Laboratory Assays of the Effects of Oxfendazole on Biological Parameters of *Galleria mellonella* (Lepidoptera: Pyralidae)[1]

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Abstract *Galleria mellonella* L. larvae were reared on a standard diet amended with varying concentrations (0, 0.0015, 0.015, 0.15, and 1.5%) of the benzimidazole-derivative antihelmintic drug oxfendazole. Survivorship, developmental times, longevity, fecundity, and fertility were monitored over the treatments. Relative to the untreated control, exposure to diet containing 1.5% oxfendazole significantly decreased survivorship in larval, pupal, and adult stages, prolonged the time to reach the adult stage, and reduced adult longevity. Oxfendazole at all concentrations significantly lowered egg production per female and decreased egg hatch. These results demonstrate that this antihelmintic may be explored as a candidate for insect pest control.

Key Words *Galleria mellonella*, oxfendazole, survival, development, fecundity

Chemical control of pest insects largely relies on applications of synthetic, broad-spectrum pesticides that pose a threat to nontarget organisms and the environment (Charriere and Imdorf 1997). These problems, coupled with concerns of human health risks, have focused attention to less-toxic and lesser-used compounds such as clinically important antibacterial, antiviral, and antifungal chemistries (Büyükgüzel 2001a,b; Büyükgüzel and İçen 2004; Büyükgüzel and Kalender 2007). Recently, we have been ascertaining the effects of anthelmintic drugs on insects (Büyükgüzel and Kayaoğlu 2014; Kılıç et al. 2015).

Oxfendazole, a benzimidazole anthelmintic drug, has long been clinically used to treat parasite infections in human and animal digestive and respiratory tracts, livers, and other internal organs (Asquith and Kulwich 1980; Marriner and Bogan 1981). Oxfendazole toxicity studies with insects as targets have been directed toward survival rates of dung insect fauna exposed to feces excreted by animals treated with the anthelmintic (Hennessy et al. 1992, 1993). Lumaret and Errouissi (2002) showed that fecal residues or metabolites of benzimidazole drugs are relatively harmless to dung fauna, contrary to the activity of other anthelmintics such as couaphos, dichlorvos, phenothiazine, and most macrocyclic lactones against such insect fauna. Moreover, oxfendazole exhibits low toxicity to laboratory rats and mice and has little, if any, negative environmental impacts (Marriner and Bogan 1981). It

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has been proposed that, like other benzimidazole anthelmintics, oxfendazole inhibits polymerization of tubulin proteins and, hence, interferes with the uptake of glucose causing mortality due to energy depletion (McKellar 1997; Prichard 1970). Thus, it is active against both larval and adult parasites. Metabolism of oxfendazole by hepatic microsomal cyctochrome P450 or degradation in the intestine is often associated with the production of febendazole and its derivatives, which damage parasite tissues (Delatour et al. 1985; Murray et al.1992). Aukštikalnienė et al. (2005) reported that increasing doses of some anthelmintics damaged epithelial cells in the intestine of a parasite, *Toxocora canis* (Werner), will cause increased mortality. However, oxfendazole-induced impacts on the biological fitness of insects have been not established.

Galleria mellonella L. larvae are convenient models for assessing the virulence of microbial pathogens and the efficacy of antimicrobial drugs, giving results comparable to those that can be obtained using mammals (Jacelyn et al. 2013; Li et al. 2013). Although we studied the effects of antibacterial and antifungal antibiotics on the biological and biochemical response of *G. mellonella* as a model insect, there are limited studies determining effects of anthelmintic drugs on insects (Büyükgüzel and Kayaoğlu 2014). We hypothesized that the anthelmintic agent induces dietary impairment leading to deteriorated biological fitness of *G. mellonella*. Herein, based on our previously reported data on effects of some antihelmintic drugs on *G. mellonella*, we further studied the effects of oxfendazole on survival, development, longevity, fecundity, and egg hatching rate of the insect reared on artificial diets under laboratory conditions.

Materials and Methods

Galleria mellonella larvae and pupae were collected from honeybee, *Apis mellifera* L., hives in apicultural areas in Zonguldak, Turkey, and returned to the laboratory. The newly-emerged adults were used to establish the stock colony. The insects were reared in 1-L glass jars with an artificial diet (Bronskill 1961) at 30 ± 1°C and 65 ± 5% relative humidity in constant darkness. The standard diet was composed of 420 g bran, 150 ml filtered honey, 150 ml glycerol, 20 g ground old dark honeycomb, and 30 ml distilled water. Newly emerged 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. Methods used to prepare and dispense diets, obtain eggs, and place hatched larvae onto diets were previously described by Büyükgüzel et al. (2010).

Oxfendazole, methyl 5-(phenylsulfinyl)-benzimidazol-2-carbamate (>98%) (Abdi İbrahim Medicine Company, Istanbul, Turkey), was incorporated into the artificial diets at concentrations of 0.0015, 0.015, 0.15, or 1.5% (w/w) based on results of Büyükgüzel and Kayaoğlu (2014) with niclosamide and with preliminary testing with oxfendazole. Larvae reared on diets without oxfendazole were used as controls. Treatments were replicated four times with 15 larvae per replicate. Using standard laboratory rearing conditions, the effects of oxfendazole on *G. mellonella* survivorship, development, adult longevity, egg production (fecundity), and egg hatchability (fertility) were monitored and recorded.

First instars placed on the diets were reared through adult emergence on the oxfendazole-amended diets. Survivorship in the seventh larval stadium, the pupal stage, and the adult stage, as well as the time to reach to those stages, was recorded. Surviving seventh instars were transferred into a jar lined with a filter paper where pupation and adult emergence occurred. The filter paper was used to provide a dry surface for pupation. Numbers of pupae and adults were recorded and their developmental times were calculated for each replicate. Newly emerged adults were then transferred into 30-ml plastic cups (OrLab® Ltd., Ankara, Turkey) covered with screened lids. Average adult longevity was determined by recording the number of dead adults in each treatment each day until all adults had died.

Fecundity and fertility were determined in separate tests conducted in the same manner as previously described except that females emerging from pupae reared on the oxfendazole-amended diets were placed in 30-ml plastic cups with screened lids and allowed to oviposit for 2 d (our experience with egg laying in the stock colony showed that newly emerged females laid most of their eggs within the first 2 d). We paired one male with one female that emerged on the same day and let them lay eggs until the death of all individuals. Adults were removed from the cups and eggs were transferred into Petri dishes using a fine brush. Total numbers of eggs laid per female per day were recorded as fecundity. Egg counts were performed in the Petri dish using a black background to make the eggs more visible. Eggs were held in the dishes for hatching, and the numbers of eggs hatched per female per day were recorded to calculate fertility (percentage of total eggs that hatched). Females were not provided with food during the oviposition period. Each treatment was replicated four times with 15 females per replicate. Egg production and larval hatching were monitored continuously from the first oviposition day until experiments were halted.

Data obtained in these tests were subjected to one-way analysis of variance (ANOVA). To determine significant differences between means, the least significant difference (LSD) test (SPSS 1997) was used. Survivorship data were compared by a chi-square test (χ^2; Snedecor and Cochran 1989). When the F and χ^2 estimate exceeded the probability of 0.05, the differences were considered statistically significant.

Results

Survivorship and development. Oxfendazole at the concentrations evaluated in this study significantly decreased the survival of *G. mellonella*. At the seventh larval stadium, 43.0 ± 1.5% (mean ± SE) of the larvae had survived on the 1.5% concentration of oxfendazole while 94.9 ± 1.5% survived on the untreated check. Similar responses were observed in survivorship to the pupal and adult stages. On the control diet, 91.6 ± 1.4% survived to pupation and 84.9 ± 1.4% survived to emerge as adults. On the diet amended with 1.5% oxfendazole, only 36.7 ± 1.5% survived to pupation while 34.9 ± 1.4% survived to the adult stage (Table 1).

Developmental time from egg to the seventh larval stage was not significantly affected by the oxfendazole-amended diets; however, the developmental time from egg to pupation was significantly prolonged with the 1.5% concentration of oxfendazole in comparison to the control diet and the other concentrations of the

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

Oxfendazole Concentration (%)	% Survival to Seventh Larval Stage	Time (days) to Seventh Larval Stage	% Survival to Pupal Stage	Time (Days) to Pupal Stage	% Survival to Adult Stage	Time (Days) to Adult Stage
0.0	94.9 ± 1.45 a	23.5 ± 0.67 a	91.6 ± 1.45 a	30.1 ± 0.71 a	84.9 ± 1.42 a	36.3 ± 0.26 a
0.0015	79.9 ± 4.08 b	22.3 ± 0.50 a	71.7 ± 3.63 b	28.9 ± 0.66 a	64.9 ± 1.42 b	37.1 ± 1.06 a
0.015	66.1 ± 2.01 b	22.0 ± 0.35 a	56.5 ± 3.83 b	30.6 ± 0.86 a	53.3 ± 4.70 b	36.7 ± 0.85 a
0.15	61.6 ± 2.75 bc	22.2 ± 0.30 a	60.0 ± 3.32 b	30.7 ± 1.13 a	51.5 ± 4.38 b	38.5 ± 0.45 a
1.5	43.0 ± 1.50 c	23.5 ± 0.90 a	36.7 ± 1.67 c	33.8 ± 0.90 b	34.9 ± 1.45 bc	39.9 ± 0.54 ab

* Means within a column followed by the same lowercase letter are not significantly different ($P > 0.05$); four replicates per treatment with 15 larvae per replicate.

Table 2. Mean (±SE) longevity, egg production per female, and percentage hatch of eggs of *G. mellonella* in response to increased concentrations of oxfendazole in larval diets.*

Oxfendazole Concentration (%)	Longevity (Days)		Number of Eggs (per Day/Female)	% Eggs Hatched
	Males	Females		
0.0	8.8 ± 0.06 a	9.6 ± 0.21 a	93.8 ± 1.72 a	88.9 ± 1.92 a
0.0015	10.1 ± 0.17 b	9.5 ± 0.20 a	61.4 ± 1.03 b	74.7 ± 2.20 b
0.015	10.4 ± 0.51 b	9.1 ± 0.18 a	56.3 ± 0.87 c	52.8 ± 1.90 c
0.15	8.8 ± 0.10 a	8.9 ± 0.20 a	45.6 ± 0.72 d	60.9 ± 0.83 d
1.5	7.0 ± 0.12 c	6.5 ± 0.36 b	30.8 ± 0.94 e	53.7 ± 1.43 c

* Means within a column followed by the same lowercase letter are not significantly different ($P > 0.05$); four replications with 15 larvae per replicate.

anthelminthic (Table 1). Oxfendazole-amended diets did not significantly affect the developmental time from egg to adult emergence.

Longevity, fecundity, and fertility. In comparison to the control diet, male longevity and female longevity were significantly shortened when larvae were fed on the diet containing 1.5% oxfendazole. Interestingly, male longevity was significantly prolonged with the 0.0015 and 0.015% concentrations, but male longevity was not significantly affected by the 0.15% concentration. In addition, female longevity was not signficantly affected by concentrations of 0.0015, 0.015, or 0.15% (Table 2).

The number of eggs oviposited per female was inversely related to oxfendazole concentrations in the artificial diet with the highest concentration (1.5%), exhibiting a mean (±SE) of 30.8 ± 0.94 eggs produced per female. This was about 33% less than the number of eggs (93.8 ± 1.72) produced by each female that had been fed the control diet during larval development (Table 2). The percentage of eggs that hatched also was significantly decreased by oxfendazole. The percentage of hatch was 53.7 ± 1.43% in the treatment with the highest level of oxfendazole (1.5%) while percentage hatch in the controls was 88.9 ± 1.92%; hatch percentage was intermediate for the remaining concentrations tested (Table 2).

Discussion

Anthelmintics are already considered valuable in agriculture for their use in managing parasites that infest in some economically important livestock. This study reported herein, however, demonstrates that benzimidazoles cause deterioration of developmental and biological parameters of *G. mellonella* that consumed oxfendazole during larval development. These results, therefore, indicate that anthelmintics could possibly be used as alternatives to traditional chemical insecticides to manage insect pests.

Results of this study support our hypothesis that dietary oxfendazole treatments influence the biological fitness of *G. mellonella* larvae. The highest concentration of oxfendazole (1.5%) tested decreased adult longevity and survivorship and prolonged developmental time of *G. mellonella*. A previous study showed that niclosamide, another anthelmintic, negatively impacted the life table parameters of *G. mellonella* that fed on that chemical (Büyükgüzel and Kayaoğlu 2014). It has been suggested that niclosamid and other chemicals might be candidates for evaluation to chemically control *G. mellonella* larvae (Büyükgüzel et al. 2013; Durmuş and Büyükgüzel 2008; Hyrsl et al. 2007). Our results from the present study suggest that oxfendazole should be added to those chemicals for further evaluation. In comparison to untreated controls, *G. mellonella* reared on a diet containing 1.5% oxfendazole had only 35% of adults successfully emerge.

Büyükgüzel (2001a,b) further demonstrated that the sublethal effects of an antibiotic-amended diet may depend on altered larval feeding rates, perhaps due to interaction of the antibiotic with dietary nutrients. Additional evidence confirming this suggestion includes previous studies showing that dietary components interact to biologically impact insects in artificial rearing systems (Yazgan 1972, 1981; Yazgan and House 1970). Previous efforts also have demonstrated that the nutritional and physical quality of larval food are factors that may affect the biological performance of adult insects (Krams et al. 2015; Slansky and Scriber 1985; Zucoloto 1988). In agreement with these findings, our data presented herein demonstrated decreased adult fitness in terms of extended longevity and decreased fecundity and fertility of *G. mellonella* adults exposed to sublethal doses of oxfendazole in larval diets. We surmise that the observed increased toxicity of dietary oxfendazole at sublethal concentrations may result from increased consumption of this anthelmintic. We need further experiments to test this suggestion by comparing diet consumption per treatment. Aukštikalnienė et al. (2005) reported that increasing doses of some anthelmintics damaged epithelial cells in the intestine of a livestock parasite, *T. canis*, causing increased mortality of the parasite population. A similar mechanism may occur in the midgut of *G. mellonella* larvae that consume oxfendazole, as reported following exposure to different insecticides (Adamski et al. 2014; Büyükgüzel et al. 2013).

Another possible mode of action may be the impact of oxfendazole on the populations of symbiotic bacteria in the digestive tract of *G. mellonella*. Several studies have demonstrated that symbiotic bacteria contribute to nutritional needs for normal development and performance of insects (Bucher and Williams 1967). Petri (2011) notes that oxfendazole is poorly absorbed through the mammalian intestine and into the bloodstream. Jawetz et al. (1984) also report that antibiotics synergize or antagonize their effects depending on different absorption rates in the intestine. If this holds true for the insect midgut, it is reasonable to suggest that oxfendazole, not being absorbed through the gut wall, will remain in the host gut for longer periods of time and at higher concentrations to impact symbiotic bacteria residing there. Also, oxfendazole has antibacterial effects, as do other benzimidazole anthelmintics (Lingala et al. 2011). These factors may play significant roles in morphology of the larval midgut following consumption of oxfendazole and, thus, the observed effects of this anthelmintic agent in decreased survival and development.

While the mode of action of oxfendazole in insects remains unknown, our results show that deteriorated life table parameters are associated with increasing dietary

concentrations of the anthelmintic. Oxfendazole prevents polymerization of tubulin to form microtubules by binding these proteins and also inhibits fumarat reductase (Prichard 1970) and glucose transport (McKellar 1997) in nematodes, thus leading to death due to energy deprivation. Future research should explore the nutritional or physiological impacts of oxfendazole on life history parameters of *G. mellonella* and other insects.

In conclusion, our study showed that oxfendazole has potential for development in the management of insect pests, and that its role in pest management is not limited to direct lethal effects due also to dietary impairment in preadult and adult life stages and modes of activity that reduce the survival and delay development. Negative effects on insect survival, development, and adult fitness including longevity, fecundity, and fertility may prove important in understanding this continued process. Investigations are in progress to determine the mode of action of this anthelmintic in *G. mellonella*.

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