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The diversity and abundance of visitor insects on pummelo (C*itrus maxima* (burm) Merr) cv. nambangan

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Abstract
Insects are an effective pollinator to increase agricultural products. Knowledge of the diversity of plant pollinating insects is needed for strategy development of crop production. Pummelo (*Citrus maxima* (Burm) Merr.) is a monoecious plant with perfect flower. Most pummelo varieties have self-incompatibilities and require insects as pollinating agents. The research aims to study the diversity and abundance of pollinating insects of pummelo cv. Nambangan in East Java, Indonesia. Results showed that 17,401 individuals of insects belong to twelve species visited pummelo flowers. Nine species of visiting insects were potential as pollinator agents of pummelo, i.e. honey bee (*Apis cerana*), stingless bee (*Tetragonula laeviceps*), *Ceratina* sp., carpenter bees (*Xylocopa confusa* and *Xylocopa latipes*), butterflies (*Papilio demoleus, Catopsilia pyranthe,* and *Catopsilia pomona*) and unidentified dipteran. Three species, wasp (*Vespa affinis*), ants (*Dolichoderus thoracicus* and *Oecophylla smaragdina*) are categorized as predatory insects that have an important role in biological control.

Keywords: Bee, environment, pollinator, predator, pummelo

Introduction
Insects have an important role in helping pollination on agricultural land [1]. Pollinator insects are mainly belongs to order Hymenoptera, Diptera, Lepidoptera, and Coleoptera [2]. Pollination by insects has a significant effect on fruit productions [3]. Species richness and abundance of pollinator insects increase yield in some crops [4]. Bees are the most important insects for pollination, but non-bee species also have an equally important in crop production [2]. The role of pollinating insects in the self-pollination of horticultural crops was less attention [5].
The pummelo *(Citrus maxima* (Burm.) Merr.) is one of the cultivated plants that has a high economic value, large fruit size, and fruit weight ranging from 1-3 kg [6]. Pummelo flowers are perfect, having both sexes on the same flower so that self-pollination can occur [6]. However, most pummelo varieties probably have self-incompatibility (SI). Self-incompatibility occurs because the pollen attached to the pistil but cannot germinate or pollen tubes cannot grow properly because of the genetic barrier to inbreeding [7]. The primary advantage of SI has avoided the harmful effects of inbreeding with recognize and reject their own pollen (self-fertilization) [8]. Self-incompatibility has disadvantages such as reduced gene transmission in the next generation and pollination will be disrupted if no pollinators are unavailable [9].
The cross-pollination can overcome SI, thereby increasing the weight of seeds and fruits [10]. Self-incompatibility occurs in some plants such as *Nicotiana alata, Petunia inflata, Solanum tuberosum, Lycopersicon peruvianum* [11]. The use of insects in plant pollination is the most consistent and effective strategy [12, 13]. Pummelo flowers are easily damaged causing the formation of fruit does not occur properly. Therefore, the presence of pollinating insects is very important. However, the publication of the diversity of insect pollinators in pummelo is still very limited. This research aims to study the diversity of pollinators insect of pummelo in East Java, Indonesia.

Materials and methods
Study Site
The research was conducted at Sukomoro (270 masl), Magetan, East Java, Indonesia as the center of pummelo plantations, from October-December 2019. Pummelo used in the research was *Citrus maxima* (Burm) Merr cv. Nambangan in the same age (10 years old), 5-6 m in height with canopy width of 4-5 m. Pummelo cv. Nambangan is a native variety of Magetan, Indonesia [14].

Characteristics of pummelo cv. Nambangan flowers

Biology of the pummelo flower was observed i.e. shape, color, number of sepals, petals, stamens, pistil, size, flowers position, flowers blooming, and length of flowers bloom [15]. Flowers and insects were examined and observed with a stereo light microscope Olympus coupled with Optilab camera and Panasonic Lumix G-85 camera. The sugar content of pummelo was measured using a refractometer [16]. Measurement of sugar content was done on fresh flowers at 07.00 am, 12.00 am, and 05.00 pm using handheld refractometer ATC HT214.

Observation of the diversity of pollinator insects

Observations of pummelo insect diversity were conducted by using a scan sampling method [17]. Observations were conducted in an area of 15x25 m by walking slowly and recording the number of individuals and species visited the flower [18]. The observation was done every 15 minutes with an interval of one hour, starting at 07.00 am-17.00 pm. Environmental parameters (air temperature, relative humidity, light intensity, and wind velocity) were measured at 07.00 am-05.00 pm using Lutron LM-8010. Some individuals of insects were caught by using an insect net for the identification process. Specimens identification of insects were conducted at the Laboratory of Animal Biosystematics and Ecology, Department of Biology, IPB University, and Laboratory of Entomology, Indonesian Institute of Sciences.

Data Analysis

The diversity of visiting insects was analyzed by using the Shannon diversity index (H), evenness index (E), dominance index (D), and Sorensen similarity (Cs) [19]. The correlation between environmental parameters and number of individuals of visiting insects were analyzed by using Spearman correlation and Principal Component Analysis (PCA) in the Paleontological Statistics (PAST) Program 3.20.

Results and discussions

Characteristics of pummelo cv. Nambangan flowers

This research was conducted in Magetan, East Java, which has two seasons (dry season and rainy season). The flowering of pummelo cv. Nambangan is generally influenced by water stress and induction of the tree with the pruning process. The duration and number of flowering depend on irrigation, drought stress, environment temperature, health, and maturity of the tree [20]. Most of the pummelo orchards in Magetan are traditional plantations so irrigation depends on the rainy season. These conditions cause flowering cannot occur throughout the year except for pummelo trees that are close to water sources. The peak of flowering usually occurs at the beginning of the rainy season in around of October or November.

Based on observations, pummelo cv. Nambangan have a compound flower (inflorescence) with the axillary position in the leaf armpit. Each inflorescence stalk consists of 8-13 flowers (Fig. 1A). Each flower has radial symmetry consists of 5-6 sepals, 5 petals, 1 pistil, and 26 stamens (Fig. 1B). The sepals (calyx) are light green with white dots, joined to form a bowl-like structure, attached above the receptacle (superior) with 0.8-1.2 cm in length (Fig. 1C). The petals are white with 3.0-3.5 cm in length and will turn brown 2-3 days after the flowers bloom (Fig. 1B).

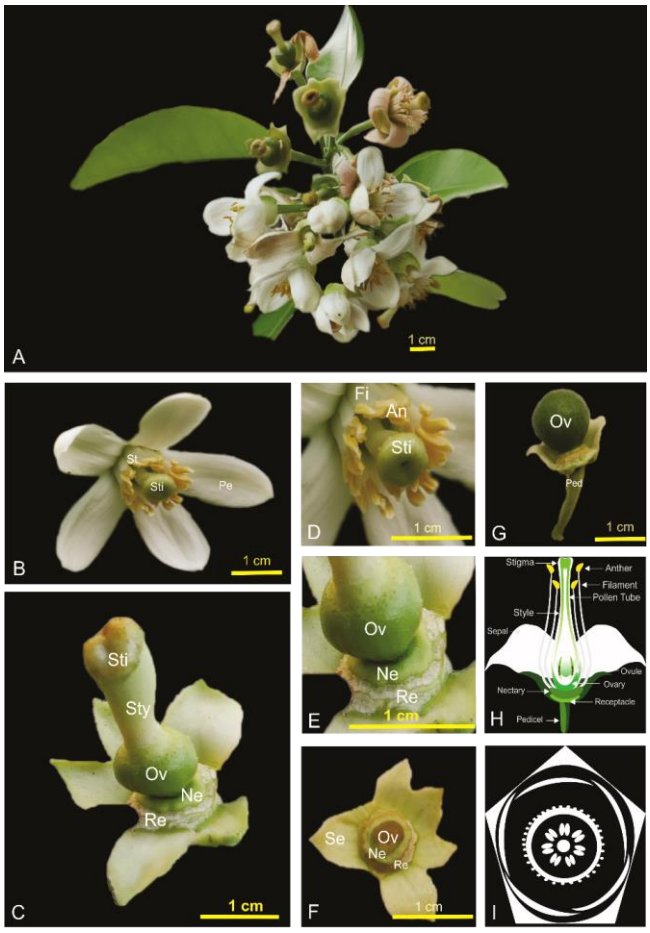

Fig 1: Flowers of pummelo cv. Nambangan : **A.** group of flowers (compound flower); **B.** single flower; **C.** female sex organ (pistil); **D.** male sex organ (stamen); **E.** lateral view of nectary; **F.** dorsal view of nectary; **G.** fertilized embryo; **H.** flower structure; **I.** floral diagram; Pi-pistil, Sti-stigma, Sty-style, St-stamen, Fi-filament, An-anther, Ov-ovary, Ne-nectary, Se-sepal, Pe-petal, Re-receptacle, Ped-pedicel

The single pistil is in the center of each flower consists of a light green stigma at the tip, white style, and green ovary (Fig. 1C). The surface of the stigma is coated by a sticky thick liquid. The stigma and ovary are connected by a white style that forms a knob. On the inside of the ovary, there are ovules that will develop into seeds and stick to the ovary walls. At the bottom of the ovary, there is an organ that produces nectar (nectary). The stamens attach to the outside of the ovary with circular position. The stamens consist of white filaments and anthers with yellow pollen (Fig. 1D). The flowering period of each pummelo tree occurred 2-4 weeks. The flowering period takes place at the beginning of the rainy season. The flowering period can take place off-season (off-bloom) for plants that are close to water sources.

The pummelo flowers can bloom in the morning, noon, or night, regardless of time. Flower anthesis occurred 12-14 days after the green bud (flush bud) appear with 2-3 days anthesis duration. Pummelo flowers are easy to fall by touching, vibration, wind, or rain. The sugar content in the nectar of pummelo cv. Nambangan flowers ranged from 14-15.5%. During the blooming period, the flowers release a strong fragrant. The bloom flowers in the evening will fresher because they are not exposed to the sun. Exposure of sunlight will make the flowers dry up, smell-less, and turn brown in color. Constant exposure to high temperatures damages petals, pollens, and gynoecium [21]. Changes in temperature affect plant fitness which can affect the attractiveness and number of visitors.

Diversity of visitor insects of pummelo

The flowers of pummelo cv. Nambangan has a very strong fragrant and bright color that attracts insects to visit. The flower shape, size, nectar position, and pollen are suitable for flower visitors. Results showed that total of 17,401 individuals consist of twelve species of visiting insects on pummelo flowers were found (Table 1). Those insects belong to three orders, i.e. Hymenoptera, Diptera, and Lepidoptera (Fig. 2). The Hymenoptera had the highest percentage of visitors (68.65%) followed by Lepidoptera (26.73%), and Diptera (4.61%) (Table 1). Shrestha [22] reported that Hymenoptera is a major flower visitor and has the largest contribution to increase agricultural production. The diversity of visiting insects in the study area was moderate (H=2.03), data showed that evenly distributed (E= 0.69), and almost no dominant species (D=0.15) (Table 1).

Table 1: Diversity of visiting insects in pummelo flowers

Order Family, Subfamily	Species	Number of individuals	Percentage (%)
Hymenoptera			
Apidae, Apinae	*Apis cerana*	3,256	18.71
	Tetragonula laeviceps	2,725	15.66
	Ceratina sp.	358	2.06
Apidae, Xylocopinae	*Xylocopa confusa*	645	3.71
	Xylocopa latipes	1,307	7.51
Vespidae, Vespinae	*Vespa affinis*	409	2.35
Formicidae, Dolichoderinae	*Dolichoderus thoracicus*	1,067	6.13
Formicidae, Formicinae	*Oecophylla smaragdina*	2,179	12.52
Diptera	Unidentified species	803	4.61
Lepidoptera			
Papilionidae, Papilioninae	*Papilio demoleus*	1,040	5.98
Pieridae, Coliadinae	*Catopsilia pyranthe*	1,334	7.67
	Catopsilia pomona	2,278	13.09
Number of species	12		
Number of individuals	17,401		
Shannon index diversity (H')	2.03		
Evenness index (E)	0.69		
Dominance index (D)	0.15		

The flowers visitor insects were grouped into several categories i.e. pollinator, predator, pest, and parasitoid insects [23]. Results showed nine species of insects were grouped as pollinator (*A. cerana*, *T. laeviceps*, *Ceratina sp.*, *X. confusa*, *X. latipes*, *unidentified dipteran*, *P.demoleus*, *C. pyranthe*, and *C. pomona).* Three species were grouped as predatory insects (*V. affinis*, *D. thorachicus* and *O. smaragdina*) (Fig. 2).

The Pollinator Hymenoptera, *A. cerana* was found with the highest number of individuals (18.71%). Three species of solitary bees, namely *Ceratina* sp., *X. confusa*, and *X. latipes* were also visited pummelo flowers (Table 1). *Ceratina* sp. was found with the smallest number of individual (2.06%) and only visits flowers in the morning. In Mexico, *Ceratina* has been reported as a pollinator of orange (*Citrus sinensis*) and lemon (*Citrus limon*) [24]. Carpenter bees, *X. confusa* and *X. latipes* were found in the morning and noon. Both species of *Xylocopa* have potential as a pummelo pollinator. However, both species have large body size so that they can damage the flowers of pummelo. Thapa [25] reported *Xylocopa* is a pollination agent of various plants, such as eggplant (*Solanum melongena*), broccoli (*Brassica oleracea*), *Cucumis sativus*, and okra (*Abelmoschus esculentus*).

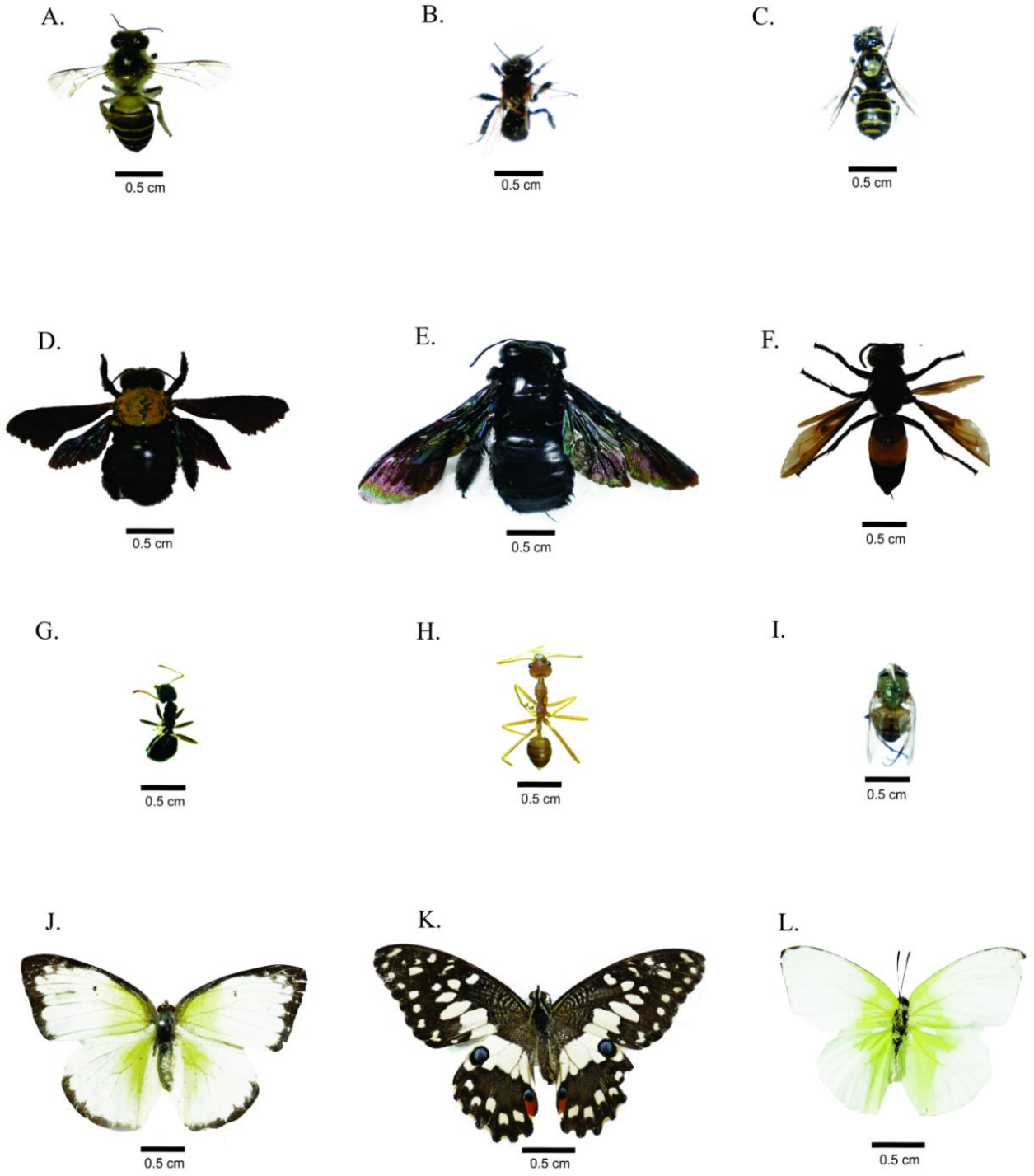

Fig 2: Diversity of visiting insects of pummelo cv. Nambangan flowers: **(A)** *A. cerana*, **(B)** *T. laeviceps*, **(C)** *Ceratina* sp., **(D)** *X. confusa*, **(E)** *X. latipes*, **(F)** *V. affinis*, **(G)** *D. thoracicus*, **(H)** *O. smaragdina*, **(I)** unidentified dipteran, **(J)** *P. demoleus*, **(K)** *C. pyranthe*, **(L)** *C. pomona*

The Predatory Hymenoptera, i.e.wasp (2.35%), and ants (18.65%) were also found during this study. Wasp (*V. affinis*) and ants (*D. thorachicus* and *O. smaragdina*) are predators with no have special structures to transfer pollens and have less body hairs. Nevertheless, ant foraging activity allows the process of pollinating plants [26]. Three species of butterflies i.e. *P. demoleus*, *C. pyranthe*, and *C. pomona* were found during this study. Adult butterflies are potential as pollinators of pummelo flowers. Pollens of pummelo are easy to adhere to the insect bodies and possible for cross-pollination. Previously, *P. demoleus* was reported as flowers visitor of lemon (*C. limon*) and avocado (*Persea americana*) [27]. Two species of pierids, *C. pyranthe* and *C. pomona* also were reported as insect visitors on some plants, such as fenugreek [28], *Coriandrum sativum* L. [29], and *Pavetta tomentosa* [30]. The number of pummelo visitors were high in the morning, decreases in the noon, and increases again in the afternoon (Fig. 3). The condition was probably caused by the increasing number of predatory Hymenoptera at noon (Fig. 3). Predatory Hymenoptera was high at 11.00-12.00 am and low at 03.00-05.00 pm (Fig. 3). Tsuji [31] reported that the presence of ants reduces the visiting rate of other insects. The ants visit the flowers to eat nectar as a nutrition source for their larvae [32].

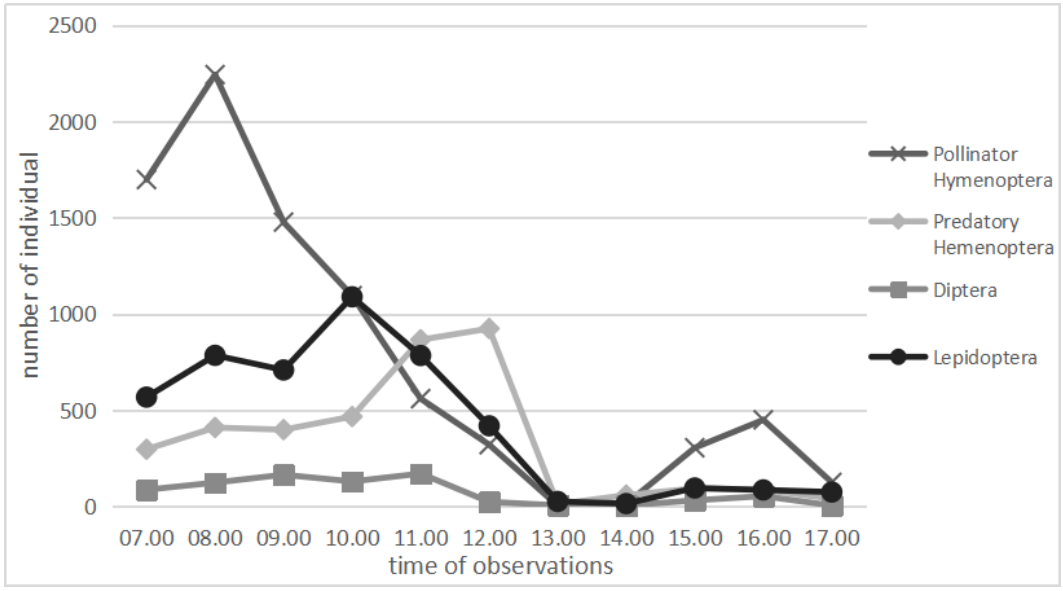

Fig 3: Number of individuals of visiting insect (order) on pummelo flowers

Some species, such as *T. laeviceps*, *D. thoracicus*, *O. smaragdina*, *P. demoleus*, *C. pyranthe*, *and unidentified dipteran* were found visited pummelo flowers at each time of observations. Solitary bee, *Ceratina* sp. only was found at 07.00-11.00 am during the observations. The diversity of insects during each observation time was lowest at 02:00 pm (H=1.77) (Fig. 4). Data of visiting insects showed that evenly distributed and almost no dominant species (D=0.25) (Fig. 4).

The abundances of pollinating insects in an area were affected by the number of flowers, size, shape, color, volume, and quality of nectar, pollens, and environmental factor [33]. Sorensen index that the similarity of visiting insects was highest in the morning-afternoon (Cs=61.54%) followed by morning-noon (Cs=56.47%), and noon-afternoon (Cs=55.22%) (Fig. 5).

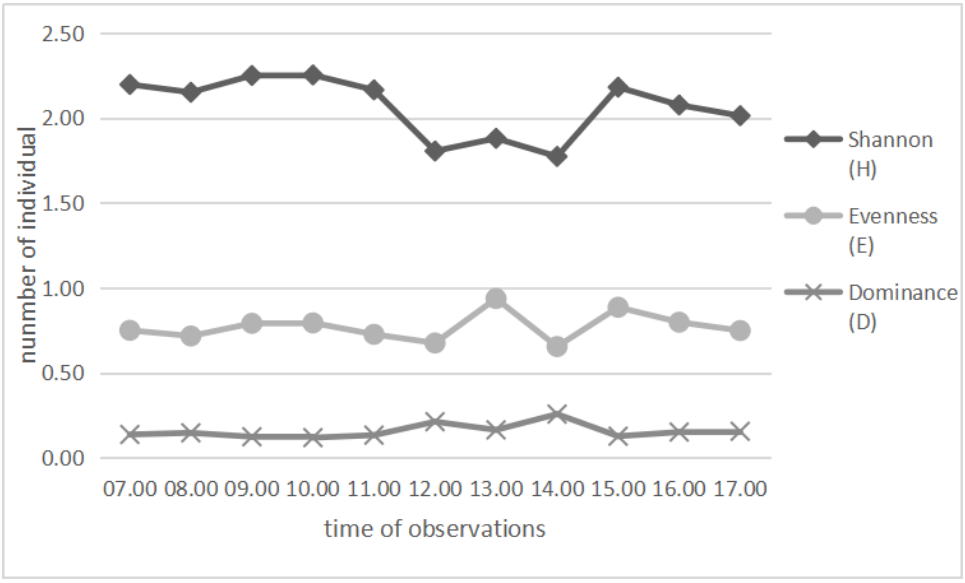

Fig 4: Shannon diversity, evenness, and dominance index of visiting insects in pummelo flowers based on the time of observation

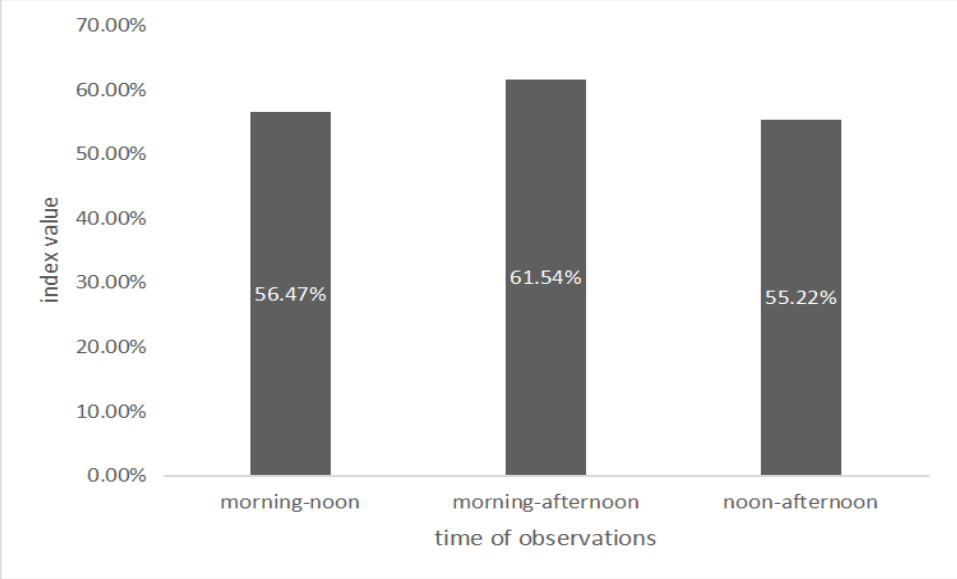

Fig 5: Similarity of visiting insects (Sorensen similarity) on pummelo flowers in the morning (07.00-10.00 am), noon (11.00 am-02.00 pm), and afternoon (03.00-05.00 pm)

The environmental parameters during insect observations were air temperature ranged 27.79-36.26^{0}C, relative humidity 31.09-79.89%, light intensity 7116-17237 lux, and wind velocity 0.89-2.64 m/s. The peak activity of insects visiting occurred in the morning (08.00 am) with air temperature, relative humidity, light intensity, and wind velocity were 29.4^{0}C, 72.77%, 9585.21 lux, and 1.28 m/s, respectively. Spearman correlation analysis and Principal Components Analysis (PCA) showed air temperature, light intensity, and wind velocity were negatively correlated, while the humidity was positively correlated with the number of visiting insects (Table 2, Fig. 6).

Table 2: Correlations between environmental factors and the number of visiting insects

Correlations	Air temperature (^{0}C)	Relative humidity (%)	Light intensity (lux)	Wind velocity (m/s)
Coefficient	-0.502**	0.811**	-0.420**	-0.379**
Sig. (p value)	3.38E-11	3.19E-37	5.99E-08	1.28E-06
N	154	154	154	154
**. Correlation is significant at the 0.01 level (1-tailed).				

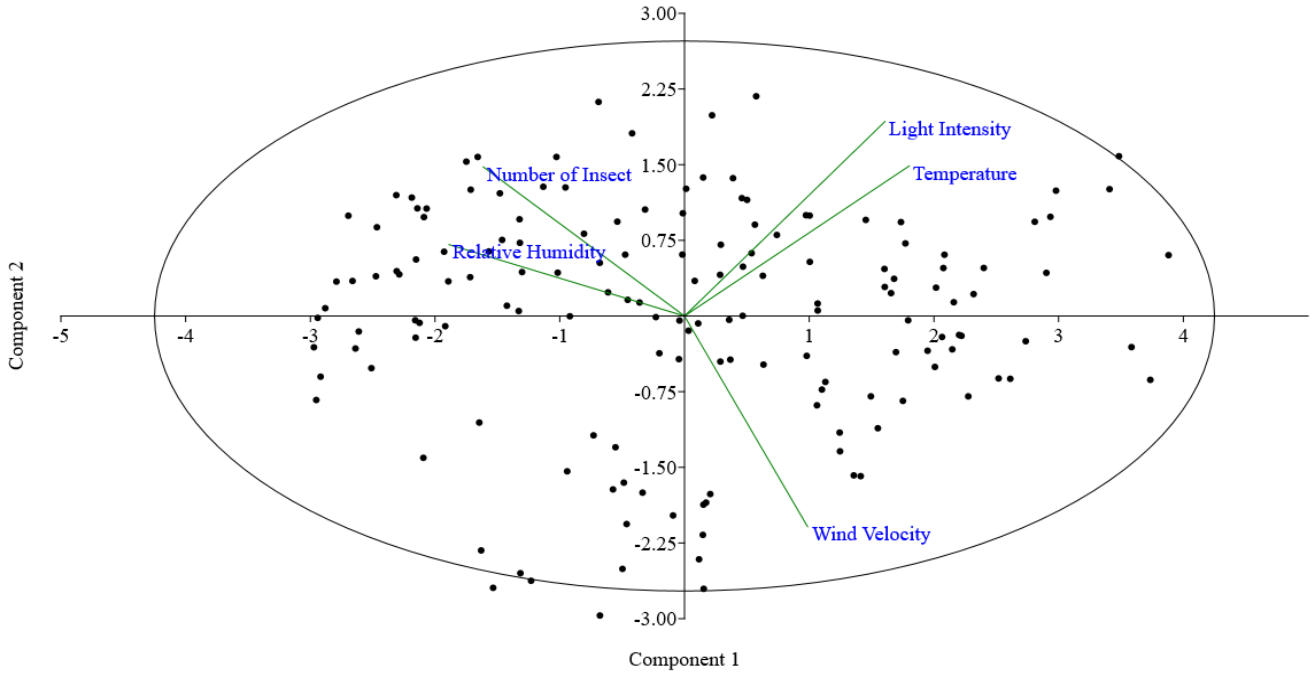

Fig 6: Relationship between visiting insects of pummelo flowers and environmental parameters based on the Principal Component Analysis (PCA)

This result didn't support Taha [34] that reported temperature and light intensity to have a positive correlation, while relative humidity and wind speed have negative correlation with the number of visitor insects. The peak number of visiting insects occurred at 08.00 am with air temperature of 29.41^{0}C and humidity of 72.77% and decrease when the temperature above 30^{0}C. *Apis cerana* and *Tetragonula laeviceps* are the most visitors so the numbers will affect the analysis. Putra and Kinasih [35] reported that the activity of honey bees and stingless bee increased at temperatures around 30°C and temperatures above 30°C decrease their activity. The peak activity of *A. cerana* occurred in temperature 15.5-21^{0}C and *A. mellifera* in temperature 21-25^{0}C [36], *A. florea* in temperatures 24.5-28^{0}C, and relative humidity 45-70.5% [37]. Stingless bee, *T. carbonaria* prefers warm flowers when the ambient temperature is cold, but it changes preference when the ambient temperature is more than 30˚C [38].

Conclusion

Twelve species of pummelo flower visitors were found during the research. Nine species of insects were potentially as pollinators, i.e. *Apis cerana*, *T. laeviceps*, *Ceratina* sp., *X. confusa*, *X. latipes*, *P. demoleus*, *C. pyranthe*, *C. pomona* and unidentified dipteran. Three species were categorized as predatory insects, i.e. *V. affinis*, *D. thoracicus*, and *O. smaragdina*. The number of pummelo visitors were high in the morning, decreases in the noon, and increases again in the afternoon. The peak activity of insects visiting occurred in the morning (08.00 am) with air temperature, relative humidity, light intensity, and wind velocity were 29.4^{0}C, 72.77%, 9585.21 lux, and 1.28 m/s, respectively.

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