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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.1138.1145

Research Article

Effect of Wood Tar on Some Biochemical Aspects and Morphological Changes of the Head Lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae)

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Abstract

Background and Objective: The head lice, *Pediculus humanus capitis* is considered one of the most important parasites that attach human body and causes many diseases infestation of humans worldwide. This insect could be found between children during the warm months in developed and developing countries. This study is aimed to evaluate the insecticidal effect of wood tar oil against the adult of head Lice *Pediculus humanus capitis.* **Materials and Methods:** Adult insects were collected from school children that do not have been treated with any applications whether chemical or natural treatments. Adult insects were treated with 3, 5 and 7% of wood tar. Acetylcolinesterase, glutathione peroxidase and superoxide dismutase activities were evaluated. In addition, total soluble protein in treated insects was quantified using Bradford technique. Alterations in adult insects were evaluated using scanning electron microscope. **Results:** Observed data demonstrated significant effect of three concentrations 3, 5 and 7% of wood tar where the recorded mortalities were 60, 90 and 100% after 6 h, respectively. Treatments with wood tar caused inhibition in acetylcholine esterase level, it also reduces the total protein activity in treated insects. The GPX and SOD activities increased gradually in the adult of *P. humanus capitis* after exposure with tar concentrations. Scanning electron microscope showed several distortions of the body structure, spiracles, hairs and body segments of dead lice exposed to wood tar. **Conclusion:** Treatment with tar caused oxidative and morphological damages in insects. Wood tar could be a good insecticidal agent against head Lice *Pediculus humanus capitis.*

Key words: Head lice, *Pediculus humanus capitis,* wood tar, natural product, scanning electron microscope

Citation: Jamila S. Al-Malki and Rania S. Rashwan, 2020. Effect of wood tar on some biochemical aspects and morphological changes of the head lice *Pediculus humanus capitis* (phthiraptera: pediculidae). Pak. J. Biol. Sci., 23: 1138-1145.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The head lice, *Pediculus humanus capitis* is considered one of the most important parasite that attach human body and causes many diseases infestation of humans worldwide[1]. This insect could be found between children during the warm months in developed and developing countries[2-5].

During the louse feeding, they may cause pruritus, excoriation, conjunctivitis and bacterial inflammation. Transmission of insects may be direct, from one head to another when contacting together or indirect, through common use of hair brushes, clothing as hats, caps, jackets, , combs[6] and so on.

Head lice could be transmitting by contact[7]. The main symptoms associated with infestation are constant itching, which caused by biting the skin and the secreting saliva during the feeding. This parasite, may lead to infection pyoderma[8].

In addition, lice infestation may cause psychological distress, social stigma disrupt learning performance of the formers[9].

Different formats from Pediculicide drug products are marketed as Over The Counter (OTC) Drugs in the form of shampoo, lotion, washable creams, powder, gels and so on. Lindane, Malathion, Carbaryl and Permethrin are examples of such products, whereas other organochlorines, organophosphorus are no longer in use due to their side effects including emergence of resistant head lice populations[3].

Many problems are caused by using chemical insecticides such as hazards to human health and increasing the resistance of pests, so it is urgent to use alternative applications as natural product such as using botanical and bio-agent insecticides against pests[10]. Kim *et al.*[11] and Aslan *et al.*[12] evaluated the efficacy of some botanical oils products in controlling some insect pests.

Wood tar oil could be extracted from trees and it considered as natural product that used in several area. Tar is dark, brightened, oily material and has strong smell, it produced by slow pyrolysis of wood, coal or peat.

It could be used as a preservative for utensils made from woods and objects against rot, in addition are used for wood protection, shining agents. It also could use as a flavor in candies and food, a perfume and an anti-dandruff agent in shampoo and as a component of cosmetics[13].

The aim of the present study was to investigate the insecticidal potential of using tar against head lice *Pediculus humanus capitis* under laboratory conditions and its effect on some biochemical aspects of treated adults. Distortions of the body structure were recorded by direct observation by using Scanning Electron Microscopes (SEM) for better understanding the mechanisms of action on the morphological changes of treated insects.

MATERIALS AND METHODS

Study area: The study was carried out at Biology Department, Entomology Lab., during the summer of 2018.

Collecting insects: Adult insects were collected from school children that do not have been treated with any applications whether chemical or natural treatments. Collecting insects were done through the summer of 2018 (June-August) by using plastic comb with sharp teeth on the children's head and then collected insects were removed into clear small tubes (2×2.5 cm). Head lice samples were transported to laboratory[14].

Preparing tar solution: Wood tar could be obtained by pyrolysis of lignin, which is a complex mixture of aromatics and oxygenated compounds mainly composed of aromatic hydrocarbons[15]. After transporting the samples, treatments with three concentrations of wood tar were started within 10 min after collecting the samples, by using Petri dishes lined with filter paper in contact toxicity bioassay. Three concentration of wood tar were prepared by mixing with olive oil. These concentrations were 3, 5 and 7%. Three replicates for each concentration, each replicate consist of ten adult insects, these insects were equal in size for similar testing. Filter paper with 8.5 cm diameter was applied with the concentrations, after 3 min of treatment, filter paper was transferred to the bottom of Petri dish with diameter 9 cm, 10 adult insects were placed on Petri dish. For the positive control, adult insects were transferred to filter paper that saturated only with olive oil while negative control, insects were transferred to filter paper without any treatments. The experiments were kept under lab conditions (27.5±2.3°C and 70±5% (RH). Mortalities were recorded each 2 h under stereomicroscope.

Biochemical assay: After treatment with three concentrations, dead insects were collected into a chilled Eppendorf tubes, insects were rinsed with 5 mL acetone to remove surface residues, tissues were homogenized in phosphate buffer by adding Teflon homogenizer with pieces of ice. Homogenate tissues were centrifuged at 6000 rpm for 20 min at 4°C. Supernatant of treated and untreated (control) were immediately assayed to determine the following:

Acetylcolinesterase (AChE): The activities of acetylcholine-esterase (AChE) was evaluated by using acetylcholine bromide[16].

Determination of Total Soluble Protein (TSP): Colorimetric determination of total soluble protein in total homogenate *Pediculus humanus capitis* adults was carried based out as described by Bradford[17]. Biuret reagent with amount of 5 mL was added to 0.2 mL of adult homogenate as volume and then it incubated for 30 min at 20-25°C. The absorbance of the sample against a blank Biuret reagent was measured at wavelength of 546 nm.

Determination of glutathione peroxidase (GPX): The GPX activity was evaluated according to Paglia and Valentine[18], where the activity was measured by H_2O_2. Oxidation was observed when adding NADPH at 340 nm for 3 min. A control of the non-enzymatic oxidation of NADPH demonstrated as blank without homogenate.

Determination of superoxide dismutase (SOD): Superoxide dismutase (SOD) activities were evaluated by using the technique[19]. One unit of superoxide dismutase activity was measured as the amount of protein that causing 50% inhibition of pyrogallol auto-oxidation. Activity was measured as units/mg of protein. A control was considered as a blank without homogenate.

Scanning electron microscope: Adults of *P. humanus capitis* were kept in glutaraldehyde till examination, preparing method for scanning electron microscopy was described by Harisson[20]. The specimens were washed many times in distilled water. Samples were fixed on disc with double side carbon tape[21], samples were inspected by using SEM Model Quanta 250 FEG, pictures were taken at different magnification powers of SEM and resolution for Gun.1n, analytical SEM at the Mineral Resources Center in Egypt.

Statistical analysis: Mortalities were analyzed with ANOVA test by using SAS program[22]. Significant different were calculated by using least significant differences (Fisher's LSD test, $p \leq 0.05$), Lethal Concentration (LC_{50} and LC_{90} values) was calculated by probit analysis.

RESULTS AND DISCUSSION

Efficiency of different concentrations of tar oil against adult of *Pediculus humanus capitis*: The results in Table 1 demonstrated the effect of tar solution with three concentrations against *P. humanus capitis*. The data investigated that the percentage mortality of adult had positive correlation with tar concentrations.

Mortality of insect was increased with the increase of treatment concentration as well as exposure time. These data revealed that treated with 5% caused 100% mortality after 8 h compared with positive control which achieved 20% mortality at the same time. Similarly, 100% mortality was observed with concentration 7% which occurred at earlier time[23] of 6 h, clarified slow demolition of components in the soil during agriculture application also, developing methods as novel bio-control technologies. Rahmat *et al.*[24] investigated that treatment of wood vinegar resulted the highest mortality of maize weevil compared to untreated.

The probit statistics estimates of LC values of tar were calculated, where data indicated that the toxicity of tar varied according to the concentration and exposure period. Data in Table 2 showed the calculated LC_{50} and LC_{90} of wood tar after 2, 4, 6 and 8 h against adults of *P. humanus capitis.* Takano-Lee *et al.*[25], tested olive oil for controlling head lice. Heukelbach *et al.*[26] recorded quite effective in their study when using tea tree as a natural product. Shrivastava *et al.*[27] recorded stopping of the adult movements after half an hour when treated with undiluted lemon. Tashakori *et al.*[28] recorded different mortalities when treated insects with three controlling methods.

Activities of some biochemical components

Acetylcholine esterase level in *Pediculus humanus capitis* homogenate: Acetylcholine esterase is considered the main source for controlling the acetylcholine levels in the nervous

Table 1: Adult mortality (Mean±SD) of head lice *Pediculus humanus capitis* induced with three concentrations of tar oil

	Exposure period							
Concentration (%)	2 h	Mortality (%)	4 h	Mortality (%)	6 h	Mortality (%)	8 h	Mortality (%)
3	2±0.94	20	4±0.47	40	6±0.81	60	7±1.2	70
5	5±0.47	50	7±0.81	70	9±0.47	90	10±0.1	100
7	6±0.47	60	8±0.47	80	10±0.81	100	-	-
Positive control (Olive oil)	0	0	0	0	1±0.1	10	2±0.01	20
Negative control	0	0	0	0	0	0	0	0

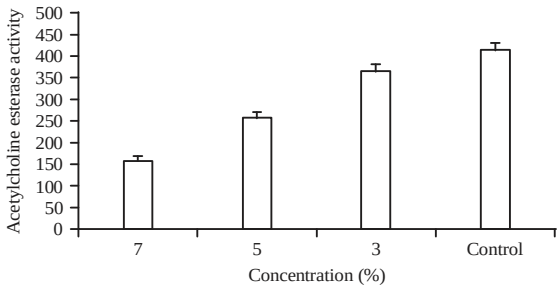

Fig. 1: Effect of different concentrations of tar on acetylcholine esterase activity in the adult of *Pediculus humanus capitis*

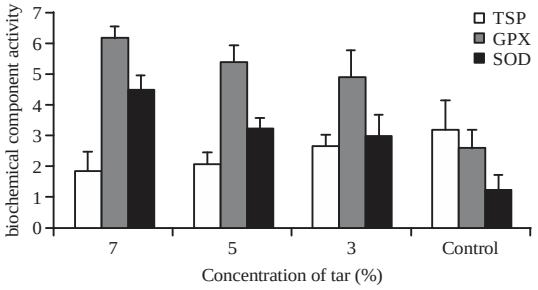

Fig. 2: Effect of different concentrations of tar on TSP, GPX and SOD activity in the adult of *Pediculus humanus capitis*

TSP: Total soluble protein, GPX: Glutathione Peroxidase, SOD: Superoxide dismutase

system. As shown in Table 3 and Fig. 1, the inhibition of enzyme recorded lowest value when insects treated with the concentration 7%, which recorded 158.8 mmol. Significant differences were demonstrated between three considered concentrations and exposed periods where ($p \leq 0.05$). This treatment decreased and inhibition of acetylcholine esterase level which reflect the increasing level of acetylcholine in post synapse, that led to accumulation of acetylcholine and the synaptic nerves become continuously on. Sigh and Sohi[29] explained the reduction of AChE level and its sensitivity to pesticides was explained by Al-Barty *et al.*[30], who demonstrated significant decrease in serotonin and acetyl-cholinesterase levels in *Oryzaephilus surinamensis* when treated with garlic oil as compared to untreated beetles of garlic oil. Senthil-Nathan *et al.*[31] calculated lethal concentration values of goniothalamin, as an effective botanical insecticide which led to releasing AChE in low levels compared to control. Zibaee and Bandani[32] monitored the levels of acid phosphatase, alkaline phosphatase, acetylcholine esterase when treated *Eurygaster intefriceps* with a methanolic extract of *Artemisia annua* as medical plant,

Table 2: Estimated LC_{50} and LC_{90} for wood tar against *P. humanus capitis* after exposure four periods

	Exposure periods (h)			
LC values	2	4	6	8
LC_{50}	5.4	4.5	1.1	0.8
LC_{90}	12.1	6.0	2.4	1.5

obtained results showed that when increasing the concentrations of extracts, significant decreasing of tested components were observed. Also inhibition of acetylcholine esterase was investigated in *Periplaneta americana* when treated with goniothalamin[33]. Decrease in AchE could suggested by Gamil *et al.*[34], who explained the reduction of AchE as a result of blocking sited of nervous system.

Total soluble protein (TSP): Total proteins are very important for growing and many vital activities for living organisms. Decreasing the quantity of total proteins was detected in treated adult of *P. humanus capitis* after exposure to different concentrations of tar, the highest significant decrease was observed in insects that treated with the concentration 7% followed by 5 and 3% which it recorded 3158.8, 259.6 and 368.2, respectively (Table 3 and Fig. 2).

In the treated adults, decrease the activities of total protein content could suggested by increase in transaminase activities and increasing in free amino acids, these results were agreed with Shafeek *et al.*[33], who evaluated the effect of Indoxacarb 15% EC in controlling *S. littoralis* which considered one of the most destructive insect pest that attacking many crops[35] and Hussain *et al.*[36] recorded depletion in total proteins levels in larvae of *Bombyx mori* and adults of *Tribolium castaneum* respectively when exposed to treatments.

Determination of antioxidant defense enzymes activity: Data in Table 3 and Fig. 2 investigated GPX and SOD activities to be highly increased in the adult of *P. humanus capitis* after exposure to tar concentrations, the highest recorded increasing of GPX and SOD activities were demonstrated at 7% concentration, while when treated adults with 3 and 5% concentrations, they recorded less values. Increasing production of these enzymes occurred because they have an important role in detoxification mechanism in tissues insects so, they were increasing after application treatments. Huang *et al.*[37], Dubovskii *et al.*[38] and Kamata and Hirata[39] mentioned that insecticides cause's oxidative stress that leads to producing free radicals and reactive oxygen species that effect on antioxidant enzyme activities which considered as defense system in insects against insecticides.

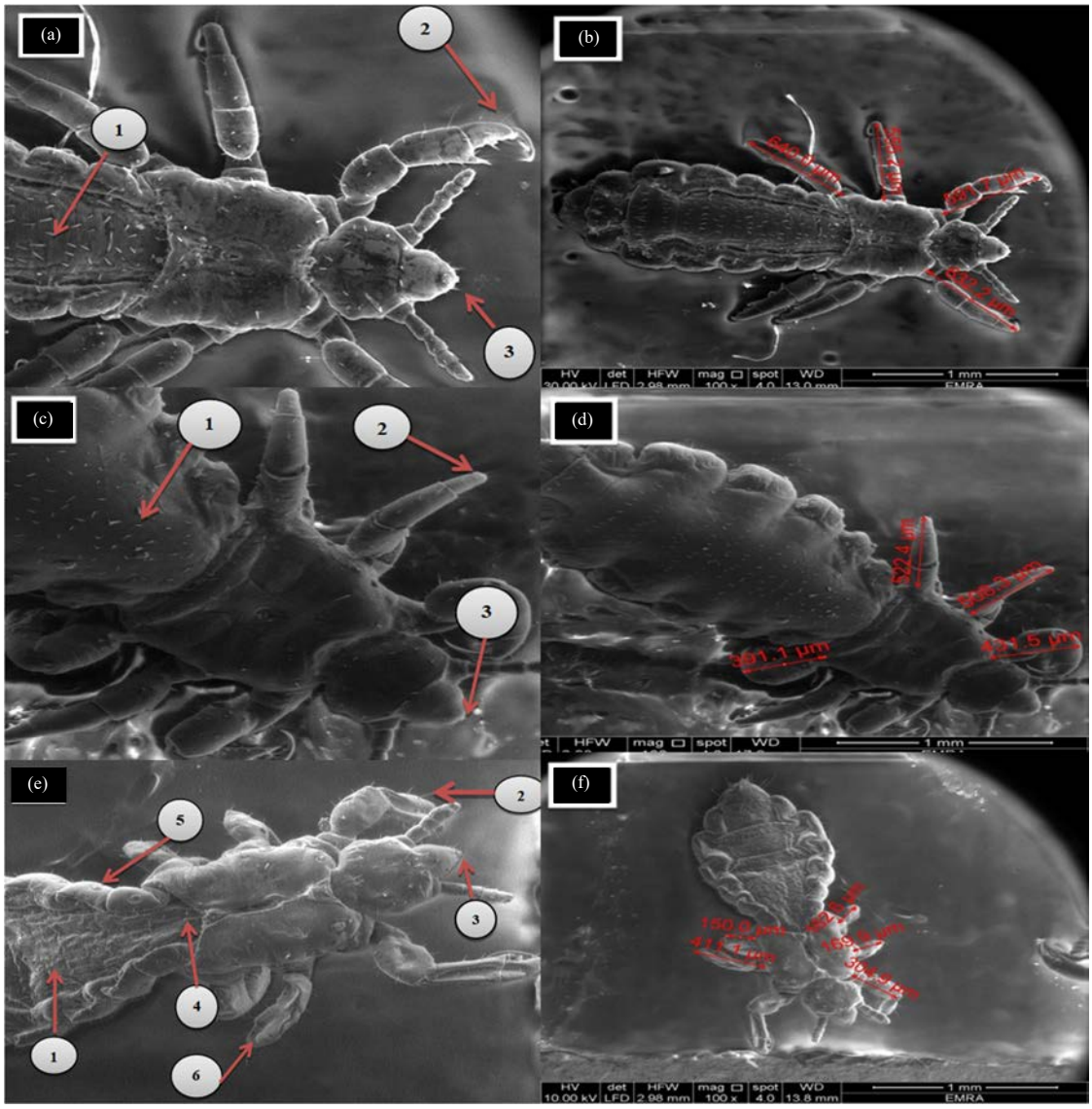

Fig. 3(a-f): Scanning electron microscope for adult of *Pediculus humanus capitis*, (a-b) Negative control and (c-d) Treated insects with tar at 3 and 5% and (e-f) Insects treated with tar at 7%

1: Abdominal hairs and seta, 2: Hook of terminal legs, 3: Sensory hairs of the head anteriorly, 4: Contraction of abdominal segments, 5: Dipping of respiratory spiracles in the chitin, 6: Shrinking of legs

Increasing activities of GPX and SOD were observed in homogenates tissues of insects after treatment with three concentrations. Treatment with tar caused oxidative stress in insects when generating reactive oxygen species in the insect tissues. Yankanchi and Gadache[40] and Harisson *et al.*[20] demonstrated that treatments with insecticides cause lipid peroxidation and increasing gradually in the antioxidant enzymes, which play an important role in oxidative stress defense. Also, previous study[41] investigated the effect of radiant 12 sec that showed significant differences alternations in SOD and CAT levels- as antioxidant enzymes after 2 days of application as compared to untreated in *Sitophilus oryzae.* Generating these enzymes protect the insect from insecticidal stress. Copping and Menn[42] reported that an increase in super oxide dismutase and glutathione peroxidase levels is responding to the increasing of radical oxygen species in the red blood cells in treated rats. Nehare *et al.*[43] and Wang *et al.*[44] observed over production of glutathione S-transferase levels in larvae of *Plutella xylostella* after treated with indoxacarb.

Table 3: Measurements of biochemical components (Mean±SD) induced in *Pediculus humanus capitis* as a result of treatment by three concentrations of tar

Concentration (%)	Acetylcholinesterase (mmol/min/mL)	Total Soluble Protein (TSP) (mg dL^{-1})	Glutathione peroxidase (GPX)	Superoxide dismutase (SOD) (U mg^{-1} protein)
7	158.8±9.52[d]	1.871±0.62[c]	6.21±0.36[a]	4.52±0.45[a]
5	259.6±11.14[c]	2.103±0.37[b]	5.41±0.52[b]	3.26±0.33[b]
3	368.2±12.73[b]	2.689±0.36[b]	4.93±0.84[c]	3.01±0.68[b]
Control	416.4±19.04[a]	3.213±0.96[a]	2.63±0.59[d]	1.26±0.47[c]

Different letters in the same column expressed significant differences between three tested concentrations

Scanning electron microscopic examination: The observed morphological changes that occurred in the outer structure of the body insects after treatments were recorded by using scanning electron microscope as shown in Fig. 3.

Scanning electron microscope for control lice (*Pediculus humanus capitis*) (Fig. 3a,b) showed a conical head, ventral constriction and blunt abdomen. At the anterior end of the head it appeared with three pair of hairs. Thorax could be distinguished as prothorax, mesothorax and metathorax. Movable hook-like claws are located at the end of tarsus. Abdominal segments are clear and distinctive with observed respiratory spiracles on the two sides of abdomen hairs are spreading on the body cuticle. Adults of negative control were still alive during all the experiments, these results were in agreement with finding of Dutra *et al.*[21], who recorded the changes occurred in dead adult lice and found that the body structure were kept at the normal shape, size and other features. Also, Shrivastava *et al.*[27], Takano-Lee *et al.*[25] and Heukelbach *et al.*[26] recorded the same results. Treated lice with tar concentrations illustrated damaged and disfigured bodies. As shown in Fig. 3c and d for the concentration 3 and 5%, specimens of adult insects loss some hairs and seta that located on the body as thorax, abdomen and legs, when compared to control, also three sensor pairs of hairs on the anterior end of the head were loss. Claws of legs are shriveled, disfigured and distorted where it lost the outer smooth architecture as well as tibial spurs. These changes appeared more clearly with the highest concentration 7% (Fig. 3e,f), where addition to the previous mention changes, all the body was contracted and shrank as compared to control, legs are damaged and apparently flaccid, damage the chitinous of the anterior abdominal segments, respiratory spiracles were dipping in the chitin where the malformation increase. These results were similar with the finding of El Akkad *et al.*[45] who showed disfigured and damaged bodies of lice when treated with olive oil, lemon juice and tea tree oil.

According to this finding, treatment with wood tar caused oxidative damage in insects by producing ROS in the tissues. The change in GPX and SOD activities which play an important role as defense enzymes were observed in the homogenate's tissues after treatment with three concentrations. Wood tar also caused morphological damages in insects. Wood tar could be a good insecticidal agent against head Lice *Pediculus humanus capitis.* The obtained results indicated that wood tar could use as a natural insecticidal agent against head lice *Pediculus humanus capitis.* Moreover, it contribute reducing environmental pollution instead of using chemical insecticides.

CONCLUSION

The present study demonstrated that treatment of wood tar against adults of head lice *Pediculus humanus capitis* have positive effective on the survival of insects, in addition; it affected on the biochemical components and the morphological features of insects, which recommends as a promising natural alternative controlling this medical human pest.

SIGNIFICANCE STATEMENT

This study discovers the possibility of using natural product as wood tar in controlling head louse, that can be beneficial for reducing the side effect of using chemical insecticides. This study will help the researcher to uncover the ability of applying wood tar in many purposes. Thus, a new theory on this botanical insecticide and understanding its safety effect on human and environment may be arrived at.

ACKNOWLEDGMENTS

Our appreciations and thankful to the staff member of Biology Department, Faculty of Science, Taif University for advices, positive cooperation and introducing all the facilities to complete our study in the best way. Many thanks to the Egyptian mineral resources authority, central laboratories sector for helping in scanning the insect samples.

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