PERFORMANCE EVALUATION ON NATURALLY MATED AND ARTIFICIAL INSEMINATION OF QUEEN BEES APIS MELLIFERA LINNAEUS, 1758 IN FIELD COLONIES

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Abstract

Artificial insemination (AI) known as instrumental insemination, is a practice that is popularity among queen breeders. It provides a valuable tool to control the random mating process and is essential for bee breeding and research requiring specific crosses. Three study sites have been chosen as site (I) in Shan State (Aung Ban), site (II) in Bago Region (near Taungoo University) and site (III) in Mandalay Region (Yamethin). Study period lasted from December, 2017 to December, 2018. The study sites were chosen based on the migratory beekeeping method by flowering plants and seasonal changes condition of Myanmar. Artificial Insemination tool was composed of artificial insemination device, microscope, CO₂ as anesthesia. Artificial Insemination process have inseminated on 30 virgin queen bees in 2018, totally. In site (I) in Taung Lay Lone, Shan State, AI process have inseminated on ten virgin queen bees, but none is survived and oviposition due to severe weather condition. Artificial insemination process has inseminated on ten virgin queen in site (II), of near Taunggoo University, Bago Region. Result has succeeded six AI mated queens in this area. In site (III) of Yamethin, Mandalay Region, AI process have inseminated on ten virgin queen bees have recorded seven AI mated queens. Comparison on the survival rate of Naturally Mated (NM) queen and Artificially Inseminated (AI) queen bees were carried out. The comparison of the oviposition rate on Artificially Inseminated (AI) queen was also done in three study site. The relationship of oviposition rate and mean temperature, mean relative humidity and mean rainfall is carried out in three study sites. SPSS (Statistical Package for Social Science) version 25 was utilized for the statistical tests. The Nonparametric ANOVA test (Kruskal Wallis) was used.

Keywords: Artificial Insemination Technique, Artificially Inseminated and Normally mated Queen bees, Drone

Introduction

Many animals, especially insects, are very effective pollinators. Among them, bees are the most effective and reliable pollinators due to their dependence on flowers for their brood food. Several social and solitary bees can be utilized for the pollination of crops (Sihag, 1997).

Honeybees are the main insects which help in pollination of different species of plants. Honeybees play an important ecological role as pollinators of many plant species, and their products are the basis for a multi-million dollar commercial industry around the world. They are major agricultural pollinators around the world and are the keystone pollinators in tropical ecosystems.

Honeybees are beneficial to both agriculture plant and most wild plants as pollinator. For mankind they provided a lot in agriculture, medicine and food.

Throughout the world, the honeybee is a domestic animal and the great majority of the hundreds of species of bees live in solitary and colony. All honeybee species are social insects.

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In Asia, a total of 11 honeybee species were recorded (Michener, 2000). Some species are being domesticated and employed as pollinator and production of bee product such as honey, beeswax, bee pollen etc; were used in various ways (McGregor, 1973).

In Myanmar, (Petersen, 2005) have reported to have six species of honeybees. Five of them, *Apis dorsata, Apis laboriosa, Apis cerana, Apis florea* and *Apis andreniformis* are indigenous species. One of them, *Apis mellifera*, the European honeybee, a commercial species, large-scale importation of exotic species into Myanmar was done under an FAO project stared in 1979. Under this project, about 500 packages of Italian honeybee *Apis mellifera lingustica* was introduced in 1979 from Australia (Morse, 1982). *Apis mellifera* is now preferred for beekeeping. They are the main bee pollinators worldwide and pollination of plants and production of honey.

A colony of honeybee consists of a queen, several thousand workers and in a certain season of the year- a few hundred drones. Among the members of the colony there is a division of labour and specialization in the performance of biological functions. The structure of the comb of all honeybee species is especially similar: it consists of adjoining hexagonal cells made of wax secreted by worker's wax glands.

The queen, a true mother bee, is the only female that is completely developed sexually. This is a result of a total diet of royal jelly during a developmental period. She is distinguished by her long, slender appearance, due to the full development of the ovaries in her abdomen. In the colony, she is found in the area of the brood nest. The developmental time of the queen, 16 days, is the shortest.

Workers are females that are not fully developed sexually. They do the work of the colony and maintain it in good condition. Workers have special structures and organs which are associated with the duties they perform. The adult worker emerges from the cell 21 days after the egg is laid.

Drones, the males of the colony, are produced from unfertilized eggs. The queen can control whether or not the egg is fertilized as she lays it. The body of the drone is larger than that of the worker or queen. The eyes are large and cover practically the whole head. The end of the abdomen is blunt and is covered with a tuft of small hairs. Drones cannot sting. As the sting is a modified structure of the female genitalia, drones do not have stings. They also do not have any of the structures necessary to collect nectar and pollen. The developmental period of drones is 24 days (Yadav *et al.*, 2017).

Artificial insemination (AI) known as instrumental insemination, is a practice that is popularity among queen breeders. It also becomes more popular as beekeepers realize that traditional technique results in low quality queen bees, unlike the systematic practice of artificial insemination. Controlled mating is essential to achieve the goals of any breeding program. Honey bees present a unique challenge, because queens mate in flight with an average of 15 to 20 drones and therefore mating is difficult to control. Artificial Insemination (AI) provides a valuable tool to control the random mating process and is essential for bee breeding and research requiring specific crosses. Artificial insemination is an essential tool that provides complete control of honey bee mating for research and breeding purposes. The technique requires specialized equipment to anesthetize and immobilize the queen and to collect and deliver semen from the drones.

Artificial insemination is a valuable breeding technique that offers a way to utilize the desirable traits. It allows types of mating that are not possible with natural mating such as mating a queen to a single drone or to a few specific drones, mating mutant queens and drones and mating a queen to her own male offspring (Harbo, 1986a). Naturally, mated queens could mate with drones from unknown origins that could result in bees with undesirable characteristics. Artificial insemination enables bee breeders to design breeding programs in which complete genetic isolation is maintained with the ability to produce consistent, high quality queens selected for a specific trait and high brood viability (Page and Laidlaw, 1982 and Page *et al.*, 1983).

Taking these facts into consideration, the present work was conducted with the following objectives.

- to study in detail on Artificial Insemination of honeybee in Apiculture including field and practical lab works
- to compare on the survival rate of the Naturally Mated queen and Artificially Inseminated queen bees
- to investigate on the oviposition rate of the queen bee by the Artificial Insemination method

Materials and Methods

Study Sites

The present study was carried out in an apiary situated in the experimental farms including Site I near Taung Lay Lone village, Shwe Nyaung township, Shan State situated at $20^{\circ}44'47.50$ "N and $96^{\circ}54'19.19$ "E and Site II near Taunggoo township, Bago Region situated at $18^{\circ}57'4.18$ "N and $96^{\circ}25'57.60$ "E and Site III near the Pya Tike village, Yamethin, Mandalay Region situated at $20^{\circ}25'6.63$ "N and $96^{\circ}8'15.57$ "E (Plate 1).



Site I (Taung Lay Lone, Shan State)



Site II (Taunggoo, Bago Region)



Site III (Pya Tike village, Yamethin, Mandalay)Plate 1 Map of study sites (Source Google Earth, 2019)

Study Period

The field survey and data analyses were conducted from December, 2017 to December, 2018.

Preparation of Beekeeping

In general, honey beekeeping was conducted with two methods (1) stationary beekeeping (2) migratory beekeeping. In the present study, the migratory method was based on the accessibility of food sources for pollination of crops and production of honey. A single bee colony composed of one queen, approximately one hundred drones and nine thousands worker bees was kept in a wooden box containing six vertical wooden square frames with two parallel steel strings to facilitate the construction of beehive. Requirements for beekeeping – (1) Wooden box ($61cm \times 43cm \times 34cm$) (2) Wooden frames (3) Smoker (4) Hive tool (5) Bee brush (6) Bee veil (7) Uncapping knife (8) Extractor (9) Pollen trap (10) container. Data collection was carried out from 10 nucleus colony beehives at three study sites during study period.

Preparation of Project Design

Experimentation was used on the selected from the six days old virgin queen, mature drones and instrument of artificial insemination. There will be three distinct period in a year, wet season and cool season and dry season based upon the migratory method of available designated locations and places in Myanmar as outlined in this proposal. Breeding of virgin queens were prepared in the research apiary field. Before Artificial Insemination process, *Apis mellifera* L. queens were reared in process of queen rearing by the Doolittle grafting method (Doolittle, 1889).

Queen Rearing

One or two days old of young larvae were grafted to queen cups supplied with royal jelly, then transferred to a common cell builder queenless colony. The larvae were originally taken from met frames of selection breeders of the apiary that maintained by the beekeepers at study sites. After maturation, queen cells were individually placed in three frame nucleus colonies. One group of 15 queen cells was randomly selected and allowed to mate naturally. The second group of 15 queen cells was confined to cages and instrumentally inseminated.

Drone Rearing

The strong colonies of honeybee for drone rearing were used. A high population of adult drones depends on a continuous supply of surplus quality pollen coming into the colony. Only pollen is essential for drone rearing. For management of drone rearing, six kinds of wooden frames are prepared for drone rearing (circle-shape, triangle shape, saw- tooth shape, plane sheet foundation, free frame). These frames are kept in the selective colonies respectively.

Procedure

Artificial Insemination (AI) process was begun in apiary and available laboratory.

- (a) Ten virgin queen of six days old were selected for Artificial Insemination.
- (b) Drones are selected from 16-24 days old.
- (c) One day before artificial insemination, each selected virgin queen is treated with CO_2 for 5-7 minutes.
- (d) After that, when the queen returned to conscious state, she was replaced into the original hive.
- (e) The queen was taken out of the hive on the next day.
- (f) The semen was collected from the selected drones.
- (g) As the mating time of bee is very specific and different time zone of the native country, care must be taken that the time at which semen collected.
- (h) Actually, the semen of 15-20 drone is sufficient for one queen to be inseminated.
- (i) The virgin queen was placed in an inverted position keeping the end of abdomen upwards.
- (j) Then, the vagina of virgin queen was opened and 1mm of semen was introduced first.
- (k) The queen was allowed to rest for 2 minutes, then, observation was made on whether the spermatheca was activated or not.

- (1) If the spermatheca was activated, the remaining 1cm of semen was inserted into the vagina.
- (m) Then, one third of the wings were cut off to prevent the mating flight.
- (n) The queen was placed into the queen cage and she was left for 3 minutes under observation
- (o) After that she was replaced back into the original hive
- (p) And then, monitoring was done whether the worker bees were accepted the replace queen or not
- (q) Care must be taken for duration of insemination which should be not more than 20 minutes so as to avoid the unnecessary damage to the queen.
- (r) After about the six days, it was checked whether the eggs are oviposited or not
- (s) Then, the queen was left in the original hive under observation for 3 months

Data Collection and Identification

Data collection was made and recorded by visiting to apiary near study site during the rearing of queen, drone, selected sample queen and drone. The same experiment was conducted in the three study sites throughout the study period. Collected data were identified according to the methods described by Bingham (1897).

Statistical Methods

Statistical analysis was conducted using SPSS (Statistical Package for Social Science) version 25. The Non-parametric ANOVA test (Kruskal Wallis) was used to test if there were significance differences of survival rates resulted from two methods, Normally Mated (NM) and Artificially Inseminated (AI) queens.



A. Equipment of Artificial Insemination



B. Insemination of Queen bee



C. Equipment of Artificial Insemination



D. Nurse Hive



E. The selected virgin queen is treated with CO₂ before AI Process



F. Collection of DronesPlate 2 Data collection of experiments and fields



(A) AI queen of after Insemination



(B) AI queen bee and workers who accept the AI queen





(C) Inseminated AI queen covered by queen confider (D) AI mated queen and workers



Plate.6 (E) After Inseminated AI queen



(F)Together with AI mated queen and workers



(G) AI Mated Queen (Yamaethin, Nay Pyi Taw)

Plate 3 Recorded on the survival rate of AI Mated Queen



Plate 4 Maintained for Mated queens in field

Results

Classification of Apis mellifera Linnaeus, 1758

Kingdom	- Animalia
Phylum	- Arthropoda
Class	- Insecta
Order	- Hymenoptera
Family	- Apidae
Genus	- Apis
Species	- Apis mellifera Linnaeus, 1758
Common name	- European honeybee

Survival Rates of Queen bees by Normally Mated (NM) and Artificially Inseminated (AI) Queenbees

In the three study site, total mated queen bees were recorded that 22 Normal Mated (NM) and 13 Artificially Inseminated (AI) queens from December, 2017 to September, 2018 (Table. 1 and 2). Comparison of study based on survival rates of queen bee both Normal mated (NM) and Artificially Inseminated (AI) supersede rates and longevity.

In the study site (I) of Taung Lay Lone, Shan State, colonies of 10 Normal Mated (NM) and 10 Artificially Inseminated (AI) queens were established in December, 2017. By February 2018, 8 out of the 10 Normal Mated (NM) queens were survived whereas no survived due to unfavorable weather condition in Shan State at that time. The process of Artificial Insemination was failed in December, 2017. In the study site (II) of near Taunggoo University, Bago Region, colonies of 10 Normal Mated (NM) and 10 Artificially Inseminated (AI) queens were established in March, 2018. By May 2018, 7 out of the 10 NM queens and 6 out of the 10 AI queens were established in July, 2018. By September 2018, 7 out of the 10 NM queens and 7 out of the 10 AI queens were recorded.

The percentage survival rate in three study site were recorded as 22 NM queens out of 30 queens (73.3 %) and AI queens with 13 out of 30 queens (43.3 %) during 3 month study (Figure. 1 and Table. 1, 2, 3). Between study sit (I) Shan State and study site (II) Bago Region, survival rates were higher in Normal Mated (NM) than Artificially Inseminated (AI) queens as well as between Shan State and study site (III) Mandalay Region. They have showed significant differences in study sites (II and III) of relative with study site (I), respectively. At Bago Region, survival rates were slightly higher in NM than AI queens during study period. At Mandalay Region, survival rate was the same level in NM and AI queens in study period.

TI:	S	Shan State			Bago Region			Mandalay Region		
Number	Dec, 17	Jan, 18	Feb, 18	March, 18	April, 18	May, 18	July, 18	August, 18	September, 18	
H1					Х	Х				
H2		\checkmark		\checkmark	\checkmark	\checkmark		Х	Х	
H3		\checkmark	Х	\checkmark				\checkmark		
H4		\checkmark		\checkmark	\checkmark	\checkmark		\checkmark		
H5		х	Х	\checkmark	\checkmark	\checkmark		\checkmark		
H6		\checkmark			\checkmark	\checkmark			Х	
H7		\checkmark			х	х			Х	
H8		\checkmark		\checkmark	\checkmark	\checkmark		\checkmark		
H9		\checkmark		\checkmark	\checkmark	\checkmark		\checkmark		
H10		\checkmark	\checkmark	\checkmark	х	х		\checkmark	\checkmark	
Survival Number	10	9	8	10	7	7	10	9	7	
Survival Rate (%)		80%			70%			70%		

 Table 1 Survival Rates of Normally Mated Queen Bees

 Table 2 Survival Rates of Artificially Inseminated Queen Bees

Uivo	Shan State			Bago Region			Mandalay Region		
Number	Dec,	Jan,	Feb,	March,	April,	May,	July,	August,	September,
Tumber	17	18	18	18	18	18	18	18	18
H1		Х	х			\checkmark			\checkmark
H2		Х	х	\checkmark		\checkmark		\checkmark	\checkmark
H3		Х	Х	Х	Х	Х		Х	Х
H4	\checkmark	\checkmark	х		\checkmark	\checkmark		\checkmark	\checkmark
H5	\checkmark	Х	х					\checkmark	\checkmark
H6	Х	Х	х				X	х	Х
H7			х					\checkmark	\checkmark
H8	\checkmark		х		\checkmark	х		\checkmark	\checkmark
H9	Х	х	х		х	х		\checkmark	Х
H10	\checkmark	Х	х		\checkmark	х		\checkmark	\checkmark
Survival	Q	3	0	0	Q	6	0	Q	7
Number	0	3	0	9	0	0	9	0	7
Survival		0%			60%			70%	
Rate (%)		070			0070			7070	,

Survival rates resulted from two different methods, Artificially Inseminated (AI) and Normal Mated (NM) were tested separately for the respective states, Shan, Bago and Mandalay. The survival rates of AI and NM between the Shan states was found to be statistically significant at the level of 0.05. However, the survival rates resulted from two methods in the states, Bago and Mandalay, were not significantly at 0.05 significant level.

Significance difference of the survival rates among the three states, Shan, Bago and Mandalay were tested using non-parametric ANOVA test (Kruskal Wallis) at the level of 0.01.

The significant difference was observed (Table 1 and 2). In order to get the exact specific significance test, further pair wise test was conducted by doing Bonferroni correction on the significance value (Table 1 and 2).

Non-parametric Tests (Method = AI)

_	Hypothesis Test Summary							
	Null Hypothesis Test	Sig.	Decision					
	Independent- The distribution of Number is the Samples same across categories of State. Kruskal- Wallis Test	.001	Reject the null hypothesis.					

Asymptotic significances are displayed. The significance level is .05.

Sample1-Sample2	Test Statistic [⊕]	Std. Error ⊜	Std. Test⊜ Statistic	Sig.	Adj.Sig.
Shan-Bago	-16.500	5.409	-3.050	.002	.007
Shan-Mdly	-18.000	5.409	-3.328	.001	.003
Bago-Mdly	-1.500	5.409	277	.782	1.000

Each row tests the null hypothesis that the sample 1 and sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is 0.05. Significance values have been adjusted by the Bonferroni correction for multiple tests.

The results revealed that the difference between the states, Shan and Bago and Shan and Mandalay were found to be significant. However, the two states between Bago and Mandalay were not significantly different.

Non-parametric Tests (Method = NM)

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Number is the same across categories of State.	Independent- Samples Kruskal- Wallis Test	.537	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

In the case of Normally Mated (NM), the significance differences among the three states were not significantly different at the 0.05 level.

Study Sites	Normally Mated	Artificial Insemination Mated	
Shan State	8	0	
Bago Region	7	6	
Mandalay Region	7	7	
Totally Survival Queen Number	22	13	
Mean <u>+</u> SD	7.3 <u>+</u> 0.58	4.3 <u>+</u> 3.76	

 Table 3 Comparison on the Survival Rates of Normally Mated and Artificially Inseminated Queen Bees



Figure 1 Comparison on the Survival Rates of Normally Mated and Artificially Inseminated Queen Bees



Plate 5 Artificially Inseminated (AI) mated queen laying eggs

Oviposition Rate of Artificially Inseminated queens

In study site (I) in Shan State, 0% oviposition rate were observed in December, 2017 as the weather condition in severely bad after insemination. In Shan State, the process of Artificial Insemination was failed and weather condition is so bad in inserted period.

At study site (II) in Bago Region, the highest percentage of oviposition rate was recorded as 41.6, 52.3, 39.7 and 47.8 in hive number of 2, 4, 6 and 7, respectively after three months (Figure 2 and Table 4).

At study site (III) in Mandalay Region, the highest percentage of oviposition rate was recorded as 51.2, 45.2, 50.3 and 33.5 in hive number of 2,5,8 and 10, respectively after three months (Figure 3 and Table 5).

Uino Numbor	Ovipo	osition Rate	(%)
nive Nulliber	March	April	May
H1	20	21.8	23.02
H2	20	32.5	41.6
H3	20	18.9	13.2
H4	23	33	52.3
H5	20	20	18.3
H6	21.5	30.9	39.7
H7	22	31.28	47.8
H8	18	12	0
H9	17.8	13.45	0
H10	13.5	10.39	0
Mean Total Oviposition Rate (%)	19.58	22.422	24.54
Mean Temperature (°C)	37.48	38.35	35.36
Mean Relative Humidity(%)	42.23	43.2	60.58
Mean Rainfall (mm)	0	12.67	19.58

 Table 4 Oviposition Rate of AI mated queens found in Taunggoo, Bago Region



Figure 2 Oviposition Rate of AI mated queens found in Taunggoo, Bago Region

Hivo Numbor	Ovi	Oviposition Rate (%)			
Inve Number	July	August	September		
H1	19.3	25.57	27.8		
H2	20	29.05	51.2		
Н3	15	11.5	9.23		
H4	21	24.89	26.5		
H5	25	30.6	45.2		
H6	16.5	15	7.1		
H7	20	19.5	28.6		
H8	20	28.4	50.3		
Н9	20	14	12		
H10	20	27	33.5		
Mean Total Oviposition Rate (%)	19.68	22.551	29.143		
Mean Temperature (°C)	32.47	32.22	33.22		
Mean Relative Humidity(%)	70.09	71.61	68.8		
Mean Rainfall (mm)	6.92	11.78	11.7		

 Table 5 Oviposition Rate of AI mated queens found in Yamethin, Mandalay Region



Figure 3 Oviposition Rate of AI mated queens found in Yamethin, Mandalay Region

Relationships between Oviposition Rate of Artificially Inseminated queens and Weather conditions in three Study Sites

At study site (I) in Shan State as oviposition rate was observed 0 percent due to severe weather condition. At study site (II), in Taunggoo, Bago Region, oviposition rate increased in May as the mean temperature decreased from 37.48 (°C) to 35.36 (°C). Regarding relative humidity, the higher the relative humidity in May was 60.58(%), the greater the oviposition rate, which was 24.54%. For the mean rainfall, the oviposition rate increased to 24.54% as the mean rainfall increased from 0 mm to 19.58mm (Fig 4 and Table 4).





Figure 4 Relationships between Oviposition Rate of Artificially Inseminated queens and Weather conditions at Study Sites (II) in Bago Region

At study site (III) in Yamethin, Mandalay Region, oviposition rate increased in September as the mean temperature increased from $32.47(^{\circ}C)$ to $33.22(^{\circ}C)$ were recorded. Reguarding relative humidity, the lower the relative humidity in September was 68.8(%), the greater the oviposition rate, which was 29.14%. For the mean rainfall, the oviposition rate increased as well as the mean rainfall increased from 6.92 mm to 11.7 mm (Fig 5 and Table 5).



(A) Temperature





(C) Rainfall

Figure 5 Relationships between Oviposition Rate of Artificially Inseminated queens and Weather conditions at Study Sites (III) in Yamethin, Mandalay Region

Discussion

In study period, the results of this study indicate similarity in performance levels of field colonies headed by Normally Mated (NM) and Artificially Inseminated (AI) queens.

At study sites (II) and (III), the survival rate Normally Mated (NM) queens and Artificially Inseminated (AI) queens more or less similar in their field performance. The results revealed that the difference between the states, Shan and Bago and Shan and Mandalay were found to be significant. However, the two states between Bago and Mandalay were not significantly different. In the case of Normally Mated (NM), the significance differences among the three states were not significantly different at the 0.05 level. However, the survival rate of Normally Mated (NM) queens is slightly higher than of Artificially Inseminated (AI) queens. At study site (I) in Shan State, no survival rate in both Normally Mated (NM) and Artificially Inseminated (AI) queens due to severe weather condition. This indicates that the weather condition is greatly influence on the behaviour of drones and Normally Mated (NM) queens which inhibit mating. It is also influence on Artificially Inseminated (AI) queens as they are sensitive to ambient temperature, humidity and rainfall.

Although NM queens showed higher oviposition rates no significant differences were found between the two groups. NM queens showed a slightly higher survival rate than AI queens during the three month study.

In study sites (II) of Bago Region, the highest number of oviposition rates as 41.6, 52.3, 39.7 and 47.8 in hive number of 2, 4, 6 and 7, respectively after three months were recorded. In study sites (III) of Mandalay Region, the highest number of oviposition rates as 51.2, 45.2, 50.3 and 33.5 in hive number of 2,5,8 and 10, respectively after three months were recorded. In study site (I) of Shan State, the process of Artificial Insemination was failed in December, 2017.

<u>Nelson and Laidlaw (1988)</u> found that brood area and honey weight were similar in colonies headed by NM and AI queens. NM queens were significantly heavier than AI queens only on arrival; two months later no differences were found. <u>Wilde (1989)</u> found that only in the first year of a two-year study, NM and AI queens were similar in their production of brood and

honey. In the second year, AI queens produced significantly higher brood and honey than NM queens.

In this study, Artificial Insemination queens were recorded to have many problems and changeless with initial introduction and acceptance. The results indicates that the optimum temperature for high oviposition rate assume to be between 33.22 °C and 35.36 °C depending on the locality and food sources. Regarding relative humidity and rainfall, the more relative humidity and rainfall, the higher the oviposition rate of both NM and AI queens in both study sites (II) and (III) were recorded.

Artificial Insemination technique is widely employed in queen breeding programs for the improvement of honeybee races to have best colony performance. Because of conflicting reports in the literature, research is still needed to study factors that affect rearing, insemination and introduction of AI queens that would lead them to have better evaluations. The present study has been undertaken to compare the performance of naturally mated and instrumentally inseminated queens for survival and oviposition rate.

Nelson and Laidlaw (1988) suggested that AI queens perform as successfully as NM queens and these findings contrary to this might be due to the lack of some special beekeeping and/ or insemination procedures given to AI queens. Conditions that had led to have AI queens exceed NM queens in field performance (Wilde, 1989; Szalai, 1995) should be utilized and improved.

The present study was conducted only within short duration due to migratory behavior of honeybee, limited time for available food sources and sensitive to ambient weather conditions. However, the main aim of the present study is to promote the high quality queens to small scale beekeeping of area by Artificial Insemination technique.

Conclusion

Field performance of AI queens is influenced by many factors, problems and changeless. Nevertheless, the present investigation shows that successful insemination and high performance levels can be achieved when careful attention is given to queen rearing conditions, pre- and post-insemination treatments, semen doses and quality and technique of insemination utilized. Controlled mating is essential to improve honeybee-breeding stock and to maintain high performance in field colonies. Artificial insemination is an essential tool that provides complete control of honey bee mating for research and breeding purposes. This research of breeding high quality of queen bee by the Artificial Insemination techniques that one can apply to promote the quality of Myanmar honey product. As part of the research study, the proposer believes that this work will contribute to the local beekeepers and Department of Apiculture, Ministry of Agriculture, Livestock and Irrigation in Myanmar. Artificial insemination is a viable and insured method that bee breeders and beekeepers can rely on for that purpose.

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References

Bingham, C.T., (1897). The fauna of British India, including Cevlon and Burma.

Doolittle, G.M., (1889). Scientific Queen-rearing. Wicwas Press, Ithaca, NY., ISBN-10: 1878075241.

- Harbo, J.R., (1986). Oviposition rates of instrumentally inseminated and naturally mated queen honey bees (Hymenoptera: Apidae). Ann. Entomol Soc. Am., 79: 112-115.
- Nelson, D.L. and H.H. Laidlaw, (1988). An evaluation of instrumentally inseminated queens shipped in packages. Am. Bee J., 128: 279-280.
- Mcgregor, S.E., (1976). *Insect pollination of cultivated crop plant*. Agricultural Handbook. Washinton D.C. USDA-ARS.NewYork.496 pp.

Michener, C.D., (2000). The Bee of the World. The John Hopkins University Press. Baltimore, M. D. USA. 913pp.

- Morse, R.A., (1982). Honeybee resource: Biology and management Exotic Apismellifera: 222.
- Page, Jr.R.E. and H.H. Laidlaw Jr., (1982). Closed population honeybee breeding. 2. Comparative methods of stock maintenance and selective breeding. J. Apic. Res., 21: 38-44.
- Page, R.E., H.H. Laidlaw and E.H. Erickson, (1983). Closed population honey bee breeding: The distribution of sex alleles with gyne supersedure. J. Apic. Res., 22: 184-190.
- Petersen, S.F., (2005). Beekeeping study tours Chin and Kachin States, Myanmar. Press, Cambridge Massachusetts.
- Roberts, W.C., (1946). Performance of the queen. Am. Bee J., 85: 185-186.
- Sihag, S., (1997). Pollination biology: basic and applied principles. Rajendra Scientific Publisher, Hisar.
- Szalai, E., (1995). Results of instrumental insemination of queen honey bees in Hungary. Pszczelnicze Zeszyty Nnaukowe English Summery, 39: 61-69.
- Wilde, J., (1989). Development and productivity of honeybee colonies with naturally mated and artificially inseminated queens. Proceedings of the 31st International Apimondia Congress, (IAC`89), Apimondia Publishing House, Warsaw, pp: 442-444.
- Yadav,S., Kumar, Y. and Jat, B.L., (2017). *Honeybee diversity, Castes and Life Cycle*. Omkar(ed.), Industrial Entomology.