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Studies on insect infestation in chocolates

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Abstract

The ability of stored-product pests including the cigarette beetle, Lasioderma serricorne, the sawtoothed grain beetle, Oryzaephilus surinamensis, the rust-red flour beetle, Tribolium castaneum, and the almond moth, Ephestia cautella, to infest chocolates under packaged and unpackaged conditions was investigated in the laboratory at $25 \pm 1\,^{\circ}C$ and $65 \pm 5\%$ r.h. Four types of chocolates were investigated: milk, nut, dried fruit and nut, and wafer chocolates. Adults (beetles only, 20 per replicate) or eggs (30 per replicate) were released on unpackaged and packaged chocolates and infestation levels (number of living adults and larvae) were determined 45 days later. When adult beetles were released on unpackaged chocolates, the degree of infestation varied depending on the species and the type of chocolate. The highest infestation observed in unpackaged chocolate was that of O. surinamensis in wafer chocolate (mean 138.4). When eggs were released on unpackaged chocolates, the most numerous species was E. cautella in dried fruit and nut chocolate (mean population = 180.8). With packaged chocolates exposed to adults or eggs, insect infestation was nil or negligible (mean population <6.0). Although infestation levels were low, infestations were found in 50% of treatments over all. Damage to the packaging material along the folds or edges was observed in infested chocolates. The study has shown that milk, nut, dried fruit and nut, and wafer chocolates can support insect infestation and therefore, insect-proof packing of the chocolates and storage under hygienic conditions are important to avoid customers' complaints.

Keywords: Chocolates; Insect infestation; Ephestia cautella; Oryzaephilus surinamensis; Lasioderma serricorne; Tribolium castaneum

1. Introduction

Chocolate products in many forms and flavours are relished by people of all ages for their sweetness and richness in nutrition. The global confectionery market including chocolate products has been estimated to exceed \$73.2 billion per annum and the annual global consumption of chocolate confectionery has been estimated as 6.5 million tonnes (CAOBISCO, 2004). Chocolate is prepared from a mixture of one or more of the following ingredients: cocoa bean/ nib/ press cake, cocoa dust, sugars, cocoa butter, milk solids and flavouring agents. Depending on its composition, chocolate is known as plain chocolate, milk chocolate, white chocolate, blended chocolate, bitter chocolate or composite chocolate. Cocoa, the basic ingredient in these chocolates varies from 20% to 60%. The type of packaging material used for chocolates varies; generally, aluminium foil, composite films, paper or plastic trays are used. The packaged chocolates are known to keep their quality up to 5 months when stored at 10–18 °C and 60–70% r.h. The actual storage period, however, may extend for a longer duration in the distribution network/ retail market.

Insect infestation in chocolates may occur at any stage from production to consumption. Insect infestations in and around chocolate manufacturing facilities have been reported in some countries (Bowditch and Madden, 1997; Wohlgemuth, 1992). It is recommended that chocolate products are stored in hygienic conditions in well-ventilated locations at 18–20 °C and <50% r.h. (ICCO, 2000). In the retail market, however, hygiene varies and accordingly the products are prone to infestation. When insects are found in the chocolates, consumers often direct their complaints to the manufacturer and the image of the company is affected (Highland, 1984). Customers'

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Table 1
Details of the types of chocolates used for the experiments

Chocolate type	Unit weight (g)	Moisture content (%)	Packaging material used	
			Inner	Outer
Milk chocolate	42	1.1	12 μm foil/25 μm polyethylene	Printed paper
Nut chocolate	35	2.8	25 μm biaxially oriented polypropylene	Printed carton
Dried fruit and nut chocolate	80	3.2	12 μm foil/25 μm polyethylene	Printed paper
Wafer chocolate	15	2.4	Nil	12.5 μm polyethylene terephthalate/metallisation/17.5 μm biaxially oriented polypropylene

complaints about insect infestation in chocolates in the retail market in both developed and developing countries have been reported (Scheurer and Dubau, 1999).

Data on the infestability of different types of chocolates under packaged and unpackaged conditions by insect pests are lacking. Experiments, therefore, were conducted to study the infestability of different types of chocolates under packaged and unpackaged conditions by four common stored-product insect pests viz., the rust-red flour beetle, Tribolium castaneum (Herbst), the sawtoothed grain beetle, Oryzaephilus surinamensis (L.), the cigarette beetle, Lasioderma serricorne (L.), and the almond moth, Ephestia cautella (Walker), over a storage period of 45 days.

2. Materials and methods

2.1. Chocolates

Milk chocolate (ingredients: milk solids, cocoa butter, cocoa solids, sugar, emulsifiers etc.), nut chocolate (ingredients: almonds + other ingredients as in milk chocolate), dried fruit and nut chocolate (ingredients: raisins, cashew nuts, apricot kernels + other ingredients as in milk chocolates) and wafer chocolate (ingredients: wheat flour, hydrogenated vegetable oil, edible starches + other ingredients as in milk chocolates) of the same brand were obtained from the local market. The type of packaging material used on the chocolates and their unit weight varied. The chocolates were not checked for damage or defects in the packaging materials prior to use in the experiment. The moisture content of the chocolates as determined by the toluene distillation method ranged from 1.1% to 3.2% (Table 1).

2.2. Insects

Tribolium castaneum and L. serricorne cultures were maintained on whole wheat flour + 5% yeast, O. surinamensis on broken wheat + rolled oats + yeast at a 5:5:1 ratio and E. cautella on broken wheat + 5% yeast + 5% glycerol in the laboratory at 25 ± 1 °C and 65 ± 5% r.h. From these cultures, unsexed 1–2 week old adults of T. castaneum and O. surinamensis and 2–3 day old adults of L. serricorne were taken for infestation studies. For the collection of eggs of T. castaneum, L. serricorne and O. surinamensis, wheat flour was passed through an 85-mesh standard sieve (pore size 180 μm). Into the sieved flour (200 g) c. 250 adults were released for oviposition for 48 h. At the end of 48 h, the adults were removed from the flour using standard 25- or 44- mesh sieves (pore sizes 600 and 335 μm, respectively). The flour was subsequently sieved through an 85-mesh sieve to separate the eggs, which were then placed on the chocolates. To collect E. cautella eggs, newly emerged adults were released into a metal cage having a mesh bottom, which allows eggs to pass through. Eggs, 0–2 days old, were collected in a tray under the cage and then placed on the chocolates. Viability of eggs of individual species was checked as described in Rajendran et al. (2004). Eggs (four replicates per species) were placed individually in the wells of ELISA plates that were cut into half so as to have only 48 wells and egg hatch was observed under a binocular microscope, daily, until there were no more emergences. Viability (% hatched) of eggs was: T. castaneum (91.0 ± 5.9), L. serricorne (79.2 ± 12.3), O. surinamensis (86.8 ± 7.8), and E. cautella (76.1 ± 14.5).

2.3. Infestation studies

The studies were carried out on chocolates (70–84 g per replicate) under packaged conditions as well as without any packaging materials. Chocolates were placed in bottles (size 13 cm × 9 cm) for 45 days at 25 ± 1 °C and 65 ± 5% r.h. There were 2 sets of experiments. In the first set, adults (20 per replicate) were released on to chocolates with and without packaging material. In the second set, 1–2 day old eggs (30 per replicate) of the individual species were placed on both unpackaged and packaged chocolates. There were 5 replicates for each type of chocolate. After the addition of insects, the bottles containing chocolates and reference diets were covered with pieces of cloth, tied with rubber bands and held at 25 ± 1 °C for 45 days. At the end of 45 days, insect populations (live larvae and adults) in chocolates were recorded. In packaged chocolates, whenever infestation was found, the packaging materials of the

Table 2
Populations of Tribolium castaneum, Lasioderma serricorne, Oryzaephilus surinamensis and Ephestia cautella in unpackaged chocolates at the end of a 45-day storage period at 25±1 °C

Insect	No. of insects (mean±SD) recorded in different types of chocolates[c,d]			
	Milk	Nut	Dried fruit and nut	Wafer
Adults[a]				
Tribolium castaneum	23.0±8.7ax	17.4±13.3ax	22.8±10.8ax	11.6±2.6ax
Lasioderma serricorne	58.2±23.0bx	45.2±12.3bx	86.0±15.5bx	79.0±75.6bx
Oryzaephilus surinamensis	53.2±5.0bx	52.0±6.0bx	95.6±20.3by	138.4±22.5cz
Eggs[b]				
Tribolium castaneum	9.2±4.7aby	10.6±8.2ay	11.6±4.2ay	3.6±3.7ax
Lasioderma serricorne	16.0±5.1by	11.2±7.7ay	17.4±2.8aby	6.2±4.9ax
Oryzaephilus surinamensis	6.8±6.0ax	8.4±7.4ax	27.4±10.0by	5.4±5.4ax
Ephestia cautella	18.6±4.1bx	71.6±45.4by	180.8±50.9cz	14.8±6.2bx

[a]20 adults per each of 5 replicates released.
[b]30 eggs per each of 5 replicates released.
[c]Means in the same column of adults or eggs followed by different letters (a, b, c) differ significantly according to Duncan's new multiple range test ($P<0.05$).
[d]Means in the same row of adults or eggs followed by different letter (x, y, z) differ significantly according to Duncan's new multiple range test ($P<0.05$).

chocolates were examined for the presence of damage or defects.

2.4. Data analysis

Data on insect populations in the four types of chocolates were transformed to logarithmic (for unpackaged chocolates) or $\sqrt{(x+0.5)}$ (for packaged chocolates) values and subjected to ANOVA followed by Duncan's New Multiple Range test to determine significant differences in infestation (of the individual species) between chocolate types.

3. Results

In unpackaged chocolates, all four species could infest but the degree of infestation varied between chocolate types and insect species (Table 2). In general, populations were greatest in dried fruit and nut chocolate. Where adults were released in the chocolates, O. surinamensis (average counts, 52–138) and L. serricorne (45–86) populations were higher than T. castaneum (12–23). Oryzaephilus surinamensis infestation levels in the chocolates were: wafer < dried fruit and nut < milk = nut. No significant differences in the levels of T. castaneum and L. serricorne infestation between chocolate types were observed.

When eggs were used, high population levels of E. cautella (180.8±50.9) were noted in dried fruit and nut chocolate. Among the beetle pests, high average counts were recorded of O. surinamensis (5–27) and L. serricorne (6–17). The levels of infestation when eggs of O. surinamensis were placed in unpackaged chocolates was: dried fruit and nut < milk = nut = wafer, whereas, for L. serricorne as well as T. castaneum the infestation levels were: milk = nut = dried fruit and nut < wafer.

With packaged chocolates exposed to adults or eggs, insect infestations were nil in 50% of the treatments and in the remaining treatments very low infestation levels (mean population < 6.0) were observed (Table 3). No significant differences in the level of infestation between species and between chocolate types were noted. No infestations were found from T. castaneum adults released on wafer or nut chocolates nor from eggs of either O. surinamensis or E. cautella in dried fruit and nut, nut or wafer chocolates. Damage to inner or primary packaging material (12 μm foil/ 25 μm polyethylene) along the folds or edges was observed in milk and dried fruit and nut chocolates infested with the adults of O. surinamensis as well as L. serricorne and with the eggs of E. cautella. Damage to the packaging material (12.5 μm polyethylene terephthalate/metallization/ 17.5 μm biaxially oriented polypropylene) along the seal was also noticed in wafer chocolate infested with the adults of O. surinamensis.

4. Discussion

Stored-product insects have different food preferences. Chocolates contain various ingredients such as cocoa, tree nuts, dried fruits and wheat flour that are favourable for the growth and multiplication of insects. Lasioderma serricorne is a major pest on cocoa and cocoa products and can breed readily in all four types of chocolates in addition to tobacco. Oryzaephilus surinamensis, a common pest on processed foods and tree nuts, also breeds readily on chocolates (Table 2). The omnivorous T. castaneum, however, produced only low-level populations in the chocolates tested. Population levels of E. cautella, a pest on a variety of foodstuffs including cereals, cereal products, cocoa, oilseeds, tree nuts and dried fruits were

Table 3
Populations of Tribolium castaneum, Lasioderma serricorne, Oryzaephilus surinamensis and Ephestia cautella in packaged chocolates at the end of a 45 days storage period at $25\pm1\,^{\circ}C$

Insect	No. of insects (mean ± SD) recorded in different types of chocolates			
	Milk	Nut	Dried fruit and nut	Wafer
Adults[a]				
Tribolium castaneum	0.8 ± 1.1	0	3.6 ± 8.0	0
Lasioderma serricorne	0	0.2 ± 0.4	1.6 ± 1.8	5.4 ± 9.9
Oryzaephilus surinamensis	5.8 ± 13.0	0	0.6 ± 1.3	1.0 ± 2.2
Eggs[b]				
Tribolium castaneum	1.8 ± 2.2	0.2 ± 0.4	0	0
Lasioderma serricorne	0	0	0.8 ± 1.8	0.4 ± 0.9
Oryzaephilus surinamensis	0.2 ± 0.4	0	0	0
Ephestia cautella	1.8 ± 3.6	0	0	0

No significant differences were observed between columns or rows of adults or eggs according to Duncan's new multiple test ($P<0.05$).
[a]20 adults per each of five replicates released.
[b]30 eggs per each of five replicates released.

high on the chocolates particularly in fruit and nut as well as nut chocolates.

The susceptibility of the ingredients used in chocolate manufacture to insect pests such as E. cautella and the Indian meal moth, Plodia interpunctella (Hübner) has been studied (Locatelli and Garavaglia, 1995). It was noted that raw hazel nuts used in the manufacture of chocolates were more susceptible than the basic ingredients of the chocolate.

Insect infestation in chocolates may depend on (1) storage conditions, (2) storage temperature, (3) type and thickness of the packaging materials, and (4) storage period (Collins, 2003). The extent of infestation also depends on the insect pest complex occurring in the premises. The storage period of manufactured chocolates generally varies from 3–6 months in the retail market. During longer storage, more insects are likely to invade or penetrate the packaging material causing more damage to the chocolates. The longer storage period facilitates insect development to a life stage, i.e., larva or adult that is capable of damaging the packaging material and/or the insects have sufficient time to chew or damage the packaging material (Kelly, 2004). Rapid turnover of the stocks will discourage insect pest attack during storage (Anonymous, 1984).

Packaging plays an important role in maintaining the shelf life of chocolates and as a physical barrier against the entry of insect pests. The penetrability of the packaging material by insect pests varies between species and among the active stages of a particular species (Cline, 1978). Stored-product insects have been classified as invaders and penetrators of packed foodstuffs. Invaders (e.g. adults as well as larvae of O. surinamensis) can gain entry into the product through pinholes/punctures or imperfect sealing, whereas penetrators (e.g. adults of L. serricorne, larvae of E. cautella) with powerful mouthparts can pierce or damage the packaging materials. In the current study, damage to the packaging material along the folds or edges due to insects (O. surinamensis, L. serricorne and E. cautella) was noticed. In the present experiment on packaged chocolates, out of a total 28 treatment combinations, 50% of treatments had at least one infested replicate (Table 3). Although infestation levels were low, any infestation is important from a retailer or customer's point of view.

While examining the barrier properties of different packaging materials that were used for chocolate products, Schmidt (1979) observed that even aluminium foil was not 100% insect proof. In the present study, insects could penetrate along the folds or edges of 12 μm aluminium foil combined with 25 μm polyethylene packaging material in milk and dried fruit and nut chocolates. Insect pests could gain entry into the packaged products through minute holes and defective sealing of the packaging material. Sealing defects are likely to occur during manufacture of chocolates. For the entry of insects (adults or larvae), depending on the species, minute holes ranging from 560 to 1700 μm are enough for penetration (Cline and Highland, 1981). Due to variations in the extent of damage/defects in the packaging materials of the four types of chocolates and invasion potential of individual insect species, infestation in packaged chocolates was sporadic in the present study (Table 3).

Food aroma/volatiles escaping from defective sealing or damaged packaging material may attract insects for oviposition or feeding. In laboratory studies, Mowery et al. (2002) showed that female adults of O. surinamensis were attracted towards packaged products for oviposition. In another study, it was noted that E. cautella females were attracted towards the grain odour, which stimulated their oviposition (Barrer and Jay, 1980). In laboratory tests on insect resistance to packaging materials, it was shown that P. interpunctella, an important pest on chocolate products, could distinguish sealed packets with and without food items for oviposition (Mullen, 1994).

Insects such as T. castaneum (24 days as adults and 9–12 days as larvae) and L. serricorne (9 days as adults as well as larvae) are highly tolerant of starvation (Cline, 1978; Koura and El-Halfawy, 1973). Starved insects have a greater tendency to attack packed products than normal insects. In laboratory tests it was observed that starved larvae are more aggressive in penetrating into the packaging material (Cline, 1978). Therefore, in the absence of any other food commodities, insects present in the vicinity of chocolates would be more likely to penetrate the packaging material of the products. In the present study it was noted that a few adults of T. castaneum survived during the storage period (45 days) probably by feeding on the packaging material of the chocolates.

5. Conclusion

Chocolates have ingredients such as cocoa, tree nuts and dried fruits and therefore are susceptible to insect infestation during storage. The present study has shown that all four stored-product species tested could establish different levels of infestation on four types of chocolates. In packaged chocolates, however, only negligible levels of infestation were established. To discourage infestation, there must be rigorous quality checks on packaging materials and their sealing during production and the chocolates must be stored under hygienic conditions away from infested foodstuffs.

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