Dynamics of nectar secretion, honey production potential and colony carrying capacity of *Coffea arabica* L. (Rubiaceae)

TURA BAREKE[1*], ADMASSU ADDI[1], KIBEBEW WAKJIRA[1], TOLERA KUMSA[1]

[1]Oromia Agricultural Research Institute, Holeta Bee Research Center, Ethiopia

**Correspondence details: trbareke@gmail.com*

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Abstract: Coffee is an important export commodity for the Ethiopian economy and also used for honey production. This study was aimed to identify the effect of temperature and humidity on nectar volume, the nectar secretion dynamics, honey production potentiality, and the number of bee colonies required to be placed in a hectare of coffee plantation for optimum honey production.

The study was conducted in Gera District western Ethiopia. One day before nectar measuring, 5 inflorescences were covered with fine mesh bags on a different part of the tree. From covered inflorescences, twenty flowers per tree were randomly selected and nectar volume was measured using micropipettes. Accordingly, nectar volume and concentration, temperature, and air humidity were measured with an interval of one hour. The average nectar volume (µl) per flower in 24 hours, sugar amount per tree (g), expected honey yield per tree (kg) and honey (kg) production potential per hectare for *C. arabica* were 3.3 $\pm$ 0.2, 0.040 $\pm$ 3, 0.050 $\pm$ 4 and 125 kg (25-275 kg), respectively. The actual harvestable amount of honey is half of the potential (62.5 kg/ha). If a kilogram of *C. arabica* honey would be valued at 6.25 USD, the total financial to be expected is $ 390.63 per hectare. The mean nectar volume and concentration have significant differences ($p<0.05$) at different hours of the day. Nectar volume was positively correlated with humidity while concentration positively correlated with temperature. One hectare of productive trees of *C. arabica* holds 4 or 3 or 2 honeybee colonies for traditional or transitional or frame hives respectively. From this study, it is concluded that Coffee is not economically valuable only for its seeds, but also for honey production. Therefore, the integration of coffee plantations with beekeeping is recommended.

Keywords: *Nectar, volume, concentration, honey potential*

Introduction

Bee plants are those plant species that provide nectar and/or pollen for honeybees (Addi and Bareke, 2019; Admassu *et al.*, 2014). Among these, *Coffea arabica* L. is one of the important plants widely grown in the tropics. It is self-fertile cash crop in many tropical countries (Klien *et al.*, 2003). Studies conducted by many authors have indicated that honeybee pollination improved the yield of *C. arabica* by 12-50% as compared to self-pollination (Roubik, 2002; Klien *et al.*, 2003; Ngo *et al.*, 2011). After pollination *C.*

arabica flowers wither in 1 or 2 days. If not pollinated it remain open for up to 5 days (Free, 1993). Honeybees are the major visitors of *C. arabica* while Stingless bees *Trigona spinipes* and *Partamona testacea,* Carpenter bees (*Xylocopa* species) are the minor visitors (Veddeler *et al.*, 2006; Vergara *et al.*, 2008). Honeybees provide pollination service for coffee flowers while coffee flowers supply nectar for honeybees (Ngo *et al.*, 2011). Coffee is a perennial crop that belongs to the family Rubiaceae originated from Ethiopia (DaMatta, 2004; Belitz *et al.*, 2009). It is the most important cash crop for trading and exports since the 18th century, providing jobs for millions of families worldwide.

In Ethiopia, Coffee grows in a wide range of agro-ecological conditions mainly from the range of altitudes 1200 to 3000 m (Muleta *et al.*, 2011; Moat *et al.*, 2017). Formerly, it was assumed to be endemic in the south-western forests of Ethiopia and it has now spread through trade first into southern Arabia and from there to the East Indies by Dutch traders late in the 17th century. Coffee is planted throughout the tropics, but particularly in the east and West Africa and central and later to South America and the West Indies (Admassu *et al.*, 2014). It can be propagated from seeds. The flowering period (anthesis) of *C. arabica* is varied from region to region and generally, it flowers from January to April (Ngo *et al.*, 2011) based on the rainfall of the area. *C. arabica* has a short flowering length with the range of 5-7 days. It gives flowers profusely after rains but some coffee trees are found with flowers at any time of the year. Coffee is a major honey source in the southern, western, and southwestern parts of Ethiopia (Bareke and Addi, 2019a, b; Bareke and Addi, 2018). Honeybees play an important role in increasing seed yields through pollination and honey production by collecting nectar. Floral nectar is a reward offered by flowering plants to visiting pollinators, reflecting co-evolution between the plants and their pollinators (Galetto, 1997, Ning-Na *et al.*, 2015; Power *et al.*, 2018). It plays a central role in plant reproduction by mediating plant-pollinator interactions due to its inherent features such as sugar concentration, volume, viscosity, and chemical composition (McDade and Weeks, 2004). Thus, in Ethiopia, it is suggested that coffee growers should keep honeybee colonies in their coffee plantations (Admassu *et al.,* 2014). However, to integrate coffee plantations with beekeeping, there is a lack of information about the potential of coffee plants for honey production for recommending the optimum number of honeybee colonies required to put in a hectare of the coffee plantation. In this regard, knowledge of nectar production dynamics of this plant is fundamental to understand the nectar secretion potential and dynamics such as the amount of nectar it offers, the time patterns, frequency of visit to the plant by bees, that are among others important factors to be understood for planning (Galetto and Bernardello, 2004).

Many authors have determined the honey production potential of bee plant species, based on their nectar secretion potential (Kim *et al.*, 2011; Adgaba *et al.*, 2012; Abdulaziz *et al.*, 2015; Adgaba *et al.*, 2016 and Bareke *et al.*, 2020). However, this important information is still lacking and its significance for honey production has not yet been documented for this the most economically important cash crops of Ethiopia. Therefore, this study was aimed to determine the nectar secretion dynamics, honey production potential, number of honeybee colonies required to be placed in a hectare of the coffee plantation as well as to identify the effect of temperature and humidity on the nectar secretion of *C. arabica.*

Materials and Methods

The study was conducted in Jimma Zone, Gera District, Ethiopia. Gera District comprises substantial coverage of natural forest and known as a key biodiversity hotspot area for *C. arabica* conservation and potential area for beekeeping (Kitessa, 2007; Bareke and Addi, 2019). Integration of coffee plantation with beekeeping is used to boost the seed yield of coffee and simultaneously coffee honey which can be sold at premium prices. The climate of study area is cool and humid with 1906.3 mm average annual rainfall which is unevenly distributed throughout the year. The texture of the soil of the study area is classified as sandy clay soil (Tilahun *et al.*, 2021). Fertilizer and other pesticide chemicals are not used.

Phenology

Observations were made on three coffee plants to identify the timing of anther dehiscence and nectar secretion. From each plant, five flower buds (a total of 15) were labeled and observed (Bareke *et al.*, 2020).

Determining the number of flower heads per tree

Eighteen productive plants with massive flowers were selected randomly to get the average number of flower heads per plant (Bareke *et al.*, 2020). Each plant had a similar age. Coffee flowers are white in color (Figure 1)

Figure 1 - Coffee flowers

Determination of nectar volume and concentration

Five (5) inflorescences were covered with fine mesh bags (40 x 40 cm) on different parts of the plant, one day before nectar collection (Bareke *et al.*, 2020). From different inflorescence whorls, flowers were collected randomly (Wyatt *et al.*, 1992). Fifteen plants were used for nectar measurement. Twenty (20) flower heads per tree were randomly selected. The volume of nectar produced in 24 hours was determined directly upon sampling from 100 flowers with micropipettes (10µl).

Determination of nectar secretion dynamics

The nectar volume, nectar concentration as well as temperature, and humidity were measured using a hygrometer at one-hour intervals from 7:00-18:00 hours, simultaneously. Nectar was collected from three flowers at each time measurement for 5 consecutive days (3 Flowers*12 times*5 days=180 flowers). Additionally, 5 flowers were isolated and measured daily from the beginning to end of nectar secretion to determine the amount of nectar (mg) secreted throughout the lifetime of the flower.

Determination of sugar amount in nectar per flower

The amount of sugar found in nectar was calculated from the nectar volume, concentration, and sucrose density. The readings in most refractometers are in sucrose equivalents expressed as milligrams of sugar per 100 mg of solution. They were converted to milligrams of sugar per flower by converting the measured sucrose equivalent to g/liter and multiplying this value by the nectar volume (Bolten *et al.*, 1979). The conversion of sucrose concentration to density was using Pry-jones and Corbet (1987) equation and the amount of sugar was calculated using Dafni (1992) equation.

Estimation of honey production potential

The honey production potential (HPP) of the plant was estimated by using the following formula: HPP= the average number of flower heads per plant * the average amount of sugar per flower head* nectar secretion length (days). This gives the average amount of sugar per tree /flowering season (Bareke *et al.*, 2020). The average amount of sugar per tree converted to honey was calculated.

At the international market the average acceptable honey moisture content is 18% from 1 kg of honey whereas 82% is a total dissolved sugar. This is used to convert the mean mass of sugar produced by a single plant per flowering season to honey. The estimation of the number of plants per hectare was based on the recommended space required per this species.

Estimating optimum honeybee colony carrying capacity

The optimum number of traditional (TH), intermediate (IH), and modern hives (MH) for the given area was estimated as follows:

TH = [Expected potential honey yield per area/ (honey yield of well managed traditional hive) *2]

The optimum number of intermediate hives

IH == [Expected potential honey yield per area/ (honey yield of well managed intermediate hive) *2]

The optimum number of modern hives

MH= [Expected potential honey yield per area/ (honey yield of well managed modern hive) *2] (Al-Ghamdi *et al.*, 2016; Bareke *et al.*, 2020).

Dividing the honey productivity potential by two is to consider the assumption that for every 1 kg of harvestable honey, bee colonies may consume 1 kg of honey to fulfill nutrient requirements of maintenance and reproduction (Chaudhary, 2009).

Data analysis

Data was analyzed using One-way ANOVA and prior to analysis, homogeneity of the variances of the data was checked using Levene tests. Tukey used for mean separation among the treatments. In addition to this, a linear regression model was computed using

R-software to see the effect of temperature and humidity on nectar volume and concentration

Results

Nectar secretion length

Nectar volume of *Coffee arabica* was significantly different between the start and end of the secretion date (Figure 2). As the age of the flower increased, the amount of nectar secreted was decreased. Accordingly, the peak nectar secretion was recorded on day 2 while the lowest was recorded at the end of secretion (day 5). On the 6th day, it was difficult to measure nectar volume and this was considered as the plant almost stopped secretion. This suggests that *C. arabica* can be considered as a species with a short flowering length.

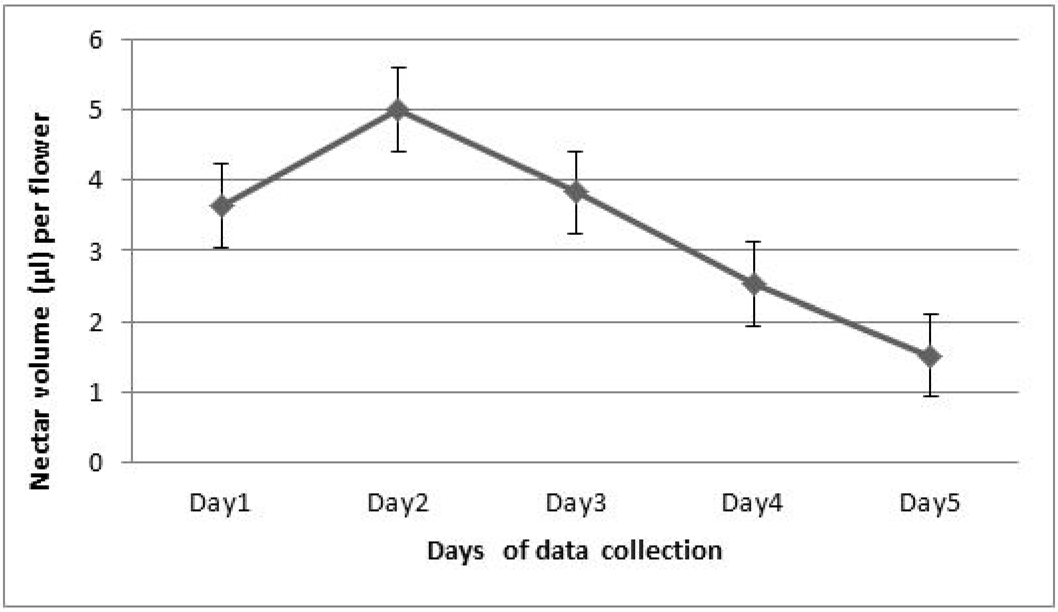

Figure 2 -Nectar secretion length and volume of Coffea arabica flower from start of secretion to end (repeated collection daily) (N=15 flowers daily from the start to end)

Nectar secretion dynamics

The mean nectar concentration and volume were significantly different across times of the days ($p<0.05$). The lowest mean nectar concentration was recorded at 7:00 pm and the highest nectar concentration recorded at 16:00 pm. On the other hand, the highest mean of nectar volume was recorded at 7:00 am while the lowest was at 17:00 pm. This is due to high humidity and low temperature in the morning (7:00) and vice versa in the study area. The mean amount of sugar present in nectar was not significantly different ($p<0.05$) across times of the day (Table 1).

Table 1 - Mean nectar secretion dynamics: Mean nectar concentration (%), nectar volume (µl) and amount sugar (mg) in nectar per flower in 1hour intervals per flower with ± standard error (SE) of C. arabica in 7:00 to 18:00 hours of the day

TIME (HOUR)	AVERAGE NECTAR CONCENTRATION (%) ± SE	AVERAGE NECTAR VOLUME (µL) ± SE	AVERAGE SUGAR AMOUNT PER FLOWER/1HR INTERVALS
7:00	11.1 ± 2.58d	6.6 ± 1.1a	3.3 ± 0.7a
8:00	14.1 ± 2.01cd	4.0 ± 0.74bc	2.8 ± 0.68a
9:00	18.6 ± 2.17abcd	4.7 ± 0.71abc	4.3 ± 0.66a
10:00	17.2 ± 2.85bcd	5.3 ± 0.41ab	4.6 ± 0.88a
11:00	19.9 ± 1.82abc	3.7 ± 0.7bc	3.8 ± 0.91a
12:00	18.8 ± 1.84abcd	3 ± 0.7bc	2.8 ± 0.69a
13:00	20.7 ± 3.33abc	3.0 ± 0.33bc	3.0 ± 0.54a
14:00	21.7 ± 3.75abc	3.1 ± 0.85bc	3.6 ± 1.23a
15:00	24.3 ± 1.71ab	2.5 ± 0.88c	3.1 ± 1.16a
16:00	25.9 ± 1.64a	3.2 ± 0.7bc	4.1 ± 0.89a
17:00	23.4 ± 2.14ab	2.7 ± 0.54c	3.3 ± 0.89a
18:00	25.4 ± 2.69a	3.2 ± 0.96bc	4.6 ± 1.69a

Note: Different letters show significant differences

The effect of temperature and humidity on nectar secretion

During the study period, air humidity was found in the range of 21-84% whereas temperature of the day was in the range of 14-33.9 ^{0}C. The highest nectar volume was recorded at the lowest temperature. On the other hand, the highest nectar volume was recorded at the highest values of humidity. This shows the indirect relationships of nectar volume with temperature as well as the direct relationship of nectar volume with humidity (Figure 3).

The highest value of nectar concentration was recorded above 25 ^{0}C while the lowest was at less than 20 ^{0}C. Thus, nectar concentration has direct relationships with temperature of the study area (Figure 3).

On the other hand, the highest nectar concentration value was recorded at less than 50 % humidity whereas the lowest recorded above 55%. This indicates that as humidity increased the values of nectar concentration decreased. The lowest concentration was recorded at the lowest humidity (figure 4).

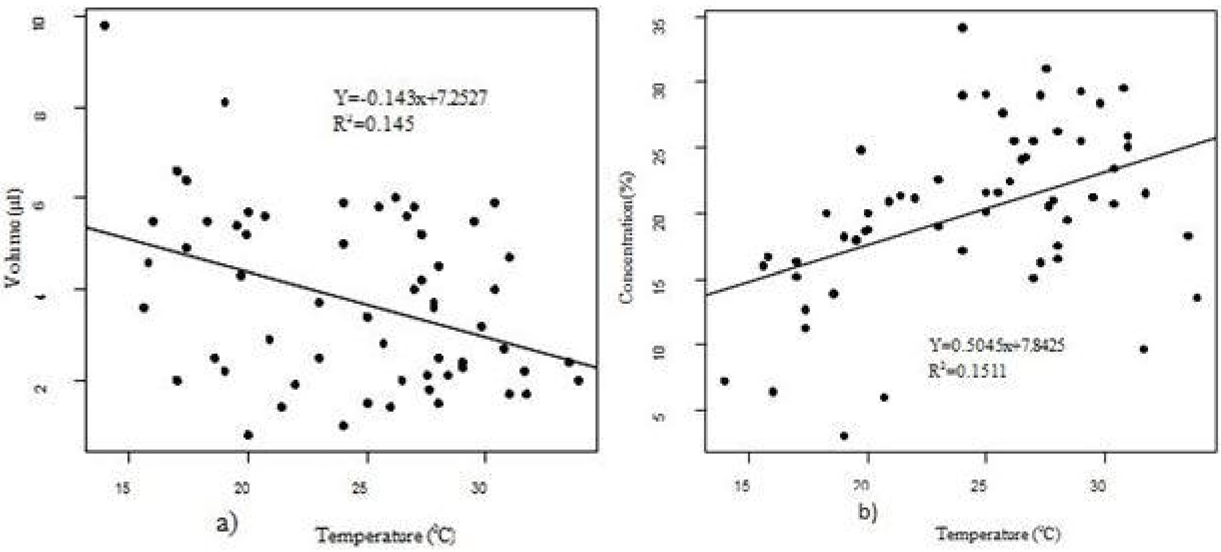

Figure 3 - Effect of temperature on nectar volume (a) and concentrations (b) of C. arabica

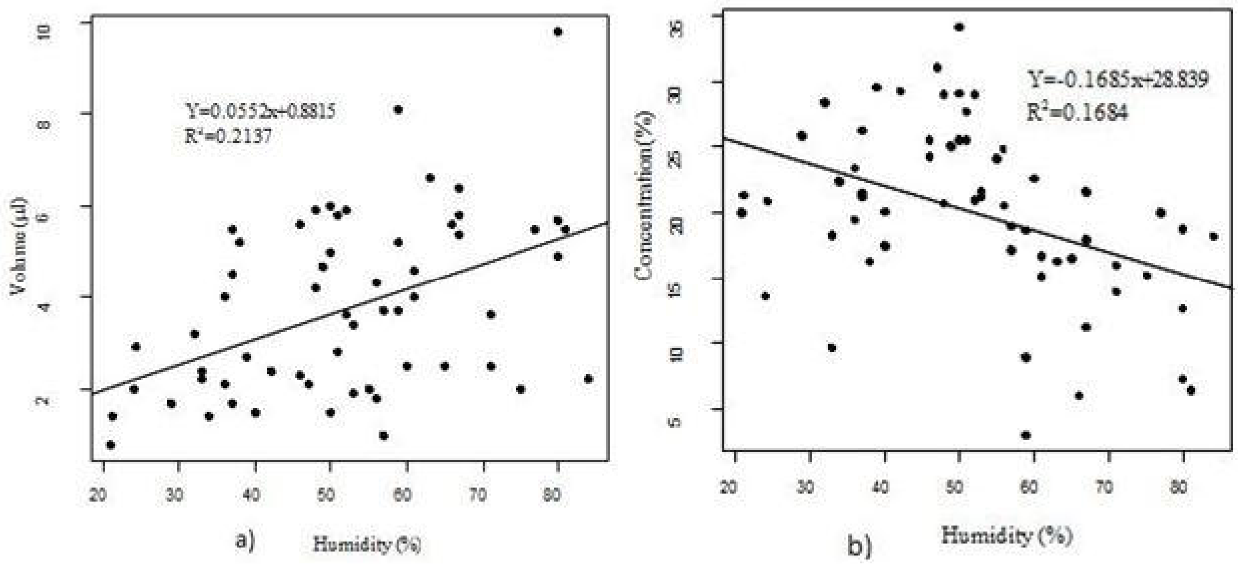

Figure 4 - Effect of humidity on nectar volume (a) and concentrations (b) of C. arabica

Since sugar amount is calculated from nectar volume and concentration, temperature and humidity have no significant effect on the amount of sugar (Figure 5). Because the effect of temperature and humidity on nectar volume and concentration opposite of each other (figure 6). This means as temperature increased, humidity decreased and as temperature decreased humidity increased. However, the highest amount of sugar value was estimated from 20-30 ^{0}C temperature, and 45-55% humidity (Figure 5). So, the appropriate temperature and humidity for coffee honey production are ranges (20-30 ^{0}C) and 45-55% respectively.

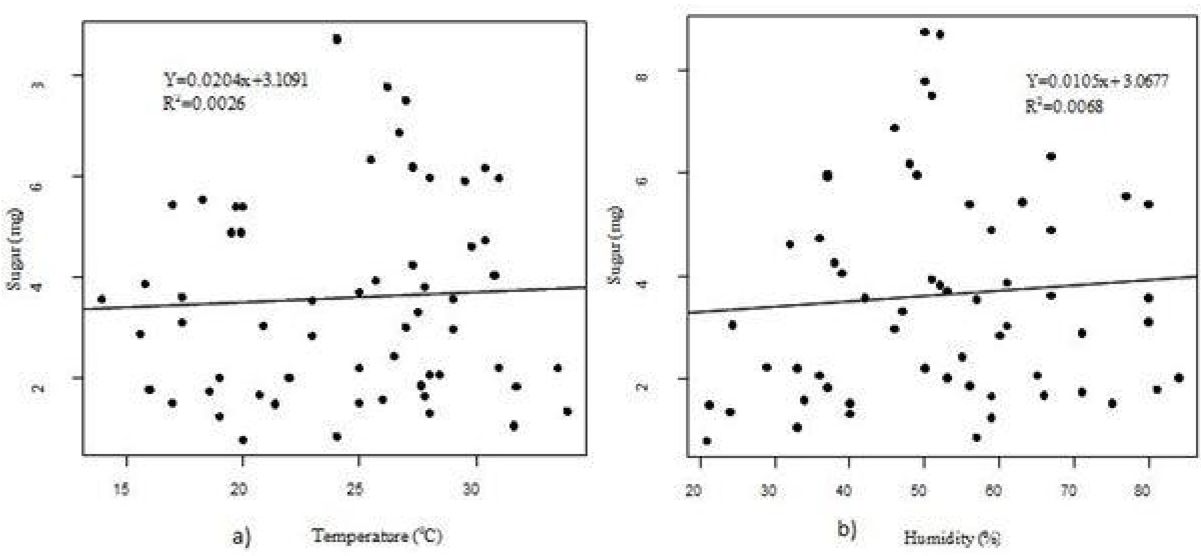

Figure 5 - Effect of temperature (a) and humidity (b) on sugar amount of C. arabica

On the 2nd day, the highest air humidity was recorded due to rainfall while on the 3rd day; both temperature and humidity were reduced compared to other days due to the prevailing wind during the study (Figure 6). This indicates that the value of temperature and humidity can also be affected by the windy condition of an area.

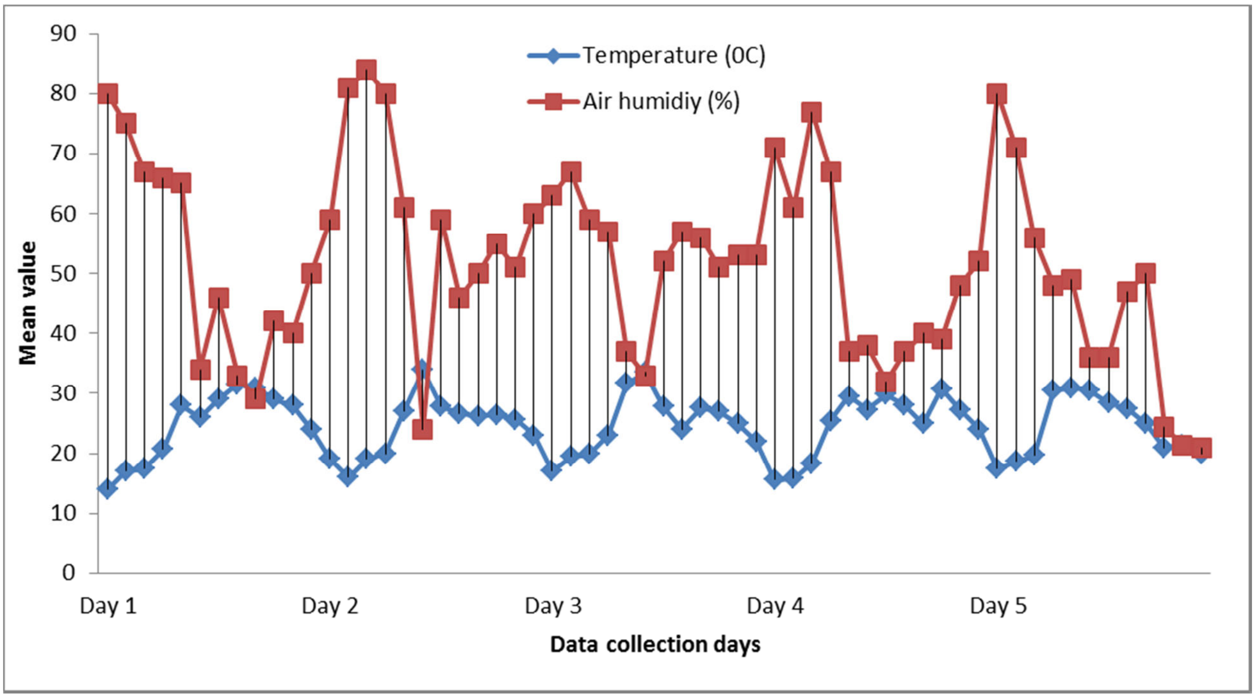

Figure 6 - Distribution of air humidity and temperature during nectar data collection

Honey production potential

The average number of *C. arabica* flowers per plant was 1044 (Table 2). Each flower was observed to provide nectar for at least 5 days. The mean amount of sugar per flower was 3.6 ± 0.3 mg (ranges from 0.8 to 8.72 mg), and therefore, the average mass of sugar produced per plant is estimated to be 0.04 kg (range from 0.008-0.09 kg).

Table 2: Mean number flower heads/plant (N=18 plant), mean Nectar volume in 24 hours (µl) (N=100 flowers) and mean sugar amount per flower life cycle (mg) (N=100 flowers) of Coffea arabica in Gera District

TREATMENTS	MEAN ± SE	MINIMUM	MAXIMUM
Mean number flower heads/plant	1044 ± 135	485	2330
Mean Nectar volume in 24 hours (µl)	3.3 ± 0.20	0.70	7.30
Mean sugar amount per flower life cycle (mg)	3.6 ± 0.30	0.80	8.72
Mean sugar amount per plant (kg)	0.04 + 0.003	0.008	0.09

Given that 1 kg of honey with 18% moisture content (wt/wt) contains 820 g of total dissolved sugar, the mean mass of sugar produced by a single tree of *C. arabica* (0.04 kg) per season is estimated to produce 0.05 kg of honey (range 0.01 – 0.11 kg). Space between coffee plants was 2 meters and therefore, the total number of Coffee plants per hectare of land is about 2500. Therefore, under ideal conditions, the average honey production potential per hectare of coffee plantation area per flowering season would be about 125 kg, which ranges from 25 to 275 kg. The actual amount of honey that can be harvested from the hive is half of the estimated potential which is 62.5 kg per hectare.

Financial implication from a hectare of C. arabica through honey production potential

The expected harvestable honey yield from a hectare of productive trees of *C. arabica* with the massive flowers was 62.5 kg. If a kilogram of *C. arabica* honey would be valued at USD 6.25, the total financial gain from one hectare of coffee plantation would be 62.5 kg * 6.25 = 390.625 USD. This indicates how much this plant is economically valuable, in addition to seed sales.

Determining optimum number of honeybee colonies that can be place in a hectare of C. arabica for honey production

The honey yield of well managed honey bee colonies from different types of bee hives are 16 kg (Shenkute *et al.*, 2012); 25 kg and 45 kg (Tullu, 2014) per traditional, transitional and frame hive in Ethiopia, respectively. Even though the bee forage resources are similar for all bee hive types but the volume of the hive, population size of honey bee colony and the way of management between the hive types are varied (Bareke *et al.,* 2020). For instance, the size and number of honey bee population of traditional hives are small as compared to transitional and frame hives which result in variation in honey yield. In addition to this, the traditional hive is not suitable for internal honey bee colony inspection. Based on honey production potential (125 kg) of coffee per hectare, the optimum number of managed honey bee colonies to be introduced per hectare during its flowering period can be 4 traditional, 3 transitional or 2 modern frame hives. So, the number of honey bee colonies to be placed in a hectare of *C. arabica* are differs between the hive types.

Discussion

Nectar secretion of Coffea arabica

Coffee flowers provide nectar from 7:00 to 18:00. Similarly, a study conducted by Adgaba *et al.* (2012) on Ziziphus *spina-christi* also indicates that it provides nectar the whole day. In addition to this, *Lavender* species also secrete nectar the whole day (Adgaba *et al.,* 2015). On the other hand, *Croton macrostachyus* secrets nectar from 8:00

to 15:00 hours (Bareke *et al.*, 2020). The significant variations in the amount and patterns of nectar secreted by the different honey source plants could be due to the variations in biotic and abiotic factors associated with the different plant species in their respective environments (Al-Ghamdi *et al.*, 2016). This indicates that nectar secretion time is varied from plant species to species. The causes of nectar secretion variability between flowers on the same plant are due to position on the flowering stem to the microclimate of the area (Macukanovic *et al.*, 2004; Jakobsen and Kristjansson, 1994; Petanidou *et al.*, 1996). In addition to this, day to day variation in weather may cause shifts in the pattern of nectar characteristics, morphological and phenological characteristics have an effect on nectar secretion.

Effect of temperature and humidity on nectar secretion of C. arabica

As the temperature increased the nectar volume of *C. arabica* decreased. On the other hand, nectar volume was increased as the humidity of the area increased. This indicates how much nectar volume is affected by the humidity and temperature of the particular area. Similarly, a study conducted by Bareke *et al.* (2020) on *C. macrostachyus* also indicates that nectar volume increased at the lowest temperature. However, study conducted by Kim *et al.* (2017) on *Crataegus pinnatifida* in Korea and Chinese indicates that nectar volume was positively correlated with both temperature and relative humidity. This shows the effect of environmental factors on the amount of nectar secreted is varied from plant species to species.

Nectar concentration has direct relationships with temperature of the study area. Similarly, the study conducted on *Thymus capitatus* (Petanidou and Smets, 1996) and C. *macrostachyus* (Bareke *et al.*, 2020) indicate that their nectar concentration (solute amount) have positively correlated with temperature. In addition to this, a study conducted by Adgaba *et al.* (2015) on *Lavandula dentata* and *L. pubescens* in southwestern Saudi Arabia indicates that for both species the nectar concentration was significantly increased with an increase in temperature. On the other hand, the amount of nectar in *L. dentata* tended to increase with an increase in relative humidity. But, for *L. pubescens* the nectar volume tended to decrease with an increase in relative humidity. Therefore, the effect of temperature and humidity on the nectar secretion is varied based on the plant species. For some plants, temperature and humidity highly affected the nectar secretion potential while no effect on some other species

As the humidity of the area increased the values of nectar concentration of *C. arabica* decreased. Study conducted on *Antigonon leptopus* and *Theretia peruviana* by Adjaloo *et al.* (2015) also indicated that the nectar concentration was negatively correlated with the humidity. The peak of temperature coincided with the lowest relative humidity, indicating an inverse relationship between the two environmental parameters (Adjaloo *et al.*, 2015).

Honey production potential

The average mass of sugar per coffee plant (0.04kg) is lower than the average amount of sugar of *Croton macrostachyus* per plant (Bareke *et al.*, 2020). The common nectar variables relevant to pollination are its concentration, volume and sugar. Nectar collection method is primarily dictated by the flower size, nectar volume and solute concentration (Dafni, 1992). The common method is to extract the nectar with micropipettes for volumes above 0.5 µl and concentration below 70%. Nectar collection from small flowers needs special techniques (Dafni, 1992).

The actual amount of honey that can be harvested from the hive is half of the estimated potential of the plant. When bees collect and transport the nectar to the hives they definitely consume a certain amount of sugar for their flight energy. In addition to this, due to rapid crystallization, all the nectar secreted may not be available to honeybees

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(Adgaba *et al.*, 2012; Bareke *et al.,* 2020). The estimated honey production potential that can be obtained from *C. arabica* plantation per hectare was 125 kg. These results are comparable to the reports made for different annual plants and trees such as *C. macrostachyus in* range of 234 kg - 1770kg /hectare (Bareke *et al.,* 2020), Lime species (*Tilia* spp.) (90 to 1200 kg honey/ha) (Crane *et al.,* 1984), and *Ziziphus spina-christi* (550-1300 kg of honey/ha) (Adgaba *et al.*, 2012), *Brassica juncea* and *Sinapis alba* crops 65.5 kg and 71.2 kg/hectare, respectively (Masierowska, 2003). Monofloral honey of *C. arabica* is produced in some parts of Ethiopia. For example, from western Ethiopia it is produced in Gera District (Bareke and Addi, 2019). Since the flowering period of *C. arabica* is short, the beekeepers should apply seasonal colony management following flowering calendar to harvest the monofloral honey of this plant.

In general, trees were more productive in nectar secretion due to their larger biomass, dense flowers, deep roots and resistance to moisture stress (Adgaba *et al.,* 2017). Furthermore, in most trees, the flowers are not colorful and are expected to secrete more nectar to strongly attract sufficient pollinators (Schemske and Bradshaw, 1999). However, *C. arabica* has white color which can attract honeybees and other insect pollinators.

Optimum honeybee colony carrying capacity of C. arabica for honey production

Knowledge of bee colony carrying capacity is very important to utilize the floral resource of a given area. Some beekeepers cited that the apiary site is nearby the forest area where the diversity of bee forage species is high and the strength of honeybee colony is good throughout the year. However, honey obtained from such area is very low (Bareke and Addi, 2018). This is due to less abundant bee forage plants flowered at the same time and many of them flowered in different time of the year. In such areas, estimation of honeybee colony carrying capacity is very important to use the resource effectively. Harmonizing a number of honeybee colonies with the available resource is used to increase the productivities of honeybee colonies by overcoming the problem of colony overstocking (Al- Ghamdi *et al.,* 2016; Adgaba *et al.,* 2017). Study conducted by Esteves *et al.* (2010) also indicated that the optimum distribution of honey bee colonies minimizes overpopulation with the consideration of the available honey bee plants within the maximum flight distance of the honey bees.

Conclusion and recommendation

C. arabica is a good producer of nectar and significantly contributes to honey production in Ethiopia. Both temperature and humidity have significant effects on nectar volume and concentration of the flowers. Amount of nectar volume and concentration varied in different times of the day. One hectare of *C. arabica* plants has a potential to produce 125 kg of honey of which 62.5 kg is expected to be harvestable. The honey production potential of *C. arabica* holds a maximum colony carrying capacity for 4 traditional, 3 transitional or 2 modern frame hives honeybee. Monofloral honey can be produced from this species at areas where it is abundantly found. The current study clearly indicates that coffee is not only economically valuable for its seeds, but also used for honey production. Therefore, integration of coffee orchard with beekeeping is recommended to produce honey, as well as to boost the seed yield of coffee.

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