Effects of Dietary Additives in Artificial Diets on Survival And Larval Development of Cnaphalocrocis medinalis (Lepidoptera: Crambidae)

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EFFECTS OF DIETARY ADDITIVES IN ARTIFICIAL DIETS ON SURVIVAL AND LARVAL DEVELOPMENT OF *CNAPHALOCROCIS MEDINALIS* (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Cnaphalocrocis medinalis (Guenée, 1854) (Lepidoptera: Crambidae) is an important defoliating insect of rice crops in Asia, and is difficult to rear on artificial diets. The effects of dietary additives in artificial diets on survival and larval growth of *C. medinalis* were evaluated. Antibiotics, ascorbic acid and vitamin mixture in the medium were indispensable for the survival and growth of *C. medinalis* larvae. The neonates of *C. medinalis* did not survive for 5 days on the artificial diets that lacked antibiotics and an antioxidant, and they did not develop to the pupal stage on the diets without the addition of vitamin mixtures. Addition of antibiotics, antioxidant and vitamins at a low level facilitated larval survival and growth. Supplementation with a vitamin mixture promoted larval growth and shortened the duration of larval development. Additions of cholesterol, and sucrose to the artificial diets improved the development of *C. medinalis* larvae, but excessive amounts of cholesterol and sucrose resulted in inhibition of larval growth. Incorporation of rice or corn leaf powder and plant oils increased larval mortality, and reduced pupation. The addition of Wesson's salt to the artificial diet did not improve larval survival and growth. Therefore, leaf powder, plant oils and Wesson's salt could be omitted from the medium. In addition, the water content had significant effects on larval survival and growth of *C. medinalis*. Eighty percent water content was the most favorable for larval development, lesser or greater proportions were disadvantageous.

Key Words: *Cnaphalocrocis medinalis*; artificial diet; rearing technique; rice leaffolder

RESUMEN

Cnaphalocrocis medinalis (Guenée, 1854) (Lepidoptera: Crambidae) es un insecto defoliador importante de los cultivos de arroz en Asia, y es difícil de criar con dietas artificiales. Los efectos de los aditivos alimentarios en las dietas artificiales sobre el crecimiento de las larvas de *C. medinalis* fueron evaluados. Los antibióticos, ácido ascórbico y una mezcla de vitaminas en el medio fueron indispensables para la sobrevivencia y crecimiento de larvas de *C. medinalis*. Los neonatos de *C. medinalis* no sobrevivieron por 5 días sobre las dietas artificiales que carecía de antibióticos y un antioxidante, y no desarrollaron la etapa de pupa en las dietas sin la adición de la mezcla de vitaminas. La adición de antibióticos, antioxidantes y vitaminas en un nivel bajo facilitaron la sobrevivencia y crecimiento de las larvas. La suplementación con una mezcla de vitaminas promovió el crecimiento de las larvas y acortó la duración del tiempo de desarrollo de las larvas. Las adiciones de colesterol y sacarosa a las dietas artificiales mejoraron el desarrollo de larvas de *C. medinalis*, pero las cantidades excesivas de colesterol y sacarosa resultó en la inhibición del crecimiento de las larvas. La incorporación de arroz o polvo de hojas de maíz y aceites de plantas aumento la mortalidad de las larvas y redujeron las pupas. La adición de sal de Wesson a la dieta artificial no mejoró la sobrevivencia y el crecimiento de las larvas. Por lo tanto el polvo de la hojas, los aceites vegetales y sal de Wesson podrían ser omitidos del medio. Además, el contenido de agua tuvo efectos significativos sobre la sobrevivencia de las larvas y el crecimiento de *C. medinalis*. El contenido de agua de 80% fue el más favorable para el desarrollo de las larvas, proporciones menores o mayores fueron desventajosas.

Palabras Clave: *Cnaphalocrocis medinalis*; dieta artificial; técnica de crianza; doblador de hoja de arroz

The rice leaffolder, *Cnaphalocrocis medinalis* (Güenée) (Lepidoptera: Crambidae), is one of the most important insect pests on rice in Asia. Frequent and serious outbreaks have been reported in many Asian countries, including India, Korea, Japan, China, Malaysia, Sri Lanka, and Vietnam (Zheng et al. 2011). However, the mass rearing of the rice leaffolder with an artificial diet is difficult. In past years, many attempts had been made to improve the artificial diets (Khan 1987; Furuta et al. 1998; Ohmura et al. 2000; Tsuda et al. 2005; Li et al. 2011; Ke et al. 2011). The key problem in rice leaffolder rearing with an artificial diet is the high mortality of the neonate larvae (Furuta et al. 1998; Li et al. 2011). Furuta et al. (1998) suggested that rice leaffolder neonates should be reared on rice seedlings for the first week and then transferred onto the artificial diet. In order to improve the artificial diet, scientists in China analyzed the chemical composition of rice leaves (Xu et al. 2013), and the nutrient requirements for larval development and feeding preferences of *C. medinalis* larvae were also analyzed recently (Guo et al. 2012; 2013).

In our previous work, we found that sorbic acid and methylparaben as dietary antifungal agents at 1000 ppm had detrimental effects on *C. medinalis* larvae and caused high mortality of *C. medinalis* neonates fed on these diets. When screening for safer antifungal agents for rice leaffolder rearing was carried out in our laboratory, the ternary combination of natamycin, sorbic acid and methylparaben were the best for rearing of the rice leaffolder (Su et al. 2014). The main nutrient components of an artificial diet modified from those of Parasuraman & Kareem (1988), Li et al. (2011) and Ke et al. (2011) were optimized by applying the design of quadratic orthogonal rotational combinations (Wang et al. 2013). However, the diet recipe after proportion optimization of main nutrients (rice leaf powder, wheat germ powder, soybean powder, corn powder, casein and yeast) did not give very satisfactory rearing results. Larval mortality remained high, and pupation remained low compared with that when reared on corn seedlings.

It is well documented that the proportions of dietary additives play an important role in the growth and development of many insect species (Sivapalan & Gnanapragasam 1979; Cohen 2004). The present research was undertaken to study the effects of water content and of dietary additives including antibiotics, sucrose, cholesterol, vitamin mixture, rice leaf powder, plant oils and others, in the artificial diet on the growth and development of *C. medinalis* larvae.

Materials and Methods

Insects

The moths of *C. medinalis* were caught from rice fields with sweep nets and released into a net cage with tillering rice plants for oviposition. Moths were provided with 10% sucrose solution in moistened cotton. The tillering rice plants grown in pots were changed daily to obtain eggs of uniform age, and the rice plants with eggs were kept in a climate-control chamber at 27 °C, 60% RH and a 16:8 h L:D. Neonate larvae that hatched within 8 h were used for experiments.

Composition and Preparation of the Artificial Diet

The basal diet was adopted from Wang et al. (2013) and the proportion of components maintained constant while individually varying the concentration of each component. The diets were prepared as follows: ingredients were added to a beaker in the order in which they are listed in Table 1 and thoroughly mixed before being heat sterilized (120 °C, 20 min). Antibiotics, natamycin, Vanderzant vitamin mixture, ascorbic acid and yeast were added when the mixture had cooled to 55 °C, and were blended in the beaker. Finally, each diet was poured into sterile Petri dishes (9 cm diam) and allowed to solidify at room temperature.

Fresh leaves of rice or corn were collected from the tillering stage of the rice variety 'Shanyou' or the elongation stage of the corn variety 'Suyu', and lyophilized overnight. The dried leaves were ground and the resultant powder was screened through a fine sieve (ca. 80 mesh), then stored at 4 °C until use. Wesson's salt and the Vanderzant vitamin mixture were prepared according to Cohen (2004). Other dietary ingredients such as wheat germ, agar, soybean and yeast were available from a local food supermarket. The chemicals were analytic-grade reagents.

Experimental Procedures

The newly prepared diets with varying proportion of ingredients (according to Tables 1-9) were

TABLE 1. COMPONENTS OF THE BASAL DIET FOR *CNAPHALOCROCIS MEDINALIS* (ADOPTED FROM WANG ET AL. 2013).

Components	Quantity (g)
1. Distilled water	100
2. Agar	1.50
3. Wheat germ	6.00
4. Soybean powder	8.00
5. Casein	2.00
6. Methyl paraben	0.10
7. Sorbic acid	0.10
8. Yeast	4.00
9. Natamycin	0.03
10. Antibiotics	0.03 (Table 3)
11. Ascorbic acid	0.10 (Table 4)
12. Vitamin mix	0.10 (Table 5)

sliced into pieces and transferred into the sterile glass vials (8 cm height × 2 cm diam), and 20 vials were prepared for each diet, and 5 neonates were transferred to each vial with diet. All the vials were closed with cotton plugs and placed in an incubator at 27 ± 1 °C, 60-70% RH and 14:10 h L:D. The diets were changed every 5 days. Larval survival was checked daily until pupation, the pupae were removed from rearing vials, and weighted individually, and then kept in plastic boxes for emergence. Culture conditions were the same as described above. Four vials of 5 larvae constitute a replicate, the experiment was replicated 5 times, and a total of 100 neonates were used for each diet.

Statistical Analysis

The effects of varying the proportions of ingredients on the growth and development of the rice leaffolder were evaluated by recording the survival rates of neonates, duration of the larval stage, pupation percentage and pupal weight. All the data were subjected to analysis of variance (IBM Corp. 2011), significant differences between treatments were identified using Duncan's multiple range test.

Results

Effects of Artificial Diet Water Content on Larval Survival and Development

Different water contents in artificial diets were evaluated for the effects on larval survival and development of *C. medinalis*. Water contents in diets had a strong influence on the larval survival. The larval survival rate at the 5th day after eclosion and the pupation rate were obviously higher on diet containing 80% water than that on the other diets with lesser or greater water contents (Table 2). Low water content (< 75%) delayed larval development and increased the duration of the larval stage. Water contents less than 77.5% and greater than 82.5% are not suited for larval survival and development of the rice leaffolder. Therefore, 80% water content in diets is optimal for *C. medinalis* rearing.

Effects of Antibiotics in the Artificial Diet on Larvae and Pupae

Antibiotics were required to suppress bacterial contamination in the diets, because *C. medinalis* larvae did not survive for 5 days on the diets without antibiotics (Table 3). Larval survival was significantly higher on the diets with 125-250 ppm of chloramphenicol or spectinomycin than on other diets, and the pupation rates were obviously high on the diets with 125-250 ppm chloramphenicol or 125 ppm spectinomycin. Concentrations of antibiotics over 500 ppm were detrimental to larvae of *C. medinalis*. High concentrations of antibiotics also extended the duration of the larval stage and reduced pupal weight.

Effects of Added Ascorbic Acid on Larvae and Pupae

Ascorbic acid is commonly used as an antioxidant in insect diets and was required for *C. medinalis* rearing on an artificial diet. On the diets without ascorbic acid all the rice leaffolder larvae died before pupation and the addition of ascorbic acid increased the larval survival and pupation (Table 4). However, both high and low concentrations of ascorbic acid had negative effects on neonate survival and pupation rates. No obvious effects were observed on the duration of the larval stage and pupal weight by ascorbic acid addition to the diet. An ascorbic acid concentration of 0.16% resulted in the highest rate of pupation.

Effects of the Vanderzant Vitamin Mixture on Larvae and Pupae

Addition of Vanderzant vitamin mixture was indispensable for larval survival, and no larvae developed to the pupal stage on the diet lacking the Vanderzant vitamin mixture. Neonate sur-

Table 2. Effects of water content in the artificial diet on the development of the rice leaffolder.

Water content	Percent larval survival at the 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
72.5%	41.7 ± 4.2 d	31.5 ± 0.5 a	3.3 ± 1.9 bc	19.5 ± 0.1 c
75.0%	55.0 ± 5.0 bc	29.3 ± 1.1 ab	5.0 ± 1.7 bc	23.8 ± 2.9 a
77.5%	60.0 ± 3.8 bc	28.8 ± 0.4 b	13.3 ± 4.7 b	21.9 ± 0.4 ab
80.0%	78.3 ± 5.0 a	28.5 ± 0.8 b	31.7 ± 7.9 a	20.8 ± 0.3 bc
82.5%	65.0 ± 5.0 b	28.9 ± 0.4 b	8.3 ± 1.7 bc	20.0 ± 1.4 bc
85.0%	48.3 ± 5.7 cd	0	0	0
87.5%	23.3 ± 1.9 e	0	0	0

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

TABLE 3. EFFECTS OF ANTIBIOTICS IN THE ARTIFICIAL DIET ON THE DEVELOPMENT OF THE RICE LEAFFOLDER.

Antibiotics	Content (ppm)	Percent larval survival at 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
Erythromycin	125	58.3 ± 6.9 cd	29.0 ± 0.6 c	13.3 ± 4.7 b	18.9 ± 0.3 abc
	250	41.7 ± 1.7 ef	32.2 ± 1.4 a	8.3 ± 3.2 b	19.0 ± 0.3 abc
	500	36.7 ± 5.8 f	32.1 ± 1.0 a	8.3 ± 1.7 b	16.3 ± 1.4 d
	1000	30.0 ± 3.3 f	0	0	0
Chloramphenicol	125	85.0 ± 4.2 a	28.6 ± 0.6 c	35.0 ± 4.2 a	19.8 ± 0.6 a
	250	83.3 ± 4.3 a	28.2 ± 0.7 c	35.0 ± 5.0 a	19.5 ± 0.2 ab
	500	63.3 ± 5.8 bcd	31.5 ± 0.5 ab	6.7 ± 2.7 b	17.8 ± 0.3 cd
	1000	55.0 ± 3.2 de	0	0	0
Spectinomycin	125	76.7 ± 7.9 ab	29.7 ± 0.4 bc	36.7 ± 3.3 a	18.4 ± 0.5 abc
	250	71.7 ± 1.7 abc	27.5 ± 1.6 c	13.3 ± 4.7 b	17.8 ± 0.9 bcd
	500	58.3 ± 6.9 cd	31.3 ± 1.3 ab	6.7 ± 0.0 b	17.4 ± 0.9 cd
	1000	55.0 ± 5.7 de	0	0	0
CK	0	0	0	0	0

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

vival and pupation rate were high on the diet with 0.16% Vanderzant vitamin mixture (Table 5), but higher contents were unfavorable to larval survival and pupation. Moreover, increasing the vitamin content accelerated larval development and shortened the duration of the larval stage significantly. Thus, the addition of 0.16% Vanderzant vitamin mixture appears to be optimal for *C. medinalis* rearing based on the higher neonate survival and pupation rate at this concentration.

Effects of Added Sucrose on Larvae and Pupae

The addition low concentrations (0.8% and 2.4%) of sucrose in diets improved larval survival and the pupation rate of the rice leaffolder. However high concentrations of sucrose (≥4.0%) decreased larval survival and the pupation rate, delayed larval development, and reduced the pupal weight (Table 6). The addition of about 2.4% of sucrose in the diet is suggested for rice leaffolder rearing.

Effects of Added Cholesterol on Larvae and Pupae

Addition of a small amount of cholesterol (≤0.24%) did not affect neonate survival of rice leaffolder compared with the control. However, the addition of 0.32% of cholesterol in the diet reduced neonate survival (Table 7). Pupation rate was significantly high on the diets with 0.08-0.16% of added cholesterol. The effect of cholesterol on the duration of the larval stage and pupal weight were not obvious.

Effects of Added Plant Oils on Larvae and Pupae

The addition of linseed oil, corn germ oil and rice bran oil had adverse effects on larval survival and development (Table 8). Neonate survival and pupation rate were decreased significantly even by addition of moderate amounts of these 3 plant oils. Therefore, linseed oil, corn germ oil and rice bran oil are not necessary components of the diets.

TABLE 4. EFFECTS OF ASCORBIC ACID ADDED TO THE ARTIFICIAL DIET ON THE DEVELOPMENT OF THE RICE LEAFFOLDER.

Ascorbic acid content	Percent larval survival at 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
0	70.0 ± 5.8 a	0	0	0
0.08%	81.7 ± 4.2 a	28.8 ± 0.7 a	10.0 ± 6.7 b	18.5 ± 0.5 b
0.16%	85.0 ± 5.0 a	29.3 ± 0.2 a	31.7 ± 6.4 a	18.5 ± 0.2 ab
0.32%	38.3 ± 5.7 b	28.0 ± 0.0 a	13.3 ± 9.4 b	18.8 ± 0.2 ab
0.48%	28.3 ± 5.0 b	29.7 ± 1.0 a	11.7 ± 6.4 b	19.7 ± 0.6 a

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

TABLE 5. EFFECTS OF VANDERZANT VITAMIN MIXTURE ADDED TO AN ARTIFICIAL DIET ON THE DEVELOPMENT OF RICE LEAFFOLDER.

Vitamin mixtures	Percent larval survival at the 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
0	65.0 ± 10.0 bc	0	0	0
0.08%	80.0 ± 2.7 ab	30.0 ± 0.7 a	11.7 ± 11.7 bc	17.6 ± 0.1 b
0.16%	83.3 ± 3.3 a	28.4 ± 0.3 b	33.3 ± 12.2 a	19.5 ± 0.7 a
0.24%	65.0 ± 3.2 bc	27.8 ± 0.7 bc	20.0 ± 5.4 ab	20.2 ± 0.8 a
0.32%	60.0 ± 4.7 c	27.1 ± 0.5 c	23.3 ± 12.8 ab	19.3 ± 0.6 a

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

Effects of Plant Leaf Powder on Larvae and Pupae

In this experiment, various percentages of rice or corn leaf powder were incorporated into the diets (Tables 1 and 9); water content was 80%, and the other ingredients were reduced accordingly. Addition of rice leaf powder significantly reduced neonate survival, pupation rates, and pupal weights. Similarly, increasing the concentrations of corn leaf powder in diets adversely affected neonate survival and pupation rates (Table 9). Therefore, neither rice leaf powder nor corn leaf powder in an artificial diet is beneficial for rearing *C. medinalis*.

Effects of Wesson's Salt on Larvae and Pupae

Increasing the contents of Wesson's salts (> 0.32%) in the artificial diet had detrimental effects on neonate survival, pupation rates and pupal weights (Table 10). Because there were no obvious differences in neonate survival, pupation rates, and pupal weights between the diets containing 0 and 0.16% Wesson's salts, their addition is unnecessary.

DISCUSSION

It is well known that the rice leaffolder is difficult to culture on artificial diets. Ohmura et al (2000) reported an artificial diet consisting of dried powders of rice leaves and INSECTA F-II at the ratio of 3:7 on which more than 80% of larvae developed into adults; however this artificial diet is not available in China and other Asian countries. Thus, the culture and maintenance of populations for this species in the laboratory has relied on rearing the insect on corn or rice seedlings (Park et al. 2006; Liao et al. 2012). The central problems encountered in the rearing of rice leaffolder with an artificial diet are the high larval mortality and the low pupation rate. In this investigation, the effects of serial concentrations of dietary additives in artificial diets on larval development of rice leaffolder were evaluated.

Based on the water contents in rice leaves at tillering and elongation stages, Xu et al. (2013) suggested that artificial diets should have 70-80% water content. Water content in artificial diets was compared for the effects on neonate survival, pupation rate, and pupal weight. Eighty percentage of water content in diets was the most favorable for neonate survival, and lesser or greater water contents increased *C. medinalis* neonate mortality.

Frequently host plant materials are incorporated in the artificial diets of insects (Brun et al. 1993; Blossey et al. 2000; Wheeler & Zahniser 2001). Incorporation of the natural plant host of insects in an artificial diet may benefit the rearing of insects, probably due to the feeding stimuli or cryptic nutrients in natural plant host (Cohen 2004). Several investigators reported that addition of rice leaf powder into the artificial diet improved the cultures of rice leaffolder (Ohmura et

TABLE 6. EFFECTS OF SUCROSE ADDED TO THE ARTIFICIAL DIET ON THE DEVELOPMENT OF THE RICE LEAFFOLDER.

Sucrose content	Percent larval survival at the 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
0	61.7 ± 8.8 b	28.4 ± 0.8 b	16.7 ± 3.8 b	18.5 ± 0.5 a
0.8%	80.0 ± 2.7 a	28.5 ± 1.0 b	25.0 ± 13.7 ab	17.6 ± 0.7 abc
2.4%	81.7 ± 3.2 a	30.2 ± 0.6 ab	30.0 ± 3.8 a	18.4 ± 0.4 ab
4.0%	41.7 ± 5.0 c	30.0 ± 0.0 b	1.7 ± 3.3 c	16.6 ± 0.0 c
5.6%	38. 3 ± 4.2 c	32.0 ± 0.0 a	1.7 ± 3.3 c	17.2 ± 0.0 bc

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

TABLE 7. EFFECTS OF CHOLESTEROL ADDED TO THE ARTIFICIAL DIET ON THE DEVELOPMENT OF THE RICE LEAFFOLDER.

Cholesterol content	Percent larval survival at the 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
0	85.0 ± 8.8 a	29.3 ± 0.7 ab	13.3 ± 5.4 b	17.5 ± 1.0 a
0.08%	85.0 ± 1.7 a	30.1 ± 0.7 ab	28.3 ± 3.3 a	18.9 ± 0.5 a
0.16%	86.7 ± 4.7 a	30.7 ± 0.4 a	28.3 ± 3.3 a	19.1 ± 0.2 a
0.24%	85.0 ± 3.2 a	28.3 ± 0.6 b	20.0 ± 5.4 b	18.7 ± 0.5 a
0.32%	63.3 ± 8.4 b	30.3 ± 1.0 ab	13.3 ± 5.4 b	18.4 ± 0.3 a

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

al. 2000; Li et al. 2011; Ke et al. 2011). However, our research showed that addition of either rice leaf powder or corn leaf powder into artificial diets did not enhance larval survival or pupation rates of this species. On the contrary, the incorporation of rice or corn leaf powder into artificial diets increased larval mortality and decreased the pupation rate. Khan et al. (1989) reported that rice leaves (including susceptible and resistant varieties) had antibiosis effects on the larvae of *C. medinalis*, but we found that larval survival was significantly less on the diets in which leaf powder of a susceptible rice variety was incorporated than on the control without rice leaf powder. Also larval development was delayed in diets with leaf powder compared to that on the control diet. According to our research and Khan et al. (1989), rice leaf powder or corn leaf powder is not recommended for artificial diets of *C. medinalis*.

Similarly, plant oils such as linseed oil, rice bran oil and wheat germ oil incorporated into artificial diets reduced the larval survival and pupation rates. Sivapalan & Gnanapragasam (1979) reported that the fatty acids, linoleic and linolenic, are critical dietary supplements for the successful adult emergence of the tea tortrix, *Homona coffearia* (Nietner) (Tortricidae) on artificial diets. Linseed oil, rice bran oil and wheat germ oil are believed to rich in unsaturated fatty acids. The 3 plant oils used in this study were brought from a local food supermarket, their chemical compositions were not identified, and the reasons for their adverse effects on larval survival are not clear. Because of the adverse effects of these 3 oils, and because insects on the control diet developed normally, we do not recommend that these 3 plant oils be incorporated into artificial diets of *C. medinalis*. The addition of Wesson's salt is also unnecessary, and the inorganic salts in wheat germ, soybean powder, yeast and other ingredients appear to be sufficient for development of the rice leaffolder.

Sucrose is a feeding stimulus for some insect species and also has nutrient effects for larval development (Cohen 2004; Glendinning et al. 2007; Guo et al. 2012). The addition of sucrose proved to be beneficial for rearing of *C. medina-*

TABLE 8. EFFECTS OF PLANT OILS ADDED TO THE ARTIFICIAL DIET ON THE DEVELOPMENT OF THE RICE LEAFFOLDER.

Plant leaf powder	Content	Percent larval survival at the 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
CK	0	85.0 ± 3.2 a	29.6 ± 0.6 bc	30.0 ± 4.3 a	19.3 ± 0.4 bcd
linseed oil	0.2%	80.0 ± 5.4 ab	27.5 ± 0.5 de	25.0 ± 5.7 ab	20.3 ± 0.6 b
	0.4%	66.7 ± 2.7 bc	27.9 ± 1.4 cde	16.7 ± 4.3 bc	17.5 ± 0.5 de
	0.6%	38.3 ± 3.2 ef	33.0 ± 1.5 a	5.0 ± 1.7 def	12.7 ± 1.0 f
	0.8%	28.3 ± 3.2 f	0	0	0
Corn germ oil	0.2%	58.3 ± 8.8 cd	29.3 ± 0.3 c	15.0 ± 5.7 bcd	23.0 ± 0.9 a
	0.4%	35.0 ± 1.7 ef	29.0 ± 1.0 cd	3.3 ± 1.9 ef	19.3 ± 1.0 bcd
	0.6%	30.0 ± 4.3 f	28.5 ± 1.5 cde	3.3 ± 1.9 ef	18.9 ± 1.0 bcd
	0.8%	25.0 ± 5.0 f	29.5 ± 1.5 c	3.3 ± 1.9 ef	16.2 ± 3.8 e
Rice bran oil	0.2%	63.3 ± 5.8 c	26.7 ± 0.7 e	13.3 ± 6.1 cde	19.4 ± 0.4 bcd
	0.4%	55.0 ± 3.2 cd	31.3 ± 0.7 ab	6.7 ± 2.7 cdef	19.5 ± 1.9 bc
	0.6%	46.7 ± 8.2 de	27.0 ± 0.0 e	1.7 ± 1.7 f	19.0 ± 0.0 bcd
	0.8%	35.0 ± 4.2 ef	28.5 ± 0.5 cde	3.3 ± 1.9 ef	17.7 ± 0.2 cde

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

TABLE 9. EFFECTS OF EITHER RICE LEAF POWDER OR CORN LEAF POWDER ADDED TO ARTIFICIAL DIETS ON THE DEVELOPMENT OF THE RICE LEAFFOLDER.

Plant leaf powder	Content	Percent larval survival at the 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
Rice leaf	1.6%	63.3 ± 6.9 bc	25.3 ± 0.2 c	28.3 ± 5.0 ab	18.4 ± 0.1 b
	3.2%	70.0 ± 1.9 abc	25.2 ± 0.7 c	20.0 ± 0.0 bcd	18.3 ± 0.7 b
	4.8%	71.7 ± 6.9 abc	28.0 ± 0.3 b	11.7 ± 5.7 de	17.5 ± 0.6 b
	6.4%	78.3 ± 5.7 ab	31.8 ± 1.3 a	8.3 ± 1.7 ef	17.7 ± 0.7 b
Corn leaf	1.6%	81.7 ± 6.3 a	30.1 ± 0.8 a	23.3 ± 3.3 abc	17.5 ± 0.4 b
	3.2%	70.0 ± 3.3 abc	29.9 ± 0.4 ab	16.7 ± 3.3 cde	17.7 ± 0 b
	4.8%	70.0 ± 3.3 abc	0	0	0
	6.4%	60.0 ± 3.8 c	0	0	0
CK	0	81.7 ± 7.9 a	30.1 ± 0.5 a	31.7 ± 5.7 a	19.8 ± 0.2 a

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

lis by artificial diet because at 2.4% of the diet, it increased neonate survival and pupation rates. But high content of sucrose (≥ 4.0%) in the diets is detrimental to rice leaffolder neonates.

Antibiotics, ascorbic acid, and the Vanderzant vitamin mixture were indispensable for rearing the rice leaffolder on artificial diet, and larvae did not develop into pupae on diets lacking these ingredients. Diet spoilage caused by microbial contamination affected the larval feeding, and led to neonate mortality and delayed development. The addition of antimicrobial agents is necessary to inhibit the growth of microbial in the artificial diets, as is good sanitation in the laboratory and sterilization of diets and appliances. In our previous work, we reported the combination of sorbic acid, methylparaben and natamycin prevented the growth of *Aspergillus* sp. on the artificial diets for 20 days and was safe for the larval development of *C. medinalis* (Su et al. 2014). This antimicrobial combination is effective for control of fungi; however, it is not very effective in bacterial control. The commonly used antibiotics, streptomycin and aureomycin, had negative influences on *C. medinalis* larval development. Chloramphenicol and spectinomycin had fewer adverse effects on larval survival and pupation rates than erythromycin, and we recommend the incorporation of a low concentration of chloramphenicol or spectinomycin (120 ppm) into diets for bacteria control. Ascorbic acid is included in almost all artificial diets of insects as an antioxidant (Cohen 2004). Our study showed this antioxidant is also essential in the artificial diet of *C. medinalis*. Addition of 0.16% ascorbic acid enhances neonate survival and larval development, but the addition of greater amounts had adverse effects on immature of rice leaffolder.

By integrating these findings with our previous investigations, we propose the following artificial diet recipe to rear *C. medinalis*: agar 1.2%, wheat germ 4.8%, soybean powder 4.8%, casein 1.6%, yeast 3.2%, sucrose 2.4%, cholesterol 0.08%, methylparaben 0.08%, sorbic acid 0.08%, ascorbic acid 0.16%, natamycin 0.024%, Vanderzant vitamin mixture 0.16%, chloramphenicol 0.0125%, add water to make 100%. Currently, the continuous rearing of rice leaffolder with this artificial diet is under evaluation in our laboratory.

TABLE 10. EFFECTS OF WESSON'S SALT ADDED TO THE ARTIFICIAL DIET ON THE DEVELOPMENT OF THE RICE LEAFFOLDER.

Wesson's salt	Percent larval survival at the 5th day	Duration (days) of the larval stage	Pupation rate (%)	Pupal weight (mg)
0	80.0 ± 2.7 a	29.5 ± 0.5 ab	30.0 ± 5.8 a	18.8 ± 0.3 ab
0.08%	66.7 ± 9.0 ab	29.9 ± 1.2 ab	16.7 ± 3.3 b	19.1 ± 0.5 a
0.16%	78.3 ± 6.3 ab	26.7 ± 1.1 b	31.7 ± 6.9 a	19.0 ± 0.2 ab
0.32%	78.3 ± 4.2 ab	30.0 ± 0.9 a	16.7 ± 1.9 b	18.5 ± 0.3 ab
0.48%	61.7 ± 5.7 b	29.6 ± 1.2 ab	11.7 ± 4.2 b	18.1 ± 0.4 b
0.64%	35.0 ± 4.2 c	29.1 ± 2.0 ab	13.3 ± 2.7 b	16.5 ± 0.2 c

Values are means ± SD. Values in a column followed by the same letter do not differ significantly ($P > 0.05$, Duncan's multiple range test).

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