

Evaluating the efficacy of a new lice treatment shampoo containing Quassia Amara using physical and tolerance approaches

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Abstract

Many methods for evaluating head lice treatment products through field reports and chemical methods have been reported, and these results are incomparable due to several physical limiting factors, including hair type, application protocols and traditional hygiene practices. The present study investigated physical factors related to the efficacy of a new louse treatment with 5% Quassia Amara using a factorial model. The model terms included hair type (≤ 5 or > 5 cm), age (6-18 years), stroke caused by shampoo (2-6) or comb (1-4), and duration of exposure (10-20 min) according to the factorial model. A Lice stress resistance assessment technique was used to comparatively assess the effectiveness of the new product named Go Away Lice and two commercial louse treatment products. The results of the factorial model showed that the new shampoo product containing Quassia a. had a significant effect on the number of dead lice and nits at different ages and on different hair types ($P < 0.05$). Dead lice and nit were significantly associated with the interaction influence of age and hair size ($P < 0.05$). The stress tolerance limits of the two commercial head lice brands and the local brand were comparable to that of the most effective commercial brand in terms of killing capacity ($P > 0.05$). It was concluded that a reduction in the lice population is significantly influenced by age and hair size. The Goaway Lice Shampoo brand demonstrated potential as a cosmetic for head lice treatment.

1. Introduction

Head lice (*Pediculus humanus capitis*) infestations in children and teenagers are common problems in many societies. These lice only parasitize humans and nourish themselves by sucking blood. The louse eggs (nits) are long and oval (0.3–0.8 mm) and reddish brown or gray in color (Hansen, 2004). Several reports have noted that on one day, these lice can lay up to 10 eggs, which totals 300 eggs in a period of 30–40 days (Mumcuoglu et al., 1999 and McCage et al., 2002). Larvae normally hatch after 7–10 days, and nymphs (immature lice) die within 24 hours if no human blood is available. Sexually mature louse can survive without blood for 8–10 days. New generations of lice are born in 18–24 days (Sokoloff 1994).

The Food and Drugs Association (FDA) has approved these agents for head lice infection, such as lindane, malathion, and permethrin crème. The increase in resistance of head lice has led to the consideration of other treatments, such as trimethoprim, sulfamethoxazole, malathion, ivermectin and carbamyl (Hypolito et al., 2001). It is evident that the reference drug is permethrin, which is known to have the greatest efficacy, although the irrigative effect is 2.1–5.9% (Andrews et al., 1992). Today, the main chemical agents used are those reported in various studies (Sokoloff 1994, Lindsay and Peek 1993, Courtiade et al., 1993). In developed countries such as the U.S., Lindane is designated a second-line treatment if other first-line treatments fail (Heo et al., 2000); however, in Europe, lindane-containing medications are banned from the market (Mary et al., 2021). In a developing country such as Vanuatu, such medication is expensive, and people are afraid of exposing themselves to products containing chemical agents, although no basic data are available on the use of louse treatment products. Previous research has shown that the forehead scalp is highly permeable to scabies when compared to the

amount of insecticide applied. Thus, some research has shortened the duration of treatment to prevent toxic risk through skin absorption (Moody and Ritter 1989).

Although chemical treatment agents are considered safe for occasional use, they can also pose a risk of cumulative toxicity. The failure of chemical agents to kill embryos in eggs, environmental and food safety, and chemical toxicity of these agents are some of the reasons to investigate alternative products. There is a need to understand the conditions under which alternative chemical free compounds could be actively used as lice and egg-killing agents.

Various studies have tested the efficacy of essential oils combined with vinegar and water and have shown improved results by weakening the hold and enabling removal (Veal 1996). Another method involves so-called dry suffocation-based-pediculicide (Pearlman et al., 2004). It was reported that dimethicone killed head lice in 5 mins through low viscosity silicone penetration into the spiracle, causing asphyxia and death (Oliveira et al 2007). Several new studies of this compound have reported this mode of action (Scanni and Bonifazi, 2005; Heukelbach et al., 2008 & 2009). Head lice worldwide have been reported to develop resistance to insecticides, prompting the need to find alternative treatments, including plant extracts (Burgess 2022).

The new Shampoo product (Goaway Lice) of Volcanic Earth under study was developed to treat the hair and scalp afflicted by nits and head lice of children older than 5 years and adults. It does not contain insecticides but rather contains an active ingredient from plant extracts. The product is entirely natural (activate extract from plants with no silicone). It combines two active principles that affect both lice and eggs at the same time. The lice are asphyxiated by coconut oil so that they are dead or become weak and easy to collect, comb away or wash away, and the Quassia Amera (5% inclusion) plus Apple Cider Vinegar are used to dissolve the chitin in the nits so that they are killed. The other components that demonstrate antilice efficacy include essential oils, peppermint, lavender and rosemary.

The objectives of this study were to assess the efficacy of this plant extract shampoo product based on the duration of exposure, hair length, age, combination of stroke and shampoo stroke, as well as its optimal effects on lice and nits, as well as its adverse effects on patients, and to compare its efficacy with that of commercial brands of the lice treatment shampoo.

2. Materials and Methods

2.1 Quassiava Amera product and ethics

The active ingredient of the lice treatment was mainly Quassia americana leaves, which were included at a level of 5%. The shampoo was produced by Volcanic Earth Natural Skin Care Ltd., under the brand name “Go away lice,” and exported to different countries. This study was approved by the Ethics Committee of the Ministry of Health, Government of Vanuatu.

2.2 Selection criteria for participants

The study was conducted by the Vanuatu Bureau of Standards on a new head louse treatment product, the “Go away lice shampoo.” Approximately 72 participants were recruited from 2 schools based on a factorial design following strict criteria. The selected schools were the Pango English School in Pango and the Saint Joseph School at Numbatu Lagoon in Port Vila. The participants included in the model development were aged between 6 and 16 years. Age was used as a factor and divided into two categories: 6–12 years as cat1 and 13–16 years as cat2. The size of the hair (short > 5 cm, long < 5 cm) is another factor that is specific for each of the two age categories. Other conditions included comb stroke (complete combing over the hair), shampoo stroke (complete rubbing of shampoo over the head right to the scalp) and time of exposure.

Prior to the survey and head washing, the parents of each participant provided consent through endorsement of the consent form. The eligibility criteria were developed based on the initial assessment of lice within 5 mints where a person is qualified if a minimum of 3 lice or nits were spotted in 5 mints. The exclusion criteria included people affected by scalp disorders, irritation sensitivity to medication and other conditions listed in Mary et al. (2012).

To assess the efficacy of the product, all people received the shampoo following the factorial method, as shown in Table 1, based on systematic randomization to predict the optimized conditions.

2.3 Application of Shampoo

Lice treatment was performed on day 0 and repeated on day 2, and sampling was performed on day 4. This prevents eggs from hatching prior to sampling, as it was noted that eggs hatch at 6–10 days after ovipositional activity (Buxton 1939). The treatment was applied over the whole scalp, covering the hair roots, behind the ears and on the neck after the hair was moistened with water. The duration of each treatment varied depending on the design, i.e., between 10 and 20 minutes. Thereafter, the hair was rinsed with water completely before drying with a towel. The nits and lice were combed and thrown into trash and burned. The effectiveness of the shampoo was judged based on the absence of lice or nits after day 2 of treatment or day 4 of the overall period, including a high number of weak or dead lice and nits during sampling.

The primary cure rate will involve the numbers of weak and dead lice or nits after the trial. Visual inspection was conducted on nymphs, hatching nymphs, and adult lice (with a ×10 magnifying lens), using a head lice detection comb.

2.3 Tolerance evaluation

To comparatively assess the acute tolerance of lice exposed to the Go Away Lice shampoo, the method of Moe et al., 2004, was used. The two commercial louse treatment shampoos with the brand name KP24 RAPID Shampoo (Oystershell NV, Nijverheidsweg 10, 9820 Merelbeke Belgium) and Essential Oil LICE ATTACK (Primer Cct Warana, Qld, Australia) were used for comparison. The stress tolerance technique was used to evaluate the effectiveness of each shampoo based on the number of deaths of the lice over time. A product causing 50% death of lice in a short period of time was considered most effective. This

technique evaluates the efficacy of each product in killing lice, irrespective of the factors associated with the application of shampoo to participants. Briefly, a minimum of 8 lice (per treatment group) of equal sizes were collected from each participant and confirmed to be alive via microscopy. The healthy lice (active) were divided equally into Petri dishes in duplicate for exposure. No control was used in this study since comparisons were made between commercial and local products only. A total of 0.15 g of shampoo was diluted in 25 ml of water and shaken to dissolve. The solution was transferred onto each Petri dish containing lice from each respective group and allowed to soak in shampoo for 10 minutes. Thereafter, the solution was gently poured from the Petri dishes, and the lice were left to die over time. The time from when the solution was cleared was designated as time 0. Louse movement was observed every 30 minutes with gentle knocking on the Petri dish to trigger movement. After 70 minutes of exposure, the above steps were repeated for another 10 minutes. The time used in the process was added to the exposure period for observation. The sections were observed with a microscope. Death was noted when no leg or body movement was observed.

The time to 50% death was calculated according to Ren et al. (2007) and Moe et al. (2004) using log₁₀ values in the following equation.

$$Y = aX + B$$

where $Y = \log_{10}(\text{survival, assumed to be } 100\%) = 2$, $X = \text{time to individual death of fish (min)}$, and $LT_{50}(X)$ was obtained when $Y = 1.7$ since $\log_{10}(50) = 1.7$. Higher values indicate greater tolerance against stress.

2.4 Statistical analysis

The factorial randomized design was generated using Minitab Statistical Software. A stepwise regression model was used to predict the relationships between factors and predictors. The significance level was determined following the method of Lenth's pseudo (Edwards and Mee 2008). $P < 0.05$ indicated statistical significance, and an optimization tool was subsequently used to predict the responses. The accuracy of the model was determined by observing the coefficient of determination R^2 . A principal component analysis (PCA) was performed with XLSTAT software. The relationships between factors and between variables are displayed for interpretation. Two-way analysis of variance (ANOVA) was also conducted using XLSTAT to identify interactive terms that significantly influence numeric variables such as lice, nits and dead lice and nits. One-way ANOVA was used to determine differences in the effectiveness of the 50% mortality model between 2 commercial head louse shampoos and the local Go Away Lice shampoo.

3. Results

3.1 Effects of factors on the responses

The standardized effects of the reduced model terms are in decreasing order of their absolute values. A significant effect was observed for dead nits and lice for the combined effect of age and hair type and comb and age, respectively ($P < 0.05$). The dead lice model term showed no significant lack of fit ($P > 0.05$).

The two-way analysis of variance (ANOVA) was used to evaluate the significance of the interaction between age at death and hair type (Table 1). The results showed that individuals aged older than 12 years with long hair tended to have more deaths than did those aged 6 to 12 years or 12 years and older with short hair ($P < 0.05$).

Table 1
Interactive effect of age and hair size on mortality

Model parameters (Dead nits):						
Source	Value	Standard error	t	Pr > t	Lower bound (95%)	Upper bound (95%)
Intercept	0.000					
Age-1	0.000	0.000				
Age-2	6.471	1.777	3.641	0.001	2.926	10.016
Hair type-1	0.000	0.000				
Hair type-2	4.471	1.777	2.516	0.014	0.926	8.016

The two-way analysis of variance (ANOVA) was used to evaluate the significance of the interaction effects between death, age and hair type (Table 2). The results showed that individuals aged older than 12 years with long hair tended to have more dead lice than did those aged 6 to 12 years or 12 years and older with short hair ($P < 0.05$).

Table 2
Interactive effect of age and hair size on dead lice

Model parameters (Dead lice):						
Source	Value	Standard error	t	Pr > t	Lower bound (95%)	Upper bound (95%)
Intercept	0.000					
Age-1	0.000	0.000				
Age-2	1.353	0.654	2.069	0.042	0.049	2.657
Hair type-1	0.000	0.000				
Hair type-2	1.412	0.654	2.159	0.034	0.107	2.716

The optimization of factors following the reduce model term is shown in Fig. 2. Dead lice and nits counts were maximized to obtain the most potential interactive effects of the model terms. The maximum number of dead lice collected in a minimum of 7 louse counts was 3. A maximum count of 10 dead nits is observed when the total nit count is above 18. These figures were obtained following the conditions of desired curability, such as 2 shampoo strokes and 4 comb strokes, in children aged 6 to 12 years with short hair (> 5 cm) (Fig. 2).

The relationships between the quantitative variables are displayed in Fig. 3. The numerical variables did not show any correlation; however, the number of comb strokes explained 22.8% of the relationship, and 20% of the relationship was explained by comb strokes.

The relationships between categorical variables used in the model development are shown in Fig. 4. Age group 2 (13–16) and Hair type 2 (> 5 cm) were strongly correlated, as they were both explained by the positive axis of factor 1, with a 22.8% score. Age group 1 and hair type 1 did not significantly differ.

A loading plot showing the interaction between quantitative and qualitative variables is displayed in Fig. 5. The incidence of shampoo stroke is strongly associated with age and hair type (size). These 3 parameters explained 22.80% of factor 1. The number of comb strokes and duration of exposure explained 20.25% of factor 2.

The tolerance of head lice to stress after exposure to the 3 tested lice products is displayed in Fig. 7. Interestingly, the local brand, Go Away Lice, died at a rate comparable to that of the commercial brand KP24 Rapid Shampoo (142.1 ± 13 min and 120.8 ± 19 min, respectively) ($P > 0.05$), while the commercial brand Essential Oil Lice had the least mortality, with a duration of 207.6 ± 32 min ($P < 0.05$). Lice were less resistant to Go Away Lice Shampoo than to the Essential Oil Lice attack product ($P < 0.05$).

4. Discussion

Currently, the products used for controlling head lice include DDT or organochloride insecticides. These products could be more toxic than other products and may cause toxicological problems, resulting in the need to discontinue their use (Buxton et al., 1950). In recent decades, researchers have reported head louse resistance to pediculicides, particularly permethrin (Meiking et al., 2002; Connolly et al., 2009). Furthermore, cross-resistance between products such as permethrin and DDT or bioallethrin was noted, although permethrin was used for louse control for a while (Mumcuoglu et al., 1999). Pyrethrine was effective in the last century; however, recently, its effectiveness has been reduced to 50% (Heukelbach et al., 2004). A similar failure rate was reported for permethrin (Maunder 1981). Resistance has also been reported for DDT, lindane, pyrethrins, permethrin, malathion, and carbamethoxazole (Mac-Mary et al., 2012). Other physical factors also influence the effectiveness of lice for treating shampoo, as reported elsewhere (Burgess 2022).

Many factors may play a role in lice resistance to the synthetic lice treatment shampoo. Naturally, active ingredients have recently been recognized as alternatives to the conventional louse treatment shampoo

(Burgess 2022). The composition of the tested product is free of insecticides, and the product physically affects lice (asphyxiated by the Andiroba oil) and nits (quassia vinegar damages the chitin of the nits), with interesting results. Products containing Quassia are rarely known for any resistance phenomena and have a far lower risk of toxicity than comparable insecticides (Mac-Mary et al.,2012).

In clinical studies of lice, treatments often burden patients and families, as some patients shampoo may need to remain on the scalp for a long time before being washed out (MacMary et al.,2012). The tested product showed an effective treatment time of less than 20 minutes per session. Older patients aged 13 years and older with long hair had more dead lice than younger patients (Fig. 1, a & b; Tables 1 & 2). This may have been due to the high shampoo retention capacity of older patients with long hair compared to younger patients. Suffocating agents such as essential oils may also contribute to the killing of lice (Mumcuoglu 1999). The physical factors associated with the application of this product can be adjusted to achieve the best possible results, as demonstrated in the stepwise regression model. Patients interested in using this product may consider adjusting conditions based on physical factors to achieve the best results (Fig. 2). Independent quantitative factors such as the number of comb strokes, duration of exposure and incidence of shampoo stroke did not significantly influence the number of death lice or nits (Fig. 3). It is evident that the number of shampoo strokes, size of hair and age have combined influences on the efficacy of the product, while the number of comb strokes and duration of exposure are independent of these effects (Fig. 4). The new product of shampoo should be applied two times for 2 days between treatments. The study revealed that two treatments are appropriate for less severe cases, while 3 treatments are necessary for complete relief in severe cases. The side effects are unusual; in rare cases, there may be redness, dry skin or flakiness.

The tolerance of commercial products and this product to stress showed that the new product is within the range of killing capacity for lice (Fig. 5). Preference in this regard would be for a less toxic product, in this case, the new product. The stress tolerance technique is a relatively new assessment technique and may be used to evaluate the efficacy of lice treatment shampoo.

Conclusion

It may therefore be concluded that this new head louse treatment, shampoo, is a beneficial or valuable addition to the existing ones considering the exclusion of chemical insecticides, absence of toxic risk to classic pediculicides, and patient-friendly use instructions. This product is particularly beneficial for children, who are the major population with louse infestations.

Declarations

Acknowledgment

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Author Contribution

R.S.S. designed and analyzed the study and wrote and reviewed the manuscript. K.K.K. analyzed the data, wrote the main text of the manuscript and reviewed the manuscript. Similarly, L.L., D.T., H.T., L. T. and M.F.EB. wrote the main text of the manuscript and reviewed and edited the manuscript. All the authors have read and agreed to publish the manuscript.

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Data Availability and Materials

The datasets generated and analyzed during the current study are not publicly available due to property rights protection but are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Ministry of Health, Government of Vanuatu. Consent to participate was obtained from all participants involved including parents and school teachers.

Consent for publication

Yes, all the authors have provided consent for publication.

Competing interests

The authors declare no competing interests.

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Figures

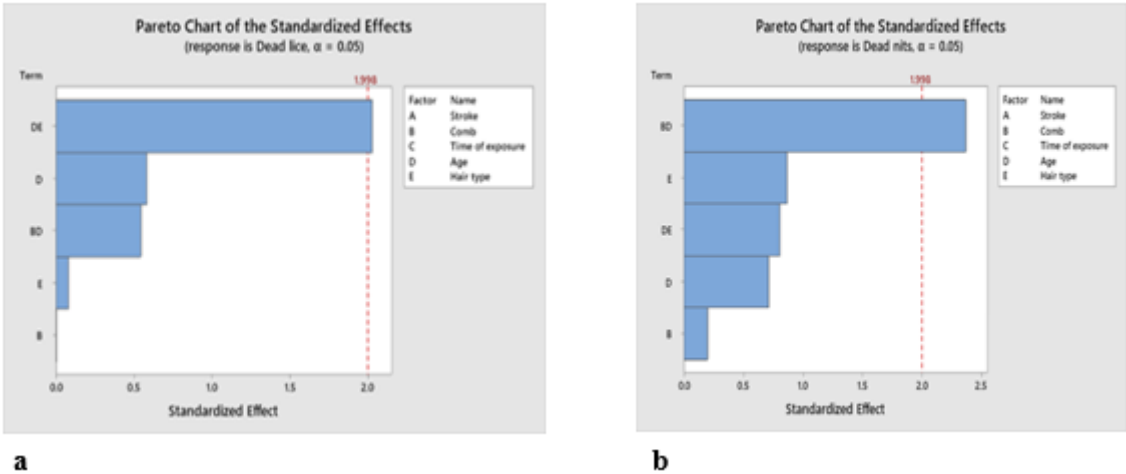


Figure 1

The reduced model term for each significant factor and their interactions, where a is the dead lice and b is the dead nits.

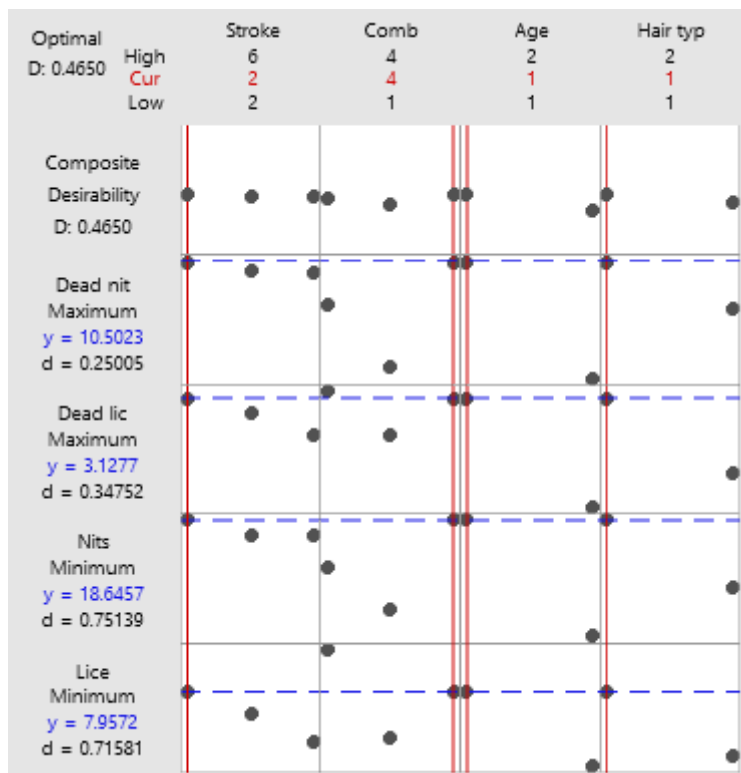


Figure 2

Factor optimization plot for the reduced model terms

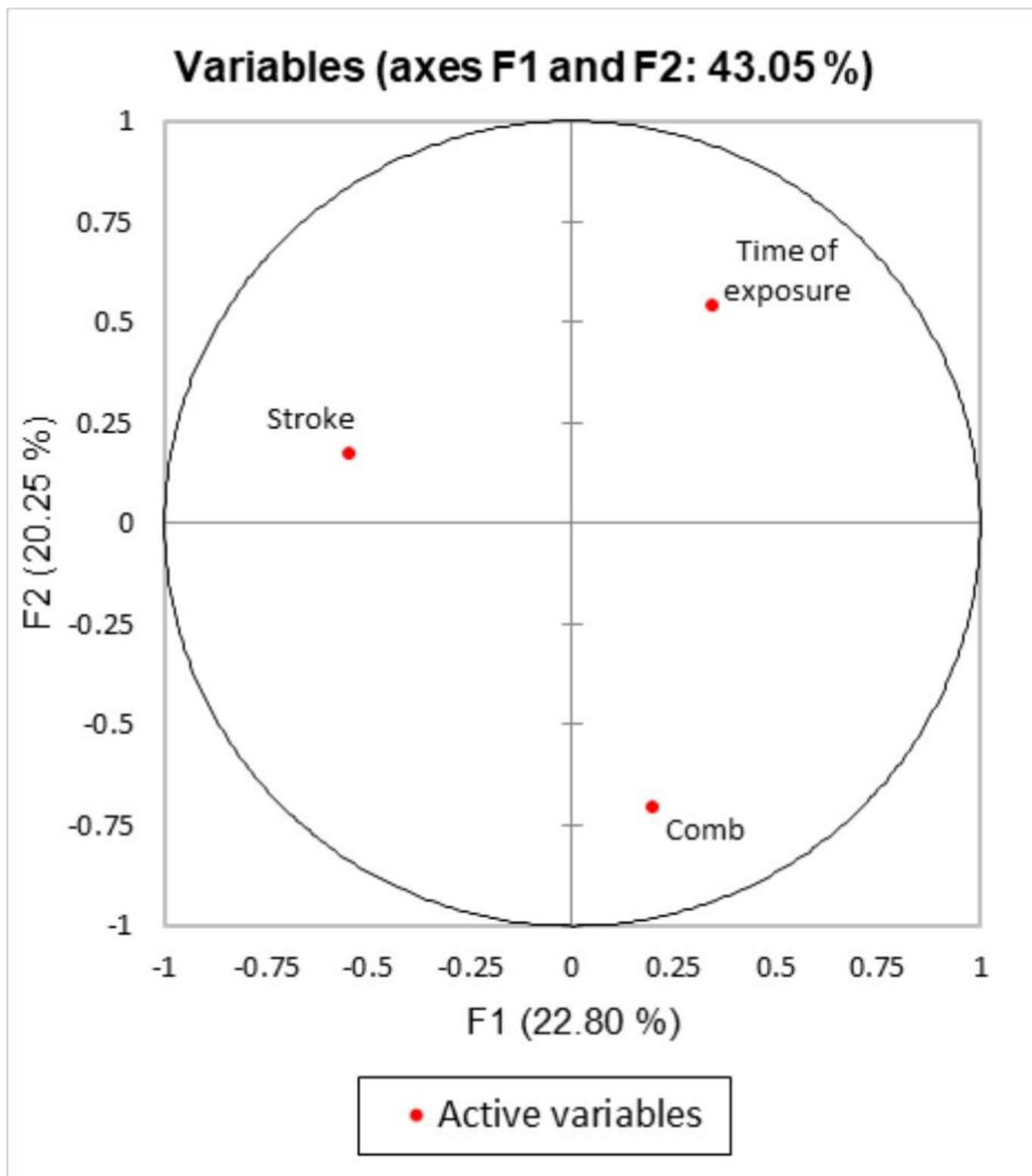


Figure 3

PCA of quantitative variables used in the model

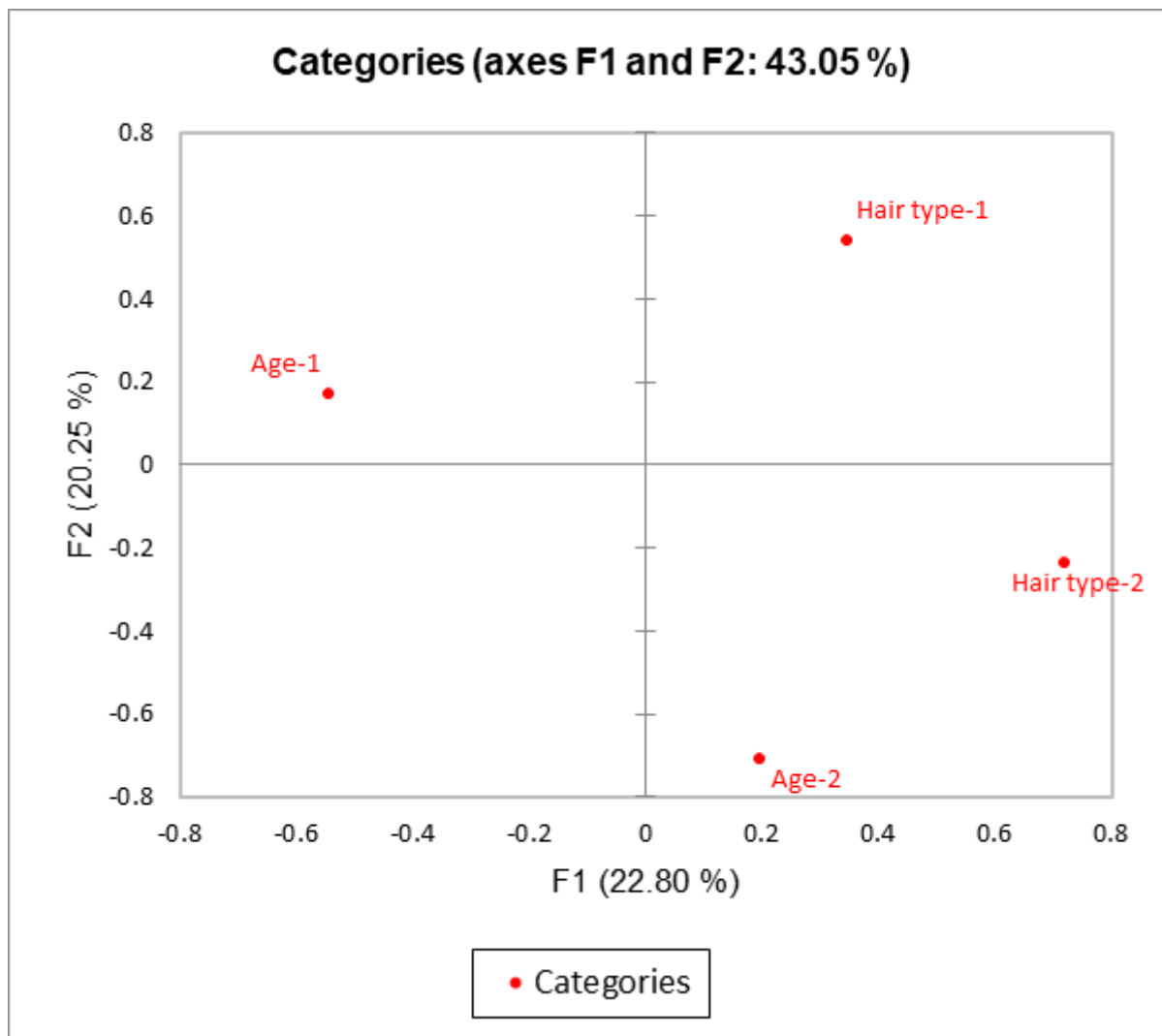


Figure 4

PCA of qualitative variables used in the model

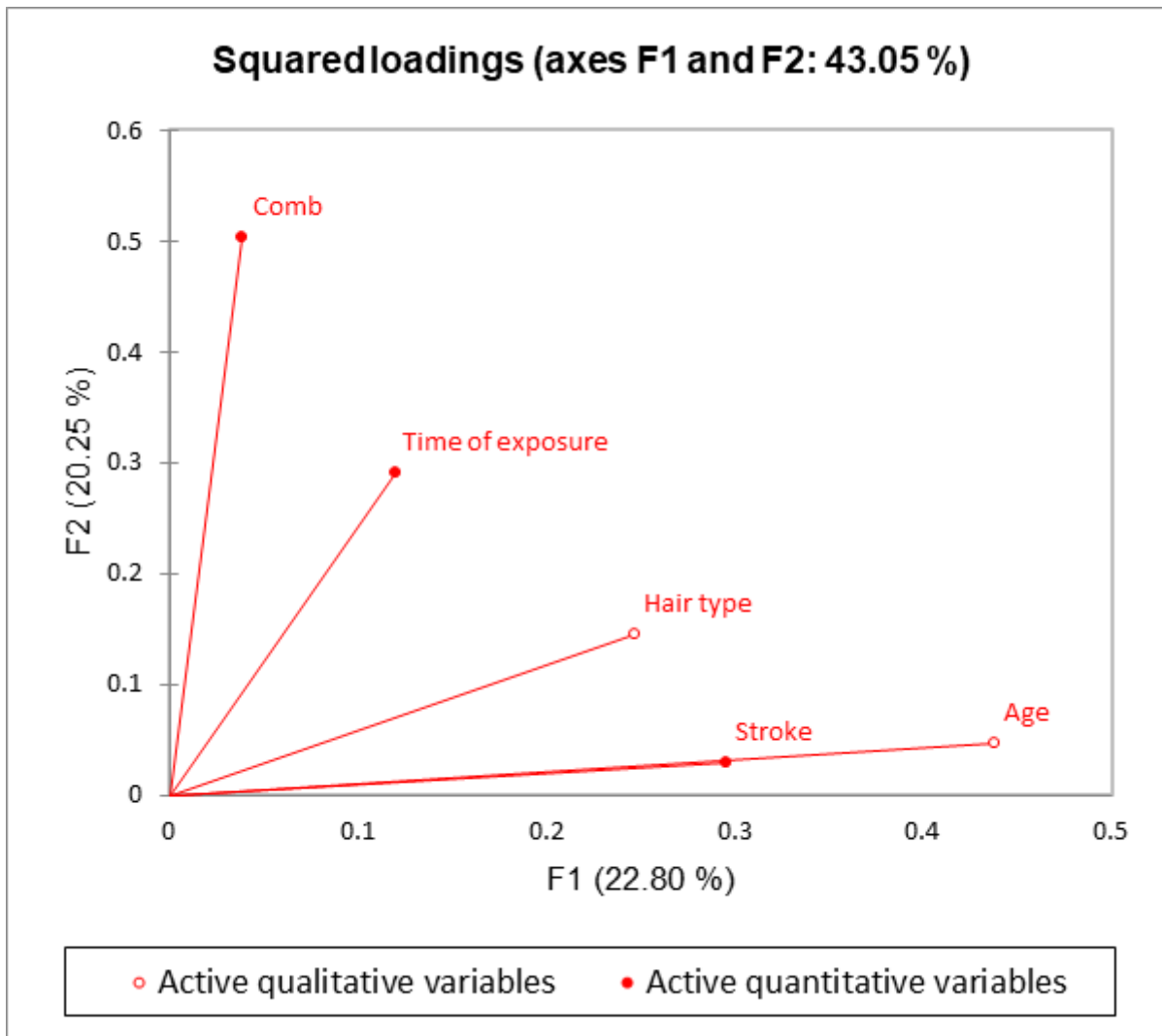


Figure 5

A square loading plot of the variable interaction

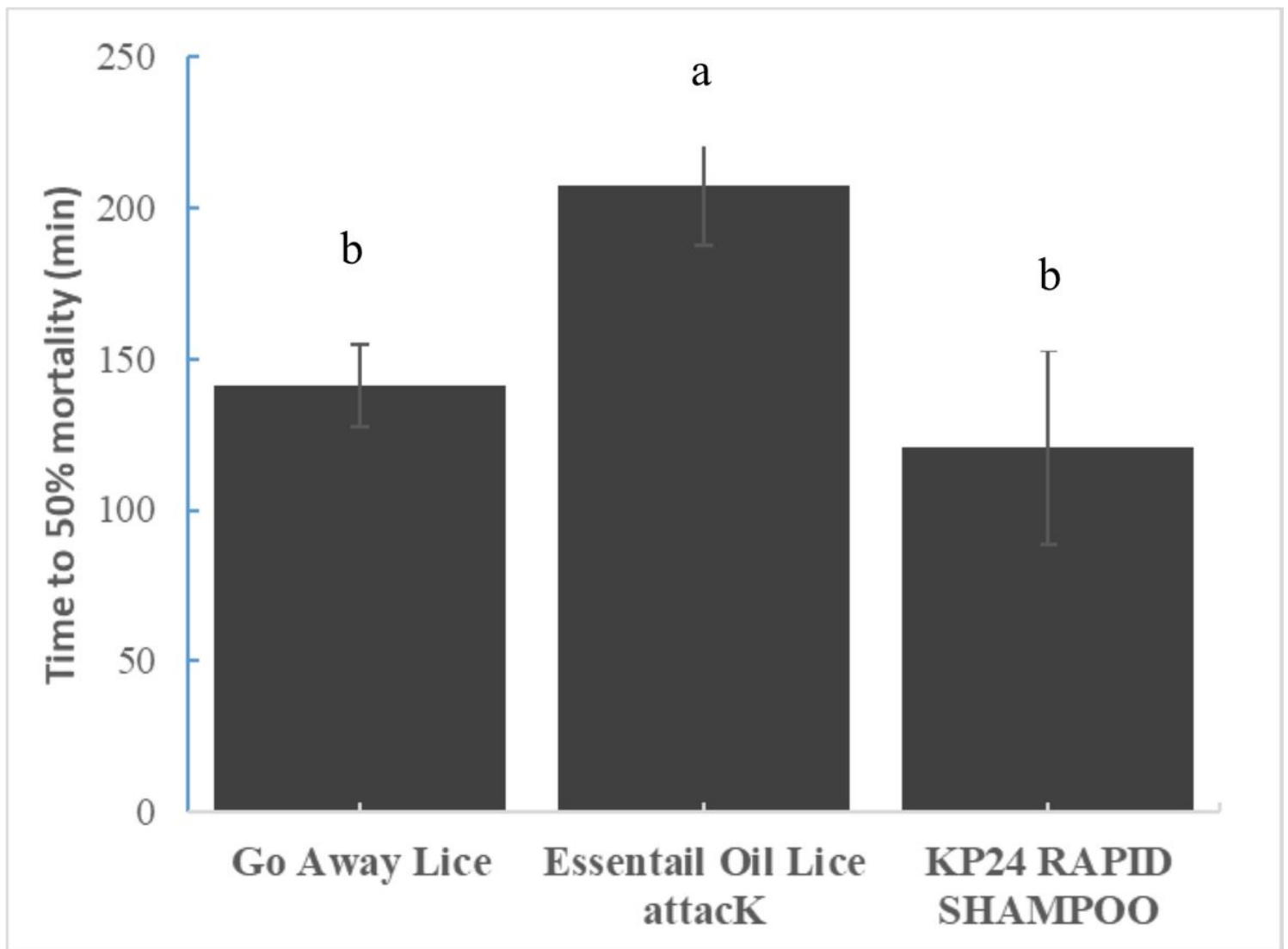


Figure 6

Time to 50% death of head lice treated with 3 Lice treatment: shampoo, the local brand 'Go Away Lice,' and commercial brands, namely, Essential Oil Lice attack and KP24 Rapid Shampoo. The bars represent the standard error (SE ±). Different letters represent significant differences between groups ($P < 0.05$).