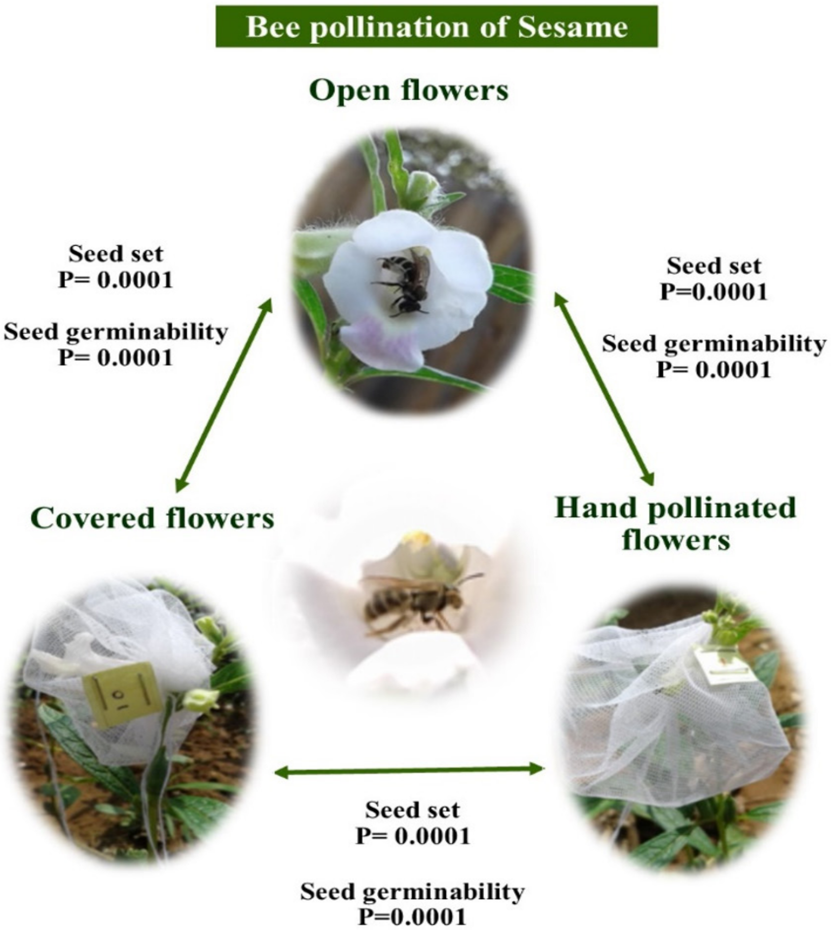
RESEARCH ARTICLE

Native Sri Lankan bees enhance pollination of sesame, *Sesamum indicum*

E.M.D. Ekanayake and W.A.I.P. Karunaratne*



Highlights

- Six bee species visited sesame flowers, *Gnathonomia nasicana* being the most dominant.
- Seed number, seed weight, and seed germinability resulted from three trials were significantly different.
- Bee pollinated flowers had significantly higher seed number and germinability compared to covered flowers.
- Native bees of Sri Lanka enhance seed number, seed weight, and seed germinability in *S. indicum*.

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Native Sri Lankan bees enhance pollination of sesame, *Sesamum indicum*

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Abstract: Decline of bee pollinators results in insufficient crop pollination, lowering the quality, and quantity of crop yields. In Sri Lanka, there is a lack of research on the importance of local bees in sesame pollination. The current study investigated the role of Sri Lankan native bees in pollinating sesame and increasing crop productivity. The research was carried out at Meewatura Farm, located in the Kandy District of the University of Peradeniya, Sri Lanka. Three pollination trials were conducted to determine the contribution of bees to enhance seed number, weight, and germinability of sesame, selecting 100 sesame flower buds randomly for each trial. One set of flowers buds was covered to prevent bee visits; another set was kept open to enable bee visits, while the third set was pollinated by hand. Six bee species; *Systropha tropicalis*, *Gnathonomia nasicana*, *Lasioglossum serenum* (F: Halictidae), *Apis cerana*, *Amegilla comberi*, and *Ceratina binghami* (F: Apidae), were identified as visitors of sesame flowers. Period of stigma receptivity (0700 - 1100 h) and pollen availability (0640 - 1000 h) coincided with the highest bee activity period from (0830 - 1030 h). Significant differences ($p < 0.05$) in seed number, seed weight, and seed germinability were detected among the three trials. Open flowers had significantly higher seed number ($p = 0.0001$) and seed germinability ($p = 0.0001$) than covered flowers. Although there was no significant difference in seed weight between seeds produced from open flowers and covered flowers, the seed weight of hand-pollinated flowers was significantly higher than the other two treatments ($p = 0.0001$). This pioneering study highlights the role of native bees of Sri Lanka to enhance production in *S. indicum* in terms of seed number and quality.

***Keywords*:** Abundance; bee pollination; crop yield; Sri Lankan native bees; sesame.

INTRODUCTION

Pollination is the movement of pollen grains from anthers of a flower to the stigma of the same or a different flower. It is considered one of the essential plant-animal interactions ensuring the reproduction of plants (Murcia, 1996). As a result of animal pollination, around 75% of crops yield more fruits. Among the insects, bees (Hymenoptera: Apoidea) serve as an important group of pollinators, visiting more than 90% of crop species (Klein *et al*., 2007). Bees visit plants to collect pollen and nectar as food for adults and larvae (Michener, 1974; Roubik, 1992). Bees tend to confine their visits to a single flower species during each journey, ensuring pollen transmission from one plant to another of the same species, which is a vital behavioural adaptation for plants (Free, 1993).

According to Garibaldi *et al*. (2014), the fruit quantity of most crop species is known to be increased by bee pollination. Though pollination improves the yield of most crop species and contributes to one-third of global crop production, comprehensive benefits, including crop quality, are yet to be identified. As native bee pollinator decline affects fruit and seed quality and quantity of many crops in many regions, the demand for pollination services is increasing (Klein *et al*., 2007; Wester and Lunau, 2017). Many investigations have attempted to approximate the value of crop pollination and pollinator dependency on almonds, coffee, cacao, tomato, canola, watermelon, and sunflower (Southwick and Southwick, 1992; Losey and Vaughan, 2006; Gallai *et al*., 2009; Costanza *et al.*, 2014).

Sesame (*Sesamum indicum* L.) is a flowering plant belonging to the family Pedaliaceae. It is native to Asian (India and China), and African (Egypt and Central Africa) countries (Dossa *et al*., 2016), and these countries account for over 90% of the world's sesame cultivated land. According to Andrade *et al*. (2014), in 2014, the global sesame production is predicted to be 390 kg ha^{-1}. Sesame cultivation has a high demand because of its seeds containing high oil content and unsaturated fatty acids, proteins, and antioxidants (Bahrami *et al*., 2012). Sesame seeds are also rich in minerals (calcium, iron, phosphorus) and vitamins (vitamin A, thiamine, and riboflavin) (Obiajunwa, 2005). Sesame seeds are consumed as food, as well as in pharmaceutical and chemical industries (Hwang, 2005; Namiki, 2007) due to their high therapeutic and nutritional values (Liu *et al*., 2011; Morcos *et al*., 2013; D'Souza *et al*., 2016; Dissanayake *et al.*, 2017).

Sesame has hermaphrodite zygomorphic blooms with a 3 - 4 mm long pendulous tubular corolla in varied colors of purple white. The androecium has two long and two short stamens, while the gynoecium has a superior ovary, a multi-carpelar style, and a long stigma with a bifid stigma. The nectary disks are grouped around the ovary in a circle (Free, 1993). Though sesame is a self-pollinating crop (autogamous) (Khidir, 1972; Abdel All *et al*., 1976), its blossom structure facilitates cross-pollination (allogamous) (Andrade *et al*., 2014). Differing rates of

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cross-pollination have been reported (Abdel All *et al*., 1976; Yermanos, 1980; Free, 1993), while bee pollination was known to enhance the yield of sesame seeds from 22% to 33% (Mahmoud, 2012; Blal *et al*., 2013). Nevertheless, previous studies reported that the cross-pollination rates ranged from 1 to 68% (Abdel All *et al*., 1976; Free, 1993; Ashri, 2007), indicating the need for further investigations in different sesame growing countries.

Sesame is a major oilseed field crop in Sri Lanka (Weeraratna and Weerasinghe, 2009), and the total cultivation area is around 13,120 ha (Dissanayake *et al*., 2017). Sesame is grown at different scales, mainly in the Yala season (from March to August) under rain-fed conditions (Rajapaksha, 1998; Gunasena, 2001; Dissanayake *et al.*, 2017) in 23 districts of which 14 belong to the dry zone. However, comprehensive studies on sesame pollination are also lacking in Sri Lanka, and practically all sesame growers get their pollination services free from nature. Due to the lack of information on the role of native bees in the pollination of sesame, this study was conducted with the specific objectives of (i) to determine the time of stigma receptivity, anther dehiscence, and number of pollen grains per sesame flower, (ii) to identify different bee species that visit *S. indicum*, their abundance, and floral resources (pollen / nectar), and (iii) to determine the contribution by bees to pollinate sesame.

MATERIALS AND METHODS

Study site

The study was conducted at Meewatura in Kandy District (geographical coordinates; 7° 15′ N, 80° 45′ E), central Sri Lanka, for eight months (from December 2018 to July 2019). This site with sandy loam soil having an organic content of 4.50 - 4.60% (Jayarathne *et al*., 2020) is a semi agricultural research field of the University of Peradeniya, located in the western valley of the Mahaweli River. The mean annual temperature, wind speed, humidity, and rainfall at the site were 44 °C, 0.94 m s^{-1}, 75%, and 700 mm, respectively (source: Natural Resources Management Center, Peradeniya).

Preparation of a cultivated plot of *S. indicum*

The plot for the cultivation of sesame was prepared in an area of 3×1.5 m^2. Dry cow dung and compost were mixed with the loose soil in the ratio of 1:3:2, respectively. Seeds were planted within sixty holes (6 holes×10 rows with equal inter-plant and inter-row distance of 25 cm and 30 cm, respectively) to have two sesame seeds in each hole. The plot was watered once in a two-day interval. The study area was surrounded by a fence made of asbestos sheets (about 1 m tall) to avoid the invasion of wild animals.

According to the preliminary study, *Sesamum indicum* seedlings emerged within 3-4 d after planting seeds. After 25-30 d of maturity, plants reached the reproductive stage, during which the flower buds appeared. Fruit maturity occurred within 55-60 d after flower appearance. On average, the plants took nearly 80-90 d to produce mature fruits starting from the seedling stage. Flowers fully opened around 0700 h, wilted around 1400 h, and fell on the following day. The lifespan of a flower was about seven hours.

Determination of the time of stigma receptivity, anther dehiscence, and number of pollen grains per sesame flower

Time of stigma receptivity was recorded in freshly opened five flowers by observing the stigma using a hand lens at every 10-minute intervals from 0630 h. Flowers began to open around 0630 h and fully opened around 0700 h. The stigma was touched with a needle tip to check for stickiness which was considered the time of stigma receptivity (Wanigasekara and Karunaratne, 2012).

The time of anther dehiscence was recorded by shaking five randomly selected flowers onto a white paper at 10-minute intervals from 0630 h and was observed through a hand lens. The time at which pollen was released and collected onto the white paper was considered the time of anther dehiscence.

To investigate the number of pollen grains per anther and flower, ten fresh flowers in which the anthers that were not yet split open were selected. One anther was removed from each flower and placed in a solid watch glass. The anther was sliced lengthwise with a sharp needlepoint, and pollen grains were removed onto a solid watch glass containing one milliliter of 50% alcohol (Sowunmi, 1968). The number of pollen grains was counted and recorded, using a Hemocytometer. This number was multiplied by the number of anthers per flower to get the total number of pollen grains produced by each flower. Finally, the mean number of pollen grains per anther and flower was calculated.

Identification of different bee species, frequency of visits, and floral resources

Flower visiting bee species were observed once a week for 14 consecutive weeks of a similar climate. Thorough observations were made by walking around the sesame plot having more than 100 sesame plants from 0700-1100 h to record different species of bees visiting sesame flowers in bloom. Observations were taken until no new species were recorded during the period of stigma receptivity and anther dehiscence. Representative specimens (2-3) of the different species of bees visiting the sesame flowers were collected using a sweep net and were curated using standard entomological techniques. Identification of the bee species was made using the key developed by Karunaratne and Edirisinghe (2008). Identified specimens were cross-checked with the reference collection of bees lodged at the Invertebrates Systematics and Diversity Facility (ISDF) in the Department of Zoology, University of Peradeniya.

The frequency of visits of each bee species was recorded by observing the number of visits to flowers. During the period of stigma receptivity and anther dehiscence, randomly selected five flowers were observed for bee visits at a time, from 0630 h to 1100 h at 10-minute intervals. The bee species and their numbers visiting each flower were recorded. The observations were made for 14 sunny days. The behavior of each bee at flowers, whether collecting pollen on anthers or nectar from the nectaries was also recorded, but no attempt was made to quantify their percentage.

Determination of the contribution by bees to pollinate *S. indicum*

Three pollination trials; T1: Open flowers to enable bee visits, T2: Covered flowers to stop bee visits, and T3: Hand pollinated flowers were conducted to determine the contribution of bees to pollinate sesame. Randomly selected 100 flower buds ready to open were tagged and kept open for bees to visit. Another similar set was tagged and covered by fine mesh cloth bags to prevent visits of bees. After the petals of the flower fall, the mesh bags were removed allowing any pods to mature. Another similar set of flower buds was kept closed by fine mesh bags and was hand-pollinated as soon as the stigma becomes receptive. Pollen grains from another freshly dehisced anther of a flower from a different plant were collected onto a fine paintbrush and applied on the receptive anthers of the selected flowers. After that, they were again covered by a fine mesh bag to prevent bee visits. After pod formation, the mesh bags were removed to allow fruit maturity. The tags were kept until the pod's maturity. Mature pods from the three treatments were harvested with their tag numbers, and the number of seeds per fruit was counted. Seeds were weighed using a digital balance (Readability; 0.01 g, Weighing capacity; 410 g, Linearity; ± 0.002 g, METTLER PM 400, Toledo, USA).

Determination of the seed germinability

All seeds produced from each pod of the three pollination trials were allowed to germinate on paper towels in Petri dishes (9 cm diameter). Petri dishes were sterilized by wiping with 70% alcohol. Subsequently, a paper towel was placed on the bottom. Each Petri dish was labeled with the flower number, type of pollination treatment, and date of germination test conducted. Thereafter, 4 ml of distilled water was added to the paper towel on which the seeds from each pod were spread separately on the surface, leaving a distance between seeds at least three to five times the seed diameter (Rao *et al*., 2006). Thereafter, the Petri dishes were covered with the lid and placed at room temperature (~ 25 °C). The paper towels were moistened regularly several times during the experiment. After four days, the number of germinated and non-germinated seeds was recorded.

Data Analysis

The normality of three treatments tested using the Shapiro-Wilk test revealed that the data were normally distributed. One-way ANOVA was performed to compare the equality of mean values of three treatments; whether there is a difference in the number of seeds produced per fruit and the number of germinated seeds per fruit with respect to three treatments using Minitab 14.0. In addition, a two-sample t-test was carried out to determine for any significant differences in mean values of seed number, seed weight, and seed germinability between treatments.

RESULTS

Stigma receptivity, anther dehiscence, and number of pollen grains per *S. indicum* flower

The stickiness of stigmas started around 0700 h and remained until about 1100 h. This period was considered the stigma receptive period. Anthers dehisced between 0640 h and 0710 h, and pollen grains were available in anthers until 1100 h. Thereafter, the anthers dried up and changed colour from white to yellow and then brown. The mean number of pollen grains per anther and the per flower was 495.4 ± 62.1 and 1982 ± 249, respectively.

Bee species that visited flowers of *S. indicum*

Six species of bees; *Systropha tropicalis* (Cockerell, 1911), *Gnathonomi anasicana* (Cockerell, 1911), and *Lasioglossum serenum* (Cameron, 1897) of the family Halictidae, *Apis cerana* (Fabricius, 1793), *Amegilla comberi* (Cockerell, 1911), and *Ceratina binghami* (Cockerell, 1910) of the family Apidae visited *S. indicum* flowers in the site at Meewathura during the 14 sunny days.

Frequency of each bee species that visited sesame flowers and their floral resources (pollen / nectar)

As demonstrated in Figure 1, the frequency of different bee species that visited a flower of *S. indicum* in an average day varied during the study period. The most frequent bee species was *G. nasicana*, with 25 visits, followed by *S. tropicalis* (4 visits). *Ceratina binghami* was the least visited species in sesame flowers. Observations on bee behaviour at flowers revealed that all bee species visited sesame for pollen while only *S. tropicalis* visited for both nectar and pollen.

Figure 2 gives the mean frequency occurrence of each bee species that visited *S. indicum* flowers per day from 0630 h to 1100 h in the site at Meewathura for fourteen days. According to Figure 2, the mean frequency of all bee species visiting *S. indicum* flowers gradually increased from 0630 h to 1000 h and decreased thereafter. *Gnathonomia nasicana* was the most frequent bee on *S. indicum* flowers, and its maximum mean frequency was found during 0940 h and 0950 h. The maximum mean frequency of *S. tropicalis* and *A. comberi* on *S. indicum* flowers was from 1000 - 1010 h. The maximum mean abundance of *A. cerana* was found during 0940-0950 h, while that of *L. serenum* was from 1010-1020 h. The least common bee, *C. binghami* was observed on *S. indicum* flowers from 0940-0950 h. The frequency of occurrence of each bee species coincides with the period of pollen availability (from 0640 - 1100 h) and anther dehiscence (from 0640 - 0710 h).

Contribution of bees to pollinate sesame

The total number of seeds produced by the 100 open flowers was the highest (4981), while it was the lowest (3627) in hand-pollinated flowers. The number of seeds per pod is given in Figure 3(a). The average seed weight ± SD of fruits produced from open flowers, covered flowers, and hand-pollinated flowers were 0.12427 ± 0.03936 g,

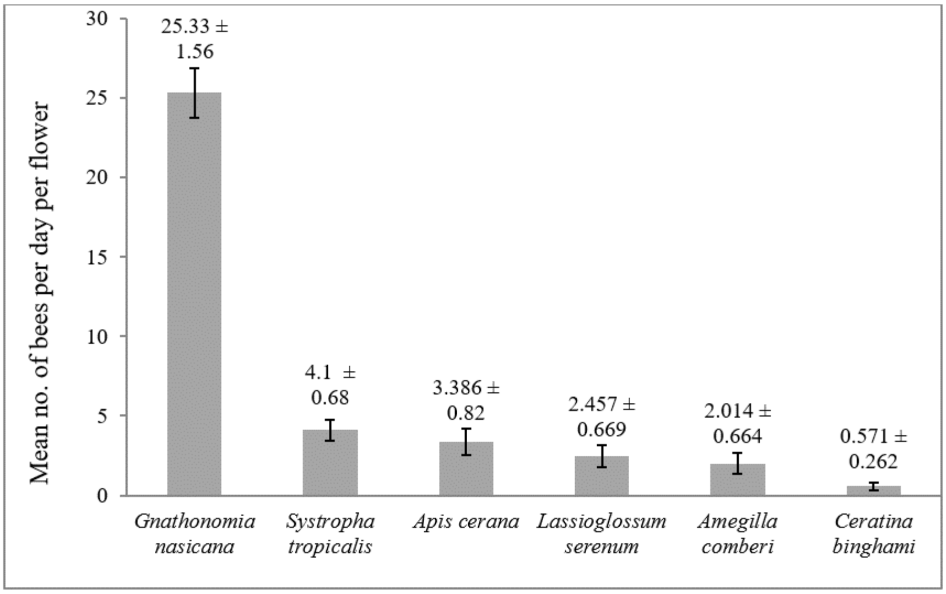

Figure 1: Mean number of visits of the six bee species ± SE per *S. indicum* flower per day at the Meewathura site (visits of the six species within 14 d from 0630 h to 1100 h are averaged).

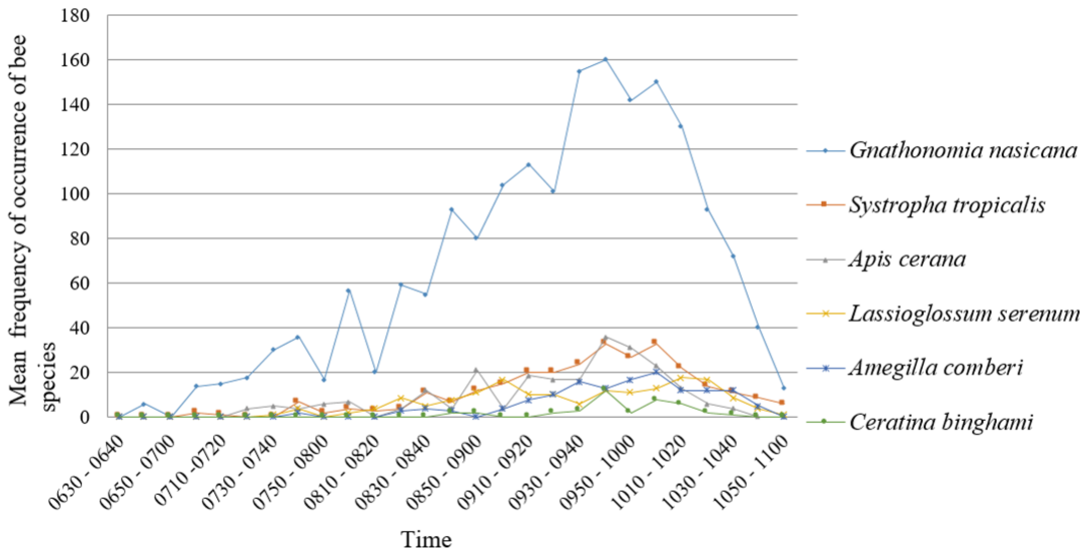

Figure 2: Mean frequency of occurrence of the six species of bees (*G. nasicana*, *A. cerana*, *S. tropicalis*, *L. serenum*, *A. comberi*, and *C. binghami*) that visited five *S. indicum* flowers during the fourteen sunny days during 10-minute intervals from 0630 h to 1100 h at Meewathura site.

0.11220 ± 0.09669 g, and 0.07958 ± 0.05619 g, respectively (Figure 3(b)). As shown in Figure 3(c), the average number of germinated seeds per pod from open flowers, covered flowers, and hand-pollinated flowers were 50%, 39%, and 27%, respectively. Furthermore, of the 4981 seeds formed from the open flowers, 95% were germinated. Of the 4482 seeds formed from covered flowers, 82% germinated, while 71% of the 3627 seeds formed from hand-pollinated flowers germinated.

The two-sample t-test performed for the number of seeds formed from flowers kept open for bee visits and flowers pollinated by hand, revealed a significant difference (p = 0.0001), as shown in Table 1. Similarly, a significant difference was observed in the number of seeds formed from flowers kept open for bee visits versus flowers kept closed for bee visits (p = 0.0001). Furthermore, the number of seeds formed from hand-pollinated flowers versus covered flowers also showed a significant difference (p = 0.0001).

There was a significant difference in the weight of seeds formed from open flowers vs hand-pollinated flowers and covered flowers vs hand-pollinated flowers (p = 0.0001), but no significant difference (p = 0.262) in the weight of seeds formed from open vs covered flowers. The quantity of germinated seeds produced by open flowers was much higher than that of hand-pollinated flowers, as indicated in Table 1. Similarly, the number of germinated seeds produced from the open flowers was significantly

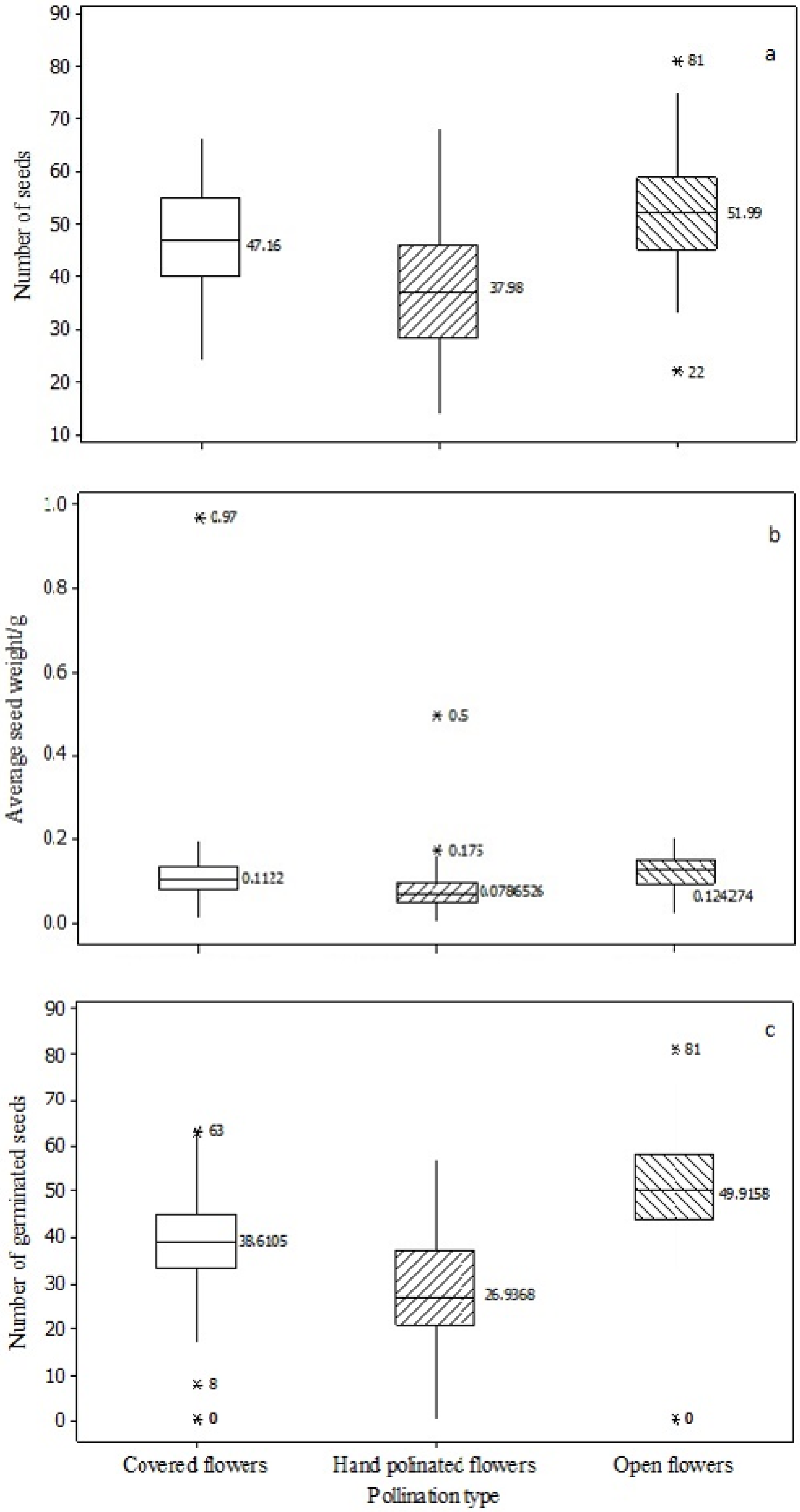

Figure 3: (a) Number of seeds produced per pod, **(b)** average weight of seeds produced per pod and **(c)** average number of germinated seeds produced per pod from open flowers, covered flowers, and hand-pollinated flowers of *S. indicum.*

Table 1: Comparison of the three pollination trials; open flowers, covered flowers, and hand-pollinated flowers of *S. indicum* in relation to seed set, seed weight, and seed germinability using a two-sample t-test.

	Seed set			Seed weight			Seed germinability		
	T-Value	P-Value	DF	T-Value	P-Value	DF	T-Value	P-Value	DF
Open vs. Hand pollinated flowers	9.27	0.0001	191	6.50	0.0001	168	12.32	0.0001	184
Open vs. Covered flowers	3.56	0.0001	197	1.13	0.262	124	6.67	0.0001	187
Hand pollinated covered flowers	6.10	0.0001	190	-6.10	0.0001	190	-6.41	0.0001	181

higher than that of covered flowers (p = 0.0001) while that of hand-pollinated flowers was also significantly higher than that of covered flowers (p = 0.0001).

DISCUSSION

In the present study, the findings of the period of stigma receptivity (from 0700 - 1100 h) and the time of anthesis (from 0640 - 0710 h) are in close agreement with Andrade *et al*. (2014). Andrade *et al*. (2014) indicated that pollination of *S. indicum* can occur at any time from anthesis to 10.00 h but is most effective from 0700 h to 0900 h. Abdel All *et al*. (1976) stated that the stigma of *S. indicum* was receptive before flower opening and remained viable for 24 h after anthesis. Yermanos (1980) reported that the receptivity of the stigma of *S. indicum* flowers was lost within 14 h after flower opening, while Free (1993) states that the stigma of *S. indicum* flowers becomes receptive two hours after flower opening. These variations may probably be due to the differences in environmental conditions in different study locations (Andrade *et al*., 2014).

The findings of the present study showed that *S. indicum* flowers attracted six species of bees. Among them, *G. nasicana* and *L. serenum* belong to the Halictidae family, while *A. cerana*, *A. comberi*, and *C. binghami* belong to the Apidae family. These observations corroborate those of McGregor (1976), Pashte and Shylesha (2013), and Kumar and Lenin (2000), all of whom found the same species of bees visiting *S. indicum* flowers. Studies conducted in different locations of Sri Lanka from 2001 to 2004 (Karunaratne, 2004; Karunaratne *et al*., 2005) listed 13 species of bees visiting flowers of *S. indicum,* mainly from the country's dry zone. Of them, the present study recorded only three bee species, *G. nasicana*, *A. cerana* and *A. comberi* to visit *S. indicum* flowers. This could be owing to the study site's unique environmental conditions, as well as the lack of cultivated and natural flora that could serve as food sources and nesting sites for bees. *Systropha tropicalis* was a new record to *S. indicum* flowers in Sri Lanka. Karunaratne and Edirisinghe (2006) reported 32 bee species in 11 genera from *S. radiatum* grew in the same study site, which has been altered with time due to the construction of several buildings and invasion by the tall grass, *Panicum maximum*. These alterations may have resulted in the reduction of the diversity of flowering weeds and bee nesting sites. All bee species collected pollen from *S. indicum*, while only *S. tropicalis* collected both nectar and pollen. According to Rahman (2014), honey bees are the primary visitors of *S. indicum* flowers, which attract various bees and other insects that feed on pollen or nectar.

The abundance of different bee species per sesame flower per day varied during the study period. Halictidae bees were the dominant flower visitors, of which *G. nasicana* was the dominant visitor while *C. binghami* was the rarest visitor. Enabling cross-pollination, the highest bee activity coincided with pollen availability and anther dehiscence. In a study conducted by Bhagawati *et al*. (2016), *A. cerana* was recorded as the most abundant bee species, while *Xylocopa leucothorax* was recorded as the rarest. Kamel *et al*. (2013) revealed *A. mellifera* and *Ceratina tarsata* as the dominant bee species of *S. indicum* flowers in Egypt. According to Sachdeva *et al*. (2003), *A. dorsata* was the dominant bee species of *S. indicum* flowers, and maximum bee activity was observed during 10.00 11.00 h in India.

In the present study, the flowers kept open may have pollinated through means of cross and self-pollination. The open flowers showed the highest seed set, seed weight, and germinability, which were significantly higher (p < 0.05) from the other two pollination trials indicating that the frequency of visits of bees is vital for a significant increase of yield and quality of seeds in sesame. Furthermore, seeds from open flowers germinated more readily (number of germinated seeds) than seeds from covered flowers and hand-pollinated flowers. Andrade *et al*. (2014) also revealed that the yield of sesame is high in Brazil when flowers are kept open for bee visits. The flowers that were kept covered may have produced seeds entirely due to self-pollination while the hand-pollinated flowers through both cross and self-pollination methods. The covered flowers produced the second-highest seed number, weight, and germinability per fruit. The lowest seed number, weight, and germinability per fruit in hand-pollinated flowers than in open flowers and covered flowers may be due to the damages that may have been caused to pollen grains and stigmas during their transfer using a fine paintbrush (Young and Young 1992).

According to Roubik (1992), seed number and seed germinability are key markers of plant reproductive success. A higher number of seed outputs increases the possibilities of a species' perpetuation, implying that bee pollination aids in improving *S. indicum* productivity in the study area. The covered flowers may have ensured the perpetuation of the species by self-pollination when no partners or cross-pollinating agents were present in the vicinity. This condition is more evident in species that inhabit harsh habitats with fewer biotic pollinators in the vicinity (Free, 1993). Though self-pollination is less advantageous in nature as it does not favor new genetic combinations, sesame is considered selecting self-pollination (Andrade *et al*., 2014). The present study highlights that self-pollination and cross-pollination mediated by bees have a greater potential to increase the yield of *S. indicum*.

CONCLUSION

According to the findings, bee pollination enhances the seed number, weight, and germinability compared to hand pollination and covered flowers in sesame at Meewathura farm. These findings highlight the importance of the management of bee pollination services in sesame and thereby recommend the conservation of bee populations in man-made habitats.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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