Effects of herbal and chemical detergents on *Dermatophagoides farina, Dermatophagoides pteronyssins* and *Blomia trobicalis*

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Abstract: House dust mites in clothing and bedding are the source of major allergen. Based on studies of *Dermatophagoides pteronyssinus* only, weekly washing in hot water is recommended to kill dust mites and remove allergen from clothing and bedding (DiAnn *et al.*, 2002). However, in the Saudi Arabia, washing is most often done in warm or cold water, and other mite species are involved. Most stages of *D. pteronyssinus*, *Blomia trobicalis* and *Dermatophagoide farinae* cannot survive when exposed to 45°C- 55°C for at least 48hrs. Survival of the house dust mite *D. farinae* and *D.pteronyssinus* at specific ambient conditions, was undertaken to determine the possibility of chemical and herbal detergent and temperature manipulation as a control method.Three studies were performed. The first compared combinations of 2 laundry agents and 2herbal agents (**Nabk** leaf, Bay Laurel solutions, detergents with enzymes, and detergent without enzymes) and water alone, constant temperature (50°C), same extraction time.The second study examined two types of laundry agent (waters, Chlorine bleach and sodium hypochlorite 45°C) at 25°C and 45°c for 4 hours. The third study compared 2detergents (water alone, soap with sodium hypochlorite, soap with chlorine bleach) in all experiments water are used as control. Each experiments has been applied several times in several different days. Washing by using regular chemical commercial detergents at ≥55°c is recommended to kill dust mite.However, this recommendation is made based on a study of *D.pteronyssinus, D.farinae* and *B. trobicalis* survival in hot, warm, or cold water with and without chemical commercial detergents and in the other hand using of **Nabk leaf and** Bay Laurel showed significant effect in dust mite mortality. If washing is to be a recommendation to kill dust mite, it is important to determine the survival of all species of dust mite in hot, warm, and cold wash water with and without chemical and herbal detergent.
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1. Introduction

Several important allergens that are commonly associated with asthma are found indoors. These include house dust mite, cat, cockroach and smoke of Incense. In addition, some allergens that originate outdoors, such as pollen and some fungal spores, can also accumulate indoors and provide high levels of exposure (Mitakakis *et al*., 2000, Tovey *et al*., 2001). The house dust mite *D. farina*, *D.pteronyssinus* are the major sources of indoor allergens and are therefore considered important health problems worldwide.In humid climates, most homes contain one or more species of these house dust mite. In Jeddah city most homes contain both *D.farinae*, *D. pteronyssinus* and some have *Blomia trobicalis*. House dust mites can be found in significant numbers living in textile garments, and therefore development of optimal washing conditions for delicate textile represents an important aim for domestic mite control (Bischoff *et al*.,1998).Laundry washing provides the most effective method of removing allergens from washable items such as bedding and clothing (Euan *et al*.,2001).

Nabk leaf (***Zizyphus spina* Christi)** and Bay Laurel (*Laurus nobilis*) solutions have been prepared in fixed concentration, and used for all species by dissolving one gram of **Nabk** dry leaf powder, one gram of Laurel Bay soap (made in Turkey) in 20ml of running water to make two different types of solutions. Study 1: showed that detergents with enzyme and Bay Laurel, mortalities more than either soap without enzyme, **Nabk** dry leaf or water alone and that. Study 2 Showed that running water, Chlorine bleach (commercial) and sodium hypochlorite (ore) performed variation in mite mortalities. Study 3 showed that the presence of detergents with chlorine bleach and soudium hypochlorite) in formulations produce a significant effect on the mortalities of mites. In light of the lack of data on laundry and herbal agents in the discrepancies in the literature about allergen extraction in Saudi Arabia, we have examined the extraction of the three major domestic dust mite associated with asthma, perennial rhinitis, and eczema in a simulated detergents materials.

The purpose of this study was to systematically investigate survival of

D.pteronyssinus, *D.farinae* and B. *trobicalis* when soaked in selected detergent (detergents with enzymes, and detergent without enzymes chlorine bleach, sodium hypochlorite, **Nabk** leaf, Bay Laurel and running water) in three different Experiment in cold, warm and hot water specified lengths of time to determine whether these additives would increase mortalities during washing. Herbal detergents showed effective influence in mites mortalities.

2. Material and Methods:

D.farinae, *B.trobicalis* and *D.pteronyssinus* used for experimentation were obtained from thriving pure laboratory culture at 75% RH and 25°C, as described by Arlian (1977). Culture medium consisted of a mixture of bakers' yeast and human hair (Edrees, 2006).

Effects of laundry detergents on mite Survival:

Male and female mites were selected randomly from thriving culture and confined in cages in groups of approximately 25 mites. The cages consisted of glass tubes (4mm IDx25mm) closed with 35µm nylon mesh as previously describe. These test were performed as previously described commercially available detergents and herbal detergents solutions were added to the glass of running water. All detergents were liquids.Three studies were performed. The first compared combinations of 5 laundry agents (running water, **Nabk** dry leaf, Bay Laurel solutions, detergents with enzymes, and detergent without enzymes) at temperatures (50°C), and one extraction time (120 minutes). The second compared of 3 laundry agents (running water, Chlorine bleach and sodium hypochlorite) at (30°C) and one extraction time (4 hours). The third compared of 3 laundry agents (running water, soap with chlorine bleach and soap with sodium hypochlorite) at one temperature (30°C) and one extractions time (2 hours). Each experiment included 12 duplicate each experiment was conducted seven different times in seven different days.

Mites male and female (Apart) were selected randomly from thriving culture and confined in cages in groups of approximately 25 mites. The cages consisted of glass tubes (4mm IDx25mm) the ends were plugged with a nylon mesh screen by inserting a Teflon washer. These test were performed as previously described Commercially available detergents were added to the glass of regular water, herbal solutions were added as described before. All detergents were liquids. In turn, these bottles were placed in a BOD (Biochemical Oxygen Demand) incubator to provide the desired temperature. Survival counts were made.

Statistical analysis:

The data in tables are presented as mean ±. The statistical analysis between the mites species and the different sex of each species were performed using paring " t – test " Armitage,1974. All statistical were computed by SPSS 14.

3. Result:

The result of the experiments with chemical and herbal detergents are in Table (1- 6)

Survival of *D.farinae* in various Temperatures between 30°C and 50°C comparing to control. Survival times were inversely related to the water temperature and the solution type (Diagrams 1-6).

Experiment 1: Survival of *D.farinae*, *D.pteronyssinus* and *B. trobicalis* in Nabk dry **leaf powder, Bay Laurel soap, detergents with enzymes, and detergent without enzymes**

Soaking in pure water and four different solutions and running water at (50°C) exhibited different rate of mortality within mites in two different sex of each species. Mortalities among all three species were significant (Table1:6). Generally, females of *D.pteronyssinus* seemed to be the most tolerant in all solutions, followed by *D.farinae* and *B.trobicalis* respectively.

Mortalities in *D.farinae*, *D.pteronyssinus*, and *B.trobicalis* were greater using detergents with enzymes containing the recommended concentrations of most of the laundry detergents followed by Bay Laurel, **Nabk** leaf and detergent without enzymes respectively (diagrams 1-6).

Experiment 2: Survial of *D.farinae*, *D.pteronyssinus*, *B.trobicalis* in waters, Chlorine bleach and sodium hypochlorite 30°C)

Mortalities among all three species after 4-hours soaks in (water, Chlorine bleach and sodium hypochlorite) at 30°C were significant. likewise, mortalities were low in the recommended concentration of Chlorine bleach comparing to the soda solution.In comparison, *D.farinae* and *B.trobicalis* exhibited high rate of mortalities under these same conditions comparing to *D.pteronyssinus* (Table 2).

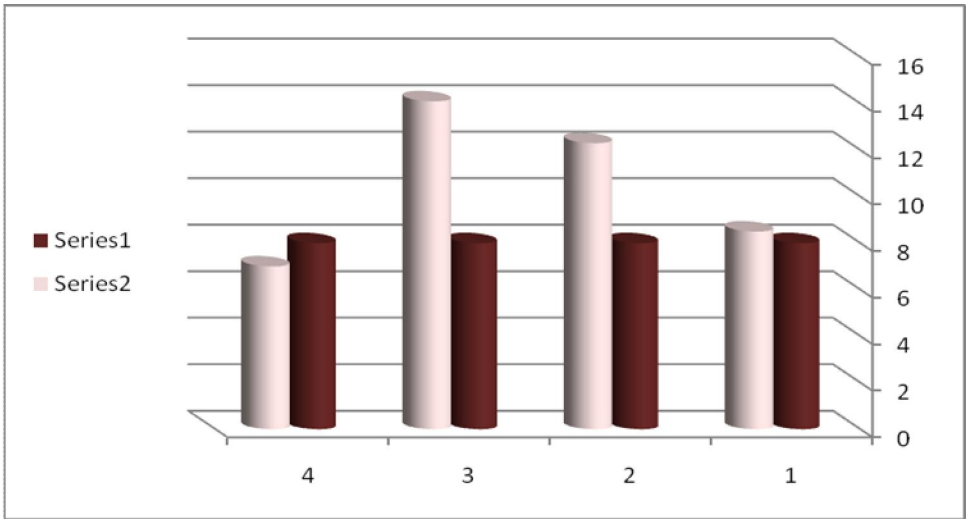
The third compared of 3 laundry agents (water, soap with chlorine bleach and soap with sodium hypochlorite) in 30°C

D.farinae and *D.pteronyssinus* were soaked in selected detergent solutions, soap with chlorine bleach and soap with sodium hypochlorite, and running water at 30°C. four-hours soaks of *D.farinae* and *D. pteronyssinus* in two detergents showed high mortality in soap with sodium hypochlorite, soap with chlorine bleach respectively whereas water alone caused no mortality. A 2-hours soak in a disinfecting solution of sodium hypochlorite caused

high mortality in *B.trobicalis*,*D.farinae* and *D.pteronyssimus* respectively (Table 3). Each experiment included 12 duplicate each experiment was conducted seven different times in seven different days.

Table 1: Mortality rate of *D. pteronyssinus* (female) after soaking for 120 minutes in various detergents (Running water, Bay Laurel, Nabk **leaf, detergents with enzymes, and detergent without enzymes)**

Detergents type	Mean	N	Std. Deviation	Std. Error Mean	Sig.
con2	0.8000	12	1.13529	0.35901	0.000
Nabk leaf powder	8.5000	12	7.01189	2.21736	0.000
Bay Laurel soap	12.3000	12	6.66750	2.10845	0.000
detergents with enzymes	14.1000	12	7.27935	2.30193	0.000
detergent without enzymes	7.0000	12	4.94413	1.56347	0.000



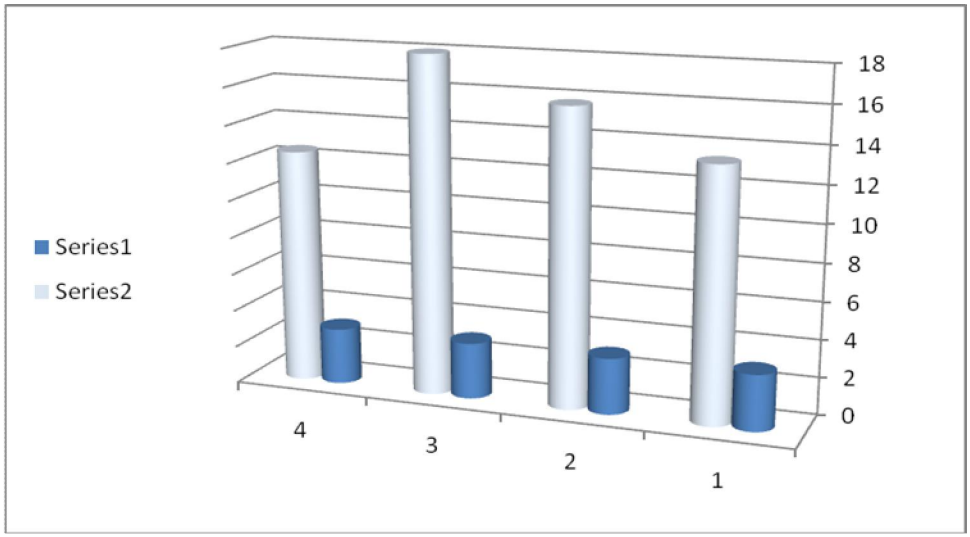
Digram1: Mortality rate of *D.pteronyssinus* (female) after soaking for 120 minutes minutes in various detergents (Running water, Bay Laurel, Nabk leaf, detergents with enzymes, and detergent without enzymes)

Table 2: Mortality rate of *D.pteronyssinus* (male) after soaking for 120 minutes minutes in various detergents (Running water, Bay Laurel, Nabk **leaf, detergents with enzymes, and detergent without enzymes)**

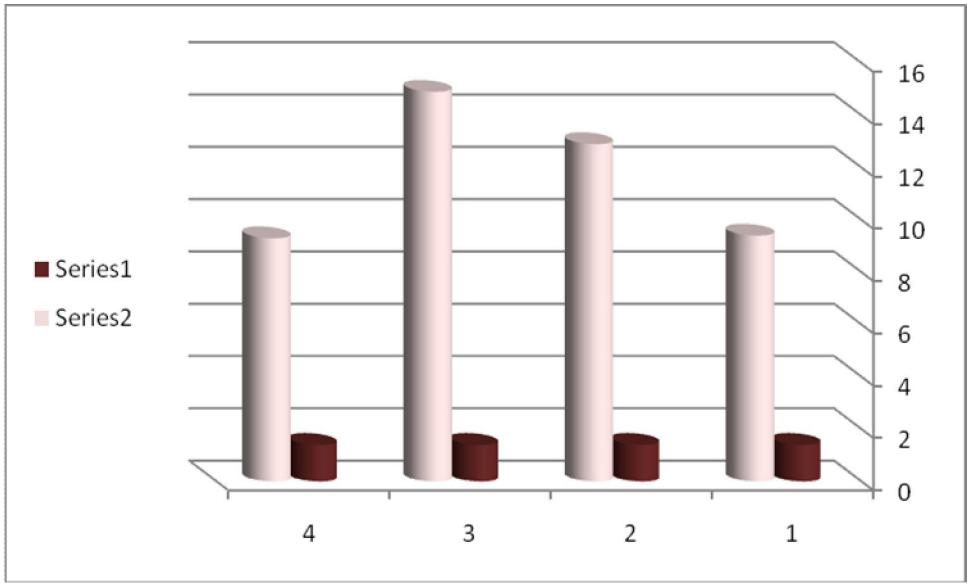
Detergents type	Mean	N	Std. Deviation	Std. Error Mean	Sig.
cont	3.0000	12	1.90693	0.55048	0.002
Nabk leaf powder	13.4167	12	6.15642	1.77721	0.002
Bay Laurel soap	15.0833	12	6.31676	1.82349	0.002
detergents with enzymes	18.0000	12	5.84652	1.68775	0.002
detergent without enzymes	12.5833	12	5.85364	1.68980	0.002

Table 3: Mortality rate of *D. farinae* (female) after soaking for 120 minutes minutes in various detergents (Running water, Bay Laurel, Nabk leaf, detergents with enzymes, and detergent without enzymes)

Detergents type	Mean	N	Std. Deviation	Std. Error Mean	Sig.
con4	1.4000	12	2.06559	0.65320	0.000
Nabk leaf powder	9.4000	12	5.23238	1.65462	0.000
Bay Laurel soap	12.9000	12	4.74810	1.50148	0.000
detergents with enzymes	14.9000	12	6.04520	1.91166	0.000
detergent without enzymes	9.3000	12	4.85455	1.53514	0.000



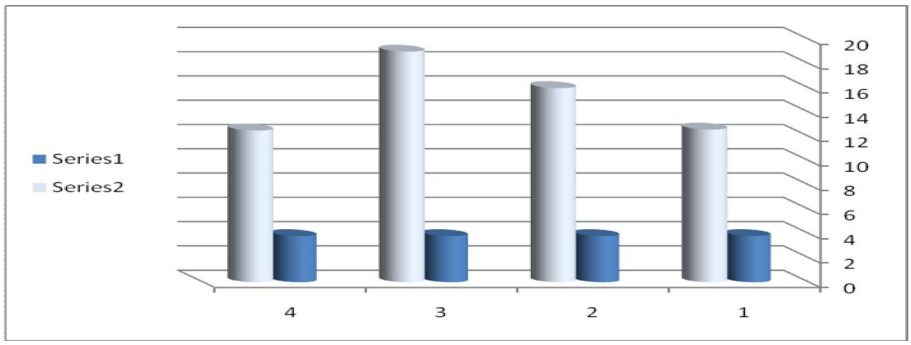
Digram 2: Mortality rate of *D.pteronyssinus* (male) after soaking for 120 minutes minutes in various detergents (Running water, Bay Laurel, **Nabk** leaf, detergents with enzymes, and detergent without enzymes)



Digram3: Mortality rate of *D.farinae* (female) after soaking for 120 minutes in various detergents (Running water, Bay Laurel, **Nabk** leaf, detergents with enzymes, and detergent without enzymes)

Tables 4: Mortality rate of *D. farinae* (male) after soaking for 120 minutes in various detergents (Running water, Bay Laurel, Nabk leaf, detergents with enzymes, and detergent witout enzymes)

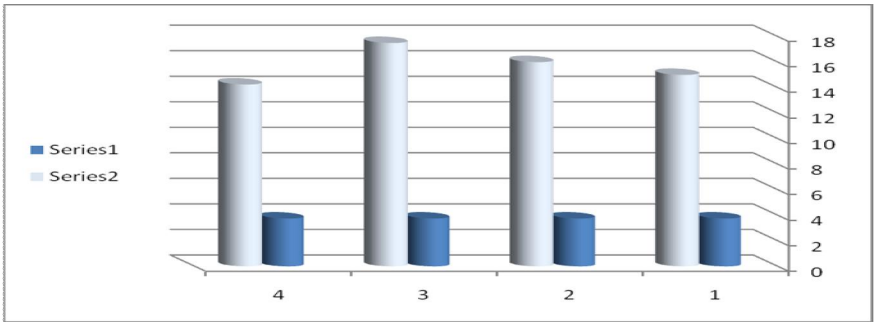
Detergents type	Mean	N	Std. Deviation	Std. Error Mean	Sig.
con3	**3.8333**	**12**	**4.26046**	**1.22989**	**0.000**
Nabk leaf powder	**12.5833**	**12**	**6.09707**	**1.76007**	**0.000**
Bay Laurel soap	**16.0000**	**12**	**5.16984**	**1.49241**	**0.000**
detergents with enzymes	**19.0000**	**12**	**4.67099**	**1.34840**	**0.000**
detergent without enzymes	**12.5000**	**12**	**3.65563**	**1.05529**	**0.000**



Digram 4: Mortality rate of *D.farinae* (male) after soaking for 120 minutes in various detergents (Running water, Bay Laurel, Nabk **leaf, detergents with enzymes, and detergent without enzymes)**

Table 5: Mortality rate of *B.trobicalis* (male) after soaking for 60 minutes in various detergents (Running water, Bay Laurel, Nabk **leaf, detergents with enzymes, and detergent without enzymes)**

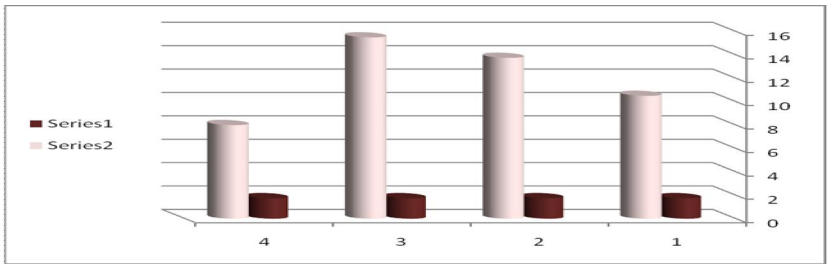
Detergents type	Mean	N	Std. Deviation	Std. Error Mean	Sig.
con5	3.7500	12	4.33013	1.25000	0.000
Nabk leaf powder	15.0000	12	5.16984	1.49241	0.000
Bay Laurel soap	16.0000	12	6.09023	1.75810	0.000
detergents with enzymes	17.5000	12	5.83874	1.68550	0.000
detergent without enzymes	14.2500	12	4.80766	1.38785	0.000



Digram 5: Mortality rate of *B.trobicalis* (male) after soaking for 60 minutes in various detergents (Running water, Bay Laurel, Nabk **leaf, detergents with enzymes, and detergent without enzymes)**

Table 6: Mortality rate of *B.trobicalis* (female) after soaking for 60 minutes in various detergents (Running water, Bay Laurel, Nabk **leaf, detergents with enzymes, and detergent without enzymes)**

Detergents type	Mean	N	Std. Deviation	Std. Error Mean	Sig.
CON6	1.7500	12	2.13733	0.61699	0.000
Nabk leaf powder	10.5000	12	6.86890	1.98288	0.000
Bay Laurel soap	13.7500	12	6.90356	1.99289	0.000
detergents with enzymes	15.5000	12	7.25509	2.09436	0.000
detergent without enzymes	8.0000	12	5.16984	1.49241	0.000



Digram 6: Mortality rate of *B.trobicalis* (female) after soaking for 60 minutes in various detergents (Running water, Bay Laurel, Nabk **leaf, detergents with enzymes, and detergent without enzymes)**

Table 7: Mortality rate of *D.pteronyssinus, D.farinae and B.trobicalis* (male & female) after soaking for 4houres in various detergents (Running water, Chlorine bleach and sodium hypochlorite)

Second experiment							
Detergents type	**specimens**	**Mean**	**N**	**Std. Deviation**	**Std. Error Mean**	**Correlation**	**Sig.**
	con	**0.0000**	**8**	**0.00000**	**0.00000**		
Chlorine bleach	**D.F MALE**	**5.8750**	**8**	**2.90012**	**1.02535**	**0.0**	**0.0**
sodium hypochlorite	**D.F MALE**	**7.1250**	**8**	**3.31393**	**1.17165**		
	con	**0.0000**	**8**	**0.00000**	**0.00000**		
Chlorine bleach	**D.f FEMALE**	**3.4444**	**8**	**2.18581**	**0.72860**	**0.0**	**0.0**
sodium hypochlorite	**D.f FEMALE**	**5.2222**	**8**	**2.86259**			
	con	**0.0000**	**8**	**0.00000**	**0.00000**	**0.0**	**0.0**
Chlorine bleach	**D.P male**	**4.4444**	**8**	**2.78887**	**0.92962**		
sodium hypochlorite	**D.P male**	**6.0000**	**8**	**2.95804**	**.098601**		
	con	**0.0000**	**8**	**0.00000**	**0.00000**	**0.0**	**0.0**
Chlorine bleach	**D.P female**	**5.0000**	**8**	**3.04138**	**1.01379**		
sodium hypochlorite	**D.P female**	**6.0000**	**8**	**3.24037**	**1.08012**		
	con	**0.0000**	**8**	**0.00000**	**.00000**		
Chlorine bleach	**B.t Male**	**9.2222**	**8**	**2.72845**	**90948**	**0.0**	**0.0**
sodium hypochlorite	**B.t male**	**11.2222**	**8**	**3.23179**	**1.07726**		
	con	**0.0000**	**8**	**0.00000**	**0.00000**	**0.0**	**0.0**
Chlorine bleach	**B.t Female**	**7.7778**	**8**	**2.77389**	**0.92463**		
sodium hypochlorite	**B.t Female**	**9.6667**	**8**	**3.42783**	**1.14261**		

Table 8: Mortality rate of *D. pteronyssinus, D.farinae and B.trobicalis* (male & female) after soaking for 4houres in various detergents (Running water, soap with chlorine bleach and soap with soudium hypochlorite)

Detergents type	**specimens**	Mean	N	Std. Deviation	Std. Error Mean	Correlation	Sig.
con	**con**	.0000	8	.00000	.00000		
Chlorine bleach	**D.F MALE**	16.0000	8	3.46410	1.15470	0.0	0.0
sodium hypochlorite		18.0000	8	3.08221	1.02740	0.0	0.0
con	**con**	.0000	8	.00000	.00000	0.0	0.0
Chlorine bleach	**D.f FEMALE**	14.6667	8	4.44410	1.48137	0.0	0.0
sodium hypochlorite		17.0000	8	4.33013	1.44338	0.0	0.0
con	**con**	.0000	8	.00000	.00000	0.0	0.0
Chlorine bleach	**D.P male**	14.7778	8	3.38296	1.12765	0.0	0.0
sodium hypochlorite		16.6667	8	3.50000	1.16667	0.0	0.0
con	**con**	.0000	8	.00000	.00000	0.0	0.0
Chlorine bleach	**D.P female**	12.0000	8	5.09902	1.69967	0.0	0.0
sodium hypochlorite		15.7778	8	4.23609	1.41203	0.0	0.0
con	**con**	.0000	8	.00000	.00000	0.0	0.0
Chlorine bleach	**B.t male**	19.5556	8	2.65100	.88367	0.0	0.0
sodium hypochlorite		21.2222	8	2.90593	.96864	0.0	0.0
con	**con**	.0000	8	.00000	.00000	0.0	0.0
Chlorine bleach	**B.t Female**	18.1111	8	2.75882	.91961	0.0	0.0
sodium hypochlorite		18.6667	8	3.08221	1.02740	0.0	0.0

4. Discussion:

There is limited information about the removal of dust mites by laundry washing, In contrast to no studies done on detergents derived from herbs to control domestic mites in Saudi Arabia nor the world.The three species of the mite family Pyroglyphidae that occur most commonly in house dust *D.pteronyssinus, D.farinae* and *B.trobicalis* have been to produce similar allergens, whereas species of other families occasionally found in dust are sources of different allergens (Spieksma *et al*., 1971). different washing-detergents were used in these experiments. There are differences in the ingredients used in the different formulations, Bay Laurel, **Nabk** leaf, detergents are natural detergent, there are possible that these may influence the mite killing effect or the ability of the washing detergent to remove mites.

In this study we found that the detergents death effect of *B.trobicalis* was lower than those for

D.farinae and *D.pteronyssinus*, soak in chemical and herbal detergents, where killed at 50°C in different quantity depend on the mites species. This suggests that with a 3-hours soak, the best mites killer is Laurel, **Nabk** followed by Bay Laurel soap, detergents with enzymes, detergent without enzymes and, **Nabk** leaf respectively, and therefore could effectively eliminate this species with worm -water washing at 50C°. In contrast, only low mortalities were achieved for *D.pteronyssinus*, *D.farinae* and *B.trobicalis* soaked for 4 hours at 30°C. A lower water temperature of 30°C for 2 hours was necessary to get moderate mortalities of *B.trobicalis*, D.farinae and *D.pteronyssinus* respectively, in the third experiments.

Normal washing at any temperature usually includes a detergent or laundry additive. Our results showed that use of the Bay Laurel soap and detergent without enzymes in hot water (50°C) greatly increased mortalities of *B.trobicalis, D.farinae and D.pteronyssins* respectively compare with detergent without enzymes and**, Nabk** leaf respectively and respectively water alone at comparable wash water temperatures. Bay Laurel soap increased the mortality of *D. pteronyssins* compared with the mortality observed using the recommended concentrations. Particularly after exposures of certain times (Tables 1-6). In contrast mite in Chlorine bleach and sodium hypochlorite was less much affected by detergent and mortalities (Table 7). Mortalities for all species in herbal and chemical detergent or water alone (hot water) were significant after 120minute. However, 4-hours soaks in Chlorine bleach and sodium hypochlorite detergents results in moderate mortality(Table7). however soaks in soap with chlorine bleach and soap with sodium hypochlorite results in moderate mortality, and showed a more effective impact compared with the second experiment detergents (Table8).Therefore, long soaks in warm water containing Bay Laurel soap and detergents with enzymes have high benefit. If the washing of bed linens with long presoaks of bed linens with long presoaks were done weekly, one would expect that the live mite population would decline exponentially over time.This goes with DiAnn *et al*., 2002 study which mentioned that the chlorine bleach or sodium hypochloride can inactivate common allergens It evaluated to what extent regular house cleaning with bleach can influence the risks of respiratory and allergic diseases in children.

Two previous studies conducted by Anderson and Roesen, 1989, in Denmark and McDonald and Tovey, 1992, in Australia examined the survival of *D.pteronyssinus* in water and detergent solutions. Anderson and Roseen, 1989, report 87.2 to 99.7% mortality for *D. pteronyssins* at 10 to 50°C regardless of whether or not soap was used. Thus, they attributed most of the mortality to drowning. McDonald and Tovey 1992, using detergents available in Australia, found that after 10 minutes in water at 55°C and 50°c,100 and 47% mortalities, respectively,were induced for *D. pteronyssinus*, which is consistent with results of our study. The different detergent in the 50°C-soak increase the proporation of mites killed compared with the running water control. In fact mortality was higher in the detergent(herbal and detergent with enzymes solutions) compared with other detergents combinations suggested these products had a protective effect near the critical temperature. In contrast to the results of these previous studies, we found that detergents in hot water (50°C) greatly increased mortalities of *B.trobicalis*, *D.farinae* and *D.pteronyssins* respectively. Bay Laurel soap resulted in greater mortality for *B.trobicalis*, *D.farinae* and *D.pteronyssinus* compared with water alone. Bay Laurel soap increased the mortality of *D.pteronyssinus*. we attribute the different results to the fact we were using detergents sold and used in Saudi Arabia market and used in its people culture.

In contrast soaking mites in chlorine bleach in water at 30°C had a slight effect. After 4-hours soaks in a recommended concentration of chlorine bleach and also in bleach plus selected mortalities were observed in warm water. However, *D.pteronyssinus* have tulorent to affected by the chlorine bleach. In the other hand, long soaks in warm water containing soap with chlorine bleach and soap with soudium hypochlorite is much effective in killing *B.trobicalis*, *D. farina*, but slightly effective in killing *D.pteronyssinus*.

Our experiments determined the mortalities of *D.pteronyssinus*, *D.farinae* and *B.trobicalis* under controlled laboratory conditions. However, our results depending on other studies should be good representations of what would happen in a washing machine. Even though air bubbles may be trapped in clothing and bedding while washing, the live mites adhere to the surface of fabrics and would be submerged in a film of detergent solution.(DiAnn *et al*., 2002). The air temperature in bubbles likely equilibrates with temperature of the wash water. Studies by Chang *et al*, coupled with our study, show that the thermal death temperature for mites in air and water are the same. Additional studies are needed to determine whether water hardness influences mite survival. three common house dust mites found in houses were removed quickly, with great efficiency, and at moderate temperatures through normal domestic laundering by all of the detergents tested. Launry is a simple, inexpensive, and extremely

effective method of massively reducing allergen reservoirs (Tovey *et al*.,2001).

Recommendations: It is to do more studies on the Bay Laurel soap and **Nabk** leaf and used as an alternative natural detergents, to reduce the chemical soap damages in causing allergic reactions, and maintaining of tissues fibers (textile garments).

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