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ORIGINAL PAPER

# Insect pollination and pollinator supplementation enhances fruit weight, quality, and marketability of avocado (*Persea americana*)

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**Abstract**
Avocado is a pollinator-dependent crop rich in fiber, monounsaturated oils, vitamins, and minerals, which is seeing an increase in global demand. While some studies have shown that insect pollination improves avocado fruit set, the effects of pollinators on fruit quality and the nutritional profile remain unclear. Furthermore, it remains untested whether a potential pollination deficit can be compensated by the supplementation of farms with extra pollinators. We quantified the contribution of insect pollination on fruit and seed weight, and oil, protein, carbohydrate, and phytochemical (flavonoid and phenolics) contents, and assessed if the supplementation of pollinators on farms can improve fruit parameters. The experiment was conducted in 36 smallholder avocado farms of which 18 farms were supplemented with two *Apis mellifera* bee hives, while 18 were used as controls. Four manipulative pollination treatments were conducted: hand, open, self- and wind pollination. We observed that avocado fruit weight was significantly higher (213.7 g) for insect pollinated flowers than in case of wind- (107.8 g) or self-pollination (95.1 g).

Insect pollination resulted in heavier seeds (29.5 g) as well as higher oil contents, clearly showing that insect pollination was required to reach a high seed yield and quantity of oil. Honey bee supplementation on farms enhanced the avocado fruit weight by 18%, and increased avocado oil content by 3.6%. As the marketability of avocado directly depends on fruit size and oil content, a sustainable management of bee communities may be of key importance for the long-term profitability of avocado agriculture.

**Keywords** Crop pollination · Crop yield · Ecosystem service · Fruit weight · Nutritional proximate · Pollination deficit

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## Introduction

Pollination is crucial for global food security and human nutrition, contributing to approximately 35% of world food crop production (Klein et al. 2007; Chaplin-Kramer et al. 2014). However, there is increased evidence of pollinator declines for both managed and wild insect pollinators (Potts et al. 2010; Cameron et al. 2011; Dicks et al. 2021), which is putting pollination-dependent crops at great risk (Dainese et al. 2019). Pollination-dependent crops such as fruits and vegetables form a substantial component of human food and contribute significantly to a healthy diet by supplying essential nutrients such as vitamins, antioxidants and fiber, which help to reduce micronutrient deficiencies (Eilers et al. 2011). There is lack of sufficient nutrient supply especially in sub-Saharan Africa (Bain et al. 2013). Hence, nutrient supply is not a matter of production quantities alone, but rather depends on the quality of agricultural products, which has become a major challenge. Several factors such as soil quality, pests and diseases, and climatic conditions affect quality in crop production (Liliane and Charles 2020).

Some studies have shown that decreased yield losses emerge from fruit deformations and undeveloped fruits caused by pollination deficits, adversely affecting the marketability of fruits (Ariza et al. 2011). Few studies have been conducted on the effect of pollination on fruit quality parameters. For instance, pollination increased fruit weight and shelf life in strawberries (*Fragaria ananassa*) (Klatt et al. 2013), while in oilseed rape (*Brassica napus*), pollination enhanced seed quality (heavier seeds) and oil content but lowered chlorophyll content (Bommarco et al. 2012). Pollination also influenced the nutritional composition in almonds (*Prunus dulcis*) whereby fat and vitamin E content of the nuts were highly increased by pollination (Brittain et al. 2013). Furthermore, effective pollination was found to decrease malformations in apples (*Malus domestica*) and strawberries (*Fragaria ananassa*) (Matsumoto et al. 2012; Wietzke et al. 2018), whereas in oriental melon (*Cucumis melo*), pollination was found to increase flavor-enhancing elements such as sugars and acids (Shin et al. 2007). However, there is scanty literature on the effect of insect pollination on avocado fruit quality and nutritional composition.

Avocado is a unique fruit with high nutritional value. It is a rich source of monounsaturated oleic acid, and it contains important lipid-soluble antioxidants and high levels of phytochemicals such as carotenoids, polyphenols, chlorophylls, tocopherols, and phytosterols (Pieterse et al. 2003; Duarte et al. 2017). The oil content in avocado is a critical quality attribute which affects market acceptance both for industrial and culinary use. Oil content is also used as an index of maturity in avocado fruit. Currently, there is increasing evidence of the health benefits of avocado, which has led to increased production and consumption (Bhuyan et al. 2019). Consumption of avocado fruits aids in promoting cardiovascular health, weight management, and healthy aging (Carvajal-Zarrabal et al. 2014; Weschenfelder et al. 2015; Johnny et al. 2019). However, several factors affect avocado production and quality, among them pests and diseases, poor soils, and pollination deficits (Alcaraz and Hormaza 2009). Avocado requires insects for successful pollination with honey bees being the most common pollinators (Evans et al. 2011, Sagwe et al. 2022). Other potential avocado pollinators include the hoverfly (*Phytomia incisa*), tropical African latrine blowfly *(Chrysomya putoria*), and polistine wasps (*Polistes* sp.) (Mulwa et al. 2019; Okello et al. 2021; Sagwe et al. 2022).

Fruit size and weight are among the most important quality parameters for market evaluation and consumer preference of avocado fruits (Migliore et al. 2018). During the packaging and marketing process, avocados are classified into different grades based on size. For instance, according to OECD and UNECE standards for avocados (OECD 2011; UNECE 2017) and Codex Alimentarius Standards for avocados (CODEX, 2021, STAN 197–1995), market grading of fresh fruits is classified according to size codes 1 to 30 with a minimum weight of 80 g for avocados of variety Hass. In Europe, the preferred size for Hass avocados is 16 (227 to 274 g) to 18 (203 to 243 g) and for the variety Fuerte, 14 (258 to 313 g) to 16 (227 to 274 g), with bigger fruits generally generating higher market values.

Some studies have been conducted on the dependence of avocado fruit quality on pollination (Can-Alonzo et al. 2005; Altendorf 2019). Nevertheless, major knowledge gaps exist linking pollination services to fruit quality aspects, which in turn have an economic impact on avocado production. Thus, in-depth knowledge of fruit quality parameters is essential to deliver avocado fruit of excellent quality to the global market. This study aimed to investigate the impact of insect pollination on various aspects of avocado fruit quality, including fruit size, proximate composition, and phytochemical contents. Additionally, we examined whether the supplementation of honey bees would affect fruit quality parameters, nutritional composition, and phytochemical contents.

## Materials and methods

### Study site

The study was carried out in smallholder avocado farms in Murang'a County, Kenya (S 0°43'0", E 37°9'0") during August – September 2019. We selected 36 smallholder avocado farms (0.2 – 0.4 ha) for our study. Half of the farms (18) were selected for pollinator supplementation with two colonies of the Western honeybee *A. mellifera* per farm and the other half (18) were the non-supplemented controls. Honeybee colonies were housed in standard Langstroth hives with ten frames and headed by a naturally mated queen. The treatment option (pollinators and control) depended on the farmer's willingness to implement (Adan et al. 2021). To avoid overlapping, the farms with pollination supplementation were at least 1.5 – 3.5 km away from each other informed by the foraging range of the honeybee (Hagler et al. 2011) and at least 3.5 km apart from the control farms. The *A. mellifera* colonies were introduced just before avocado blooming. The experiment included two dominant avocado varieties, Fuerte and Hass. Among the surveyed farms, Hass was grown in 21, Fuerte in 12 farms, and three farms cultivated both varieties.

## Pollination treatments

At each farm, three trees were randomly selected. The trees had a minimum distance of 10 m from the edge of the farm to minimize the edge effects. On each tree, four east-orienting branches (pointing eastwards) at a similar height were randomly selected, leading to a total of 432 branches for the experiment. Bags for manipulating flower access were put in place 1–6 days before flowering. Four manipulative pollination experiments were conducted: (i) self-pollination (self), using woven bags that were impermeable to wind, allowing only autogamous self-pollination; (ii) wind pollination and spontaneous selfing (self + wind), whereby insects were excluded by coarse mesh gauze with $0.8 - 1.0$ mm openings (Sacchi and Price 1988; Willmer and Stone 1989); (iii) open pollination (open), in which all insects had access to flowers (insect pollination); and (iv) hand pollination (hand), pollen was transferred to stigmas with a paint brush and flowers covered with very fine nylon mesh gauze (10 µm), according to Willmer and Stone (1989) as a positive control for optimal pollen transfer. To test the role of genetic diversity in enhancing fruit set, cross-pollination was conducted between two avocado varieties, Hass and Fuerte. In farms where these varieties were not intercropped, pollen was collected in neighbouring farms. The hand pollination method involved manually transferring pollen from the male reproductive structures (stamens) of one avocado flower to the female reproductive structure (pistils) of another flower. This process typically entailed identifying avocado flowers with open stamens and gently tapping the stamen to release pollen onto a small paintbrush. The pollen was then carefully brushed onto the receptive stigma, ensuring an even distribution. This meticulous procedure aimed to increase the likelihood of successful pollination. To mitigate the potential influence of insects on the experimental results, we applied sticky glue on the branch beneath the bagged flowers to eliminate any insects, such as ants, that could have entered the bags and potentially influenced the pollination process.

For each treatment, six weeks after bagging, the number of green fruits per branch was counted to assess the initial fruit set and determine the success of pollination and fruit development. Two months after the initial fruit set, a follow-up assessment was conducted to evaluate fruit drops during the development phase. All retained fruits were counted to determine the rate of fruit drop, providing insights into factors influencing fruit retention and stability (Sagwe et al. 2021). After five months from the initial fruit set, the fruits from each treatment were harvested to analyse the effect of pollination on fruit quality.

## Fruit physical quality parameters

### Size and weight

All avocado fruits from experimental inflorescences were harvested two weeks before commercial harvest from each of the farms (late April to early May 2020). Avocados were labelled and bagged individually according to the pollination treatment, tree and farm number, and then transported to the laboratory at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya, for quality assessment. Within 24 h of harvest, we ensured that all physical quality measures had been taken since the fruit moisture content decreases over time. Quality measures included fruit fresh weight taken on an analytical weighing balance sensitive to the nearest 0.0001 g (Mettler Toledo MS403TS/00, Scales Plus LLC, Michigan, United States), maximum width, measured using a tape measure, and height using calipers sensitive to 0.05 mm. After five days of ripening of the fruits at room temperature, the avocado flesh was removed, and the seed weight was measured.

## Fruit nutritional and phytochemical quality parameters

### Preparation of avocado fruits (samples)

Seven fruits were sampled from each of four control and supplemented farms, these farms were chosen because they contained most of the fruits from all the pollination treatments during the harvesting stage, except from one supplemented farm and one control farm where either six and eight fruits, respectively, were sampled. Hence, in total 57 fruits were sampled for fruit nutritional and phytochemical quality parameters (30 fruits from four control farms and, 27 fruits from four supplemented farms). Avocado fruit samples were left to ripen naturally in the dark at room temperature (23–25°C), for seven days. Each avocado fruit was peeled, and its flesh was cut into small unform pieces that were dried for 12 h at 60°C using an oven (Memmert AtmoControl, Memmert GmbH, Schwabach, Germany). The dry flesh was then grinded into fine powder and homogenized using a mixer grinder (Philips HL7756/00 Mixer grinder, Philips &Co, Eindhoven, Netherlands).

### Moisture content

The moisture content was calculated from the difference in mass between the initial and dried sample as follows:

$$Moisture\ content\% = (wet\ weight - dry\ weight)/wet\ weight\ x\ 100.$$

### Oil extraction

From each fruit sample, in three replications, 2 g of avocado powder were placed into a 15 mL falcon tube, then 10 mL of hexane was added and mixed rigorously using a vortex (Vortex-Genie 2 Mixer, Scientific industries, New York, USA) at 2,500 rpm for 5 min. The mixture was filtered using a 0.45 µm microfilter before concentrating the resulting filtrate (extracted lipids) using a concentrator (Eppendorf Concentrator plus, Merck, Hamburg, Germany). The lipid content in the sample was determined by weight difference:

$$Oil\,content\,(\%) = amount\,of\,oil\,extracted\,(g)\,/weight\,of\,original\,sample\,(g) \times 100$$

### Total carbohydrate content

From each fruit sample, 0.1 g of avocado powder were mixed with 5 mL of 2.5 M hydrochloric acid (HCl) and placed in a boiling water bath for 1 h. Sodium carbonate ($Na_2CO_3$) was added until effervescence stopped; then 10 mL of distilled water were added. This solution was centrifuged at 4,200 rpm for 5 min. From the solution, 1 mL was placed into a 20 mL test tube, before 1 mL of phenol solution (50% w/v) was added followed by 5 mL of concentrated sulphuric acid ($H_2SO_4$) in a fume hood. Subsequently, 500 µL of solution was removed into 20 mL test tube and diluted with 3 mL of water. The absorbance was measured at 490 nm against a blank (all reagent minus the sample). The calibration curve was made using a glucose standard at a range of (0–0.1 µg mL$^{-1}$) using five data points that were generated in replicates. This assessment was replicated three times.

### Total protein determination test

The total protein (TP) content was determined by the Bradford method with minor modifications (Bradford 1976), with three replicates. To 0.3 mL avocado fruit solution (5 mg/mL in Tris/EDTA buffer; 15.76 g of Tris and 2.92 g of EDTA in 1 L), 5 mL of Bradford reagent (100 mg of Brilliant Blue G-250 dye, 50 mL of absolute ethanol and 100 mL of 85% phosphoric acid, made up to in 1 L with distilled water) was added. After 2 min, the absorbance was measured at 595 nm against the blank (the reactive solution without the sample) using a spectrophotometer (6850 Jenway, Kobian, Nairobi, Kenya). Bovine serum albumin (BSA) was used to generate the calibration curve ($0 - 300$ µg mL$^{-1}$). The TP content was calculated and expressed as g of BSA equivalent E/100 g or %.

### Total flavonoid content

Total flavonoid content (TFC) was determined using a colorimetric method adapted by Zhishen et al. (1999) with minor modifications. An avocado fruit sample (1 g) was diluted with 10 mL of 50% (v/v) methanol. One mL this solution was then mixed with 4 mL distilled water. At the baseline, 0.3 mL of 5% (w/v) $NaNO_2$ was added. After 5 min, 0.3 mL of 10% (w/v) $AlCl_3$ was added, followed by addition of 2 mL 1 M NaOH 6 min later. The final volume of 10 mL was achieved by the addition of 2.4 mL distilled water. The mixture was vortexed to ensure adequate mixing and the absorbance was read at 510 nm. A calibration curve was created using a standard solution of quercetin done in triplicates in the range of (10–200 µg mL$^{-1}$) with five data points. The result was expressed as mg quercetin equivalents (QE/100 g). This experiment was replicated three times.

### Determination of total phenolic content

Total phenolic content was evaluated by the Folin-Ciocalteu method as described by Singleton et al. (1999) with minor modifications. One g of avocado sample was diluted with 10 mL of 50% methanol, then 1 mL of the solution was mixed with 5 mL of 0.2 N Folin-Ciocalteu reagent for 5 min. After adding 4 mL of 75 g/L sodium carbonate, the mixture was incubated at room temperature for 2 h, then the absorbance of the reaction mixture was measured at 760 nm against a water blank. Gallic acid was used as standard to yield the calibration curve using five data points done in triplicates in the range of (0–250 µg mL$^{-1}$). The total phenolic content was expressed as mg of gallic acid equivalent (mg GAE/100 g) and this experiment was replicated three times.

## Statistical analysis

Linear mixed effect models (LMM) implemented in the lmer function of the *nlme* package (Pinheiro et al., 2016) were used to analyze the effects of pollination treatments on the avocado fruit quality (weight, width, and height). Pollination treatment was used as a fixed effect to test for differences in the mean weight of fruits between hand, open, self + wind, and self-pollination. Farm ID and variety were used as random terms. In the control farms (without honey bee supplementation) we compared if there was a significant difference in fruit weight between hand-pollinated and open-pollinated flowers. Furthermore, we analyzed if farms supplemented with honey bee colonies, enhanced fruit weight by comparing hand versus open pollination. The overall effect of pollination treatment on the nutritional quality was assessed using analysis of variance (ANOVA). Pairwise comparison of means were performed using the *lsmeans* package (Lenth 2016) and with the Tukey method for adjustment of p-values. The differences were summarized using *multcompView* package (Graves et al., 2019).

Marketability of fruits for open pollination and pollinator exclusion treatments was evaluated using the market grading classification of UCECE standards for avocados (2017) and Codex Alimentarius Standards for avocado. The economic benefits of pollination services were estimated by comparing the fruit quality in open pollination with pollinator exclusion treatments. The yield gap loss was calculated as the difference between the mean fruit weight in open and self + wind pollination treatments.

All analyses were carried out in R statistical software (v 4.0.2, R Foundation for Statistical Computing, Vienna, Austria).

## Results

### Size and weight of avocado fruits

The exclusion of pollinators had a significant effect on the avocado fruit quality parameters (Fig. 1). The mean weight of avocado fruits in the open flower treatment (mean ± SE fruit weight = 213.7 ± 7.4 g) was significantly higher ($Z = 4.26$, $p < 0.001$; $Z = 8.63$, $p < 0.001$) than in treatments where pollinators were excluded from flowers

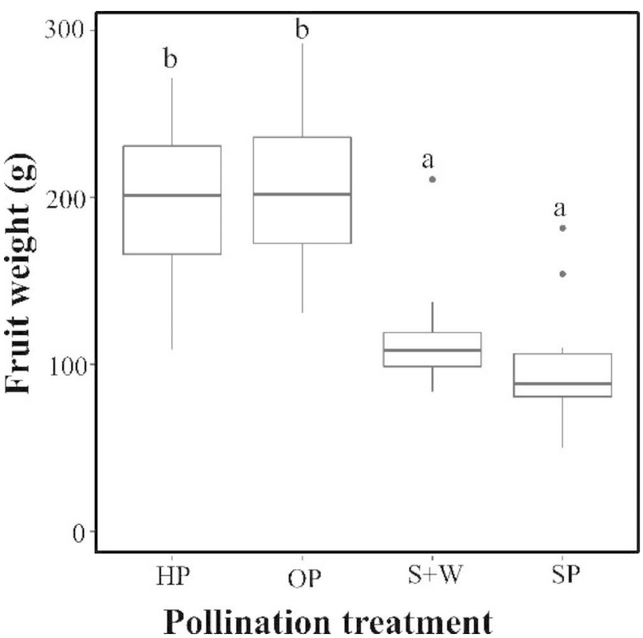


**Fig. 1** Boxplot showing the effect of pollination treatments (hand-, open-, self + wind-, and self-pollination) on avocado fruit weight. Means with different letters are significantly different in pairwise comparisons (Tukey's HSD, $P < 0.05$). Dots show the outliers. Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers)

[self + wind pollination (107.8 ± 31.8 g) and self-pollination (95.1 ± 27.2 g), respectively]. The same relationship was found in avocado seeds whereby the open pollination treatment resulted in significantly larger seeds (mean ± SE seed weight = 29.5 ± 1.6 g) than seeds from pollinator exclusion treatments; self + wind pollination (14.7 ± 3.9 g) and self-pollination only (13.3 ± 3.8 g) (Fig. 4). Additionally, a study by Sagwe et al. (2021) reported a very high reliance of avocados on insect pollinators, with a significantly lower fruit set observed for self- and wind-pollinated flowers (17.4%) and self-pollinated flowers (6.4%) compared to insect-pollinated flowers (89.5%). This indicates the importance of insect pollination for successful fruit development. No significant differences of avocado fruit weight in control farms were observed between hand and open pollination ($Z$ = -2.07, $p = 0.17$) (Fig. 2) as well as between self and self + wind pollination treatments ($Z = 0.91$, $p = 0.80$). On the contrary, there was a significant difference of avocado fruit weight in supplemented farms between hand and open pollination ($Z$ = -2.65, $p = 0.047$) (Fig. 2). Separating the different avocado varieties, the mean weight (± SE) for Hass fruits in the open flower treatment equaled 221.54 ± 9.58 g, in treatments with pollinator exclusion (self + wind pollination) 124.28 ± 8.47 g, and in the self-pollination treatment 105.95 ± 10.11 g. The Fuerte variety fruits in the open flower treatment had a mean weight of 267.68 ± 10.07 g, in self + wind pollination 124.08 ± 9.02 g, and in the self-pollination treatment 104.24 ± 7.48 g. There was no significant difference in avocado fruit weight in open pollination between Hass and Fuerte varieties ($Z$ = -1.65, $p = 0.09$).

### Avocado nutritional quality

The exclusion of pollinators affected the oil concentration of avocado (Table 1). There was a significant difference in oil concentration in the control farms between open and self + wind pollination ($Z = 4.01$, $p = 0.003$) but no significant effect was observed between open and self-pollination ($Z = 1.08$, $p = 0.70$), hand and open ($Z = 0.44$, $p = 0.97$), and between self and self + wind pollination ($Z = 2.33$, $p = 0.12$) (Fig. 3). The oil concentration was highest in both hand (40.1 ± 1.7%) and open (38.1 ± 1.9%) pollination treatments followed by self- (33 ± 6.0%) and was least in self + wind (20.7 ± 4.3%) pollination. There was no evidence that insect pollination affected other nutritional parameters; protein ($F = 0.17$, $p = 0.69$), carbohydrates ($F = 0.54$, $p = 0.48$), moisture ($F = 2.08$, $p = 0.19$), flavonoid ($F = 0.18$, $p = 0.68$) and phenolic ($F = 3.3$, $p = 0.1$) contents (Table 1). Honeybee supplementation appeared to increased oil concentration by 3.6% (mean ± SE oil concentration = 41.7 ± 1.9%), although the increment was not significant ($F = 1.56$, $p = 0.23$) when compared with control farms (mean ± SE oil concentration = 38.1 ± 1.9%) in the

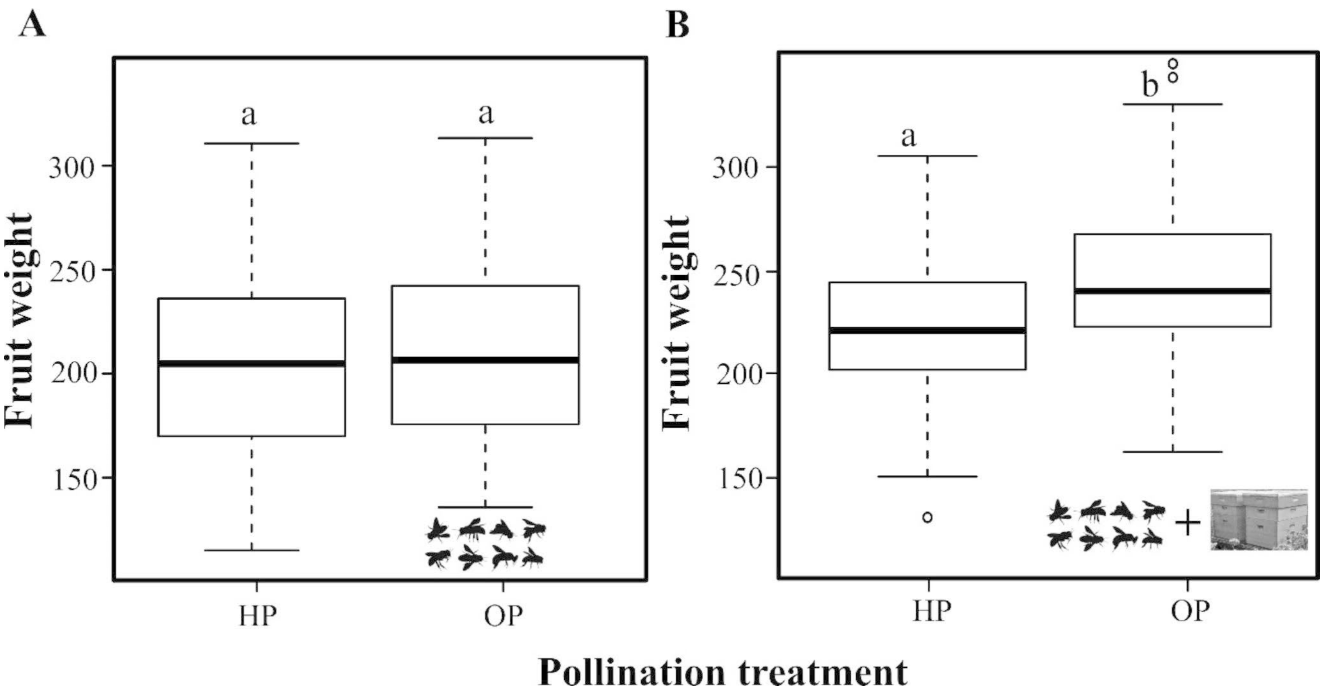


**Fig. 2** Fruit weight observed in hand (HP) and open (OP) pollination treatments. Control farms that were not supplemented with honey bees (**A**). Farms supplemented with two *A. mellifera* colonies per farm (**B**). Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers)

**Table 1** Effect of pollination treatments on avocado nutritional qualities. Shown are parameter estimates (mean ± SE) from linear mixed effects models shown ($n = 57$).

| Treatments | oil (%) | protein (%) | carbo-hydrates (%) | moisture (%) | TFC (mg QE/100 g) | TPC (mg GAE/100 g) |
|---|---|---|---|---|---|---|
| Hand | $40.1 \pm 1.7^{b}$ | $1.7 \pm 0.1^{a}$ | $2.7 \pm 0.3^{a}$ | $70.2 \pm 1.3^{a}$ | $7.6 \pm 2.5^{a}$ | $387.5 \pm 38.9^{a}$ |
| Open | $38.1 \pm 1.9^{b}$ | $1.7 \pm 0.1^{a}$ | $2.8 \pm 0.4^{a}$ | $70.2 \pm 0.8^{a}$ | $8.6 \pm 3.0^{a}$ | $442.6 \pm 48.8^{a}$ |
| Self | $33.0 \pm 6.0^{ab}$ | $1.9 \pm 6.0^{a}$ | $2.0 \pm 0.2^{a}$ | $72.3 \pm 2.3^{a}$ | $4.7 \pm 0.9^{a}$ | $482.1 \pm 90.0^{a}$ |
| Self + wind | $20.7 \pm 4.2^{a}$ | $1.5 \pm 0.1^{a}$ | $1.7 \pm 0.2^{a}$ | $72.3 \pm 1.5^{a}$ | $7.3 \pm 2.0^{a}$ | $331.3 \pm 4.7^{a}$ |

The mean values for the fruit parameters within a column with different superscript letters are significantly different (P < 0.05) according to Tukey test. TFC (total flavonoid content), TPC (total phenolic contents), QE (quercetin equivalent), GAE (gallic acid equivalent). The nutritional proximate is based on the dry matter content

open treatments. In addition, there was very little variation in the oil content between the two avocado cultivars ($Z = -0.51$, $P = 0.61$), where the average oil percentage in Hass cultivar was 36.0% while it was 34.0% in Fuerte. The avocado moisture and lipid contents were negatively correlated and only marginally significant ($R = -0.24$, $P = 0.08$).

## Economic valuation of pollination services

Avocado fruits from open pollination treatments (mean ± SE fruit weight = 213.7 ± 7.4 g) and hand pollination (200.3 ± 9.8 g) were heavier compared to self + wind (107.8 ± 31.8 g) and self-pollination (95.2 ± 27.2 g). Therefore, fruits from open pollination fall under grade 18 (203–243 g) and those from hand pollination under grade 20 (184–217 g), which are the most preferred market grade for international market according to UNECE standards for avocados and Codex Alimentarius Standards for avocados (UNECE 2017; CODEX, 2021, STAN 197–1995). Self-pollination and wind pollination resulted in lower quality avocados, falling under grade S (less than 123 g), which are less preferred in the international market. However, when honey bee supplementation was introduced, the average fruit weight increased to 253.0 g, corresponding to grade 16 (227–274 g) according to the UNECE standards for avocados (UNECE 2017). Grade 16 is also a top preferred market grade in the international market. The honey bee supplementation revealed a significant economic impact on avocado producers. Our findings indicated that by introducing honey bee colonies to avocado orchards, there was an increase in the overall size of the yield by 18%. Utilizing the method employed by Gallai et al. (2009) to evaluate the economic value of pollination, we are able to determine the surplus obtained by smallholder farmers

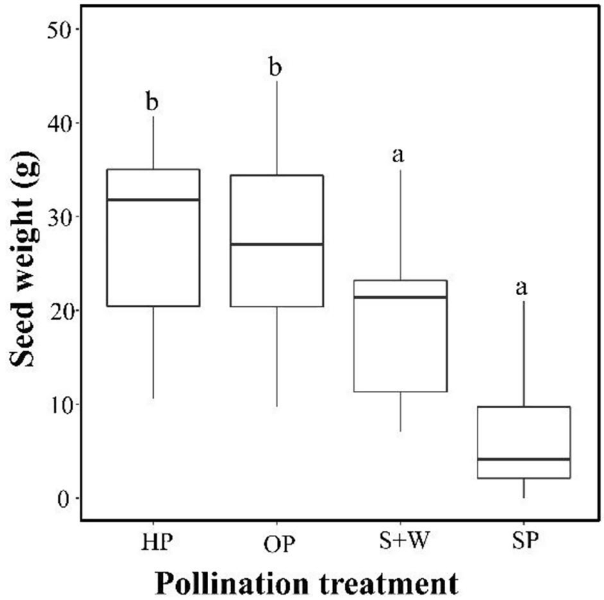


**Fig. 4** Boxplot showing the effect of pollination treatments (hand-, open-, self+wind-, and self-pollination) on avocado seed weight. Means with different letters are significantly different in pairwise comparisons (Tukey?s HSD, P<0.05). Dots show the outliers. Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers)

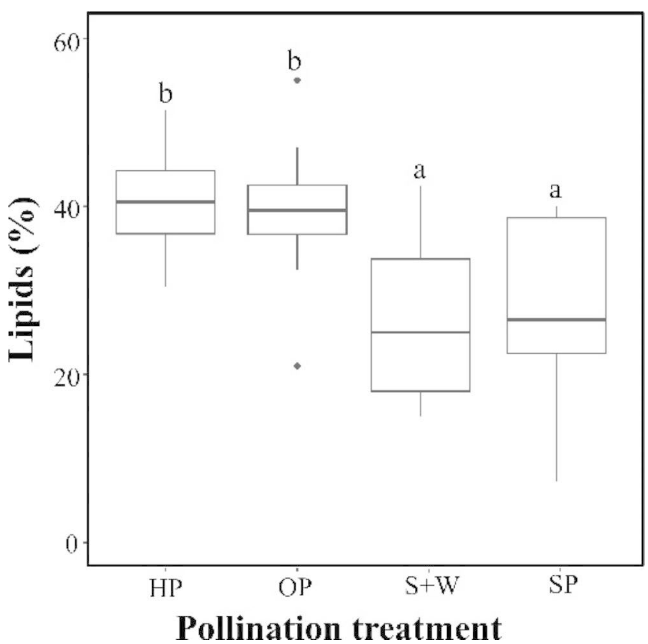


**Fig. 3** Boxplot showing the effect of pollination treatments (hand-, open-, self+wind-, and self-pollination) on avocado oil. Means with different letters are significantly different (Tukey's HSD, P<0.05). Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers)

due to the implementation of honey bee colonies on their farms. We focus exclusively on the benefit due to increased pollination services not taking the production of beehive products into account. We assume based on the data derived on smallholder farms in Murang'a County that the average farm has ten avocado trees, which produce 280 fruits of a total weight of 61.7 kg per tree (Toukem et al. 2022; Sagwe et al. 2021). The production costs of 1 tonne avocado were USD 217.40 (FAOSTAT 2021). This translates into USD 134.14 of production costs for the whole average smallholder farm (10 trees each 61.7 kg, total of 617 kg). Due to a pollination dependency of 0.4–0.9 (mean 0.65) according to Klein et al. (2007) or 0.72 on the smallholder farms in Murang'a county (Sagwe et al. 2021) the total economic value added through pollination is USD 87.19 (pollination dependency (PD) of 0.65) or USD 96.58 (PD 0.72). Implementing two honey bee colonies on a farm will lead to an increase in fruit weight by 18% which translates to USD 102.88 (PD 0.65) or USD 113.96 (PD 0.72). Due to the implementation of bee colonies farmers might incur a cost of around USD 140 (two hives, bee suite, smoker, and hive tool), while the increase in production costs is at USD 24.14. The benefit through sales depends on the market access (local vs. export) and many other factors. Overall, pollination benefits might be much larger as smallholder farms usually plant several different commodities and beehive products can also contribute to additional income. Furthermore, supplementation of honeybees leads to 3.6% increase in oil concentration.

## Discussion

The present study showed that avocado fruit physical parameters were significantly affected by pollination treatments, where insect pollination of avocado flowers increased fruit weights and lipid content significantly. Furthermore, the supplementation of avocado farms with honey bee colonies increased the fruit weight significantly compared to hand and open pollination.

Fruits from open and hand pollination treatments, showed an increase in avocado physical quality through increased fruit and seed weight compared to fruits from pollination exclusion treatments (self- and self+wind pollination), thereby improving the market value. These results confirm findings of other authors, whereby Mulwa et al. (2019) reported a significantly higher fruit yield and larger seeds from unbagged than bagged treatments. Moreover, Ish-Am and Lahav (2011) found that wind contributed only slightly to avocado yield and strongly recommended the use of honey bees for pollination. The low yield observed in self- and self+wind pollination treatments was perhaps a result of lower fertilization success. However, the study also acknowledges some limitations in achieving complete exclusion of pollinators, particularly in self-pollination experiments. While efforts were made to prevent wind and insect interference, it is possible that some minimal levels of wind or small insects, such as thrips, may have still affected the self-pollinated flowers. This limitation is noteworthy because the observed fruit set of 6.4% in self-pollinated flowers may not represent true self-pollination, but rather residual effects of unintended pollination. The mesh probably obstructed the wind and excluded larger arthropods but favoured microarthropods, which might have influenced the observed fruit set. This result implies that avocado farmers will be experiencing reduction of yield if insect pollinators are not present. Because avocado is a major horticultural fruit currently making a very significant contribution to the Kenyan income and revenue (Amare et al. 2019), significant yield and income gains could be made by farmers through improved pollinator management.

Further, our results clearly showed that insect pollination contributes significantly to high seed quality (weight and size). Similar results have been reported by Mulwa et al. (2019) in avocado seeds whereby insect pollination treatments produced heavier seed than pollinator exclusion treatments. The seed size has an indication of high seed germination rate to growers. For example, a study conducted by Olorunmaiye et al. (2011) in mango seeds found that heavy seeds produced a greater number of seedlings, seedlings with high vegetation structure, and dry matter accumulation. Moreover, Massimi (2018) indicated that large and medium seed-size of barley classes had a significantly higher standard of germination percentages than small size classes. Seed size is an important indicator of physiological quality which affect seed germination and seedling growth especially under stressful conditions (Steiner et al. 2019). This data also supports our early findings showing that insect pollination increases fruit retention since non-viable cause fruit abortion (Sagwe et al. 2021). Further, seed size has been reported to affect crop development and performance in the field (Adebisi et al. 2013). A possible explanation for this could be caused by higher food and other energy reserves, which are essential factors to improve the expression of germination and initial growth of seedlings (Shahi et al. 2015). Therefore, for successful seed germination and crop performance seed size play an important role. However, from a consumer perspective, seedlessness in avocados can be desirable since it means more fruit flesh and less wasted space occupied by the seed. Seedless avocados could provide a higher yield of edible portions, potentially increasing the market value for certain consumer preferences.

Despite the effect of pollination in enhancing the quality of avocado fruits, other agronomic factors such as irrigation and resource availability influence the general health of the tree which in turn have a greater influence on fruit size (Kremer-Köhne and Köhne 1995). Besides, it is evident that increasing cross-pollination in orchards needs to be considered because it affected some quality aspects of avocado. To effectively increase cross-pollination in the orchards there is a need for high species richness and abundance of pollinators (Samnegård et al. 2019). Our former study (Sagwe et al. 2021) indicates that avocados heavily rely on insect pollinators for successful fruit set. However, the study also acknowledges some limitations in achieving complete exclusion of pollinators, particularly in self-pollination experiments. While efforts were made to prevent wind and insect interference, it is possible that small insects, such as thrips, may have still affected the self-pollinated flowers. This limitation is noteworthy because the observed fruit set of 6.4% in self-pollinated flowers may not represent true self-pollination but rather residual effects of unintended pollination. The presence of even a small number of pollinators or wind could have influenced the observed fruit set.

In the present study, oil concentration was significantly affected by pollination treatments whereby hand and open pollination treatments had higher oil content compared to pollinator excluded treatments. In addition, the farms that were supplemented with honey bees increased oil concentration by 3.6% although the oil concentration did not differ significantly with the control farms. Similar effects have been reported in other crops, for example, oilseed rape had higher oil and lower chlorophyll contents when adequately pollinated (Bartomeus et al. 2014). Another study conducted by Fries and Stark (1983) in oilseed rape production indicated that honey bees increased the percentage of oil content of seed. Additionally, Silva et al. (2018) showed that bee pollination in sunflower enhanced unsaturated fatty acids by 0.3%. Oil in avocado is the most outstanding quality parameter linked to the market price, it also contains healthy fatty acids, which exert many cardiovascular benefits (Forero-Doria et al. 2017). Apart from oil content, none of the nutritional proximate was affected by pollination treatments. Very small quantities of carbohydrates were reported in our study, an indication that avocado has low sugar content making it more beneficial for people with diabetes (Del Toro-Equihua et al. 2016; Tramontin et al. 2020). Both protein and phytochemicals play an essential role in the human diet. For instance, phytochemicals play an important role in cancer prevention, and in scavenging free radicals that have been reported as the root cause of many pathological conditions (Manaf et al. 2018). The oil content was negatively correlated with the moisture content, which was in agreement with other studies such as (Lee et al. 1983; Osuna-García et al. 2010).

Our results confirmed that pollination can make a significant financial contribution to farmers in terms of higher fruit yield. Marketability is one of the most important aspects linked to fruit quality. Our results showed that open pollination treatments produced grade 18 (203–243 g) fruits and honey bee supplementation farms produced grade 16 (227–274 g) fruits both of which are among the market valued grades in the international market according to UNECE standards for avocados (2017) and Codex Alimentarius Standards for avocados (CODEX, 2021, STAN 197–1995). Additionally, we examined if the introduction of honey bee colonies to avocado orchard can increase farmer's profit. The results on the economic valuation highlighted a significant increase in profit for farmers. Our findings indicated that by introducing honey bee colonies to avocado orchards there was a total overall size yield increment of 18%. This revealed a higher economic impact when only wild pollinators are present, study conducted by Peña and Carabalí (2018) showed that farms that were supplemented with honey bee colonies resulted in higher yield than the control farms (without bee colonies). Similar results have been reported by Geslin et al. (2017) in apple fruits whereby, the presence of high-quality honey bee colonies increased farmer's profits by 70%. A study conducted by Gajc-Wolska et al. (2011) on cucumber (*Cucumis sativus* L.) indicated that introducing bumblebees resulted in higher fruit yield, firmness, and better quality characteristics. Therefore, introducing honey bees into the field during blooming periods can compensate for the absence of pollinators. However, it is also important to promote orchard management practices that enhance the presence and effectiveness of natural pollinators to achieve higher crop yields, as several wild bees and flies are also efficient pollinators of avocado (Sagwe et al. 2022).

## Conclusions

Our findings suggest that insect pollination is a key driver for both the physical and nutritional quality of avocado, hence higher avocado production. Supplementation with honey bee colonies resulted in a significant increase in fruit weight. Furthermore, oil content was higher in open pollination treatments than other treatments. However, it is important to recognize that avocado oil content can vary significantly based on other factors such as avocado variety, growing conditions, pollen source, rootstock, and agricultural practices. In a scenario of pollination service decline in agricultural landscapes farmers will encounter economic losses. Thus, pollination services are an essential factor in avocado management decisions across different landscapes. This will further lead to excellent fruit quality which can compete favorably in the global market. Our results highlight the need to improve pollination services in pollinator dependent crops, because pollination services are at risk from various anthropogenic threats thus advocating the conservation of insect pollinators. Furthermore, our study establishes the need to integrate food quality, nutrition and pollination through international policies and conservation strategies.

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## Declarations

**Competing interests** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



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