

In Vitro Investigation of *Allium Sativum* as Anti-Head Lice in Jeddah, Saudi Arabia



Biological Science

KEYWORDS : Head lice, Garlic juice, Ovicidal agents, Repellent agent.

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ABSTRACT

Pediculus humanus capitis are considered as the most worldwide feeding blood parasites parasitizing mainly in children. Control of head lice encourages researcher to discover new and safe anti-pediculosis capitis. Head lice are estimated to be abundant in Jeddah city mainly between school girl students, this study was conducted to evaluate effect of garlic juice as natural pediculicide. The approval of its activity was through three consequent stages conducting in vitro tests. Head lice were checked for activity in intervals 30, 60, 90 and 180 minutes in preliminary test. Garlic juice approved high pediculicidal activity from 30-180 minutes. LC50 and LC90 of garlic juice were estimated in secondary screening as 0.62 ml and 1.02 ml after 30 minutes of exposure. The best concentration of garlic juice was tested as ovicidal and repellent agents and its effect regardless the head lice's starving was tested.

INTRODUCTION

One of the blood feeding ectoparasites on mammals is *Pediculus humanus capitis* (Durden, 2001), its treatment options are included wet combing, topical agents and oral therapy (Elston, 1999). Using an effective pediculicide is considered as the most procedure that followed by removing nit manually (Burgess, 2006). Pediculicidal activity of *Dichrostachys cinerea* was evaluated (Vijayalakshmi *et al.*, 2010). Approximately 86% of head lice were killed by using alcoholic extract of *Myristica fragrans* (Surendra *et al.*, 2013). Citrus limon juice was proved in killing human adult lice in 3 hours (Shrivastava *et al.*, 2010). Activity of synthesized silver nanoparticles using leaf aqueous extract of *Tinospora cordifolia* gave LC_{50-12.46} mg/L (Jayaseelan *et al.*, 2011).

All extracts of *Pongamia pinnata* leaves were resulted as ovicidal agents (Samuel *et al.*, 2009). Licatack have an effect as repellent agent for only 7 hours (Semmler *et al.*, 2010). Green tea was proved as pediculocidal and larvicidal substance (Sherwani *et al.*, 2013). The repellent and fumigant properties *Myrcianthes cissplatensis* oil was the most effective oil (Toloza *et al.*, 2006).

Wood tar solution showed good activity after 30 minutes, LC₅₀ and LC90 were 47% and 62% and the best concentrations gave encouraging results as ovicidal and repellent agents (Al-Zanbagi and Al-Hashdi, 2015).

Garlic has scolicidal activity in hydatid disease (Moazeni and Nazer, 2010), also it has great effects on *Schistosoma mansoni* harbored in albino mice and in in vitro test (Riad *et al.*, 2013, Mantawy *et al.*, 2012), while the lowest concentration of garlic extract failed to inhibit *Trichomonas vaginalis* parasite multiplication (Ahmed, 2010).

MATERIALS AND METHODS

Head lice were collected from untreated girls attending elementary schools in Jeddah city using white paper then were put in plastic boxes to transfer immediately to the laboratory.

Fresh Garlic clove were peeled and squeezed to be used as concentrated juice and gradient dilutions. Lcid shampoo was used as positive control.

The experiments protocol was conducted in sequences screening at room temperature of 29±0.5 °C and humidity of 70 ± 1%.

In vitro tests of primary screening following filter paper diffusion bioassay (Shrivastava *et al.*, 2010), 10 head lice were placed in filter paper and 1 ml of garlic juice was spread, triplicate tests were done. Head lice were observed for 30, 60, 90 and 180 minutes, lice were considered dead if no activity symptoms were vis-

ible. Positive control was spread by Lcid while negative control was covered by distilled water.

In secondary screening, garlic juice gradient dilutions (0.2 ml-1ml) were tested following same design in primary screening. In recovery period, 1 ml of distilled water was added and monitoring for another one hour. Dead head lice were counted and time concentration relationship was documented.

In tertiary screening, known age nits were examined under microscope and the best concentration of garlic juice and Lcid were tested using filter papers assay, 1 ml of different materials was added one time on nits. Petri dishes were incubated for 14 days at 29±0.5 °C with humidity 70±1%. Nits were checked on days 6th and 14th. Embryo mortality criteria with closed operculum or nits with embryo and opened operculum, emerged nymphs were counted.

In repellent activity, 3 samples of artificial hair tufts were cut (Semmler *et al.*, 2010). Two were exposed to the best concentration of garlic juice and Lcid, third tuft was control. 10 adult head lice were placed aside hair tufts and its behavior was noticed visually for 3 hours.

To determine garlic effectiveness regardless starving of head lice that were kept after collecting in plastic container and let them starved for 7 hours, then were placed on researcher leg for 10 minutes to have their human blood meal. Head lice were collected and conducted directly in experiment as that in preliminary screening.

Median effective concentrations were given (Leitchfield and Wilcoxon, 1949). Activity of tested materials was analyzed using ANOVA tests.

RESULTS

Head lice mortality was evaluated after exposure for 3 hours to garlic juice in triplicate examinations (Table 1, Fig. 1), garlic and Lcid results were differed significantly as from distilled water ($P= 0.00$). There is no significant difference between garlic and Lcid ($P= 0.074$).

Table 1: Effect of Garlic juice and Lcid against adult head lice after exposing to the tested materials for 3 hours

Garlic juice	head lice/exp	Exp. 1		Exp. 2		Exp. 3		Mean	
		Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
30 min.	10	8	2	7	3	7	3	7.3	2.6

60 min.	10	9	1	9	1	9	1	9	1
90 min.	10	10	0	10	0	10	0	10	0
3 hours	10	10	0	10	0	10	0	10	0
Licid At all time period	10	10	0	10	0	10	0	10	0
Dis. water At all time period	10	0	10	0	10	0	10	0	10

Garlic juice caused death of 8 head lice in 1 ml after 30 minutes – 3 hours (Table 2). The LC50s and LC90s responsible for killing 50% and 90% of head lice were almost decreasing by increasing in exposure times (Fig. 2).

Table 2: Gradient concentrations in 1 ml from Garlic juice against adult head lice after 3 hours as exposure period

Tested materials	Conc. (1 ml)	Total head lice	30 min.		60 min.		90 min.		3 hours	
			Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
Garlic juice	1	10	8	2	8	2	8	2	8	2
	0.9	10	8	2	7	3	8	2	8	2
	0.8	10	5	5	6	4	7	3	7	3
	0.7	10	6	4	6	4	7	3	7	3
	0.6	10	6	4	6	4	6	4	7	3
	0.5	10	3	7	7	3	8	2	8	2
	0.4	10	4	6	5	5	5	5	5	5
	0.3	10	1	9	4	6	5	5	5	5
	0.2	10	0	10	2	8	2	8	2	8
	Dis. water	0	10	0	10	0	10	0	10	0

Using garlic caused average of hatching eggs as 0.33, non-hatching eggs as 9.66 on 6th day and 14th day of inspection (Table 3, Fig. 3), which unlike distilled water control. There was no significant difference between garlic and Licid (P= 0.282) after 14th day.

Table 3: Effect of Garlic juice and Licid against head lice nits after exposure period of 14 days

Materials Used	Dead nits on 6 th day						Mean of dead nits		
	Exp. 1		Exp. 2		Exp. 3				
	hatch	no hatch	hatch	no hatch	hatch	no hatch	hatch	no hatch	
Garlic juice	0	10	0	10	1	9	0.33	9.66	
Licid	0	10	1	9	2	8	1	9	
Dis. water	1	9	5	5	8	2	4.66	5.33	
Materials Used	Dead nits on 14 th day						Mean of dead nits		
	Garlic juice	0	10	0	10	1			9
	Licid	0	10	1	9	2	8	1	9
	Dis. water	10	0	9	1	10	0	9.66	0.33

Head lice positioned in close to treated hair tufts gave different behavior (Table 4, Figure 4).

4: Efficacy of Garlic juice as repellent materials for head lice after 3 hours as exposure period

Tested Materials	head Lice used	After 1 hour			After 2 hours			After 3 hours		
		+	+/-	-	+	+/-	-	+	+/-	-
Garlic juice	10	7	2	1	9	0	1	8	2	0
Untreated hair	10	0	0	10	0	0	10	0	0	10

Results demonstrated in (Table 5) showed that the best concentration of garlic juice caused pediculicide efficacy when tested in triplicate against head lice take blood meal from human after starving for 7 hours, mortality rate was evaluated in 3 hours and after 60 to 180 minutes, no movement was observed. In distilled water, head lice movement was observed even after 180 minutes.

The researcher was suffered from head lice feeding on her blood that little skin irritation was happened after 2-6 hours from feeding, after 24-48 hours of exposing to head lice saliva, skin irritation in form of papular rash and moderate to severe itching was recorded (Figure 5).

Table 5: Effect of the best concentration of Garlic juice against the starved adult head lice for 3 hours

Garlic juice	head lice used/exp.	Exp. 1		Exp. 2		Exp. 3		Mean	
		Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
30 minutes	10	8	2	7	3	8	2	7.6	2.3
60 minutes	10	10	0	10	0	10	0	10	0
90 minutes	10	10	0	10	0	10	0	10	0
3 hours	10	10	0	10	0	10	0	10	0
Dis. water at all time period	10	0	10	0	10	0	10	0	10

DISCUSSION

Predictions for new and ecologically tolerable pediculicide are still in progress using filter paper tests in Petri dishes for bioassay technique which affords informative and comparable results.

Garlic juice killed head lice 100% in 90 minutes which was similar with wood tar solution (Al-Zanbagi and Al-Hashdi, 2015). Also it was better than other results recorded by (Carpinella et al., 2007) and (Surendra et al., 2013). Samuel et al. (2009) and Sherwani et al. (2013) stated that 100% head lice killed in 18 hours by using petroleum ether extract of *Pongamia pinnata* and green tea respectively, both was lower than garlic result in 90 minutes.

Jayaseelan et al. (2011) estimated the LC50 of synthesized silver nanoparticles as 12.46 mg/L, while garlic results was 0.5-0.31 ml after exposure times of 30-180 minutes as well as garlic concentrations was in ml while the synthesized silver used nanoparticles, although LC50 and LC90 of garlic resembled that recorded for wood tar (Al-Zanbagi and Al-Hashdi, 2015).

Garlic juice as ovicidal agent was better than tea tree result (Campi et al., 2012) *Melaleuca alternifolia* oil induced 50% eggs failure to hatch at 1% concentration after 4 days. Also it better than green tea crude extracts that infusion caused non hatching ratio as 90.4% (Sherwani et al., 2013). Two trials were better in killing 100% head lice nymph by using spinosad and petroleum ether extract of *Pongamia pinnata* leaves (Cueto et al. [28] and Samuel et al. [11]). Finally, garlic ovicidal result was better than wood tar (Al-Zanbagi and Al-Hashdi, 2015).

Garlic juice efficacy as repellent material was better than *Mentha piperita* oil (Toloza et al., 2006), *Eucalyptus* and Lavender activity (Cueto et al., 2006), tea tree oil, peppermint, DEET and neem products (Canyon and Speare, 2007) and wood tar solu-

tion (Al-Zanbagi and Al-Hashdi, 2015). Combination of *Vitex agnuscastus* seeds extract and paramenthan-3,8-diol gave 100% repellent activity (Semmler *et al.*, 2010) was better than garlic repellency result.

Result of head lice starving effect showed good pediculicide at 30-180 minutes, this finding was superior on reporting this point so there was no previous result to discuss.

In conclusion, Garlic juice proved as strong natural pediculicide as well as ovicidal and repellency agent in low concentrations at short exposure periods. Garlic has specific properties that it is cheap, easy obtainable and medicinally substance with no toxicity.

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Figure 1: Effect of Garlic juice and Lcid against adult head lice after exposing to the tested materials for 3 hours

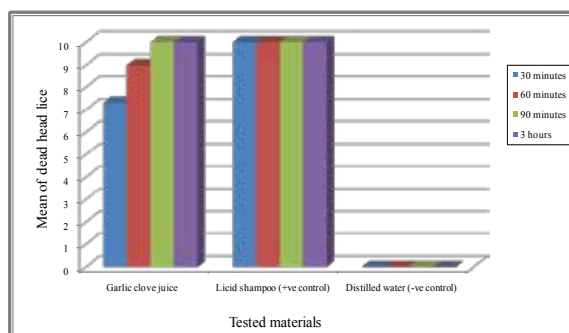


Figure 2
LC₅₀ and LC₉₀ of garlic juice with distilled water (equivalent to 1 ml) for head lice after exposure time of three hours

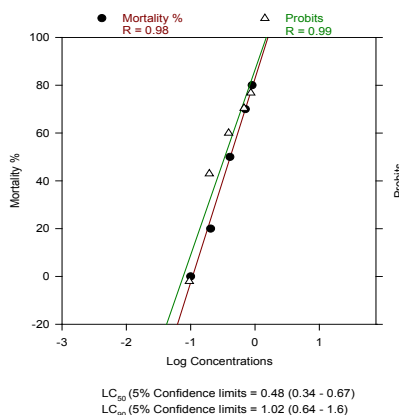


Figure 3: Effect of Garlic juice and Lcid against head lice nits after exposure period of 14 days

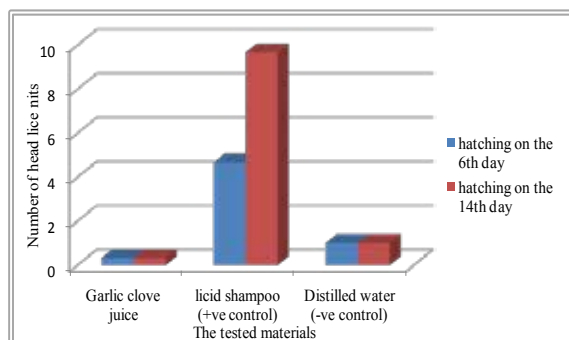


Figure 4: Efficacy of Garlic juice as repellent material for head lice after 3 hours as exposure period

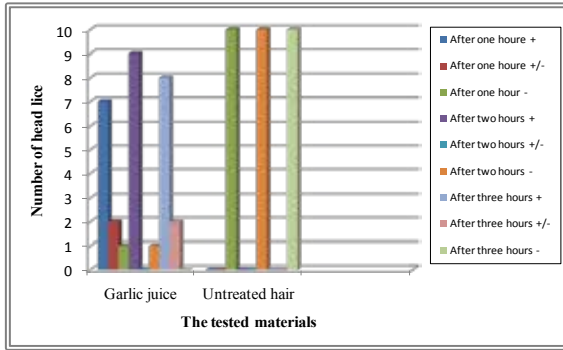


Figure 5: (A) Human skin irritation occurred directly after head lice feeding - (B) Sever itching after 24-hours from blood feeding

