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Research article

Comparison of common green bottle flies (*Lucilia sericata* Meigen) and stingless bees (*Tetragonula laeviceps* Smith) as pollinating agents for imported true shallot (*Allium cepa* L.) seed production

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Abstract

Importance of the work: The pollination process for true shallot seeds (TSSs) is usually conducted based on high-cost hand pollination that could be replaced by insects.
Objectives: To investigate two insect species as possible replacements to hand pollination for TSS production.
Materials & Methods: Common green bottle flies (*Lucilia sericata* Meigen) and stingless bees (*Tetragonula laeviceps* Smith) were used as pollinating agents for shallot plants in cages. The applications consisted of 100 flies, 300 flies, 500 flies, 1 colony of stingless bees, hand pollination, open pollination and a control. Variables observed were insect activity, pollination success and seed quality.
Results: Green bottle flies visited more flowers (15.2 ± 4.5 flowers/min) and spent, significantly, more time on flowers (135 ± 46 s/flower) than stingless bees (5 ± 3 flowers/min and 81 ± 18 s/flower, respectively). The highest pollination success and seed quality values were recorded for the hand pollination group (61.91% and 1.22 g, respectively) though these were not significantly different from the results using 500 green bottle flies (60.56% and 1.09 g, respectively). However, the seeds produced using stingless bee pollination had a significantly higher germination rate.
Main finding: Green bottle flies and stingless bees could be applied (partially or totally) as a replacement for hand pollination for imported TSS production. However, due to the possible negative consequences of the mass production of green bottle flies, the application of stingless bees as pollinating agents for TSS production would be preferable.

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Introduction

Shallot (*Allium cepa*) is one of the main herbs utilized as traditional food and medicine and the main agricultural product for some regions in Indonesia although this plant is not native there (Sun et al., 2019). During 2015–2017, total shallot production, in Indonesia, increased from 1.3 million to 1.5 million (Badan Pusat Statistik, 2017). Increasing market demand adds more pressure to the demand for seedlings for cultivation. In Indonesia, shallot cultivation is highly dependent on the bulb as the source of the seedling, with the bulb having some weaknesses as a seedling source due to high disease susceptibility, high cost (as the seedling bulb replaces a bulb designated for consumption), low shelf life and uneven quality (Palupi et al., 2015). The alternative to overcome these weaknesses is true shallot seed.

Due to some problems with the quantity and quality of seed produced domestically, Indonesia imported about 1,190 t of shallot seeds costing about USD 1.15 (Badan Pusat Statistik, 2017). An increase in demand for seed and decreasing monetary exchange rates are causes for concern. Some studies have been conducted to produce seeds of local cultivars of shallot with varying success rates (Saidah et al., 2020).

Some of the biggest challenges in true shallot seed production are flower production and the success rate of pollination as shallot requires cross-pollination to produce desirable seed quantity and quality (Mayer and Lunden, 2001) and is highly dependent on pollinating agents because of the difference in maturity between male and female flowers (Currah and Proctor, 1990; Chandel et al., 2004), while the lack of cross-pollination impacts greatly on the seed production rate that can be lower than 10% (Ewies and El-Sahhar, 1977).

Other studies have shown considerable variation (20–70%) in the pollination success of local shallot cultivars; although the quality of produced local seeds was promising, the high variation of the seeds prevented large-scale production (Palupi et al., 2015; Saidah et al., 2020). This condition could relate to the variation in the genetic source and production regime. Thus, the shallot seeds available in the market are the seeds from imported cultivars.

In this study, we succeeded to produce the flowering of the imported cultivar in Indonesia by imitating the production condition of the origin, in South Africa, by applying low-temperature stress (a process called vernalization) and specific growth hormone to the bulb and human workforces were utilized for pollination. The main challenge for local shallot seed production is lowering the cost to an affordable level by local farmers. This study focused on the important cost production regarding pollination.

Shallot features small, unspecialized green, yellow, or white flowers in umbels, with a diverse assemblage of insects, including bees, flies, wasps and beetles, contributing to their pollination in the open field (Howlett et al., 2005; Rader et al., 2009; Gaffney et al., 2011). Studies on seed production of shallot cultivars showed the importance of insects for cross-pollination (Mayer and Ludgen, 2001; Saidah et al., 2020). Among these tropical insects, local honeybees (Family: Apidae), such as *Apis cerana* F., *Apis dorsata* F. and *Apis florea* F. and imported European honeybees (*Apis mellifera* L.) are the most dominant pollinator supplied by the surrounding agroecosystems (Jadhav, 1981; Sajjad et al., 2008; Gure et al., 2009; Hosamani et al., 2019).

However, consistently maintaining populations of these insects is difficult due to their large colony size. Furthermore, their aggressive behavior makes them undesirable in an enclosed production system. An alternative is to utilize insect species that are easy to handle, low in maintenance, less aggressive and locally available, such as green bottle flies and stingless bees. The common green bottle fly (*Lucilia sericata* Meigen), known as one of the major pollinators of shallot (Currah and Ockendon, 1984; Sajjad et al., 2008; Palupi et al., 2015), has a short life, can be produced in large numbers in indoor facilities and is applicable as an indoor pollinator (Cook et al., 2020). The stingless bee (*Tetragonula laeviceps* Smith) is known for its high pollination efficiency with small-sized flowers, low colony maintenance and less aggressive behavior (Putra and Kinasih, 2014; Putra et al., 2014; Putra et al., 2017; Ramadani et al., 2021). Both these genera have been in use as part of local shallot seed production and have shown promising results (Palupi et al., 2015). However, information is lacking regarding imported cultivars and there is a possibility of different responses due to interactions between non-native plants and native pollinators that may be positive (Matteson and Langellotto, 2010; Gibson et al., 2013) or negative (Menz et al., 2011). Based on these facts, the current study investigated the possibility of using either of these insects as a replacement for hand pollination.

Materials and Methods

Research location and period

Cultivation of shallot was conducted at the production laboratory of East West Indonesia, Lembang regency, West Java, Indonesia located more than 1,250 m above sea level. The study area was considered suitable for shallot seed production (Hilman et al., 2014). The average daily temperature of the study area was 16–20°C with relative humidity of 70–90%. The study was conducted during the dry season (April–July), consisting of a pollination study for 2 mth (April–May) and harvesting and post-harvesting activities (June–July).

Insects

The laboratory colonies of green bottle flies used in this study were obtained from the Toxicology Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia. The stingless bee species used in this study was *Tetragonula laeviceps* Smith which originated from a local stingless bee farm. Colonies of 200–500 bees were kept inside a bamboo tube. All colonies were acclimated for 3 wk before application and only replaced weekly during the study.

Pollination study

The shallot cultivar used in this study was the Lokananta cultivar, an imported cultivar of shallot sold as seed in Indonesia. Shallot plants were divided into seven groups, each consisting of 20 plants that were kept inside designated 1 m × 1 m × 1.2 m screen cages for the insect pollination and self-pollination groups (Fig. 1A).

The groups consisted of: 1) self-pollinated plants covered with a screen cage; 2) open pollinated plants not covered with a screen cage; (3) hand pollinated plants conducted by manual rubbing of the umbels; 4) 100 green bottle flies (GBF 100) on plants kept inside a screen cage that were restocked to the designated number of green bottle flies weekly; 5) 300 green bottle flies (GBF 300) on plants kept inside a screen cage and restocked to the designated number of green bottle flies weekly; 6) 500 green bottle flies (GBF 500) on plants kept inside a screen cage and restocked to the designated number of green bottle flies weekly; and 7) pollination using stingless bees on plants kept inside a screen cage and replaced with a new bee colony weekly. Four replications were used. The choice of the number of green bottle flies applied was based on the results of studying rearing techniques for the flies that showed 500 as the highest population for maintaining a healthy green bottle fly population in a 1 m × 1 m × 1.2 m screen cage (Putra, unpublished data). For the stingless bee colony, a photograph of the egg section area was taken and compared after 1 wk to decide whether the colony should be refreshed from a nearby bee farm, reused or replaced with a new colony. The replacement was done when new eggs were not produced.

Pollinator activity and efficiency

Pollinator activities were directly recorded by two observers. One observer was responsible for collecting the data and the other acted as the timekeeper. Data collection was conducted during 0800–1500 hours local time twice a week.

The observed variables were: 1) insect pollinator abundance: defined as the total number of insects visiting any umbel per plant within 1 min; 2) visitation rate: defined as the number of florets (Fig. 1B) visited during 60 s (Dafni, 1992) and this was applied to individuals of both the green bottle fly and stingless bee; 3) handling time: defined as the time spent by one insect at an umbel, measured in seconds per umbel (Gingras et al., 1999); and 4) pollination efficiency: measured by the percentage of flowers that produced capsules.

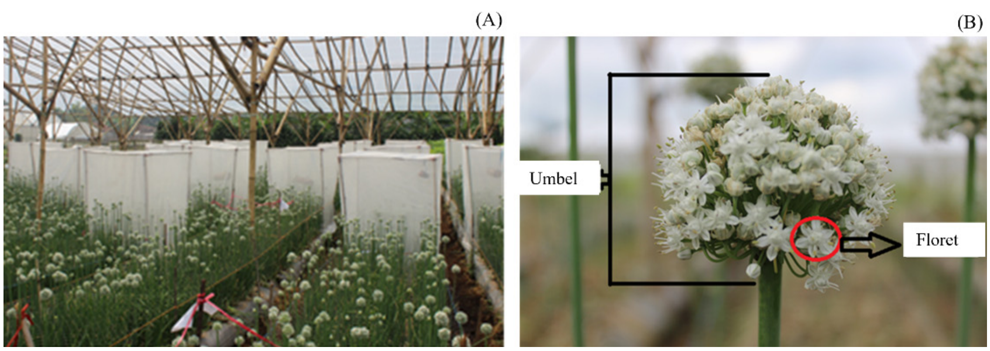

Fig. 1 (A) screen cage of pollination study; (B) components of shallot inflorescence

During observation, the temperature was recorded using a data logger to observe the possibility of temperature as a limiting factor for the application of insects as pollinating agents for TSS production.

Seed number and quality

Seeds were extracted from capsules produced from the pollination process using the inflorescence. The effect of the pollination treatment on seed production and quality was determined by calculating the total number of seeds, the weight of seeds per umbel, the weight of 100 seeds and the germination rate of the seeds.

Data analysis

Before analysis, data normality was analyzed based on a one-sample Kolmogorov-Smirnov test that indicated all the data were normally distributed. These data were analyzed using one-way analysis of variance, with Duncan's test as a *post hoc* test of significance at $p < 0.05$. Differences in handling time and foraging rate between the two species were analyzed using an independent t test at $p < 0.05$. All analyses were conducted using the SPSS 22.0 software (IBM Corp. Released, 2013). The correlation between temperature and foraging rate of both insects was analyzed based on Pearson's correlation conducted using the PAST 4.08 software (Hammer et al., 2001).

Result and Discussion

Pollinator visitation rate

The abundance of insects visiting flowers could affect the efficiency of pollination (Sajjad et al., 2008). In the current study, the significantly highest pollinator abundance was recorded for GBF 500. On the other hand, the average abundance of stingless bees on flowers was relatively high and similar to the abundance recorded for GBF 300, while GBF 100 had the lowest abundance (Table 1).

Only 5% of green bottle flies visited the flowers while the others spend their time on the screen surface. Large numbers of flies died during the study which may have been related to the environmental conditions and lack of energy sources for the flies. This condition was perceived as a major weakness of using green bottle flies as pollinators because many new replacement insects were required frequently.

On the other hand, no dead stingless bees were found during the study. Food reserves in the colony provided energy sources for forager bees that prevented the death of foragers. However, a symptom of colony deterioration (lower number of new eggs) was detected after 1 wk (personal observation).

Single pollinator activities

Observation on pollinator activity showed that a single green bottle fly had a significantly higher visitation rate and handling time than a single *T. laeviceps* (Table 2).

The higher visitation rate of single green bottle flies to shallot flowers in the current study agreed with Currah and Ockendon (1984). A higher visitation rate increases the possibility of flowers being pollinated. However, unlike the stingless bees, the visitation pattern of the green bottle flies was random and they showed a high preference for larger umbels.

A longer handling time was also recorded for green bottle flies, which could improve the probability of pollen being deposited on the surface of the insect body (Palupi et al., 2015). The length of flower-handling time could be related to time-dependent behavior such as nectar extraction (Harder, 1986; Mitchell and Waser, 1992), efficiency at flower manipulation (Ivey et al., 2003) and a possible preference to nectar. The short flower-handling time of the stingless bees at shallot flowers may have been related to the fact that the nectar of onion is high in potassium and becomes thick with increasing temperature which made it unfavorable for some bees species (Voss et al., 1999).

Table 1 Average number of insect pollinator individuals visiting umbels

Pollination treatment	Average visitation (individuals/plant/min)	% of total individuals
GBF 100	8.1±3.4^{A}	8.1
GBF 300	16.2±9.3^{B}	5.4
GBF 500	20.9±11.7^{C}	4.2
Stingless bees (one colony)	13.9±5.7^{B}	13.9

GBF = green bottle flies, with number indicating individuals used in pollination
Mean±SD superscripted with different uppercase letters are significantly ($p < 0.05$) different

Table 2 Pollinator activity of single individuals on umbels

Pollinator	Number of florets/ umbel/min	Handling time/ umbel (s)
Green bottle flies	15.2±4.5^{B}	134.5±45.9^{B}
Stingless bees	5.4±2.7^{A}	80.6±17.4^{A}

Mean±SD within each column superscripted with different uppercase letters are significantly ($p < 0.05$) different

Environment conditions and foraging rate

Both insect species showed a positive correlation with temperature though the correlation was much stronger and was significant for the green bottle flies (Pearson's correlation = 0.004) than for the stingless bees (Pearson's correlation = 0.07). Observation indicated that the stingless bees were less active at any temperature although they had a wider temperature range of activity than the green bottle flies (Fig. 2).

The green bottle flies tended to spend their time warming their bodies on the screen surface, while the stingless bees directly foraged. It seemed that the acclimatization of bees at lower temperatures helped the development of their foraging behavior at low temperatures. However, the low activity level of the stingless bees at low temperatures would be another challenge as most of the TSS farms are located at higher altitudes where low temperatures are common (Hilman et al., 2014).

Pollination efficiency

The pollination efficiency rates of hand pollination and from using 500 green bottle flies were significantly higher than for the other pollination regimes, with hand pollination producing better efficiency than GBF 500 but not by a significant difference. On the other hand, the effectiveness of stingless bee pollination was similar to the efficiency rates for GBF 100 and 300, with these population numbers being more likely to be produced in small rearing facilities that could be sustained and would have a low risk of conflict with surrounding human activities (Table 3).

The current results highlighted some important outcomes: 1) the lack of assisted pollination significantly reduced seed number (as reported for other onions by Woyke, 1981; Rao and Lazar, 1983; and Kumar et al., 1985); 2) there was lack of natural pollinators in the cultivation area that could explain the requirement for additional supplies of pollinating insects (Mayer and Lunden, 2001; Witter and Blochtein, 2003; Adel et al., 2013); 3) stingless bees could be used as an alternative to green bottle flies where using a large number of flies was impractical; 4) the efficiency of hand pollination could be matched by using a large number of green bottle flies that provided a high pollination intensity (high visitation rate and handling time) and reduced the adverse effect of limited pollen supplies (Ashman et al., 2004; Knight et al., 2005).

Number and weight of seeds per inflorescence

The number of seeds produced using hand pollination was the highest although it was not significantly different from the values using GBF 500 and GBF 300 or from using stingless bees (Table 4).

Table 3 Pollination efficiency of true shallot pollination using different treatments

Pollination treatment	Pollination efficiency (%)
Hand pollination	61.9 ± 10.4^{D}
GBF 500	60.6 ± 12.4^{D}
GBF 300	51.5 ± 13.8^{C}
GBF 100	48.3 ± 13.6^{C}
Stingless bees	49.4 ± 14.6^{C}
Open pollination	35.2 ± 11.2^{B}
Self-pollination	1.9 ± 1.7^{A}

GBF = green bottle flies, with number indicating individuals used in pollination
Mean±SD superscripted with different uppercase letters are significantly ($p < 0.05$) different

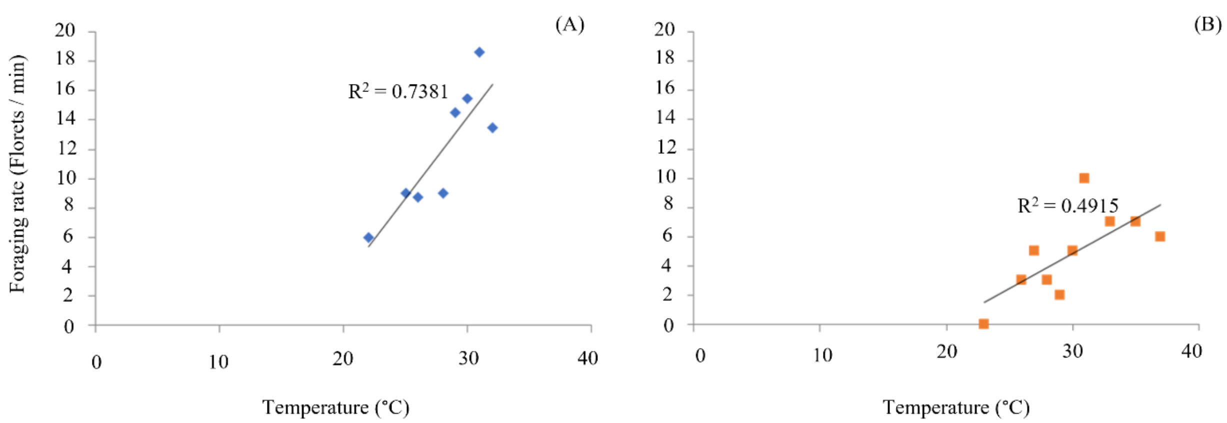

Fig. 2 Correlation between temperature and foraging rate of: (A) green bottle flies; (B) stingless bees

Table 4 Number of true shallot seeds per umbel and seed quality (seed weight per umbel, weight 0f 100 seeds and germination rate) produced using different pollination treatments

Pollination treatment	Number of seeds/umbel	Seed weight/umbel (g)	Weight of 100 seeds (g)	Germination rate (%)
Hand pollination	444.3±163.6^{C}	1.2±0.3^{D}	0.39±0.06^{A}	86.0±0.1^{B}
GBF 500	436.1±150.6^{BC}	1.1±0.1^{CD}	0.36±0.02^{A}	85.5±0.2^{B}
GBF 300	378.7±154.3^{BC}	0.8±0.1^{BC}	0.34±0.01^{A}	50.0±0.2^{E}
GBF 100	345.0±140.2^{B}	0.8±0.2^{B}	0.35±0.01^{A}	83.5±0.3^{B}
Stingless bees	392.9±135.9^{BC}	0.8±0.1^{BC}	0.36±0.03^{A}	89.5±0.1^{A}
Open pollination	355.1±139.9^{B}	0.6±0.02^{B}	0.36±0.02^{A}	82.0±0.2^{C}
Self-pollination	10.9±13.9^{A}	0.04±0.01^{A}	0.37±0.01^{A}	75.5±0.0^{D}

GBF = green bottle flies, with number indicating individuals used in pollination
Mean±SD superscripted with different uppercase letters are significantly ($p < 0.05$) different

Important results based on the data on the number of seeds per inflorescence were: 1) assisted pollination was required to produce true shallot seeds; 2) a high diversity of visiting insects may produce a similar number of seeds as from the application of a specific pollinating insect (Kamal and Akand, 2017); (3) although the number of seeds produced using hand pollination was the highest, there was variation in numbers among the replications that indicated inconsistency in pollination by hand.

On the other hand, the total weight of seeds produced using hand pollination was the highest although not significantly different to the GBF 500 group (Table 4). The current study also agreed with other studies that reported the importance of an abundance of pollination agents to improve the seed weight (Rao and Lazar, 1983; Kumar et al., 1989; Ahmad et al., 2003; Tolon and Duman, 2003; Adel et al., 2013).

Weight of 100 seeds

The weights of 100 seeds among pollination treatments in the current study (Table 4) were consistent with Kumar et al. (1985) and Palupi et al. (2015). Seed weight is a critical characteristic of the life history of a plant, which is widely used as an indicator of seed quality (Ellison, 2001; Li et al., 2015). The current results may have indicated that the weight of 100 seeds was more likely to be affected by agronomic and environmental factors, such as bulb size (Asaduzzaman et al., 2012), plant spacing (Haile et al., 2017), bulb planting time (Tesfaye et al., 2018) and soil fertility (Hossain et al., 2017) when pollinators are available (Adel et al., 2013).

Germination rate

The germination rate of seed produced by stingless bees was the highest, while the lowest was recorded for the GBF 300 group (Table 4). This result agreed with the previous study reported by Tolon and Duman (2003). In general, all true shallot flowers that received pollination assistance either by hand or insects produced germination rates higher than 80% (except for GBF 300 group), which is the minimum standard for commercialization (The Central Seed Certification Board, 2013).

On the other hand, the germination rates of the seed (50–89.5%) in the current study were better than the germination rates of local seeds (68–76%) produced using similar procedures nearby (Palupi et al., 2015). The differences in germination rates could have been due to genetic makeup (Hatzig et al., 2015; Sudha et al., 2019), storage method (Kirmizi et al., 2017), planting period (Tesfaye et al., 2018) or pollination intensity (Labouche et al., 2016). The low germination rate of GBF 300 was probably related to more flowers being produced through self-pollination than cross-pollination as one of the characteristics of pollination using flies (Currah and Ockendon, 1984) that needs to be confirmed by further studies.

The current results showed that stingless bees, in terms of seed quality, provided the best pollination service for seed fertilization compared to the other pollination regimes. Successful seed fertilization is usually related to a higher germination rate caused by perfect pollen numbers and deposition time on stigma. The low pollination intensity of the stingless bees might have been prevented by using a higher number of colonies although the health and wealth of colonies should be considered.

The current study investigated both flies and stingless bees as forced pollinators in much smaller screens than used in other studies (Currah and Ockendon, 1984; Palupi et al., 2015) and produced better seed germination rates which could have been caused by the limited space and alternative resources. The application of both these insects in natural conditions would be challenging due to the temperature-dependent behavior of both insects. Flies respond to the low temperature in the cultivation area by moving to another area to avoid extreme temperature (Currah and Ockendon, 1984). This temperature regulation may reduce the activities of flies regarding movement between flowers; frequent replenishment with large numbers of younger, active flies may counter this. On the other hand, bees are partially endothermic through greater control over their body temperature using their physiological and social behavior. However, this mechanism requires a large amount of energy from nectar. Bees probably will reduce visits to shallots in preference to other flowers having a greater energy content in their nectar.

In conclusion, both green bottle flies and stingless bees have potential as alternative pollinating agents for true shallot seed production. Stingless bees may be suitable as pollinators for seed production of non-native plants. On the other hand, the application of green bottle flies, which are relatively easier to produce and multiply than stingless bee colonies, should be investigated using a larger scale to further study the health, safety, environmental, economic and possible human conflict aspects, as this species is known as a vector of human diseases (Rahimi et al., 2021).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Adel, M.R., Namaghi, H.S., Hosseini, M. 2013. Effects of insect pollinators on onion seed production quality and quantity. J. Crop Prot. 2: 395–402.

Ahmad, S., Muhammad, T., Karim, A., Jabbar, A., Ahmad, G.H. 2003. Effect of pollinators and insecticides on seed setting of onion (*Allium cepa* L.). J. Biol. Sci. 3: 305–308.

Ashman, T.L., Knight, T.M., Steets, J.A., et al. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. Ecology 85: 2408–2421. doi.org/10.1890/03-8024

Asaduzzaman, M., Hasan, M.M., Hasan, M.M., Moniruzzaman, M., Howlander, M.H.K. 2012. Effect of bulb size and plant spacing on seed production of onion (*Allium cepa* L.). Bangladesh. J. Agric. Res. 37: 405–414.

Badan Pusat Statistik. 2017. Shallot production. Statistic Indonesia, Jakarta, Indonesia. https://www.bps.go.id/, 19 July 2017. [in Indonesian]

Chandel, R.S., Thakur, R.K., Bhardwaj, N.R., Pathania, N. 2004. Onion seed crop pollination: A missing dimension in mountain horticulture. Acta Hortic. 631: 79–86. doi: 10.17660/ActaHortic.2004.631.9

Cook, D.F., Voss, S.C., Finch, J.T.D., Rader, R.C., Cook, J.M, Spurr, C.J. 2020. The role of flies as pollinators of horticultural crops: An Australian case study with worldwide relevance. Insects 11: 341. doi.org/10.3390/insects11060341

Currah, L., Ockendon, D.J. 1984. Pollination activity by blowflies and honeybees on onions in breeders' cages. Ann. Appl. Biol. 105: 167–176. doi.org/10.1111/j.1744-7348.1984.tb02812.x

Currah L., Proctor, F.J. 1990. Onions in Tropical Regions, Vol. 35. Natural Resource Institute. Chatham, UK.

Dafni, A. 1992. Pollination Ecology: A Practical Approach. Oxford University Press. Oxford, UK.

Ellison, A.M. 2001. Interspecific and intraspecific variation in seed size and germination requirements of Sarracenia (Sarraceniaceae). Am. J. Bot. 88: 429–437.

Gaffney, A., Allen, G.R., Brown, P.H. 2011. Insect visitation to flowering hybrid carrot seed crops. N. Z. J. Crop Hortic. Sci. 39: 79–93. doi.org/10.1080/01140671.2010.526619

Gibson, M.R., Pauw, A., Richardson, D.M. 2013. Decreased insect visitation to a native species caused by an invasive tree in the Cape Floristic Region. Biol. Conserv. 157: 196–203. doi: 10.1016/j.biocon.2012.07.011

Gingras, D., Gingras, J., De Oliveira, D. 1999. Visits of honeybees (Hymenoptera: Apidae) and their effects on cucumber yields in the field. J. Econ. Entomol. 92: 435–438.

Ewies, M.A., El-Sahhar, K.F. 1977. Observations on the behaviour of honeybees on onion and their effects on seed yield. J. Apic. Res. 16: 194–196. doi: 10.1080/00218839.1977.11099886

Haile, A., Tesfaye, B., Worku, W. 2017. Seed yield of onion (*Allium cepa* L.) as affected by bulb size and intra-row spacing. Afr. J. Agric. Res. 12: 987–996. doi: 10.5897/AJAR2014.9249

Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4: 1–9.

Harder, L.D. 1986. Effects of nectar concentration and flower depth on flower handling efficiency of bumblebees. Oecologia 69: 309–315. doi.org/10.1007/BF00377639

Hatzig, S.V., Frisch, M., Breuer, F., et al. 2015. Genome-wide association mapping unravels the genetic control of seed germination and vigor in *Brassica napus*. Front. Plant Sci. 6: 221. doi.org/10.3389/fpls.2015.00221

Hilman, Y., Rosliani, R., Palupi, E.R. 2014. The effect of altitude on flowering, production, and quality of true shallot seed. J. Hort. 24: 154–161. [in Indonesian]

Hosamani, V., Reddy, M.S., Venkateshalu, Hanumanthaswamy, B.C., Lingamurthi, K.R., Ravikumar, B, Ashoka, N. 2019. Pollinator diversity, abundance and their stay time in onion, *Allium cepa* L. J. Entomol. Zool. Stud. 7: 158–161.

Hossain, M.M., Khatun, K., Haq, M.E., Ahmed, M., Al-Maruf, M.S. 2017. Macro and micro nutritional effect on seed yield of onion. Adv. Res. 12: 1–9.

Howlett, B.G., Donovan, B.J., McCallum, J.A., Newstrom, L.E., Teulon, D.A.J. 2005. Between and within-field variability of New Zealand indigenous flower visitors to onions. N. Z. Plant Protect. 58: 213–218. doi.org/10.30843/nzpp.2005.58.4275

IBM Corp. Released. 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corp. Armonk, NY, USA.

Ivey, C.T., Martinez, P., Wyatt, R. 2003. Variation in pollinator effectiveness in swamp milkweed *Asclepias incarnata* (Apocynaceae). Am. J. Bot. 90: 214–225. doi.org/10.3732/ajb.90.2.214

Jadhav, L.D. 1981. Role of insects in the pollination of onion (*Allium cepa* L) in the Maharastra state, India. Indian Bee J. 43: 61–63.

Kamal, M., Akand, M.M. 2017. Foraging activity of different pollinators for seed setting and maximizing seed yield of onion. J. Agric. Vet. Sci. 10: 88–96. doi: 10.9790/2380-1005018896

Kirmizi, S., Guleryuz, G., Arslan, H. 2017. Effects of environmental and storage condition on the germination of *Allium* species. Fresen. Environ. Bull. 26: 3470–3478.

Knight, T.M., Steets, J.A., Vamosi, J.C. et al. 2005. Pollen limitation of plant reproduction: pattern and process. Annu. Rev. Ecol. Evol. S. 36: 467–497. doi.org/10.1146/annurev.ecolsys.36.102403.115320

Kumar, J.R, Mishra, R.C., Gupta, J.K. 1985. The effect of mode of pollination on *Allium* species with observations on insects as pollinators. J. Apic. Res. 24: 62–66. doi.org/10.1080/00218839.1985.11100650

Kumar, J.R, Mishra R.C., Gupta, J.K. 1989. Effect of honey bee pollination on onion (*Allium cepa* L.) seed production. Indian Bee J. 51: 3–5.

Labouche, A.M., Richards, S.A., Pannell, J.R. 2016. Effects of pollination intensity on offspring number and quality in a wind-pollinated herb. J. Ecol. 105: 197–208. doi.org/10.1111/1365-2745.12659

Li, Z., Lu, W., Kong, X., Deng, X. 2015. Seed weight and germination behavior of the submerged plant *Potamogeton pectinatus* in the arid zone of northwest China. Ecol. Evol. 5: 1504–1512. doi.org/10.1002/ece3.1451

Matteson, K.C., Langellotto, G.A. 2010. Determinates of inner city butterfly and bee species richness. Urban Ecosyst. 13: 333–347. doi.org/10.1007/s11252-010-0122-y

Mayer, D.F., Lunden, J.D. 2001. Honey bee management and wild bees for pollination of hybrid onion seed. Acta Hortic. 561: 275–278. doi.org/10.17660/ActaHortic.2001.561.41

Menz, M.H.M., Phillips, R.D., Winfree, R., Kremen, C., Aizen, M.A., Johnson, S.D., Dixon, K.W. 2011. Reconnecting plants and pollinators: Challenges in the restoration of pollination mutualisms. Trends Plant Sci. 16: 4–12. doi.org/10.1016/j.tplants.2010.09.006

Mitchell, R.J., Waser, N.M. 1992. Adaptive significance of *Ipomopsis aggregata* nectar production: Pollination success of single flowers. Ecology 73: 633–638. doi.org/10.2307/1940769

Palupi, E.R., Rosliani, R., Hilman, Y. 2015. Increasing of true shallot seed production and quality by pollinator introduction. J. Hort. 25: 26–36. [in Indonesian]

Putra, R.E., Kinasih, I. 2014. Efficiency of local Indonesia honey bees (*Apis cerana* L.) and stingless bee (*Trigona iridipennis*) on tomato (*Lycopersicon esculentum* Mill.) pollination. Pak. J. Biol. Sci. 17: 86–91. doi: 10.3923/pjbs.2014.86.91

Putra, R.E., Permana, A.D., Kinasih, I. 2014. Application of Asiatic honey bees (*Apis cerana*) and stingless bees (*Trigona laeviceps*) as pollinator agents of hot pepper (*Capsicum annuum* L.) at local Indonesia farm system. Psyche 2014: 687979. DOI:10.1155/2014/687979

Putra, R.E., Subagio, J., Kinasih, I., Permana, A.D., Rosmiati M. 2017. Pattern of wild pollinator visitation and effect of inundation by *Trigona* (Tetragonula) *laeviceps* Smith colonies to pollination success of kabocha (*Cucurbita maxima*). J. Entomol. Indon. 14: 69. [in Indonesian]

Rader, R., Howlett, B.G., Cunningham, S.A., Westcott, D.A., Newstrom-Lloyd, L.E., Walker, M.K., Teulon, D.A.J., Edwards, W. 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. J. Appl. Ecol. 46: 1080–1087. doi.org/10.1111/j.1365-2664.2009.01700.x

Rahimi, S., Khamesipour, A., Akhavan, A.A., Rafinejad, J., Ahmadkhanih, R., Bakhtiyari, M., Veysi, A., Akbarzadeh, K. 2021. The leishmanicidal effect of *Lucilia sericata* larval saliva and hemolymph on *in vitro* Leishmania tropica. Parasites Vectors 14: 40 doi.org/10.1186/s13071-020-04543-y

Ramadani, R.F., Raffiudin, R., Ariyanti, N.S., Biagioni, S., Treanore, E., Behling, H. 2021. Stingless bee foraging behavior and pollen resource use in oil palm and rubber plantations in Sumatra. Jurnal Entomologi Indonesia 18: 81. doi.org/10.5994/jei.18.2.81 [in Indonesian]

Rao, G.M., Lazar, M. 1983. Studies on bee behavior and pollination in onion (*Allium cepa* L.). In: Proceedings of the 2nd International Conference on Apiculture in Tropical Climates. New Delhi, India, pp. 580–589.

Saidah, Wahyuni, A.N., Muchtar, Padang, I.S., Sutardi. 2020. The growth and yield performance of true shallot seed production in Central Sulawesi, Indonesia. Asian J. Agric. 4: 18–22. doi.org/10.13057/asianjagric/g040104

Sajjad, A., Saeed, S., Masood, A. 2008. Pollinator community of onion (*Allium cepa* L.) and its role in crop reproductive success. Pakistan. J. Zool. 40: 451–456.

Sudha, G.S., Ramesh, R., Sekharb, A.C., Krishna, T.S., Brambachari, P.V., Riazunnisa, K. 2019. Genetic diversity analysis of selected Onion (*Allium cepa* L.) germplasm using specific RAPD and ISSR polymorphism markers. Biocatal. Agric. Biotechnol. 17: 110–118. doi.org/10.1016/j.bcab.2018.11.007

Sun, W., Shahrajabian, M.H., Cheng, Q. 2019. The insight and survey on medicinal properties and nutritive components of Shallot. J. Med. Plant Res. 13: 452–457.

Tesfaye, M., Belew, D., Dessalegn, Y., Shumye, G. 2018. Effect of planting time on growth, yield components, seed yield, and quality of onion (*Allium cepa* L.) at Tehuledere district, northeastern Ethiopia. Agric & Food Secur. 7: 28. doi.org/10.1186/s40066-018-0178-0

The Central Seed Certification Board. 2013. Indian Minimum Seed Certification Standards. The Central Seed Certification Board, Department of Agriculture & Co-operation, Ministry of Agriculture, Government of India. New Delhi, India.

Tolon, B., Duman, Y. 2003. The effect of pollination by honeybee (*Apis mellifera*) on onion (*Allium cepa*) seed production and quality. In: The 38th Apimondia International Apicultural Congress. Ljubljana, Slovenia, pp. 554.

Voss, R.E., Murray, M., Bradford, K., Mayberry, K.S., Miller, I. 1999. Onion Seed Production in California. ANR Publication 8008. University of California Division of Agriculture and Natural Resources. Davis, CA, USA.

Witter, S., Blochtein, B. 2003. Effect of pollination by bees and other insects on the production of onion seeds. Pesqui. Agropecu. Bras. 38: 1399–1407.

Woyke, H.W. 1981. Some aspects of the role of the honeybee in onion seed production in Poland. Acta Hortic. 111: 91–98. doi.org/10.17660/ActaHortic.1981.111.12