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# COMBINATIONAL EFFECTS OF SOME ANTIMICROBIAL AGENTS ON THE SURVIVAL AND DEVELOPMENT OF THE ENDOPARASITOID *PIMPLA TURIONELLAE* L. (HYMENOPTERA: ICHNEUMONIDAE)

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## ABSTRACT

The effects of some antibiotics and antifungal agents in different levels and combinations on the survival and development of the hymenopterous endoparasitoid, *Pimpla turionellae* were investigated by rearing the larvae aseptically on chemically defined synthetic diets. These effects varied with the kinds and levels of the antimicrobial agents in the combinations. The effects of antimicrobial agents tested were mostly exerted during the post-larval developmental stages. Some of the combinations also exerted their effects on the larval survival and development. In general, except a combination of nystatin with methyl *p*-hydroxybenzoate, the other tested combinations of two antimicrobial agents had negative effects on the post-larval survival of the insect.The diet with 45mg of nystatin and 2.5 mg of methyl *p*-hydroxybenzoate caused a significant increase in the number of survivors in the post-larval stages and shortened the developmental time for these stages. When added 30 mg of nystatin to the diet with a combination of penicillin and rifampicin (20:2.5 mg) the post-larval survival was significantly increased. The other tested combinations of three antimicrobial agents had no noticeable effects on the insect.

## INTRODUCTION

In recent years utilization of antimicrobial agents for control of microbial contaminations has greatly increased in the artificial rearing of parasitic hymenopterous insects. Some antimicrobial agents have been reported to have had some detrimental effects on the insects. The attempts were tended to determine the levels of antimicrobial agents which have no apparent effects on the insects. The first effort was made to use some antimicrobial agents such as triburon and streptomycin sulfate for control of microorganisms in the diet of *Itoplectis conquisitor* Say (Yazgan, 1972). No effects of these agents on the larvae of this insect was noted. Subsequently, some progress has been made in using of some antibiotics and antifungal agents in artificial diets for several *Trichogramma* species

(Xie et al. 1986; Grenier and Liu, 1990; 1991). They reported some negative effects of these antimicrobial agents on the survival, development, egg production and hatchability in these egg parasitoids. Our preliminary work with a pupal endoparasitoid, *P. turionellae* L. showed that some antibiotics such as penicillin, streptomycin and rifampicin had adverse effects on the survival and development according to their kinds and dietary levels (Büyükgüzel and Yazgan, 1996). In all this work antimicrobial agents alone have been used in the diets of parasitic hymenopterous insects. There has been no report about their combinational effects on the survival and development of these insects. Such a work would also be of great practical importance to control of co-contaminations of bacteria with fungi in the diets for mass-rearing of parasitic hymenopterous insects in laboratory conditions. Therefore, it is necessary to determine the levels of combinations which have no apparent effects on the insects. The combining of the antimicrobial agents would also be important in reducing or eliminating the adverse effects of antimicrobial agents on the insects.

The present work was conducted to investigate the combinational effects of some antibiotics and antifungal agents on the survival and development of the larvae of an endoparasitoid hymenopterous insect, *P. turionellae*.

## MATERIALS AND METHODS

*P. turionellae* adults used in the experiments were reared on the pupae of *Galleria mellonellae* L. in a laboratory with 23 ± 1°C, 75 ± 5 % r.h. and 16 h. photoperiod. Adults were fed with 50 % honey solution. The adults were also fed with *G. mellonellae* pupae for sucking haemolymph to provide sufficient protein every other day.

The synthetic diet described by Yazgan (1981) was used for rearing the larvae of *P. turionellae* in laboratory conditions. The diet consisted of an amino acid mixture, lipid mixture, an inorganic salt mixture, glucose, RNA and a mixture of water soluble vitamins. The methods used in the preparation of-stock solutions of dietary components, diet from these stocks, sterilization techniques, adding sterilized vitamin-glucose and antimicrobial agent solutions into diet, dispensing the diets into test-tubes, obtaining the sterilized larvae and their inoculation onto diets, preparation of gelatine capsules and transferring of the mature larvae to these capsules for pupation, and employed equipment, techniques and handling during feeding experiments were extensively described in the our preliminary work (Büyükgüzel and Yazgan, 1996).

Antimicrobial agents used in these feeding experiments were obtained from different firms; penicillin G and streptomycin sulphate were from Claus & Huth Ltd., rifampicin was from Koçak Medicine Ltd., nystatin was from Bristol - Myers Squibb Inc., Methyl *p*-hydroxybenzoate was from Koch - Light Lab., cycloheximide was from Sigma Chemical Co.

The concentration of agents tested were expressed as mg/100 ml of diet. Reguired amounts of the antimicrobial agents were dissolved in a certain volume of water, then sterilized through 0.22 μm pore-sized membrane filter and added into diet before gel formation. The volume of the diet was established by adjusting the amount of water in the diet. One newly hatched larva was inoculated into test-tubes that contained a given diet of about 0.5 mL. Ten larvae were used for each diet and each experiment was replicated three times. The feeding experiments were done under the same laboratory conditions as mentioned for rearing of the adults of *P. turionellae.*

The effects of six antimicrobial agents were investigated by addition of these agents into diets as combinations containing two or three agents (binary or ternary combinations). Firstly, both levels of each antimicrobial agents which have the most positive and the most negative effects on the post-larval survival of the insect according to control diet were individually determined. By using these levels of each antimicrobial agent the binary combinations were prepared and then tested to investigate their combinational effects on the insect: The first binary combination contained the levels of two antimicrobial agents which have the most positive effects jointly. The others contained the level of one antimicrobial agent which has the most positive effects plus the level of another agent which has the most negative effects or inversely. Each one of the antimicrobial agent (their levels which have the most positive effects) in the tested combinations alone was also tested in the same experimental group for comparison of the effects of the binary combinations. The antimicrobial agents of the binary combinations were either antibiotics (Table 1, 2, 3) or antifungal agents (Table 4, 5, 6).

The ternary combinations were prepared by addition of a third antimicrobial agent (its level which has the most positive effects) to above some binary combinations which had the most positive effects according to the tested agents alone and tested to investigate their effects on the insect. Each one of the binary combination in the tested ternary combinations alone were also tested in the same experiment for comparison of the effects of ternary combinations. The antimicrobial agents of these combinations were either two antibiotics plus a antifungal agent or two antifungal agents plus a antibiotic (Table 7, 8).

The effects of the tested diets with different levels of antimicrobial agents, alone and in combination, on insect were measured by determining the rate of development (average time to reach fifth, pupal and adult instar) and survival (number of survivors in these instars) of the larvae. Data on the rate of development were evaluated by analysis of the variance (Snedecor and Cochran, 1967). To determine significant differences between means Duncan's (1955) multiple range test was used. Data on survival were compared by $\chi^2$ test (Snedecor and Cochran, 1967). When F and $\chi^2$ exceeded their 0,01 value the differences were considered significant.

## RESULTS

### Effects of combinations containing two antimicrobial agents

#### Penicillin – streptomycin

**Table 1.** The effects of penicillin and streptomycin alone and in combination on the survival and development of *P. turionellae*.

| penicillin: | streptomycin (mg / 100ml of diet) | Initial No of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D.)[#] | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D)[#] | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D)[#] |
|---|---|---|---|---|---|---|---|---|
| 10 | - | 26 | 96.2 | 9.0 ± 0.0a | 34.6 | 22.2 ± 0.6a | 34.6 | 29.1 ± 0.1a |
| - | 20 | 27 | 96.3 | 9.0 ± 0.0a | 37.0 | 21.6 ± 0.5a | 33.3 | 29.2 ± 0.2a |
| 10 | 20 | 26 | 100.0 | 10.0 ± 0.3b | 46.2 | 23.0 ± 0.0b | 46.2 | 31.1 ± 0.5b |
| 10 | 40 | 27 | 70.4 | 10.0 ± 0.0b | 18.5 | 22.0 ± 0.0a | 18.5 | 30.6 ± 0.7c |
| 20 | 20 | 28 | 82.1 | 10.3 ± 0.5b | 28.6 | 22.0 ± 0.0a | 28.6 | 30.6 ± 0.7c |

* : Average of 3 replicates; 10 larvae per replicate
# : Values followed by the same letter are not significantly different from each other, $P > 0.01$

The effects of penicillin and streptomycin alone and in combination are shown in Table 1. The diets with these antibiotics alone had no significant effects on the survival and development of the insect when compared with each other. The diets with different combinations of penicillin and streptomycin prolonged the average time to reach the fifth and adult instar according to diets with these agents alone. Pupal development was retarded by only the diet containing 10 mg of penicillin plus 20 mg of streptomycin when compared with other tested levels of agents alone and in combination. All tested levels of antimicrobial agents, alone and in combination, had no significant effects on the survival of the insect.

**Penicillin - rifampicin**

**Table 2.** The effects of penicillin and rifampicin alone and in combination on the survival and development of *P. turionellae*.

| Penicillin: rifampicin (mg / 100ml of diet) | | Initial No. of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D. )# | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D )# | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D )# |
|---|---|---|---|---|---|---|---|---|
| 10 | - | 29 | 100.0 | 9.0 ± 0.3a | 31.0 | 23.0 ± 0.0a | 31.0 | 30.0 ± 0.0a |
| - | 2.5 | 27 | 81.5 | 10.0 ± 0.0b | 29.6 | 23.7 ± 0.7a | 29.6 | 30.0 ± 0.0a |
| 10 | 2.5 | 27 | 92.6 | 8.0 ± 0.0c | 14.8 | 21.0 ± § | 14.8 | 29.0 ± § |
| 10 | 10.0 | 23 | 52.2 | 13.0 ± 0.0d | - | - | - | - |
| 20 | 2.5 | 23 | 95.7 | 8.0 ± 0.0c | 17.4 | 19.0 ± § | 17.4 | 28.0 ± § |

* : Average of 3 replicates; 10 larvae per replicate
\# : Values followed by the same letter are not significantly different from each other, P > 0.01
§ : From two replicate

Data in Table 2 show that the diet with 2.5 mg of rifampicin retarded larval development according to the diet with penicillin alone. The diets containing 2.5 mg of rifampicin with 10 mg of penicillin significantly shortened the average time to reach fifth instar when compared to diets with these agents alone. Increasing of the rifampicin level from 2.5 to 10 mg in this combination significantly retarded the larval development. No larvae fed on the diet with this combination could produce pupae.

**Streptomycin-rifampicin**

**Table 3.** The effects of streptomycin and rifampicin alone and in combination on the survival and development of *P. turionellae*.

| Streptomycin: rifampicin (mg / 100ml of diet) | | Initial No. of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D. )# | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D )# | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D )# |
|---|---|---|---|---|---|---|---|---|
| 20 | - | 27 | 100.0 | 9.1 ± 0.2a | 40.7 | 23.1 ± 0.4a | 40.7 | 30.2 ± 0.7a |
| - | 2.5 | 27 | 96.3 | 10.0 ± 0.4b | 29.6 | 22.1 ± 0.1b | 29.6 | 29.9 ± 0.6a |
| 20 | 2.5 | 24 | 91.7 | 9.9 ± 0.3b | 25.0 | 21.8 ± 0.5b | 20.8 | 30.0 ± 0.0a |
| 20 | 10.0 | 29 | 89.7 | 9.0 ± 0.2a | 6.9 | 22.0 ± § | 6.9 | 30.0 ± § |
| 40 | 2.5 | 24 | 91.7 | 9.0 ± 0.0a | 12.5 | 22.0 ± 0.0b | 12.5 | 30.0 ± 0.0a |

* : Average of 3 replicates; 10 larvae per replicate
\# : Values followed by the same letter are not significantly different from each other, P > 0.01
§ : From two replicate

The diet with 2.5 mg of rifampicin retarted the larval development but shortened the time to reach the pupal stage in significant degree according to the diet with 20 mg of streptomycin (Table 3). The diet with the former levels (2.5:20 mg) of these agent in combination had similar effects on development of these stage to those of 2.5 mg of rifampicin alone. But this negative effects on the larval development were overcome with increasing of the level of streptomycin from 20 to 40 mg in this combination. When added 10 mg of rifampicin to the diet with 20 mg of streptomycin, the post-larval survival was significantly decreased.

## Nystatin - Methyl *p*-hydroxybenzoate

**Table 4.** The effects of nystatin and methyl *p*-hydroxybenzoate (methyl *p* HB) alone and in combination on the survival and development of *P. turionellae.*

| Nystatin: methyl *p* HB (mg / 100ml of diet) | | Initial No. of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D.)# | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D)# | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D)# |
|---|---|---|---|---|---|---|---|---|
| 30 | - | 28 | 100.0 | 10.0 ± 0.0a | 39.3 | 22.0 ± 0.0a | 39.3 | 30.0 ± 0.2a |
| - | 2.5 | 25 | 92.0 | 10.0 ± 0.0a | 36.0 | 22.4 ± 0.1b | 24.0 | 31.0 ± 0.0b |
| 30 | 2.5 | 27 | 70.4 | 8.1 ± 0.3b | 29.6 | 19.9 ± 0.5c | 25.9 | 28.2 ± 0.6c |
| 30 | 10.0 | 22 | 100.0 | 9.0 ± 0.0c | 13.6 | 22.0 ± 0.0a | 9.1 | 30.0 ± § |
| 45 | 2.5 | 26 | 92.3 | 7.0 ± 0.0d | 73.1 | 20.1 ± 0.4c | 57.7 | 28.4 ± 0.3c |

* : Average of 3 replicates; 10 larvae per replicate
\# : Values followed by the same letter are not significantly different from each other, $P > 0.01$
§ : From two replicate

The tested levels of these antifungal agents, alone and in combination, and their effects are shown in Table 4. The post-larval development was significantly retarded by the diet containing 2.5 mg of methyl *p*-hydroxybenzoate according to the diet containing 30 mg of nystatin.The nystatin in combination with methyl *p*-hydroxy-benzoate (30 mg:2.5 mg) accelerated the development of the insect. Increasing of the level of nystatin to 45 mg in this combination had the most positive effects on post-larval survival and development when compared to other tested combinations of these agents. The diet with this combination produced a yield of 73.1 per cent pupae and 57.7 per cent adults.

## Nystatin - cycloheximide

**Table 5.** The effects of nystatin and cycloheximide alone and in combination on the survival and development of *P. turionellae.*

| nystatin: cycloheximide (mg / 100ml of diet) | | Initial No. of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D. )# | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D )# | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D )# |
|---|---|---|---|---|---|---|---|---|
| 30 | - | 25 | 100.0 | 10.0 ± 0.0a | 44.0 | 23.0 ± 0.0a | 44.0 | 30.1 ± 0.6a |
| - | 0.002 | 26 | 73.1 | 10.0 ± 0.0a | 38.5 | 23.0 ± 0.0a | 23.1 | 30.8 ± 0.5a |
| 30 | 0.002 | 28 | 89.3 | 7.8 ± 0.3b | 28.6 | 20.0 ± 0.0b | 21.4 | 28.0 ± 0.0b |
| 30 | 0.008 | 27 | 96.3 | 8.0 ± 0.0b | 11.1 | 19.3 ± § | 7.4 | 29.0 ± ‡ |
| 45 | 0.002 | 24 | 95.8 | 8.9 ± 0.3c | 8.3 | 20.5 ± § | 4.2 | 30.0 ± ‡ |

* : Average of 3 replicates; 10 larvae per replicate
# : Values followed by the same letter are not significantly different from each other, P > 0.01
§ : From two replicate
‡ : From one replicate

Data in Table 5 show that the diets with tested antimicrobial agents alone had no effects on the survival and development. But according to these diets, the diets with these agents in different combinations significantly shortened the time required for the larval development. Addition of 0.002 mg of cycloheximide to the diet with 30 mg of nystatin had also positive effects on the post-larval developmental rate but no effects on the survival. When the level of nystatin was increased from 30 to 45mg in this combination the post-larval survival was significantly decreased.

## Methyl *p*-hydroxybenzoate-cycloheximide

**Table 6.** The effects of methyl *p*-hydroxybenzoate and cycloheximide alone and in combination on the survival and development of *P. turionellae.*

| Methyl *p* HB: cycloheximide (mg / 100ml of diet) | | Initial No. of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D. )# | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D )# | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D )# |
|---|---|---|---|---|---|---|---|---|
| 2.5 | - | 28 | 85.7 | 10.0 ± 0.0a | 28.6 | 23.0 ± 0.0a | 28.6 | 30.9 ± 0.6a |
| - | 0.002 | 26 | 88.5 | 9.9 ± 0.2a | 23.1 | 23.2 ± 0.6a | 19.2 | 31.0 ± 0.0a |
| 2.5 | 0.002 | 27 | 85.2 | 9.0 ± 0.0b | 7.4 | 20.0 ± § | - | - |
| 2.5 | 0.008 | 24 | 87.5 | 9.0 ± 0.0b | 29.2 | 20.8 ± 0.6b | 16.7 | 27.8 ± 0.5b |
| 10.0 | 0.002 | 24 | 70.8 | 13.0 ± 0.0c | - | - | - | - |

* : Average of 3 replicates; 10 larvae per replicate
# : Values followed by the same letter are not significantly different from each other, P > 0.01
§ : From two replicate

The effects of the diets with methyl *p*-hydroxybenzoate and cycloheximide alone and in combination were shown in Table 6. The diets with these agents alone were not effective on the survival and development according to each other. The development was significantly accelerated by 2.5 mg of methyl *p*-hydroxybenzoate in combination with 0.008 mg of cycloheximide when compared with the tested levels of these antifungals alone. But the larval development was significantly retarded by a combination of these agents (10 mg:0.002 mg) according to the tested agents, alone and in combination. No larvae fed on the diet with this combination were able to reach subsequent stages.

**Effects of combinations containing three antimicrobial agents**

The effects of addition of a third antimicrobial agents to the diets with penicillin-streptomycin or with nystatin-methyl *p*-hydroxybenzoate combinations were shown in Table 7.

**Table 7.** The effects of combinations containing two and three antimicrobial agents on the survival and development of *P. turionellae.*

| Pen: strep.: nys.: methyl *p-HB*: (mg / 100ml of diet) | | | | Initial No. of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D )# | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D )# | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D )# |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 20 | - | - | 26 | 100.0 | 10.1 ± 0.3a | 42.3 | 21.0 ± 0.0a | 34.6 | 28.4 ± 0.1a |
| - | - | 45 | 2.5 | 26 | 92.3 | 8.0 ± 0.0b | 61.5 | 19.8 ± 0.1c,d | 53.8 | 27.2 ± 0.6b |
| 10 | 20 | 30 | - | 26 | 92.3 | 8.0 ± 0.0b | 50.0 | 19.9 ± 0.4c,d | 46.2 | 27.1 ± 0.4b |
| 10 | 20 | - | 2.5 | 26 | 84.6 | 8.1 ± 0.3b | 50.0 | 20.0 ± 0.0b,d | 50.0 | 27.2 ± 0.1b |
| 10 | - | 45 | 2.5 | 23 | 95.7 | 8.0 ± 0.0b | 65.2 | 20.1 ± 0.4b,d | 60.9 | 27.4 ± 0.2b |
| - | 20 | 45 | 2.5 | 27 | 96.3 | 7.9 ± 0.3b | 44.4 | 20.3 ± 0.3b | 44.4 | 27.9 ± 0.2b |

* : Average of 3 replicates; 10 larvae per replicate

\# : Values followed by the same letter are not significantly different from each other, $P > 0.01$

pen.; penicillin, strep.; streptomycin, nys.; nystatin, methyl *p*-HB; methyl *p*-hydroxybenzoate

Development was significantly shortened by nystatin-methyl *p*-hydroxybenzoate combination when compared with penicillin-streptomycin. According to this binary combination of antibiotics, the development of the insect was also significantly accelerated by all tested combinations containing three agents. However, neither the survival nor the development was affected by these ternary combinations when compared with each other.

The effects of addition of a third antimicrobial agent to the diets with penicillin-rifampicin or with nystatin-cycloheximide were shown in Table 8.

**Table 8.** The effects of combinations containing two and three antimicrobial agents on the survival and development of *P. turionellae*.

| Pen: rif.: nys. : cyc. (mg / 100ml of diet) | | | | Initial No. of larvae | Survival to fifth instar (%) | Time to fifth instar, days (mean* ± S.D )# | Survival to pupal instar (%) | Time to pupal instar, days (mean* ± S.D )# | Survival to adult instar (%) | Time to adult instar, days (mean* ± S.D )# |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 2.5 | - | - | 24 | 95.8 | 8.0 ± 0.0a,d | 16.7 | 20.0 ± 0.0a | 16.7 | 26.0 ± 0.0a |
| - | - | 30 | 0.002 | 25 | 100.0 | 8.1 ± 0.0a,d | 28.0 | 20.0 ± 0.0a | 20.0 | 27.2 ± 0.6b,c |
| 20 | 2.5 | 30 | - | 24 | 91.7 | 8.0 ± 0.0b,d | 54.2 | 20.0 ± 0.5a | 45.8 | 27.3 ± 0.6b,c |
| 20 | 2.5 | - | 0.002 | 26 | 96.2 | 8.7 ± 0.3b,c | 46.2 | 19.9 ± 0.4a | 42.3 | 27.1 ± 0.4b |
| 10 | - | 30 | 0.002 | 27 | 96.3 | 9.1 ± 0.2c | 40.7 | 20.0 ± 0.4a | 33.3 | 27.3 ± 0.5b,c |
| - | 2.5 | 30 | 0.002 | 27 | 88.9 | 9.0 ± 0.0c | 44.4 | 20.5 ± 0.2a | 33.3 | 27.6 ± 0.6c |

* : Average of 3 replicates; 10 larvae per replicate

\# : Values followed by the same letter are not significantly different from each other, $P > 0.01$

pen.; penicillin, rif.; rifampicin, nys.; nystatin, cycl.; cycloheximide

Between tested combinations of two antimicrobial agents nystatin-cycloheximide combination prolonged the time to reach the adult instar. Addition of 30 mg nystatin to the diet containing penicillin-rifampicin combination caused a significant increase in the number of survivors in the pupal and adult stage.

## DISCUSSION

This work showed that the presence of the antimicrobial agents at different levels and combinations in the diets caused a high variability in their effects on the survival and development of *P. turionellae*. Particularly the effects of combinations containing two antimicrobial agents were more severe and irregular than those of other tested combinations. In addition to post-larval survival the larval survival appeared to be susceptible criterion for evaluation of combinational effects of these agents on the insect. A previous work demonstrated that antimicrobial agents alone were effective on mostly post-larval developmental stages of the insect in question (Büyükgüzel and Yazgan, 1996). But in this work some tested combinations of the antimicrobial agents were shown to be effective on the larval survival as well as developmental time for this stage of the insect. For example 10 mg of penicillin in combination with 10 mg of rifampicin decreased the number of survivors in the fifth instar by approximately fifty per cent (Table 2). This combination also unreasonably retarded the larval development and enabled no larvae to complete its development to subsequent stages. Similar effects on the larval development were obtained with a combination of methyl *p*-hydroxybenzoate and cycloheximide but this combination had no effects on the survival (Table 6). These results clearly showed that the data on the active feeding stages must also be considered to ascertain the nutritional importance of the combinations of antimicrobial agents in artificial rearing of the insect. Parasitoid insects have a very important growth rate in their larval stages. They require well-balanced food to prevent toxic product accumulation and thus decrease of yield in their developmental stages (Grenier et al. 1986). These negative effects on the larval stages may be attributed to nutritional impairment of the larvae. The tested combinations consequently may have impaired the nutritional balance or quality of the diet and their contribution to the feeding activity of the larvae. Singh and House (1970) stated that some antimicrobial agents may be effective on the feeding activity of the insects because of their repellant or determent effects.

In general, most of the tested combinations exerted their effects on mainly nonfeeding developmental stages. In other words, the post-larval developmental stages of the insect was more susceptible to the tested combinations of antimicrobial agents than the larval stages. Moreover, the effects of combinations of two antimicrobial agents were more severe than those of combinations containing three agents. Except a combination of nystatin with methyl *p*-hydroxybenzoate (45:2.5mg), the other combinations of two agents had generally negative effects on the survival and positive effects on the development of the insect. A combination of penicillin with streptomycin caused an insignificant increase in the survival but prolonged the time required for fifth and adult instar (Table 1). The post-larval survival was significantly decreased when added 10 mg of rifampicin to the diet with 20 mg of streptomycin (Table 3). Similar result was obtained with a combination containing 45 mg of nystatin and 0.002 mg of cycloheximide (Table 5). It was interesting that the detrimental effects of the combinations containing two antimicrobial agents were greatly overcome by addition of a third antimicrobial agent to these binary combinations. For example when added 30 mg of nystatin to the diet with a combination of penicillin and rifampicin (20:2.5 mg) increased the pupal and adult yields by approximately three-fold (Table 8). These results indicate that the combinational effects on both the larval and post-larval developmental stage varied with the kinds, levels and even the number of agents in the combinations. The differences in the effects of the tested combinations on these stages may also be attributed to alimentary canal of the hymenopterous insects (Yazgan, 1972). *P. turionellae* has no opportunity to excrete the antimicrobial agents or the products of their metabolism in the larval stages, because it has blind gut until the end of the larval maturity. Thus the absorption rate of other nutrients may be changed as a result of overaccumulation of the antimicrobial agents in the gut of the insect. Some antibiotics have been known to be effective on the intestinal absorption of the higher economically important animals (Patrick and Schaible, 1980).

There has been no report concerning the combinational effects of antimicrobial agents on the hymenopterous insects and other entomophagous ones. Some work has reported that antimicrobial agents in combination were effective on the phytophagous insects. Most of these deal mostly with combinations of antifungal agents which are used for control of fungal contaminations in the artificial diets of these insects. For example a combination of methyl *p*-hydroxybenzoate and formaldehyde significantly shortened the time required for larval and pupal development of the pink bollworm, *Pectinophora gossypiella* (Saunders) when compared to methyl *p*-hydroxybenzoate alone (Ouye, 1962). Methyl *p*-hydroxybenzoate and sorbic acid combination decreased the survival of the fringed beetle, *Graphognathus sp.* (Bass and Barnes, 1969) and the corn earworm, *Heliothis zea* (Boddie) (Brazzel, 1961) but had no effects on the cabbage looper, *Trichoplusia ni* (Hübner) (Kishaba et al., 1968). Moore et al. (1967) reported that a combination of methyl *p*-hydroxybenzoate and potassium sorbate in the larval diet of the boll weevil, *Anthonomus grandis* Boheman had no adverse effects. When decreased the levels of these antifungal agents by fifty per cent the larval development was accelerated and the number of survivors in adult stage was increased. Methyl *p*-hydroxybenzoate in combination with streptomycin had no effects on the survival but prolonged the time to reach adult instar of the maize stalk borer, *Chilo partellus* (Swinhoe) (Siddiqui et al., 1985). Whereas, a combination of this antifungal with aureofungin retarded the development and decreased the yield of adults of this insect (Siddiqui et al., 1986). Similar results were also obtained with the tested combinations of antimicrobial agents depending on their kinds and levels on an entomophagous insect, *P. turionellae*. These results also indicated that the severity of the combinational effects varied with the insect species. In general, the combinations of antimicrobial agents appeared to have a common effectiveness on the survival and development of the insects. Furthermore, positive effects of some tested combinations on these phytophagous insects and our insect indicate that these antimicrobial agent combinations will be of a nutritional importance in insect nutrition when their kinds and levels are carefully determined. Some antibiotics increased food consumption body weight, growth rate and silk production of some silkworm species which are economically important insects (Govindan et al. 1993, Saha et al. 1995).

Another result obtained from this work is the high variability in the effects of the tested combinations on the insect. For example 45 mg of nystatin in combination with 0.002 mg of cycloheximide caused a significant decrease in the post-larval survival (Table 5). When cycloheximide was replaced by 2.5 mg of methyl *p*-hydroxybenzoate in this combination the survival was greatly increased (Table 4). On the other hand the combinations of penicillin with streptomycin at different levels had no negative effects (Table 1) but its combination with rifampicin had severe effects on the survival and development (Table 2). These detrimental or beneficial effects may be a result of the presence of some interactions between the components of the combinations. These interactions may be effective on the amount of the diet eaten and thus intake of dietary antimicrobial agents. It was suggested that feeding rate can determine the intake of an antimicrobial agent (Singh and House, 1970). It is possible that these interactions may interfere with the feeding rate and consequently the effects of antimicrobial agents in the combination. A physiological interaction between the combinational antimicrobial agents during their absorption in the gut may account for the effects of the tested combinations. The combinational agents may competitively inhibit or increase the absorption of each other (Catnach et al. 1994). On the other hand, the effects of an antimicrobial agent varied directly with its absorption rate in the gut (Boreham et al., 1986). It has been known that the effects of combinations of antimicrobial agents on different organisms from primitive eucaryotes such as some parasitic protozoa (Gingras and Jensen, 1992; 1993) to higher eucaryotes including human (Appel et al., 1986; Toyoguchi et al., 1997) varied with the kinds and levels of antimicrobial agents.

Rifampicin (Table 2, 3) and cycloheximide (Table 5, 6) alone in binary combinations with other tested agents appeared to be more effective for *P. turionellae.* Rifampicin inhibits procaryotic RNA synthesis. Cycloheximide affects the protein synthesis in eucaryotes. Both of these agents are potent and toxic agents to a wide spectrum of organisms (Gottlieb and Shaw, 1967; Jawetz et al., 1984). The detrimental effects of the combinations of these agents may be a result of their direct effects on the insect. The metabolic system of the insect may be affected biochemically by toxic properties of these agent. The effects of these agents alone have already been known in the some metabolic process of the insects (Ishikawa, 1984; Weeks et al., 1993; Walters et al., 1994). The feeding experiments might be supported by the biochemical analysis to determine their effects on chemical composition of insects. Because, to use data concerning only survival and development sometimes may be insufficient to determine the nutritional suitability of any dietary nutrient (House and Barlow, 1965; Thompson, 1986).

Among the tested combinations, nystatin-methyl *p*-hydroxybenzoate (45:2.5 mg), and penicillin-rifampicin-nystatin (20:2.5:30 mg) appeared to be the best antimicrobial agent formulations for *P. turionellae.* The combination of nystatin and methyl *p*-hydroxybenzoate had the most positive effects on both the survival and development. The diet with this combination produced a yield of 73.1 per cent pupae and 57.7 per cent adults. The high pupal and adult yields may be a result of improved nutritional quality of the diet by these agents in the combinations. The quality of larval food is a factor that may affect the post-larval stages of most insects (House, 1962; Slansky and Scriber, 1985). A successful attempt was made to increase the pupal yield by using of nystatin alone in the diet for a parasitic dipterous insect, *Hypoderma lineatum* (Villers) (Chamberlain and Scholl 1991). We suggest that antimicrobial agent in combination can be used in artificial diets for efficient mass-production with high yields of the parasitic hymenopterous insects which are strong candidates of biological control programmes and other parasitic insects. The binary combinations should be carefully used and prepared with the minimum levels of less detrimental antimicrobial agents owing to their severe and irregular effects on the insect.

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