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Original Article

Bio-risk in stingless bee honey: an assessment of microbial air quality surrounding meliponiculture farm with IMA standard at Marang and Kuala Nerus, Terengganu, Malaysia

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Abstract

Bacterial contamination in a product of stingless bee is a major risk factor for the increased incidence of honey contamination by pathogenic bacteria. The main aim of the study was to compare microbial air contamination in the different meliponiculture farm by settling plate method. In this study, the quality of air in the form of bacterial load in meliponiculture site was monitored. Two meliponiculture farms were studied weekly for a month and were divided into two factors; i) radius distance from the hive, and ii) time of harvesting. There was a comparable amount of bacterial load measured between both farms. Range of index of microbial air contamination (IMA) value of Farm I and Farm II were around 26-50 and above 76, indicating fair and poor performance of its air quality respectively. Thus, the location of meliponiculture farms that located near to the road and construction site could pose a threat to the stingless bee product by its airborne-risk.

Keywords: Settle plate method, Index of microbial air contamination, Pathogenic bacteria, Meliponiculture

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Introduction

Bioaerosols are airborne particles that originate from biological sources including animals, plants, fungi, bacteria, protozoa, and viruses (Lindsey et al., 2017). This airborne-risk particle is ubiquitous and can be isolated from indoor and outdoor using a variety of methods that either enumerate viably or a collection of viable and non-viable bioaerosols. In the outdoor environment, bacteria mainly come from water, soil and plants and are associated with the presence of humans and animals. Bodies of water can dissipate bacteria into the air by aerosolization, just like the emissions from certain industrial processes and cooling units. Bacteria come mainly from the occupants because bacteria make up the natural flora of the skin and mucous membranes.

Inside the indoor environment, the species are more numerous, and the concentrations are above those of the outdoor environment. Some workplaces such as barns, breeding farms, waste and wastewater treatment

plants, and food plants facilities are themselves conducive to the presence and growth of bacteria. This type of environment is where Gram-negative bacteria are more likely to be measured. The majority of bacteria naturally present do not cause adverse health effects. Some bacteria are even essential to both the human body and the environment. Health risks appear when the concentrations of some species become abnormally high. High concentrations of thermoactinomycetes bacteria may cause hypersensitivity pneumonitis such as farmer's lung (Goyer et al., 2001).

Several studies have identified human activities like talking, sneezing and coughing (Kalogeraskis et al., 2005), while other human activities such as vehicular transportation and human movements, washing in homes and business centres, flushing of toilets and sewage, sweeping of floors and roadsides can generate bioaerosols indirectly (Kalogeraskis et al., 2005; Chen and Hildermann, 2009). Since microorganisms can lodge in/on dust particles, dust, therefore, is a potential source of bioaerosols.

Despite of huge attention towards worker respiratory health, particularly in microbial contaminated environments (Eduard et al., 2012; Nazaroff, 2016), bioaerosol assessment also suggested to be measure in-line with other active tool for quality assurance and all the programs of the Good Manufacturing Practices. According to Kornacki (2014), it is prudent to monitor airborne microbial populations for hygienic indicators and take appropriate corrective actions when exceeding acceptable levels.

Honey easily gets contaminated during the process of its production by bees and activities of man including equipment, containers, wind and dust (Olaitan et al., 2007). For this reason, meliponiculture farm assessment on environmental control procedures can be effective tools in reducing the risk of pathogenic bacteria occurrence in stingless bee honey, pollen and propolis. Evaluation of the level of air microbial contamination of meliponiculture farm is considered to be a basic step towards prevention huge complication on consumer health.

Air samples can be collected in two ways: 1) by active air sampler. 2) By passive air sampling (settle plates). In this study, passive air sampling was performed using settle plate method. It is a simple and inexpensive, economical readily available method. Petri dishes containing a solid nutrient medium are left open to air for a given period of time. Microbes carried by inert particles fall onto the surface of the nutrient under the influence of gravity, with an average deposition rate of 0.46cm/s being. Settle plates reflect the bacterial load nearest the sampling location without creating any turmoil (Whyte et al., 2016).

The aim of the study was to estimate microbial air contamination of meliponiculture farm by settle plate method (passive sampling). And the objectives include monitoring the quality of air in the form of bacterial load which aligns to the IMA standard.

Material and Methods

This study was conducted in an apiary of Marang (Farm I) and apiary of Kuala Nerus (Farm II), in the Terengganu state (Figure 1). The detail characteristics of the farm were stated in Table 1. The apiary was selected as there were active stingless beekeeping and honey production activities by the local people. Approximately 38 colonies of stingless bees (*Trigona itama*, *Trigona thoracica* and other species) were reared there.

Table-1: Characteristics of the meliponiculture farm.

Characteristics	Farm I	Farm II
Location	Hilly area, forest	Hilly area, village
Nearby factor	Large vegetable farm, unexplored land	Large construction site, rubber estate, stone quarry, busy road
Temperature	AM : 25-28^{o}C PM : 28-30^{o}C	AM : 26-29^{o}C PM : 28-34^{o}C

Passive air sampling was performed in duplicates by exposing two 9 cm Petri dishes containing PCA agar medium to the air according to the 1/1/1 scheme (Pasquarella et al., 2000). The sterile plates were then transported to meliponiculture farm in sealed plastic bags. The plates were labeled with sample number, time and date of collection and point of sampling as being drawn in Figure 2.

In total, 64 duplicates of air samples were collected from 4 points of sampling for two times per day. For initial assessment, regular sampling is taken once in a week for a month. The time of sampling was kept uniform at all the stands between 9 am to 11 am (morning section) and 1 pm to 3 pm (afternoon section). All petri dishes were left exposed to air accordingly and restored after sampling, marked and shipped to the food microbiology laboratory in short times.

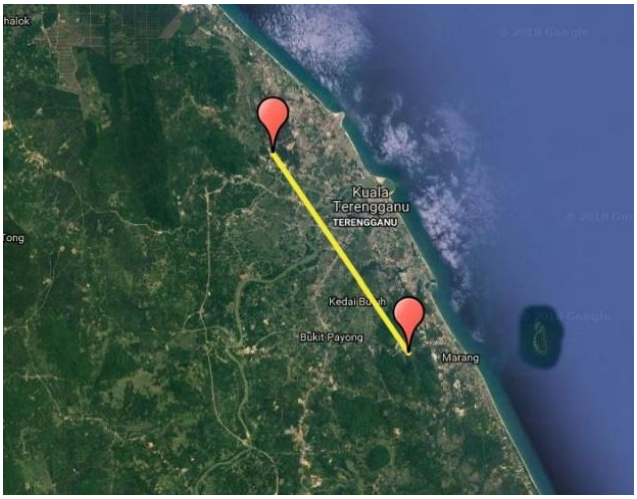

Figure-1: Location of meliponiculture farm at Bukit Kor (Farm I) and Bukit Berangan (Farm II).

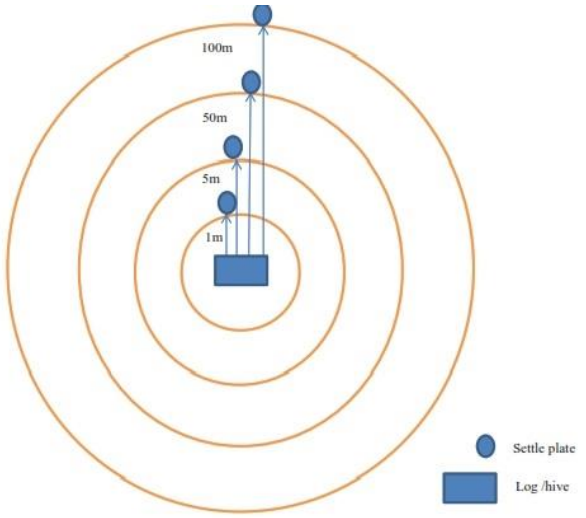

Figure-2: Point of sampling of petri dish at sampling site.

The plates were directly incubated at 36±1°C for 48 hours and no of cfu's were counted. The concentration of airborne bacteria was expressed as colony forming units per meter square per hour. For a 9 cm plate (surface area, 58 cm2) exposed for 1 hour, the settling rate was calculated and expressed as cfu/dm2/hour. Results of cfu/dm2/h can be expressed in IMA values, allowing comparisons with international standards.

Results and Discussion

Total 64 duplicate plates were studied, out of 10 plates (15.63%) was classified under poor performance according to IMA standard (Pasquarella et al., 2000). The amount was not substantial but shows the current ability of bioaerosols to pollute bee products at different outdoor environment. According to the Table 3, settling rate at Farm I during the morning session was compared to the afternoon session, showed a higher rate of contamination during an early day, 6 to 131.5 cfu/dm2/h and 2.5 to 41 cfu/dm2/h at noon. The maximum microbial settling rate at this meliponiculture farm was recorded at morning session during first week, 131.5 cfu/dm2/h; followed by 65.2 cfu/dm2/h, recorded during second week of the sampling. From this result, no other settle plate reached poor air performance at Farm I.

Meanwhile at Farm II, both sessions of sampling showed very poor performance after recorded 12 to 187 cfu/dm2/h and 7 to 130.5 cfu/dm2/h in the morning and afternoon session. According to Table 2, the maximum settling rate was 187 cfu/dm2/h, followed by 150 cfu/dm2/h; both from morning session at third week of sampling. In contrast, a minimum number of microbial settling rate recorded during afternoon at approximately 7.5 cfu/dm2/h. In overall, afternoon session showed less air contamination except sampling on the third week that recorded 67.5 to 130.5 cfu/dm2/h.

Table-2: IMA classes and their application

Grade*	Performance	IMA value	cfu/dm^2/h	In places at risk
A	Very good	0-5	0-9	Very high
B	Good	6-25	10 to 39	High
C	Fair	26-50	40 to 84	Medium
D	Poor	51-75	85 to 124	-
E	Very poor	≥75	≥ 125	-

*Revised/modified from Table X by Pasquarella et al., 2010.

Table-3: Microbial settling rate (cfu/dm^2/h) at Farm I and Farm II.

Radius in metre (Farm I)	Mean of cfu/dm^2/h							
	AM				PM			
	W1	W2	W3	W4	W1	W2	W3	W4
1	8.8	65.2	30.3	68.7	17.0	25.2	29.3	41.7
5	131.5	12.5	17.5	11.5	2.5	2.5	11.5	12.0
50	6.0	12.0	12.5	34.0	3.0	7.0	8.0	21.0
100	7.5	23.5	14.0	19.0	5.0	17.5	19.0	15.0
Radius in metre (Farm II)	**Mean of cfu/dm^2/h**							
	AM				**PM**			
	W1	**W2**	**W3**	**W4**	**W1**	**W2**	**W3**	**W4**
1	64.7	73.5	147.3	74.5	73.7	59.2	67.5	43.0
5	24.5	14.5	103.0	43.5	7.0	39.5	79.0	47.0
50	114.0	12.0	187.0	102.0	7.5	23.5	130.5	21.0
100	56.0	57.5	150.0	100.5	48.5	71.5	105.0	62.5

*AM-morning, PM-afternoon, W-week, cfu/dm2/h-microbial settling rate.

Table-4: Percentage of settle plate according to the grade of IMA standard.

Grade	Farm I		Farm II	
	No. of plate at AM (%)	No. of plate at PM (%)	No. of plate at AM (%)	No. of plate at PM (%)
A	3(18.8)	6(37.5)	-	2(12.5)
B	10(62.5)	9(56.25)	3(18.8)	3(18.8)
C	2(12.5)	1(6.3)	6(37.5)	9(56.25)
D	-	-	4(25.0)	1(6.3)
E	1(6.3)	-	3(18.8)	1(6.3)
Total	16(100)	16(100)	16(100)	16(100)

*AM-morning, PM-afternoon

From Table 4, only afternoon's sampling at Farm I showed a fair air quality value with the most settle plate was graded in the class of good performance (56.25%). Majority of the settle plates that tested in the morning session also showed good performance (62.5%) of its air quality and only a plate (6.3%) that classified under a very poor performance. This various result of air qualities performance may be due to the location of the meliponiculture farm and its nearby factors, as described in Table 1. Activities of farmers closed to the meliponiculture farm could lead to the contamination of the air quality. A farmer usually maximized their workload at morning session and avoids working at afternoon due to inconvenience of the high-temperature condition. Hence, there was no settle plate recorded below poor performance during afternoon session.

According to Pasquarella et al. (2000), the maximum IMA level included in the classification was 76. From Table 4, the percentage of the settle plate that had listed in the grade E from Farm I and Farm II was recorded during morning session at 6.3%, and 18.8% respectively. Whereas, only one plate showed higher IMA level at Farm II (6.3%) and none was recorded at Farm I. Majority of the settle plates on morning and afternoon showed a similar fair air quality value at Farm II, 37.5 and 56.25% respectively. However, Farm II provides a higher risk of becoming a source of microbial contamination towards stingless bee product as the percentage of microbial settling on the plate were apparent under grade D and E.

In general, Table 2 showed decreasing trend of microbial settling rate as the distance increased from 01 metre to 100 metres at Farm I. The opposite trend showed at Farm II as higher microbial settling rate once the distance of settle plate increased from the hive. The factor of distance from stingless bee's hive showed a correlation between a number of microbial settles on the plate and level of exposure by that place from bioaerosol contamination. Thus, an industrialized city was expected to bring higher microbial settling rate compared to an unexplored land.

From a recent study, there were a few justifications behind this result. Farm I had seen as a better site for meliponiculture activities than Farm II based on recent grading of IMA level. Air microbial contamination was recorded larger during morning session as farmers were active in that period. There was also a rear animal that grazing along with that vegetable farm during the sampling period. This nearby factor must be taken into account for causing larger IMA level at morning session and kept below the maximum level on the afternoon. According to OSHA (US), farmworkers were exposed to respiratory hazards such as organic dust, microorganisms, endotoxins and chemical toxicants. That fact showed an existed potential risk of microbial air pollution in the vegetable farm and it was parallel with the result obtained at Farm I.

However, Farm II had unlikely emerged as a more suitable location for meliponiculture activities as its qualitative assessment of the air showed it's associated higher risk of microbial contamination to the stingless bee product. This location was susceptible to harbor more microorganisms in the air due to its nearby factors. Airborne microorganisms are usually derived from various natural sources such as soil, animals, and humans. Human activities such as sewage treatment, plants and animal rendering, fermentation processes and agricultural activities tend to release microorganisms into the air (Hansen et al., 2010; Gheorghe et al., 2016; Tarigan et al., 2017). Record of the prevalence of fungal and bacteria trapped in the air at the agricultural sector had been apparent and had been previously studied in many aspects (Salustiano et al., 2003; Eduard et al., 2012; Adell et al., 2014). Thus, both meliponiculture farms had the tendency of harboring with a microbial population in the air and could settle down in stingless bee product of honey, pollen and propolis.

In this recent study, IMA measurement by settle plates, related as it is to the level of the microbial contamination of the surrounding atmosphere. It immediately gives an objective and accurate representation of both meliponiculture locations. The general acceptance of the IMA in this study would allow the comparison of results obtained by different persons in different places in the study of the microbial air contamination, which currently is not possible. Simultaneously, this result could provide an easy and generally valid parameter for official guidelines, particularly in view of the low cost and the ease of the test. People that interested in doing meliponiculture could seriously learn from this guideline in order to produce a high quality of stingless bee honey, pollen and propolis.

Conclusion

In conclusion, air quality within meliponiculture farm area was important and cannot be neglected in reference to the occurrence of pathogenic bacteria in the product of stingless bee. Future studied could be done on identifying microorganism that had growth in the PCA agar in this study. It is also suggested for every related party in this country to revise and plans out carefully before starting their meliponiculture project for the benefit of everybody.

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Contribution of Authors

Chilek TZT: Mentoring and guiding the research ideas and formulation of research questions
Razak SBA: Guiding the research ideas and formulation of research questions
Khalid MI: Conducted laboratory work, data analysis and writing
Ahmad F: Guiding the research ideas and formulation of research questions
Amin ATM: Technical expert for data analysis and assisting in article writing

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