Effects of a DNA Gyrase Inhibitor, Novobiocin, on the Biological Parameters of *Galleria mellonella* (Lepidoptera: Pyralidae) Larvae[1]

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Abstract *Galleria mellonella* L. larvae were reared on a standard artificial diet amended with various concentrations (0.5%, 1.0%, 1.5%, and 2.0%) of the aminocoumarin DNA gyrase inhibitor antibiotic drug novobiocin. The effects of the inhibitor on survival and development of the larvae were measured. Survival rates for 7th instars, pupae, and emerging adults were 90.0%, 86.2%, and 76.2%, respectively, in the control diet. These rates were decreased to 15.0%, 7.5%, and 6.2% for larvae fed the diet with the highest novobiocin concentration (2.0%). High concentrations of the antibiotic caused prolongation in the larval, pupal, and adult developmental periods. Our results indicate that this gyrase inhibitor can be used as dietary additive in artificial rearing of *G. mellonella* when its concentrations are ascertained.

Key Words *Galleria mellonella*, novobiocin, artificial diets, survival

The artificial rearing of insects started long ago with the goal to conduct studies on the biology, physiology, and behavior to develop alternative chemicals for pest management (Grenier 2012). Pest management programs often rely upon applications of broad-spectrum pesticides. The problem associated with these chemicals has shifted attention to less toxic antibiotics and lesser-used compounds such as clinically important antibiotics including DNA gyrase inhibitors (Büyükgüzel 2001a, 2001b; Büyükgüzel and İçen 2004) and some antihelmintics (Büyükgüzel and Kayaoğlu 2014, Sefer and Büyükgüzel 2018). Our pioneering study with some DNA gyrase inhibitors showed that novobiocin, nalidixic acid, and oxolinic acid at higher concentrations have adverse effects on the development and survival of ichneumonid endoparasitoid *Pimpla turionellae* L., whereas some of them at certain concentrations have positive effects on this parasitic wasp (Büyükgüzel 2001b).

Despite these negative implications of these antibiotics on this insect, their effects or even mechanism of action on insects have remained obscure. The aminocoumarin antibiotic which is a gyrase inhibitor, novobiocin, affects DNA synthesis by inhibiting the gyrase function (Maxwell 1993). This agent is widely used in the management of urinary tract infections in humans because of its low

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distribution in tissues and excretion mainly into the urine (Rubin et al. 1992). Novobiocin at high levels was also effective on some tissues of humans and other eukaryotic organisms (Francke and Margolin 1981, Pate et al. 1986). Novobiocin was first incorporated into artificial diet fed to some agricultural pests including the aphid *Myzus persicae* (Sulzer) to ascertain its impairment of growth and development (Mittler 1971). However, there is no information dealing with effects of the potent agent on greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), except for some other chemicals (Sertçelik et al. 2018). The greater wax moth is a serious pest of honey bee, *Apis mellifera* L., feeding on combs, wax, and honey in beehives, leading to financial losses in apiculture. Management of this wax moth has traditionally depended on the application of insecticidal fogs and fumigants such as phosphine gas and methyl bromide which directly act through the nervous system (Charriere and Imdorf 1997).

The present work has been directed towards the use of antibacterially more active antibiotics at low levels in the artificial diet. On the basis of this information, we hypothesized that one mechanism of DNA synthesis inhibitors action in insects is through its deteriorative effects on insect life. Therefore, this work deals mainly with the effects of a bacterial DNA gyrase inhibitor, novobiocin, on the life-history parameters of *G. mellonella* larvae reared on an artificial diet.

Materials and Methods

Insect stock culture. *Galleria mellonella* larvae were reared in 1,000-ml glass jars with an artificial diet (Bronskill 1961) to adult stage at 30 ± 1°C and 60% relative humidity in constant darkness. The standard diet was composed of 420 g of bran, 150 ml of filtered honey, 150 ml of glycerol, 20 g of ground old dark honeycomb, and 30 ml of distilled water. Fifteen newly emerged adult females were placed in the jars and provided a piece of old honeycomb on the diet for egg deposition and feeding of newly hatched larvae. The methods used to prepare and dispense diets into containers, and the methods used to obtain eggs and larvae and their placement onto diets were described previously (Büyükgüzel and Kalender 2007).

Assays. The novobiocin (Upjohn Co., Kalamazoo, MI) and sodium salt (micronized form, 907 μg/mg; Warner-Lambert Research Institute, Morris Plains, NJ) used in these assays were donated by the Eczacıbaşı Medicine Company Ltd. (Istanbul, Turkey). The feeding assays were conducted under the same conditions as for rearing the stock culture. The antibiotic was tested for its effects on the survival and development of the insect by the addition of the agent directly into the diets. Four diets containing increasing concentrations of the antibiotic were tested and compared with a control diet (without antibiotic). Novobiocin at levels of 0.5, 1.0, 1.5, and 2.0 g/100 g of diet were tested for its effects on the insect.

Developmental time from 1st instar to 7th instar, pupal stage, and adult stage and survivorship in each of these stages were recorded as life-history parameters. The 7th-instar larvae were transferred into another jar lined with filter paper for pupation and then adult emergence. Number of pupae and adults were recorded, and their developmental times were calculated for each replication. Each

experiment including four antibiotic concentrations and one control was replicated four times with 20 larvae each.

Statistical analysis. Rate of development data were evaluated by one-way analysis of the variance (Snedecor and Cochran 1989). The least significant difference test (Statistical Package for the Social Sciences, Version 10, Chicago, IL) was used to determine significant differences among treatment means. Data on survivorship were compared by a chi-square test (Snedecor and Cochran 1989). When the F and χ^2 estimate exceeded the probability of 0.05, the differences were considered significant.

Results

Novobiocin at high concentrations (1.0–2.0%) significantly decreased survival rate while the highest concentration (2.0%) significantly increased developmental period from 1st instar to 7th instar by approximately 9 d (Table 1). The control diet produced 90% of 7th instars as were initially placed on the diet. The highest concentration of novobiocin significantly decreased that larval survival rate to 15%. We recorded a similar trend in the postlarval developmental stages reared on this gyrase inhibitor. This antibiotic, at all concentrations, decreased pupation and adult emergence rates, and except for low antibiotic concentration, other concentrations significantly decreased larval survival. Novobiocin at the lowest concentration did not significantly influence developmental period of 1st to 7th instars, pupal stage, or adult stage, while high concentrations of the antibiotic caused longer pupal and adult developmental periods. At all dietary concentrations, the antibiotic resulted in decreased survival rates in both the pupal and adult stages in a concentration-dependent manner. While the survival rates for pupation and adult emergence were 86.2% and 76.2%, respectively, in the control diet, these rates were decreased to 7.5% and 6.2% in the diet with the highest dietary antibiotic concentration. The effectiveness of the antibiotic on the development was increased through adult emergence that only the highest antibiotic concentration (2.0%) increased the larval development while other concentrations were effective to increase pupal (1.5–2.0%) and adult (1.0–2.0%) developmental periods.

Discussion

DNA gyrase inhibitors prevent activity of gyrase enzyme, which is responsible for the introduction of negative DNA supercoils. For this reason, they are used in treatments of bacterial infections in human and animals (Reece and Maxwell 1991). Although there are several studies concerning the effects of antibiotics on some metabolic process of insects, there is limited information on the effects of DNA gyrases on insect life table parameters. For example, some traditional antifungal antibiotics which inhibit cytoskeleton development or sterol biosynthesis have been shown to affect the biological characteristics of the western tarnished plant bug, *Lygus hesperus* (Knight) (Alverson and Cohen 2002). Rifampicin, an RNA synthesis inhibitor, significantly increases the adult emergence time of a whitefly species, *Bemisia tabaci* (Gennadius), and decreases adult emergence (Ruan et al. 2006). Moreover, we found that some first-generation gyrase inhibitors, novobiocin,

Table 1. Mean (± SE) survivorship and developmental time of *G. mellonella* in response to increasing concentrations of novobiocin in larval diets.*

Novobiocin Concentration (%)	% Survival to 7th Larval Stage	Time (d) to 7th Larval Stage	% Survival to Pupal Stage	Time (d) to Pupal Stage	% Survival to Adult Stage	Time (d) to Adult Stage
0.0	90.0 ± 3.9 a	27.2 ± 3.5 a	86.2 ± 3.6 a	33.5 ± 2.9 a	76.2 ± 3.6 a	37.2 ± 3.5 a
0.5	81.2 ± 5.1 a	30.7 ± 4.1 a	36.2 ± 5.6 b	34.5 ± 3.0 a	35.0 ± 6.3 b	39.2 ± 3.4 a
1.0	35.0 ± 3.0 b	29.7 ± 4.3 a	15.0 ± 3.0 c	33.2 ± 3.2 a	15.0 ± 3.0 c	45.0 ± 3.9 b
1.5	32.5 ± 3.7 b	27.7 ± 3.2 a	17.5 ± 3.7 c	38.5 ± 3.3 b	17.5 ± 3.7 c	46.5 ± 4.0 b
2.0	15.0 ± 1.7 c	36.2 ± 4.4 b	7.5 ± 1.2 c	39.2 ± 3.2 b	6.2 ± 1.0 cd	44.2 ± 3.9 b

* Means within a column followed by the same lowercase letter are not significantly different ($P > 0.05$); four replicates per treatment with 20 larvae per replicate.

nalidixic acid, and oxolinic acid at high dietary concentrations, resulted in deteriorated survival and development of endoparasitoid *P. turionellae*. In this study, we try to determine secondary mechanisms that contribute to deteriorative effects of these three gyrase inhibitors on life table parameters of the insect (Büyükgüzel 2001a, 2001b). Similarly, our results showed that the gyrase inhibitor aminocoumarin and the antibiotic novobiocin decrease survival capacity of *G. mellonella* as well.

The present study showed novobiocin, at high dietary concentrations, decreased survival and increased developmental period of *G. mellonella* larvae reared on artificial diets. These results raise the possibility that the gyrase inhibitor may interact with dietary nutrients, or act as a physiological toxicant. There is no study dealing with determining the effects of gyrase inhibitor antibiotics on survival and developmental response of *G. mellonella*. However, life-history parameters are important for potential use in determining the biological response of preadult stages to these antibiotics as in response of adult stages as changes in longevity, fecundity, and fertility of *G. mellonella* to a new-generation gyrase inhibitor, gemifloxacin (Hız et al. 2016). This suggestion is further supported from previous studies dealing with the effects of some gyrase inhibitors on survival and development of various insects (Büyükgüzel 2001a, 2001b; Büyükgüzel and İçen 2004; Mittler 1971; Streett et al. 2008). Changes in survivorship and developmental times have been observed with the effects of antibiotics on insects as their nutritional suitability in the larval diet was evaluated (Büyükgüzel and Yazgan 1999, 2002). These studies clearly indicated that survival and development through the adult stages are more sensitive to antibiotics than those of the adult stage. Moreover, the effects of antibiotics were mostly exerted on the larval and pupal stages and their effects on adult stage were more or less similar to those of the pupal stage. During metamorphosis, fat body tissue of lepidopteran insects changes from a larval stage to adulthood. Larval cells are differentiated into basic structures of adult insects with extensive morphological changes through pupal development. Although some histolysis of the fat body has been suggested (Dutkowski 1974), the change in function of the fat body during metamorphosis includes transformation of cellular activity at the genetic level during reorganization (Haunerland 1995, Ray et al. 1987). Therefore, the larval and pupal stages of the wax moth may be of potential interest for the study of relationship between gyrase inhibitors and life-history parameters. Our previous study showed that a gyrase inhibitor, novobiocin, at the lowest level significantly increased the survival in the pupal stage but its high levels markedly decreased the survival in this stage of *P. turionellae* (Büyükgüzel 2001b). Similarly, the present study demonstrated that high levels of novobiocin decreased survival and increased the development time of *G. mellonella* except for dietary lowest level of novobiocin. The significance of these findings is that the effects of the antibiotic are complex and dependent on their dietary levels, and that the effect on survivorship is inversely related to dietary levels. The inverse relationship may be attributed to changed level or rate of various metabolic process by the antibiotic in the diets. Slansky (1982) stated that many insects are able to reduce variation in their growth by considerably altering their food consumption and utilization in response to variation in the nutritional suitability of their food. In supporting our suggestion, Singh and House (1970) showed that antibiotics affect the feeding activity of *Agria affinis* (Fallen) larvae because of

deteriorative effects on diets. Therefore, insect diet studies confirm that, although there are no effects on survival, antibiotics can be precautionarily used for any purposes such as control of microbial contamination or preservation of the insect diets (Büyükgüzel and Yazgan 1999).

Life-history parameters are also potentially important for determining prooxidant response in terms of damaged biomolecules and changed antioxidant enzymes in different life stages to another gyrase inhibitor, gemifloxacin (Erdem et al. 2016). Dietary antibiotics may produce oxidative stress in insect tissues, leading to decreased survival and increased developmental time. We found high concentrations of novobiocin had negative effects on survival and development of *G. mellonella*, suggesting that this gyrase inhibitor causes oxidative stress leading to deteriorated life table parameters. However, further studies must examine this suggestion further.

Our study also showed that the levels of the antibiotic in the larval diet should be carefully balanced considering their effects on the survival and development, and possibly other life table parameters, for example, adult fitness parameters. This is supported by the finding of an antiviral antibiotic, acyclovir, incorporated into artificial diet of *G. mellonella* to suppress microbial contaminations (Büyükgüzel and Büyükgüzel 2016). Based on the results of its effects on the survival of *M. persicae* (Mittler 1971), *L. hesperus* (Streett et al. 2008), and the endoparasitoid *P. turionellae* (Büyükgüzel 2001a, 2001b), our results indicate that this gyrase inhibitor might affect survival by its dietary impairment. This result is supported by Streett et al. (2008), who stated that at least low levels of novobiocin could be used to reduce the cost for diet preservation without a decrease in the quality of insects produced in the mass-rearing facility.

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