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Contents

Original articles

- Experimental study on mechanical behaviors of polyoxymethylene woven tube-reinforced cementitious composites 311
Fayun Wei, Fan Yang, Kai Feng, Zhiqian Yang, Bin Yu, Hailou Wang and Wei Zhang
- Quantitative analysis of the relationship between the male body shape and block pattern based on the three-dimensional human model 323
Ming Xia, Hongyv Lu, Xiao Xu and Lixiang Zhang
- Electrospinning drafting system for polyamide 6 and polyamide 6.6 nanofiber production 341
NJR Belino, TRA Barata and Roshan Paul
- Efficient purification of high-salt rural groundwater in the areas along the Yellow River in Henan Province, China, by hollow fiber membrane distillation process 357
Hongbin Li, Linmao Li, Mengjie Fan, Wenying Shi and Jie Liu
- Effect of fabric properties on the laundry movement, mechanical action, and motor current of front-loading washers 375
Eugene Song, Mingyeong Kim, Seokhee Chang, Heewon Choi, Siyeon Lim, Sungmin Kim and Jooyoun Kim
- The effects of the support panel of compression leggings on the provision of low pressure for foot progression angle correction 390
Hye Suk Kim
- Tensile theoretical modeling and auxetic behavior analyzing of negative Poisson's ratio yarn 401
Junli Chen, Xiaojing Wen, Xiang Liu and Zhaoqun Du
- Optimization design of functional running pants and evaluation of thermal-humid comfort capabilities 415
Ning Guo, Zhijiang Li, Nanliang Chen, Hong Dai, Kaixuan Liu and Peihua Zhang
- Titanium dioxide release behavior during wash of UV-protective garments by total reflection X-ray fluorescence 427
Diego de Dio Ferreira, Fábio Luiz Melquiades and Carlos Roberto Appoloni
- The effects of fixed and graduated application percentage-type compression leggings in adult women with mild in-toeing gait 434
Hye Suk Kim
- The construction of a three-dimensional human body based on semantic-driven parameters to improve virtual fitting 451
Wenqian Feng, Xin Rong Li, Xingxing Li, Yuzhuo Li, Jiaqi Wen, Hansen Li and Jingqiu Fang
- Investigation of some properties of yarns and fabrics made with different blend ratios of flame-resistant fibers 463
Sibel Şardağ and Dilhan Erkurt
- 7th new insect species with high potential to be used in the production of a natural red dye: *Dactylopius opuntiae* (Cockerell, 1896) (Hemiptera: Dactylopiidae) 477
Mustafa Genc and Bengu Aydin

Review articles

- Fiber movement during twisting in air vortex spinning 484
Qing Wang, Xushan Lv, Shuai Dang, Gaoxiang Liang, Yuefu Jiang and Tiantian Zhao

BaFe ₁₂ O ₁₉ /graphene/polyaniline coatings for flexible fabrics with enhanced microwave absorption <i>Liu Jie, Zhang Zhaohuan, Dong Zijiang, Chen Min, Sun Runjun, Dai Jiang, He Caiting, Zhu Binghui and Li Yunyu</i>	494
Recent advances in recycling technologies for waste textile fabrics: a review <i>Rivalani Baloyi Baloyi, Oluwatoyin Joseph Gbadeyan, Bruce Sithole and Viren Chunilall</i>	508

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7th new insect species with high potential to be used in the production of a natural red dye: *Dactylopius opuntiae* (Cockerell, 1896) (Hemiptera: Dactylopiidae)

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Mustafa Genc and Bengu Aydin 

Abstract

There is a total of six insect species belonging to four families from the order Hemiptera, used in the production of red dye as a natural dyestuff. Carmine is obtained from *Dactylopius coccus* (Costa, 1829) (Hemiptera: Dactylopiidae) feeding on prickly pears (*Opuntia* spp.). The genus *Dactylopius* includes a total of 11 species, and none of them except *D. coccus* were declared as a dye insect. The reported use of *Dactylopius opuntiae* (Cockerell, 1896) as a natural dye insect, was more widespread than *D. coccus*, and advantages versus *D. coccus* were announced for the first time in this study. To prove the hypothesis that *D. opuntiae* could be used as a natural dyestuff, silk fabric samples were dyed with *D. opuntiae*, naturally distributed in Cyprus. The light, wet and dry friction fastness values of the dyed fabric samples were tested. Also the carminic acid values of *D. opuntiae* were analyzed by high performance liquid chromatography analysis and surface imaging analysis (scanning electron microscope equipped with energy dispersive X-ray spectrometer). The results of the fastness values of the dyed fabric samples and the high carminic acid content of *D. opuntiae* showed that this species has a very high potential to be used as the 7th natural dye insect in the world.

Keywords

Fastness values, HPLC, natural dyestuff, new dye insect, SEM-EDX

Natural dyes have been used for coloring of fibers like silk, cotton and wool as well as food substrate and leather from prehistoric times. Due to increasing environmental awareness, people now avoid some hazardous synthetic dyes that cause water pollution and waste disposal problems. Natural dyes used in textiles have nonallergic, nontoxic and environmentally friendly properties, therefore the use of natural dyes in textiles have become a matter of significant importance.^{1,2}

Natural dyes are extracted from a variety of natural substances such as some plants (e.g. daisy, indigo, and saffron), some animals (e.g. mollusks or shellfish), some minerals (e.g. clay, ferrous sulfate, and ochre), and some insects (e.g. cochineal beetles and lac scale insects) without any chemical treatment.³ A spectrum of elegant natural colors ranging from yellow to black exists in the natural dyes.⁴ In this spectrum, the red color obtained from insects has a special place and importance. There are six insect species that are used in the production of red dye as a natural dyestuff. Well known examples are the reds based on the ‘laccic acids/lac-dye’ from lac insects (*Kerria lacca* (Kerr, 1782) and *Kerria chinensis*

(Mahdihassan, 1923) (Hemiptera: Kerridae)), ‘kermesic acid/kermes’ from kermes (*Kermes vermilio* (Planchon, 1864) (Hemiptera: Kermesidae)), and ‘carminic acid/cochineals’ from cochineal (*Dactylopius coccus* (Costa, 1829) (Hemiptera: Dactylopiidae), *Porphyrophora polonica* L. 1758, *Porphyrophora hamelii* (Brandt, 1833) (Hemiptera: Margarodidae)).^{5,6} The female lac insects, Indian lac *K. lacca* and *K. chinensis*, secrete a red resin, stick-lac, from which is obtained both the lac dye and the shellac resin while in both cochineal (*D. coccus*) and kermes (*K. vermilio*) the red dye is obtained from the eggs of the female insects.^{6–9} Red or scarlet dyes are derived from the species of *Porphyrophora* spp. (e.g. Polish cochineal *P. polonica* (L. 1758) and Armenian cochineal *P. hamelii* (Brandt, 1833)). Although species of *Porphyrophora* also contain carminic acid, *D. coccus*

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which has been cultivated has a much higher content (15–20%) of the dye, compared with only 0.8% and 0.6% for the Armenian and Polish species, respectively.^{6,7}

Carmine, used in the production of red dye, and also known as cochineal, is obtained from *D. coccus* feeding on prickly pears (*Opuntia* spp.) and distributed in South and North America. Carminic acid, which gives a reddish and purplish color, obtained from dried female *D. coccus* individuals, was an important export product used to color fabrics in the 16th century, but now it is a natural dyestuff that is frequently used in food and cosmetics.⁹

As a result of the discovery and spread of synthetic dyes in the late 19th century, the use of natural dyes decreased. Environmental problems arising from the use of synthetic dyes have brought about hesitation regarding the safety of their use. The negativity brought about by the use of unnatural dyestuffs has made natural dyestuffs obtained from cochineal attractive again, and *D. coccus* cultivation has become widespread, especially in Peru. Currently, in addition to Peru, Mexico, Chile, Argentina and the Canary Islands are among the major natural dyestuff producing countries.¹⁰

The genus *Dactylopius* includes a total of 11 species, with *Dactylopius gracilipilus* newly described, and none of them except *D. coccus* were declared as a dye insect.^{11,12}

Dactylopius opuntiae (Cockerell, 1896) spreads in Africa (Cape Verde, Kenya, Madagascar, Mauritius, Morocco, Reunion Island, South Africa, and Zimbabwe), Asia (India, Israel, Jordan, Lebanon, Pakistan, and Sri Lanka), Europe (Cyprus, France, and Spain), North America (Jamaica, Mexico, and the United States), South America (Brazil) and Australia.^{13–24} *D. coccus* as well as *D. opuntiae* feeds on cactus species of the genus *Opuntia*, which includes species called prickly figs. These *Opuntia* species are known as ‘Eşek inciri’, ‘Hint inciri’, ‘Frenk inciri’, ‘Dikenli inciri’ and/or ‘kaynanadili’ in Turkish and ‘Babutsa’ in Cyprus (Figure 1). No scientific studies have been conducted on the possibilities of using *D. opuntiae* as a natural dye insect.

In this study, *D. opuntiae*, which is naturally distributed in Lefkoşa (Nicosia) Turkish Republic of Northern Cyprus and the main pest of *Opuntia* spp., was used as a natural dye and silk fabric samples were dyed with this natural dyestuff. The ‘light’, ‘wet friction’, and ‘dry friction’ fastness values of the dyed fabric samples were tested. In addition, the carminic acid values of this species were analyzed by high performance liquid chromatography (HPLC) analysis. The high carminic acid content of *D. opuntiae* and the results of the fastness values showed that this species has a very high potential to be used as the 7th natural dye insect in the world. This study has shown



Figure 1. Nymphs and adult females of *Dactylopius opuntiae* covered by wax on *Opuntia* spp.



Figure 2. Wild cochineal (*Dactylopius opuntiae*) colony and the carminic acid it produces.

for the first time that *D. opuntiae* is a new insect species that can be used as a natural dyestuff.

Materials and methods

Materials

Cochineal (*D. opuntiae*) was used as the natural dye material and *D. opuntiae* specimens were provided from Lefkoşa, Turkish Republic of Northern Cyprus by the first author in September 2022 (Figure 2). Alum [$KAl(SO_4)_2 \cdot 12H_2O$] was used as a mordant material from Labor-Technical Laboratory Materials Industry and Trade Joint Stock Company, Istanbul, Turkey. Knitting silk fabric was used for dyeing. Dyeing was carried out with tap water with a pH of 7.4.

Methods

Silk fabric weighing 12 g was boiled with 1.2 g alum in 1.5 l of water in a glass beaker at 85°C for 1 h.

Afterwards, the silk fabric samples were washed in cold water, rinsed and left to dry, then 18 g of dried *D. opuntiae* samples were crushed into flour in a ceramic bowl (Figure 3).

The natural dyestuff turned into flour was boiled in 1.5 l of water in a glass beaker at 85°C for 1 h. This boiling solution was left to stand for 1 day. The mordanted silk fabric samples were boiled in the dye solution, which was left for 1 day, at 85°C for 1 h (Figure 4). The dyed silk fabric was washed in cold water and left to dry, and the dyeing process was completed.

Fastness properties. Fastness tests, washing fastness, dry and wet rubbing fastness, and light fastness of dyed



Figure 3. Dried *Dactylopius opuntiae* specimens before it was crushed into flour in a ceramic bowl.

fabrics, were conducted in the DATU Cultural Heritage Preservation and Natural Dyes Laboratory in Istanbul, Turkey.

The washing fastness, dry and wet rubbing fastness, and light fastness of dyed fabrics were determined according to ISO105: C06 (A1S), ISO105-E04, ISO105-X12, and ISO105-B02 standards, respectively. The ISO 105: C06 A1S fastness test was carried out at 40°C for 30 min containing 10 steel balls. The dyed samples were exposed to the light for 48 h from a xenon arc lamp (250 W).

Analysis

HPLC analysis. Chromatographic measurements were carried out using an Agilent 1200 series system (Agilent Technologies, Hewlett-Packard, Germany). HPLC analysis was performed to determine carminic acid and its ratio in dyed fabric patterns and compare with the standard carminic acid curve.

SEM-EDX analysis. The dyed silk fabrics were analyzed by a scanning electron microscope equipped with an energy dispersive X-ray spectrometer (SEM-EDX) for surface imaging analysis with a secondary electron (SE) detector. The samples were placed on staples with C tape to ensure conductivity. low vacuum mode was used. The chamber pressure where the samples were placed was determined as 60 Pascals.

Results and discussion

Fastness properties

The results of light fastness, dry and wet rubbing fastness, and washing fastness of dyed fabrics are given in Table 1.

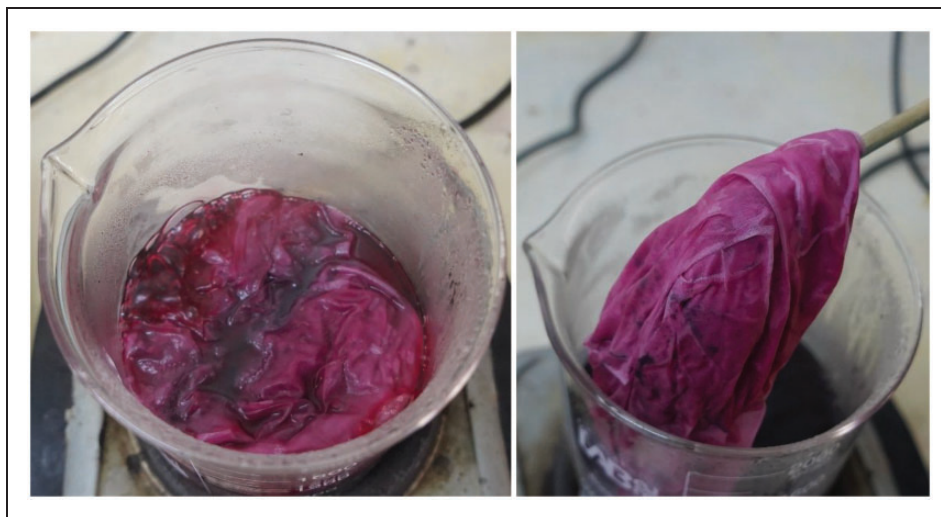


Figure 4. Dyeing and boiling process image of mordanted silk fabric samples with natural dyestuff, *Dactylopius opuntiae*, (left), color obtained before washing process (right).

Table 1. Light, rubbing and washing fastness test results of silk fabric patterns dyed with *Dactylopius opuntiae*

Light fastness	Rubbing fastness		Washing fastness – staining					
	Dry	Wet	Acetate	Cotton	Nylon 6.6.	Polyester	Acrylic	Wool
5	4–5	4	4	4–5	4–5	4–5	4	4–5

Fastness characteristics: 1: little; 2: medium; 3: good; 4: fairly good; 5: very good.

ISO105: C06 (AIS), ISO105-B02, ISO105-X12, and ISO105-E04.

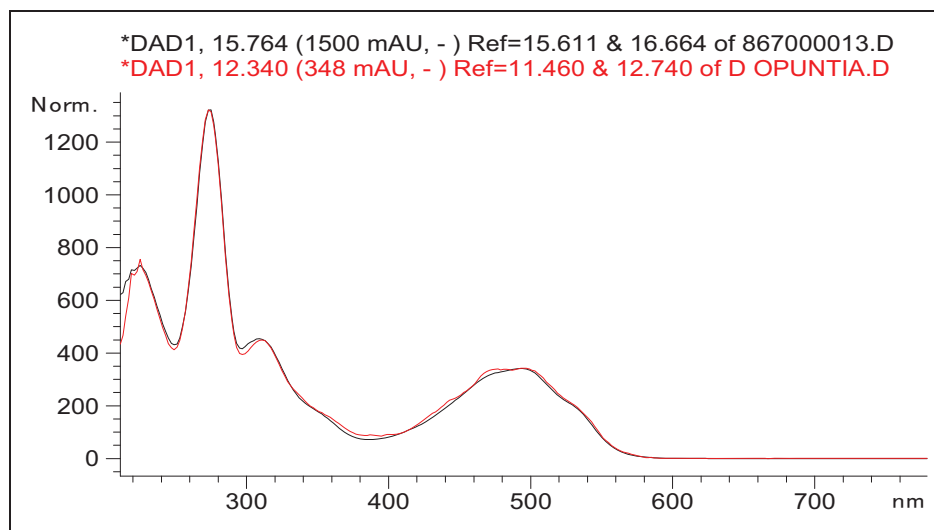


Figure 5. Comparison standard carminic acid curve (black line) with determined carminic acid in dyed fabric with *Dactylopius opuntiae* (red line).

The results of previous studies with *D. coccus* show that the light fastness is in the range of 4 to 5.²⁵ Analysis results showed that the light fastness values of fabric samples dyed with *D. opuntiae* reached a maximum value of 5. When the light fastness values of fabric samples dyed with *D. opuntiae* were compared with *D. coccus*, it was determined that the light fastness value of *D. opuntiae* was found to be higher than *D. coccus*.²⁵ Results of rubbing and washing fastness test results of silk fabric patterns dyed with *D. opuntiae* were found to be very similar to those of *D. coccus*. These results show that silk fabrics dyed with *D. opuntiae* are as effective as *D. coccus*.

HPLC analysis

HPLC analysis was performed to determine the similarity of carminic acid in dyed fabric samples obtained by using *D. opuntiae*, compared with the standard carminic acid curve (Figure 5).

HPLC analysis results show that the standard carminic acid curve and the carminic acid curves obtained from fabric samples dyed with *D. opuntiae* overlap (Figure 5). According to this result, it was revealed that the carminic acid curve contained in *D. opuntiae* was quite close to the standard carminic acid curve.

Also, HPLC analysis results show that silk fabrics dyed with *D. opuntiae* contain 95% carminic acid and 5% dc (II) and dc (IV). The literature shows that dc (II) and dc (IV) are natural dyestuffs found together with carminic acid belonging to the cochineal. We think that dc (II) and dc (IV) belong to the waxy layer secreted to protect itself from natural enemies found in the cochineal. The waxy layer of wild cochineal (*D. opuntiae*) is covered with cottony wax, and fine cochineal (*D. coccus*) is covered with powder wax.²⁶ One of the steps for obtaining dye from *D. coccus* is the sieving method. In the sieving process, the waxy layer on *D. coccus* is removed from the female individual. Because the waxy layer on *D. coccus* is powder wax, most of this waxy layer is easily separated from the female during the sieving and sun drying phases. However, it is not easy to remove the waxy layer from *D. opuntiae* by sun drying and/or sieving. This is because the waxy layer on the female individuals of *D. opuntiae* is cottony and this cottony waxy layer is very attached to the female body (Figure 6). dc (II) and dc (IV) dyestuffs obtained as a result of HPLC analysis were estimated to come from the cottony waxy layer of *D. opuntiae*. For this reason, we thought that there was no need to separate the cottony waxy layer, which was



Figure 6. Colony of *Dactylopius opuntiae* (wild cochineal) covered with cottony wax (left) (photographed by the first author), *Dactylopius coccus* (fine cochineal) covered with powder wax (right) (© Frank Vincentz).

already very difficult to separate, from the body of the insect female and that these components were already dyestuffs.

The fact that there is no need to do the sieving method applied in obtaining the insect to be used as a dyestuff and the cottony waxy layer can be used directly as a dyestuff increases the advantage of *D. opuntiae* versus *D. coccus*.

SEM-EDX analysis

Carminic acid is more soluble in the alum aqueous solution than in the water. SEM-EDX analysis results are given in Table 2 and Figure 7.

According to the elemental analysis results, it is seen that the sum of carbon (C), oxygen (O), and nitrogen (N), which is the important criterion of organic structures, of the fabric samples dyed with cochineal is 97.13%. In addition, EDS analysis revealed that fabric samples dyed with cochineal contains sodium (Na), aluminium (Al), sulphur (S) and potassium (K) elements apart from C, O, and N.

EDS analysis results of fabric samples dyed with *D. coccus* (0.3%) showed similar results to our study and the C and O ratio was found to be 69.70 and 29.59, respectively.²⁵ This result shows that the C and O ratio of *D. opuntiae* is slightly higher than that of *D. coccus*.

Conclusions

This study has revealed for the first time that *D. opuntiae* is a new insect species that can be used as a natural dyestuff. The possibilities of using *D. opuntiae* as a natural dyestuff were tried to be confirmed by some analysis.

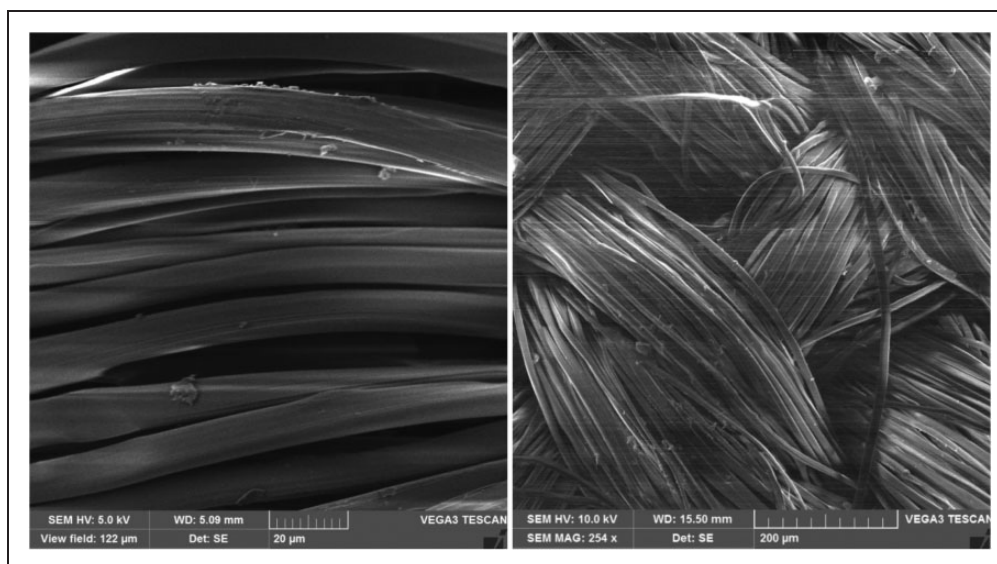
Results of rubbing and washing fastness tests of dyed silk fabrics with *D. opuntiae* were found to be similar to those of *D. coccus*. Also, the light fastness values of fabric samples dyed with *D. opuntiae* were compared with *D. coccus* and it was found that the light fastness value of *D. opuntiae* was higher than that of *D. coccus*. These results show that silk fabrics dyed with *D. opuntiae* are as effective as *D. coccus*. The HPLC analysis method confirmed that the carminic acid curve in fabric samples dyed with *D. opuntiae* overlapped with the standard carminic acid curve. In addition, the HPLC analysis result shows that dc (II) and dc (IV) are natural dyestuffs found together with carminic acid belonging to the cochineal. We think that these dyestuffs obtained from the cottony waxy layer on the body of female individuals do not need to be removed from the body by the sieving method. It has been proved by this study that it is possible to carry out the dyeing process without removing the waxy layer from the insect. The fact that the cottony waxy layer does not need to be further removed from the female body gives *D. opuntiae* an advantage over *D. coccus*. EDX analysis revealed that dyed fabrics with *D. opuntiae* had similar element ratios to those of *D. coccus*.

D. opuntiae, which has a high potential to be used as a natural dyestuff, is advantageous over *D. coccus* since it has spread in many parts of the world such as Africa, Asia, Europe, America, and Australia. *D. opuntiae* is considered the main pest on cactus in many countries. For this reason, many countries are struggling with this pest because it feeds on the cactus, which generates economic income. Unfortunately, pesticide applications are at the forefront of the control methods against this pest. If *D. opuntiae* can be cultivated for a natural

Table 2. Results of structure determination and elemental analysis (carbon coated) with SEM-EDX

Element	AN	Series	Unn. C. (wt.%)	Norm. C (wt.%)	Atom. C (at.%)	Error (1 sigma) (wt.%)
C	6	K-series	63.44	63.44	70.45	7.06
O	8	K-series	33.59	33.59	28.00	4.04
Na	11	K-series	1.97	1.97	1.14	0.15
Al	13	K-series	0.32	0.32	0.16	0.04
S	16	K-series	0.34	0.34	0.14	0.04
K	19	K-series	0.24	0.24	0.08	0.04
N	7	K-series	0.10	0.10	0.02	0.05
		Total	100.00	100.00	100.00	

SEM-EDX: scanning electron microscopy with energy dispersive X-ray spectroscopy.

**Figure 7.** The scanning electron microscopy (SEM) image of dyed fabric with cochineal (left 500× and right 254×).

dyestuff source and grown in a controlled manner, it will make a great contribution to the world economy. *D. opuntiae* has also an advantage due to the proximity to the importing countries of natural dyestuffs.

If *D. opuntiae* can be cultured and grown in a controlled manner, it can be used as a natural dye in cosmetics and food products. Thus, *D. opuntiae* can be eliminated from the pest category and turned into an economically valuable crop.

Declaration of conflicting interests

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