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Research Article

Effects of Antimicrobial Agents on the Survival and Development of Larvae of *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) Reared on an Artificial Diet

Kemal BÜYÜKGÜZEL
Department of Biology, Faculty of Science-Art, Karaelmas University, 67100, İncivez, Zonguldak-TURKEY
Şevki YAZGAN
Department of Biology, Faculty of Sciences, Ankara University, 06100, Beşevler, Ankara -TURKEY

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Abstract: The effects of 13 antimicrobial agents that have different structures and modes of action, on the survival and development of the hymenopterous endoparasitoid, *Pimpla turionellae* L., were investigated by rearing the larvae aseptically on chemically defined synthetic diets. These effects varied according to their kind and dietary levels. The agents tested exerted their effects generally during the post-larval development of the insect. The post-larval survival and development were negatively affected by most of the agents tested at certain levels. The antimicrobial agents tested also affected the larval development but had no significant effects on the larval survival of the insect. The larvae showed a wide tolerance against both the kind and dietary levels of the agents. None of the agents tested were toxic to the larvae. Sodium benzoate and nystatin at low levels had positive effects on the food consumption of the larvae.

Key Words: *Pimpla turionellae*, Antimicrobial agents, Insect, Endoparasitoid

Bazı Antimikrobiyal Ajanların Sentetik Besinde Yetiştirilen *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) Larvalarının Yaşama ve Gelişmesine Etkileri

Özet: Endoparazitoid zarkanatlı türü, *Pimpla turionellae* L., kimyasal yapısı bilinen sentetik besin ortamlarında aseptik şartlarda beslenerek, farklı yapı ve etkiye sahip onüç antimikrobiyal ajanın böceğin yaşama ve gelişmesine etkileri incelendi. Bu etkiler denenen antimikrobiyal ajanın çeşidine ve miktarına göre değişmektedir. Denenen ajanların etkileri çoğunlukla larva sonrası evrelerde ortaya çıkmış olup bu evrelerdeki yaşama ve gelişme olumsuz yönde etkilenmiştir. Antimikrobiyal ajanlar aynı zamanda larval evredeki gelişmeyi de etkilemiş ancak bu evredeki yaşama üzerinde önemli bir etki yapmamıştır. Larvalar antimikrobiyal ajanların hem çeşidine hem de besinsel miktarına karşı geniş bir tolerans göstermiştir. Denenen ajanların hiçbiri larvalar üzerinde toksik etki yapmamıştır. Sodyum benzoat ve nistatinin düşük miktarları larvaların besin tüketimine olumlu etkide bulunmuştur.

Anahtar Sözcükler: *Pimpla turionellae*, Antimikrobiyal ajanlar, Böcek, Endoparazitoid

Introduction

Because of their predatory nature, parasitic hymenopterous species can be used as potential tools in biological control programs. Many attempts have been made to use these insects in these programs. One of these was to cultivate these insects permanently in a laboratory. To do so, some chemically defined synthetic diets and rearing techniques were developed for some species. By using these diets and techniques, some parasitic hymenopterous species were successfully reared from egg to adult instar in laboratory conditions (1-9). Chemically defined diets that have been developed to rear parasitic insects are highly suitable for the development of microorganisms. Various antimicrobial agents were used in insect diets to prevent microbial contamination and propagation (2,10-16), but a very limited amount of work has been done on the effects of these agents on parasitic hymenopterous species (17-20). Some hymenopterous insects, especially social ones, produce some natural substances which function as antimicrobial

agents (21-25). For example, hymenopterous parasitoid *P. turionellae* larvae discharge such a substance into their host. This acts as an antimicrobial agent during the larval development of the insect (26). Some antimicrobial agents can permanently or temporarily change the mode of reproduction of the thelytoky form of some parasitic hymenopterous insects to the arrhenotoky, which is a normal system of reproduction in the hymenoptera (27-32). These indicate the importance of antimicrobial agents in the development of hymenopterous insects. This work is important in understanding the nutritional suitability of the antimicrobial agents as a dietary additive substance in the artificial rearing of these insects. Such work might also be of great practical importance in the control of bacterial and fungal contaminations, which are a very serious problem in artificial diets for the mass-rearing of *P. turionellae* and other pupal endoparasitoid hymenopterous insects. Contamination is often a major factor making their rearing unsatisfactory and sometimes impossible. This problem in the artificial rearing system of our insect mostly causes time consumption and extravagancy of nutrient chemicals. However, *Pimpla* larvae overcome the problem by discharging an anal secretion which has antibiotic function during their development in their hosts to protect themselves (26). To rear healthy adults for biological control, it is necessary to control microbial contaminations. Some work has been done on antifungal agents in artificial diets but very little deals with antibiotics or even with pupal endoparasitoids

The work which has been done deals with the individual effects of some antifungal agents and antibiotics on the survival and development of the larvae of a pupal endoparasitoid hymenopterous insect, *Pimpla turionellae*, which is a potential biological control agent of many lepidopterous pest insects. Therefore, we tried to determine the levels of these antimicrobial agents which have minimal or no detrimental effects on the insect.

Materials and Methods

P. turionellae adults used in the experiments were reared in the laboratory on the pupae of the greater wax moth, *Galleria mellonella* L., at 23±1ºC, 75±5% r.h. and with a 16 h illumination period. Adults were fed daily with 50% honey solution and *G. mellonellae* pupae hemolymph.

The synthetic diet described by Yazgan (3) was used for rearing the larvae of *P. turionellae*. 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 ingredients, the diet from these stocks, the sterilization techniques, the adding of sterilized vitamin-glucose and antimicrobial agents solution to the diet, the dispensing of the diets into test-tubes, the obtaining of the sterilized larvae and their inoculation onto diets, and the preparation of gelatine capsules for pupation were mostly the same as those described elsewhere (20).

Some of the antimicrobial agents used in this work such as tetracycline hydrochloride (968 µg/mg), chloramphenicol (980 µg/mg), erythromycin (Free base, 820 µg/mg), trimethoprim (99%), cycloheximide (98%) and sodium benzoate (99%) were obtained from Sigma Chemical Co. The others were obtained from different firms such as penicillin G (potassium salt, 1600 units) and streptomycin sulphate from Claus & Huth Ltd., methyl p-hydroxybenzoate (pure, crystalline) from Koch-Light Lab., rifampicin (-SV, 95%) from Koçak Medicine Co., lyncomycin hydrochloride (877 µg/mg) from Eczacıbaşı Medicine Co., and cephradine monohydrate (942 µg/mg) and nystatin (5300 units/mg) from Bristol-Myers Squibb Inc. Most of the antimicrobial agents tested in this work were selected from commonly used ones in the larval diets for artificial rearing of some entomophagous insects (12,17-20,33,34). In addition to these agents, trimethoprim, lincomycin and cephradine, which have not been previously used in any artificial rearing diet, were also tested. Thus, it was planned to test one or two agents from each group of antimicrobial agents with different structures and modes of action. Our previous work showed that some antimicrobial agents tested such as penicillin and streptomycin exerted their main effects at a level of 50 mg of 100 ml of diet, whereas rifampicin at this level was highly effective on the post-larval survival and development of *P. turionellae* (20). Therefore, in the present work, the tested levels of the antimicrobial agents were considered to center below or around a level of 50 mg of 100 ml of diet. Some preliminary experiments were conducted to determine the range of the 13 agents. Based on the results of these experiments, penicillin and streptomycin at levels of 10, 20, 30 and 40 mg; rifampicin, methyl p-hydroxybenzoate and sodium benzoate at levels of 2.5, 5.0, 7.5, 10.0 mg; cycloheximide at levels of 0.002, 0.004, 0.006, 0.008

mg; and the rest of the antimicrobial agents at levels of 15, 30, 45, 60 mg/100 ml of diet were tested. Thus, four levels of each agent were tested and compared with a control (without antimicrobial agents). Only bidistilled water was used as the diluent for all the antimicrobial agents. We obtained a quite stable suspension by vigorously shaking the mixture in the preparation of solutions of low soluble agents in water such as nystatin, rifampicin and methyl *p*-hydroxybenzoate. No special solvents or protective substances were used for dilution of such agents to avoid possible changes in the chemical and physical composition of the diets and thus to prevent their possible contribution to the effects of antimicrobial agents on the insect.

The agents tested were expressed as mg/100 ml of diet. Their desired amounts were dissolved in bidistilled water, then sterilized through 0.22 µm pore-sized membrane filter and added to the 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 each test-tube containing about 0.5 ml of diet with increasing levels of antimicrobial agents. This amount of the diet is apparently more than necessary in the nutritional requirements for the complete development of the larva. This excessive amount of diet was dispensed onto a large area through the inside bottom of the test-tube. This was done because we wanted to keep permanently on the diet the larva in order to continue its feeding with antimicrobial agents during the active feeding period. Early observations in this work and our previous work with antimicrobial agents (20) demonstrated that most newly inoculated larvae had a tendency to abstain from the diet during the first 24 or 48 hours and they escaped from the diet at once when inoculated on a low amount of diet dispensed onto a small area. Therefore, the customarily used amount of diet which allows normal development of the larva (0.25-0.30 ml) in the general artificial rearing works (2,3) was increased by approximately two-fold in this work. Ten larvae were used for each diet and each test was repeated three times. The feeding experiments were done under the same laboratory conditions as mentioned for the stock culture of the *P. turionellae*.

The effects of the tested diets with differents amounts of antimicrobial agents were measured by determining the rate of development (average time to reach fifth, pupal and adult instars) and survival (number of survivors in these instars) of the larvae. Data on the rate of development were evaluated by analysis of the variance (35). To determine significant differences between means, Duncan's (36) multiple range test was used. Data on survival were compared by X^2 test (35). When F and X^2 exceeded their 0.01 value, the differences were considered significant.

Results

The effects of the most effective antimicrobial agents on the survival and development of *P. turionellae* larvae are shown in the Table.

The lowest level of rifampicin had no significant effect on either survival or development, but 5 mg and 10 mg of it significantly decreased the postlarval survival. The adult development of the insect was also significantly retarded by the diet which contained only 5 mg of rifampicin. The highest level of this antibiotic also retarded the larval development by approximately four days. Chloramphenicol, at all tested levels, did not affect the larval survival and development of the insect. This agent had negative effects on the postlarval development and survival. Development was retarded by all tested levels and the number of survivors was decreased by the diets with 30 mg and 60 mg of chloramphenicol. However, neither survival nor development was affected by low levels of cycloheximide. The rate of development was significantly retarded by high levels of this agent. These levels also significantly decreased the number of survivors in the pupal and adult instar. Diets with 30 mg and higher levels of penicillin significantly shortened the average time to reach the pupal stage but this positive effect was not exerted on the development of the larvae to adult stage. Only the 30 mg level significantly decreased the postlarval survival of the insect. None of the tested levels of lincomycin affected the development of the insect. Diet with 45 mg of this agent caused a significant decrease in the number of individuals reaching the pupal and adult stage. None of the levels of cephradine tested affected the rate of development. With 45 mg of this agent, the number of survivors in both the pupal and adult stage was significantly decreased when compared to the control diet. Similarly, 15 and 45 mg of trimethoprim caused a significant decrease in the pupal and adult yield. Although the diet with high levels of this

Table The effects of antimicrobial agents on the survival and development of the larvae of *P. turionellae*.

Levels (mg/100 ml 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.)#	Adults emerged (%)	Time to adult instar, days (mean* ± S.D.)#
Rifampicin							
00 (+)	24	100.0a	9.2 ± 0.3a	58.3a	22.1 ± 0.5a	54.2a	29.9 ± 0.6a
5.0	22	100.0a	9.7 ± 0.4a	13.6b	22.3 ± 0.6a	13.6b	31.3 ± 0.7b
10.0	8	37.5a	13.7 ± 0.4b	12.5b	25.3 ± ‡	12.5b	33.0 ± ‡
Chloramphenicol							
00 (+)	26	80.8a	10.1 ± 0.07a	50.0a	25.4 ± 0.4a	42.3a	34.4 ± 0.5a
30	24	75.0a	10.2 ± 0.5a	11.6b	26.7 ± 0.8b	8.3b	37.5 ± §
45	20	70.0a	10.3 ± 0.6a	20.0a	27.0 ± 0.0b	20.0a	36.2 ± 0.9b
60	23	82.6a	10.7 ± 0.4a	8.7b	28.0 ± §	8.7b	36.0 ± §
Cycloheximide							
0.000 (+)	26	84.6a	11.4 ± 0.3a	40.5a	25.6 ± 0.1a	38.6a	35.6 ± 0.8a
0.006	26	46.2a	12.7 ± 0.6b	19.2b	27.0 ± 0.0b	11.5b	37.3 ± 0.7b
0.008	27	37.0a	14.7 ± 0.4c	11.1b	30.7 ± 0.1c	7.4b	42.0 ± §
Penicillin							
00 (+)	29	69.0a	9.7 ± 0.4a	41.4a	26.6 ± 0.3a	41.4a	35.3 ± 0.7a
30	29	62.1a	10.1 ± 0.3a	10.3b	25.0 ± 0.0b	10.3b	35.0 ± 0.0a
Lyncomycin hydrochloride							
00 (+)	29	96.5a	10.1 ± 0.1a	31.0a	25.1 ± 0.7a	27.6a	33.4 ± 0.8a
45	23	95.6a	10.6 ± 0.3a	4.3b	26.0 ± ‡	4.3b	33.0 ± ‡
Cephradine monohydrate							
00 (+)	30	86.7a	9.4 ± 0.4a	50.0a	24.1 ± 0.7a	50.0a	34.4 ± 0.7a
45	26	88.5a	10.2 ± 0.04a	11.5b	24.7 ± 0.7a	11.5b	34.7 ± 0.7a
Trimethoprim							
00 (+)	27	96.3a	9.0 ± 0.0a	59.3a	22.3 ± 0.2a	55.5a	32.5 ± 0.7a
15	29	96.6a	9.5 ± 0.3b	13.8b	23.7 ± 0.3a	13.8b	33.3 ± 0.7a
45	29	62.1a	10.6 ± 0.8c	17.2b	24.2 ± 0.8a	13.8b	34.5 ± 1.1a

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

agent significantly retarded the larval development, this negative effect was overcome through the adult emergence.

None of the tested levels of tetracycline, erythromycin and sodium benzoate affected the survival or development of *P. turionellae* larvae. However, the lowest level of tetracycline caused a slight and insignificant increase in the percentage of survivors in the pupal stage. It was observed that the larvae fed on the diet with low levels of sodium benzoate were larger than those fed on the control diet, but they produced neither larger pupae nor adults. Streptomycin, nystatin and methyl p-hydroxybenzoate at only their highest levels significantly retarded the development of the larvae to adult stage. None of the tested levels of this agent affected on the survival of the insect. Even 5 mg of methyl p-hydroxybenzoate increased the survival by about 16 percent but this increase was not significant when compared to the control diet.

Discussion

The results of this work show that the antimicrobial agents tested exert their effects mainly during the post-larval developmental stages of *P. turionellae*. No significant effects on the survival were recorded in larval

development. The antimicrobial agents were tolerated at levels within the range tested by the larvae of the insect. However, the dipterous parasitoid *Agria affinis* auct. nec Fallén (12) had a wider tolerance for the range of antimicrobial agents compared to our insect. For example, a range from 200 mg to 1600 mg of penicillin G was tolerated by this insect. Although they are entomophagous insects, this variable tolerance may be attributed to their differential alimentary canals. In contrast to this sarcophagid insect, *P. turionellae* larva has a closed midgut at its rear (37). The larvae can defecate the content of the gut only at the end of larval maturity or close to pupation. For *A. affinis* larvae, the safe level of methyl p-hydroxybenzoate, which is commonly used in rearing diets of insects to control fungal contamination, was 10 mg/100 ml of diet (12). For a tachinid insect, *Phryxe caudata* ROND. larvae, it was below 2 mg (14), whereas for our insect this level was 5 mg per 100 ml of diet. Moreover, this antifungal agent at very low levels inhibited the hatching of the eggs of a hymenopterous egg parasitoid Trichogramma pretiosum Riley (17). These results also demonstrate that the tolerance of insects to the levels of antimicrobial agents varies with insect species as well as the kinds and dietary levels of the antimicrobial agents. These variations may be attributed to the differential physiological conditions of the insect species. As Grenier et al. (38) previously suggested, the same species may require very different physiological conditions in its different developmental stages.

Thus, of 13 antimicrobial agents including antibiotics and antifungal agents added in increasing levels to the larval diet, many at certain dietary levels decreased the survival and retarded the development of *P. turionellae*. Particularly the pupal stage appeared to be more sensitive to the antimicrobial agents tested. The main effects were exerted mostly on the pupal stage and remained fairly constant throughout the adult emergence. The diets with rifampicin, chloramphenicol and cycloheximide at levels of 5, 10 mg; 30, 60 mg; and 0.006, 0.008 mg/100 ml of diet respectively produced significantly lower yields of adults than the control diet. These agents also affected development. Chloramphenicol significantly retarded only post-larval development, whereas rifampicin and cycloheximide had similar effects on both the larval and post-larval development of the insect. Consequently, the average time to reach the adult stage was significantly prolonged by the diets with these agents compared to the control diet.

Penicillin, lincomycin, cephradine and trimethoprim at certain dietary levels had significant effects only on survival. For example, when compared to the control diet, both the yields of pupae and adults were significantly reduced from 50 to 11.5 percent by 45 mg of cephradine. Penicillin and trimethoprim at levels of 30 mg and 45 mg respectively produced 10.3 and 13.8 percent of adult yields. Moreover, lincomycin significantly decreased both the pupal and adult survival to a percentage as low as 4.3. These four agents had no effects on the development to adult instar. Tetracycline, erythromycin and sodiumbenzoate had no effect on either the survival or the development of the insect. However, only the highest levels of streptomycin, nystatin and methyl p-hydroxybenzoate had a negative effect on adult development.

The results obtained from this work show large variableness in the effects of the antimicrobial agents tested and even of the dietary levels of each agent on survival. The inefficacy of some antimicrobial agents at certain levels indicates that the negative effects may be related to their nutritional implication in the diet rather than to their direct effects on the insect itself. These antimicrobial agents may have impaired the nutritional value or quality of the diet. Parasitoid larvae require a good quality, well-balanced diet to produce high yields of individuals in the post-larval developmental stages (38). Furthermore, the quality of the larval diet or natural food may affect the adult stage of most insects (39). The antimicrobial agents may deter or reduce the feeding activity of the larvae (34). Some larvae were observed to be prevented from feeding by the diets with the highest levels of some antimicrobial agents such as rifampicin, cycloheximide, sodium benzoate and methyl *p*-hydroxybenzoate. For example, of a total of 30 newly inoculated larvae on the diet with the highest level of rifampicin, only 8 were able to survive, of which 37.5 percent were able to reach fifth instar and the others died within 48 hours. As a result of this initial mortality, the percentage of larval survivors may not be significantly different from that of the control diet. This initial mortality is attributed to the changed flavor of the diet as rifampicin at high levels imparted a reddish color to the diets and to the larva fed on these diets. However, at the highest level of rifampicin, a significant retardation of

over 4 days was observed compared with the control. The high level of cyclohexmide also significantly retarded the larval development. Our observation with these agents seems to be consistent with the suggestion of Zucoloto (40), that an insect chooses a diet on the basis of its nutritional value and its flavor. On the other hand, this situation may be attributable to the repellent effect of these agents. Singh and House (33) stated that the possible direct toxic or repellent effect of antimicrobial agents on the insect itself was an important problem in their use to control microbial contamination of artificial rearing diets of insects. Thus, such compounds at high levels appear to be noxious and unsuitable for practical use in the artificial rearing of *Pimpla* larvae.

Some other work has reported that antimicrobial agents affect other parasitic insects. For example, the survival of the egg parasitoid *Trichogramma dendrolimi* Matsumura was decreased by each one of the antifungal agents nystatin, cycloheximide and sodium benzoate. They also decreased egg hatching in this insect (18,19). In another hymenopterous parasitoid, *T. pretiosum* Riley, egg hatching was inhibited by methyl p-hydroxybenzoate (17). Antimicrobial agents also affected the survival and development of some Sarcophagid dipterous species. Work done with *Agria affinis* (= *A. housei* shewell), *Phryxe caudata* ROND. and *Phaenicia sericata* (= *Lucilia sericata*) showed that antimicrobial agents had negative effects on these insects (12,14,33,34,41).

Among the antimicrobial agents tested, only methyl p-hydroxybenzoate at a level of 5 mg caused a small and insignificant increase in the percentage of survivors in the adult stage compared to the control. This tested level of the antifungal agent also had no effect on the development of the larvae to adult instar. Also, streptomycin and nystatin at low levels had no significant effects on post-larval development. Similarly, none of the tested levels of tetracycline, erythromycin or sodium benzoate had any effect on the survival and development of the insect. These results indicate that these antimicrobial agents at low levels may be suitable candidates for practical use in the artificial rearing diet of *P. turionellae*. They might be used to control microbial contamination of the diet for artificial mass-propagation of our insect. Probably the low levels of these agents allowing the larvae to survive to adult instar may be unable to control contamination in the diets. Further investigation is needed to clarify this important question. Consequently, this work showed that none of the antimicrobial agents alone had significant positive effects both on survival and development. However, 2.5 mg of methyl p-hydroxybenzoate in combination with 45 mg of nystatin added to the larval diet of *P. turionellae* produced 73.1% of pupae and 57.7% of adults. These high yields were significant according to those of individually tested levels of these antifungal agents (42). Survival to pupation of young third stage larvae was increased from 5% to 73% when nystatin and chloramphenicol alone were present at low levels in the artificial diet of a parasitoid dipterous insect, Hypoderma lineatum (Villers) (43).

In general, the antimicrobial agents tested did not show regular effects. Many factors might be responsible for these irregular effects and even for the main effects of the antimicrobial agents tested on the insect. First of all, the dietary levels of an agent may influence the amount of diet consumed by the insect. Antimicrobial agents may have accumulated and distended the midgut and thereby decreased feeding. This suggestion is supported by our observation of the presence of an apparent accumulation inside the middle region of the abdominal segments of the larvae reared on the diets with some antimicrobial agents such as rifampicin, chloramphenicol and cycloheximide. This accumulation may be a result of an increase in intake of the antimicrobial agents. These agents were shown to be the most effective both on the survival and development of *Pimpla* larvae among the other tested agents in this work. This raised the possibility that the effects of these agents may be dependent on their increased accumulation in the gut of the insect. Some antibiotics including cycloheximide, erythromycin, tetracycline, chloramphenicol, rifampin and penicillin G were also demonstrated to have detrimental effects on the survival of a eucaryotic invertebrate organism, the malarial parasite *P. falciparum*. Except cycloheximide, the agents are effective on the prokaryotic organisms (44). Thus, the negative effects of most of these antibiotics on the survival of this parasitic protozoan were attributed to their increased concentration inside the parasite (45). Tested antimicrobial agents are expected to be effective on the osmotic pressure and pH of the midgut of the insect. The midgut is mainly responsible for absorption of nutrients in parasitic wasps. Its osmotic pressure is much lower than other parts of the alimentary canal of the

larvae and pH is nearly neutral (37). The amount of a special antimicrobial agent eaten may change the pH and osmotic pressure of the gut. These changes consequently would affect the absorption of this agent. Most antimicrobial agents, especially antibiotics, are capable of ionization under physiological conditions. The degree of ionization of an antibiotic is one of the most important factors determining its absorption from the intestine and thus its effects in higher animals (46). On the other hand, the absorption of antimicrobial agents depends on their intestinal amounts and their contribution to the osmotic pressure and pH of the intestine (47- 49).

The low dietary levels of nystatin and sodium benzoate appear to have positive effects on the diet consumption of *P. turionellae* larvae. It was observed that larvae fed on diets with low levels of either of these agents were apparently larger and more distended than those fed on the control diet. A little residue of the diet remained within most of the test-tubes containing the diets with low levels of these antifungal agents and even the whole diet had been consumed by a few larvae when they reached maturity. Similar observations on some larvae were made with a diet with a level of 50 mg of streptomycin in the larval diet of *Pimpla* in our previous work (20). However, none of these larvae produced pupae. These observations indicate that these antimicrobial agents at certain levels may have positive effects on the food consumption of the larvae. Ethanol had similar effects on *Xyleborus ferrugineus* (F.) (50). Another agent which had positive effects on the insect was nystatin. Adults emerging from the diet containing higher levels of nystatin were active and rigorous. These diets produced very animated adults with normally expanded wings and high flying performance. However, most of the adults that emerged from the diet with low levels of nystatin were feeble and some of them were incapable of escaping the adult exuvium. The positive effects of this agent may be attributed to its polyene structure. It is well known that substances with a polyene structure have various physiological functions in animals (51). Cycloheximide was the only antimicrobial agent that caused body malformation. Larvae fed with high levels of this agent had articulated bodies. Similarly, an antifungal agent, acriflavine, caused abnormal formation of the adult structure in *Heliothis zea* (Boddie) (52).

The antimicrobial agents may have biochemical effects on metabolic system of the each developmental stage of the insect. The present work is an important attempt dealing with only the dietary implication of some antimicrobial agents. Therefore, it would be reasonable to suggest that the effects of the antimicrobial agents on survival and development may be related to their effects on the nutritional value of the artificial diet. Although they have detrimental effects mostly on the post-larval developmental stages at certain levels, none of the agents tested were toxic to the insect. These agents were tolerated at a low range by *P. turionellae* larvae. These data mostly indicate that antimicrobial agents at low levels may be of practical importance in artificial mass rearing of pupal hymenopterous endoparasitoids. A wider variety of antimicrobial agents should be screened for use in the larval diet of the insect. It is also important to choose nontoxic agents to eucaryotic organisms and their minimal levels in order to lessen the nutritional impairment of larvae and thus the detrimental effects on the quality of insect reared for biological control when they are used to control microbial contamination during the artificial rearing of *P. turionellae*. Many agents are detrimental for insects and especially for parasitoids. Endoparasitoid insects are known to be more sensitive than their hosts, which are free living insects (14). If antimicrobial agents added into artificial diet of *P. turionellae* larvae weaken the performance of adult individuals, this may become a more important problem in biological control programs. So although the low levels of most antimicrobial agents tested did not affect the survival and development of the insect, further work is needed to determine their effects on the traits of adults.

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