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Mortality effects of Amphotericin B on House Fly, *Musca domestica* (Diptera: Muscidae) under Laboratory Conditions

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Abstract

House fly, *Musca domestica*, is a fly of the suborder Cyclorrhapha, (Diptera: Muscidae). It is a cosmopolitan pest that carries pathogens on its body, faeces and can cause food borne illnesses. The purpose of this study was to investigate the effect of Amphotericin B on house flies using specific tools such as poison baits. The concentrations of poison baits used were 0.5%, 1%, 1.5% and 2%. Amphotericin B -based bait was used as positive control while the no bait (water, sugar syrup and granular sugar only) was used as negative control. Post feeding, 9.88%, 10%, 12.2%, 12%, and 12.23% of house flies were found dead after 3, 4, 5, 6, and 7 days follow up respectively. Amphotericin B significantly reduced the density of house flies. We recommend that the effectiveness of Amphotericin B on wild strains of house flies and other nuisance insects such as cockroaches be investigated in future.

Keywords: *Musca domestica*, Amphotericin B, Insectarium, Transmission, Mortality

1. Introduction

Musca domestica (house fly) is a cosmopolitan pest to both humans and animals. It transmits infectious disease of public health importance. House flies are not only nuisance, but they can also vector some diseases, especially in tropical areas [1]. This common fly (house fly) originated from central Asia, but now lives in many climates from tropical to subtropical regions [2]. House flies are considered to be mechanical and biological vectors of pathogenic microbes [3, 4]. This species do not bite but transmits many pathogens such as bacteria, fungi, viruses, and parasitic worms. Previous studies showed that house flies are reservoirs of some pathogens and diseases such as *Campylobacter*, *Chlamydia*, *Escherichia coli*-labile toxin, *Enterococcus*, *Salmonella*, *Bacillus anthracis*, Shigellosis, tuberculosis, typhoid bacilli, *Vibrio Cholera*, intestinal and urogenitaly worms [5-8].

Amphotericin B is an antifungal polyene antibiotic obtained from a strain of *Streptomyces nodosus*. This drug is used primarily for the treatment of patients with progressive and potential life-threatening fungal infections [9]. On the other hand, this drug has toxic side effects [10].

There are more practical and common control measures for house flies other than Amphotericin B such as sanitation, use of traps, insecticides, biological control, and garbage sanitation [11]. Poison baits are effective if located in suitable, attractive, and odor free places [12]. Previous studies showed some chemical compounds used as poison baits such as Tetrodotoxin nervous system blocker caused rapid and violent death amongst house flies [13-15]. Other chemical agents include disinfectants, plant extracts, organic acids, and antifungal drugs, e.g. AmB. However, veterinarian regulations do not allow the use of antibiotics in the treatment of *Nosema spp.* in Europe, there is an increasing problem with the invasion of *Nosema ceranae* in Europe and there are bee's populations that are kept and must not be lost [16-18].

In order to safely use AmB on humans and also to enable it restricted use on bees (the lesser evil), we have to learn more about the functions and mechanism of action of this drug in living organisms.

Sub-lethal effects of tetrodotoxin (TTX) on cockroaches have been investigated and the study suggested that TTX can be very useful in the analysis of sub-cellular nature of sodium channels

in nerve membrane [2]. In another related study, TTX blocked the blocked the sodium channel activity of German cockroach, *Blattella germanica*. The study showed that TTX provided partial protection from the toxicity of type I pyrethroids, carbamates, and DDT, but little protection from type II pyrethroids, oregano phosphates, nicotine, or muscarine. Pretreatment with sub-lethal doses of permethrin or cypermethrin resulted in 1.7- to 2.6-fold synergism of bendiocarb, but it did not substantially change the toxicity effect of chlorpyrifos or malathion [19]. The study also showed that AmB shortened the life span of the honey bees and reduced the level of global DNA methylation compared to the control [20]. Therefore, in this investigation the effect of Amphotericin B on house flies was carried out using specific tools such as poison baits

2. Materials and methods

Study Area

This study was conducted in the Research Center Insectary of the School of Public Health, Tehran University of Medical Sciences, Iran during 2015.

Selection of susceptible strains of *Musca domestica*

Susceptible strains of *Musca domestica*, were selected for both the treatment and control groups and were divided and grouped according to WHO standard techniques

Rearing

The insectary condition temperature was 24 ± 2 °C, relative humidity of 40%, and daily light /dark period was 12:12 hrs during to 2015. The optimal temperature for larval development was between 35 to 38 °C though larval survival was between 17 to 32 °C. Larvae completed their development in 4 to 13 days at optimal temperatures, but required 14 to 30 days at temperatures of 12 to 17 °C [21-25].

Treatment

Treatment was carried out based on the principle described by Bailey and Keiding [26, 27]. The treatment group was divided into four subgroups. Fifty pupae were transferred to each cage. In the same group for each cage of control, 50 pupae

were transferred. Amphotericin B was diluted with powder concentration and administrated orally with water, granular sugar, sugar syrup [20] and dry milk. Low amounts of lipid concentrations were given at 5%, 10%, 15%, and 20% of poison baits, respectively. Poison baits were kept in special sheet and accessible to house flies after immersing from pupal stage. In order to add water supplement, water-impregnated woolen was used. Mortality was recorded after 1, 2, 3, 4, 5, 6, and 7 day intervals. Repeated tests were conducted four times in each group. In the same way, water and granular sugar were used as control for the control group.

Statistical Analysis

The data was analyzed statistically. SPSS ver. 16, and one way ANOVA test were used to compare mortality of *Musca domestica* between two groups and between exposure times. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

Table 1 summarizes the result of poison baits with 0.5% Amphotericin concentration after 1, 2, 3, 4, 5, 6, and 7 days intervals. Mortality occurred after 48 hrs exposure in the treatment group. Significant statistical association was observed between the mortality of common flies in day 1, and post feeding in others days ($P < 0.05$). However, mortality rate increased to 5 days follow up. Mortality was more than 5%, 3 days post-feeding and onward follow up respectively.

Significant differences were observed between the mortality in the first day and other follow-up days using Amphotericin B 1% ($P < 0.05$) (Table 2). and in days 1, 2, and other follow up days in the treatment group using Amphotericin B 1.5% ($P < 0.05$) (Table 3). In the control samples, no mortality was observed except in the fourth and fifth follow-up days. The total number of *Musca domestica* alive and those that died following feeding on poison baits treated orally with Amphotericin B 2% were compared both in the treatment and control groups (Table 4). Significant differences were observed between mortality in the first, second, and other follow up days ($P < 0.05$) (Table 5).

Table 1: Number of *Musca domestica*, Tehran strain fed on poison baits treated orally with Amphotericin 0.5% compared with control group, 2015.

Day	Treatment				*Statistic Analysis	Control				*Statistic Analysis
	Alive	Dead	Total	Mortality %	<i>P</i> value	Alive	Dead	Total	Mortality %	<i>P</i> value
1	40	0	40	0	$P < 0.05$	28	0	28	0	0.1
2	132	5	137	3.64		32	0	32	0	0.01
3	118	11	129	8.52		36	1	37	2.8	0.11
4	110	13	123	10.6		35	0	35	0	0.001
5	97	15	112	13.4		34	0	34	0	0.01
6	87	9	96	9.4		32	1	33	3.1	0.02
7	38	6	44	13.7		33	0	33	0	0.001

*The mortality of common flies in 1 day, and post feeding others days ** The mortality of common flies in treatment versus control groups

Table 2: Number of *Musca domestica*, Tehran strain fed on poison baits treated orally with Amphotericin B 1% compared with control group, 2015.

Day	Treatment				*Statistic Analysis	Control				*Statistic Analysis
	Alive	Dead	Total	Mortality %	<i>P</i> value	Alive	Dead	Total	Mortality %	<i>P</i> value
1	123	0	123	0	$P < 0.05$	37	0	37	0	0.1
2	119	4	123	3.3	0.045	37	0	37	0	0.01
3	116	11	127	8.7	0.033	38	0	38	0	0.005
4	82	11	93	11.83	0.14	38	1	39	2.6	0.001
5	68	10	78	12.9		35	2	37	5.5	0.01
6	61	10	71	14.1	0.15	35	0	35	0	0.02
7	51	8	59	13.6		34	0	34	0	0.001

* The mortality of common flies in 4, 5 and 6,7and other days, and post feeding others days ** The mortality of common flies in treatment versus control groups

Table 3: Number of *Musca domestica*, Tehran strain fed on poison baits treated orally with Amphotericin 1.5% compared with control group, 2015.

Day	Treatment				*Statistic Analysis	Control				*Statistic Analysis
	Alive	Dead	Total	Mortality %	P value	Alive	Dead	Total	Mortality %	P value
1	148	0	148	0	0.1	32	0	32	0	0.1
2	148	0	148	0		32	0	32	0	0.11
3	128	20	148	13.6	0.011	36	1	37	3.8	0.005
4	113	19	132	14.4	0.01	33	0	33	0	0.001
5	107	9	116	7.8	0.029	32	1	33	3.1	0.01
6	105	12	117	10.3	0.033	32	1	33	3.1	0.02
7	97	9	106	8.5	0.031	31	1	32	3.2	0.001

* The mortality of common flies in 1, 2 days, and post feeding others days ** The mortality of common flies in treatment versus control groups

Table 4: Number of *Musca domestica*, Tehran strain fed on poison baits treated orally with Amphotericin 2% compared with control group, 2015.

Day	Treatment				*Statistic Analysis	Control				*Statistic Analysis
	Alive	Dead	Total	Mortality %	P value	Alive	Dead	Total	Mortality %	P value
1	14	0	14	0	0.1	4	0	4	0	0.1
2	28	0	28	0		9	0	9	0	0.15
3	53	5	58	8.7	0.011	15	0	15	0	0.005
4	65	9	74	12.2	0.01	17	0	17	0	0.001
5	88	15	103	14.6	0.03	19	0	19	0	0.02
6	92	4	96	14.2	0.023	20	0	20	0	0.03
7	73	11	84	13.1	0.021	21	0	21	0	0.002

* The mortality of common flies in 1, 2 days, and post feeding others days ** The mortality of common flies in treatment versus control groups

Table 5: Mortality percent of *Musca domestica*, Tehran strain after post feeding 0.5, 1, 1.5, and 2 Amphotericin orally compared with control group, 2015.

Day	Concentrations								Reang & Average (%)	*Statistic Analysis
	0.5%	Control	1%	Control	1.5%	Control	2%	Control		P value
1	0	0	0	0	0	0	0	0	-	0.1
2	3.6	0	3.3	0	0	0	0	0	-	0.15
3	8.52	2.8	8.7	0	13.6	3.8	8.7	0	8.7- 13.6= 9.88	0.005
4	10.6	0	11.83	2.6	14.4	0	12.2	0	10.6-14.4= 10.01	0.001
5	13.4	0	12.9	5.5	7.8	3.1	14.6	0	7.8-14.6= 12.2	0.02
6	9.4	3.1	14.1	0	10.3	3.1	14.2	0	14.2-14.1= 12	0.03
7	13.7	0	13.6	0	8.5	3.2	13.1	0	13.7-8.5= 12.23	0.002

* The mortality of common flies in 1 day, and post feeding others days

4. Discussion

AmB is a drug used in patients with impaired immunity, for example, in transplant recipients and patients with cancer or AIDS [28, 29]. The selective action of AmB is related to the fact that the antibiotic has a greater affinity for membranes containing ergosterol (cells of fungi and certain protozoa) than to membranes containing cholesterol (animal cells) [30, 31]. This is the first report on the effectiveness of AmB feeding on poison baits against susceptible strains of *Musca domestica* Linnaeus compared with wild strains. A study was carried out on the effectiveness of insect growth regulators on common flies in Iran [32]. Amphotericin B was confirmed to be an anti-*Nosema spp* protector of honeybees and is a life-saving antibiotic in human mycosis [20]. AmB is toxic to humans and forms concerns in the liver, kidneys, and brain [33]. In a study which was carried out by Chapman on fish hemoflagellate *Cryptobia salmositica* it was found that Amphotericin is effective in un-inhibiting the fish *C. salmositica*. He used 0.0625 µg Amphotericin B/mL. Amphotericin on the *C. salmositica* for 5 weeks when they were incubated in vitro [34]. In another study done by Jafari on the leishmanicidal effect of different extract of *Euphorbia bungei* Boiss aerial part, the positive effect of Amphotericin was established. It shows that Amphotericin B (0.5 mg mL⁻¹) killed all parasites [35]. A study of Myriocin significantly increased the mortality of a non-mammalian model host during *Candida* pathogenesis, Amphotericin B and Fluconazole were used in combination with Myrocin for suppressing *Candida albicans* a major human pathogen, and these resulted in insects pretreated with the drug followed by *C. albicans* inoculation, MYR and *C. albicans* significantly increased mortality to 93% from 67% with *C. albicans* alone 48 h post-infection whilst Amphotericin B with *C. albicans*, Fluconazole and *C. albicans* only showed 26% and 0% mortalities, respectively. Myrocin combinations with other

antifungal drugs in vivo also enhanced larval mortalities, contrasting the synergistic antifungal effect of the Myrocin with Amphotericin B combination in vitro [36].

In another study by Dunne shows that Administration of Amphotericin B after exposure to *A. fumigatus* or *A. terreus* significantly reduced mortality in the *G. mellonella* insect model [37]. Amphotericin B has para-sympathetic, cholinergic, heart electro cardiogram effect and also causes increase in blood pressure and peripheral blood system. The present study shows significant mortality among house flies, interruption of the infectious process and mechanical diseases. The result indicates significant difference between mortality of house flies post feeding with concentrations between day 1, 2, and other follow-up days ($P < 0.05$). It was found out that between the ranges of 8.7-13.6 of 2% poison baits house flies died 3 days post feeding with most mortalities occurring 5 days after feeding. The lowest mortality was seen between the first and second day after feeding with Amphotericin B at concentrations of 0.5%, 1%, 1.5%, and 2%. House flies died after days 3, 4, 5, 6, and 7 of follow-up at percentages of 9.88, 10, 12.2, 12, and 12.23 respectively. No side effect of Amphotericin B was observed, and none was reported from the laboratory subjects during the follow-up study. Amphotericin B significantly reduced the density of house flies. We recommended that in future, the effectiveness of Amphotericin B on wild strains of house flies and other nuisance insects such as cockroaches be investigated.

5. Acknowledgements

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