The Use of Transferable Breeding Container for Population Enrichment of Pollinator, *Forcipomyia* spp. in the Cocoa Ecosystem

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***Abstract*—The study aims to investigate the potential use of breeding substrates (cocoa pod husk, banana stump, and combination of cocoa pod husk and Cocoa pod borer infested pods) for an enhancement population of pollinators, *Forcipomyia* spp. (Diptera: Ceratopogonidae). The numbers of pollinators was monitored at intervals of two weeks at four distances (2, 5, 8 and 12 m) from the breeding sites. This observation was carried out for the period of 94 days. Field study demonstrated that the emergence of pollinators was higher at Day 52 (2.875a), and significantly different with Day 66 (1.563bc), Day 80 (1.250bc), Day 12 (1.042bc), Day 94 (0.792c) and Day 1 (0.792c). The numbers of male (1.063a) and female (1.813a) pollinators were recorded highest at Day 52. Cocoa pod husk recorded the highest number of male (1.115a) and female pollinators (0.792a), followed by banana stump, control and combination of cocoa pod husks and CPB's infested pods. Higher numbers of pollinators were recorded at a distance of 2 m (1.760a) from the breeding sites, followed by 12 m (1.740a), 8 m (1.480a) and 5 m (1.323a). The overall observation suggests that cocoa pod husk is the most suitable breeding substrates for enhancement of cocoa pollinator's population.**

***Index Terms*—*Theobroma cacao*, cocoa pollinator, *Forcipomyia* spp., transferable breeding container**

I. Introduction

Cocoa, a fruit product of *Theobroma cacao* L. (Malvales: Sterculiaceae), is the third most important agricultural crop in Malaysia. The cocoa trees bearing fruits throughout the year, and the development of cocoa fruit started after pollination of the cocoa flowers. Cocoa is a cauliflorous or truncate plant, which their flowers are formed on the trunk or branches. Cocoa trees produce a large number of flowers, unfortunately only 1 to 5% of flowers will develop mature fruits [1]. The cocoa trees produce flowers all year-around, but reaching peaks occur in early wet seasons. The flowers are small with an average diameter of 15mm, and strictly dependent on insect to pollinate the flowers, due to their anthers and sticky pollen are each enclosed by a folded petal.

Crop pollination by pollinators is an important ecosystem service as shown in coffee and cocoa, two major insect-pollinated tropical plantation crops. Both crops formerly known as self-compatible, however, after went through a series of breeding process, they become strictly self-incompatible crops. Cocoa is among 13 leading crops whose production would be reduced by over 90% in the absence of pollinators [2]. Incompatibility in cocoa occurs when certain trees could not set fruit with their own pollen nor with one another's. It is very clear that an agent is required, which can pass freely between trees to overcome incompatibility, and deposit forty or more pollen grains on the very small style of flowers. Incompatibility occurs in the style or stigma of flowers which preventing the development of pollen tubes in most of the plants, but different mechanism was observed in cocoa. The pollen tubes normally develop, but when the mating is incompatible, the male gamete does not fuse with the female gamete [3]. The proportion of non-fusion between the gametes will up to 100%, and in all cases the ovary will fail to develop and the flower falls off three or four days after pollination.

As an incompatible species, the role of pollinator in the process of depositing pollen on the flower's style was very crucial for successful pollination. Pollination in cocoa has been evaluated to be the most significant limiting factor in cocoa yield than agronomic resources. The most important group of pollinating insects belonging to several genera of the family Ceratopogonidae (Nematocera: Diptera), especially the genus of *Forcipomyia*. Ceratopogonids or commonly known as midges is a small insect; approximately 2-3 mm long, having few setae on the thorax to facilitate their movement in the narrow flower space as in cocoa flowers. Most of the observations on the pollinators were from genus *Forcipomyia* spp. due to only this midges demonstrated great pollinator importance to a cocoa plantation by their visitation rates and massive deposition of pollen grains on the stigma [4]. *Forcipomyia* spp. was proven as true cocoa pollinators, which can deposit a minimum of 35 pollen grains on the stigma of cocoa flowers for effective pollination to occur [5]. The life cycle of these midges is about 28 days and the population builds up during rainy season [3]. Reference [6] recorded four species of ceratopogonids comprised of genus *Forcipomyia* spp., *Dasyhelea* spp., *Brachypogon* spp.,

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and *Atricopogon* spp. in Peninsula Malaysia. In addition to their previous research, ref. [7] listed 17 species of genus *Forcipomyia* as important cocoa pollinators worldwide and 7 species was recorded in Perak, Malaysia.

Cocoa yield is likely to increase with an increase of successful pollination of the cocoa flowers, however it may not be achievable if the population of pollinators remains the same or insufficient. Under natural conditions, *Forcipomyia* spp. and other ceratopogonid midges breed and complete their life cycle in a variety of rotting organic substrates [8]. The lack of pollination due to an insufficient population of pollinators can be one of the constraints in limiting the production of cocoa [7]. Aside of limiting factors such as environmental heterogeneity, extensive use of chemicals, the lack of phonological synchronization between the pollinators and flowering phenology; the lack of breeding substrates may highly affect on the pollinator population. Pollinators highly dependent on decaying organic materials, and lack of the substrates in the cocoa field may limit their population to built-up, especially during long dry period. Reference [8] has suggested that the pollinators highly depend on moist habitats and long dry seasons can affect their natural populations.

The need to increase the population of pollinator at the right time of the year, especially during flowering season is important, and subsequently will increase successful level of pollination. Pollinators' abundance may be synchronized through environmental manipulation, by providing suitable breeding sites in the cocoa field [6]. Providing supplemental and additional breeding substrates may promote the development of temporarily-stable pollinator populations [9]. The breeding substrates may be supplemented during the period of maximum flowering period for population increment. Several substrates that suitable for pollinator to breed is decaying cocoa pod husks, rotten banana stumps, rotting jackfruit and rotting leaf litter [6], [8], [10]. The banana will serve as a breeding place for the pollinators, either when they are still alive or after they die and rot [7]. However, the use of banana stumps as a breeding substrate was less efficient when the data collection was undertaken during prolonged dry seasons [11]. Rotten pod husks also colonized by the midges, and the amount of pod husk is plenty especially during cropping season. However, at the cocoa plantation where the infestation of Cocoa Pod Borer (CPB), *Conopomorpha cramerella* Snellen (Lepidoptera: Gracillariidae) is endemic, it is not recommended to scatter the cocoa pod husks on the field. The larvae of CPB having insidious characteristics, where spending approximately 14 to 17 days inside the cocoa pod. Scattering the infested cocoa pod husk may increase the population of CPB when the risk of larva survival is high inside the pod husk. It was due to infested pod husks may still harbor a residual number of mature larvae and have the potential in inflicting further damage if the CPB is emerging and survive. Later, this becomes more nuisances to the cocoa growing areas. Random placement of infested cocoa pods within the field will promote never ending CPB's problem in cocoa [9].

With the aims to provide a breeding substrate for pollinator enhancement, the use of transferable breeding container was taken into consideration. These containers can be moved within the block, either at selected trees or at a low population of pollinators. Ideally, it is very important to encourage prolonged moist conditions, especially in long dry seasons, and it is advocated to serve a breeding substrate inside suitable transferable containers. To create similar conditions with cocoa closed canopy, the breeding substrates must be placed inside the closed container. The container must be closed-lids, not transparent to provide a cool, dark with appropriate moist conditions for pollinators to breed. Putting the substrates which can attract the pollinators and placed in a container, so the substrates will be left undisturbed. The containers must be high, so the heaps of substrates should be piled at least a meter so the bottom of the piles remains moist especially during the dry season.

The selection of Cocoa Pod Husks (CPH), a banana stumps combination of cocoa pod husks and CPB's infested pods (CPH+CPB) as breeding substrates is due to their availability in the cocoa field. All the selected substrates also play roles in the nutrient supply to the cocoa trees. The substrates were placed inside a black container, with the height of 1 meter. All substrates were heaped to ensure greater moisture retention, especially at the base of the containers. These close-lids containers were selected in order to provide an optimal moist condition for population built-up, and must avoid the drying up of the substrates especially during dry seasons. In the beginning of the study, it was predicted that the provision of suitable breeding substrates for cocoa pollinators would increase their population.

Observations on the pollinators were carried out from 7.30 a.m.to 9.30 a.m. due to number of midges found to visit the cocoa flowers between 6.00 a.m. to 9.00 a.m. was high compared to that between 3.00 p.m. to 5.00 p.m. [3]. Cocoa flowers begin to open in the late afternoon and the opening is complete early the next morning. Anther dehiscence occurs between 6.00 a.m. and 9.00 a.m., and the staminoides flare outward from the style, thus increasing chances of pollinator access to it. Numbers of pollinators were recorded throughout the study, and were used to evaluate the efficacy of transferable breeding containers with three different breeding substrates in enhancing their population in the cocoa field.

II. Materials and Methodology

The population of cocoa pollinators was monitored, both in the cocoa field (Block 4B) and in the Entomology laboratory at Cocoa Research and Development Center (CRDC), Malaysian Cocoa Board Bagan Datuk, Sungai Sumun, Perak, Malaysia (Longitude E.100 M, 52' 0', Latitude N3 53' 42) for the period of six months. Block 4B contained mature cocoa trees with the planting systems of 2 m x 3 m and *Gliricidia macculata* as a shade tree. The block was adjacent to other cocoa blocks which harbored almost similar agro-ecosystems and planting systems.

The transferable breeding substrates were constructed using black containers, approximately 1 m height and 45 cm in diameter. A number of small holes (0.5 cm in diameter) were made in the plastic container for ventilation and discharged liquid waste from rotten substrates. Three different raw materials were prepared as a breeding substrate and approximately 70 - 80% of the substrates were placed inside the closed lids-containers. The experiment was set up in a randomized complete block design, with four treatments and three replicates. The substrates were cocoa pod husk, banana stumps and combination of cocoa pod husk and CPB's infested pods. The containers were then placed randomly along the cocoa rows, and the number of pollinators visited cocoa flowers were monitored at four distances from the containers, which are at 2, 5, 8 and 12 m. Three cocoa trees were selected as a replicate for each range, including untreated cocoa trees served as control. Presumably, control trees had less population of pollinators compared to those with breeding substrates. Thus, a total of 48 trees were assessed at intervals of two weeks in the field study, and this observation was carried out for the period of 94 days (Days 1, 12, 24, 38, 52, 66, 80 and 94).

Collection of pollinators was carried out using an aspirator by sucking the insects perching on flowers, cocoa cushion, dried cherelles, leaf-tips as well as those that were in flight (Fig. 1). The use of transparent aspirator is important due to the midges is very small, and difficult to observe directly particularly in the gloom of cocoa plantations. Each tree was sampled for five minutes by three trained staff, from 7.30 to 10.00 am due to pollination takes place more in the morning and in late afternoon. The staffs were trained to sort out pollinators in the laboratory using the dipteran taxonomic identification key. Captured pollinators in the field were identified for their sexes. A laboratory experiment was conducted to determine which substrate supported the highest population of *Forcipomyia* spp. The study has three replicates for each substrate with complete randomized design. Approximately 100 grams of substrate were filled in the transparent containers, and number of pollinators emerged were monitored at intervals of 5 days and recorded for 50 days (Fig. 2). The data from both observations were arranged and pooled for analysis in Excel® program and SAS® (a SAS® system for Windows® V8). Analysis of variance was carried out to determine the significance level of the study at $\alpha = 0.05$.

Figure 1. Pollinators captured using aspirator in the field.

Figure 2. Emergence of pollinators in the laboratory.

III. Results and Discussion

The experiments were carried out for 94 days in the field and 52 days in the laboratory. The number of pollinators collected from banana stumps (5.625a) in the laboratory study was highest among the treatments, although it was not significantly different ($p \leq 0.05$) with cocoa pod husk (CPH) 1.813a, and combination of cocoa pod husk and CPB's infested pods (CPH + CPB), 1.813a (Fig. 3). Highest number of pollinators emerged in the banana stumps container may suggest that the rotting process banana stumps was faster compared with the other two substrates. This findings support the previous studies by several researchers which demonstrated *Forcipomyia* spp. preferred to Musaceae family as oviposition sites because of shorter decomposition period. In view of that, the abundance of pollinators can be attained by adding discs of rotten banana stumps on the ground litter of cocoa plantation [8]. Meanwhile Reference [12] in their study observed that the rotten banana pseudostem recorded the highest population of pollinators after 56 days, followed by cocoa pod husk and cocoa leaf litter. Results on the use of CPH, and CPH + CPB shows the decomposition process of these substrates are time consuming and harbored less moisture content compared with banana stumps.

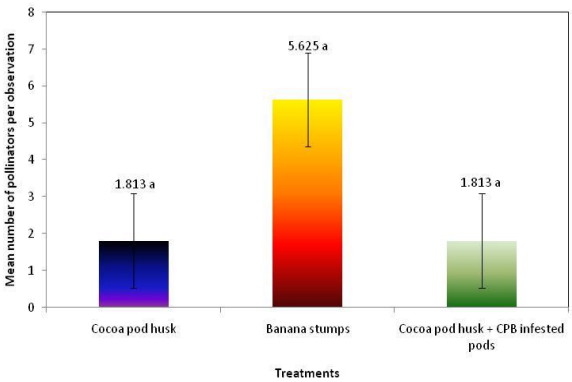

Figure 3. Number of pollinators emerged from different substrates in laboratory assessment.

Overall number of pollinators captured in the cocoa field for a period of 94 days using an aspirator is shown in Fig. 4. Field study demonstrated that the emergence of pollinators was higher at Day 52 (2.875a), and significantly different with Day 66 (1.563bc), Day 80

(1.250bc), Day 12 (1.042bc), Day 94 (0.792c) and Day 1 (0.792c). The results may suggest that the decomposition process of the tested substrates started from Day 24 to Day 52. The mean number of pollinators emerged after Day 52 gradually decreased thereafter. This could be due to the beginning of drying process of the substrates inside the transferable breeding containers, thus did not provide suitable conditions for pollinators to breeds.

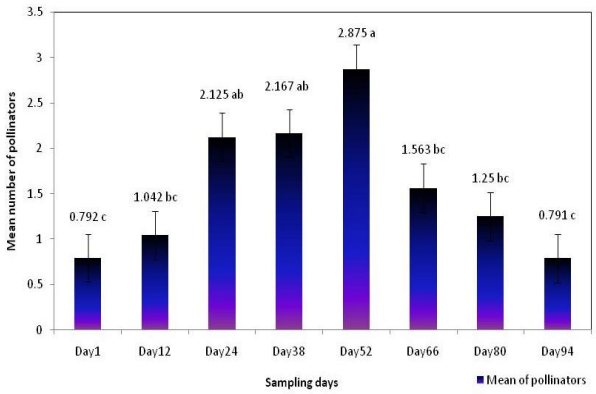

Figure 4. Mean number of pollinators recorded in the cocoa field for 94 days observation.

As in the observation on the overall number of pollinators, the number of both females and male pollinators captured produce almost similar results. The numbers of males (1.063a) and females (1.813a) pollinators were recorded highest at Day 52 (Fig. 5). Number of females exceeded the number of male pollinators in all data collections. Female pollinators are far more abundant as cocoa flower visitors than males, and both sexes crawl inside enclosed floral parts associated with reproductive behavior in cocoa [13].

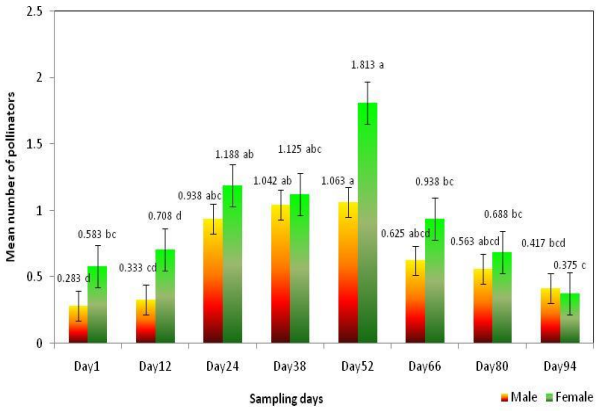

Figure 5. Males and females pollinators recorded in the cocoa field.

Numbers of male and female pollinators observed at three cocoa trees adjacent to the transferable breeding substrates is shown in Fig. 6. Cocoa trees adjacent to the CPH containers recorded the highest number of females (1.115a) and male pollinators (0.792a), followed by banana stumps, control and CPH + CPB. Results showed that the number of pollinators recorded lowest at the trees nearby the CPH + CPB containers regardless of their sex.

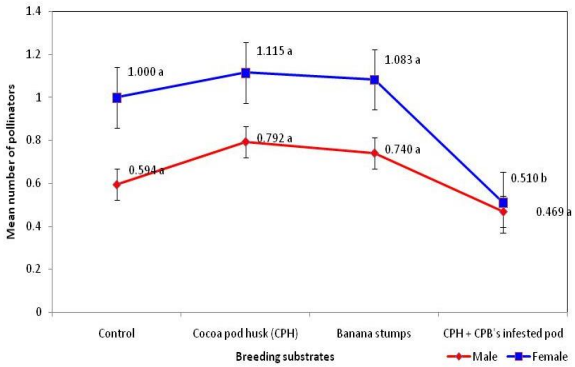

Figure 6. Males and females pollinators recorded inside different substrates.

Numbers of pollinators were recorded at four different range (2, 5, 8 and 12 m) from the transferable breeding substrates is shown in Fig. 7. Highest number of pollinators were recorded at a distance of 2 m (1.7600a), followed by 12 m (1.740a), 8 m (1.480a) and 5 m (1.323).

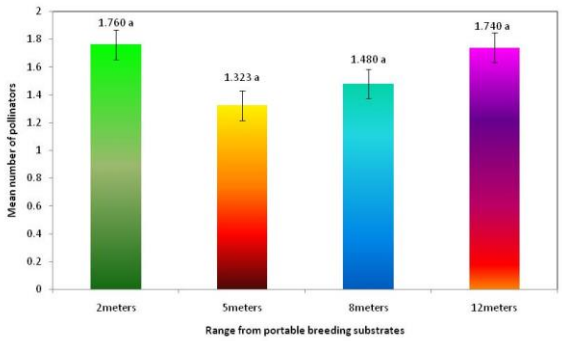

Figure 7. Number of pollinators recorded at four different ranges from transferable breeding substrates.

Numbers of pollinators were recorded at several distances from banana stumps containers, CPH containers, CPH + CPB containers and control is shown in Fig. 8, Fig. 9, Fig. 10 and Fig. 11, respectively. The results obtained from all observations denoted that the number of pollinators recorded highest at Day 52. There are a significant difference ($P \leq 0.05$) for Day 52 and Day 1 for banana stumps' containers, as well as Day 52 and Days 80 and 94 for CPH's containers. There was no significant difference between Day 52 and other sampling occasions for control and CPH + CPB's containers.

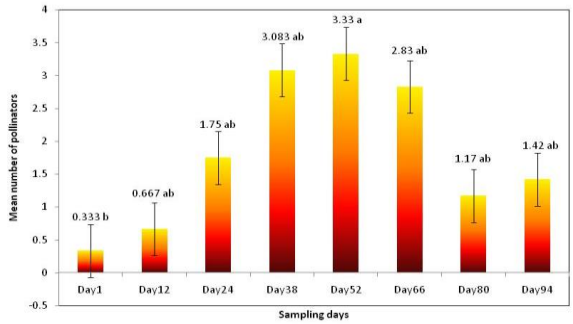

Figure 8. Number of pollinators recorded at four different ranges from banana stump containers.

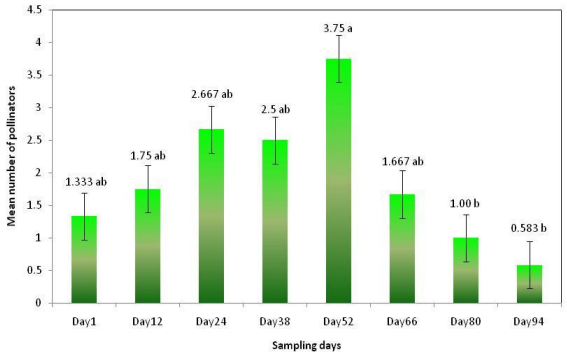

Figure 9. Number of pollinators recorded at four different ranges from cocoa pod husks containers.

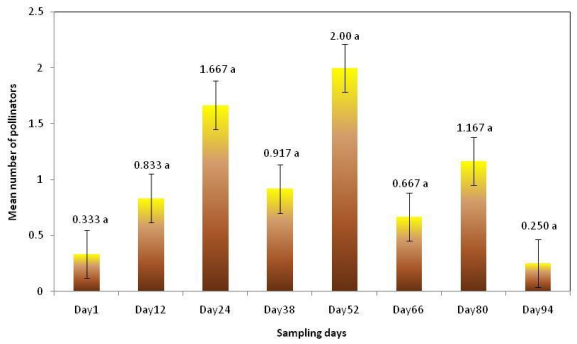

Figure 10. Number of pollinators recorded at four different ranges from cocoa pod husks + CPB containers.

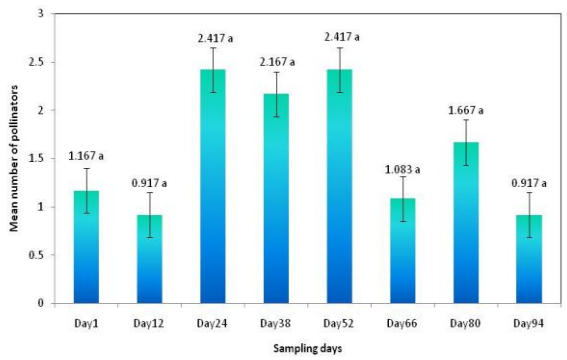

Figure 11. Number of pollinators recorded at four different ranges from cocoa pod husks + CPB containers.

Results obtained from a field study showed that the number of female exceeded male pollinators in all sampling occasions. Female *Forcipomyia* have a few good specific characters, and the males may often be identified with the characters of the peculiar genitalia [14]. This observation was in agreement with previous findings from Reference [6] that denoted females frequently visited cocoa flowers more than male pollinators. Adult females were observed to actively pollinate cocoa flowers while the males were only observed perching on flower petals. Reference [3] also denoted that both sexes are involved in pollinating cocoa flowers, however the females are more effective than the males. Meanwhile Reference [15] recorded that only female midges have been clearly implicated in cross-pollination, and [10] denoted that males either did not pollinate or only very rarely pollinated cocoa flowers.

Number of pollinators captured throughout the study for both male and female pollinators was highest at Day 52. All substrates harbored highest number of pollinators captured at Day 52, compared with other sampling dates. This may suggest that during the early rotting process of all substrates, number of pollinators were low, due to low moist contents inside the containers. However, the moisture gradually increased through the rotting process, thus provides more suitable places for pollinators to breed. Based on the behavior of Ceratopogonids that require cool, shaded and moist habitat for various activities [6], the micro-environment inside the containers may suitable for their breeding activity. After Day 52, the substrates start to dry, and from all observations, it was clearly seen that the number of pollinators caught were decreased over time.

Placement of transferable breeding containers near to the cocoa trees will harbored higher number of pollinators, compared if the containers were placed far from cocoa trees. Number of pollinators captured was highest at a range of 2 m from breeding containers, compared with 5, 8 and 12 m. Cocoa pollinator is believed to fly for short distances up to 6 meters, although their foraging range can be enlarged up to 35 meters by wind [2]. The populations are expected to depend on adjacent habitats closed to the available breeding sites due to their limited flying capability. As the sampled blocks consists of mature cocoa trees, the air-movement became less impeded the average distances flown by the midges between pollinators increased, though distances remain shorter in the dense canopy than below the canopy [15]. Reference [15] also suggests that as the cocoa trees became taller, the influence of local convection currents becomes less than of general air-movements determined by conditions over a much wider area. The flights between pollinations must often be short yet must at times be considerable due to this small midges carry and deposit mixtures of pollen derived from previously-visited flowers. Some foreign pollen may be deposited at least 75 meters into a plot, and pollen may be carried over much greater distances. Thus, the cocoa canopy must be pruned to allow easier flying for these very small midges. Flight distances seem greater in open conditions below the canopy of mature cocoa than within the dense canopy, suggesting that the pollinators are dependent on air-movement within the cocoa plantations.

Containers with cocoa pod husks harbored highest number of pollinators, followed by banana stumps and control for males and females pollinators. This finding was contradicted with the result by Reference [12], which found that the populations of pollinators were significantly greater under rotten banana pseudostem compared to cocoa pod husks, both field and laboratory studies. Surprisingly, the number of pollinators recorded at control trees was higher than the combination of cocoa pod husks and CPB's infested pods. This might be due to infested pods may have less nutrient content, compared with non-infested pods thus less preferred by the

pollinators to breed. The results suggest that scattering CPB's infested pods may not provide significant impact on the population of pollinators. Furthermore, leaving large amounts of CPB infested pod husks near cocoa trees will conflicts with the management of CPB, therefore infested pods should be removed from the cocoa field, to deter their life cycle.

Providing of breeding substrates inside transferable breeding containers may helps in population enhancement in the cocoa field. Each of the substrates used in these studies was easily found on the cocoa field and had significance in the cocoa ecosystem, as they all harbored the *Forcipomyia* spp. However, in most of the cocoa plantation in Malaysia, oversimplification of mono-cocoa ecosystem with *Gliricidia macculata* was planted as permanent shade trees, while banana only planted during the early stage of planting cocoa trees. Thus, it was suggested that banana trees can be also planted in the mature cocoa field at specific locations [9], and this will provide continuous breeding sites throughout the years. Standing plantain and banana in cocoa farms at boundaries could thus be manipulated to augment the midge population especially during dry season.

The overall observation after 94 days has suggested that cocoa pod husk can be a good breeding substrate for pollinators. The supplement of the breeding materials was successful to increase their population by placing the fresh substrates and allow them to rot later to be colonized by the ceratopogonids [15]. However, rotting process of the pod husks inside the containers takes longer period before it becomes suitable substrates for cocoa pollinators compared if the substrates left on the ground. As we know, if the substrates was placed scattering in the cocoa field, drying up process become faster, hence depriving the pollinators from colonizing them as a breeding substrate. Placement of banana stumps longitudinally through the block harbored low number of both sexes of the pollinators [11]. She suggested that banana stumps should be placed together as a heap and stumps should be piled a meter or so high, thus the bottom of the piles remains moist during dry season. Under natural conditions in the field, rotten substrates may become too moist as a consequence of heavy rainfall, especially in the rainy seasons will deter pollinators from breeding on it [9]. In contradict, the small holes that made for each breeding container may reduce excessive moisture, leaving appropriate conditions inside the containers. Greater retention of moisture, especially at the bottom of the heaps inside the containers by stacking the substrates may help in providing more suitable places for the pollinators to breed. In addition, the organic matters in all the transferable breeding containers will dry up faster during dry period, and then kill the developing pollinator populations.

In general the capture rates of pollinators in this study were low every sampling occasion. It may occur due to their small sizes, flying behavior and timing of attending the flowers. The number of midges may be higher if the observation will carry out in the middle of the day when the pollinators appear to be resting [15], however this needs destructive sampling of the cocoa flowers, thus may reduce the production of cherelle later on. In addition, low number of pollinators recorded in this study may also affected by a long dry season throughout the sampling occasions. As reported by References [4], [13], the midge population was high during the rainy season and was very much reduced toward the end of the dry season when intensively flowering occurs.

The maintenance and encouragement of major breeding sites is important in stabilizing the population of pollinators, especially during dry seasons [10]. Suitable breeding substrates inside the containers will allow pollinators to lay their eggs in the moist decomposing materials, and the larva will obtained foods from the molds and fungi that develop on the rotting materials [8]. The study concluded that although low population of midges was recorded throughout sampling occasions, significant higher number of pollinators at the cocoa trees was observed near the transferable breeding substrates. Consequently, in accordance to the higher number of pollinators captured within 2 m, the use of transferable breeding containers stuffed with cocoa pod husks and placed near the cocoa trees should be considered as the most appropriate action in enhancing the population of *Forcipomyia* spp. in the cocoa field.

IV. Conclusions

As we know that additional substrates should be provided on the cocoa field for population enhancement of cocoa pollinators, thus the substrates must be practical and easily found in the cocoa field. The results denoted that by providing breeding substrates in suitable containers may help in the population of *Forcipomyia* spp. to built-up. The use of transferable breeding substrates which are dark, closed-lids were served as suitable breeding containers due to the avoidance of direct exposure from the sunlight, which might dry up the heap more quickly as compared if just by scattering the substrates in the cocoa field. Consequently, in accordance with the results, cocoa pod husks should be considered as the most appropriate substrate for pollinator activity in the cocoa plantation. Continuously replaces the substrates inside the transferable breeding containers, and manual movements of the containers throughout the cocoa block could be encouraged. In conclusion, the implementation of 'waste to wealth' concepts with the use of debris from the cocoa fields filled in the transferable breeding containers may help in increasing the population of cocoa pollinators. As a result, the chances of cocoa flowers being pollinated are increased and subsequently improved the productivity later on. Moreover, the technique is cheap, less technical know-how and can be easily adopted by cocoa growers.

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