The effect of different staking on the growth and yield of the climbing beans (HV/Namulenga) in Bushumba, Kabare-Nord, the South Kivu province, east of the Democratic Republic of Congo

INTRODUCTION

Beans are considered a very important commodity in the world. It is eaten in the form of seeds (fruit vegetables) and leaves (leafy vegetables). Bean seeds can be eaten green and are preferred almost anywhere in the world. At this state, they contain a lot of vitamins. Beans are grown for food in parts of Africa and Latin America. In developed countries, alongside the limited production of home gardens for home consumption, this crop has grown in open fields over large areas while constituting a source of capital and a pillar of development (Iripi, 2005).

Beans are one of the main crops in eastern DR Congo (Civava et al., 2013). In Kabare territory, it is seen along with soybeans, as an important component of the cropping system through restoring soil fertility and providing protein to small farmers. With the appearance of bacterial banana wilt in the area, which has had disastrous consequences on the economy of agricultural households, it seems more important to intensify this crop given the nutritional and agronomic advantages it offers (www.N2Africa.org, February 2021).
Despite genetic progress, the optimum production potential is rarely expressed by cultivated bean varieties because of the biotic and abiotic constraints involved in the development of the yield components (Mako et al., 2010). In addition, there are poor land management practices leading to nutrient depletion of the soil, but also increasing population pressure leading to a drastic reduction in cultivable land (FAO, 2000).

Over the past 30 years, there has been a dramatic increase in population in Kabare. Anable land has become scarce. Day by day, there are land issues between peasants. Some wealthy people who acquired large plantations during the period of Belgian settlers pursuing their own interests do not exploit them for the benefit of the peasant population in the annual report of the Bushumba area (2014). Climate change and its serious consequences mean that rains are becoming rare in many areas, including our own. All this results in the scarcity of cultivable land, land conflicts between neighbors, the drop in production and yield, the overexploitation of land with numerous consequences such as famine, rural exodus, malnutrition, lack of employment, juvenile delinquency, serious immorality,...(Civava et al.,2013 ; Muhigwa, 2010).

With all these constraints, it seems important to study and promote bean production techniques adapted to local conditions. Depending on how the varieties grow, beans are subdivided into bush and climbing. Climbing beans are also more resistant to fungal and root rot diseases (Mcharo and Katafire, 2014), and have a better potential to fix nitrogen (Bliss, 1993; Ramaekers et al., 2013; Wortmann, 2001). Although climbing beans produce more than bush beans, it is the latter that are preferred in field crops. However, several authors Nyabyenda (2005), van der Burg et al.(2005) and www.N2Africa.org (February 2021) show that staking increases the production of twining bean from 29 to 100%. To tie 2, 3 or 4 long stakes together or tripod increases in doubles or triples the climbing beans production of that of bush beans for the same unit of area.

In Kabare territory precisely in the Bushumba area, the main constraint to the extension of climbing bean remains the availability of stakes. This low availability is the result of the deforestation which accompanied the massive arrival of Rwandan refugees in 1994. The finding is that when the stakes are used, they rarely exceed 1.5 m. In most cases, they broadcast beans and / or on small mounds, rarely in rows. Do the different staking methods have a significant impact on climbing bean production? What would be the best method to popularize for climbing bean staking? However, the application of 3 m stakes or other staking methods combined with good cultural practices and practices would improve the yield of climbing beans.

The main aim is to contribute to the improvement of the production of climbing beans in Kabare territory South Kivu province by evaluating the effect of the various techniques of stakes on the growth and the yield of climbing bean and offer the most efficient and economically profitable staking method.

**METHODS**

**Study area**

The test was conducted in Bushumba area Kabare territory at the Mushweshwe experimental site. This site is located between 28° 33’ 32’’ longitude and 2° 18’ 58’’ latitude at an altitude of 1538 m. It is separated from Katana by 15 km and from Birava by 7 km. It is bounded to the north by Luhiihi area to the south by Lugendo area and village of Buhehe and to the west by village Nyabulongwe, to the east by village de Buhehe. The agricultural domain of Mushweshwe is located in the village of Buhehe and separated from it by neighboring trees planted by the Belgian Congolese and German cooperation.

The Bushumba area is 97 km. It is part of 14 groups (Bushumba, Luhiihi, Lugendo, Ishungu, Bugorhe, Bugobe, Irambi-Katana, Miti, Mudaka, Mudusa, Mumoshos, Kagabi and Cirunga) which make up the Kabare chiefdom collectivity which bears the same name of the territory. The territory of Kabare is located between 27° 45’ and 28° 55’ longitude and 2° 30’ and 2° 50’ latitude. It includes two communities (chiefdom); the chiefdom community of Kabare and the chiefdom community of Nindja. Bushumba is located about 30 km from Bukavu, the capital of South Kivu province. The Bushumba area is administratively made up of six villages Murama, Muganzo, Cishoke 1 et 2, Lwangoma, Nyabulongwe, Buhehe in the report of the Bushumba area (2010).

The vegetation consists of savannah varying from wooded savannah to grassy savannah under the multiple effects of agriculture. Thus the forest is replaced by this, the original *Albizia grandibactectera* forest has disappeared (Furaha et al., 2013). Today the vegetation consists of trees, shrubs and grasses. The grass usually or mostly consists of grasses (*Hyparrhenia spp*) which are used as pasture for cattle. The latter are much threatened by farmers who lack arable fields.

We can add to this plant flora, afforestation trees like *Eucalyptus grandis, Leucaena glora* and *L. Leucocephala*, *Grevelea robusta*, *Cedrela serrata*, *Maesopsis emini, Markhamia lutea*, and some endangered species since the advent of Rwandan shelters. The latter taught the populations to exploit and to exploit wood (manufacture of embers) almost all large trees and even the means to small trees have been decimated and many hills remain bare and should be reforested in order to bypass them. Heavy consequences on crops. We cannot but cite bananas despite the presence of bacterial wilt and industrial crops coffee,
tea, sugar cane, cinchona, etc. which are distributed in different corners in Kabare, but the largest plantations are those of coffee trees than fruit trees and fields of vegetable crops (food crops etc.). The fodder species observed are mainly grasses such as *Hyparrhenia diplandre, H. filipendula*, etc. and legumes such as *Leucaena* spp, etc.

Mushweshwe soil would be ultisol according to the American classification. It is similar to that of ISDR / Bukavu. It is poor soil but can be alleviated with farmyard manure, compost and good soil conservation and protection methods. Mushweshwe soil has two origins volcanic and forest. Its pH would be variable from acidic soil to basic soil from 4.1 to 7 (GIZ, 2016). This soil is neutral (pH = 7) and alkaline (4.1) in the bottom. The result of the soil analyzes of the soil samples carried out at the UCB / Kalambo soil laboratory is in table 1.

**Table 1.** Chemical composition and texture of the soil sample from the experimental site at the UCB / Kalambo soil analysis laboratory on January 28, 2017.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Main morphological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. pH-H₂O</td>
<td>5.7 à – 6</td>
</tr>
<tr>
<td>2. % C Organique</td>
<td>3,787</td>
</tr>
<tr>
<td>3. % N Total</td>
<td>0,374</td>
</tr>
<tr>
<td>4. % C/N</td>
<td>10,124</td>
</tr>
<tr>
<td>5. Phosphore assimilable</td>
<td>15,12 ppm</td>
</tr>
<tr>
<td>6. Texture</td>
<td>argileuse</td>
</tr>
</tbody>
</table>

The area benefits from a humid tropical climate characterized by two seasons including 3 months of dry season (June to August) and 9 months of rainy season (September to May). A small dry season begins from mid-January to mid-February, lasting 3 to 4 weeks. The mean annual temperature of the area varies between 19 and 20 °C. Relative humidity ranges from 68 to 75% and annual rainfall is 1,500 mm (CRSN Climate Service – Lwiro, 2016). Long periods of drought (dry season) are observed in our environments and disrupt the agricultural calendar and the crop cycle. The dry season has become long 3 to 4 months or sometimes even 5 months compared to the rainy which has become 7 months instead of 9 months. There is a significant increase in temperature following this global warming.

This hydrography is dominated by Lake Kivu which makes its natural limit towards the eastern part. Then there are large rivers which take their sources in the PNKB and flow into the lake – Kivu. We mention Mushweshwe, Karhungulu, Karhanywa, Mpugwe, Cindrier, Langa, Lwiro, Bidagarha, Cirhagabwa, and Nyabanongo form the natural boundary of Kabare and Kalehe territory. Mushweshwe, Kanhungulu rivers and Lake Kivu surround the Mushweshwe Agricultural domain. The ITAV / Mushweshwe contains several water sources in village of Buhehe 3 sources of drinking water in the planned plots of the ITAV (10, 6, 8 and 20 and 22) and in Buhehe the sources of Lyamuzungu towards Lake Kivu are Lukondogolo, Kamabale, Lyamabiba I, Lyamabiba II, etc. in the lower Karhungule (ITAV / Mushweshwe report, 2016).

**Climbing bean variety HV / Namulenga**

The characteristics of this climbing bean variety HV / Namulenga are presented in table 2. This variety was purchased from INERA/ Mulungu for the test.

**Table 2.** Characteristics of the variety HV / Namulenga (PNL/INERA Mulungu, 2018)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Main agronomic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Plant habit</td>
<td>Reams at climbing bear</td>
</tr>
<tr>
<td>2. Growth type</td>
<td>Undetermined</td>
</tr>
<tr>
<td>3. Leaf color</td>
<td>Dark green</td>
</tr>
<tr>
<td>4. Color of flowers</td>
<td>Violet</td>
</tr>
<tr>
<td>5. Roughness of trifoliate leaf</td>
<td>Average</td>
</tr>
<tr>
<td>6. Terminal leaflet shape</td>
<td>Trapezoidal</td>
</tr>
<tr>
<td>7. Seed color</td>
<td>Black and white</td>
</tr>
<tr>
<td>8. Half-flowering time (days)</td>
<td>39 to 45</td>
</tr>
<tr>
<td>9. Flowering time (days)</td>
<td>47</td>
</tr>
<tr>
<td>10. Half-maturity duration (days)</td>
<td>100 to 102</td>
</tr>
<tr>
<td>11. Duration of maturity (days)</td>
<td>101</td>
</tr>
<tr>
<td>12. Parameters</td>
<td>Main agronomic characteristics</td>
</tr>
<tr>
<td>13. Weight of 100 seeds (g)</td>
<td>126 to 130</td>
</tr>
<tr>
<td>14. Fe mineral content</td>
<td>(70 to 75ppm); Zn (31ppm)</td>
</tr>
<tr>
<td>15. Yield (Kg / ha)</td>
<td></td>
</tr>
<tr>
<td>16. In a controlled environment</td>
<td>2500 to 3500 ± 4000</td>
</tr>
</tbody>
</table>
In a real environment:
Mushweshwe
INERA/Mulungu
1200 to 1600
1391 to 1598, sometimes 1700

Resistance to diseases
Ascochytosis, Anthracnose, Rusts

Insect resistance
Resists many insects but sensitive to bruchus.

Color: black - white, good productivity with a stake of 3 m or more, good taste and rapid cooking, its vigor depends on the soil (Soil and climate) and the staking method

Device: Complete Random Block
The field experimentation method was used and carried out in growing season A, from November 2nd, 2020 to February 15th, 2021. The experiment took place in the agricultural field of Mushweshwe at the ITAV/ Mushweshwe, an expanse of land located at the bottom of the guest house (Guest house) of senior executives and Administrative staff of the ISEAV, next to the Kanyenye Primary School. The experimental field is located between 02 ° 18'04'' S latitude and 028 ° 53' 03'' E longitude on an elevation of 1508 m.

The experimental set-up was a complete random block (Figure 1). This device was subdivided into 5 blocks and each block into 5 plots, for a total of 25 (5 blocks x 5 treatments / block). Each plot has a dimension of 2 m x 1.75 m or 3.5 m², at a distance of 50 cm between the lines. The length of 11 m and the width of 9 m is the total surface of 99 m² with a useful surface of 87 m². Each treatment is repeated once and only once per block. All the treatments were monitored at the same time and staking was carried out on November 20th, 2020. Figure 1 shows the experimental design below.

<table>
<thead>
<tr>
<th>Repetition</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetition 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repetition 2</td>
<td>C</td>
<td>P1</td>
<td>P2</td>
<td>P3</td>
<td>P4</td>
</tr>
<tr>
<td>Repetition 3</td>
<td>P2</td>
<td>P3</td>
<td>P4</td>
<td>C</td>
<td>P1</td>
</tr>
<tr>
<td>Repetition 4</td>
<td>P3</td>
<td>C</td>
<td>P1</td>
<td>P2</td>
<td>P1</td>
</tr>
<tr>
<td>Repetition 5</td>
<td>P4</td>
<td>P3</td>
<td>C</td>
<td>P1</td>
<td>P2</td>
</tr>
</tbody>
</table>

Figure 1. Experimental design

Legend
P1 = stake of the tripod: Tie 3 long stakes together. Tying stakes together increases their strength.
P2 = Outside T stakes + string + spike or a rope or stake is tied horizontally between 2 strong single stakes. Our ropes are made from sisal.
P3 = Single stake of 3 m.
P4 = Crossed stakes
C = Single stake of 1 m considered as control treatment

Leaf area, plant height, diameter data were measured for a total of 15 plants. The diameter data was collected by the diagonal and median method at the rate of 5 plants per diagonal and 3 plants per median, a total of 10 plants (for 2 diagonals) and 6 plants (for 2 medians). It was measured using a caliper. The branches were counted per plant to get the number of ramifications. Plant height was measured using a decameter and leaf area using a graduated slat. We took the length of the sheet and the width and the regression coefficient. The bean correction index is 0.3. The number of seeds per pod was counted during the observations also the number of pods for each treatment. The yield was obtained by extrapolation of the plot production per hectare.

Statistical analysis
Data encoding was done on the laptop computer using Microsoft Office Excel 2010 (Curties, 2010) and statistical analyzes using SigmaPlot 12.0 software.

RESULTS
Collar diameter
Figure 1 shows the collar diameter of climbing bean following all treatments below. Indeed, the outside T stakes + string + spike or a rope (P2) has a large diameter at the collar with 0.109 cm than the following: (P1) stake of the tripod (0.084 cm), (P3) single stake of 3 m (0.055 cm), (P4) crossed stakes (0.084 cm) as well as (C) single stake of 1 m (0.0545 cm). Then stake of the tripod (P1) and crossed stakes (P4) have the same diameter 0.08 cm respectively and the single stake of 3 m and of 1 m than too (0.05 cm).
One way analysis of variance shows a significant difference of collar diameter \( F = 13.585; p < 0.001 \) i.e. the vigor of climbing bean plants differs into systems of staking using. Comparison of means using the Smallest Significant Difference method (or Fisher LSD Method) shows that (P4) crossed stakes and (P3) single stake of 3 m are so vigorous than (P2) rope, (P1) stake of the tripod and single stake of 1 m (C). (P1) stake of the tripod, (P2) rope and (C) single stake of 1 m have the same collar diameter so these plants have the same vigor.

### Plant height

The crossed stake plants (P4) have an 8.185 cm of height than the other of the remaining treatments following with the rope (P2) which has 7.334 cm of height than the stake of the tripod (P1), single stake of 3 m (P3) and single stake of 1m (C). Stake of the tripod (P1) are tall with 5.319 cm than those single stake of 3 m (P3) and single stake of 1m (C). Also, single stake of 3 m (P3) are giant than rope (P2), stake of the tripod (P1) and single stake of 1m (C). Stake of the tripod (P1) and rope (P2) have the same height so these plants have the same size but are large compared to single stake of 1m stake or Control (C).

### Number of ramifications

Figure 3 shows the number of climbing bean branches of the different treatments. The treatment of crossed stakes (P4) has 40 ramifications than the other treatments. Single stake of 3 m (P3) has 32 ramifications, followed of rope (P2) with 26 ramifications and stake of the tripod (P1) with 24 ramifications and at the end single stake of 1m (C) with 8 ramifications.
Figure 3. Number of ramifications of climbing bean

One way analysis of variance shows a significant difference between the number of ramifications (F = 13,567.023; p < 0.001) i.e. the ramification of climbing bean plants differs with staking systems. Comparison of means using the method of the Smallest Significant Difference (or Fisher LSD Method) shows crossed stakes (P4) have more branching than single stake of 3m (P3), stake of the tripod (P1) and single stake of 1m (C). Single stake of 3 m (P3) has more ramification than rope (P2), stake of the tripod (P1) and single stake of 1m (C). Rope (P2) has more ramification than stake of the tripod (P1) and single stake of 1m (C). Stake of the tripod (P1) has more ramification than single stake of 1m (C).

Leafarea (cm²)

In figure 4 below, the leaf area differ between treatments, the crossed stakes (P4) has broad leaves with 13.476 cm², single stake of 3 m (P3) with 13.238 cm² respectively the rope (P2) and stake of the tripod (P1) have 12.441cm² and finally single stake of 1m (C) with 11.782 cm².

Figure 4. Variation of leaf area of climbing bean

The analysis of variance shows no significant difference between the leaf areas of staking treatments (F = 2.023; p = 0.118). The leaf area of climbing bean is the same for all applied stake systems.

Number of seeds per pod

Figure 5 shows the number of seeds per pod according to the different treatments. The crossed stakes (P4) has 8 seeds per pod than the other treatments. Then, the rope (P2) has 7 seeds per pod and single stake of 3 m (P3) and stake of the tripod (P1) have respectively 6 seeds. The control or single stake of 1 m (C) has 4 seeds per pod.
The ANOVA shows a significant difference in the number of seeds per pod (F = 6.044; p = 0.002): the number of seeds per pod differs from each other. Comparison of means using the method of the Smallest Significant Difference (Fisher LSD Method) shows crossed stakes (P4) have a higher number of seeds per pod than single stake of 3 m (P3), stake of the tripod (P1) and single stake of 1 m (C). While they have the same number of seeds per pod as those outside T stakes + string + spike (P2). Then, stake of the tripod (P1) respectively have the same number of seeds per pod as crossed stakes (P4) which have a higher number than control or stake of 1 m (C).

**Figure 5. Number of seeds per pod of climbing bean**

The ANOVA shows that a significant difference in the number of pods per treatment (F = 50.792; p <0.001), the number of seeds per pod differed between them. Comparison of means using the method of the Smallest Significant Difference (Fisher LSD Method) shows that crossed stakes (T4) has a higher number of pods than the others (T0, T1 and T2) but equal single stake of 3 m (T3). The outside T stakes + string + spike (T2) stakes has the same number of pods per treatment as the stake of the tripod (T1) which have a large number than the control.

**Figure 6. Number of pods per treatment**

**Yield**

Figure 7 shows deference in treatments of the yield of climbing beans. Single stake of 3 m (P3) has a higher yield (5.072 t / ha) than the other treatments. Then, the outside T stakes + string + spike (P2) raised (4.744 t / ha) and crossed stake (P4) with 2.852 t/ha, the stake of the tripod (P1) with 2.486 t / ha and single stake of 1 m (C) has a yield of 0.968 t / ha.
The ANOVA shows a significant difference in climbing bean yield ($F = 108.608; p < 0.001$); climbing bean yields differ from each other. Comparison of means using the method of the Smallest Significant Difference (Fisher LSD Method) was already revealed that planting practices on the single stake of 3 m (P3) has the same yield as outside T stakes + string + spike (P2) which has a high performance than stake of the tripod (P1), crossed stakes (P4) and, single stake of 1m (C). Stake of the tripod (P1) and crossed stake (P4) have the same yield which is high compared to the control.

**DISCUSSION**

Crosse stakes (P4) and single stake of 3 m (P3) are so vigorous than rope (P2), stake of the tripod (P1) and single stake of 1 m (C). Stake of the tripod (P1), rope (P2) and single stake of 1 m(C) have the same collar diameter so these plants have the same vigor. The length of the stakes explained these. Nyabyenda (2005) shows that the ideal stake length is 2 m.

Crossed stake plants (P4) are taller or giants than single stake of 3 m (P3), rope (P2), stake of the tripod (P1) and single stake of 1 m (C). Single stake of 3 m (P3) are giant than rope (P2), stake of the tripod (P1) and single stake of 1m (C). Stake of the tripod (P1) and rope (P2) have the same height so these plants have the same size but are large compared to single stake of 1m stake or Control (C). Our results are similar to those obtained by Nyabyenda (2005) and Ramaekers (2001). These could be explained by the staking method used too.

Our results are the same with those of Nyabyenda (2005) and Ramaekers et al., (2013) which shows that the climbing bean must be well staked (density and length of the stakes) to produce well. Additionally, climbing beans show their full production potential, they must be staked before tendrils form.

The leaf area of climbing bean is the same for all applied stake systems. It is a hereditary varietal trait (Ramaekers, 2001).

Crossed stakes (P4) have a higher number of seeds per pod than single stake of 3 m (P3), stake of the tripod (P1) and single stake of 1m (C). While they have the same number of seeds per pod as those outside T stakes + string + spike (P2). Then, stake of the tripod (P1) respectively have the same number of seeds per pod as crossed stakes (P4) which have a higher number than control or stake of 1m (C). These could be explained by the staking method used (Nyabyenda,2005; Ramaekers,2001).

Crossed stakes (T4) has a higher number of pods than the others (T0, T1 and T2) but equal single stake of 3 m (T3). The outside T stakes + string + spike (T2) stakes has the same number of pods per treatment as the stake of the tripod (T1) which have a large number than the control. Our results are similar to those obtained by Nyabyenda (2005) and Ramaekers (2001). These could be explained by the staking method used too.

Single stake of 3 m (P3) has the same yield as outside T stakes + string + spike (P2) which has a high performance(4-5 t / ha) than stake of the tripod (P1), crossed stakes (P4) and, single stake of 1m (C). Stake of the tripod (P1) and crossed stake (P4) have the same yield which is high compared to the control. Our results are similar to those obtained by Nyabyenda (2005) and Ramaekers (2001). These could be explained by the staking method used too.

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Crossed stakes (P4) have a higher number of seeds per pod than single stake of 3 m (P3), stake of the tripod (P1) and single stake of 1m (C). While they have the same number of seeds per pod as those outside T stakes + string + spike (P2). Then, stake of the tripod (P1) respectively have the same number of seeds per pod as crossed stakes (P4) which have a higher number than control or stake of 1m (C). These could be explained by the staking method used (Nyabyenda,2005; Ramaekers,2001).

Crossed stakes (T4) has a higher number of pods than the others (T0, T1 and T2) but equal single stake of 3 m (T3). The outside T stakes + string + spike (T2) stakes has the same number of pods per treatment as the stake of the tripod (T1) which have a large number than the control. Our results are similar to those obtained by Nyabyenda (2005) and Ramaekers (2001). These could be explained by the staking method used too.

Our results are the same with those of Nyabyenda (2005) and Ramaekers et al., (2013) which shows that the climbing bean must be well staked (density and length of the stakes) to produce well. Additionally, climbing beans show their full production potential, they must be staked before tendrils form.

The leaf area of climbing bean is the same for all applied stake systems. It is a hereditary varietal trait (Ramaekers, 2001).

Crossed stakes (P4) have a higher number of seeds per pod than single stake of 3 m (P3), stake of the tripod (P1) and single stake of 1m (C). While they have the same number of seeds per pod as those outside T stakes + string + spike (P2). Then, stake of the tripod (P1) respectively have the same number of seeds per pod as crossed stakes (P4) which have a higher number than control or stake of 1m (C). These could be explained by the staking method used (Nyabyenda,2005; Ramaekers,2001).

Crossed stakes (T4) has a higher number of pods than the others (T0, T1 and T2) but equal single stake of 3 m (T3). The outside T stakes + string + spike (T2) stakes has the same number of pods per treatment as the stake of the tripod (T1) which have a large number than the control. Our results are similar to those obtained by Nyabyenda (2005) and Ramaekers (2001). These could be explained by the staking method used too.
has the same yield as outside T stakes + string + spike which has a high performance (4-5 t / ha) than stake of the tripod, crossed stake and, single stake of 1m. For a better future of our households, and also for a good economy and development, we recommend to the practice of climbing beans by sowing in rows and using the single stake of 3 m and the outside T stakes + string + spike or a rope.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: We declare that there is no conflict of interest with the publication of this manuscript. No human/animal participants were involved in the preparation of this manuscript.

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