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Research Article

Effect of additional pollen supplement on fruit setting in *Moringa oleifera*

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ABSTRACT

Moringa oleifera Lam. is a woody species with hermaphrodite flowers cultivated for its fruits and seeds for oil production. The fruit production of the species remains below producers' expectations.The current study was carried out from July to October along two consecutive years (2019 and 2020) at Komsilga (12°18'48.2"N and 1°64'60.2"W) and at the INERA experimental station in Ouagadougou (12°22'801"N and 1°30'295"W) both located in the northern Sudanian zone of Burkina Faso. Our study aimed at testing whether additional pollen on the flower stigma could improve pod and seed yields. For this purpose, a sample of 10 flowering trees and 20 inflorescences/tree was randomly selected at each site. Per tree, 10 newly flowering inflorescences were left to open pollinated and 10 others received pollenmanually. Results show that hand-pollinated inflorescences produced an average of 0.19 and 0.17 fruits, compared to 0.16 and 0.14 for open-pollinated inflorescences at INERA and Komsilga respectively (p= 0.7720). The mean seed weight was 63 (±11.2) and 50 (±7.4) g for the manualand open pollinated treatment, respectively. The Kruskal-wallis test of mean seed weight shows that the smallest fruits were obtained in the open pollination which differed significantly from manual pollination (p< 0.0001). The addition of pollen significantly improved Moringa fruit production.

Keywords: *Moringa oleifera*; Burkina Faso; fruit; pollen; seed

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INTRODUCTION

Plant-host/visitor insect interactions are critical to the success of sexual reproduction in flowering plants (Diallo, 2001). Among these interactions, cross-pollination occurs, which begins with the transfer of pollen from the stamens to the stigma of the flower (Richard, 1997). Several ecological factors are likely affect the pollination success of many species in the Sudano-Sahelian zone, including pollen shortages caused by pollinator decline, high parasitism, habitat degradation and loss, and climate change (Stout *et al.*, 2017).

Increased diversity of pollen available in the environment improves yields in terms of number and weight of seeds produced, thus positively affecting their nutritional and commercial values (Bommarco *et al.*, 2012; Brittain *et al.*, 2014; Garibaldi *et al.*, 2016). Studies on pollination patterns have mostly focused on coffee (Roubik, 2002), avocado and mango (Johannsmeier, 2001). However, little is known about leguminous cash species such as *Moringa oleifera*. Moringa is a woody species that is widely consumed by Burkinabe populations and up to date is being popularized as a vegetable plant by local communities. According to Diouf *et al.* (1999), growing Moringa in vegetable gardens for its leaves and seeds is economic activity for young and poor producers in rural areas and generates more and more significant income. However, the pod yield in quantity and quality is below the expectations of producers and buyers (Dao *et al.*, 2015).

The flowers are hermaphroditic and sexual reproduction, mostly of the cross type, is achieved through the transportation of pollen through several hordes of insects that led to fruits productions. *Apis mellifera*, *Anthophora pilipes* and *Bombus* sp, are involved in the cross-pollination of moringa in Dano, a locality in the Sudano-Sahelian zone in Burkina Faso (Krieg *et al.*, 2017) and in India (Sowmiya *et al.*, 2018). Fruit production of this species is still not improved for local producers. The objectives of this study are to compare the fruit set and fruit set of Moringa plants receiving supplemental pollen (i.e., those receiving a saturating amount of pollen from conspecific individuals) to control plants (i.e., those receiving pollen from free pollination of the surrounding environment). The hypothesis that supports this study is that: Moringa yields are higher with the extra pollen addition compared to open-pollinated flowers (i.e., in the setting flowers are assumed to be pollen-limited).

MATERIALS AND METHODS

Study area

The study was carried out from July to October of the years 2019 and 2020 in the northern Sudanian zone at two sites located in Komsilga, a rural commune in the province of Kadiogo in Burkina Faso (12°18'48.2"N and 1°64’60.2’’W) and at the INERA experimental station in Ouagadougou (12°22'801"N and 1°30'295"W).

Plant materials

The plant material consisted of Moringa seedlings direct sown at a distance of 2m × 2m. The Moringa plants are over 3 years old and have all previously flowered and fruited for at least 2 years. Maintenance consists of manual weeding to eliminate weeds and promote plant development. The amendment of the plants was made with organic fertilizer. The trees were irrigated only in the dry season.2.3 Methodology.

Effect of supplemental pollen on fruit and seed yields

The methodology consisted of randomly selecting a total sample of 200 inflorescences at the stage of elongated flower buds in early bloom of 10 flowering trees. These inflorescences were subjected to the following treatments:

Treatment 1 or control: 100 labeled inflorescences left to pollinate freely, i.e., 10 inflorescences/tree,

Treatment 2: 100 other inflorescences whose newly opened flowers received an additional manual pollen supply, i.e., 10 inflorescences/tree.

In the present study, we proceeded according to the protocol described by Burd (1994) and Bommarco *et al*. (2012). Pollen from hand pollination was obtained from anthers of newly opened flowers collected from Moringa plants located in other areas far enough away to promote cross-pollination (Ferland, 2014). Anthers bearing pollen grains were crushed into a cardboard sheet and then rubbed onto the stigma of the recipient flower. This method has the advantage of depositing enough pollen grains directly on the stigma to initiate fruit set (Cane & Schiffhauer, 2003; Javorek *et al.*, 2002; Ferland, 2014). All open flowers in inflorescences marked in treatment 2 were manually pollinated on three consecutive days from the opening of each flower. At fruit maturity, fruiting counts of the different treatments were made by infrutescence at the end of October 2019 and 2020 and pods were weighed. Seeds extracted from pods by crushing were counted and weighed.

Data analysis

The average fruit production and average seed weight per infrutescence in each treatment were calculated. For each test, the Kruskall-Wallis analysis (McKight & Najab, 2010) was performed and then the residuals were checked for normality, in order to compare the median values of the pollination types (open and pollen addition) and the different sites. The Tukey test was used for the separation of means at the 5% threshold. All analyses and graphs were performed in the R4.1.1 software environment (R Core Team, 2021).

RESULTS AND DISCUSSION

Effects of production site on fruiting

Table 1 shows the effects of production site on the measured parameters (number of seeds, number of reproductive organs in the fruit set, fruit weight and average seed weight). From this table, it can be seen that for the two consecutive years of collection, the production site did not have a significant effect at the 5% threshold on different parameters such as fruit set, number of seeds, fruit weight and average seed weight. After 8 to 12 months, Moringa starts flowering. Its production becomes more important between 1 and 3 years with an average production of 500 fruits/tree (ECHO, 2007).

Table 1: Effects of production site on the measured parameters

Parameter	F	Probability
Harvesting	1.3041	0.253
Number of fruits	0. 013	0. 312
Fruit weight	0. 024	0. 877
Number of seeds	0. 024	0. 877
Average weight of seed	0. 0045	0. 947

Effects of pollen limitation on fruiting

In the two consecutive years of collection, the results of the Kruskall-Wallis test show that there is a highly significant difference between the organs involved in fruit set and seed weight for open pollination and hand pollination but not significant for the other parameters measured at the 5% threshold (Table 2)

Moringa produce the maximum of these seeds mainly from allopollination at the expense of self-pollinated seeds. Note in passing that this strategy has an energetic cost to the plant. In our study, Moringa would have a flowering strategy that could attract enough floral visitors to be efficient in transporting pollen, but also to detect from the stigma the nature of pollen

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received during insect visits. These floral resources (allopollen) are not sufficiently available in time and space in quantity and quality in nature. To this, Lloyd (1979), Lande and Schemske (1985), Bawa & Buckley (1989), Lesaffre & Billiard (2019) suggest that the benefit to the energetic plant would be the maintenance of heterozygosity and diversity that guarantee its sustainability. As long as allopollen is carried by insect pollinators, Moringa would minimize the allogamous mode of sexual reproduction including inbreeding within the plantations studied. Moringa insect pollinators therefore played a major role in the production of good quality fruit. Other studies have confirmed our results. Through their pollinating activity, insects improve fruiting rate, seed weight, average number of seeds of cherimoya (*Annona cherimola*) in Morocco (Ezzahouani *et al.*, 1996) and of *Glycine max* in Cameroon (Taimanga & Tchuenguem, 2018). Similar results according to Faegri *et al* (1979); (Mazi *et al.*, 2019) indicated that potential pollinators (*Xylocopa violaceae*) contribute to the increase of fructtification rate by 27.49%, number of seeds per pod by 45.43% and normal seed percentage by 89.38% in *Phaseolus coccineus*.

Table 2: Effects of pollination type on the measured parameters

Parameter	Degree of freedom	Sites		Free and added pollen	
		Resid. Dev	Pr (>Chi)	Resid. Dev	Pr (>Chi)
Harvesting	1	212.231	0.761	216.33	**<0.0001**
Number of fruit	1	309.078	0.091	218.128	0.091
Fruit weight	1	341.711	0.413	124.721	0.123
Number of seeds	1	211.510	0.9113	163.404	0.113
Average seed weight	1	650.122	0.061	123.342	**0.008**

The structure of the values of the parameters measured during the two consecutive years of monitoring compared according to the types of pollination (open and by manual pollen) are presented in Figure 1.

The inflorescences with manual pollination produced an average number of 0.19 ± 0.007 fruits against 0.15 ± 0.005 for those with open pollination. The rate of fruiting inflorescences was higher with pollen addition (46%) compared to natural pollination (30%). The average fruit weight was 8.2 (±1.78) g and 7.3 (±1.35) g in hand and open pollination, respectively. The average seed weight was 50.2 (±7.4) g and 63.3 (±11.2) g in the open and manual pollination treatment, respectively ($p<0.0001$). Our results also showed that the knotting potential obtained from open cross-pollination through insects could be further improved when the flowers received more allopollen in Moringa. The number of gnarls increased as well as the seed weight with the additional addition of allopollen to the flowers. We can explain these results by the high rate of fertilized ovules because the type of pollen received would have triggered in the plant, the germination of a maximum of pollen grains deposited on the stigma, leading to the formation of a maximum of pollen tubes. This resulted in the development of a large number of pods with full, well developed seeds containing large kernels. Hence the increase in seed weight. Another explanation would be that the seeds from the additional addition of allopollen would accumulate more nutrient reserves and thus ensure the development of fertilized ovules until pod and seed maturation. Seed quality has been improved by hand addition pollination making the seeds heavier (Bommarco *et al.*, 2012) showing that a given amount of pollen is needed to increase yield and seed quantity. The more pollen grains a flower receives, the more potential it has to develop into fruit (Mazi *et al.*, 2019). Improving the production of fruit trees by the additional addition of allopollen has

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been reported by Ezzahouani *et al.* (1996) on Annona cherimola in Morocco, by Stout *et al.* (2018) on shea in Burkina Faso.

The low fruiting rate of open cross-pollination due to insects compared to that of artificial addition of allopollen indicates that other biological variables than pollen limitation, such as stigma receptivity may be equally important factors for fruiting success (Abome, 2002). The success of hand pollination by pollen addition remains mainly dependent on stigma receptivity (Ezzahouani *et al.*, 1996; Saavedra, 1977). Indeed, even if the pollinator is efficient in disseminating or adding pollen, the deposition of allopollen outside the receptivity phase of the stigma in mainly cross-pollinated plants will result in a lower chance of fertilization and thus of fruiting.

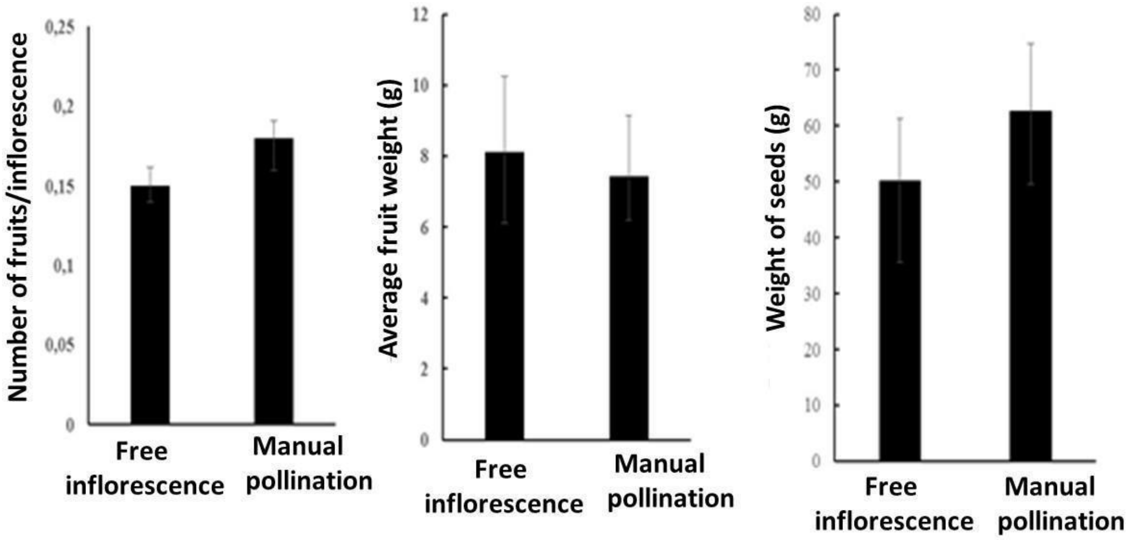

Figure 1: Structure of the values of the measured parameters compared according to the types of pollination (free and by manual pollen)

Conclusion

Moringa oleifera is a leguminous plant that benefits from entomophilic pollination. By comparing the yield of plants with open pollination and with pollen addition, it appeared that the additional pollen supply improves the average fruit production and seed weight. Under these conditions, the quantitative supply of pollen grains is necessary to increase fruit yields. To improve the fruit yield of *M. oleifera*, it is advisable to preserve non-harmful flowering insects in Moringa plantations by avoiding chemical pesticide treatments during the flowering period.

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Authors' contribution

BAZIE Babou Frédéric conceived the idea, designed the experiment, analyzed the data and wrote the manuscript, DAO Madjelia Cangré Ebou supervised the research as a biologist and

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guided the data analysis. All authors read and approved the final manuscript.

Conflict of interest

There is no conflict of interest associated with this publication.

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