screening of large number of segregating populations and breeding lines for resistance to the groundnut rosette disease. Ninety-nine (99%) percent success rate in spreading the disease to susceptible plants has been recorded. Test plants were placed 30cm away from the infector row. Two weeks after emergence, the infector row (JL 24) was inoculated by placing three viruliferous aphids on each plant. There was no direct inoculation of aphids onto the test plants. Watering was done by pouring water straight onto the soil to avoid draining aphids off the plants.



Figure 1. Experiment site at ICRISAT Malawi.



Figure 2. Aphid colonization on test plants.

## 2.3. Management of the Experiment

Soils used in this experiment were collected from low lying virgin lands near Chitedze Research station and were sterilized via heat. Phosphorus fertilizer was applied as  $P_2O_5$  at the rate of 200kg/ha. Before inoculation, all test and infector row plants were sprayed against fungal infection using BRAVO® 720 (contains chlorothalonil 720 g/l (Reg. no. L7005, Act no. 36 of 1947) of Syngenta Group Company. Once inoculation had been done, there was no more chemical spraying to avoid killing aphids. Plants were watered twice per day, in the morning and late afternoon through the base or directly onto the pot to avoid washing aphids from the leaves. Frequent monitoring was undertaken to ensure the experiments were weed free.

## 2.4. Data Collection

Data collected included; date of first colonisation by aphids, no of aphids per plant (collected twice a week for five weeks, date to first symptoms and rosette severity. Severity rating was done on a 0-5 scoring scale where; 0 - 1.5 was resistant, 1.6-3.9 moderate resistant 4-5 susceptible.

## 2.5. Statistical Analysis

Quantitative data was subjected to analysis of variance (ANOVA) using GenStat, 22<sup>nd</sup> edition

(<https://vsni.co.uk/software/genstat>), to assess genotype and vector colonization differences and interaction effects on groundnut rosette disease development. The number of aphids was log transformed ( $\log_{10}$ ) to normalize their distribution before subjecting them to ANOVA. Figures were generated using Microsoft excel. Where significant differences among variables were found, means were compared using Fishers Protected Least Significant Difference (LSD) at 5% level of probability or using the standard error of the difference of means (SED).

### 3. Results and Discussion

#### 3.1. Aphid Colonization and Rosette Severity

Significant differences ( $P < 0.05$ ) were observed for aphid infestation as well as rosette severity on the test genotypes (Table 2). Results for severity concur with field ratings as all genotypes rated resistant had severity score of  $< 1.39$  and all genotypes rated moderate resistant, had scores  $< 1$  while susceptible genotypes recorded scores  $> 2.8$ . All susceptible genotypes; CG 7, JL 24, ICGV-SM 06711 and ICGV-SM 07599

had an average aphid population of 40 per 12 plants under observation. Among these varieties, CG 7 had the highest number of 124 followed by ICGV-SM 07599 with 117 aphids. The resistant and moderate resistant genotypes constituting 68% of the test plants had the lowest number ( $< 15$ ). Genotype ICG 12991 attracted the least number of aphids confirming its status as an aphid resistant variety. Only one genotype, ICGV-SM 01709 that was moderate resistant in the field, had high aphid population similar to that of the susceptible genotypes.

The implication of the results was that, the ability of host plants to allow minimal aphid colonization is an important factor for plants to check disease progression. It may also infer that, attracting high aphid population may result into inability for a plant to resist infection, and this concur with findings by [2, 9], who reported that resistance to groundnut rosette virus is controlled by two recessive genes responsible for the production of antiviral substances by plants, but when these plants are subjected to massive inoculum pressure from viruliferous aphids, the resistant plants could be infected with GRV. Genotype ICGV-SM 01709 may be an example of those genotypes that produce sufficient amounts of antiviral substance hence being resistant despite the heavy aphid load.

**Table 2.** Aphid population infestation by genotypes.

Genotype	Description	Aphid accumulation	Disease severity
CG 7	Susceptible	123.5	2.9
Chalimbana	Susceptible	40.4	2.8
ICG 12991	Resistant	1.9	1
ICGV-SM 01514	Resistant	3.8	1
ICGV-SM 01709	Moderate resistant	42.8	1
ICGV-SM 01731	Resistant	11.3	1
ICGV-SM 01739	Moderate resistant	6.0	1
ICGV-SM 03710	Resistant	14.8	1
ICGV-SM 06637	Moderate resistant	3.2	3.8
ICGV-SM 06711	Susceptible	67.3	3
ICGV-SM 07544	Moderate resistant	4.3	1
ICGV-SM 07599	Susceptible	116.7	4.1
ICGV-SM 08503	Resistant	11.6	1
ICGV-SM 90704	Resistant	6.7	1.4
ICGV-SM 99568	Moderate resistant	5.15	1
JL 24	Susceptible	105.7	3.7
Mean		35.3	1.8
Fpr		$< 0.001$	$< 0.001$
SED		13.86	0.3751

### 3.2. Trend of Aphid Infestation on Six Selected Genotypes

Aphids appeared on both groups (three (3) resistant and three (3) susceptible genotypes) in the second week after inoculating the infector rows. At the onset, more aphids were recorded on ICGV-SM 08503, ICGV-SM 01514 and ICG 12991, the three resistant genotypes than the susceptible ones (Figure 3). This trend was followed by a sharp decline in the level of aphids for the genotypes stabilizing for two weeks at low levels before a second wave of high population especially among the susceptible genotypes. Susceptible genotypes had a high population of aphids throughout the experimental period. Only ICGV-SM 01514 from the resistant group attracted more aphids by the fifth week of experimentation, but reduced quickly after two weeks.

Highly contrasting trends were observed between JL 24, a

susceptible check and ICG 1299, an aphid resistant check. The aphid population declined sharply 14 Days After Inoculation (DAI) for ICG 12991 leveling off to zero confirming its status as an aphid resistant genotype. The study revealed two cycles of aphid population in JL 24, with the first cycle ending 21 DAI and high population building up at 28 DAI. This shows that JL 24 is an aphid susceptible genotypes. These observations tally with findings by [15, 5], who carried out experiments exposing groundnut genotypes including ICGV-SM 90704, JL 24 and ICG 12991 to viruliferous aphids, whereby 10 DAI, increased numbers of aphids were observed on ICGVSM 90704 and JL 24 with an average of 93 and 96 aphids per plant respectively. In contrast, aphid number on ICG 12991 fell from 5 to 3 per plant. The increased number of aphids on both ICGV SM 90704 and JL 24 were an indication of susceptibility to aphids while the reduction on ICG 12991 was an indication of resistance to aphids.

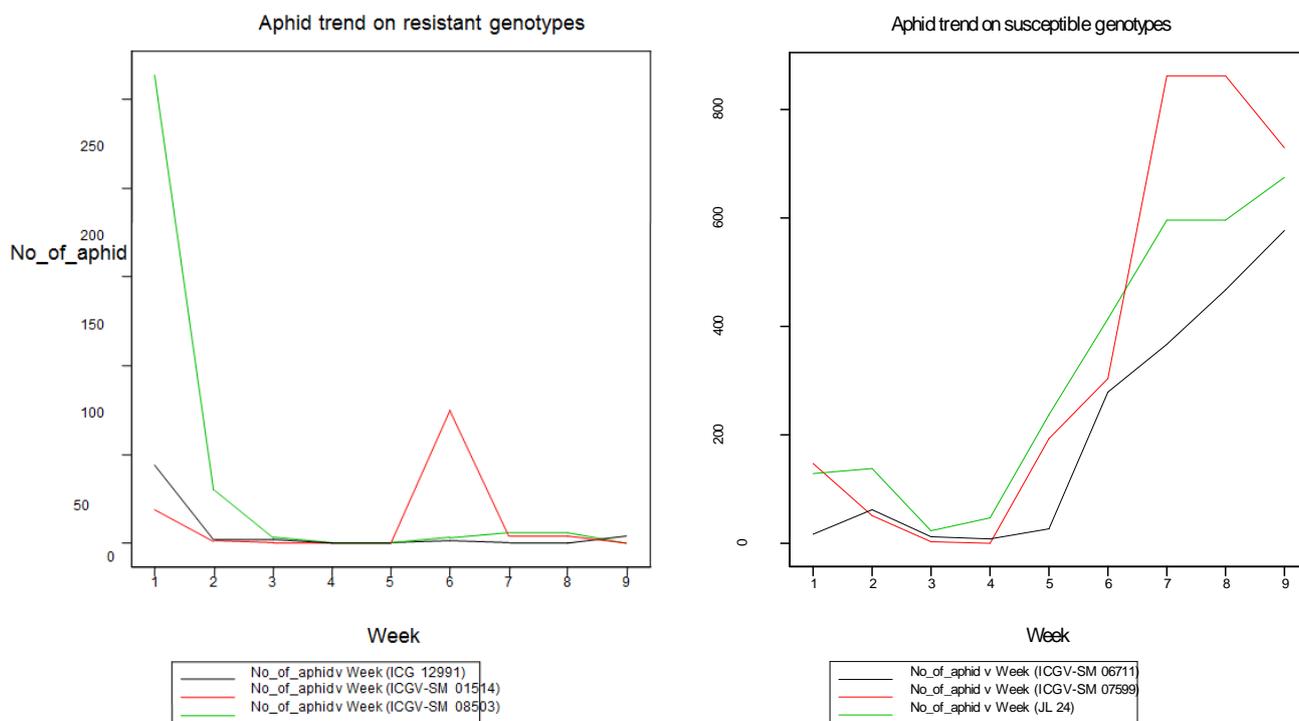


Figure 3. Aphid trend performance in susceptible and resistant genotypes.

### 3.3. Aphid and Severity Levels on Susceptible and Resistant Genotypes

The six (6) genotypes; three susceptible and three resistant, were compared to determine the general trend on aphid population and rosette disease incidence. Significant differences,  $P < 0.05$  in reaction to rosette disease for the three groups were observed (Table 3). Resistant genotypes had fewer aphid population on average (8.4) than the susceptible

(90.7), implying there is underlying mechanisms in resistant genotypes that limit aphid colonization. Further observations show genotypes being classified into three groups in relation to aphid and disease incidence namely; 1) resistant with low aphid population, 2) resistant with higher levels of aphid population and 3) susceptible without regard to levels of aphids (Figure 4). Genotypes CG7, Chalimbana and JL 24 had high levels of aphids that translated into high levels of rosette severity. The increase in the number of aphids however did not translate into high disease severity in some genotypes, for example, ICGV-SM 90704, a GRV resistant variety as

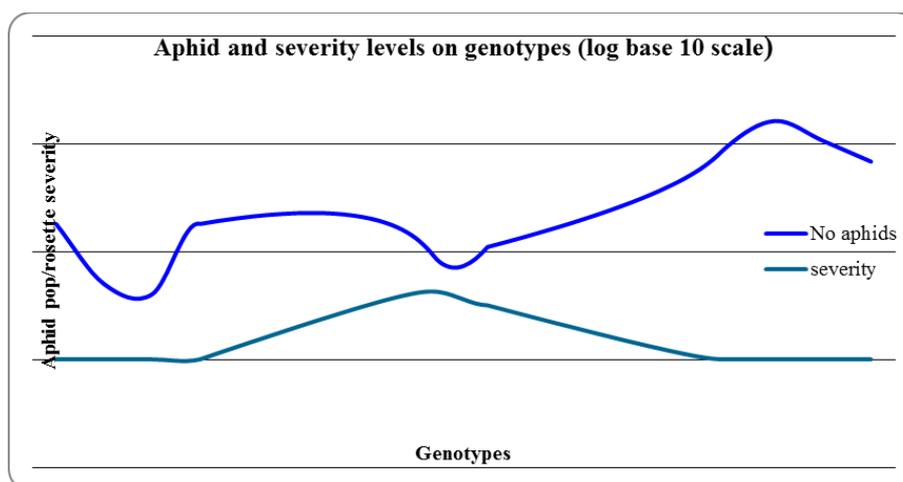
reported by [15, 5], that had considerable levels of aphids with low rosette disease severity. Four (4) other pipeline genotypes; ICGV-SM 01709, ICGV-SM 03710, ICGV-SM 08503 and ICGV-SM 01731 shared this trait, an implication that these genotypes may be conferring resistance by dealing with the virus itself (GRV) or its transmission mechanism by producing antiviral biochemical components that enable it to survive high pressure. The lowest number of aphids were on ICGV-SM 01514, ICGV-SM 06637 and ICGV-SM 07544, similar to ICGV 12991 a known aphid resistant genotype as confirmed by [10], thus it may be concluded these are aphid resistant genotypes.

**Table 3.** Effect of genotype on aphid colonization by diseases reaction category.

Reaction to rosette	Aphid accumulation
Resistant genotypes	8.4

Reaction to rosette	Aphid accumulation
Moderate resistant genotypes	12.3
Susceptible genotypes	90.7
Mean	35.3
Fpr	<0.001
SED	7.22

It is imperative that resistance associated with low aphid population may be through impediment of colonisation and multiplication of aphids, while resistance despite high aphid population is likely due to internal mechanisms dealing with the three agents of the groundnut rosette disease. The results from the current study concur with observations by [1] that suggested resistance mechanisms in groundnut deter colonization by immigrant alatae and also reduce their fecundity.



**Figure 4.** Relationship between aphid colonization and rosette severity.

### 3.4. Relationship Between Host Physical Characteristics and Aphid Infestation

Significant differences  $P < 0.05$ , were observed for aphid population with respect to colour and plant architecture

among the tested genotypes (Table 4). The results indicated that aphids were more attracted to dark green followed by green and then light green colors. This corroborates with findings by [12, 13], who reported strongest attraction for winged aphids by orange, yellow-green and green background, as opposed to red, blue, purple, white, grey and black colours.

**Table 4.** Relationship between colour and plant architecture on aphid population.

Colour of genotypes	Aphid population	Plant architecture	Aphid population
Dark green	52.6	Bunch	58.54
Green	29.8	Open	18
Light green	29.2		

Colour of genotypes	Aphid population	Plant architecture	Aphid population
Mean	35.3	Mean	38.3
Fpr	0.002	Fpr	<0.001
SED	9.45	SED	11.15

The results revealed that plant architecture does not play any role on attraction of aphids and/or selection of plants by aphids. The results show that the bunch types were either dark green or green, thus had more aphids, though colour did not necessarily influence groundnut rosette disease incidence and severity (Figure 4). Overall, the results show that there was no significant correlation (-0.005) between levels of aphid population and groundnut rosette severity among the genotypes, though within the light green coloured genotypes, increase in aphid population resulted into an increase in rosette severity, and this was so for the susceptible genotypes only. High severity in this group however, was attained even with relatively low level of aphids with the exception of ICG 12991, an aphid resistant check and ICGV-SM 01514, a new variety that has given responses similar to the aphid resistant check. These insights are the first step towards efforts to exploit more approaches to understand molecular mechanisms for sustainable aphid control strategies in tandem with proposal by [14].

## 4. Conclusions

Based on the results from the study, aphids get attracted to genotypes with greener than lighter leaf colour. However it was evident that colour had no influence on the reaction to the groundnut rosette disease. These findings disagree with general blanket observation in the fields that most light green genotypes were susceptible due to their ability to attract aphids. It is therefore important that greener genotypes that are susceptible to the rosette disease require good field management to avoid or minimise aphid populations. In addition, it was evident that plant architecture does not have any role on attraction of aphids or selection of plants by aphids. We conclude that genotypes; ICGV-SM 01514, ICGV-SM 06637 and ICGV-SM 07544 are aphid resistant similar to ICG 12991, while ICGV-SM 01709, ICGV-SM 03710, ICGV-SM 08503 and ICGV-SM 01731 are GRV resistant since their reaction was similar to that of ICGV-SM 90704, a key genetic resource in breeding for resistance. The genotype ICGV-SM 01709 proved to be resistant to the rosette diseases, despite being highly infested by aphids, the vector for the disease. This may be a helpful trait for breeders as it is an example of genotypes that produce sufficient amounts of antiviral substance as their resistance mechanism. The genotype however may be a good source of inoculum and not advisable to have it planted close to susceptible varieties. The results opens a field of enquiry as a basis for further research,

whereby there is need for further characterization of these sets of genotypes using molecular and biochemical markers to understand the physiological basis of the varied reaction to vector and disease incidence among the genotypes. This will help in understanding the biochemical basis of resistance and or susceptibility to the groundnut rosette disease. Further, sequencing the genome of the aphid species on groundnut is crucial to inform the diversity of the vector and give insights on how microbial effector proteins, host targets and plant immune receptors co-evolve.

## Abbreviations

GRD	Groundnut Rosette Disease
GRV	Groundnut Rosette Virus
DAI	Days after Inoculation

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## Author Contributions

**Mwololo James:** Conceptualization, Data curation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing

**Njoroge Samuel:** Validation, Writing – review & editing

**Munthali Wills:** Data curation, Writing – original draft, Writing – review & editing

**Okori Patrick:** Funding acquisition, Project administration

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## Data Availability Statement

The data is available from the corresponding author upon

reasonable request.

## Conflicts of Interest

There is no conflict of interest reported by authors.

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