

Henna, *Lawsonia Inermis*



Henna, *lawsonia inermis*: new leaves after a summer rain.

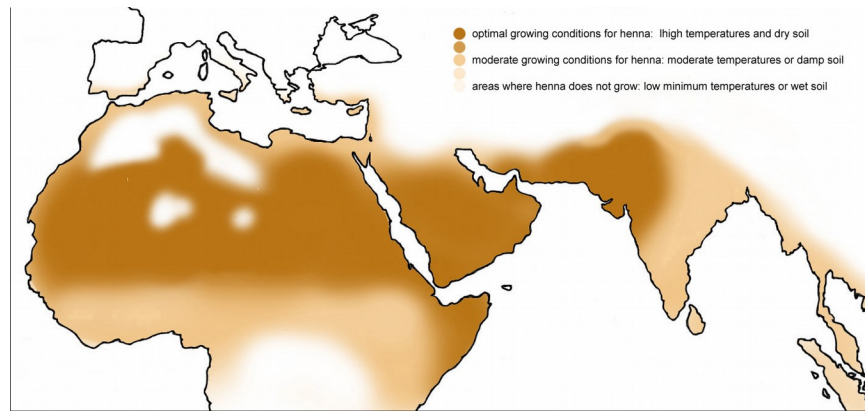
What is Henna?

Henna, *lawsonia inermis*, is a large bush or small tree native to hot, dry climates across North and East Africa, the Arabian Peninsula, the southern areas of the Middle East, and South Asia. Henna probably originated in North Africa, based on the greater genetic diversity in henna in the North African oases than in other regions.¹ Humans probably first developed their use of henna during one of the ‘moist’ phases in the region of the Sahara; the most recent ‘greening’ was from nine thousand to about five thousand years ago.

There is archaeological evidence from Egypt that henna was regularly used to treat skin ailments and to color gray hair five thousand years ago. There is evidence that brides marked their hands and feet with henna for weddings in Mesopotamia and the eastern Mediterranean.

Henna will tolerate long droughts and high heat, but not moist soil or frost. Henna does not grow in any area where there is frost. Henna does not grow in rainforest climates. Muslim traders and settlers brought henna to western Australia, and it has naturalized there. Henna has never grown in the Americas other than as an imported decorative plant, though there are a few permanently warm and frost-free areas where it could be naturalized in Mexico and Argentina.

¹ Boubaya, Anissa, Hédia Hannachi, Nidhal Marzougui, Tebra Triki, Ferdaws Guasmi, and Ali Ferchichi. 2013. "Genetic diversity assessment of *Lawsonia inermis* germplasm in Tunisian coastal oases by ISSR and RAPD markers." *Dendrobiology* 69, 31-39.



Map of henna growth range during present climate

In the 19th and early 20th centuries, most of the henna used by Europe and the USA was grown in Egypt. India is presently the largest exporter of henna. Indian governmental support of the henna industry has improved henna cultivation and technology, helping farmers to have a cash crop during years when other crops fail. The Indian government also supplies free henna plants to farmers in the arid western region to protect their soils from desert encroachment.



Henna, *lawsonia inermis*

Henna's leaves have a red-orange dye molecule, lawsone, visible in young leaves in the center vein of the leaf. The petiole, the center vein of the leaf, has the highest lawsone content.² Henna branches grow quickly after rainy spells. The small branches are then pruned back and the leaves stripped away and allowed to dry. On commercial henna plantations, little trees are often pruned of their branches back to 3' tall; the branches will regrow quickly after the next rain. Henna may be pruned for leaf harvesting up to three times a year, and may live for fifty years. In western India where there are large fields of henna, the November harvest generally has the highest lawsone content, following the rapid growth from the monsoon rains.

² The petiole is the center rib of a leaf. In Romaine lettuce, the petiole is the crunchy center part.



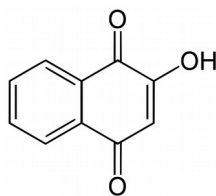
Henna, *lawsonia inermis*

The fully-grown leaves contain 0.3% to 4% lawsone content, depending on climate, weather, and soil conditions. Commercially available pure henna sold for body art typically has 1.7% to 2.4% lawsone after milling, packaging and export. The lower dye content leaves, 0.3% to 1.1% lawsone, roughly powdered and sifted, are sold to the hair dye industry.³ It is not true that the greenest leaves have the highest dye content, though brown leaves may be poor quality. Indian henna exporters often put green dye in their henna to make it appear to be better quality.⁴ Ancient Sunrise® henna does not contain green dye.

Henna leaves may be used fresh, though they are more often harvested, dried, and powdered. When pulverized henna leaves, fresh or dried, are mixed with a mildly acidic fruit juice, the lawsone molecules in the henna will be made available for dyeing. If you put henna paste on skin or hair, the lawsone molecules will migrate from the plant pulp, into keratin, leaving a red-orange stain. This action is similar to putting a wet teabag on a white tablecloth. The longer you leave the wet teabag on the tablecloth, the darker the stain. The longer you leave the henna paste on skin or hair, the more lawsone molecules will have the opportunity to migrate into the keratin, though six hours generally is enough for maximum absorption of henna. As more lawsone molecules migrate into keratin, the more saturated and rich the color.

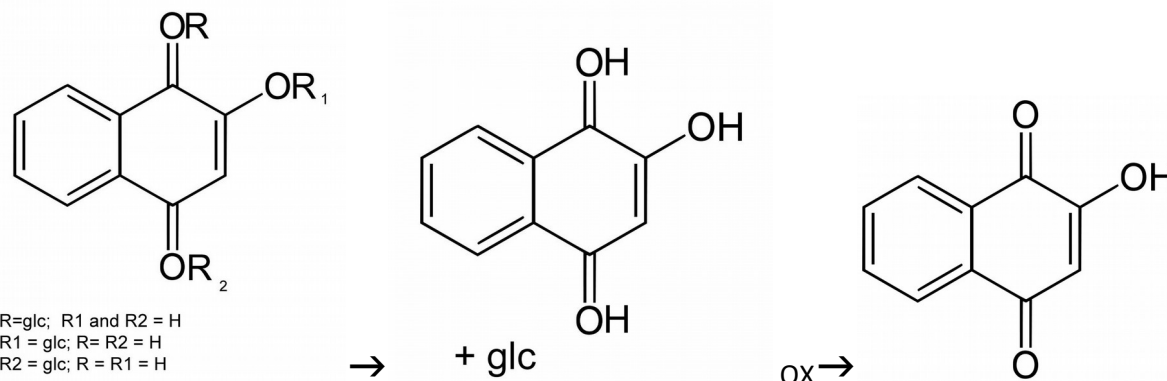
³ Between 2008 and 2014, the date of this writing, I have submitted several dozen samples of henna to an independent certified laboratory for HPLC lawsone testing. The results have ranged from 0.3% to 3.4% lawsone, all done by the same laboratory using the same methodology.

⁴ Chourasia, Sardar, Patil, and Mathew, Kanpur. "Study of Quality Characteristics of Henna." *Essential Oil Association of India*. India. 1989



Lawsone is the dominant dye molecule produced by henna.

An HPLC test of powdered henna leaves generally shows 0.5% to 3% lawsone, a red-orange naphthaquinone molecule⁵ which readily, harmlessly, binds with and stains keratin. This staining action is facilitated when the powdered henna leaf material is mixed with a mildly acidic medium; a pH 5.5 paste mix is ideal. At this mildly acidic pH, the lawsone molecule can be released from its position on the tannin and migrate from henna paste to stain keratin.⁶ A Michael Addition facilitates a non-fading stable bond of the lawsone molecule with keratin. This red-orange stain can gradually oxidize to a brownish color when bound with keratin. In alkaline conditions, the stain can oxidize to black or greenish black.



The sequence of henna dye release and binding is as follows:⁷

Lawsone is produced by hennocide precursors in the henna leaf.

The precursor is converted into the intermediate aglycone by hydrolysis in mildly acidic conditions. The aglycone intermediates will bind to keratin. Neither the precursor nor the final lawsone will bind as effectively to keratin as the aglycone intermediate.⁸ In the mildly acidic henna paste at room temperature, the aglycone will become available after about an 8 hour soak, and remain at maximum in the paste for 12 – 24 hour hours, after which the percentage of the

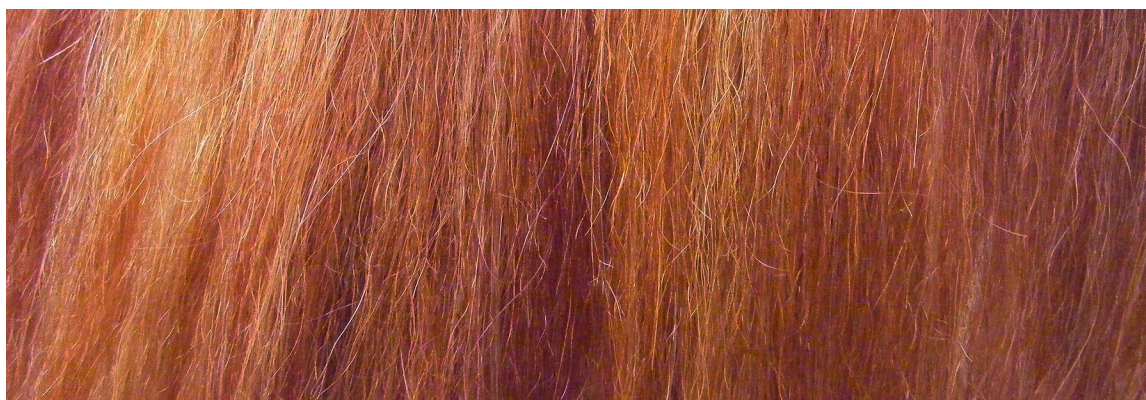
⁵ HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC, 2008 - 2016

⁶ Amro, I. H., James, K. C, and Turner, T. D.; 1993. "A Quantitative Study of Dyeing with Lawsone," *Journal of the Society of Cosmetic Chemistry*, **45**, 159 - 165

⁷ Gallo, F., Multari, G., Palazzino, G., Pagliuca, G., Zadeh, S. M. M., Biapa, P. C. N., Nicoletti, M.; 2014. "Henna through the Centuries: A quick HPTLC analysis proposal to check identity." *Revista Brasileira de Farma Cognisia*. **24**, 2

bindable aglycone form of the lawsone molecule will gradually diminish. This is termed 'demise' of the henna paste. At this point the henna paste produces diminishing stains. This transformation is gradual at room temperature. It proceeds more quickly in warm conditions and slows under cold conditions. Eventually all of the unstable aglycones will transform to the stable non-bindable form of keratin. This usually happens in about one week at room temperature; in henna work, this is referred to as demise. This demised henna paste stains keratin a weak orange color which will not darken because it can no longer bind through Michael Addition.

The acidic paste maintains the hydrogen atoms on the corners of the aglycone, the intermediate form of the lawsone molecule. In acidic mixes of henna, the intermediate form of lawsone will migrate into the keratin in hair or skin, and darken as it binds permanently with the keratin by a Michael Addition.⁹ If the henna powder is mixed only with water, the hydrogen atoms are not as well conserved. Henna mixed with water is more likely to fade from hair because unbound lawsone will gradually wash out of hair. Henna mixed with a mildly acidic mix will leave a stain in hair that is not only permanent, but will gradually darken, and continue to darken for years.



Variations of color with different lawsone content hennas and different acidic mixes, on identical samples of light colored hair

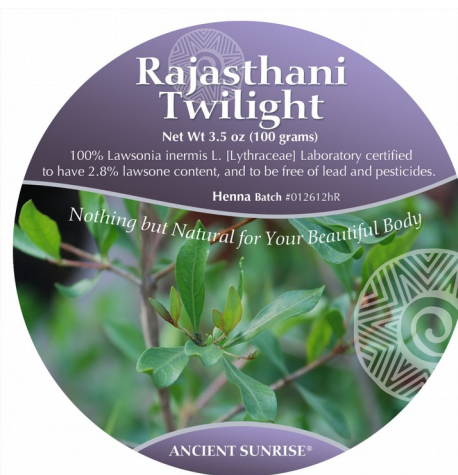
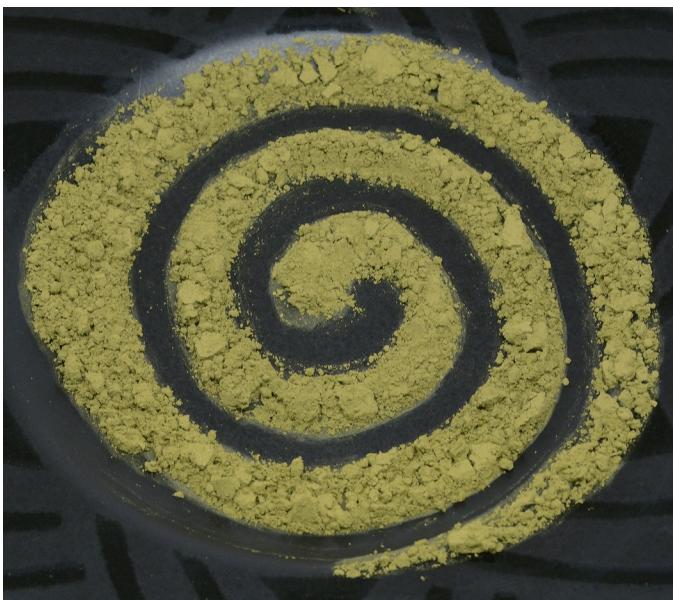
The lawsone in henna will dye hair some tone of red-orange, light coppery orange to dark auburn, depending on the base hair color, keratin structure, and the paste mixture. Every batch of henna has a slightly different level of lawsone because it is an agricultural crop. The genomes of cultivated henna plants are still close to the wild variants; the henna plant has not yet been modified, improved, and standardized. Weather and local soils affect the harvest. The harvesting, transport, storage, and milling processes have been improved over the last thirty years, producing a finer sift with water, air, and lightproof packaging, so the product stays fresh longer, but there is still a great deal of variation, even in one crop from one company.

⁸ Attempting to improve henna powder by adding lawsone powder will not improve the henna stain. Only the intermediate aglycone can effectively stain the hair.

⁹ Ruchi Badoni Semwala, Deepak Kumar Semwala, Sandra Combrinck, Catherine Cartwright-Jones, Alvaro Viljoen "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects." *Journal of Ethnopharmacology*, June 2014

Ancient Sunrise® submits every batch of henna to an independent laboratory for an HPLC lawsone test, to monitor the variability. Every batch is unique. Higher levels of lawsone are more effective for covering gray hair, mature to a browner tone. We recommend henna with higher lawsone levels those for dark-haired people who want to cover their gray hair. Lower levels of lawsone tend to stain hair lighter, brighter reds.

Henna powder smells a bit like a bale of hay. The earthy henna scent lingers in hair and on skin. People perceive the scent differently; some people love it, and some dislike it. If you dislike the scent of henna, mix ginger powder into the henna paste to reduce the odor. Cardamom powder can also mask the odor of henna. In any case, the henna scent will go away after a few shampoos.



Ancient Sunrise® Henna Powder

Pure henna, 100% lawsonia inermis, can condition and strengthen hair. The lawsone molecule, when bound into keratin, is a complete sunblock, protecting hair from UV damage. Pure henna preserves moisture in hair by making it less prone to weathering and desiccation. Hennaed hair can appear to grow faster because the ends are no longer drying and breaking off.

Pure henna, 100% lawsonia inermis, has a very high level of safety. Most sensations of itching are related to hay fever, a general mild allergic reaction to plants and pollens. Serious allergic reactions to henna are very rare but they do occur. Compound and adulterated hennas are far more likely to cause allergic reactions, particularly those which have unlisted copper salts or PPD. Ancient Sunrise® henna has purity insured by laboratory testing. If you feel intense itching or wheeze when you apply henna, rinse the henna out immediately, and never use it again. Consult your physician for further advice.



Henna with small clusters of flowers

In the Old Testament, henna is called *camphire*,¹⁰ from the Latin word for henna, henna being an Arabic/Semitic word for *lawsonia inermis*. Henna was a beloved plant growing near Ein Gedi oasis, planted as hedgerows around the vineyards. The fragrant clusters of flowers and dense brushy cover were a favorite place for lovers to spend time together, and love poetry included henna as a metaphor for a loving relationship that was as beneficial as it was enjoyable. As a hedgerow, henna protected the grapes from desert winds and sand. Present-day farmers in tropical, semi-arid zones use henna as a hedgerow to protect their fields against desert encroachment. Henna hedgerows keep wildlife and livestock out of farm gardens and define boundaries. When livestock have been nibbling at henna plants, their lips are stained red-orange as if they're wearing lipstick.

Most of the available lawsone will penetrate and bind to your hair's keratin when you henna your hair. Some lawsone will penetrate the outermost, dead layer of the scalp. About 1% of the lawsone actually in contact with skin migrates into the blood-bearing, living layer of skin. The small amount of lawsone that enters the blood stream is carried away by urine. A few people who leave their henna paste on overnight may be startled to see green urine first thing in the morning, but this seems to be absolutely harmless.¹¹ This green is the most oxidized form of the lawsone molecule, transformed from orange to green in the urine's alkalinity. There is no evidence that pure henna used as skin art or hair dye causes cancer and several medical journal articles have been published demonstrating the safety of unadulterated henna.¹² There is

¹⁰ Song of Solomon 1:14

¹¹ Asparagus will also harmlessly cause urine to be green. Fortunately, henna does not make urine smell 'skunky' as does asparagus.

¹² Kirkland, D., and Marzin, D. "An assessment of the genotoxicity of 2-hydroxy-1,4-naphthoquinone, the natural dye ingredient of Henna," *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, volume 537,

presently no contra-indication for using henna during pregnancy or nursing, though if the mother or child has homozygous G6PD deficiency, one's physician should be consulted.

Henna should not be used on children who have homozygous G6PD deficiency,¹³ a genetic blood enzyme deficiency.¹⁴ Male children are more likely to have homozygous G6PD deficiency than female children.¹⁵ This sex-based genetic characteristic is probably the reason that henna traditions are associated with women rather than men, and adults rather than children. If there is a reason for concern, such as a family history of this enzyme deficiency, a physician can run a simple blood test for G6PD.

Based on the LUKE II pesticide assays that I have run on every henna shipment for the last five years, there is minimal pesticide being used on henna, and papers from the Central Arid Zone Research Institute in Jodhpur, India, corroborate this.¹⁶ The henna in India from the Sojat region rarely has any pesticide residue, other than a trace of synthetic pyrethrum. The lead content of pure henna is rarely higher than the surrounding soil unless it is irrigated by water contaminated by industry runoff.

Henna leaves have only one dominant dye molecule, lawsone, no matter where or how henna grows. There is a narrow range of the amount of lawsone in the leaves based on the climate and soil where the henna was grown, most commonly from about .03% to 2.5% dry weight after milling and sifting. At present, there is no systematic 'in field' spectroscopic testing of lawsone content; if such could be accomplished, growers could begin plant genetic improvement and consumers would have a better end product. Leaves are the only part of the henna plant that is in

June 6, 2003.

Marzin, D., and Kirkland, B. "2-hydroxy-1,4-naphthoquinone, the natural dye of Henna, is non-genotoxic in the mouse bone marrow micronucleus test and does not produce oxidative DNA damage in

Chinese hamster ovary cells." *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 2004 May 9;560(1):41-7.

¹³ Eghbal, Fariba, Mohammad Reza Fakoorziba, Mohammad Hossein Eghbal, and Soheila Latifnia. 2012. "Survey on causes of hemolysis in Glucose-6-Phosphate Dehydrogenase (G6PD) deficient pediatric patients." *Pakistan Journal Of Medical Sciences* 28, no. 3: 666-669. Academic Search Complete, EBSCOhost (accessed February 26, 2014).

¹⁴ Fariba Eghbal et al. Survey on causes of hemolysis in Glucose-6-Phosphate Dehydrogenase (G6PD) deficient pediatric patients." *Pakistani Journal of Medical Science*. 2012 Vol. 28 No. 4

¹⁵ Homozygous G6PD deficiency is inherited in the same way as hemophilia, carried harmlessly by the mother on her x chromosome, but presenting in a male child. A physician can determine whether a person has G6PD deficiency with a simple blood test.

¹⁶ Pesticides from neighboring farmer's vegetable or cotton crop may blow over into henna and settle on the leaves. This 'pesticide drift' leaves traces that show up on LUKE II tests. Laboratory tests show henna occasionally has organophosphate pesticide drift from cotton fields; other times it has more benign drift from tomato fields. "Certified Organic" may not include residue from pesticide drift. For this reason, Ancient Sunrise® runs a full panel of tests on every shipment to insure that there is no trace of pesticide. These independent laboratory reports are also part of constructing a research database for henna, indigo, and cassia.

henna hair dye, not wood, roots, or bark.¹⁷ Henna is an earth-friendly crop. It can be grown and harvested without machinery, specialized labor, irrigation, or fertilizer. Henna cultivation requires no fossil fuel.

Demand for PPD-free permanent hair dye will increase because more people are becoming severely allergic to –diamine oxidative dyes and related chemicals. Medical research on the therapeutic potential for henna will progress. Henna plant improvement, processing, and quality should follow to supply a growing market for henna powder of standardized sift, purity, and lawsone content. ‘Mystery mixtures’ marketed as henna have contributed to a perception that henna is an inferior, problematic hair dye, due to faulty medical diagnoses and test result, as well as injuries to people using the products. Ancient Sunrise® products are all tested by an independent laboratory to insure purity for safety and assurance. Ancient Sunrise® will provide a safe way for people who are allergic to chemical hair dye to color their hair, for sensitized stylists to keep their jobs, and for the full therapeutic potential of henna to be realized.¹⁸

Pure henna can be used to relieve dandruff,¹⁹ athlete’s foot,²⁰ head lice,²¹ ringworm,²² and pain from diabetic neuropathy.²³ Pure henna can be used on a woman’s nipples to relieve thrush, and to decrease the pain of nursing. Only a physician may make a diagnosis or prescribe treatment

¹⁷ It is not unusual for exporters to claim that variously colored henna hair dye products are pure henna, with henna roots and bark added to vary the color. This is false: it is not botanically possible. These compound henna products typically include other unlisted plants and metallic salts. The countries that produce these products do not have laws requiring declaration of ingredients in henna products, and the importing countries, including the USA, often do not require that the importer examine and declare insufficiently or misleadingly declared henna products. Ancient Sunrise® sends every shipment to an independent certified laboratory to insure that there are no contaminants or adulterants in what they sell.

¹⁸ Pradhan, Rohan, Prasad Dandawate, Alok Vyas, Subhash Padhye, Bernhard Biersack, Rainer Schobert, Aamir Ahmad, and Fazlul H. Sarkar. 2012. "From Body Art to Anticancer Activities: Perspectives on Medicinal Properties of Henna." *Current Drug Targets* 13, no. 13-14: 1777-1798. Academic Search Complete, EBSCOhost (accessed February 26, 2014).

¹⁹ Berenji, Fariba, Hassan Rakhshandeh, and Homeyra Ebrahimipour. 2010. "In vitro study of the effects of henna extracts (*Lawsonia inermis*) on *Malassezia* species." *Jundishapur Journal of Microbiology* 3, no. 3: 125-128.

²⁰ Rahmoun, Nadjib, et al. "Antifungal activity of the Algerian *Lawsonia inermis* (henna)." *Pharmaceutical Biology* 51, no. 1 (January 2013): 131-135. Academic Search Complete, EBSCOhost (accessed February 26, 2014).

²¹ M. El-Basheir and Mahmoud A. H. Fouad (2002) A Preliminary Pilot survey on Head Lice, Pediculosis in Sharkia Governate and Treatment of Lice with Natural Plant Extracts. *Journal of the Egyptian Society of Parasitology*, Vol 32, No 3, December 2002, Zeinab

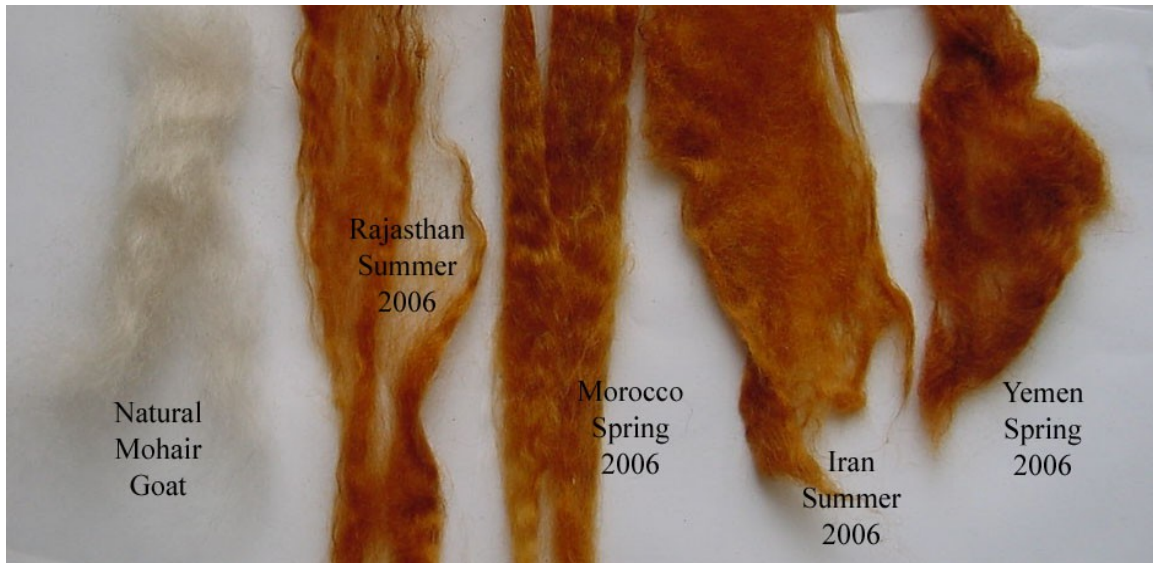
²² Bosoglu A., Birdane F., and Solmaz H., (1998) “The Effect of Henna Paste in Ringworm in Calves.”

Department of Internal Medicine, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkiye

Indian Veterinary Journal 75, January

²³ Mutluoğlu M, Uzun G., (2009) “Can henna prevent ulceration in diabetic feet at high risk?” *Experimental Diabetes Researrch*. 2009:107496.

for an illness, but with a physician's permission, pure henna can provide inexpensive and effective relief for these minor irritations.



Henna from different countries stains hair within a narrow range of color, only varying by amount of lawsone produced naturally in the leaves.



There are many wild variants of henna. The flowers may be white (*alba*), pink (*rubra*), red (*miniata*) or yellow, the leaves all contain lawsone. Only chemical additives can make henna blue, green, yellow, violet, or black.

Ancient Sunrise® Henna for Hair, Chapter 5, Plants that Dye Hair

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