JAP&T
TPA-Timisoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2021, *27* (2), 94-99

Journal of Agroalimentary Processes and Technologies

Effectiveness of some plant extracts against the different stages of the cochineal insect *Dactylopius opuntiae* on prickly pear in Syria

Imad Idris [1], Ghaleb Tayoub [2], Yaser Bakri [1]

[1] *Department of Biotechnology, Atomic Energy Commission of Syria (AECS), 17th Nissan Street, Damascus, Syria*
[2] *Faculty of Pharmacy, Tishreen University, Latakia, Halap, Syria*

Abstract

The cochineal, *Dactylopius opuntiae*, has recently become the main pest that damages the prickly cactus, *Opuntia ficus-indica*, plants in Syria. This study was conducted to evaluate the effects of juniper *Juniperus foetidissima*, rosemary *Rosmarinus officinalis*, and tobacco *Virginia tobacco* plant extracts for controlling of *D. opuntiae* under laboratory and field conditions. The results showed that the effect of *V. tobacco* plant extract was significantly superior compared to the effect of juniper and rosemary extracts on *D. opuntiae*. The 100% crude enzyme extract of *B. subtilis* had a significant effect on cochineal mortality after 1 day of treatment, but this effect was not significantly improved after 5 days of treatment. The findings of our study indicate that ethanol extract of tobacco leaves and crud enzymes produced by *B. subtilis* could be used in development and implementation of a biological control program against *D. opuntiae* under field conditions.

Keywords: biocontrol, Virginia tobacco, cochineal, juniper, rosemary

1. Introduction

Pests cause a variety of damages to plants, including shortening the life of the plant, reducing its productivity, and damaging its parts including the fruits [5,15]. Combating plant pests is essential to avoid economic losses in crop production. Farmers use synthetic insecticides to combat pests; however, biological insecticides are potential alternatives to control pests and have the advantages of low toxicity to humans and animals. Those biological insecticides might substitute synthetic pesticides [2]. Many biological control agents specific to some insects and fungal targets have been reported such as enzymes, viruses, bacteria, essential oils, and plant extracts.

Cactus crops, especially the cactus pears, have attracted more attention worldwide due to their drought resistance, salt tolerance, and ability to grow under harsh conditions [9,14]. In countries like Syria, Jordan, and Lebanon, cactus pears have long been cultivated and are widely grown in rural areas. In the countryside of Damascus, cactus pears are cultivated mainly for fruit production. The cultivation of cactus pears in Syria increased in the last two years, due to the increased demand for fruit products. Newly cultivated areas with cactus pears were established in Homs, Sweida, and the coastal region [12].

The cochineal, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Coccoidea: Dactylopiidae) is considered the most destructive pest to cactus in Syria. However, the cochineal infestation was firstly reported in the southern region of Lebanon in 2012, and later in Palestine and southern Syria in 2016 [4,17,20]. Therefore, in the past few years, cochineal, which is widespread in cactus pear farms, has caused serious damages to cactus pears farms [4]. As a result, these damages reduced fruit production which constitutesa source of income for many farmers in the region [17].

* Corresponding author: ascientific@aec.org.sy

Although more than a century has passed since Cockerell first registered *D. opuntiae* in Mexico in 1896 [8], there are no methods to control this pest, including the use of synthetic insecticides [5,9].

The importance of enzymes in controlling various insects and plant diseases is well recognized. Chitinase produced by *Bacillus subtilis* and *Bacillus atrophaeus*has was used in combating *Spodoptera litura* and *Drosophila melanogaster* larvae, respectively [2,6]. In a previous study, we reported the efficacy of the crude enzymes produced by *B. subtilis*, the local strain SY 134D, in combating *D. opuntiae* insects under laboratory conditions [10].

On the other hand, many plants contain chemicals like organic acids and alkaloids that are toxic to many organisms including insects [7]. A previous reporthas demonstrated the effectiveness of essential oils and plant extracts of some plant species in combating the potato tuber moth *Pthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) [24]. In another study, the effective role of the essential oils extracted from juniper and rosemaryin controlling Khapra beetleswas demonstrated [22].

This study compares the effects of juniper *Juniperus foetidissima*, rosemary *Rosmarinus officinalis*, and tobacco *Virginia tobacco* plant extracts and the effects of *B. subtilis*(sy134d strain) crude enzymes at 100% concentration on the life cycle of *D. opuntiae* under laboratory and field conditions.

2. Materials and Method

2.1. Plants extracts

Plant samples, rosemary leaves, and young branches of juniper plants in the flowering period were collected from Yafour rural area near Damascus in May 2019. Leaves of mature tobacco plants (Virginia variety) were collected from Latakia area on the Syrian coast.

The plant samples were dried at laboratory temperature until the stable dry weight was achieved.

Dry plant materials were pulverized into fine powder separately.

A total of 100 grams of each dried pulverized plant material was put in a separate beaker and extracted for 24 hours with 500 mL ethanol (50%).

Each extract was filtered into a separate rotary evaporator and the ethanol was removed completely.

Extracts were stored in a refrigerator at 4 °C until used.

2.2. Bacterial crude enzyme production

A strain of bacterial *B. subtilis* (SY134D) isolated from Syrian soil by Bakri et al., 2012 [3] was used in this study. This strain can use wheat bran as a substrate and produce crude enzymes under solid-state fermentation (SSF). The crude enzymatic solution was stored at -20 °C until use. Details of enzymes production by this bacterial strain were previously reported by Idris et al., 2019 [10].

2.3. Testing the effects of plant extracts and bacterial crude enzymes on cactus plants infested with cochineal under laboratory conditions

Cactus cladodes were randomly collected from a cactus farm at Kaferkuk village, 35 kilometers to the west of Damascus. Twentyfour infested cladodes of cactus plants of the same size and infestation rate were selected. Those cladodesof cactus were subjected to the following treatments (three cactus cladodes/treatment): (A, sprayed with tobacco plant extract; B, sprayed with juniper plant extract; C, sprayed with rosemary plant extract; D, sprayed with Tween-20; E, sprayed with Sycrol; F, sprayed with deltamethrina pyrethroid insecticide; G, sprayed with 100% crude enzyme solution of *B.subtilis*(SY134D); H, were sprayed with water. Treated cladodes were maintained under 70% humidity, 25°C temperature, and 12:12 light/dark cycle. After 1 day and 5 days of treatment, an area of $10cm^2$a rate of 4 squares per cladode was randomly sampled to obtain effect readings. The guidelines of Zhang (2017)[28] for stereoscopic microscopy counting of female nymphs and adults were applied before and after each treatment for all experiments.

2.4. Testing the effects of plant extracts and bacterial crude enzymes on cactus plants infested with cochineal under field conditions

Cactus plants infested with cochineal were randomly selected from an infested cactus field at Kaferkuk village. The chosen infested cactus plants were of the same size and infestation rate. Those plants were subjected to the following treatments (3 infested cactus plants/treatment): A, sprayed with tobacco extract; B, sprayed with juniper extract; C, sprayed with rosemary extract; D, sprayed with Tween-20; E, sprayed with Sycrol; F, sprayed with deltamethrin; G, sprayed with 100% crude enzyme solution of *B. subtilis* (SY134D); and H, sprayed with water.

Random cladode/plant was sampled and tested. For each treatment, 4 spots of 10 cm^2 in size were randomly selected for each cactus plant. The guidelines of Zhang (2017) [28] for stereoscopic microscopy counting of female nymphs and adults were applied before and after each treatment for all experiments.

2.5. Statistical analysis

The corrected percentage of death of nymphs and adults was calculated according to the following formula [18]:

Percentage correction of mortality = (survival rate at control plot-survival rate at treatment plot) / survival rate at treatment plot ×100

All statistical analyses were performed using STATISTICA program version 6 (Statsoft, Inc. 2007) a 5% level (P=0.05). Obtained values were subjected to analysis of variance (ANOVA; Tukey's HSD test) to determine the differences in means between different treatments.

3. Results and Discussion

The cochineal, *D. opuntiae*, has recently become the main pest that damages cactus pear *Opuntia ficus-indica* plants in Syria, and the current situation is worrying. Investigating the capability of using biological insecticides like plant extracts and bacterial enzymes to control this pest is an urgent need.

4.1. The effects of plant extracts and crude enzymes on cochineal under laboratory conditions

As shown in Table 1 there was a significant difference in mortality mean of nymphs and adult females among treatments after 1 day (DF=6, F-value=611.29, P<0.0001 and DF=6, F-value=501.08, P<0.0001, respectively), and after 5 days (DF=6, F value=672.9, p <0.0001 and DF = 6, F value=510.03, p <0.0001, respectively). There was no significant difference, after 1 or 5 days, between Tween-20 and mineral oil treatments. The results also showed that the average mortality of nymphs and adult females increased significantly after 5 days, and all treatments had significant differences except for the treatment with bacterial enzymes.

4.2. The effects of plant extracts and crude enzymes on cactus plants infested with cochineal under filed conditions

The field results were consistent with the laboratory results.Statistical analysis indicated that there was a significant difference between the average mortality of nymphs and adult females. No significant difference was observed between Tween-20 and Sycrol treatments (DF=6, F-value=234.598, p<0.0001 and DF=6, F-value=386.166, p<0.0001, respectively) (Table 2).

Table 1. Mortality of nymphs and adult female *D. opuntiae* on the 1st and 5th day after treatment under laboratory conditions

Type	Treatments	**% Mortality**			
		1 day		5 days	
		Nymphs	Adult female	Nymphs	Adult female
		SD ± x̄	SD ± x̄	SD ± x̄	SD ± x̄
A	Tobacco extract	84.42 ± 3.5 Bb	77.4 ±5 Bb	91.7 ±5.1 Ba	83.4 ±3.1 Ba
B	Juniper extract	18.5 ±2.9 Fb	15.2 ±3.3 Fb	25.0 ±3.2 Fa	21.1 ±32.2 Fa
C	Rosemary extract	36.6 ±4.3 Eb	30.6 ±2.4 Eb	45.3 ±2.0Ea	38.7 ±4.0 Ea
D	Tween-20	53.1 ± 4.5 Db	51.4 ±4.1 Db	63.0 ± 3.8 Da	55.8 ±3.4 Da
E	Sycrol (mineral oil)	54.4 ±3.5 Db	51.8 ±4.4 Db	63.7 ±2.6 Da	55.4 ±4.4 Da
F	Deltamethrin	64.6 ± 3.7 Cb	56.1 ±2.7 Cb	73.3 ± 5.6 Ca	67.5 ±2.6 Ca
G	Enzyme extract 100%	94.9 ± 2.5 Aa	85.3 ±2.2 Aa	95.0 ± 2.7 Aa	86.1 ±2.4 Aa

Means followed by different capital letters (columns) are significantly different at P < 0.05 (Tukey HSD test).
Means followed by different capital letters (rows) are significantly different at P < 0.05 (between 1 day and 5 days of nymphs and between adult females) (Tukey HSD test).

Table 2. Mortality of nymphs and adult female *D. opuntiae* on the 1st and 5th day after treatment under field conditions

Type	Treatments	% Mortality			
		1 days		5 day	
		Nymphs	Adult female	Nymphs	Adult female
		SD ± x̄	SD ± x̄	SD ± x̄	SD ± x̄
A	Tobacco extract	80.6 ± 4.9 Bb	69.8 ±9.9 Bb	87.1 ±3.8 Ba	78.5 ±3.6 Ba
B	Juniper extract	14.4 ±4.9 Fb	11.5 ±4.5 Fb	19.8 ±2.0 Fa	17.0 ±1.7 Fa
C	Rosemary extract	23.5 ±12.2 Eb	19.4 ±11.9Eb	33.5 ±3.7 Ea	25.6 ±5.1 Ea
D	Tween 20%	49.5 ± 5.5 Db	46.0 ± 6.4 Db	55.7 ±3.2 Da	51.9 ±2.7 Da
E	Sycrol (mineral oil)	48.7 ±4.2 Db	43.7 ±9.1 Db	54.8 ±2.5 Da	50.3 ±7.2 Da
F	Deltamethrin	66.5± 4.2 Cb	53.8 ± 4.8 Cb	71.2 ±3.7 Ca	62.8 ±3.0 Ca
G	Enzyme extract 100%	88.6 ± 7.4 Aa	81.5 ± 5.1 Aa	89.3 ±6.0 Aa	82.2 ±5.3 Aa

Means followed by different capital letters (columns) are significantly different at $P < 0.05$ (Tukey HSD test).
Means followed by different capital letters (rows) are significantly different at $P < 0.05$ (between 1 day and 5 days of nymphs and between adult females) (Tukey HSD test).

Similarly, after 5 days of treatment, we obtained the same laboratory results for nymphs and adult females (DF=6, F-value=1179.12, p<0.0001 and DF=6, F-value=725.632, p<0.0001). The results showed that the average mortality of nymphs and adult females increased significantly after 5 days of treatment. All treatments had significant differences except for the 100% crude enzyme extract treatment (DF=13, F-value=528.318, p<0.0001, DF=13, F-value=330.605, p<0.0001, respectively) (Table 2).

Research results showed that tobacco plant extract was highly effective in combating cochineal. This effect might be related to the presence of nicotinea concentration of 6.7% [23]. Nicotine has been widely used as an insecticide, and its dilute solution is used on fruits and vegetables to control plant aphids, whiteflies, and leafhoppers [11,13]. In this study treatment of cactus pearwith tobacco, extract increased the average mortality of nymphs and adult females of *D. opuntiae* under laboratory and field conditions after 1 and 5 days of treatment. In addition, tobacco extract offers a significantly better effect than juniper, rosemary, Tween-20, mineral oil, and Deltamethrin (Tables 1 and 2). According to a previous study, tobacco reduced the number of cochineal phases on cactus plants by 90% to 94%, 72 hours after the third spray [9]. Besides, Sarker et al., 2018 [19] reported that tobacco extract affects the eastern fruit insect *Grapholita molesta* (Busck) (Lepidoptera). Therefore, the male and female mortality rate of the insectswas 80% after 7 days of treatment and this mortality rate reached 100%.

Our results demonstrated that no significant difference was found between the treatment with Tween-20 and treatment with Sycrol. Previous studies have shown that using Tween-20 and Sycrolto control *D. opuntiae* on cactus is effective because the mortality rate of nymphs is between 82% and 99% [26]. Some research that mixing mineral oilwith pesticides or plant extracts improvestheir effectiveness against cochineal [1,27]. However, when emulsifiers (such as Tween-20) are mixed with water-based extracts (i.e., *Mentha piperita, Tagetes erecta, Dysphania ambrosioides*), treatments caused dehydration to plants, the second instar nymphs suffocate, affect the adult nymphs, and affect the survival rate of adult females and other reproductive parameters [25].

The crude enzyme extract produced by the bacterial strain SY134 D *B. subtilis* contains a large number of effective enzymes, including lipase and chitinase. Lipase can decompose the wax covering the insect, the main function of this wax layer is to prevent the sprayed liquid from entering its body. Its effect appears in a short period oftime estimated in hours. Chitinase plays a role in killing insects by analyzing chitin, which is the basic component in the external composition of insects' bodies [11,16]. The results of this study showed that although the 50% crude enzyme extract has significant advantages over other treatments under laboratory and field conditions, the average mortality of nymphs and adult females did not increase significantly after 5 days compared with other treatments (Tables 1 and 2). This is consistent with our previous finding, that there was no significant difference in mortality after 3 days of treatment with the bacterial enzymes [11]. Obviously, because enzymes are decomposed and lose their activity over time.

4. Conclusion

The results of this study highly encourage the use of crude enzymes produced by *B. subtilis* (SY134D) and tobacco plant extract in the biological control programs targeting *D. opuntiae*, because these natural alternatives to synthetic insecticides have no harmful effect on the environment.

Acknowledgements. The authors acknowledge Professor I. Othman, the General Director of Syrian Atomic Energy Commission and Dr. N. Mirali head of molecular biology and biotechnology department for their encouragement and support.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest.

References

1. Al naser, Z.; Ezz al-dden, D., Evaluation of the efficacy of chemical and bioinsecticides for controlling of cochineal scale insect, *Dactylopiuscoccus* Costa, *The Arab Journal for Arid Environments* **2018**, *2*(16), 2305-5243.
2. Al-qwabah, A.A.; Al-limoun, M.O.; Al-mustafa, A.H.; Al-zereini, W.A., *Bacillus atrophaeus* A7 crude chitinase: characterization and potential role against *Drosophila melanogaster* larvae, *Jordan Journal of Biological Sciences* **2018**, *11*, 451-459.
3. Bakri, Y.; Ammouneh, H.; El-khouri, S.; Harba, M.; Thonart, P., Isolation and identification of a new Bacillus strain for amylase production, *Research Biotechnology* **2012**, *3*, 51-58.
4. Basheer, A.M.; Asslan, L.; Saleh, A.; Diab., N.; Mohamed, E., Scale insect species (Hemiptera: Coccoidea) in Syria, *European and Mediterranean Plant Protection Organization of Bulletin* **2016**, *46*, 305-307.
5. Bouharroud, R.; Sbaghi, M.; Boujghagh, M.; El Bouhssini, M., Biological control of the prickly pear cochineal *Dactylopius opuntiae* Cockerell (Hemiptera: Dactylopiidae), *European and Mediterranean Plant Protection Organization of Bulletin* **2018**, *48*(2), 300-306, https://doi.org/10.1111/epp.12471.
6. Chandrasekaran, R.; Revathi, K.; Nisha, S.; Kirubakaran, S.A.; Sathish-Narayanan, S.; Senthil-Nathan, S., Physiological effect of chitinase purified from *Bacillus subtilis* against the tobacco cutworm *Spodopteralitura* Fab. *Pesticide Biochemistry and Physiology* **2012**, *104*, 65-71.
7. Cordell, G.A.; Geoffrey, A. Introduction to alkaloids: A biogenetic approach, John Wiley & Sons, 1981.
8. De lotto, G., On the status and identity of the cochineal insects (Homoptera: Coccoidea: Dactylopiidae), *Journal of Entomological Society of Southern Africa*, **1974**, *37*, 167-193.
9. Fitiwy, I.; Gebretsadkan, A.; Araya, A., Management of cochineal (*Dactylopius coccus* Costa) insect pest through botanical extraction in Tigray, North Ethiopia, *Journal of Drylands* **2016**, *6*(2), 499-505.
10. Idris, I.; Elkhouri, S.; Bakri, Y., Evaluation of crude enzyme produced by *Bacillus subtilis* SY134D culture as a biocontrol agent against *Dactylopius opuntiae* (Dactylopiidae: Hemiptera) on cactus pear, *Journal of Bio Innovation* **2019**, *8*(3), 289-300.
11. Ilo, International labor office, *Encyclopedia of Occupational Health and Safety*, Geneva, 3rd ed., **1983**, *2*, pp. 1440.
12. Inglese, P.; Mondragon, C.; Nefzaoui, A.; Saenz, C., Crop ecology, cultivation and uses of cactus pear. In: Crop ecology, cultivation and uses of cactus pear. Advance draft prepared for the IX International Congress on Cactus Pear and Cochineal: CAM crops for a hotter and drier world, Coquimbo, Chile, *Food and Agriculture Organization of the United Nations (FAO)*, 26-30 March, **2017**.
13. Trujillo, K.A.; Chinn, A.B., Nicotine, Drugs and the brain. California State University, USA, **1996**.
14. Las Casas, G.; Distefano, G.; Caruso, M., Nicolosi, E.; Gentile, A.; La Malfa, S., Relationships among cultivated *Opuntia ficus-indica* genotypes and related species assessed by cytoplasmic markers, *Genetic resources and crop evolution* **2018**, *65*, 759-773.
15. Mazzeo, G.; Nucifora, S.; Russo, A.; Suma, P., *Dactylopius opuntiae*, a new prickly pear cactus pest in the Mediterranean: an overview. *Entomologia Experimentalis et Applicata* **2019**, *167*(1), 59-72.
16. Meinwald, J.; Smolanoff, J.; Chibnall, A.C.; Eisner, T., Characterization and synthesis of waxes from homopterous insects, *Journal of Chemical Ecology*, **1975**, *1*, 269-274.
17. Moussa, Z.; Yammouni, D.; Azar, D., *Dactylopius opuntiae* (Cockerell, 1896), a new invasive pest of the cactus plants *Opuntia ficus-indica* in the South of Lebanon (Hemiptera: Coccoidea: Dactylopiidae), *Bulletin de la Société Entomologique de France*, **2017**, *122*, 173-178.
18. Paramasivam, M.; Selvi, C., Laboratory bioassay methods to assess the insecticide toxicity against insect pests - a review, *Journal of Entomology and Zoology Studies* **2017**, *5*, 1441-1445.

19. Sarker, S.; Lim, U.T., Extract of *Nicotiana tabacum* as a potential control agent of *Grapholita molesta* (Lepidoptera: Tortricidae), *PloS One*, **2018**, *13*(8), e0198302.
20. Spodek, M.; Ben-dov, Y.; Protasov, A.; Carvalho, C.J.; Mendel, Z., First record of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Coccoidea: Dactylopiidae) from Israel, *Phytoparasitica* **2014**, *42*, 377-379.
21. Statsoft, Inc., STATISTICA (data analysis software system), Version 7, **2007**, pp. 1984-2004.
22. Tayoub, G.; Odeh, A.; Ghanem, I., Chemical composition and efficacy of essential oil from *Juniperus foetidissima* Willd against the Khapra Beetle, *International Journal of Medicinal and Aromatic Plants* **2012**, *2*(3), 501-508.
23. Tayoub, G.; Sulaiman, H.; Alorfi, M., Determination of nicotine levels in the leaves of some *Nicotiana tabacum* varieties cultivated in Syria, *Herba Polonica* **2015**, *61* (4), 23-30.
24. Tayoub, G.; Alorfi, M.; Ismail, H., Fumigant toxicities of essential oils and two monoterpenes against potato tuber moth (*Phthorimaea operculella* Zeller), *Herba Polonica* **2016**, *62*(4), 82-96.
25. Vigueras, A.L.; Cibrian-Tovar, J.; Pelayo-Ortiz, C., Use of Botanicals Extracts to Control Wild Cochineal (*Dactylopius opuntiae* Cockerell) on Cactus Pear, *Acta Horticulturae* **2009**, *811*, 229-234.
26. Vigueras, A.L.; Cibrian-Tovar, J.; Pelayo-Ortiz, C., Use of botanicals extracts to control wild cochineal (*Dactylopius opuntiae* Cockerell) on cactus pear. In: *The VIth International Congress on Cactus Pear and Cochineal* **2007**, *811*, 229-234.
27. Zeitoun, R.; Hayar, S.; Majed, L.; El-Omari, K.; Dousset, S., Comparison of the efficacy of two insecticides for the management of *Dactylopius opuntiae* on prickly pear cactus in Lebanon and monitoring of the insecticides residues dissipation rates in fruits and cladodes, *SN Applied Sciences* **2020**, *2*(1), 1-16.
28. Zhang, Z., The effects of simulated and natural rainfall on cochineal insects (Homoptera: Dactylopiidae): colony distribution and survival on cactus cladodes, *Agricultural Forestry and Fisheries* **2017**, *6*, 45-48.