Influence of sowing dates on the population density of the fall armyworm *Spodoptera frugiperda* (JE. Smith) and its damage on maize plants in Chad

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**ABSTRACT**

Fall armyworm, *Spodoptera frugiperda*, constitutes a major challenge for maize growers and scientists, as it threatens the food security of all these countries. In order to contribute to the sustainable management of the pest in Chad, this study aimed at assessing the effect of sowing dates on the level of maize infestation in the field. Three sowing periods for maize, chosen in connection with the onset of rains, were tested (the early sowing (July 2nd), the intermediate sowing (July 16th), and the late sowing (July 30th)) with a Fall armyworm-sensitive maize variety. The results indicated a significant effect of sowing dates on the population densities of the pest, with the early sowing hosting the highest density and infestation rate of the pest, compared to the intermediate and late sowing (P < 0.0001). Moreover, the results showed a significant impact of sowing dates on grain yield (P < 0.005), with significantly higher values in the intermediate sowing while, the early and late sowing recorded lower yields. These results indicated that the intermediate sowing should be recommended for this early maturing and fall armyworm-sensitive maize variety to limit population outbreaks of this pest, together with its adverse consequences on maize yield.

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**Keywords:** agronomic control, sustainable management, fall armyworm, intermediate or normal sowing, rainfall regime.

**INTRODUCTION**

It is widely acknowledged that both biotic and abiotic factors are key elements that determine the productivity of agricultural plots (Dresselhaus and Hückelhoven, 2018; Liliane and Charles, 2020). More specifically, abiotic factors such as seasonal rainfall regimes can influence crop growth directly through the amount of water available yield build up, but also indirectly through their influence on biotic factors such as diseases and pests that interfere with the productivity of the plants. Rainfall,
especially in tropical and subtropical regions, can, indeed, alter the phenology and population density of crop pests, with consequent impact on crop yields (Anandhi et al., 2020). In that respect, a rational management of this abiotic factor is expected to significantly contribute to the reduction of pest incidence in the fields. This management requires, however, a judicious choice of crop sowing dates, in connection with the phenology of the major prevailing pest species (Showler, 2005; Shahid et al., 2014).

One such pest of importance in Africa and other parts of the world nowadays, turns out to be the Fall Armyworm (FAW), *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) (Gorgen et al., 2016). This moth causes serious damage to cereals, especially to maize, a widely consumed foodstuff in most African countries. The extent of its damage in maize fields makes it the most important threat to maize production compared with the other stem borer species previously known to infest cereal fields (Goergen et al., 2016). The strong capacity of this pest species to threaten food security in Africa calls for the need to search and implement adequate control measures against it. This is more so as in many countries under the tropics, maize accounts both as food and as cash crop (Erenstein et al., 2022).

Just upon its discovery in maize fields in Africa, the first management methods available to growers and scientists were the intensive use of chemical pesticides, despite their many environmental costs, coupled with their unaffordable financial cost (Kumela et al., 2019). The need for more sustainable control strategies against this pest has since become a challenge to both growers and scientists including decision makers. In that search, it has been reminded that a good knowledge of factors that influence the distribution and abundance of a pest could be of key importance in the development and implementation of any control strategy (Baskauf, 2003). Indeed, control methods based on agronomic management practices could represent an interesting alternative to chemical control because they are economically more affordable for smallholders with limited resources and less risky for health and the environment (Thierfelder et al., 2018). One of the most important of these agronomic practices is the careful choice of sowing date (Asante et al., 2001). Using optimal sowing dates is indeed an effective management practice for increasing maize yield (Zhang et al., 2019).

Rodríguez-del-Bosque et al. (2012), showed that the population density of *S. frugiperda* and its attack levels on host-plants are higher when the sowing was delayed (late sowing). Similarly, Ayala et al., (2013), reported in Argentina that the sowing date affected the infestation levels with the early seeding avoiding the high armyworm densities that developed later in the season. These observations prompted Baudron et al. (2019), to highlight the need for research in Africa to specifically clarify the impact of sowing dates on FAW damage in maize fields in view of integrating this agronomic practice into the control strategy package against *S. frugiperda* on the continent.
In Chad, where the pest is present and widely spreading in maize fields (Prasanna et al., 2018), no study has yet been carried out on the effect of sowing dates on the level of FAW infestation and damage on maize plants. It is, therefore, to fill this gap that the present study was initiated to determine the most favorable sowing dates of maize that could limit the infestation by *S. frugiperda* so as to integrate this parameter (optimum sowing dates) into the strategies for a sustainable control of this devastating pest in Chad and hopefully in the neighboring countries.

**MATERIALS AND METHODS**

**Study site**

The study was conducted at the Bébédjia Agronomic Research Station (8°40'34" N; 16°33'58" E; 397 m altitude). This station is located in the Sudanian zone of Chad at 524 km south of N'Djamena (the capital city). The climate here is of the Sudanian tropical type, characterized by the alternation of one rainy season which extends from April to October (4 - 5 months), and a dry season from November to March (7-8 months). The annual rainfall ranges between 600 mm and 1200 mm (DMN, 2019). Rainfall data collected at the Bébédjia meteorological station, 3 km from the experimental site, were used for this study. These data included the daily rainfall as well as the number of rainy days recorded during the study period. Overall, the average monthly rainfall recorded on the experimental site during the study was 81.78 mm while the average temperature varied between 23°C and 35°C, with the minimum in August and the maximum in October. The relative humidity during the study period varied between 79% and 86%, with an average of 82.0 ± 1.63%.

**Study materials**

The animal material consisted mainly of *S. frugiperda* larvae. As for the plant material, it consisted of the maize variety ‘TZEEW’, known to be susceptible to FAW in Chad (Mbaidiro et al., 2021).

**Experimental procedure**

The experimental setup was a Complete Randomized Block Design with three treatments and four replicates. Each block was divided into three plots separated from each other by a 1.5 m buffer while the blocks were separated from each other by a 2 m buffer. Each plot was 7 m long and 5 m wide and harbored 13 seedrows spaced by 0.6 m. The treatments were represented by the three ‘sowing dates’ as follows: T1- early sowing on July 02, 2021; T2- intermediate sowing on July 16, 2021; T3- late sowing on July 30, 2021. Note that the optimal sowing date recommended by the Chadian Ministry of Agricultural Development, for this early variety of maize is 10th-15th July of the year.

The experimental plots were plowed at the depth of 15-20 cm, followed by a harrowing to prepare the seedbed. Maize was sown at the rate of three seeds per pocket and at a spacing of 0.60 m x 0.40 m, after a significant rainfall of at least 20 mm. A first weeding was undertaken 14 days after emergence followed by a second weeding at 21 days after the first. The chemical fertilizer N, P, K (20-10-10) designed for cereals was applied as basal dressing at the dosage of 150 kg/ha in furrows dug at 10 cm nearing the seedling lines. Urea was applied as top dressing fertilizer in two events respectively, at the 10-leaf stage and at tasseling, at the dosage of 25 kg/ha. No pesticides were applied on the experimental plots.

**Data collection**

*Larval density of *S. frugiperda* and infestation of maize plots*

The population density of *S. frugiperda* larvae was estimated by directly counting the caterpillars on 25 maize plants chosen at random from the five central rows of each plot. These observations were made every 7 days from the 33rd day after sowing when the infestation peaked, until the 68th, when the plants reached their physiological maturity and that new infestation became negligible.
(Pannuti et al., 2016). The infestation rate of maize plots was then determined by calculating the ratio between the number of infested plants (i.e., those harboring the pest) and the total number of plants sampled.

**Damage of *S. frugiperda* to leaf and ear on maize plants**

The extent of leaf damage was assessed on 25 plants randomly selected from the five center rows of each plot, using a simplified damage scale 0 to 4 (Grijalba et al., 2018; Fotso Kuate et al., 2019; dos Santos et al., 2020; Toepfer et al., 2021) (Figure 1), derived from the 0-9 damage rating scale suggested by Davis et al. (1992). Leaves were individually inspected for damage and the damage scores assigned per individual leaf were then pooled together and divided by the total number of leaves examined per plant. This scale is the one generally used especially for monitoring the effectiveness of phytosanitary treatments (Toepfer et al., 2021).

Damage on ears was evaluated on a sample of 25 maize ears randomly selected on the five central rows of each plot. The extent of pest damage on each dehusked maize ear was determined using the scale of 1 to 9 suggested by Kamweru et al. (2022). This ranking is presented as follows: 1 = no visible damage to the ear; 2 = damage to a few kernels (<5) or less than 5% damage to an ear; 3 = damage to a few kernels (6–15) or less than 10% damage to an ear; 4 = damage to 16–30 kernels or less than 15% damage to an ear; 5 = damage to 31–50 kernels or less than 25% damage to an ear; 6 = damage to 51–75 kernels or more than 35% but less than 50% damage to an ear; 7 = damage to 76–100 kernels or more than 50% but less than 60% damage to an ear; 8 = damage to >100 kernels or more than 60% but less than 100% damage to an ear; 9 = almost 100% damage to an ear, as illustrated in Figure 2 (CYMMIT 200; unpublished protocol).

**Evaluation of maize yield on the different sowing dates**

For this purpose, all the maize plants of the five central lines of each plot were harvested. The ears were then detached, dehusked, dried and shelled manually. The grains were dried to reach a residual moisture content of 12% and then weighed with a Steinberg brand electronic scale (300 kg; int. precision 50 g). The production determined was thus, that of the five central lines of each plot (i.e. 21 m²). The subsequent yield was averaged per treatment and this yield was then transposed per hectare for clarity and homogeneity, thereby facilitating comparisons with other research results.

**Data analysis**

Data on FAW density and infestation rate of maize plots were subjected to the Shapiro–Wilk test for normality (Shapiro & Wilk, 1965), and Levene's test for homogeneity of variances.

These data were then compared among treatments (i.e., sowing dates) using a one-way Analysis of Variance (ANOVA). In case the data were not normally distributed, they were transformed using log10(X+1) and Arcsine√(X/100), respectively for FAW densities and infestation rates. When ANOVA revealed significant differences among treatments, treatment means were separated using the Student–Newman–Keuls (SNK) multiple range test. A linear regression analysis was performed to determine the relationship between FAW density, the cumulative rainfall and the cumulative number of rainy days recorded on the study site during the experimental period. Rainfall data collected at the Bébédiya meteorological station, located 3 km from the experimental site, were used for this purpose. All of these statistical analyses were performed using XLSTAT Version 2016.02.27444 software.
<table>
<thead>
<tr>
<th>Score</th>
<th>Damage symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No damage</td>
</tr>
<tr>
<td>1</td>
<td>Little damage (pinholes, and/or small holes, small leaf edge parts eaten, shot holes)</td>
</tr>
<tr>
<td>2</td>
<td>Medium damage (some larger holes and/or larger leaf edge areas eaten)</td>
</tr>
<tr>
<td>3</td>
<td>Heavy damage (many larger holes and/or larger leaf edge areas eaten)</td>
</tr>
<tr>
<td>4</td>
<td>Total damage (destroyed, non-functional leaves)</td>
</tr>
</tbody>
</table>

**Figure 1**: FAW leaf damage index from 0 to 4 (each leaf is assessed). Source: Toepfer et al. (2021).

**Figure 2**: Maize ear damage scores caused by *S. frugiperda* Source: CYMMIT (2020).
RESULTS

Effects of sowing dates on the larval density of *S. frugiperda* and infestation of maize plots

Among the three treatments and over the sampling period, the mean density of *S. frugiperda* larvae varied from 1.75 ± 0.43 to 8.75 ± 0.89 caterpillars/plant (Table 1). The results of the ANOVA revealed a significant effect of sowing dates on the number of *S. frugiperda* larvae per plant (df = 2, F = 32.15, P < 0.0001), with the lowest densities recorded at the intermediate, while the highest densities were recorded at the early sowing date. However, densities recorded at the intermediate and late sowing dates were not statistically different. As for the infestation rate, it averaged 5.38 ± 0.99%, 8.75 ± 1.87% and 21.75 ± 3.05%, respectively for intermediate, late and early sowing dates over the entire sampling period. The results of the ANOVA here also showed significant differences among sowing dates (df = 2, F = 16.23, P < 0.0001) with the highest rate recorded at the early sowing (21.75 ± 3.05%) while the intermediate and the late sowing dates had the lowest and statistically similar *S. frugiperda* infestation rates (Table 1).

Effects of sowing dates on *S. frugiperda* damage to maize leaves and ears

The mean total number of maize leaves per plant ranged from 9.94 ± 0.56 leaves (late sowing) to 13.65 ± 0.76 leaves (early sowing). As for the number of maize leaves attacked by *S. frugiperda* and their average damage scores, they ranged respectively, from 2.72 ± 0.09 to 8.32 ± 0.35 and 0.81 ± 0.16 to 2.50 ± 0.20 (Table 2). The results of the ANOVA revealed significant differences in the total numbers of maize leaves per plant among the three sowing dates (df = 2, F = 74.11, P = 0.0001), with the highest number on plants from the early and the intermediate sowing date while the significantly lowest number of damaged leaves was encountered on plants from the late sowing date. The ANOVA also revealed significant differences among sowing dates in the number of *S. frugiperda*-damaged leaves per maize plant (df = 2, F = 15.17, P = 0.001), with the highest number from plants of the early sowing and the lowest and statistically similar ones from plants of the intermediate and late sowing dates (Table 2). Significant differences were also shown among sowing dates regarding damages caused to maize leaves by the caterpillars (df = 2, F = 21.61, P < 0.0001). Here, the early sowing date recorded the highest damage index while the intermediate and the late sowing dates recorded the lowest and statistically similar indices. As for the ears, the damage index varied from 1.00 ± 0.25 to 3.25 ± 0.25 (Table 2). The ANOVA revealed significant differences among sowing dates (df = 2, F = 30.50, P < 0.0001), with the early sowing supporting the greatest damage followed by the intermediate sowing, while the late sowing recorded the lowest damage score (Table 2).

Relationship between *S. frugiperda* density, cumulative number of rainy days and total rainfall received by each treatment

The population trend of FAW density as a function of the cumulative number of rainy days and the cumulative rainfall over the three sowing dates (i.e. early sowing, normal or intermediate sowing, late sowing) revealed that FAW densities were overall the lowest for the intermediate sowing (i.e. normal sowing), followed by late sowing while the early sowing recorded the highest larval densities (Figure 3). It also appeared that the cumulative number of rainy days experienced by the crop was higher in the intermediate (i.e. normal) sowing than in the early or late sowing, whereas the total amount of rainfall received was the highest in the early sowing, followed by the intermediate sowing (i.e. normal), and the lowest in the late sowing (Figure 3).

The linear regression analysis respectively between the cumulative number of rainy days and FAW densities and between the cumulative total rainfall and FAW densities for
the three sowing dates taken together (Figure 4) showed a significantly negative relationship between the number of rainy days and FAW densities ($r^2 = 0.1193$, Slope = -0.1938, $P < 0.002$) whereas in contrast, the relationship between the cumulative rainfall and FAW densities was not significant ($r^2 = 0.0218$; Slope = -0.0046, $P < 0.216$). It emerged from these analysis that the frequency of rainfall negatively affected the population density of FAW while the cumulative rainfall had no significant impact on the population density of the pest (Figure 4).

**Effect of sowing date on the maize grain yield**

Among the three sowing dates, the mean (± SE) maize grain yield, estimated over 25 plants per plot varied from 1,976.2 ± 142.2 Kg/ha to 4,773.8 ± 813.3 Kg/ha (Figure 5). The ANOVA showed a significant effect of sowing dates on the yield (df = 2, $F = 10.35$, $P < 0.005$). The SNK mean separation test revealed that the highest yield was recorded with the intermediate sowing while the early and late sowing recorded the lowest and statistically similar yields (Figure 5).

<table>
<thead>
<tr>
<th>Sowing dates</th>
<th>$S. frugiperda$ larvae/plant (mean ± SE)</th>
<th>Infestation rate (%) (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early sowing (02-07-2021)</td>
<td>8.75 ± 0.89 a</td>
<td>21.75 ± 3.05 a</td>
</tr>
<tr>
<td>Intermediate sowing (16-07-2022)</td>
<td>1.75 ± 0.43 b</td>
<td>5.38 ± 0.99 b</td>
</tr>
<tr>
<td>Late sowing (30-07-2021)</td>
<td>2.63 ± 0.60 b</td>
<td>8.75 ± 1.87 b</td>
</tr>
</tbody>
</table>

In a column, the means followed by the same letter are not statistically different (SNK test)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total number of maize leaves</th>
<th>Number of leaves attacked by FAW (proportion of total)</th>
<th>Mean $S. frugiperda$ damage index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early sowing (02-07-2021)</td>
<td>13.65 ± 0.76 a</td>
<td>8.32 ± 0.35 a (0.61)</td>
<td>2.50 ± 0.20 a</td>
</tr>
<tr>
<td>Intermediate sowing (16-07-2022)</td>
<td>12.98 ± 0.63 a</td>
<td>4.72 ± 0.16 b (0.36)</td>
<td>1.00 ± 0.22 b</td>
</tr>
<tr>
<td>Late sowing (30-07-2021)</td>
<td>9.94 ± 0.56 b</td>
<td>2.72 ± 0.09 b (0.27)</td>
<td>0.81 ± 0.16 b</td>
</tr>
</tbody>
</table>

In a column, the means followed by the same letter are not statistically different (SNK test).
Figure 3: Evolution of the FAW density according to the cumulative number of rainy days and the cumulative rainfall over the three different sowing dates.

Figure 4: Relationship between FAW densities and the cumulative number of rainy days.
**DISCUSSION**

In the unrestrained search for sustainable alternatives to the chemical control of *S. frugiperda*, the present study seems to be one of the very first to have evaluated the effect of sowing dates on the population level and density of the pest and its subsequent effect on the productivity of maize plots in the Republic of Chad. The test-maize variety was one known to be susceptible to *S. frugiperda* (Mbaidiro et al., 2021) therefore, the outcome of this study could serve as an example in many other maize growing countries in the sub-region. The results showed that under the climatic conditions prevailing in Chad, the density of the pest, the level of infestation of maize plants as well as the damage inflicted to leaves and ears by the FAW on maize plants were significantly affected by the sowing dates. Thus, unlike what one could have expected, maize crops established early in the rainy season were the most severely attacked by the FAW. Possible explanations for these observations could be sought among both biotic and abiotic factors.

Indeed, from a biotic point of view, maize sown just at the onset of the rainy season appears to be the only food source available for the first generation of FAW after hatching; therefore, their caterpillars rely almost exclusively on this crop on which they massively feed to complete their developmental cycle and increase their population size. Such early attacks could explain the important pest damage observed on maize plants with subsequent negative impact on maize yield in the early sowing compared to the normal (intermediate) and late sowing. Whereas our findings are in agreement with those by Kandel and Poudel (2020), they contrast those by Sowmiya et al. (2022), who reported that the maize sown earlier harbored lower fall armyworm infestation and provided higher grain yield compared to intermediate and late sowing. However, it is generally observed that very few farmers succeed in sowing their plots early in the season; thus, the supply of food to the pest is very limited and, by virtue of volatile cues emitted by the plants, the pest can easily detect these young maize fields to forage on them (Ekholm et al., 2020). Caterpillars can, indeed, easily locate and infest maize fields by using the volatile substances generally emitted by *S. frugiperda*. 

![Figure 5: Effect of sowing dates on maize grain yield.](image-url)
infested maize plants and referred to as “Herbivore-Induced Plant Volatiles” (Szendrei and Rodriguez-Saona, 2010; Aartsma et al., 2017). This is more likely as maize plants seem to be the most preferred host-plants of S. frugiperda in Chad (Prasanna et al., 2018). In contrast, at the normal or late sowing dates, many maize fields are planted almost simultaneously, which would have limited the infestation of a particular plot by the pest, given the great availability of host-plants, thus a wider range of choice. Overall, the level of infestation of maize plants in our study plots were significantly higher in early sowing date compared to the intermediate (normal) and late sowing date.

Another implication of biotic factors may be that, with the early sowing, proven and/or potential natural enemies of FAW present in the neighborhood of maize plots may not have really settled and built up adequate populations in the fields to quickly detect the pest prey/host outbreaks to feed on them (Hatano et al., 2015). In such a situation, they are not yet able to reduce the pest populations nor their subsequent negative impact on the crop. In contrast, in the normal (i.e. intermediate) or late sowing, in contrast, interactions between maize plants and the pest have already been running over a relatively longer period, thereby having allowed natural enemies of the FAW to build-up a significant population size in the maize field to quickly detect the pest which they prey upon or parasitized; thereby limiting their negative impact on the maize plants.

Among abiotic factors, it is well known that in the tropics, temperature but especially precipitation (i.e., rainfall), are those that significantly regulate pest populations on cultivated plots (Savopoulou-Soultani et al., 2012; Thakur and Rawat, 2014). Whereas rainfall is essential for plant growth, it can also be a direct mortality factor for plant-inhabiting pests because of the washing-off caused to them. Indeed harsh rains can prompt pests drop on the ground where they can get drown or become easily available to their natural enemies (Zulucyi et al., 2002). This might explain why in the present study, the infestation rates as well as the larval densities of S. frugiperda were higher in the early sowing than in the normal or late sowing. The fact is that, at the beginning of the rainy season, rains are spaced out over time and certainly less abundant than in the middle of the rainy season. Consequently, rainfall-induced mortality of caterpillars and even of FAW eggs is generally lower at the start of the rainy season than in the middle of the rainy season. However, besides these direct impacts mentioned above, abiotic factors, particularly rainfall, can also indirectly influence the phenology and the level of pest damage to crops.

Indeed, the onset of rainfall promotes the vegetative development of maize plants, which increases the amount of food available to pests, with the potential for a rapid increase of their populations. Therefore, in absence of rainfall-induced pest mortality that reduce pest populations, one should rather expect a high S. frugiperda population and damage on the intermediate and late sowing dates. Thus, the slight damage recorded on maize leaves and ears from normal and late sowing were certainly due to the higher rainfall frequency during the first stages of the vegetative growth of maize plants, leading to an intensive washout of first and second instar larvae, thereby preventing them from settling on maize plants (Chandrasekhar et al., 2022). Our results clearly support these suggestions as they clearly indicate that the total amount of rainfall on a plot is not an important source of mortality to S. frugiperda whereas, in contrast, the frequent occurrence of rainfall kills them, as has been observed by several authors for other pests on other crops (Saminathan et al., 2001; Onzo et al., 2005; Prianka et al., 2018; Anandhi et al., 2020).

As one could expect, based on the observed effects of sowing dates on the population dynamics of S. frugiperda on maize plants, maize grain yields were significantly higher on the normal (i.e. intermediate) sowing date than on the early but also on the late sowing dates where the yields were statistically similar. This finding demonstrates once more the negative impact of S. frugiperda infestations on the productivity of maize plots. It should be noted, however, that based on our
data on pest densities and damage scores, one would have expected maize grain yields to be similar on intermediate (normal) sowing and on late sowing dates. This absence of linearity could be due to other factors such as a possible inadaptation of the maize variety tested with the environmental conditions that prevailed in the fields towards the end of the rainy season; such factors may include lower ambient temperatures, which could adversely affect the grain filling of the maize cobs, and possibly a depletion of soil nutrient reserves due to the leaching of the fertilizers, as stated by Eash et al. (2019). These high relative humidity conditions could also favor the development of fungal diseases that can negatively affect maize grain yield. In addition, indirect yield losses could also occur through defoliation that can, in return, reduce plant productivity through a decrease in the photosynthetic area on the maize plant (Capinera, 2008; Vilarinho et al., 2011).

**Conclusion**

The present study had evaluated the effect of sowing dates on the level of FAW infestation and on maize yield. In general, damage caused to maize plants by the pest was higher for early sowing (early July) than for intermediate (mid-July) and late (late July) sowing. As a consequence, the maize grain yield was better for the intermediate (i.e. normal) sowing date than for the early and late sowing dates. These results suggest that a judicious adjustment of maize sowing dates and the integration of this parameter into the control strategies against *S. frugiperda* by adapting it to the prevailing climatic conditions as well as to the type of maize variety could provide a sustainable yield through reduced pest densities and damages.

**COMPETING INTERESTS**

The authors declare that there is no competing interests.

**AUTHORS’ CONTRIBUTIONS**

MTJ designed the study, participated in data collection and drafting of the manuscript. MTJ and OA participated in the drafting of the manuscript, data acquisition and their interpretation. DA participated in the drafting of the manuscript, the statistical analysis and the interpretation of the results. MM participated in the drafting of the manuscript. All authors have read and approved the final manuscript.

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