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Extended Abstract

Biofloral Phenology, Foraging Behaviour and entpollinatological effect of honey bees in Pomegranate (*Punica Granatum*) fruit quality and yield

A. S. Tanda
School of Horticulture, Western Sydney University, Australia

Abstract

Entpollinatology or insect pollination is a key element in agribusinesses with bio mutual interactions among flowers. Both male (unfertile) and bisexual (fertile) flowers on the pomegranate (*Punica granatum*) allow it to be self-pollinated as well as cross-pollinated. Whilst the evidence suggests that insect pollinators including honey bees are of significant benefit in increasing the fruit set and quality of fruit yields. Studies have shown that generally, male flowers produced more anther numbers than bisexual flowers did. Wind played no role in the dispersal of the pollen. *A. mellifera was* the main pomegranate flower visitor and pollinator. Insect visitation rate was more at 21°C and poor on foggy rainy or cloudy days. Bee foraging activity was increased at 1200 h. Fruit set in bee-pollinated and naturally-pollinated flowers did not vary significantly. Bee-pollinated (BP) fruits were bigger in diameter dark red, succulent, very tasty full of juice best in sweetness quality followed by naturally-pollinated fruits (NP). Average yield of the pomegranate was significantly better than self-pollinated (SP) flowers.

Keywords: *Punica granatum*, entpollinatology, honey bees, Bee-pollinated, *Apis mellifera*

Introduction

The pomegranate (*Punica granatum L.*) family Punicaceae is a fruit-bearing deciduous small tree is best suited to climates where winters are cool and summers are hot. The pomegranate is native to Iran and north India (DAF 2005). Throughout Eastern Europe, Asia and the USA, it is now widely cultivated the main areas of world production being in India, Iran, Spain and California. It can be consumed as fresh fruit or used in fruit juices, teas, pharmaceutical and medicinal products and in dyes or as decoration (Keogh, et al., 2008). Recent research results have revealed that pomegranate has a wide range of beneficial effects on human health, such as prevention and treatment of some types of cancer, diabetes, hypertension, cardiovascular disease, obesity, and erectile dysfunction.

Approximately 50% of the total pomegranate production which is about 2,000,000 tonnes is grown in the states of Maharashtra and Andhra Pradesh in India. The second largest country is Iran which is producing around 35% of the global production. Pomegranate production in Spain is around 2.5% and in the USA area is around 10,000ha. Turkey and other Mediterranean countries such as Morocco and Italy as well as the Middle East and former Russian states produce the balance (RIRDC 2008).

As a consequence, pomegranate trees can be recognized as either self-pollinated or cross-pollinated by insects mainly honey bees. At least two different cultivars of pomegranate should be planted in an orchard to encourage pollination and optimal fruit set. Evaluating different modes of pollination including self, open, and cross-pollination found that pollination is necessary to increase the quantity and quality of fruits in commercial pomegranate crop. The insects realize pollination of pomegranate and main pollinator is the honeybee. To meet export market standards, the fruit has to be of a certain size which is achieved by cross pollination success and consequently to seed number.

Self-fruitful cultivars providing entpollinatological (insect pollination) services greatly enhances the yield and fruit quality of cotton, okra, crops in eggplant. Studies on the pomegranate crop in Sydney NSW area were lacking, so keeping in view the role of honey bees in various crops, observations on bio-floralphenology, foraging behavior and entpollinatological effect of honey bees in pomegranate (*Punica Granatum*) and its fruit quality and yield were recorded throughout the blooming period at Rose hill Sydney Australia.

Materials and Methods

An experiment was conducted during March-May, 2019 and 2020 in the pomegranate (*Punica granatum*) plantations at Rose hill garden NSW 2142 Sydney Australia, which has latitude:-33.82721, longitude:151.01699 in the District of Parramatta

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City of New South Wales Australia. Studies on the assessment of entpollinatological effect of honey bee (*Apis mellifera*), its foraging behavior, biofloralphenology of pomegranate (*Punica Granatum*) fruit quality and yield were under taken.

Honey bee flower visitors, native wild bees and other insect pollinators were observed and the fruit setting in bee-pollinated and naturally-pollinated plants were assessed and compared statistically. The experimental crop plants were kept free from any spray during flowering period. To study the increase in seed number, pollen grains were applied with a brush each day for 4 days after the flowers opened. Percentage increase in seed number was calculated later on. Stigmatic receptivity was also studied during pollen transfer application process.

The amount of pollen production per anther and per flower was determined using the Hemacytometric method. Five microscope glass slides (7.6 × 2.6 cm2) were coated with vaseline and placed on a 45 cm high stool close to the flower height level. On sunny days, microscope slides were set up at 8:00 A.M. before flowering and collected around 4:30 P.M., after most flowers had discharged. The collected smears of glass slides were observed for the presence of pollen grains. The relative abundance of pollinators (number of flowers visited by pollinators) was recorded randomly on selected five plants during different times of the day (8.00, 10.00, 12.00, and 18.00 hrs) during the peak blooming period. The observations on foraging behavior viz., number of flowers (Number of pollinators/m2/ 5min) visited by insect pollinators at different hours of the day, foraging rate of pollinators (mean number of flowers visited/min) were recorded by using an electronic stopwatch during flowering period.

The observations were initiated at 10 per cent flowering and continued at weekly intervals for five weeks. Fifty individuals were observed visiting pomegranate flowers. The fruit set and fruit quality in naturally pollinated, self-pollinated and honey bee cross-pollinated flowers were also investigated. Bisexual flowers were observed pollinated by honey bees.

Flowers visited by bees were marked and tagged as Bee-pollinated (BP), Naturally-pollinated flowers were marked and tagged as NP and the self-pollinated flowers were covered with paper bags, and tagged as SP. Fruits diameter were measured using a micrometer caliper accurate to 0.003 cm. Ten fruits were selected randomly and physical properties such as color texture, appearance, weight, dimensions, fruit density and geometric mean diameter of fruits were determined. Fruit weight was measured by using digital scale (sensitive to ±0.01 g). Number of arils per fruit was determined by extracting the arils carefully by hand. Fruit width was measured by a digital caliper with ±0.01 mm Accuracy. Percentage of aril was determined by using digital scale (±0.01 g). All fruits obtained were analyzed pomologically. Average fruit weight, 100 aril weight, juice percentage were measured. Fruit weight was determined in the field shortly after fruit removal, using a battery-powered top loading balance accurate to ±2mg (Derin and Eti, 2001). Data were analyzed for flower insect visitation with meteorological parameters.

Observations were made for different groups of pollinators visiting the flowers for five minutes in each square meter area from five spot during peak flowering period. The time spent by the different bee species on five flowers was also recorded. Then the data was averaged time wise and peak foraging time. Mean of all the observations were pooled. The data were subjected to statistical analysis to infer the pollinator fauna as well as the dominance of a particular group. Special care was taken in all handling operations to prevent product damage and the associated loss of vi

sual appearance, increased water loss, and increased decay. Number of fruits, healthy and malformed fruits per plant was counted and mean number of healthy and malformed fruits per plant was calculated. The average fruit weight was computed by taking weight of all fruits individually. The fruit harvested from each plant treatment were weighed and recorded during each picking and noted the yield at the end. Samples were selected randomly. During the flowering period, the atmospheric parameters near the experimental field area (Fig.1) were recorded. Weather parameters like temperature (maximum and minimum), relative humidity (%) (Morning and evening hours), wind speed (km/h) and evaporation (mm) were collected daily from the Rosehill WeatherzoneMobile during the peak flowering period.

Observations were carried out on 50 bee-pollinated flowers vs. 50 naturally-pollinated flowers and 50 self-pollinated flowers

from 10 randomly selected stems. Each experimental flower was marked with a paper tag for later harvesting. Approximately four weeks after surveying flower visitors, we collected all ripe fruits from 10 randomly selected stems. Fruits were harvested in the same stage of ripening (i.e. when detaches easily from the receptacle and colour is bright pinkish red), and kept in a freezer.

A magnifying glass was used to count the number of anthers from each flower. European honey bee (*Apis mellifera*) willingly visited flowers of all blooming pomegranate experimental plants in Rose hill and other observations on floral biology, anther dehiscence, fruit studies and bee pollination data analysis were conducted at self-developed Insect Experimental Laboratory at Rose hill NSW 2142, Australia.