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Effective Time Duration of Chinese Electronic Fly Catcher Trap Bait on the Best Performance

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Abstract—Chinese Electronic Fly Catcher Trap (CEFCT) baits are found to be the most effective baits used in attracting flies in Malaysia as has been proved by previous studies. The manufacturer of this bait failed to reveal the material components for this bait, which thus, precludes other researchers attempt to reinvent baits that are have imitates such effects as CEFCT bait. It is necessary for researchers to investigate the phenomenon why this bait remains the best bait due to a major challenge in identifying effectiveness of time duration in this bait because of some factors. It was revealed that this bait is very expensive and the supply of this bait in market is quite less. Hence, this makes this research more significantly invaluable because the researchers identify the factors that makes CEFCT bait as the most effective in attracting flies in terms of bait performance. Chemometric analysis (cluster analysis CA & discriminant analysis DA) and spread plate bacteria analysis on Nutrien Agar (NA) were carried out in order to determine the effective time duration for the best performance of the bait. Chemometric analysis was conducted by running eight sample of CEFCT baits using Gas Chromatography Mass Spectrum (GC-MS). Furthermore, for spread plate bacteria count analysis, eight samples of CEFCT bait were prepared with different solution combinations. It was plate on NA agar and bacteria that were counted on day 2, 3 and 4 only. Each time effective analysis was run by using eight discriminant variables: 30 minutes, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, control CEFCT powder, and control molasses ($p < 0.05$). Therefore, it was observed that the CEFCT bait bioassays analysis shows that CEFCT baits were very active up to 30 minutes through CFU count in day two until day four (9.28, 9.34, and 9.35 lofcfu/ml). Although, for Chemometrix analysis, CEFCT bait can achieve its time effectiveness through CA and DA according to three group of time effectiveness given by correct classification (100%, 75%, and 75%). The results indicated that chemometric techniques can be used for rapid assessment of time duration of CEFCT bait. Further researches are needed to identify low-cost bait components with similar effect of the best bait (CEFCT) in local markets in order to develop new low cost baits.

Keywords— CEFCT Bait, Chemometric, Cluster analysis, Discriminant analysis, Bacteria Plate Count.

1. INTRODUCTION

In general, insects are the group of animals, living invertebrates, or insect class, which have the highest number of species [1]. Controlling house flies in different ways give different impacts. As sanitation, exclusion, chemical means and non-chemical means can be ways of controlling flies. Therefore, baits are considered as alternative ways to control house flies effectively. An experiment conducted on different baits in Kuala Terengganu, Terengganu Malaysia found Chinese Electronic Flies Catcher Trap (CEFCT) bait as the most effective in attracting flies [2]. Mostly, CEFCT bait has the highest ability to attract house flies compared to other baits and this was probably due to a number of design factors which include weather. The main challenge encountered from this bait, is that researchers do not know the components involved in this bait and why they are effective in attracting flies. Thus, this experiment will test on the time effectiveness of CEFCT baits using bioassay analysis and chemometric analysis.

The first experiment was conducted using microbiology test in order to determine the time duration of CEFCT by using bacteria plate count. The common procedure used for enumeration of bacteria is the viable plate count [3]. In this method, serial dilutions of a sample containing viable microorganisms are plated onto a suitable growth medium [4]. The suspension is either spread onto the surface of agar plates (spread plate method), or is mixed with molten agar; poured into plates, and allowed to solidify (pour plate method) [3]. Then, the plates are then incubated under conditions that permit microbial reproduction, so that colonies develop and can be seen without the aid of a microscope [5].

It is assumed that each bacterial colony evolves from an individual cell that has undergone cell division. Therefore, by counting the number of colonies and accounting for the dilution factor, the number of bacteria in the original sample can be determined [6]. There are several drawbacks to the viable count method. The major disadvantage is that it is selective and therefore biased. The nature of the growth conditions, including the composition and pH of the medium used, as well as conditions such as temperature, determine which bacteria in a mixed population can grow [5]. Since there is no universal set of conditions that permits the growth of all microorganisms, it is impossible to enumerate all micro organisms by viable plating. Though, this same disadvantage, however, becomes advantageous when one is interested in only a specific microbial population [5]. For example, selective procedures can be designed for the enumeration of coliforms and other physiologically defined microbial groups.

Thus, using bacteria plate count method suits the purpose and is deemed appropriate for this study. The viable count is an estimate of the number of cells [5]. Because, some organisms exist as pairs or groups and due to the fact that mixing and shaking of the sample does not always separate all the cells, hence, taking a count of the "colony forming units" is thus preferred. One cell or group of cells will produce one colony, therefore when the results for a viable count are recorded, it is customary to record the results as colony forming units per ml (cfu/ml) or per gram (cfu/g) of test material [3]. For the reason that, researchers generally lack idea of how many bacteria are in a sample, therefore, it is almost always necessary to prepare a dilution series to ensure that a dilution containing a reasonable number of bacteria to count is obtained [5]. Dilutions in the range 10-1 (1/10) to 10-8 (1/100,000,000) are generally used, although with particular types of samples the range of dilutions can be restricted [3].

However, chemometric methods used has not yet been widely utilized for interpreting the performance of bait. Few studies on chemometric analysis have been used to clarify and justify the significane of bait performance [7] [8]. Thus, for this particular study, the researchers used Spread plate bacteria count analysis in order to determine the effective time for the performance of the best bait [9]. Enumeration process of CEFCT bait can be used to identify the time duration of best bait using bacteria plate count. It is because the pattern of bacteria growth can give impact to its odor. The more bacteria numbers the more effective bait. Importantly, some researchers also used this method in determining the number of cells per unit volume of a suspension under a microscope by using counting chamber in a biology lab [10]. Furthermore, this study used chemometric analysis in order to identify the time duration of CEFCT bait. Cluster analysis (CA) and discriminant analysis (DA) were used to determine the time duration of best bait chosen. Thus, the aim of

this research is to analyse CEFCT bait in order to identify the number of bacteria, and the time duration analysis.

According to [11] and [12] chemometric techniques have often been used in data analysis tools exploratory for classification of the sample. It has also been used to identify pollution sources as opined by [13]. This technique is useful for the researchers in identifying common patterns in data distribution that allow for the identification of possible factors/sources that cause baits sample hygiene problems for it to be useful as a friendly product. In many other cases, the exploratory data analysis results serve to gain an insight into: for example, the contamination situation of a certain location and make a plan for remediation or prepare more focused sampling plans. Equally, multivariate techniques consider a number of factors that control data variability simultaneously [14] and therefore, offer significant advantages over univariate techniques.

2. Material and Method

A. *Identifying Effectiveness of Time Duration in CEFCT Bait Using Microbiology Approach*

1. *Sample Preparation of CEFCT Bait*

A total of eight samples of Chinese Electronic Fly Catcher (CEFCT) bait combined with molasses were prepared to eight different times presented as A-30minutes, B-2hours, C-4hours, D-6hours, E-8hours, F-12hours, G-Control CEFCT Bait and H-Control Molasses. Each sample was according to Label saline tube of CEFCT bait for the eight different samples (A-30minutes, B-2hours, C-4hours, D-6hours, E-8hours, F-12hours, control CEFCT powder and control molasses). Each sample was combining its solution of CEFCT powder and molasses together.

2. *Dilution Process on CEFCT Sample*

Dilution process was done to make sure that the number of bacteria grow in the media can be counted. This is because the number of bacteria counted should not be less than 30 and not more than 300. Each sample was diluted using distilled water (DH_2O) for six-time dilution. Six saline tubes were labeled as 9 ml DH_2O (10^{-1}dilution), 9 ml DH_2O (10^{-2} dilution), 9 ml DH_2O (10^{-3} dilution), 9 ml DH_2O (10^{-4} dilution), 9 ml DH_2O (10^{-5} dilution) and 9 ml DH_2O (10^{-6} dilution). And this step needs to be replicated for eight types of different samples. Aseptically removed 0.1 ml of sample A with a sterile pipette and transferred it to the 9 ml DH_2O (10^{-1}dilution). Then, vortex 9 ml of DH_2O (10^{-1}dilution) tube, and continued with the transfer of 0.1 ml from 9 ml DH_2O (10^{-1}dilution) to the 9 ml DH_2O (10^{-2} dilution) tube. This step is repeated until tube 9 ml DH_2O (10^{-6} dilution) for the eight samples.

3. *Selected Media for Enumeration Process*

In this study, Nutrien Agar used as a media to grow bacteria as shown in figure 1.

Fig. 1 Images of Selected Nutrien Agar

4. *Plating CEFCT Sample into Selected Agar*

Three BHI Nutrien Agar plate were labelled as $\log10^{-4}$, $\log10^{-5}$, and $\log10^{-6}$ respectively, and so were this step repeated for all the eight types of samples. Using a new sterile pipette, a transfer was made aseptically of 1.0 ml from 9 ml DH_2O (10^{-4} dilution) tube to the plate of different samples labeled $\log10^{-4}$ A, $\log10^{-4}$ B, $\log10^{-4}$ C, $\log10^{-4}$ D, $\log10^{-4}$ E, $\log10^{-4}$ F, $\log10^{-4}$ G, and $\log10^{-4}$ H . Then, the sample is spread in the plate using hockey stick on the surface of the agar. And, the above steps were repeated from $\log10^{-5}$ and $\log10^{-6}$ sample dilution to the plates, respectively. Subsequently, the surface of the agar is then allowed to dry before the plates are moved or inverted. Each dilution was plated in triplicates, and the plates were incubated at 37˚C for 48h, 72h, and 96h. All colonies on the replicate plates were then counted and expressed as mean V cholera CFU per (g/ml). After incubation, the plates were counted on a standard colony counter as per manufacturer instructions and the results were recorded as number of colony forming units (CFU). For colonies enumeration, all colonies were counted on day two, three and four. The number of Colony Forming Units was reported as $\log_{10}$ CFU per cm^2.

5. *Counting for Microorganisms*

After incubation, the colonies on each of the plates were counted. While, holding the plate in a light source, the colonies are then counted by marking their position on the back of the petri plates with a marking pen. This method assists in keeping track of those colonies previously counted in order to avoid recounts. For a plate that has more than 300 colonies, it is recorded as TNTC (too numerous to count). From the plate count data, the concentration of bacteria is calculated in the original sample. For statistical reasons, data from plates which have between 30 and 300 colonies is used only in this calculation. Each colony forming unit (cfu) represents a single cell or a group of cells attached together that are inseparable by shaking.

Therefore, the number of cfu in the original sample is determined by multiplying the number of colonies on a dilution plate by the corresponding dilution factor. For example, if there are 200 colonies on the 10-4 plate, then there are 200 x10,000 = 2,000,000 colonies or 2 x 10^6 cfu/ml in the original sample. Generally, replicates of each dilution are plated, and the mean count is recorded. Thus, the mean of the data from all groups in the lab would be an excellent estimate of the number of bacteria in the original sample.

Fig. 2 Images of Agar Plate placed in an Incubator Machine at 37^{o}C

6. *Statistical Analysis of Bacteria Plate Count*

Statistical analyses were performed using SPSS Statistics 19. Statistical significance for all tests was determined at alpha value of $p \leq 0.05$ and descriptive statistics were calculated for all variables as appropriate. Mean bacterial counts on Bacteria Plate Count is used for enrichment, cultivation and isolation of all species of bacteria on Nutrien Agar. All indicators were compared using one-way analysis of variance (one-way ANOVA).

B. *Identifying Effectiveness of Time Duration in CEFCT Bait Using Chemometric Approach*

1. *Solid Phase Extraction*

In this phase, gas chromatographic mass spectrometry (GCMS) was used to separate all the compounds. Therefore, solution combination of CEFC bait with molasses sugar was blended together. The solution which was used to extract the bait sample is Acetone, Benzol, Chlorophorm, Hexane and Methanol [7]. This CEFCT bait solution is only extracted in Methanol. A 4.4 gram of molasses was added with 0.4 gram of bait (Chinese electronic fly catcher bait). The solution was conducted for eight different times as labelled A - H (A-30minutes, B-2hours, C-4hours, D-6hours, E-8hours, F-12hours, G-control powder sample, and H-control molasses sample). After completion, all the samples are added along with 4.0ml of methanol for extraction. These mixtures were extracted overnight using methanol solution. The extracts were held at 4˚C until analysis. After the samples were extracted overnight, it was then filtered twice. The first filtering used filter paper, while the second filtering with shrink filter. After filtering, 400µL from the sample was added to the dilution two times as log10 (3.6ml methanol) and log100 (3.6ml methanol) respectively. The samples for log100 dilution were placed into a tube and was then run in a GC-MS machine.

Fig. 2 Gas Chromatography - mass spectrometry Machine

7. *Gas Chromatographic Analysed*

A 1-µL from each extract was analysed using a trace gas chromatography - mass spectrometry (GC-MS; QP2010, AOC-205 Shimadzu Auto Sampler column (30 m × 0.25 mm inner diameter, film thickness was 0.25 µm) as shown in figure 2. The GC-MS oven temperature control comprised of an initial hold at 35˚C for 6 min, then step it up at 10˚C/min to 260˚C, and followed by a final hold for 5 mins at 260˚C. The infusion port was held at 260˚C in split less mode, while the exchange line was set at 260˚C, and the bearer gas was set to a steady flow of 1.2 mL/min.

8. *Chemometric Approach on CEFCT Bait*

Environmental data sets are usually complex and contain a large amount of information with internal relationships among variables, often in a partially hidden structure. The goal of chemometric studies is to display the most significant patterns, looking for possible groupings and sources of data variations, as well as for their temporal and geographical distributions through resolution and modelling of the raw data [8]. After data conversion into a single matrix formed by concentration values for each combination of variables and cases, a stepwise statistical approach was used employing the following exploratory techniques: cluster analysis, and discriminant analysis.

8.1.Cluster analysis

Cluster analysis, was applied to discover natural groupings within real data in terms of samples similarity and it also an unsupervised technique. According to [11], and [15], squared Euclidean distance was always used as the interval measure for clustering using distinct linkage methods: between groups linkage, within groups linkage, and Ward's method. Raw data was computed after standardization based on Z-scores by variable. Therefore, cluster analysis groups the objects (cases) into classes (cluster) based on similarities within a class and dissimilarity between different classes. The results of CA help in interpreting the data and indicating patterns [11].

8.2.Discriminant analysis

Discriminant analysis determines the variables that discriminate between two or more naturally occurring groups/clusters[16]. It constructs a discriminant function (DF) for each group [15], and [17]. DFs were calculated using Eq. 1:

$$f(Gi) = \sum_{i=1}^{n} wij\ pij$$

where *i* is the number of groups (G), *k i* as the constant inherent in each group, *n* is the number of parameters used to classify a set of data into a given group, and *w j* as the weight coefficient assigned by DF analysis to a given parameter (p j). Therefore, in this study, DA was conducted to determine whether groups differ with regards to the mean of a variable, and to use that variable to predict group membership. DA was done in order to transform data by using the standard, forward stepwise, and backward stepwise modes. These were used to construct DFs to evaluate variations of the effective time to attract house flies in CEFC bait. The identified organic contaminants were the grouping (dependent) variables, while all the measured parameters constitute the independent variables. In the forward stepwise mode, variables were included step-by-step beginning from the most significant variable until no significant changes were obtained. In the backward stepwise mode, variables were removed step-by-step beginning with the less significant variable until no significant changes were obtained.

I. RESULT AND DISCUSSION

This section presents the results of the data analysis using the data obtained from the experiment conducted on a selected method. Two types of analyses were used in order to determine the effectiveness of CEFCT bait. The first ways are bacteria plate count. The more bacteria found the more effective bait. Then it follows with chemometric analysis which contains two analyses: Cluster Analysis (CA) and Discriminant Analysis (DA). For CA analysis, it distribute parameters into certain groups while DA discriminate group of parameters.

A. Experiment Result on Microbiology Analysis

1. *Data Collected from Bacteria Growth on Nutrien Agar*

The data displayed in table 1 shows the numbers of bacteria in Nutrien Agar that were counted. Three dilution samples and bacteria growth result were prepared and collected by the researchers until the fourth day. Data collection started from day two until day four. Thus, in this research, the plates that could be counted were selected only. As shown on table 1 below, TNTC presented as (Too Numerous To Count) and TFTC as (Too Few To Count). All the data collected from this experiment were presented in figure 4.9, 4.10, and 4.11.

TABLE 1 DATA OF BACTERIA GROWTH ON NUTRIEN AGAR MEDIA FOR DAY TWO, THREE AND FOUR

NUTRIEN	**LOG/CFU BACTERIA GROW**

AGAR GROW		0.3 H	2 H	4 H	6 H	8 H	12 H	C.B.	C.M
DAY 2	Dilution of (10^{-4})	TNTC	7.20	7.20	TNTC	TNTC	TNTC	TNTC	TNTC
	Dilution of (10^{-5})	TNTC	7.35	7.97	7.69	7.40	TNTC	TNTC	TNTC
	Dilution of (10^{-6})	9.28	TFTC	TFTC	TFTC	8.82	9.06	7.96	7.80
DAY 3	Dilution of (10^{-4})	TNTC	7.38	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	Dilution of (10^{-5})	TNTC	TNTC	8.10	7.91	TNTC	TNTC	TNTC	TNTC

	Dilution of (10^{-6})	9.34	TFTC	TFTC	TFTC	8.74	8.95	8.16	7.97
DAY 4	Dilution of (10^{-4})	TNTC	7.28	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	Dilution of (10^{-5})	TNTC	TNTC	8.07	7.91	TNTC	TNTC	TNTC	TNTC
	Dilution of (10^{-6})	9.35	TFTC	TFTC	TFTC	8.79	8.95	8.28	8.00

2. Significant Growth of Bacteria in CEFCT Bait

There are various results of bacteria colonies that grow on the Nutrient Agar. Table 2 gives the descripion as seen below:

TABLE 3 MICROBIAL COUNTS (LOG CFU / ML) OF V. PARAHAEMOLYTICUS IN CEFCT BAIT IN EIGHT DIFFERENT SAMPLES. DATA REPRESENT MEAN ± STANDARD DEVIATION OF THREE REPLICATIONS. ^{A,B,C} DATA IN THE SAME COLUMN WITH DIFFERENT LETTER IS DIFFERENT SIGNIFICANTLY ($P < 0.05$)

Time of bacteria grow (hours)	Number of bacteria grow (Logcfu/ml)
0.3	$9.32^{a}\pm0.04$
2	$7.29^{g}\pm0.09$
4	$8.05^{de}\pm0.07$
6	$7.84^{f}\pm0.13$
8	$8.78^{c}\pm0.04$
12	$8.99^{b}\pm0.06$
C.B	$8.13^{d}\pm0.16$
C.M	$7.92^{ef}\pm0.11$

The significance that this study found is known to be present in characteristically different aquatic environments whereby V. parahaemolyticus is mainly distributed in coastal water (Thompson & Polz, 2006)(Austin, 2006). The prevalence of V. parahaemolyticus in CEFCT bait was determined from 0.3 hours, 2 hours, 4 hours, 6 hours, 8 hours, 12, hours, control CEFCT powder, and control molasses using Viable Plate Count and plating onto Nutrien Agar (NA) with significant difference as ($p>0.05$). Table 2 below summarizes the prevalence of V. parahaemolyticus in CEFCT bait. From the sample analyzed, V. parahaemolyticus was detected highest in 0.3 hours at 9.32 logcfu/ml, and lowest in 2 hours at 7.29 logcfu/ml. The other growth of 4 hours, 6 hours, 8 hours, 12, hours, control CEFCT powder, and control molasses recorded 8.05 logcfu/ml, 7.84 logcfu/ml, 8.78 logcfu/ml, 8.99 logcfu/ml, 8.13 logcfu/ml, and 7.92 logcfu/ml respectively. Therefore, it shows that these samples were very active during 0.3 hours and shows a fluctuation in growth starting from 2 hours up to 6 hours. The numbers of bacteria started increasing from 8 hours until 12 hours.

Results have also shown that, all the samples revealed different significance except for sample 4 hours and control molasses which found the same significance as shown in the table below. This is because each sample presents a different letter, as 0.3 hours presents letter *a*, while, 2 hours presents letter *g*, 4 hours presents these letters *de*, as 6 hours, 8 hours, 12 hours, control CEFCT powder and control molasses present letters *f*, *c*, *b* , *d* and *ef* respectively.

TABLE 2 THE NUMBER OF BACTERIAL COLONIES (THE NUMBER OF VIABLE ORGANISMS) DURING THE EFFECTIVE TIME FOR WHICH BACTERIA IS GROWN BASED ON DILUTION TYPES

Dilution	Nutrient Agar Plate	Effective Time for Bacteria Growth	Number of Bacterial Colony
Second Day ($10^{-1)}$		Early 0.3 Hours	9.28

		Early 2 Hours	7.20
Third Day (10^{-2})		Early 0.3 Hours	9.34
		Early 2 Hours	7.38
Fourth Day (10^{-3})		Early 0.3 Hours	9.35
		Early 2 Hours	7.28

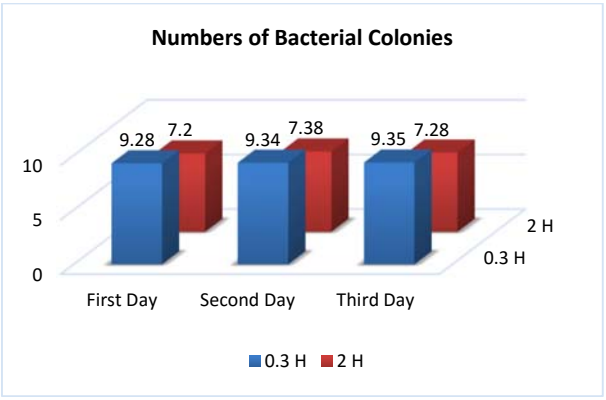

Fig. 3 The number of bacterial colonies (the number of viable organisms) during the effective time for which bacteria is grown based on dilution types.

Based on figure 3 as generated above, the results showed that for the first day 9.28 bacterial colonies were produced at the beginning of 0.3 hours or 18 minutes earlier and produced 7.20 colonies in 2 hours earlier. For the second day, the results showed that the bacteria produced 9.34 colonies early in 0.3 hours or 18 minutes earlier and produced 7.38 colonies in 2 hours earlier.

The results for the third day showed 9.35 colonies produced at the beginning of 0.3 hours or 18 minutes earlier and 7.28 colonies produced in 2 hours early. Based on the observations above, there is a comparison of production between the numbers of bacterial colonies on earliest time. 0.3 hours earlier showed that most

of the bacteria are produced compared to other periods. Hence, this proves the number of bacterial colonies (the number of viable organisms) during the effective time of bacteria growth based on dilution types is in 0.3 hours earliest.

B. Experiment Result of Chemometric Analysis

1. *Time Similarities on CEFCT Bait Performances*

The data pertaining to the distribution of time duration in the CEFCT bait sample is tabulated in Table 4 as descriptive statistical parameters. The standard deviation (SD) values related to the distribution of these time durations for CEFCT bait samples show a very high dispersion around the CEFCT concentration. Data on time duration distribution in the sample based on mean concentration in CEFCT sample shows sample H as dominant contaminant with the highest mean concentration of 2.139 ml/min, followed by sample C with 2.095 ml/min. For sample A, D, F, and G shows the same mean concentration that is 2.000 ml/min and finally followed by sample B and E with 1.999 ml/min respectively.

2. Cluster *Analysis on CEFCT Bait Performances*

CA was carried out to detect similarities in groups between the time sampling. The data sets were treated (after data scaling by z-transformation) by the Ward's method of linkage with squared Euclidean distance as a measure of similarity. The dendrogram of the time of different groups was presented in Fig. 4. It shows that the effective time for attracting flies can be grouped into three clusters. Cluster 1 is formed by the D, C, and E. This sample of time is in the middle time where solution takes place. Furthermore for second cluster, there are sample B and F. Sample B presents 2 hours solution preparation, while F presents 12 hours solution preparation. It shows that at this time it would have the same effect. Moreover these clusters show the lowest effect among all the three groups of clusters. One of the reasons for second cluster is because the lowest effect may be influenced by the number of flies in the surrounding. Final custer consists samples of H, A, and G. This third cluster shows the most effective time to attract flies. This cluster presents 0.3 hours for sample A, control CEFCT powder for sample G, and control molasses for sample H. Among all of these three samples, sample H appears with the highest impact as shown in the figure 4.

TABLE 4 BASIC STATISTICAL PARAMETERS OF TIME DURATION IN CEFCT BAIT SOLUTION (ML/MIN)

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
A	50	0	50	0.470	18.000	2.000	2.803
B	50	0	50	0.250	24.440	1.999	3.893
C	50	0	50	0.250	23.520	2.095	3.624

D	50	0	50	0.260	25.680	2.000	4.093
E	50	0	50	0.260	23.320	1.999	3.508
F	50	0	50	0.260	28.130	2.000	4.350
G	50	0	50	0.200	23.490	2.000	4.741
H	50	0	50	0.170	22.100	2.139	4.202

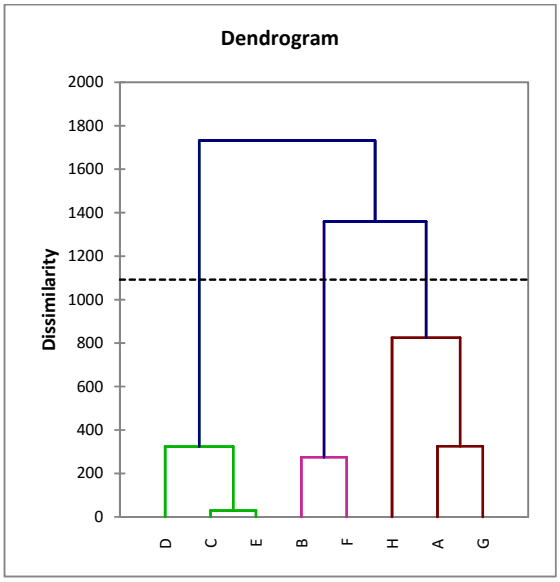

Fig. 4 Dendogram of Clustering sampling of CEFTC Bait according to Eight Sample of Different Time using Ward's Method

3. *Discriminant Analysis on CEFCT Bait Performances*

Spatial DA was evaluated using standardized data of CEFCT bait solution and was thus divided into eight samples. Clearly, the plot of discriminant functions obtained from CEFCT bait solution samples (Fig. 5) shows that the time duration of this bait can be divided into three categories: early stages, middle stages and final stages of CEFCT bait solution time preparation. Classification matrices of CEFCT bait solution data sets obtained from standard, forward stepwise, and backward stepwise modes of DA are shown in Table 5.

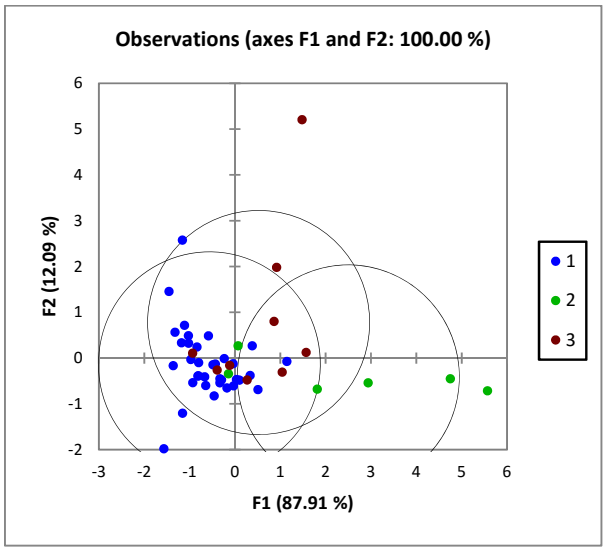

Fig. 5 Plot of Discriminant Functions Showing Three Categories of Different time effectiveness in CEFCT Bait

TABLE 5 CLASSIFICATION MATRIX OF DA FOR ALL DATA MEASUREMENTS IN CEFCT BAIT SAMPLES

Sample Sources	% Correct	Sources assign by DA			
		1	2	3	Total
Standard DA mode					
1	100.00%	42	0	0	42
2	75.00%	1	3	0	4
3	75.00%	1	0	3	4
Total	96.00%	44	3	3	50
Forward stepwise DA mode					
1	100.00%	42	0	0	42
2	75.00%	1	3	0	4
3	50.00%	1	1	2	4
Total	94.00%	44	4	2	50
Backward stepwise DA mode					
1	100.00%	42	0	0	42
2	50.00%	1	2	1	4
3	50.00%	1	1	2	4

Total	92.00%	44	3	3	50

The standard mode yielded the corresponding correlation matrices achieving 96.00% precisely using 44 variables (Table 5). The forward stepwise mode yielded 94.00% precisely as classified using only four discriminant variables (class 1, 2, and 3) whereas the percentage of cases precisely classified in backward stepwise mode is 92.00% using only three discriminant variables (class 1, 2, and 3). Thus, the DA results suggest that class 1, 2, and 3 are the significant parameters (Table 5) that discriminate time differentiation detected in CEFCT samples between the sampling parameters. The results from the analysis of the extracted CEFCT bait show many different organic components with varying functional groups. Thus, microbial and chemometric application found that extract from H sample (molasses) is the significant parameter that attracts flies. From H sample extract, six volatile compounds found were identified as Pentadecanoic acid methyl ester, Tetradecanoic acid methyl ester, Hexadecanoic acid methyl ester, Hexanoic acid, Butanoic acid and octanoic acid. All the six compounds were present throughout the first day sample period extract. Higher concentration of compounds produce smell of bait released in huge diameter. However, smell reaction did not give the behavioural criticalness of flies as to whether their capacities as a pheromone, allomone or kairomone. According to [18], it was concluded that butanoic acid incite a positive reaction from house flies, though butanoic acid elicited a positive response from house flies. Another study that was directed on unpredictability of molasses furfuryl liquor, but it is officially found in hexane and ether extracts [19]. Other similar concentrates additionally found a substantial number of esters including methyl benzoate, ethyl formate, ethyl benzoate, and others are demonstrative of microbial change item [19]. Some developments in nearness are more often than not credited to anaerobic microscopic organisms than those believer hydroxyl gatherings to methyl and ethyl esters. A few cases of conceivable mixes of esterification are found in this study incorporating Pentadecanoic acid methly ester, Tetradecanoic methyl ester, Eicosapentaenoic acid, Hexadecanoic acid and Nervonic acid. A Study conducted by found that the occurrences of pesticide including methyl ester compound in the CEFCT bait was successfully interpreted by chemometric techniques.

II. Conclusion and Recommendation

The general aim of this research is to find out which time parameter used is very effective in attracting flies. In addition, the research then continues to verify the active compounds in order for further studies to create new baits that have similarities with Chinese Electronic Fly Catcher trap (CEFCT) bait in terms of efficiency to attract flies. Moreover, this study also found that by comparing the concentrations of these compounds to the number of microbial bacteria that affect the attraction of house flies and identifying the components responsible for the attraction of house flies to fresh molasses show positive relationship. Fresh molasses has been used as an attractant either by itself or in a mixture with other ingredients such as the Beltsville Bait. This study can also be furthered to look into the ideas and standards of bait assembling from Islamic perspective. Understanding the capability of this huge Halal (Shari'ah Compliant) market, numerous manufacturer organizations have been attempting to attaining the high ground by realigning their assembling practices to be in consistence with Shari'ah (Islamic Jurisprudence) [20].

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