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Optimum levels of sorbic acid and methyl-p-hydroxybenzoate in an artificial diet for Adalia bipunctata (Coleoptera, Coccinellidae) larvae

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Kariluoto, K. T. 1978. Optimum levels of sorbic acid and methyl-p-hydroxybenzoate in an artificial diet for Adalia bipunctata (Coleoptera, Coccinellidae) larvae. — Ann. Ent. Fenn. 44, 94—97.

Five concentrations (0, 500, 1000, 1500 and 2000 ppm) of sorbic acid (SA) and methyl-hydroxybenzoate (MpHB) in 25 combinations were added to an artificial diet for *Adalia bipunctata* L. larvae. 1500 ppm of SA alone could control moulding of the diet for three weeks at +25°C, but 2000 ppm of MpHB could do the same only with 500 ppm or more of SA. Adult emergence was highest (76 %) on the diet with 1000 ppm of SA + 0 ppm of MpHB. The higher the concentrations of SA and/or MpHB, the more retarded was postembryonic development (19.0 d with 0 ppm of SA + 0 ppm MpHB and 24.3 d with 2000 ppm of SA + 2000 ppm of MpHB). The adult emergence weight was not clearly affected by the various concentrations of SA and/or MpHB.

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Index words: *Adalia bipunctata,* artificial diet, mould contamination, larval development, methyl-p-hydroxybenzoate, sorbic acid.

The antifungal agents used to control moulding of rearing media for insects are known to have detrimental effects on larval development (Ouye 1962, Kishaba et al. 1968, Bass & Barnes 1969, Singh & House 1970). Two of the agents used most frequently are methyl-p-hydro-

Table 1. Effect of different combinations of sorbic acid (SA) and methyl-p-hydroxybenzoate (MpHB) on the moulding of rearing vials by *Aspergillus niger* after A. 1 week's. B. 2 week's and C. 3 week's rearing at +25°C. Moulding rates as percentages ± S.E.M. Means, for SA and MpHB in rows and columns followed by the same letter do not differ significally according to Tukey's studentized range (P = 0.05).

MpHB ppm	A SA ppm 0	500	1000	1500	2000	Mean	B SA ppm 0	500	1000	1500	2000	Mean	C SA ppm 0	500	1000	1500	2000	Mean
0	100± 0	78± 8.7	18± 7.8	0	0	39.2 a	100± 0	90± 5.6	44± 11.1	0	0	46.8 a	100± 0	94± 3.5	44± 11.1	0	0	47.6 a
500	94± 5.6	80± 6.8	14± 8.5	0	0	37.6 a	98± 1.4	84± 6.5	20± 9.3	0	0	40.4 ab	98± 1.4	86± 7.2	22± 11.1	0	0	41.2 ab
1000	86± 7.2	26± 7.9	0	0	0	22.4 b	94± 1.4	50± 8.7	6± 3.5	0	0	30.0 bc	96± 3.5	58± 10.5	6± 3.5	0	0	32.0 b
1500	80± 8.7	10± 6.0	0	0	0	18.0 b	88± 7.1	26± 9.1	0	0	0	22.8 c	88± 7.1	36± 14.2	0	0	0	24.8 c
2000	44± 10.1	0	0	0	0	8.8 b	74± 10.6	0	0	0	0	14.8 c	74± 10.6	0	0	0	0	14.8 c
Mean	80.8 a	38.8 b	6.4 c	0 c	0 c	25.2	90.8 a	50.0 b	14.0 c	0 c	0 c	31.0	91.2 a	54.8 b	14.4 c	0 c	0 c	32.1

xybenzoate (MpHB) and sorbic acid (SA) (Singh 1974). These were included in the artificial diet developed for *A. bipunctata* larvae by Kariluoto et al. (1976), but the concentrations used (550 ppm of SA with 620 ppm of MpHB) did not inhibit mould satisfactorily. The most usual fungal contaminants were *Aspergillus niger* (van Tieghem) and *Penicillium* spp. Chlortetracyclinechloride at 46—30 ppm was used against bacteria with good success.

Material and methods

Experimental animals and rearing methods

Adult *A. bipunctata* collected in the Helsinki area in autumn 1976 were kept at room temperature (LD 18:6 h) and fed on *Acyrthosiphon pisum* (Harris). Eggs were collected during the daily feeding and stored at +10±0.5°C (RH 80—90 %, LD 0:24 h) for at most 7 days. The eggs hatched in 2 days when taken to the rearing chamber (+25±0.5°C, RH 55—65 %, LD 18:6h). The light source in the chamber was 10 fluorescent tubes (Philips TLD 30 W/32 De Luxe) located 0.8—1.1 m above the rearings.

Preparation of the rearing medium

The basic diet was that presented by Kariluoto et al. (1976) with slight modifications, the ingredients being as follows: deionized water 90.0 ml, Bacto-agar, Difco 3.7 g, deionized water 120.0 ml, wheat germ 5.0 g, brewer's yeast 7.0 g, sucrose 7.0 g, soluble casein, BDH 3.0 g, egg ca. 50 g, beef liver 50.0 g, honey 10.0 g, salt mixture (Beckman et al. 1953) 1.0 g, ascorbic acid, Merck 1.0 g, DL-alpha-tocopherol, Merck 0.3 g, vitamin stock (Ignoffo 1963) 4.0 ml, choline chloride, 15 % in water, Merck 1.5 ml, meso-inositol, Merck 0.3 g, aureomycin (chlortetracyclinechloride 55 g/kg) Cyanamide 0.3 g, sorbic acid (SA), Merck 0.00—0.72 g, methyl-p-hydroxybenzoate (MpHB) 0.00—0.72 g and ethyl alcohol, 94 w % 5.0 + 5.0 ml, all together ca. 362 g.

Five concentrations (0, 500, 1000, 1500 and 2000 ppm) of SA and MpHB were added in 25 combinations as EtOH solutions. The amounts of SA and MpHB were dissolved separately in 5.0+5.0 ml EtOH abs., so that the amount of EtOH added to every diet was the same.

The agar was first dissolved in 90 ml water in a boiling water bath. All the other ingredients, including the SA and/or MpHB solutions, and 120 ml water were ground to a fine suspension with a homogenizer (Ultra-Turrax, Janke & Kunkel KG) for 5—6 min and then heated to +60—65°C in a hot water bath. The mixture was then blended again for 5—10 min with a household mixer. During blending at least a part of the EtOH evaporated. The hot clear agar was poured into the mixture and when it was evenly blended the liquid medium was pipetted into rearing vials.

The vials (4 ml), each containing 0.8 ml of the medium were left uncovered until cold, to allow excess moisture to evaporate and then stored in plastic bags at +1°C for 3—4 days or at — 18°C for longer periods. When required for newly hatched larvae, they were taken to room temperature and left overnights upside down in their bags. In the morning the bags were removed and free water was allowed to evaporate.

Table 2. Effect of different combinations of sorbic acid (SA) and methyl-p-hydroxybenzoate (MpHB) in the larval diet of *A. bipunctata* on adult emergence (percentages ± S.E.M.). Means for SA and MpHB in rows and columns, followed by the same letter do not differ significantly according to Tukey's studentized range (P = 0.05).

MpHB ppm	SA ppm 0	500	1000	1500	2000	Mean
0	12± 5.5	66± 7.2	76± 4.7	60± 6.8	30± 8.1	48.8 a
500	10± 6.0	40± 6.0	66± 5.7	66± 9.1	38± 7.7	44.0 a
1000	12± 3.2	50± 4.0	54± 7.2	74± 7.2	52± 3.2	48.4 a
1500	18± 6.3	50± 6.8	48± 6.3	54± 3.5	40± 6.8	42.0 a
2000	20± 6.8	42± 5.5	46± 6.5	50± 8.1	32± 7.7	38.0 a
Mean	14.4 a	49.6 ab	58.0 b	60.8 b	38.4 ab	44.2

Table 3. Effect of different combinations of sorbic acid (SA) and methyl-p-hydroxybenzoate (MpHB) in the larval diet of *A. bipunctata* on the postembryonic (larval + pupal) developmental time (in days ± S.E.M.). Equation for developmental time calculated from the means of each combination by multiple linear regression: $T_{dev} = 19.71 + 0.00132 \times SA_{ppm} + 0.00104 \times MpHB_{ppm}$.

MpHB ppm	SA ppm 0	500	1000	1500	2000	Mean
0	19.0± 0.49	19.6± 0.38	21.6± 0.41	21.6± 0.43	22.7± 0.61	21.1 a
500	22.0± 0.68	21.0± 0.47	21.8± 0.40	22.2± 0.38	23.7± 0.35	22.1 bc
1000	20.0± 0.55	21.2± 0.43	21.8± 0.38	22.0± 0.30	23.7± 0.35	22.0 ab
1500	20.6± 0.52	21.9± 0.45	23.2± 0.40	23.0± 0.49	23.6± 0.32	22.7 cd
2000	22.7± 0.80	23.2± 0.41	22.7± 0.42	23.9± 0.47	24.3± 0.49	23.4 d
Mean	21.0 a	21.2 a	22.1 b	22.5 b	23.5 c	22.2

Five batches of 10 one-day-old, unfed larvae were put on every diet, one larva to each vial, and the vials were plugged with cotton. On the seventh rearing day the larvae were transferred to new vials with fresh medium, where they completed their development. The first rearing vials were left in the rearing chamber to incubate possible contaminants. The number of contaminated vials was counted after 1, 2 and 3 weeks.

Results and discussion

The results indicate that SA controls *A. niger* on the rearing medium more effectively than MpHB (Table 1). The data were subjected to analyses of variance and the means were compared using Tukey's studentized range (P= 0.05). Kishaba et al. (1968) have reported a similar difference between SA and MpHB. A parallel finding is that *A. niger* has developed strains resistant to MpHB (Gifawesen et al. 1975). *Penicillium* spp. were not common on the present medium and they were not found at all when it contained 1000 ppm or more SA.

Judged from the adult emergence percentages the optimal levels of SA and MpHB in the medium lie between 500 and 1500 ppm of SA and 0 and 1500 ppm of MpHB (Table 2). The low emergence values on diets with 0 ppm of SA may be due to the high contamination by mould (Table 1 A). Table 2 indicates that high concentrations (2000 ppm) of MpHB and SA, especially the latter, tend to lower emergence in *A. bipunctata*.

The shortest postembryonic (larval + pupal) developmental period (19 days) was on the diet with no SA or MpHB. The higher the concentrations of SA and MpHB, the longer were the developmental periods (Table 3). The effects of SA and MpHB seem to be of the same degree. (Table 3). This retardation of development accords with the results of Ouye (1962) and Singh & House (1970), who suggest that the most sensitive criterion of the effects of antimicrobial agents is the larval and pupal development.

The various concentrations of SA and MpHB did not clearly affect the mean adult weight (Table 4), but diets with no SA or MpHB produced lighter adults than the others, possibly owing to high contamination by mould (Table 1 A).

The concentrations of SA and MpHB required to control contamination of the rearing medium by mould are sometimes also lethal to the insect species itself (Prokopy 1967, Bass & Barnes 1969). The results of this study indicate that *A. bipunctata* larvae can survive satisfactorily on

Table 4. Effect of different combinations of sorbic acid (AS) and methyl-p-hydroxybenzoate (MpHB) in the larval diet of *A. bipunctata* on the weight of emerging adults (in mg ± S.E.M.). Equation for adult weight calculated from the means of each combination by multiple linear regression: $W_{ad} = 6.092 + 0.000308 \times SA_{ppm} + 0.000172 \times MpHB_{ppm}$.

MpHB ppm	SA ppm 0	500	1000	1500	2000	Mean
0	5.7± 0.30	6.2± 0.29	5.5± 0.21	6.6± 0.24	7.0± 0.40	6.2 a
500	5.3± 0.72	7.3± 0.35	6.4± 0.24	6.9± 0.23	6.7± 0.33	6.7 b
1000	6.2± 0.43	6.7± 0.22	6.7± 0.38	7.4± 0.20	7.1± 0.29	7.0 b
1500	5.9± 0.32	7.0± 0.29	6.3± 0.25	6.9± 0.50	6.3± 0.29	6.6 ab
2000	5.9± 0.37	6.6± 0.24	6.9± 0.25	6.6± 0.29	6.6± 0.37	6.6 ab
Mean	5.8 a	6.7 bc	6.3 ab	6.9 c	6.7 bc	6.6

medium with sufficiently high concentrations of SA and MpHB to inhibit mould contaminants. Tables 1—4 indicate that the optimal addition of these antifungal agents is a combination of 1000—1500 ppm of SA and 0—1000 ppm of MpHB.

Bacteria were not a serious problem, because during preparation of the medium the temperature rose to ca. +70°C and at least a part of the microbes died. Chlortetracyclinechloride was used at a concentration of about 46 ppm, which seemed to be high enough to control bacteria (cf. Gifawesen et al. 1975).

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