DEVELOPMENT OF MASS-REARING METHODS FOR THE SUGARCANE BORER *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE) II: DIET GELLING AGENTS

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Abstract

Artificial diets used to rear plant-feeding insects require a high water content, but no free water. Agar is most commonly used for compounding the diet as it mixes readily with dietary ingredients and forms a solid gel at room temperature. This chemical has, however, become increasingly expensive. As it is the most costly constituent of the diet used for the borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae), the possibility of reducing the amount of agar was investigated. Economical alternatives to agar were also sought. Results showed that autoclaving the agar solution before incorporation into the diet resulted in a 60% saving of agar. Apple pectin was found to be a suitable alternative to agar.

Introduction

The feeding habits and mouthpart structure of any insect to be reared in the laboratory largely determines the consistency and structure of the artificial diet it is to feed on. For example, a diet developed for plant-feeding insects should be solid enough for them to press their mouthparts against to enable biting. This diet should contain at least 80% water to prevent dehydration of the insect (Singh, 1977).

Agar is a carbohydrate, produced by several red seaweed species, that forms a gel when mixed into solution, even in very small quantities (Anderson, 1991). It is irreplaceable in the culturing of bacteria and fungi in medical pathology and microbiological research. For these applications it is used as a neutral medium to which nutrients can be added. Gels can be prepared with agar concentrations ranging from 0,05% (slightly viscous) to 3,0% (firm gel) (Singh and Moore, 1984). A firm gel is formed at concentrations of 1,5% or more depending on the nature of the dietary ingredients and the total water content to be added to the gel. Most diets use about 3,0% agar (Singh, 1977). Its liquidity above 40°C, solidity at room temperature, and compatibility with other foodstuffs and minerals, in addition to its low nutrient content, have resulted in its use in insect media (Singh and Moore, 1984).

In recent years the cost of agar has increased substantially and, in large insect rearing programmes such as that used for *Eldana saccharina* Walker, it has become a major expense. Steps taken to reduce the quantity of agar used and to seek economical alternatives are described here.

Materials and methods

Agar reduction

The equivalent of between four and ten grams of agar/litre of water were dissolved in 100 ml of boiling water. The cooled agar solutions were poured into multicell rearing trays and left at room temperature to set.

While mixing the agar in boiling water, it was observed that a large volume of undissolved agar particles were present. The solutions were reboiled in a microwave oven and, when cooled, poured into multicell trays and left to gel at room temperature.

The agar concentrations which gelled satisfactorily were incorporated into a 65 litre diet mix, and inoculated with first instar larvae in the routine manner described by Graham and Conlong (1988).

When the agar was dissolved only in boiling water, none of the tested quantities gelled rigidly enough for use in the diet. When the solution was reboiled, all the agar particles dissolved giving a rigid gel down to a concentration of 5 g/litre of water (0,4%).

A 65 litre diet mix was prepared using 5 g of agar/litre of water. The agar (325 g) was dissolved in 15 litres of boiling water and then autoclaved at 121°C for 15 minutes in stainless steel buckets before use. The balance of the boiling water (50 litres) was added directly to the bulk mixer. An extra 25 litre mix was prepared using the equivalent of 4 g (0,3%) of autoclaved agar/litre of water (260 g) to test gelling properties in combination with the remaining dietary ingredients, even though this quantity of agar did not gel adequately in water alone.

Eldana growth and survival on the experimental diets were checked 26 days after inoculation. A 65 litre routine diet served as control. Thirty-five days after inoculation, pupae were removed from the diets, cut from their cocoons, and separated according to their sex. A sample of 30 male and 30 female pupae from each of the treatments and from the control, were weighed.

Agar substitutes

Suitable substitutes for agar were sought. Gelatine and apple pectin were tested. Gelatine is an edible jelly produced from animal bones and pectin a substance obtained from ripe fruit. Pectin is used commercially at a concentration of 5 g/litre in jams with 50% solids. A pH of 3,7 ± 0,3 is required and gelling occurs at 50°C. The pH of the eldana diet has been shown to be optimal at 5,0 (present diet 5,2), at which microbial contamination is minimal, and in addition falls within the pH range of sugarcane (4,5 - 5,5). Pectin also needs to be mixed with sucrose to aid dispersion in water.

To test efficacy of gelling of these substitutes one litre batches of routine eldana diet, incorporating pectin in concentrations of 2,5, 5 and 10 g and a combination of 5 g agar and 5 g pectin (expressed as g/litre of water), were made up using a domestic food mixer. Dietary pH was not adjusted. Higher concentrations were not tested because they were more expensive than agar.

Concentrations which gelled satisfactorily were incorporated firstly into 15 and then 25 litre diet mixes using the bulk diet mixer, and inoculated with first instar larvae as described above.

Pectin was less soluble than agar in boiling water. In the absence of either glucose or sucrose in the diet, a high speed blender was used to ensure thorough dispersal and mixing of the pectin.

When incorporated into the eldana diet, pectin gelled satisfactorily at a concentration of 10 g/litre. For a 15 litre mix (150 g pectin), three batches of 50 g pectin were blended with one litre of boiling water each. The remaining volume of water was added directly to the food mixer. For the 25 litre mix (250 g pectin), the same quantity of agar was blended with a total of five litres of boiling water in one litre batches.

When the pectin diet surface was scarified, free water seeped into the scarifications. To avoid the possibility of the hatched larvae drowning in the water, the diet was left unscarified. Scarification of the normal eldana diet has been a routine practice to break the 'skin' formed on the diet surface. This ensured a penetration site for the hatched larvae into the diet.

Eldana growth and survival in the different experimental diets was again checked at 26 days for the pectin diets. Two routine diet mixes served as controls.

Pupae were treated as described for the reduced level of agar determinations. Samples were obtained from each of the two pectin and two control treatments.

To determine the fecundity of the insects reared, single male and female moths were each placed together in 250 ml lidded paper cups and provided with fluted paper towelling as an oviposition substrate. Thirty pairs of moths from the pectin and 30 from the routine diet were tested. The average number of eggs laid were compared between the two treatments.

Gelatine was tested by dissolving an equivalent of 10 g of gelatine in 100 ml of boiling water. This solution was increased in 5 g quantities of gelatine until it gelled satisfactorily.

Results

Agar use

Tables 1 and 2 summarise eldana survival (in terms of larval density per multicell rearing tray), and suitability of the diet for eldana (in terms of pupal weight), respectively.

There were no significant differences in the larval density and pupal weights of eldana developing in the diets containing 4, 5 and 10 g of agar/litre of water.

Agar substitutes

To obtain a suitable gel, gelatine had to be used in very large quantities (40 g/litre of water) and gelled only at very low temperatures. In contrast, food grade and commercial agars set at approximately 38°C and 45°C respectively, maintaining their form throughout eldana development at 26°C.

Tables 3 to 5 summarise eldana survival (in terms of larval density per multicell rearing tray), fecundity and suitability of the diet for eldana (in terms of pupal weight), respectively.

There were no significant differences in the larval density, pupal weights and fecundity of eldana developing in the diets containing pectin and agar.

A combination of half agar and half pectin had less water running into the scarifications, but the diet itself was less rigid than when pectin was used.

Table 1

Larval density of *Eldana saccharina* developed in diets containing 4, 5 and 10 g (control) of agar/litre of water, 26 days after inoculation

Agar (g/litre)	Larval density			
	Total larvae	Mean/tray	SD	No. of trays
4 (0,3%)	515	34,3	14,1	15
5 (0,4%)	136	27,2	5,0	5
10 (0,7%)	148	29,6	4,5	5

Table 2

Mean pupal weights of *Eldana saccharina* developed in diets containing 4, 5 and 10 g (control) agar/litre of water

Agar (g/litre)	Female pupae			Male pupae		
	Weight (mg)	SD	No pupae	Weight (mg)	SD	No pupae
4 (0,3%)	171	29	90	108	19	90
5 (0,4%)	184	28	30	112	12	30
10 (0,7%)	167	24	30	108	13	30

Table 3

Larval density of *Eldana saccharina* developed in diets containing pectin and agar (control), 26 days after inoculation

Gelling agent	Larval density			
	Total larvae	Mean/tray	SD	No. trays
Pectin	308	30,8	12,1	10
Agar	357	35,7	13,8	10

Table 4

Mean pupal weights of *Eldana saccharina* developed in diets containing pectin and agar (control)

Gelling (g/litre)	Female pupae			Male pupae		
	Weight (mg)	SD	No pupae	Weight (mg)	SD	No pupae
Pectin	181	27	60	115	19	60
Agar	184	26	60	110	18	60

Table 5

Fecundity of *Eldana saccharina* moths developed from diets containing pectin and agar (control)

Gelling agent	Fecundity		
	Ave. eggs/F	SD	No females
Pectin	278,1	109,1	30
Agar	357,8	179,2	30

Discussion

The development of a low cost, high quality diet, more like sugarcane in composition, is a major aim of the mass rearing operation. With the refinement of insect rearing operations in the Insect Unit, agar has been reduced and the volume of fibre increased in the diet (Graham and Conlong, 1988). This is common practice in diet formulation. King and Leppla (1984) report that agar can be reduced by increasing the fibre content. The latter contributes little to overall nutrition, but is essential for binding nutrients, providing bulk and giving proper dietary shape and texture.

A model diet contains 2,5% agar and 10% cellulose (fibre) (Singh and Moore, 1984). Atkinson's (1978) diet contained 1,6% agar and 2,3% fibre. By increasing fibre in the eldana diet to 17,2%, the agar was reduced to 0,7% (Graham and Conlong, 1988). This diet has since been further modified (Table 6) by decreasing the fibre content to 14,4%. This avoided blockages of the diet dispensing pump. The agar content was reduced to 0,3% by autoclaving the agar solution.

Table 6

Modified *Eldana saccharina* larval diet (final volume about 78 litres)

Ingredients	Amount	Per cent
Crushed cane	13 000 g	14,4
Chickpea	6 500 g	7,2
Yeast extract	195 g	0,2
Casein	1 114 g	1,2
Sodium propionate	594 g	0,7
Ascorbic acid	217 g	0,2
Calcium lactate	74 g	0,1
Ferric citrate	4 g	0,004
Tri-sodium citrate	149 g	0,2
Sodium chloride	37 g	0,04
Citric acid	149 g	0,2
Nipagin	130 g	0,1
Dithane	11 g	0,01
Formaldehyde	228 ml	0,3
Ethanol (70%)	2 275 ml	2,5
Agar (4 g/l)	260 g	0,3
Water (autoclave)	20 l	)
		) 72,3
Water (balance)	45 l	)

The savings in current cost by halving the quantity of agar used is given in Table 7.

Table 7

Cost of agar used in 65 litres of *Eldana saccharina* diet at concentrations of 4, 5 and 10 g of agar/litre of water (current cost R125,00/kg for 50 kg, including VAT)

Agar (g/litre)	Cost (Rands)		
	Daily	Monthly	Annually
10	81,25	1 787,50	21 450,00
5	40,63	893,86	10 726,32
4	32,50	715,00	8 580,00

In 1989 an imported food grade agar (China grass) was found at a local spice dealer, which when tested in the eldana diet gave results comparable with those of commercial grade agar. A more reliable source of the lower grade agar was obtained from Germany (via South West Africa) which met the Insect Unit demands until May 1991, when it became unavailable. This agar was initially available at half the cost of commercial agar. This left commercial agar as the only option, or an alternative, cheaper gelling agent.

The commercial agar used in the eldana diet conforms to a typical analysis as performed by suppliers, harvesters and manufacturers, as shown in Table 8 (King and Leppla, 1984). In addition the specifications for food grade agar are indicated.

Table 8

Typical analysis for standard agar used in insect diets (King and Leppla, 1984) and commercial and food-grade agars used in the *Eldana saccharina* diet

Agar product	Standard agar	Commercial agar	Food-grade agar
Gel strength at 1,5% (Kobe method)	730–800 g/cm^2	800 g/cm^2	800–900 g/cm^2
Moisture	16–17%	18%	15,5% max
pH	7,2	7,0	7,0–7,5
Total ash	1,5%	1,84%	5,0% max

Tamhankar and Dongre (1992) in India reported that the locally available crude agar (China grass), which is used in food products, is as good as any bacteriological grade agar in terms of gelling properties. This product is readily available and is cheaper than agar.

Ochieng'-Odero *et al.* (1991) substituted vanilla flavoured 'fruit-jelly' (3,9%) for the expensive imported agar for rearing *Chilo partellus* (Swinhoe), and found it to be cheaper with better keeping properties. The life cycle performance of the insects reared on this diet was as good as that of those reared on the agar-based diet.

Conclusions

The agar used in eldana diet production has, as a result of these tests, been reduced to 4 g/litre of water (0,3%).

Major food corporations have identified a growing demand among consumers for natural products to replace artificial food additives. Agar already has many uses in the food industry. As an entirely natural product it is likely to be in increasingly short supply on world markets (Anderson, 1991).

Pectin is a suitable gelling agent in the absence of agar for the eldana diet. It is widely used in the food industry and readily available should the need arise. The pectin price has not increased since May 1991 (R82,50/kg for 25 kg, including VAT).

REFERENCES

Anderson, R (1991). Harvesting Seaweed. *Veld and Flora* Sept 1991: 76-77.

Atkinson, PR (1978). Mass rearing and artificial infestation methods for *Eldana saccharina* Walker. *Proc S Afr Sug Technol Ass* 52: 143-145.

Graham, DY and Conlong, DE (1988). Improved laboratory rearing of *Eldana saccharina* (Lepidoptera: Pyralidae) and its indigenous parasitoid *Goniozus natalensis* (Hymenoptera: Bethylidae). *Proc S Afr Sug Technol Ass* 62: 116-119.

King, EG and Leppla, NC (eds) (1984). Advances and Challenges in Insect Rearing. USDA, New Orleans 306 pp.

Ochieng'-Odero, JPR, Onyango, FO, Kilori, JT, Bungu, MDO and Ambosa, EO (1991). Insect rearing management as a prerequisite in the development of IPM for sustainable food production. *Insect Sci Appl* 12 (5/6): 645-651.

Singh, P (1977). Artificial Diets for Insects, Spiders and Mites. Plenum Press, New York.

Singh, P and Moore, RF (eds) (1984). Handbook of Insect Rearing Vol 1. Elsevier, Amsterdam 488 pp.

Tamhankar, AJ and Dongre, TK (1992). Improved artificial diets for laboratory rearing of the spiny bollworm *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae). *Trop Pest Man* 38(1): 85-88.