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Preliminary tests for new low cost and reusable bulking agents in the artificial larval diet of Mediterranean fruit fly *Ceratitis capitata* and peach fruit fly *Bactrocera zonata* (Diptera: Tephretidae)

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Abstract:

A preliminary tests have been executed to evaluate replacing of the traditional wheat bran that used as bulking agent in the artificial larval diet of Mediterranean and peach fruit flies with Sackcloth (can wash and reuse) to overcome storage, handling and waste manage problems of the diet residue also replaced with fine and rough saw dust (priceless material) to reduce costs. quality control tests (Pupal weight, Pupal size, Emergence, Deformity, Sex ratio, Flight and starvation) executed to compare the traditional wheat bran based with the suggested sawdust based and sackcloth based diets and revealed that results were very close for both fruit flies (Mediterranean and peach fruit flies). For medfly, there were no significant differences between standard diet and rough sawdust diet for all quality control tests, but they differ significantly from both sackcloth and fine sawdust diets. For peach fruit fly, results revealed that diets based on the alternatives were superior to the standard diet for majority of the biological parameter tested through quality control tests.

Introduction

Fruit flies considered the most serious insect pests worldwide attacking more than 300 plant species, including several species of high commercial value (Liquido *et al*., 1991). Rearing these flies in the laboratory permits attempts at Sterile Insect Technique (SIT) programs, biological control in addition to basic researches in different areas. Sterile Insect Technique is one of the principal techniques for controlling of medfly (Hendrichs *et al*., 2002). To be effective, the SIT requires large numbers of high-quality insects be released (Dyck *et al.,* 2005). The production of insects must be timely and cost effective. Many economical alternatives for the solid bulking agents in the artificial larval diets have been used by many researchers such as, flaked agar (Maeda *et al*., 1953), dehydrated carrot powder (Mitchell *et al*., 1965), wheat shorts and middling (Nadel, 1970), sugarcane bagasse and wheat germ (Peleg and Rhode, 1970), fluid larval medium (Schroeder *et al.*, 1972) , dried carrot, wheat bran and rice bran (Awadalla and Faris, 1973), digestive bran and fresh carrot pulp (Barnes, 1976), pulverized paper (Zumreoglu *et al*., 1979), sugarcane bagasse and sugar beet bagasse (Vargas

et al., 1983), (Bruzzone and Schwarz Gehrke, 1987) studied a recycling larval rearing medium where the diet can be reused after certain treatments to readjust its features without adding any ingredients except water and Nipagin), mill feed (Vargas, 1989) and toilet paper (Kakinohana and Yamagishi, 1991). A "liquid" larval diet has been developed that discard the bulking agent (Tanaka *et al.*, 1969). All alternatives used as bulking agents in fruit fly mass-rearing facilities, suffer from some problems caused by the solid bulking agents including residues management (Disposal and tray cleaning), storage space, high costs, labor, and sanitation.

In the present work, to overcome costs, the low cost sawdust were used as an alternative to the wheat bran bulking agent, also, to overcome storage, handling and waste challenges of traditional wheat bran based diet, and reduce costs also, the reusable sackcloth were used as an alternative to the wheat bran bulking agent that can be washed and reused many and many times.

Materials and methods

1. Flies strains:

Mediterranean and Peach fruit flies lab strains (Horticultural crops insects department laboratories - Plant Protection Research Institute) that reared upon an artificial larval diet composed of sugar (As a source for carbohydrate) 82 gm., dried sterile yeast (As a source for protein) 82 gm., wheat bran (As a bulking agent) 330 gm., sodium benzoate 3 gm., citric acid 3 gm. (Preservatives) and water 500 ml. The adult flies feed normally upon water, sugar and hydrolyzed protein (4:1).

2. Bulking agents:

2.1. Sackcloth:

Sackcloth characterized by its wide meshes and high ability to keep humidity, so it presents an excellent physical medium that larvae can move easily through the fiber meshes in addition to keep humidity for longer period. Diet prepared by saturating multiple sheets of sackcloth (18 X 16 cm2 weighting 150 gm.) with the liquid diet instead of the wheat bran that used as bulking agent.

2.2. Sawdust (Fine and rough):

Traditional sawdust (As the waste residue from wood processing) sifted to fine and rough dusts. Diets prepared by replacing the wheat bran content by 207 gm. fine sawdust (Fine sawdust diet) and replaced by 330 gm. rough sawdust (Rough saw dust diet) to get the same physical feature of the diet.

3. Quality control tests Experiments details:(IAEA, 2014):

3.1. Weight of pupae:

Record average weights 10 pupae two days before emergence (Five replicates).

3.2. Size pupae:

Calculated volumetrically by counting the number of pupae per one ml using graduated cylinder and record the average size per pupa (Five replicates).

3.3. Emergence percentage:

Calculate number of successfully emerged flies for each 100 pupae and estimate the emergence percentage (Five replicates).

3.4. Ratio of deformed flies:

Calculate number of flies that failed to emerge or emerge with deformation for each 100 pupae to estimate the deformity percentage (Five replicates).

3.5. Sex ratio:

For each group of 100 emerged adult flies, The numbers of males and females are counted and recorded (Five replicates).

3.6. Flight ability:

100 pupae for each treatment placed into one PVC cylinder coated internally by talcum powder and put inside a plastic transparent bag with metal frame inside to make it

swallowed, on emergence, calculate the flies succeeded to fly and escape the cylinder to be the flier individuals (Five replicates).

3.7. Survival under stress:

A number of 100 flies are caged separately (Transparent cups with top with a fiber mish top cover to supply aeration) without any feeding material or water and calculate how long the fly can persist alive without any nutrition.

Results and discussion

1. Mediterranean fruit fly:

1.1. Pupal weight:

Results in Table (1) regarding to the pupal weight revealed that control and rough sawdust pupae have the superior weight with mean of 11.0 mg /pupa and 10.86 mg /pupa but they differ significantly from sackcloth which came as second rank with mean weight of 10.44 mg/pupa and finally with the least weight, pupae reared upon fine sawdust larval diet with mean weight of 9.48 mg/pupa.

1.2. Pupal size:

Table (1) show that there was no significant difference between control pupae and rough sawdust pupae with mean pupal size of 0.021 ml /pupa and 0.020 ml/pupa respectively while sackcloth pupae and fine sawdust pupae have the same pupal size with means of 0.0188 ml.

1.3. Emergence percentage:

Data in Table (1) showed that there is no significant difference between control pupae, fine and rough sawdust pupae with mean emergence percentages of 97.0, 96.4 and 97.6 % respectively and significantly different from sackcloth which has a lower emergence percentage mean of 94.4 %.

1.4. Deformity percentage:

Results of deformed flies (Table 1) came with no significant differences among the four treatments with mean percentage of 0.6, 0.4, 1.2 and 1.2 % for control, sackcloth, fine sawdust and rough sawdust diet, respectively.

1.5. Sex ratio:

Data obtained in (Table 1) reveled that male : female ratio came as ♂48 : ♀52 , ♂53 : ♀ 47, ♂52 : ♀48 and ♂51 : ♀ 49 for control, sackcloth, fine sawdust and rough sawdust diet respectively.

1.6. Flight ability:

Percent fliers differ significantly among the four groups, Rough sawdust came with greatest percent with mean of 84.6 % followed by fine sawdust (73.2 %) then sackcloth fliers (63.2 %) and finally, control as the least flier percentage with mean of 65.6 %.

1.7. Survival under Stress (Starvation):

Data in (Table 1) show that there were a significant differences among the four groups, rough sawdust has the longest life span (In hours) without food (Mean of 129.8 hours) followed by sackcloth (119.8 hours) then fine sawdust fliers (113.8 hours) and finally control, as the least life span with mean of 107.8 hours.

Table (1): Results of quality control tests for standard (Control), sackcloth, fine sawdust and rough sawdust diets for larval rearing of medfly.

Parameter	Control	Sackcloth	Fine sawdust	Rough sawdust
Pupal weight	11.0 ± 0.031 a1	10.44 ± 0.081 b1	9.48 ± 0.159 c1	10.86 ± 0.092 a1
Pupal size	0.021 ± 0.0003 a2	0.0188 ± 0.0008 b2	0.0188 ± 0.0002 b2	0.020 ± 0.0002 a2
Emergence	97.0 ± 0.316 a3	94.4 ± 0.245 b3	96.4 ± 0.510 a3	97.6 ± 0.245 a3
Deformity	0.6 ± 0.245 a4	0.4 ± 0.245 a4	1.2 ± 0.583 a4	1.2 ± 0.374 a4
Sex ratio	♂ 48 ♀ 52	♂ 53 47♀	♂ 52 ♀ 48	♂ 51 ♀ 49
Flight	65.6 ± 0.4 c6	63.2 ± 0.20 d6	73.2 ± 0.374 b6	84.6 ± 0.40 a6
Starvation	107.8 ± 0.2 d7	119.8 ± 0.45 b7	113.8 ± 0.374 c7	129.8 ± 0.374 a7

2. Peach fruit fly:

2.1. Pupal weight:

Results in Table (2) revealed that pupal weights differ significantly among the four diets, Rough sawdust came as the highest weight with mean of 14.82 mg., followed by pupae resulted from sackcloth with a mean weight of 13.18 mg. then, fine sawdust pupae with mean weight of 12.96 mg. and finally, standard (Control), with the least pupal weight mean of 10.86 mg.,

2.2. Pupal size:

Table (2) showed that pupae resulted from rough sawdust has the highest size with a mean of 14.82 ml that differ significantly from the rest diets. Then, as the second order, fine sawdust pupae with a mean of 0.0243 ml. while, control and sackcloth diet with no significant difference between them and mean pupal size of 0.0239 and 0.0238 ml respectively.

2.3. Emergence percentage:

Results in Table (2) revealed that percent of emergences differ significantly among the four diets, sackcloth has the highest percentage with mean of 95 %, followed by fine sawdust based diet with mean percentage of 95 %, then rough sawdust diet which has a mean of 83.4 % and finally the control diet which has the least emergence percentage of 63.8 %.

2.4. Deformity percentage:

Results of deformed flies (Table 2) came with no significant differences among the four treatments with mean percentage of 0.6 %, 1.2 %, 0.4 % and 1.2 % for control, Sackcloth, Fine sawdust and Rough sawdust diet respectively.

2.5. Sex ratio:

Data obtained in Table (1) reveled that male : female ratio came as ♂54.5: ♀ 45.5 , ♂ 55: ♀45, ♂ 54.5: ♀45.5 and ♂ 50: ♀ 50 for control, sackcloth, fine sawdust and rough sawdust diet, respectively.

2.6. Flight ability:

Diet based on sackcloth (Table 2) came with the superior flight ability with mean 80.4 % that differ significantly from the rest of the diets, followed by both of control and fine sawdust diets with no significant difference between them and means of 58.4 % and 60.4 % respectively, and rough sawdust based diet has the least flight percentage of 50.4 % mean.

2.7. Survival under Stress (Starvation):

Table (2) show that pupae resulted from rough sawdust has the highest ability to live under stress of food absence with a mean of 113.4 hours which differ significantly from the rest diets. Then, as the second order, both sackcloth and fine sawdust pupae with means of 113.6 and 104.8 hours respectively. while, control that differ significantly from the other diets, came as the least ability to survive under stress mean live span of 79.2 hours.

Table (2): Results of quality control tests for standard (Control), sackcloth, fine sawdust and rough sawdust diets for larval rearing of peach fruit fly.

	Standard (Control)		Sackcloth		Fine sawdust		Rough sawdust	
Pupal weight	10.86 ± 0.051 d1		13.18 ± 0.02 b1		12.96 ± 0.051 c1		14.82 ± 0.073 a1	
Pupal size	0.0239 ± 0.001 c2		0.0238 ± 0.001 c2		0.0243 ± 0.001 b2		0.0251 ± 0.001 a2	
Emergence	63.8 ± 0.663 d3		95 ± 0.316 a3		90.4 ± 0.510 b3		83.4 ± 0.245 c3	
Deformity	0.6 ± 0.245 a4		1.2 ± 0.20 a4		0.4 ± 0.245 a4		1.2 ± 0.20 a4	
Sex ratio	♂54.5	♀ 45.5	♂ 55	♀45	♂ 54.5	♀45.5	♂ 50	♀ 50
Flight	58.4 ± 0.510 b5		80.4 ± 0.510 a5		60.4 ± 0.490 b5		50.4 ± 0.510 c5	
Starvation	79.2 ± 0.374 c6		113.6 ± 0.245 b6		104.8 ± 0.374 b6		113.4 ± 0.245 a6	

Results of quality control tests (Pupal weight, pupal size, emergence, deformity, sex ratio, flight and starvation - for both of the

Mediterranean and peach fruit flies) revealed that traditional wheat bran based artificial larval diet was very close to the new diets based on Sackcloth, fine and rough Sawdust (Used as bulking agents) in spite of the significant differences that showed through the statistical analysis of the results but these differences will compensated through successive generations reared upon the same new diets as mentioned by Kamikado *et al.* (1987), Souza *et al.* (1988) and Economopoulos (1992) whose demonstrated that adaptation of the fruit flies to the laboratory artificial diet for colonization need several generations.

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