Evaluation of the effect of temperature, relative humidity, plants age and time on nectar volume and concentration of *Callistemon citrinus L.*

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ABSTRACT

Objective: To evaluate the effects of temperature, relative humidity, plants' age and time on nectar volume and concentration of *Callistemon citrinus L.* in and around Holeta bee research center

Methods: Nectar Volume was measured by a graduated capillary pipette or micropipette with time interval of one and half an hour. Nectar concentration was measured by pocket refractometer at time of 7:30 AM , 9:00 AM, 10:30 AM, 12:00 AM, 1:30 PM, 3:00 PM and 4:30 PM -5:00 PM , relative humidity and temperature were measured by Hygrothermometer for these three age categories of *Callistemon citrinus L. plants :* smallest plants at the age of 7 , medium at the age of 13 and oldest plants at the age of 34 in and around Holeta bee research center **.**

Results: Result indicates that there was no effect of relative humidity on nectar volume of youngest plants of *Callistemon citrinus L.* and also there was no significant effect of temperature on nectar concentration of youngest plants but for medium and oldest plants temperature has an effect and they have highest concentration at temperature of 21C^{o} that was 36.1(w/w at 5:00 Pm and medium plants have 16.1w/w nectar concentration at temperature of 24.7. They have highest volume that was 24.87µl at relative humidity of 43 for medium and oldest has highest volume that was 24.04µ at highest relative humidity of 42%**.**

Conclusions: The concentrations and volume of *Callistemon citrinus L.* is affected by plants age, time, relative humidity and temperature .For younger plants volume was not affected by relative humidity and also nectar concentration was not affected by temperature, but for medium and oldest ages concentration and volume were highly affected by age of plants , temperature ,relative humidity and time and they have high concentration 16.1w/w and 36.1w/w for medium and oldest plants respectively. .

Background of the study and justifications

Bottle brush, Callistemon lanceolatus DC (Myrtaceae), is native to Australia. In India it is cultivated as an ornamental plant(Gupta *et al.,* 1993). Plants' ability to sense their environment and respond to it is critical for their survival(Veits *et al.,* 2018). Many of the most important honey plants secrete such small amounts of nectar to the individual flower that neither of these methods is practicable. In some cases fructose and in others glucose is the dominating reducing sugar(Stoner *et al*.,2016). Floral nectar consists largely of sugars (chiefly sucrose, glucose and fructose) and water. Insects, birds and mammals take nectar, and its sugars provide energy that fuels activity or provisions the larvae(Corbet, 2003). These findings point to the adaptation and success of *T. capitatus* to the Mediterranean conditions(Petanidou & Smets, 1996).Nectar is a floral resource commonly sought after by plant visitors such as insects, birds and small mammals, because of its nutritional importance(Adjaloo, 2015). Mānuka nectar yield and composition are strongly influenced by plant genotype, flower age and the environment(M. J., *et al.,*2018).Climate change appears to be a major concern for agriculture in general and may also have worrying implications for beekeeping(Langowska *et al.,* 2017).Nectar drinkers must feed quickly and efficiently due to the threat of predation. While the sweetest nectar offers the greatest energetic rewards, the sharp increase of viscosity with sugar concentration makes it the most difficult to transport. The quantity of nectar sugar in a flower fluctuates through time as nectar is supplied by secretion(Corbet, 2003). Measurement of the sugar contained in honeydew and other carbohydrate-rich exudates is important for quantifying a variety of ecological processes since level of foraging Diptera depends greatly on temperature, as few species have the ability to warm themselves(Inouye *et al.*,2015). In certain experimental designs the flowers have previously been isolated with a tulle net in order to exclude visiting insects(covered flowers)(Farkas, 2012). Microcapillary tubes were a device used to remove nectar from the caged flowers and the sugar concentration was recorded with a hand refractometer(Gupta *et al.*, 1993).For Secretion patterns and characteristics of nectar of an Argentinian population of Pyrostegia venusta at different times of the day are described with respect to flower age(Veg *et al,* 1994).Refractometer with very closely set prisms will allow accurate concentration measurements(Dungan *et al.*,2014).The size, thermal reflectance and behaviour of ectothermic Diptera determine their heat budget and hence, their ability to withstand extremes of heat and cold(Inouye *et al.*,2015). The source of nectar components, especially sugars, is closely related to

the rate of nectar production: a high rate requires storage of reserve material in nectary parenchyma cells(Dungan *et al*.,2014). These physiological constraints on foraging Diptera help to explain observed temporal patterns of foraging activity, suitable temperatures for foraging may occur both in the morning and afternoon, with only mid-day too warm for foraging(Inouye, *et al.*,2015).The rate of nectar production these features also influence the manner and site of nectar exposure (nectar presentation)(Pacini & Nepi, 1970). Cucurbitaceae species depend on pollination by honey bees for fruit production. Nectar secretion is important for attracting insects, favoring the pollination of crops and production of nectar by the target crop facilitates maintenance of the pollinating colonies*(Das et al.*,2014). many papers have been published on the sampling of floral nectar, a paucity of information exists regarding sampling from flowers that produce low nectar volumes (,1 mL), and also on the conditions under which the resulting nectar samples should be stored(Morrant *et al*.,2008).The temporal patterns of secretion, cessation, and reabsorption, if any, define nectar production dynamics. Nectar production requires considerable expenditure of energy(Pacini & Nepi, 1970).In the male flower the nectary is located at the base of the filaments, and the bees can access the nectar through three pores. In the female flower the nectary is opened forming a ring around the base of the style(Das *et al*.,2014).Nectar concentration is highly influenced by environmental factors, especially temperature and humidity (Nicolson and Nepi, 2005). In many cross-pollinated crop plants, pollination is dependent upon bee activity which is influenced in part by nectar availability and quality(Kim *et al.*, 2011).Lacking exact knowledge of the physical condition of nectar as it passes through the membrane of the nectary epidermal cells, one can postulate two ways in which atmospheric humidity might influence the water concentration of nectar(F *et al.*,1952). The problem of nectar secretion, therefore, is of more than academic interest to the agronomist.(Whitney *et al.*, 2008). These observations suggest that, rather than the concentration of nectar sugars, the prevalence of insects was influenced by the prevailing weather and environmental conditions that could have supported insect survival and activity, and possibly other stimuli other than nectar concentration .Nectar contained three sugars: glucose, fructose and sucrose, though hexose (glucose and fructose) was dominant(Rutikanga *et al.*, 2016).No strong correlation occurred between insect population and total nectar sugar concentration or nectar volume. Insect populations were rather influenced by the weather conditions, the long rainy season. The relatively low mean nectar concentration of some plant species could be

related to the lower mean maximum temperatures and higher precipitation characteristics of their environment (Barros *et al.*, 1983).

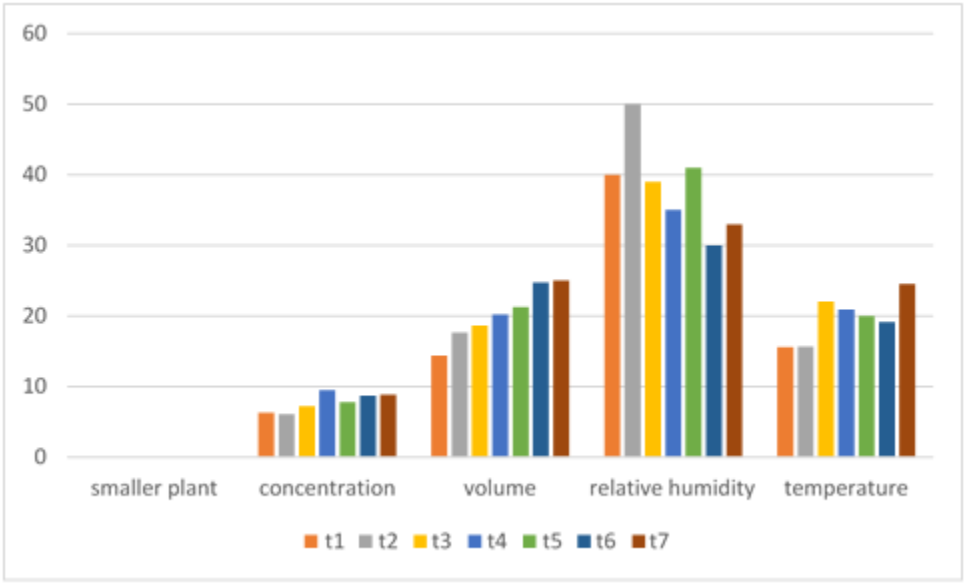
3. Material and methods

Plants was selected randomly and nine plants with three replication from those planted for bees forage in Holeta research center at Apiary site for each age categories after their 50 % of blooming periods and randomly chosen inflorescences of those plants was bagged with insect prove for 12 hours. A nectar sugar concentration was obtained as percentage (%) reading from the pocket refractometer. The *C. citrinus L* sample plants was categorized into three age categories (smallest at the age of 7, medium at the age of 13 and oldest plants at the age of 34 years at and around Holeta research center. The nectar volume and sugar concentration of flowers (50 per plant species) was measured at an hour and half intervals(Adjaloo, 2015). Different flowers of the same plant was never tested in the same day, nor in the same treatment group(Veits *et al.*,2018).Nectar volumes was determined by measuring the length of nectar columns in the capillary tubes to the nearest 0.5 mm or 0.045(Southwick, 1983).To collect the nectar samples mature flower buds was covered using insect prove 1 hr before sunset. One hour after anthesis nectar was extracted, and its volume was measured with glass capillary tubes. Nectar concentration was measured for each flower collected(Stoner *et al.*,2016).Nectar sugar concentration produced per flower was measured in the field(Das *et al.*, 2014).The flowers of *C.citrinus.L* was used to test storage of nectar concentration(Morran *et al.*, 2008). From these bagged inflorescences volume(µl) was collected by means of absorption on strips of graduated micropipette using a graduated capillary pipette and measured with time interval of one and half an hour at 7:30 AM, 9:00 AM, 10:30 AM, 12:00 AM and at 1:30 PM, 3:00 PM and 4:30 PM-5:00 .PM from fifty flower heads for each age categories nectar volume and concentration and volume was compared with environmental relative humidity and temperature to see the effect of relative humidity and temperature on nectar volume after they measured by Hygro thermo meter for all ages and periods of time. Environmental relative humidity and temperature was measured and compared with nectar concentration of three age categories of *C. citrinus* plants following these experimental periods. Recent research in nectar chemistry shows that nectar is more than water and sugars(Adjaloo, 2015). Variation in nectar production in relation to age was be done for *C. citrinus* pants following reports of these researchers for other species

Cruden, 1976; McDade & Kinsman, 1980; Bertin, 1982; Southwick & Southwick, 1983; Steiner, 1985; Martinez del Rio & Biirquez, 1986; Zimmerman, 1988.

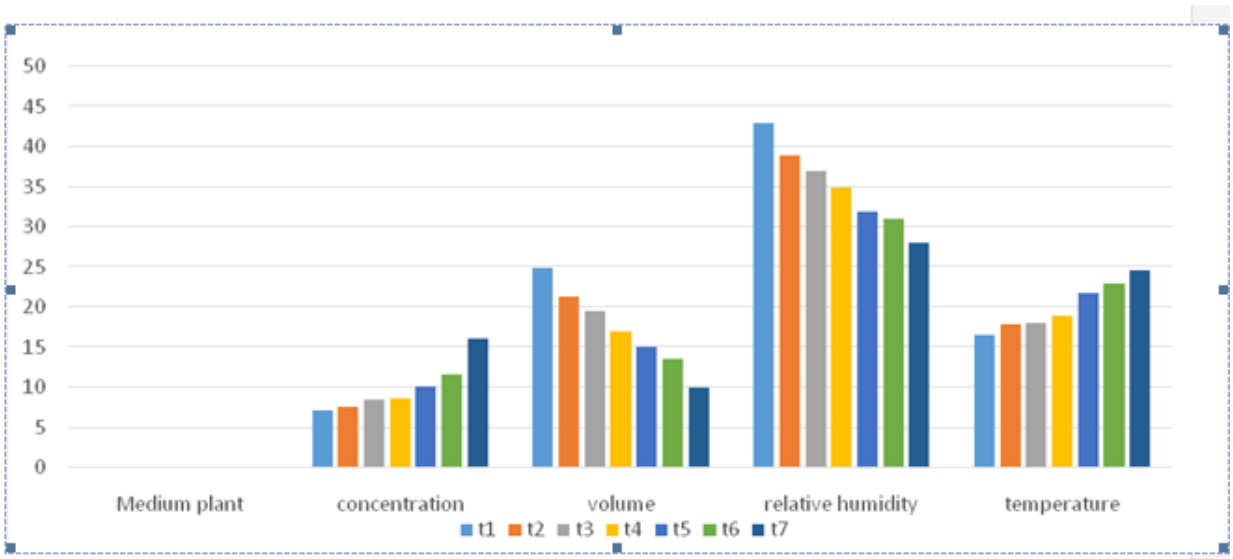
Statistical analysis and research design

The experimental design was Completely Randomised Design (CRD)with three replications (Adjaloo, 2015).For statistical evaluation of the results, the (R software version 3.5 was used for multi-level analysis)(Farkas, 2012).And Microsoft excel 2010 was used for descriptive statistics . The nectar concentration and volume obtained from three age categories of *Callistemon citrinus .L* at the seven exposure periods of the experiment was subjected to statistical analysis. The calculations were carried out by using Microsoft Excel program in order to obtain mean percentage values and to draw charts. The R software version3.5 was used to confirm statistical significance of relative humidity, temperature, time, age and concentration effect on nectar volume of *Callistemon citrinus* at 1% level ($P < 0.01$) and also time, temperature, relative humidity, volume and age have significance effect on nectar concentration at 1% level ($P < 0.01$).That is volume has highly significance effect on nectar concentration of *Callistemon citrinus L.*



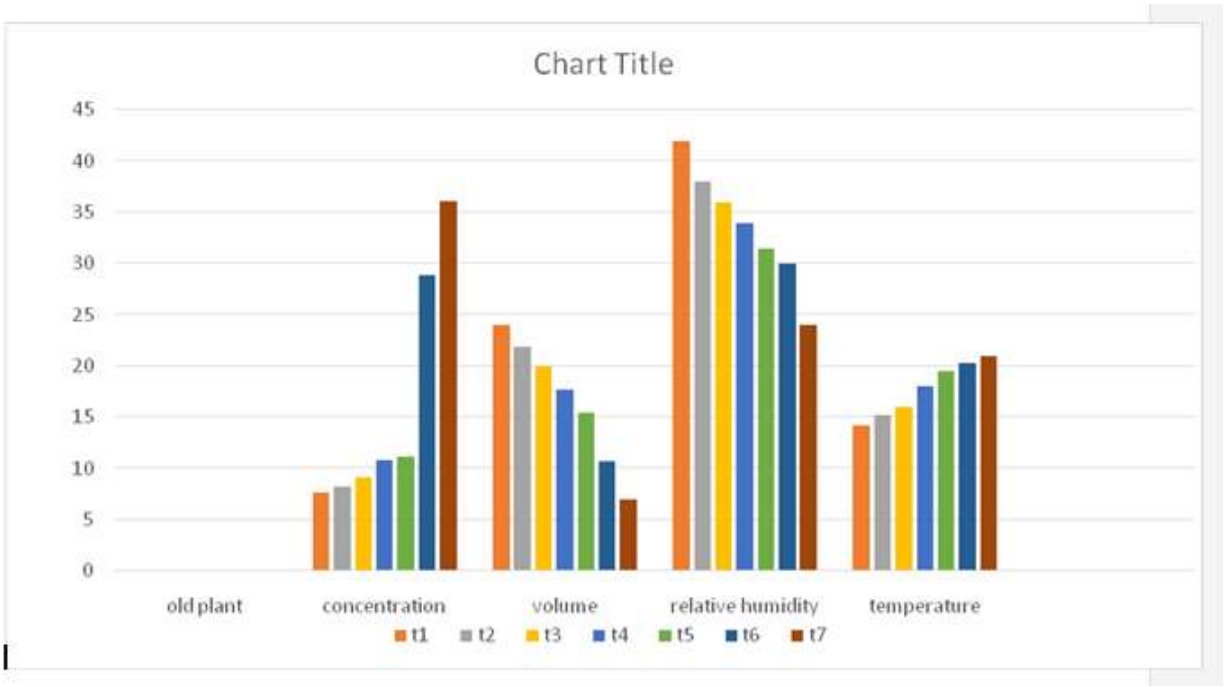
4.1. Chart of clustered column analysis of smallest (youngest) Callistemon citrinus *L.*

For younger plants of *Callistemon citrinus L.* volume increases from 14.36 at t1 -24.99µl at t7but concentration decreases and they are inversely proportional to each other. Nectar concentration was lower at t1, t2 and t3, higher at t4 and decrease at t5 and slightly increases at t6 and t7.



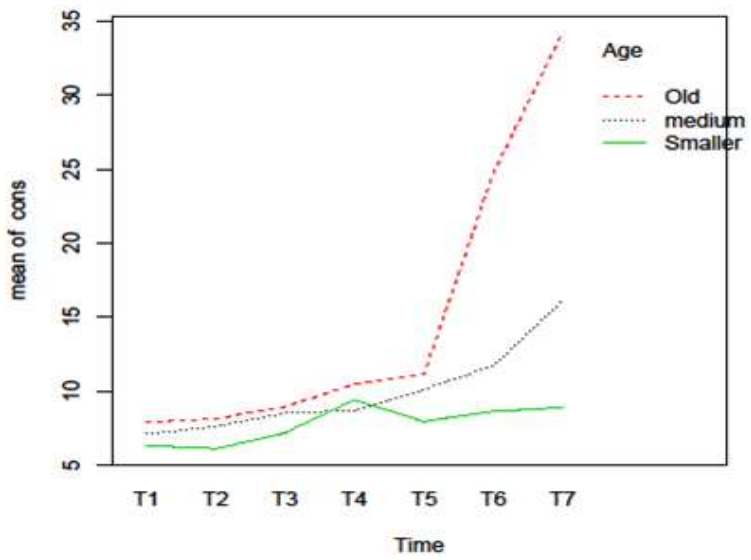
4.2. Chart of clustered column analysis of medium age of Callistemon citrinus plants.

As day time gets more heater and heater from morning 7:300 Am, 9:00 Am, 10:30Am, 12:00 Am, 1:30 Pm, 3:00 Pm and 4:00 Pm-5:00Pm nectar concentration increase continuously from 7.1w/w-16.1w/w and highest concentration was recorded at t7, but volume was highest that is 24.87µl at t1and continuously decreases from t1-t7 and 10.02 at t7 for medium plants and this shows that concentration and volume were inversely proportional to each other but volume and relative humidity were directly proportional to each other. When temperature increases nectar concentration also increases and they were directly proportional to each other for both medium and oldest plants of *Callistemon citrinus L*.



4.3. Chart of clustered column analysis of oldest *Callistemon citrinus .L* plants

As sunshine gets more heater and heater from morning 7:300 Am, 9:00 Am, 10:30 Am, 12:00 Am, 1:30 Pm, 3:00 Pm and up to 4:00Pm-5:00 Pm nectar concentration increase continuously from 7.7w/w-36.1w/w and highest concentration was recorded at t7, but volume was highest that is 24.04µl at t1 and continuously decreases from t1-t7 and 7.04 at t7 this shows that concentration and volume were inversely proportional to each other but volume and relative humidity were directly proportional to each other. When temperature increases 14.2 - 21℃ nectar concentration also increases7.7w/w-36.1w/w and they were directly proportional to each other.



4.4. Figure

For oldest plants nectar concentration increases as time increases highly from t1-t7 (1:30Am - 11:00 Pm).For medium plants also concentration increases continuously from t1-t7 but for youngest plants Concentration decreases at t1-t2 but increase from t3-t4 then decreases from t4-t5 then slightly decreases up to t7.

5. Discussion:

Sugar concentration in nectar changes from low to high along the day(D. De 2010).

Nectar concentration and volume of *Callistemon citrinus.L* are affected by factors like age of plants, relative humidity, and temperature of the environment and exposure periods of the experiment, but volume and concentration of youngest plants of *Callistemon citrinus* are not affected by time, relative humidity of the environment and temperature. Medium and oldest plants of *Callistemon citrinus'* volume and concentration are highly affected by time, temperature and relative humidity and their concentration increases with time and temperature and their volume decreases when temperature and exposure period increases, so their volume is inversely proportional to concentration .Their volume increases as relative humidity increases, so their volume and relative humidity are directly proportional to each other. **The results from this study demonstrated that nectar production in the two melliferous plant species was high in the early morning hours when temperature was low with high relative humidity**(Adjaloo, 2015)**.But this study show a difference results from this done on *Callistemon citrinus.L had low nectar concentration for all the three types of age considered in the study younger***

medium and older ages of this plants. As a result shows nectar concentration increases as time increases as sun shine gets heater and heater as figures above shows and medium ages also nectar concentration increases as time increases or environments gets heater and heater from morning 7:30 Am to 5"00 Pm afternoon but for nectar concentration decreases as increases it only at 12Am and high nectar concentration was recorded at 5:00Pm on older age that was 36.1w/w results recorded at volume of 7.04µL and at temperature of 21℃ from our results we conclude that increase in temperature can increase nectar volume for medium and older plants but not for younger plants it is in contrast to results obtained by T(Petanidou & Smets, 1996) increase in temperature induce a greater nectar secretion rate in *T.capitatus* than *in B. acetabulosa*. This is very important for the apiculture potential of the phrygana, since T. capitatus is widespread and abundant throughout the Mediterranean region ***for medium and for medium ages 16.1w/w and older plants but this support mines foe younger age of plants .this shows as nectar volume decreases nectar concentration increase they inversely proportional for medium and older ages of Callistemon citrinus L. and as age gets older and nectar concentration increases may be the tissues gets water lose and as this shows lower for younger ages may they have more water in their tissues since are young. The volume and sugar concentration of nectars are known to vary among plant species and to affect pollinator response to plants. The nectar of the most important melliferous plants in the USA, alfalfa (Medicago sativa L.)***(Das *et al.*, 2014)**. The total nectar-sugar concentration ranged from 2.3–32%,with the highest*** among dessert cultivars 'Kamaramasenge'***(AAB-genome)and'Gisukari'(AAA-genome)***(Rutikanga *et al.*,2016) ***and it is 6.1-36.1 w/w for Callistemon citrinus L. and volume of 1.5 -26µL. Daily nectar production ranged from 0.1 to 3.8 µL per flower with sugar concentrations of 25 to 50%***(Farkas, 2012)**. Nectar yield increased and declined with stage of development, as hypothesized, but there were also unexpected and predictable changes in nectar composition as the flower progressed from opening to the beginning of capsule formation***(M.J.,*et al.,* 2018)**. The concentration of sugar in the nectar produced following the exposure to sound increased by ~20% on average***(Veits *et al.*,2018)**.**further, measuring of nectar concentration at day and night will indicate daily change of environmental temperature and relative humidity for isolation important nectar sources of honey bee forage plants in the Ethiopia

6. Conclusion:

The concentrations of Callistemon citrinus.*L* is affected by plants age, volume, time, relative humidity and temperature .For youngest plants volume has direct relation with relative humidity and also nectar concentration is not affected by temperature but nectar volume and concentration of medium and oldest ages of Callistemon citrinus.*L* are highly affected by time, temperature and relative humidity and have high concentration at t7 (16.1w/w and 36.1w/w) for medium and oldest plants respectively

7. Keywords: *Callistemon citrinus L*, nectar concentration, nectar volume, temperature, relative humidity and age of plants

8. Conflict of interest statement

I declare that I have no conflict of interest and it is my original work.

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