In addition, as has also been observed earlier, we show that the development of ventral veins seems to be dependent on the inductive action of the dorsal surface veins.

Effects of *Penicillium roqueforti* on some growth parameters of *Drosophila melanogaster*.

Uysal, Handan, and Omer Faruk Algur. Department of Biology, Faculty of Science and Arts, Atatηrk University, 25240, Erzurum, Turkey.

Abstract

In this work, the effects of *Penicillium roqueforti* filtrate (PRF) on development, fecundity and dominant lethal mutation of *Drosophila melanogaster* have been investigated. For this purpose, different concentrations of *Penicillium roqueforti* filtrate (50, 100, 150 ppm) were added to the standard medium of *D. melanogaster*. The experiments demonstrated a significant increase in the mortality of the F1 generation and decrease in the number of progeny.

Introduction

Fungal contamination in laboratory *Drosophila* cultures is an undesirable condition. But, unfortunately, fungal contaminations by some mold genera, especially *Penicillium* and *Aspergillus*, have been observed although much effort has been spent for sterilization. It is probable that these molds were transferred to the culture medium by the extremities of adult individuals. Furthermore, it was shown that some secondary metabolites such as patulin, citrinin, ochratoxin A, roquefortine, rubratoxin B and penicillic acid of these molds may cause acute and chronic toxicity, especially to the kidney and liver tissues of rat, guinea pigs, mice and cockerels (Saito *et al.*, 1971; Cole *et al.*, 1972; Scott *et al.*, 1976). These findings caused new investigations on the effects of these metabolites on the developmental stages of *Drosophila melanogaster* and it was found that some extracts and metabolites of *Aspergillus* and *Penicillium* decreased survival of the larvae of *Drosophila hydei* and prolonged their development (Cole and Rolinson, 1972; Hodge and Mitchell, 1997). Similar effects have also been observed in *Spodoptera exigua* larvae (Boucias *et al.*, 1994). On the other hand, it was reported that as a result of applications of some chemicals such as dithane M-45 and ethyl methane sulphonate (EMS), the mortality has been observed usually at the early stages of the development (Vasudev and Krishnamurthy, 1982-1983; Ivanov, 1998). In addition, simplicissin obtained from *Penicillium* cf. *simplicissimum* is known to have an inhibitory effect on pollen growth of *Camellia sinensis* (Kusano *et al.*, 1997). But, no research on the effect of the extract of *Penicillium roqueforti* on the development of *D. melanogaster* was found. The aim of this research is to investigate the effects of the extract of *P. roqueforti*, which frequently contaminates our *D. melanogaster* cultures, on the development of *D. melanogaster*.

Materials and Methods

Organism:

Drosophila melanogaster Meig (Diptera: Drosophilidae) Oregon-R strain was used in the investigation. This is a laboratory wild type stock adapted into laboratory conditions by intermating

for a long time. For the growth and stock cultures, Standard Drosophila Medium (SDM) was used (Bozcuk, 1978). The stocks were maintained in an incubator adjusted to 25±1°C and 40-60% relative humidity.

Penicillium roqueforti was isolated from our stock *Drosophila* cultures. For this purpose, a part of the contaminated colony in the stock was transferred to Czapek Solution Agar (Difco) in petri dishes. After incubation at 25°C for 8 days, the colonial and microscopic examinations were made and it was identified as *P. roqueforti*. The identification of this species was made according to Raper and Thom (1949) and Ramirez (1982).

Preparation of *Penicillium roqueforti* Filtrate:

Penicillium roqueforti was grown in 500 ml of Nutrient Broth (NB) in a 1 liter erlenmeyer flask and agitated at 140 rpm at 25°C for 72h. At the end of incubation, molds were filtered with Whatman No. 1 filter paper and the filtrate was tyndalyzed at 70°C. This later solution is termed *Penicillium roqueforti* Filtrate (PRF) and used throughout the experiment.

Experimental Groupings:

For this purpose, two experiment groupings (control and experimental) were prepared. Each group was made of three parallels.

Different concentrations (50, 100 and 150 ppm) of the PRF were added to 100 ml of the SDM in culture vials and kept waiting for 24 hours for diffusion of PRF into the medium. One female and three male flies of the same age were put in each culture vial. All of the female flies selected were virgins. The culture vials containing only the SDM were used as a control. The developmental stages were followed daily. After pupa formation, the parental individuals were removed. Offspring were counted every day from the first day of eclosion and sex was noted.

Determination of Fecundity:

The small plastic vessels with 2 cm diameters containing different concentrations of the PRF in the SDM were put into 150 ml empty culture vials. One female and three male of the same age were etherised and put into these culture vials. For 10 days, the eggs in each small vessel were counted at 22 hour intervals (Strickberger, 1967) and the vessels were changed with the new ones. After egg counting was completed, the vessels were put into sterile petri dishes for completion of the metamorphosis and the eggs hatched were counted. The number of embryonic deaths were counted for calculating dominant lethals.

Regression line analysis, using the formula $y = mx + c$, was employed to determine the dose-effect relationships. The chi-square test was used for the statistical analysis of the results. Statistical comparisons are between experimental groups and the control.

Results

The effects of the PRF on the metamorphosis of *D. melanogaster* are shown in Table 1. As shown in Table 1, the PRF delayed the metamorphosis especially at 150 ppm concentration. In the control group, laid eggs were observed on the second day of mating. Then, first, second and third instar larvae, prepupae, pupae and adult individuals were formed, respectively. The first F1 adult was observed on the 9^{th} day of mating. For experimental groups with the applications of 50 and 100 ppm of the PRF, the metamorphosis was completed in the same period as the control. However, with the application of 150 ppm of PRF, while laid eggs were observed on the second day after mating, the first larvae and the first pupae were formed at the 4^{th} and 8^{th} days after mating, respectively. Furthermore, the first adult was observed on the 11^{th} day after mating.

The effect of PRF on the number of offspring of *D. melanogaster* is shown in Figure 1. In the experimental groups, the number of offspring decreased on the basis of the increased concentration of PRF. As shown in Figure 1, the numbers of offspring for control and applications (50, 100 and 150

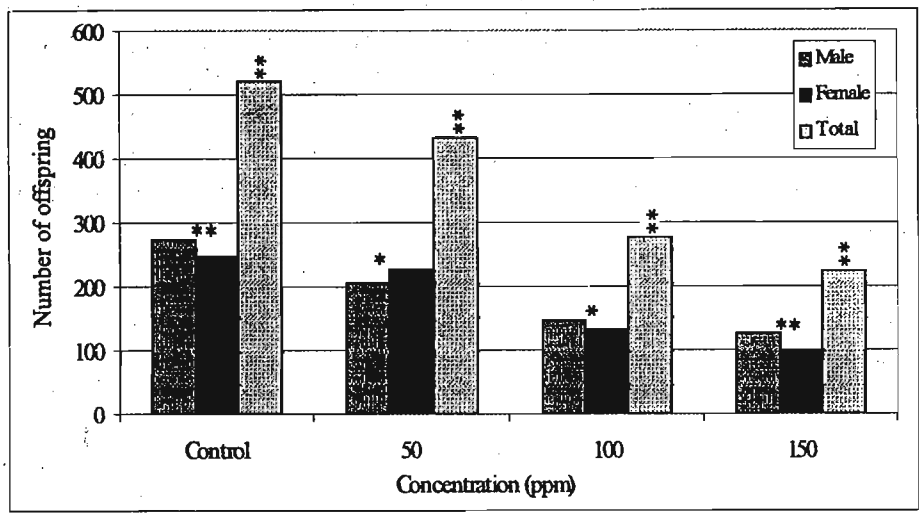

Figure 1. The effect of PRF on the number of offspring of *D. melanogaster*. Statistical comparisons are between experimental groups and the control. Sex-ratio is not important (*P > 0.05 and **P > 0.01) but the decrease in the total number of offspring is significant (*/* P < 0.01).

Table 1. The effect of PRF on the metamorphosis of *D. melanogaster*.

	Days of stages at control and applications			
Stages of metamorphosis	Control	50 ppm	100 ppm	150 ppm
Mating	1	1	1	1
Egg	2	2	2	2
1st instar larvae	3	3	3	4
2nd instar larvae	4	4	4	5
3rd instar larvae	5	5	5	6
prepupa	6	6	6	7
pupa	7	7	7	8
adult	9	9	9	11

ppm of PRF) were 522, 433, 277 and 223, respectively. The differences between the results were statistically significant ($P < 0.01$). While the number of male and female individuals of control and applications were different, it is not statistically significant ($P > 0.01$ and $P > 0.05$).

The effect of PRF on the fecundity and dominant lethality of *D. melanogaster* has been shown in Table 2. For the control group, the total number of eggs laid and eggs hatched are 257 and 243, respectively. The differences between the number of eggs laid and eggs hatched are not statistically important ($P > 0.05$). As shown in Table 2, PRF has also significantly reduced the fecundity and egg viability ($P < 0.01$). The rate of the dominant lethality increased with the increasing of PRF (Figure 2).

Discussion

As a result of PRF applications, the metamorphosis lengthened (Table 1). Prior investigations have shown that the extracts of *A. niger* and *A. flavus* have similar effects (Hodge and Mitchell, 1997). Some pesticides, such as fenbar, endrin, gamalin etc., have also lengthened the metamorphosis of *D. melanogaster* (Jayasuriya and Ratnayake, 1973). On the other hand, the PRF decreased the number of offspring of *D. melanogaster* significantly (Figure 1). It was reported that the extracts of *A. niger* decreased the number of offspring of *D. hydei* (Hodge and Mitchell, 1997). Some chemicals such as dithane M-45 and diflubenzuran also have similar effects (Vasudev and Krishnamurthy, 1978; Rup and Chopra, 1985; Kim *et al.*, 1992). Our findings are in accordance with the results of the above investigations. Although the mechanism of the process is unclear, this effect may be due to two reasons: (i) The toxic metabolites in PRF may inhibit the production of some enzymes affecting the developmental stages of *D. melanogaster*. Because, some researchers have

found that PR toxin obtained from the culture of *Penicillium roqueforti* inhibits *in vivo* and *in vitro* protein synthesis and *in vitro* nucleic acid synthesis (Scott, 1981). (ii) Some materials in PRF may have some adverse affects on the secretion of juvenile hormone (JH) *of D. melanogaster*. Because, it was reported that the chemical and physical factors affecting the secretion of this hormone also has effects on the developmental stages of insects because it has an important effect on the growth, development, reproduction and diapose of hemimetabola and holometabola (Wigglesworth, 1961; Scott and Kennedy, 1976; Bowers *et al.*, 1976).

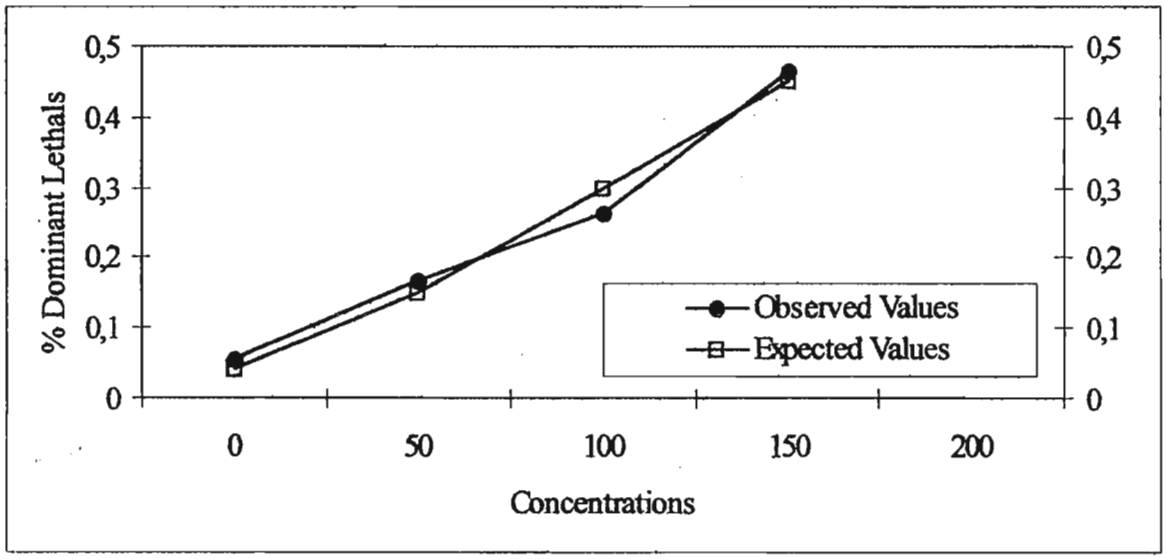

Figure 2. Dose-effect response in the induction of dominant lethals by PRF in *D. melanogaster*.

Table 2. The effect of PRF on the fecundity and dominant lethality of *D. melanogaster*.

Concentration (ppm)	Expt. no.	Total no. of eggs laid	Total no. of eggs hatched	Total no. of dominant lethals	Percentage of dominant lethals
Control	1	74	68	6	0.081
	2	94	90	4	0.042
	3	89	85	4	0.044
	1 + 2 + 3	257*	243*	14	0.054**
50	4	74	60	14	0.189
	5	64	55	9	0.140
	6	81	67	14	0.172
	4 + 5 + 6	219	182	37	0.168**
100	7	44	36	6	0.181
	8	48	36	12	0.250
	9	52	34	18	0.346
	7 + 8 + 9	144	106	38	0.263**
150	10	32	18	14	0.437
	11	22	7	15	0.681
	12	21	15	6	0.285
	10 + 11 + 12	75	40	35	0.466**

*P < 0.05 ; ** p < 0.01

Egg hatchability has been used as a measure of dominant lethality and there is considerable evidence to show that this is related to chromosome breakage (Sankaranarayanan, 1969; Vasudev *et al.*, 1994). On the other hand, it was reported that dominant lethality mutation is a major genetic damage (Auerbach, 1962) and is any newly arisen mutation resulting in immediate death of the zygote in the first generation (Ivanov, 1998). So, as it is shown in Table 2, the dominant lethality was calculated and we showed that PRF increased DLM ($P < 0.01$) significantly. The increasing of DLM caused the decreasing of offspring in the first generation in all of the different concentrations ($P < 0.01$). Unfortunately, we could not find any literature on the effect of *Penicillium* spp. extracts on dominant lethality to compare our results. But, it was reported that some chemicals also have increased dominant lethality (Datta *et al.*, 1978; Krishnamurthy *et al.*, 1984-85; Sutter *et al.*, 1991; Vasudev *et al.*, 1994; Ivanov, 1998). It is probable that some toxins in PRF may cause some mutations in the offspring of *D. melanogaster*. Although the mechanism of these processes is unclear, we showed that the PRF caused some disorders in the development of *D. melanogaster*. These disorders in the developmental stages of *D. melanogaster* are to be the source of the chromosomal aberrations. Because, in our other research, aberrations have been observed in the salivary gland of third instar larvae of *D. melanogaster* (unpublished data). Depending on chromosomal aberrations, it should not be neglected that various phenotypic abnormalities may occur in offspring.

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