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Development and Evaluation of Diets for Rearing *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) and *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) using Readily-available Materials

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ABSTRACT

Cadra cautella and *Corcyra cephalonica* are economically-important stored-product pests. Mass culturing of these species is a requirement to have them in sufficient numbers to be used in experiments. While the diet has a direct influence on the development and performance of the larvae and emerging moths, there are limitations associated with the currently-used rearing media. The objective of this research was to develop a suitable rearing medium for *C. cautella* and *C. cephalonica* using readily-available food materials at the market. Food materials were mixed in different combinations and the larvae were introduced. Nine diets for *C. cautella* and ten diets for *C. cephalonica* were tested. The eggs of a particular moth species were introduced into each replicate medium. The larvae were weighed at 17 and 21 days following egg introduction The total duration of adult emergence and the number of F_2 generation larvae produced by one adult moth in each diet were also recorded. The measurements varied across the media used. Honey and glycerol when combined with red-rice or rice-flour + rice-flakes + dog-food integration gave highest performance in *C. cautella.* For *C. cephalonica,* the best medium was honey and glycerol combined with rice-flour, rolled oats and mung bean. Our study highlights the successful development of new diets for efficient rearing *C. cautella* and *C. cephalonica* using food ingredients readily available at the local market.

1. Introduction

Serious losses occur during various operations following harvest of agricultural produce until consumed. The losses can be of different types such as quantitative, qualitative or economic; quite high; and vary with the crop, geographical location, type of operation, etc. [1]. Insects are a major destructive factor of stored food in tropical environments due to the existence of the conditions required for the growth and development of the alleged insects [2]. In Sri Lanka, insects account for nearly 80% of grain loss during storage [3]. Moths of Family Pyralidae are found during storage of paddy and other cereals under high temperature and humidity conditions. In general, the moth species damage a wide array of stored food [4] and accompany certain identical biological/ physiological adaptations including diapause [5] thus necessitating differential approach on the management strategies of stored products.

Cadra cautella (Walker) and *Corcyra cephalonica* (Stainton) are economically important stored-product insect species found throughout the globe [4]. The two species can coexist as well [6]. *Cadra cautella* larvae feed on grains, flour, nuts, dried fruits, seeds and other food products [4].*Corcyra cephalonica* infestation in rice is more evident, but it also attacks other commodities including wheat, maize, sorghum, groundnut, cotton, coffee, spices and cocoa beans [7]. Both *C. cautella*[4] and *C. cephalonica* [8] develop quickly under tropical conditions common in Sri Lanka.

Mass rearing of Lepidopterans is requiredfor various experimental purposes [9]. *C. cephalonica* is also used as a host for nematodes and mites [10]. In biological control programs where certain moth species are used for culturing parasitoids, rearing conditions determine the successful growth of moth larvae [11] and the opportunity to develop mites [12].

The ability of moths to infest stored food and the factors affecting their colonization has been widely

studied [13]. It is desirable to have a Low variation in growth and development during mass rearing of moths. [14]. In lepidopteran species, the larval diet has a direct impact on the rate of development, reproduction, and survival [15]. Issues related to obtaining sufficient amounts of progeny and their performance occur despite the availability of rearing media for moths.

In many moth diets, glycerol and protein sources such as wheat germ and brewers' yeast are included. During the maintenance of diet over time, glycerol helps to decrease development of molds [16]. However, convenient and efficient extraction of larvae from the rearing medium in a timely manner is also a requirement when the larvae have to be used in higher numbers. Also from a practical point of view, it is imperative that the developed rearing media to be cost effective. Considering these requirements, we aim to develop a suitable rearing medium using readily-available materials that facilitates growth and development of *C. cautella* and *C. cephalonica* larvae.

2. Material and Methods

2.1 Insects

Larvae of *C. cautella* were introduced to 200 g of crushed red-rice in a plastic bottle and covered with a piece of cloth. Similarly *C. cephalonica* larvae were introduced into 200 g of crushed maize in a plastic container, and the opening covered by a piece of cloth. The insect cultures were maintained inside growth chamber (FH-1200 LED T8, HiPoint Laboratory, Taiwan) at 35±0.5 °C, 65±1% relative humidity (RH).

2.2 Collection of eggs

To collect eggs, thirty unsexed adult moths of each species (*C. cephalonica* and *C. cautella*) were introduced into plastic bottles, each species separately. The adults reared in mother cultures were collected into empty plastic bottles using vacuum pump (Rocker 300, Rocker Scientific Co. Ltd., New Taipei City). Each bottle was covered by a nylon mesh and placed upside down on a Petri dish. The eggs on Petri dishes were collected and used in the experiments within 24 hrs.

2.3 Diets

The following readily-available food materials purchased from a local market were used to prepare the diets. They were red-rice, rice-flour, rice-flakes, wheat flour, soybean, groundnut, oat, mung-bean, maize, dates, raisons, dog-feed, broiler feed, honey and glycerol. Red-rice, soybean, mung-bean and maize were washed thoroughly with water and allowed natural air drying in shade condition under room condition for 2 days followed by oven drying at 42 ºC for 8 hours in order to kill possible living organisms if present. Rice-flour, rice-flakes, wheat-flour, groundnut, oat, dates, raisins, dog-feed and broiler feed were directly oven dried as mentioned before used. The food ingredients were ground as required by using a grinder. Red-rice, dog-feed, broiler starter and rice-flakes were used as brokens. Dried raisons, dates, groundnut and soybean were ground and used. Rolled oats, rice flour and wheat flour were used in the existing form without further processing. As mentioned in Table 1, first the dry ingredients were mixed well in a plastic bottle, and then liquid components honey and glycerol were added. From each diet 100 g was used in a plastic container which served as one replicate. From each diet, 4 replicates were used.

Table 1: Composition of different diets developed and tested

Diet	Honey (mL)	Glycerol (mL)	Red-rice (g)	Rice flour (g)	Rice flakes (g)	dog-food (g)	Soy-bean (g)	Broiler feed (g)	Ground nut (g)	Wheat flour (g)	Raisin (g)	Rolled oats (g)	Mung bean (g)	Dates (g)	Maize (g)
1	5.5	5.5		25	50	25									
2	5.5	5.5	100												
3	5.5	5.5					50	50							
4			50											50	
5	5.5	5.5						50	50						
6	5.5	5.5		10								60	30		
7			30					40	30						
8						25		25		50					
9					50						50				
10															100

2.4 Introduction of eggs to diets

The eggs collected as mentioned in section 2.2 were observed under a microscope (OPTICA, Triace, Italy), and only the plump eggs were used. They were gently introduced into diet media using artist's brush (No. 0) [5]. Sixteen eggs of each species (*C. cautella*or *C. cephalonica*) were gently introduced to one replicate of a particular diet in 500 mL plastic bottle and maintained inside the incubator at 35±0.5 °C, 65±1% RH. The diet samples were observed daily to detect emergence of larvae and subsequently adults.

2.5 Determination of weight of larvae and emergence of adults

Ten diets were tested for *C. cephalonica* while 9 diets (except maize) were tested for *C. cautella*. The larvae were weighed 17 and 21 days following egg introduction. For this, each diet was manually dissected to collect the larvae. The larvae were released by carefully removing the webbing, frass and food materials around without damaging them. The weight of 10 larvae was determined by using analytical balance. The average weight of a single larva was determined in each replicate diet. After determining the weight, all the larvae were placed back in the same replicate diet, and all the media were maintained inside the incubator for further development. The adults emerged in each replicate diet was recorded daily for two weeks.

2.6 Determination of emergence of larvae in F_2 generation

All the *C. cautella* and *C. cephalonica* adults emerged in a replicate diet were transferred into a fresh replicate of the same diet (100 g each) in which they emerged. Adult transferring process was practiced twice a day for two weeks following the first adult emerged in that particular replicate diet. After two weeks, the larvae emerged in each replicate were counted by dissecting the diet. Total duration of adult emergence in each diet and number of larvae of F_2 generation produced per F_1 adult emerged in each diet were determined.

2.7 Data analysis

The weight of larvae, number of days taken for adult emergence and number of larvae emerged in the second generation in different diets for *C. cautella*and *C. cephalonica* were analyzed using Proc GLM of ANOVA using Statistical Analysis System (SAS) [17]. Mean separation was done using Tukey's test and the significance was tested at P=0.05 level.

3. Results

3.1 Weight of *C. cautella* larvae reared in different diets

The weight of *C. cautella* larvae measured at both 17 and 21 days following the egg introduction significantly differed among food media in which they were reared (P<0.001). The highest average larval weight was obtained in the media comprised of dog-feed + rice-flour + rice-flakes + glycerol + honey (diet 1),red-rice + glycerol + honey (diet 2) , soybean + broiler-feed + glycerol + honey (diet 3); and did not differ significantly among these food media. In contrast, the lowest average weight of larvae was recorded in dried grapes + rice-flakes (diet 9) and did not differ significantly from wheat flour + broiler-feed + dog-feed (diet 8). The larvae reared in the media comprised of dates + red-rice (diet 4), groundnut + broiler-feed + honey + glycerol (diet 5), rolled oat + rice-flour + sprouted mung bean + glycerol + honey (diet 6) and groundnut + red-rice + broiler-feed (diet 7) recorded an intermediate weight (lower the highest maximum weight and higher than the lowest weight) (Figure 1).

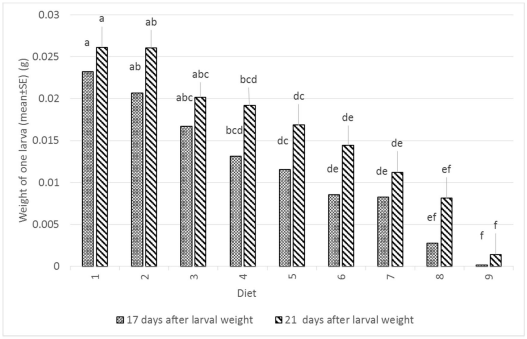

Figure 1: Average weight of *C. cautella* F_1 larvae (For a given duration, means followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA).

3.2 Weight of *C. cephalonica* larvae reared in different diets

Average weight of larvae of *C. cephalonica* at 17 and 21 days following the egg introduction significantly differed among the food media tested (P<0.001).The highest average larval weight was obtained in the media comprised of dog-feed + rice flour + rice flakes + glycerol + honey (diet 1) and rolled oat + rice flour + sprouted mung bean + glycerol + honey (diet 6), and did not differ significantly among these food media. The lowest average larval weight was recorded in media with soybean + broiler-feed + glycerol + honey (diet 3), groundnut + crushed red-rice + ground broiler feed (diet 7), wheat flour + crushed broiler feed + crushed dog-feed (diet 8), ground dried grapes + rice flakes

(diet 9) and crushed maize 100% (diet 10). The weight of larvae emerged in the media comprised of red-rice + glycerol + honey (diet 2) and ground dates + red-rice (diet 4) composition were at the intermediate level (Figure 2).

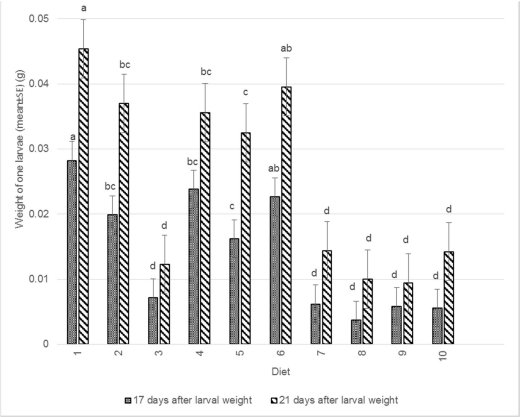

Figure 2: Average weight of *C. cephalonica* F_1 larvae (For a given duration, means followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA).

3.3 Development duration for C*. cautella* adult emergence

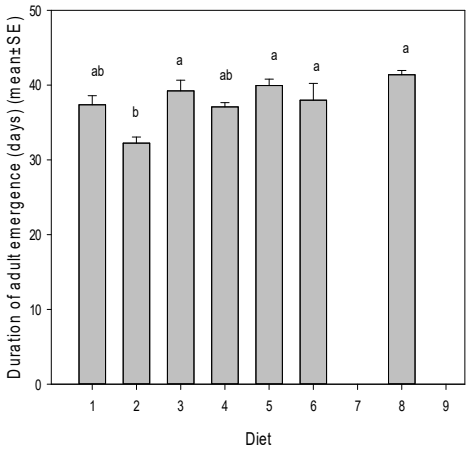

Figure 3: Duration of F_1 adult emergence in *C. cautella* (Means followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA)

Average number of days obtained to emerge adults of *C. cautella* significantly differed (P<0.001). The adults emerged in the media comprised of wheat flour + broiler feed + dog feed (diet 8), ground nut + broiler feed + honey + glycerol (diet 5), soy bean + broiler feed + glycerol + honey (diet 3), rolled oat + rice flour + sprouted mung bean + glycerol + honey (diet 6) had greater duration than red rice + glycerol + honey (diet 2). The adult emergence in the two food media with dog feed + rice flour + rice flakes + glycerol + honey (diet 1) and dates + red rice (diet 4) was in between the above two higher and lower categories and not significantly different from them (Figure 3).

3.4 Development duration for *Corcyra cephalonica* adult emergence

Average number of days of adult emergence for *C. cephalonica* significantly differed (P<0.001) among media and belonged to three broad categories. The food media dog-feed + rice flour + rice flakes + glycerol + honey (diet 1), rolled oat + rice flour + sprouted mungbean + glycerol + honey (diet 6), red-rice + glycerol + honey (diet 2)and dates + red-rice (diet 4) recorded minimum duration of adult emergence for *C. cephalonica*. In contrast, the maximum duration of adult emergence was recorded in the media comprised of dried grapes + rice flakes (diet 9) and maize 100% (diet 10). The duration of adult emergence by the larvae in the media soybean + ground broiler feed + glycerol + honey (diet 3), groundnut + broiler-feed + honey + glycerol (diet 5), groundnut + red-rice + broiler feed (diet 7), wheat flour + broiler feed + dog-feed (diet 8) were at moderate level compared to the above two

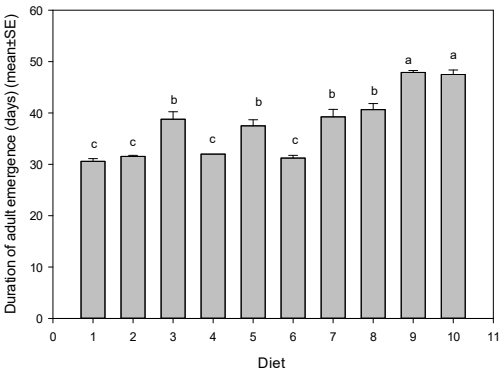

Figure 4: Duration of F_1 adult emergence in *C. cephalonica*. (Means followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA).

3.5 Emergence of *Cadra cautella* and *Corcyra cephalonica* F_2 larvae in different food media

The emergence of *C. cautella* F2 larvae differed with the diet (P<0.0001). The maximum emergence was in T_2 (red-rice + glycerol + honey). The diets T9 (raisins| + rice-flakes) and T_1 (dog-feed + rice-flour + rice-flakes + glycerol + honey) had lower emergence of F2 larvae. The lowest emergence of larvae was in T_3, T_6, and T_8. In *C. cephalonica*, the maximum larvae emerged in T6. The emergence of larvae

followed the trend as T6>T2>T1=T3=T8>T9=T10 (Figures 5, 6).

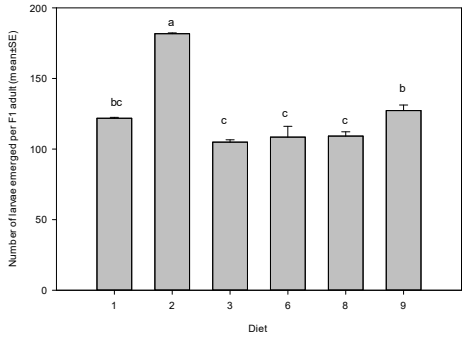

Figure 5: *Cadra cautella* F_2 larvae emerged per F_1 adult in different diets (Means followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA)

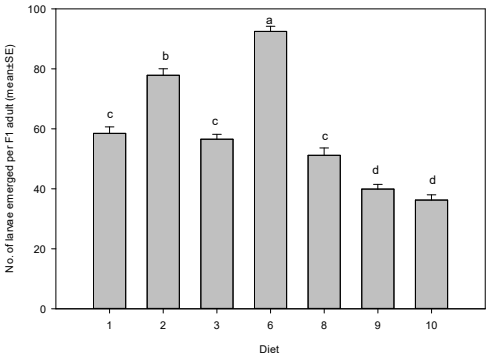

Figure 6: *Corcyra cephalonica* F_2 larvae emerged per F_1 adult in different diets (Means followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA).

4. Discussion

The composition of diet consumed by lepidopteran larvae determines their growth, development and other physiological activities during the subsequent stages of life [18]. The outcomes of our study agrees with this idea, as the *C. cautella* and *C. cephalonica* larvae developed on various diets differed in performance in terms of larval weight, development duration to adult emergence and number of larvae produced in the second generation. Adult moths in flight are attracted to water sources [19], and intake of water following mating increases egg fertility in *C. cautella* [20]. However, the presence of glycerol in the larval diet decreases hatching of eggs [18]. Observing the oscillation pattern of larval emergence in the tested food media, it can be inferred that once the adult moths were introduced to the media having honey and glycerol, they would have been attracted by the liquid medium to have the eggs laid, but the presence of glycerol had limited the emergence of larvae. This area needs to be investigated in future research. It is also evident that the incorporation of glycerol as an ingredient may alter the effects of other ingredients in a moth diet [18]. This may be why certain diets containing glycerol showed limited performance in our study.

In the current research, most occasions, the food media having honey + glycerol have promoted larval development (weight gained), adult development and number of larvae in F_2 generation. The role of glycerol in a larval diet of Pyralidae moths is critical as its absence cannot be replaced during the adult stage [18]. Glycerol also helps to maintain humidity to help larval growth [16]. Thus, the information on the role of glycerol supports comprehension of the findings of the present study.

Also, the food media with red-rice or its components have promoted growth and development of *C. cautella* and *C. cephalonica*.This can be due to the presence of nutrients in red-rice. However, the diet containing soybean showed mixed results in the present study. The soyabean diet has produced reduced larval weight of *C. cephalonica* and increased duration for emergence as *C. cautella* adults. In contrast, the same soybean diet had increased larval weight of *C. cautella*, early emergence of *C. cephalonica* adults. Further, in our study, the medium containing wheat-flour recorded low larval weight of *C. cautella* and *C. cephalonica*. Our results are comparable with the findings of early studies that the larval diets containing soybean and wheat demonstrate >80% survival and high developmental rate of *Plodia interpunctella* larvae [21]. Soybean diet show higher eggs laid than carbohydrate sources wheat and barley [21]. However, in our study, emergence of *C. cephalonica* larvae on the diets containing soybean and wheat-flour were not different. One possible reason is that each of the said two diets contain other ingredients other than the component concerned (soybean and wheat flour) which could mask the effects of the said ingredient.

Although the adult moths of Family Pyraliade including *C. cautella* and *C. cephalonica* do not consume solid food during the adult stage [22], they do consume water [18] and other liquids [20,16]. This is especially important as many food consumed by the moths contain less amount of water. Thus it is possible that *C. cautella* and *C. cephalonica* adults performed in a similar manner when introduced to the diets having honey and glycerol ensuring the successful development and subsequent emergence as adults.

5. Conclusion

Different food media showed different performance in terms of the larval weight gained, duration to complete adult emergence and emergence of larvae in the second generation in both *C. cautella* and *C. cephalonica*. These diets need to be tested for other lepidopteran stored product moth species to verify their performance as a rearing medium for those species. Future research should also focus on developing a mechanism to extract larvae easily from the medium with honey and glycerol.

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