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Pollen source foraging preference of honeybees (*Apis cerana*) in Endane biodiversity corridor of Sinharajah Forest Reserve, Sri Lanka

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ABSTRACT

Identifying the most preferred foraging plants of honeybees is a prerequisite to harvest year-round bee honey production. This study evaluated the foraging preference of pollen sources by honeybees in the Endane Biodiversity Corridor. Pollens of different plants in the study site were collected, microscopically analyzed by preparing slides. The pollen abundance of different pollen bread samples from six colonies were examined using a haemocytometer. Pollens of 54 flowering plant species belonging to 28 families were used to prepare reference slides, and among them, pollens belong to 36 plant species from 22 families were observed in collected pollen bread samples. According to the pollen analysis by Poisson regression, the abundance of pollens was significantly different ($p<0.0001$) among the bee colonies. *Memecylon umbellatum*, *Gliricidia sapium*, *Trema orientalis*, *Spondias dulcis*, *Symplocos cohinchinensis*, *Cocos nucifera*, *Elaeocarpus serratus*, and *Alstonia macrophylla* were identified as the primary pollen sources for honeybees in the study area. In addition to the flowering plants, weeds are also preferred by the honeybees for the pollen requirement.

Keywords: Honeybee abundance, Foraging preference, Pollen sources.

INTRODUCTION

Honeybees are considered one of the invaluable creatures of nature that nurture pollination and bee honey production. The nectar and pollen are collected from different flowering plants to fulfil their food growth and development requirements. Nectar provides carbohydrates and trace amounts of vitamins, minerals and amino acids (Ball, 2007). Pollens serve varying amounts of amino acids, lipids, vitamins, and minerals essential for the larval development of honeybees (Stanley and Linskens, 1974). Generally, foraging bees visit flowering plants, collect pollens and transport them to the beehive. Then other younger bees pack the pollen loads into honeycomb cells while incorporating honey, digestive enzymes and organic acids to prevent spoiling of pollens (Gillium, 1979; Fuenmayor et al., 2014; Deveza et al., 2015). These stored pollen loads are fermented by different lactic acid bacteria under anaerobic conditions and transferred into bee bread that can be used to determine the pollen sources preferred by the honeybees (DeGrandi–Hoffman et al., 2013; Kieliszek et al., 2017).

Apiculture is developing as an agro-horticultural and forest-based village industry with limited investment. The quality and quantity of honey produced by honeybees mainly depend on the abundance and occurrence of pollen and nectar sources within the surrounding area of an apiary (Free, 1980; Shubharani et al., 2013). However, the availability of these sources fluctuates due to different factors as distribution of foraging plants, flowering pattern, topography, climate (Free, 1993). Therefore, every region possesses its floral dearth periods and honey flow with short or long duration. Bees prefer to forage close to the hive and do not forage over long distances staying within a kilometre of the hive (Kevan, 1984).

Moreover, they do not depend on all flowering plants for nectar and pollen. Maintaining a great diversity of preferred flowers with significant flowering patterns leads to high pollen abundance for foragers. Hence, knowledge of bee flora is a positive impact on the effective management of good beekeeping areas.

The proximity of study area is closer to the Sinharaja Forest Reserve in Sri Lanka, endowed with biodiversity with both fauna and flora. Beekeeping is one of the promising enterprises in a nearby village, and these home gardens located within this area have a variety of flowers that are effectively utilized for sustainable beekeeping. Proper identification of the foraging plants of honeybees is essential to improve the year-round honey production in beekeeping industries. However, the information on the foraging behaviour of bees is limited under local conditions. This study intends to provide reference information on potential foraging resources for honeybees in Sri Lanka.

METHODOLOGY

Study area: This study was undertaken during the January-April months in 2021, in Uda Delvala village, where located between Walankanda Forest Reserve and Iharakanda proposed Forest Reserve that fall within the

Greater Sinharaja Forest Reserve in Ratnapura district of Sri Lanka (6° 32' 48" N, 80° 32' 03" E). Mean annual temperature ranges between 18-27 °C while mean annual rainfall ranges between 2500-3500 mm in this area (Punyawardena et al., 1999; Geekiyanage et al., 2018). The study site surrounding landscapes are dominated mainly by marginal tea lands, shrublands, woodlands and home-gardens. All the flowering plants, including weeds and their flowering pattern, were recorded during the study period, and mature flowers were collected from these plants. Pollen identification and analysis were carried out in the Plant Science laboratory, Faculty of Agriculture, Rajarata University of Sri Lanka.

Pollen identification: Pollen analysis was carried out by preparing reference slides from all the collected flowering plant specimens according to the method described by Traverse (2007) with slight modifications. The collected mature flowers were preserved in 70% alcohol for further investigation. The mature pollen grains were scraped from the anthers of preserved flowers and rinsed with isopropanol solution for few minutes to remove the oil film from pollen grains. The pollen grains were transferred to a glass slide and warmed gently on a hot plate for two minutes to dry. After cooling, the pollens were stained with safranin. Few drops of 50% glycerine solution were added to the specimen to hydrate and swell the pollens for a better view. The slides were covered with coverslips and studied under a compound light microscope (Olympus SZH52, China) in 400× magnification.

Pollen analysis: Pollen bread samples were directly collected from domesticated six honeybee hives of *Apis cerana* colonies located in the studying site. Serial dilutions of pollen bread were prepared using distilled water. A drop of safranin stain was added to each dilution and mixed using a vortex. Pollen quantification was carried out using a haemocytometer. The presence/absence and density of different pollens types were evaluated according to the size, shape, surface structure, and symmetry of the reference pollen slides. The abundance of identified different pollens in bee bread samples was analyzed using Poisson regression. SAS ver. 9.0 statistical software was used for the data analysis.

RESULTS AND DISCUSSIONS

About 54 flowering plant species belonging to 28 families (Table 1) were identified as the most abundant flowering plants in the study area, and they were used to prepare the reference slides of pollens shown in Figure 1. Only pollens belonging to 36 plant species from 22 families were observed in collected pollen bread samples. Therefore, it is clear that the honeybees highly prefer these pollens, and they do not obtain pollens from all the flowering plants available in the area. These results agree with the previous literature that polylectic species, such as honeybees, focus on few plant species and do not collect pollen from all the plant species (Dimou and Thrasyvoulou, 2007; Bauma, 2011). Because, nutrition composition and appearance of pollens differ according to the plant species and honeybees may consider different factors during foraging of the pollens (Roulston and Cane, 2000). Mainly, *Memecylon umbellatum*, *Gliricidia sapium*, *Trema orientalis*, *Spondias dulcis*, *Symplocos cohinchinensis*, *Cocos nucifera*, *Elaeocarpus serratus*, and *Alstonia macrophylla* plant species had higher pollen abundance, and thereby they could be identified as the major pollen sources for honeybees in the study area. Variation of pollen abundance: number of one type of pollens available in one mL of bee bread samples is shown in Figure 2.

Moreover, the variation of total pollen density among different six honeybee colonies is shown in Figure 3. Both parameters (total pollen density and pollen abundance) of bee bread samples collected from the six honeybee colonies were significantly different ($p<0.0001$). *Memecylon umbellatum* ('kora-kaha') and *Cocos nucifera* (coconut) pollens were recorded in all the colonies' bee bread samples. Therefore, 'kora-kaha' and coconut plants can be identified as the most attractive foraging plants. Because of that, the establishment of apiaries in coconut plantations will encourage great honey production and enable to gain additional income to the growers. In addition to that, forests and cultivated fields where already have foraging plants can be promoted as potential beekeeping areas. The bee bread samples of the fourth colony had the highest pollen diversity with 32 pollen types, followed by the fifth colony. The sixth colony had the lowest pollen diversity, with only eight pollen types. In the results of total pollen density (Figure 3), the third honeybee colony had the highest total pollen count (5.178×105 pollens) in one mL of bee bread sample. These pollen counts reveal how much pollens are used by honeybees to prepare bee bread. During the study period, all colonies were in good condition. Good and adequate pollen sources may be a reason for such good colony performance because lack of pollens leads to colony losses, poor physiological conditions and vulnerability to external threats at the individual level (Keller et al., 2005). Previous studies have suggested that a multiflora diet may build greater immune capacity in bees than a monofloral diet (Alaux et al., 2010). However, various bee species are able to overcome the effects of toxins or protective compounds present in flowers and digest different pollen types (Sedivy et al., 2011; Di Pasquale et al., 2013).

Although the observed reference pollen types had different sizes, shapes and ornamentations, plant species having higher pollen density had quite similar shape pollens and different sized pollen grains (Figure 1); means pollens having such shapes are highly preferred by the honeybees. Therefore, it can be suggested that the grain size and shape may impact the foraging behaviour. Further, previous studies have reported that the grain size and shape of the pollens are influenced to maximize the packing efficiency that means the manner of grain packing into their pollen baskets located on legs (Vaissiere and Vinson, 1994; Pernal and Currie, 2002; Lunau et al., 2015).

Table 1: List of plants used to prepare pollen reference slides

	Flowering plant		
No.	**Common name**	**Botanical name**	**Family**
1	Acasia	*Acacia melanoxylon*	Leguminosae
2	Ambarella	*Spondias dulcis*	Anacardiaceae
3	Ambilla	*Oxalis barrelieri*	Oxalidaceae
4	Ambul ambiliya	*Oxalis corniculata*	Oxalidaceae
5	Ashoka	*Saraca asoca*	Fabaceae
6	Avocado/Aligatapera	*Persea americana*	Lauraceae
7	Bo-kera/Go-kera	*Gomphia serrata*	Ochnaceae
8	Bombu	*Symplocos cochinchinensis*	Symplocaceae
9	Bovitiya	*Clidemia hirta*	Melastomataceae
10	Calianda	*Calliandra guildingii*	Fabaceae
11	Cashew/Kaju	*Anacardium occidentale*	Anacardiaceae
12	Cinnamon/Kurundu	*Cinnamomum verum*	Lauraceae
13	Coconut	*Cocos nucifera*	Arecaceae
14	Coffee	*Coffea arabica*	Rubiaceae
15	Dan	*Syzygium caryophyllatum*	Myrtaceae
16	Divi kaduru	*Pagiantha dichotoma*	Apocynaceae
17	Erabadu	*Erythrina variegata*	Fabaceae
18	Gadumba	*Trema orientalis*	Ulmaceae
19	Gandapana	*Lantana camara*	Verbenaceae
20	Ginikuru	*Alstonia macrophylla*	Apocynaceae
21	Girapala	*Commelina diffusa*	Commelinaceae
22	Guava/Pera	*Psidium guajava*	Myrtaceae
23	Heen bovitiya	*Osbeckia octandra*	Melastomataceae
24	Heen eraminiya	*Ziziphus oenoplia*	Rhamnaceae
25	Hulanthala	*Ageratum conyzoides*	Asteraceae
26	Jambola	*Citrus maxima*	Rutaceae
27	Kadupahara	*Crassocephalum crepidioides*	Asteraceae
28	Kaha karabu	*Sphagneticola trilobata*	Asteraceae
29	Kappetiya	*Croton laccifer*	Euphorbiaceae
30	Karalheba	*Achyranthes aspera*	Amaranthaceae
31	King coconut	*Cocos nucifera*	Arecaceae
32	Kiri madu / Maha madu	*Merremia umbellata*	Convolvulaceae
33	Kora-kaha	*Memecylon umbellatum*	Melastomataceae
34	Lemon	*Citrus limon*	Rutaceae
35	Lunumidella	*Melia azedarach*	Meliaceae
36	Ma dan	*Syzigium cumini*	Myrtaceae
37	Mango/Amba	*Mangifera indica*	Anacardiaceae
38	Monarakudumbiya	*Vernonia cinerea*	Asteraceae
39	Naminan	*Cynometra cauliflora*	Fabaceae
40	Nidikumba	*Mimosa pudica*	Fabaceae
41	Niyangala	*Gloriosa superba*	Colchicaceae
42	Peni thora	*Cassia occidentalis*	Fabaceae
43	Pinna	*Clerodendrum infortunatum*	Verbenaceae
44	Pomegranate/Delum	*Punica granatum*	Punicaceae
45	Rath keliya	*Litsea longifolia*	Lauraceae
46	Rubber	*Hevea brasiliensis*	Euphorbiaceae
47	Tea	*Camellia sinensis*	Theaceae
48	Wal Rubber	*Euphorbia heterophylla*	Euphorbiaceae
49	Wal te kola	*Bidens pilosa*	Asteraceae
50	Walsooriyakantha	*Tithonia diversifolia*	Asteraceae
51	Wathupalu	*Mikania micrantha*	Asteraceae
52	Weralu	*Elaeocarpus serratus*	Elaeocarpaceae
53	Wetamara	*Gliricidia sepium*	Fabaceae
54	Wild orchid / Heen binthal	*Curculigo orchioides*	Hypoxidaceae

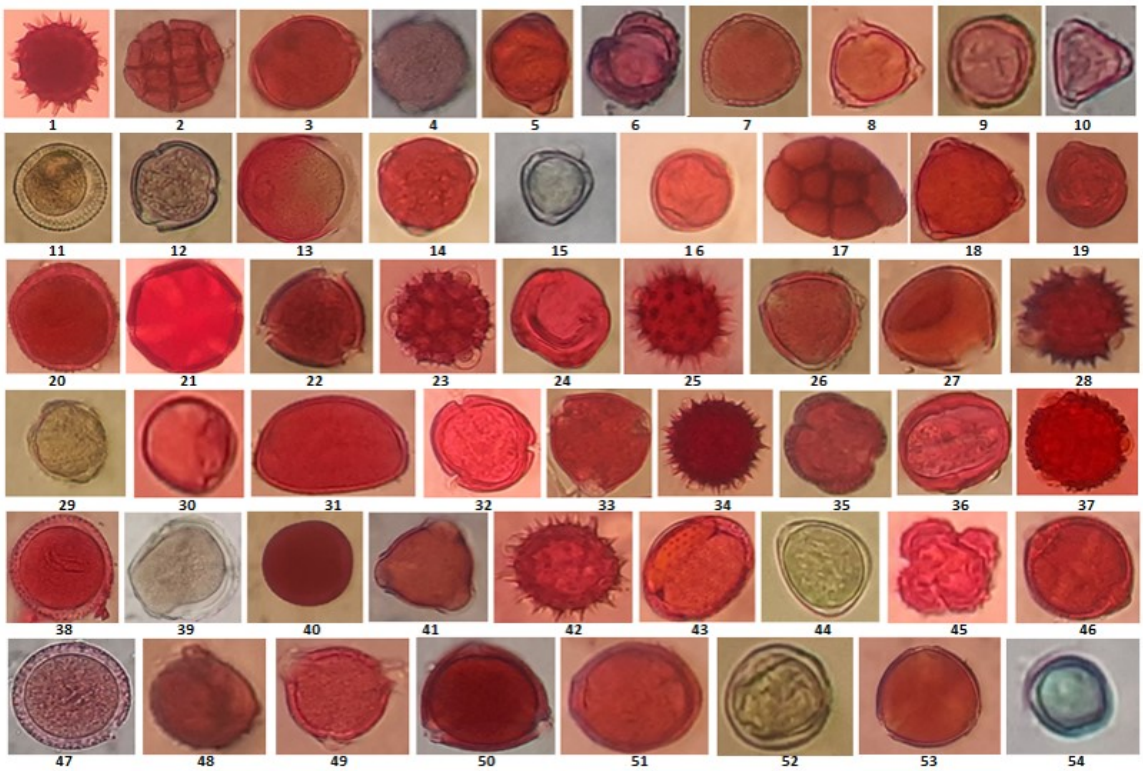

Figure 1: Morphology of pollens in different plant species under the compound light microscope (×400)

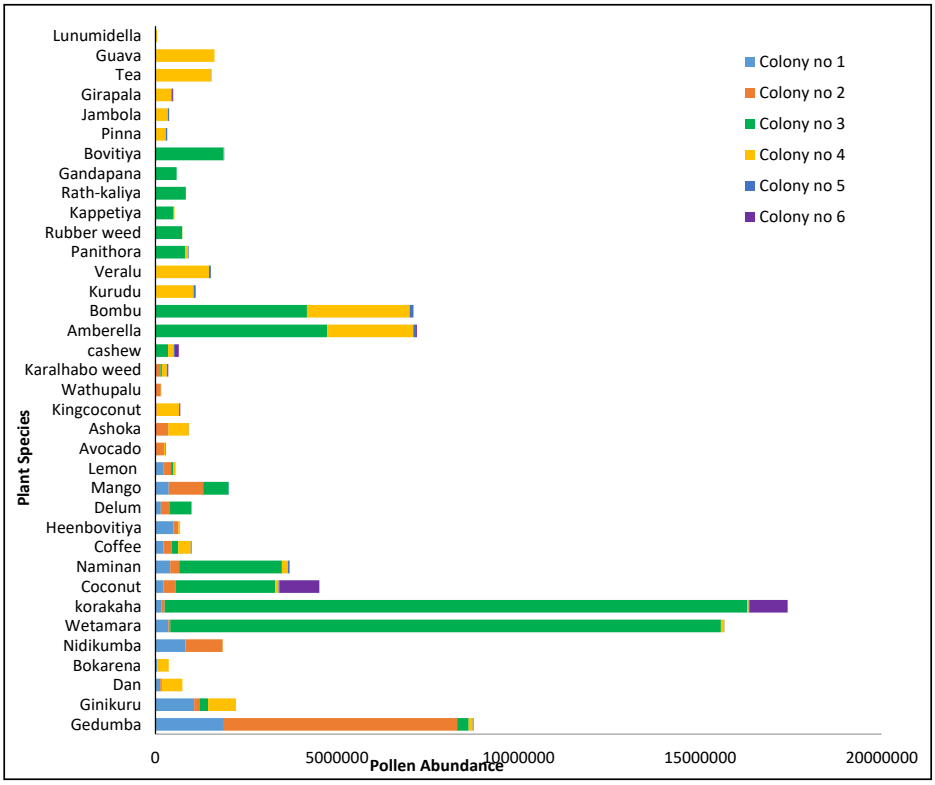

Figure 2: Graphical illustration of pollen type abundance in different honeybee colonies (number of one type pollens per one mL of bee bread sample)

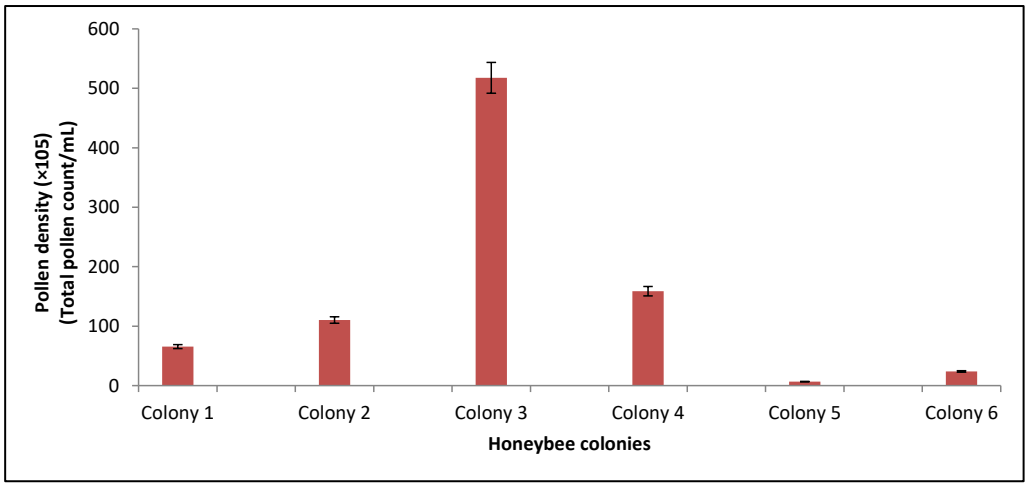

Figure 3: Graphical illustration of total pollen density in pollen bread samples of different colonies

Relatively low numbers of plants were in the blooming stage in the study area as it was a dearth period. Bhalchandra et al. (2014) reported that in the dearth period, weeds and wild flowering plants are used by honeybees as alternative food sources when agro-horticultural plants are not in the blooming stage. The highest flowering could be observed in plants such as *Symplocos cohinchinensis*, *Camellia sinensis*, *Litsea longifolia*, *Melia azedarach*, *Mallotus tetracoccus*, *Cinnamomum verum*, *Gliricidia sapium*, *Acacia melanoxylon*, *Cocos nucifera*, *Coffea arabica*, *Psidium guajava*, *Elaeocarpus serratus*, *Persea americana*, *Mangifera indica*, and *Syzygium caryophyllatum* in the study area during studied period. In addition, *Lantana camera*, *Osbeckia octandra*, *Chromalaena odora*, *Mikania micrantha*, *Ageratum conyzoides*, and *Mimosa pudika* like weeds had highest flowering. The results include *Senna tora*, *Mimosa pudika*, *Achyranthus aspera*, *Croton laccifer*, *Clidema hirta*, *Commelina diffusa*, *Mikania micrantha*, *Euphorbia heterophylla*, *Lantana camara*, *Clerodebdrum infortunatum*, and *Osbeckia octandra*, weed pollens were observed in the bee bread samples. Then, it is clear that honeybees collect pollens not only from the flowering plants, but from the weeds also. Therefore, keeping some weeds around the apiaries is crucial as they fulfil the pollen requirement of honeybees. Further, weed growth in the fields and the surrounding area would indirectly facilitate crop plant pollination because honeybees are considered the primary pollinators. Adequate pollen resources keep bee colonies in good condition, which directly influence honey production. Pollen analysis is revealed that this region has good potential for the development of beekeeping activities due to the presence of flora preferred by the honeybees, and it will support continuing beekeeping throughout the year. However, further investigations are required to get precise conclusions on the foraging preference of honeybees. Because of different factors like floral characters, the chemical composition of pollens may affect the decision making of foraging bees. This study provides the reference information of pollen grains of selected bee forage plants and beekeeping potential in the area.

CONCLUSION

The results of this study confirm the preference of honeybees for multi-floral pollen sources. At the same time, honeybees do not depend on all the foraging plants in the surrounding area to fulfil their nutrient requirement. It is advisable to establish the resulted primary pollen sources around the apiaries to ensure year-round honey production. Weeds also play a significant role as alternative pollen sources for honeybees. Therefore, it is crucial to maintain resulted plants and weeds surrounding the area of apiaries and discover more pollen sources and factors that affected the foraging preference to maintain sustainable beekeeping in Sri Lanka.

REFERENCES

Alaux, C., Ducloz, F., Crauser, D. and Le Conte, Y. (2010) Diet effects on honeybee immunocompetence. *Biology letters*, 6(4): 562-565.

Ball, D.W. (2007) The chemical composition of honey. *J. Chem. Educ.*, 84: 1643–1647.

Bauma, K.A., Rubink, W.L., Coulson, R.N. and Bryant, J.R.V.M. (2011) Diurnal patterns of pollen collection by feral honey bee colonies in southern Texas, USA. *Palynology*, 35(1): 85–93.

Bhalchandra, W., Baviskar, R.K., and Nikam, T.B. (2014) Diversity of nectariferous and polleniferous bee flora at Anjaneri and Dugarwadi hills of Western Ghats of Nasik district (M.S.). *Indian Journal of Entomology and Zoology Studies*, 2(4): 244-249.

DeGrandi-Hoffman, G., Chen, Y. and Simonds, R. (2013) The effects of pesticides on queen rearing and virus titers in honey bees (Apis mellifera L.). *Insects*, 4(1), pp.71-89.

Deveza, M.V., Keller, K.M., Lorenzon, M.C.A., Nunes, L.M.T., Sales, É.O. and Barth, O.M. (2015) Mycotoxicological and palynological profiles of commercial brands of dried bee pollen. *Brazilian Journal of Microbiology*, 46(4): 1171-1176.

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L.P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.L. and Alaux, C. (2013) Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter?. *PLoSONE*, 8, e72016.

Dimou, M. and Thrasyvoulou, A. (2007) Seasonal variation in vegetation and pollen collected by honey bees in Thessaloniki, Greece. *Grana*, 46(4): 292-299.

Free, J.B. (1980) Research and training in beekeeping development programme. *Proceedings of the 2nd International in Tropical Climates*, New Delhi, 10-15pp.

Free, J.B. (1993) *Insect pollination of crops*. Ed. 2. Academic press.

Fuenmayor, B.C., Zuluaga, D.C., Díaz, M.C., Quicazán de, C.M., Cosio, M. and Mannino, S. (2014) Evaluation of the physicochemical and functional properties of Colombian bee pollen. *Revista MVZ Córdoba*, 19(1): 4003-4014.

Geekiyanage, N. and Rathnayake, S. (2018) *Tree Flora and Soils in Proposed Endane Biodiversity Corridor Connecting Walankanda and Iharakanda Forest Reserves in the 40 Lowland Rainforest Region of Sri Lanka*, Dilmah Conservation.

Keller, I., Fluri, P. and Imdorf, A. (2005) Pollen nutrition and colony development in honey bees: part 1. *Bee world*, 86(1), pp.3-10. Kevan, P. G. (1984) Insects on flowers. Ecological entomology, 608-631.

Kieliszek, M., Piwowarek, K., Kot, A.M., Błażejak, S., Chlebowska-Śmigiel, A. and Wolska, I. (2018) Pollen and bee bread as new health-oriented products: A review. *Trends in Food Science & Technology*, 71: 170-180.

Lunau, K., Piorek, V., Krohn, O. and Pacini, E. (2015) Just spines–mechanical defense of malvaceous pollen against collection by corbiculate bees. *Apidologie*, 46: 144–149.

Pernal, S.F. and Currie, R.W. (2002) Discrimination and preferences for pollen-based cues by foraging honey bees, Apis mellifera L. *Animal Behaviour*, 63: 369–390.

Punyawardena, B. V. R. and Cherry, N. J. (1999) Assessment of predictability of seasonal rainfall in Rathnapura using the Southern oscillation and its two extremes. *J. Natn. Sci. Foundation Sri Lanka*, 27(3): 187–195.

Roulston, T.H. and Cane, J.H. (2000) Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*. 222: 187–209.

Sedivy, C., Muller, A. and Dorn, S. (2011) Closely related pollen generalist € bees differ in their ability to develop on the same pollen diet: evidence for physiological adaptations to digest pollen. *Functional Ecology*, 25: 718–725.

Shubharani, R., Roopa, P. and Sivaram, V. (2013) Pollen morphology of selected bee forage plants. *Global journal of bio science and biotechnology*. 2 (1): 82-90.

Stanley, R.G. and Linskens, H.F. (1974) Biology Biochemistry Management. Berlin: Heidelberg. Traverse, A. (2007) Palynological laboratory techniques. In Paleopalynology, Second Edition. *Topics in Geobiology Series*. Springer-Verlag New York. 28: 615-772.

Vaissiere, B.E. and Vinson, S.B. (1994) Pollen morphology and its effect on pollen collection by honey bees, Apis mellifera L. (Hymenoptera, Apidae), with special reference to upland cotton, Gossypium hirsutum L. (Malvaceae). *Grana*, 33: 128–138.