Scientific Papers. Series B, Horticulture. Vol. LXIII, No. 1, 2019
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THUJA AND LYCIUM HOMEOPATHIC BIOPREPARATIONS EFFECT ON GOJI BERRY GALL MITE (*ACERIA KUKO* KISHIDA)

Ileana RÎNDAȘU[1], Roxana CICEOI[2], Cristian Mihai POMOHACI[2], Florin STĂNICĂ[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania
[2]Research Center for Studies of Food and Agricultural Products Quality HORTINVEST, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: roxana.ciceoi@gmail.com

Abstract

The impact of invasive alien species (IAS) introduction on economic activities and biodiversity of a new territory is difficult to assess. The online trade had recently exponentially increased the risk of new IAS introductions. A typical example for Europe of such issue is Aceria kuko, the goji-berries gall mite, which was detected for the first time in UK, in 2008 and then continues to spread slowly all over the continent. In Romania the pest was detected for the first time in 2013 and intensive chemical control was applied since 2016, with unsatisfactory efficacy. For economic reasons, most of the goji producers have their fruit plantations included in the organic production system, thus commercially available systemic acaricides cannot be used. In the same time, for those who prefer intensive production, the application of these pesticides leads to important losses, as goji plants produce fruits continuously during summer and long pause intervals decrease the marketable fruit quantity. Thus, organic control measures are highly demanded, the homeopathic biopreparations being one of the very affordable measures that farmers could take. The results of Thuja and Lycium homeopathic applications on two biotypes of goji (Lycium barbarum) are presented. Promising results were obtained with Thuja CH 30 dilution and biopreparation made from host plant infested leaves. Further studies regarding the plant protection throughout the years are required, before making pest control recommendations for goji farmers.

Key words: *Lycium barbarum, Aceria kuko, goji berry gall mite, invasive alien species, Thuja CH 30*

INTRODUCTION

Traditionally known as goji berry or wolfberry, *Lycium barbarum* is one of the 70 representatives of *Lycium* genus, a perennial deciduous shrub with ellipsoid orange-red berries used since ages in Traditional Chinese Medicine. It has recently gained huge popularity due to its high therapeutic and dietary properties, especially its antioxidant activity and antiproliferative effects on different types of cancer (Georgiev et al., 2019) The goji fruits contain many polysaccharides, carotenoids, polyphenols, including caffeic acid, chlorogenic acid, p-coumaric acid, quercetin, and kaempferol (Asănică et al, 2016) and is currently consumed by people as fresh fruit, dried fruit, drinking juice, smoothies, mixed with tea energy bars, or other mixes with cereals, muffins, soups etc. In China, over 95,000 tons of goji fruits are harvested from more than 82,000 hectares (Skenderidis et al., 2019). In the last two decades, the goji berry cultivation expanded into many new countries, due to high consumers' demands for superfoods. Unfortunately, this new business opportunity triggered the online trade with plants intended for planting, an activity that had recently exponentially increased the risk of new invasive alien species (IAS) introductions. According to Dir. 2000/29/EC, the import of Solanaceous plants intended for planting from third countries, as goji, is prohibited all over Europe, in order to protect the other important Solanaceous crops. Despite this, goji plants infested with a new pest arrived on the old continent and were first detected in UK, in 2008 (Ostoja-Starzewski, 2008). The new pest, a mite named *Aceria kuko*, the goji-berries gall mite, spread rapidly in many EU countries and the eradication and containment measures that were taken failed. Until present, it was reported in UK (2008), Germany (2011), Greece (2012), Slovenia (2012), Cyprus (2013), Hungary (2014), Macedonia (2014), France (2015), Serbia (2015), Bulgaria (2016), Bosnia and Herzegovina (2016), Czech Republic (2016) but its establishment success must be verified in each country (EPPO 2017, Hrudová et Šafránková, 2018, Trajčevski, 2018; Zovko et al., 2018).

The goji-berries gall mite was first time detected in Romania in 2013 (Mencinicopschi IC, Balan, 2013, Chireceanu et al., 2015) and its eradication failed despite the intensive application of acaricides. Organic pest control measures are demanded more and more often by the farmers, as the goji producers prefer the organic system, due to higher price and consumer preferences. For the intensive production farmers, the pesticide application leads to quantitative losses because goji plants bear fruits continuously during summer and pause intervals decrease the marketable fruit quantity.

The homeopathic biopreparations could contribute to the health of goji plantation, as an affordable measure that farmers could use.

Homeopathic preparations are high dilutions of different substances, being defined as diluted and mechanically agitated (potentized) substances prescribed on the principle of similitude (Rajendran, 2019). The dilutions are named according to the ratio used at each step (1/10, 1/100, 1/50000) as decimal DH (D), centesimal CH (C) or 50 millesimal LM (LM) scales (Pawan and Archana, 2014).

Recent studies of electron microscopy (HRTEM and FESEM) show that all homeopathic dilutions, starting with centesimal 6 (C 6) or 50-millesimal 1 / LM 1 dilution, contain nanoparticles with elements from the original substance and homeopathy is a form of nanomedicine (Rajendran, 2015, Rajendran 2017, Wassenhoven, 2018). A multitude of studies were performed with high dilutions of substances on pests on different plants, the first literature review being published in 1984 (Scofield, 1984), but until now, no study was performed on *Lycium barbarum*.

An important study was carried out with *Dysaphis plantaginea* Pass. (considered a major pest in apple orchards) on apple seedlings, with *Lycopodyum clavatum* 6 C, 15 C, 30 C and a pest nosode, in double-blind and randomized independent experiments, in growth rooms, over a 17-days period. The number of offsprings was reduced after the application of *Lycopodium clavatum* 15 C and pest nosode 6 C, compared to the control group (Wyss et al, 2010). In apple seedlings, two *Staphysagria* 100 CH sprays at 12 days interval reduced mildew produced by *Podosphaera leucotricha* (Rolim et al, 2005).

The experience with *Coccinella septempunctata* preparations produced from the adult body provides the necessary protection against various pest species, such as greenhouse whitefly (Kaviraj, 2012). Another effective remedy against whitefly is represented by *Sulphur* C 200, especially if honeydew leads to colonization by fungi, producing a black colouration of the foliage (Maute, 2014). An interesting research was carried out to study the effect of high dilutions on pests of tomato crops. The treatments consisted of high dilutions of *Staphysagria*, *Arsenicum album*, *Sulphur*, *Arnica montana*, high dilutions of *Solanum lycopersicum* and *Solanum aculeatissimum*, at decimal or centesimal scales. The dilution of *Arnica montana* 12 DH increased the tomato yield in field conditions. The damage incidence was reduced with applications of *Sulphur* 12 CH (Tatiani et al, 2012).

The present study was designed mainly to highlight the effects of homeopathic bioproducts obtained from leaves of *Lycium barbarum* attacked by *Aceria kuko*, following the principle of a remedy made from leaves attacked by a pests applied on the plants attacked by the same pest. In the same time, another homeopathic remedy was tested (Thuja C 30).

MATERIALS AND METHODS

The present experiment was carried out at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, in the experimental "vegetation house", using two genotypes of *Lycium barbarum*, respectively B_1 and B_2 (Tudor et al., 2017), treated with homeopathic biopreparations at the end of vegetation season,

in 2017 (September – October 2017) and for two months, between April and May 2018.
The biological material consisted of two groups of 10 pots, of the two biotypes, marked with the symbols B1-1 to B1-5 and B2-1 to B2-5.
The pots from the two groups were treated once in a week, in 2017, from September 8 to October 28 and in 2018, from April 22 to May 26, (except abamectin solution that was used only once in 8 September 2017 and once in April 2, 2018), using 100 ml of solution per pot, with the following variants:
- V1 (variant 1) / B1-2 and B2-2 - plain water (control)
- V2 (variant 2) / B1-4 and B2-4 - 18 g/l abamectin, (chemical control)
- V3 (variant 3) / B1-1 and B2-1 - homeopathic biopreparation from *Aceria kuko* CH 6
- V4 (variant 4) / B1-3 and B2-3- homeopathic biopreparation from *Aceria kuko* CH 30
-V5 (variant 5) / B1-5 and B2-5 - homeopathic biopreparation from *Thuja* CH 30.

Figure 1. *Lycium barbarum* pots on 2nd of April 2018

The *Thuja* CH 30 solution was purchased from a homeopathic pharmacy (manufacturer - Plantextrakt, Romania) in the form of *Thuja* impregnated lactose granules; for treatments, 10 granules of *Thuja* CH 30 were used per 2 l of watering water.
The *Aceria kuko* CH 6 and CH 30 biopreparations were made using a macerate of 2 gram of *Lycium barbarum* leaves attacked by *Aceria kuko*, put in 90 degree ethyl alcohol, the leaves being kept in alcohol for 4 weeks in a closed vial (of 14 ml).

Figure 2. Macerate of *Lycium barbarum* in a 14 ml vial used in the experiment

Starting from the macerate thus obtained, dilutions were made starting with CH 1 dilution and reaching the desired dilution, respectively CH 6 and CH 30. For watering with the obtained biopreparations, 10 drops of *Aceria* solution were used for 2 l of watering water.
In order to assess the degree of attack, at the beginning of the study period (on 22 and 30 April), prior to the growth of new shoots, the number of galas was counted to the total number of leaves and then the galas on the first 5 leaves of each (May 11 and May 26).
Comparisons between variants were based on statistical analysis of differences between means using paired two samples for TTEST in SPSS software. For all the tests, the level of significance was established at $p<0,05$.

RESULTS AND DISCUSSIONS

The goji berry crop expanded in the Balcanic region in the last years, due to the increased market demand. Research made in Romania and Bulgaria in the last decade showed the possibility to obtain yields ranging between 127 - 795 kg/ha in Romania (Mencinicopschi and Balan, 2013a) and of 517 - 935 kg/ha in Bulgaria (Dzhugalov et al, 2015). Extensive researches were performed in Romania on the two goji berry biotypes by Mencinicopschi, in the period 2011 – 2013 (Mencinicopschi and Balan, 2013b). But the yields may decrease dramatically due to pests attack. *A. kuko* can completely destroy the goji plants in few years, if no control measures are taken. The mite can completely colonize the young shoots, the leaves (figure 3), but their attack can also damage the flower buds and the flowers, making impossible the hybridisation works and also affecting the quality of the fruits (figure 4).

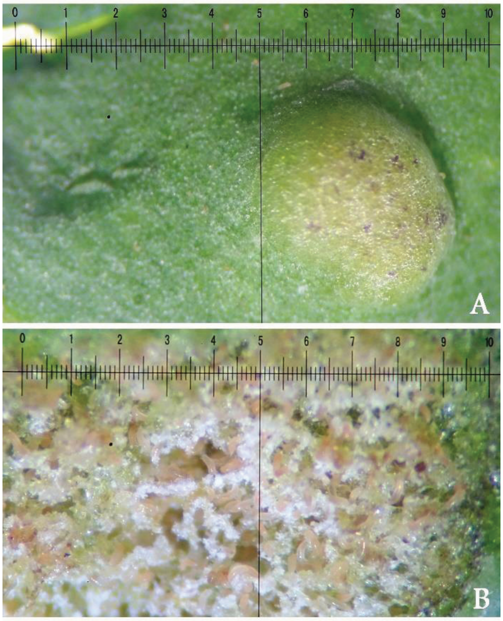

Figure 3. *Aceria kuko* galls and mites. **A**. a gall on a leaf, **B**. section through a gall

A. kuko is a difficult to control pest by contact acaricides, as they hide inside the galls (figure 3), while systemic acaricides require longer pause periods.

The assessment of the attack degree, evaluated over two months period revealed, for the genotype B1, that the water control was attacked in the highest rate, of 30.5%, followed by the chemical control, 27%, *Aceria C6* – 17,75%, *Aceria C30* - 17%, and *Thuja C30* - 9,75% (figure 5).

In average, for the genotype B2, the water control was attacked at the highest rate, of 54.25%, followed by *Aceria C30* - 50.25%, Aceria C6 – 30%, *Thuja C30* - 27.25% and the chemical control with 21% (figure 6).

There is a significant difference between the responses of the two different genotypes to homeopathic treatments.

In the first month, both goji biotypes responded well at treatments with *Thuja CH30* and *Aceria CH6* solution. For B1 biotypes, at the first count, the group *Aceria CH 6* and *Thuja CH 30* presented a lower number of galls than the chemical control.

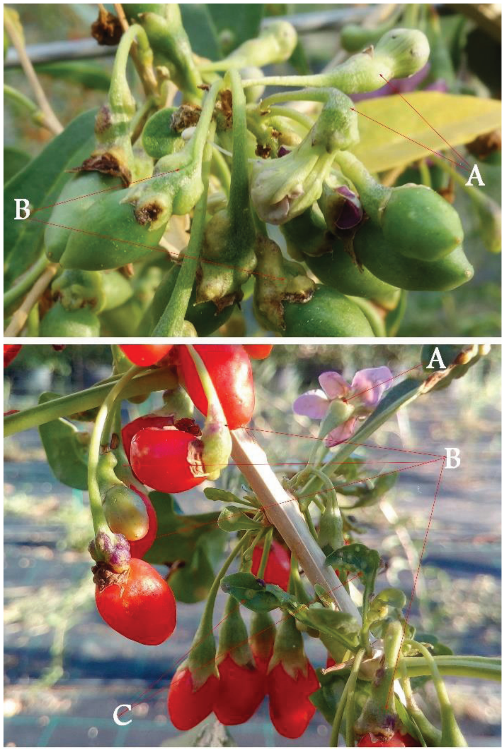

Figure 4. *Lycium barbarum* leaves, flowers and fruits with galls produced by *Aceria kuko*. **A**. galls on flower buds and flowers, **B**. galls on fruits, **C**. galls on leaves.

The B2 biotypes also had lower number of galls under the *Aceria CH 6* and *Thuja CH 30* applications, with values close to the chemical control, but significant different than the *Aceria CH 30* and the water control applications.

In the second month, the plants reaction to the homeopathic treatments changed. Still, the best results at B1 biotypes were recorded with *Aceria CH 30* and *Thuja CH 30* treatments, but the differences are insignificant between all three homeopathic remedies. The biotypes B1 plants treated with *Thuja CH 30* had the smallest number of galas compared to all other variants. At the same time, biotypes B2 showed similar values in plants treated with *Thuja CH 30* and *Aceria CH 6*, comparable to the chemical control.

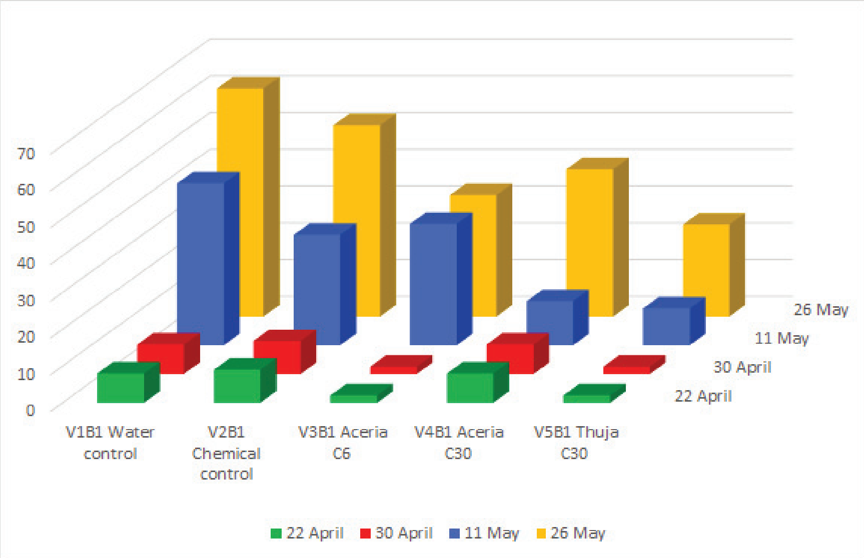

Figure 5. Attack rate of *Aceria kuko* mite on leaves of *Lycium barbarum* biotype **B1** - spring 2018

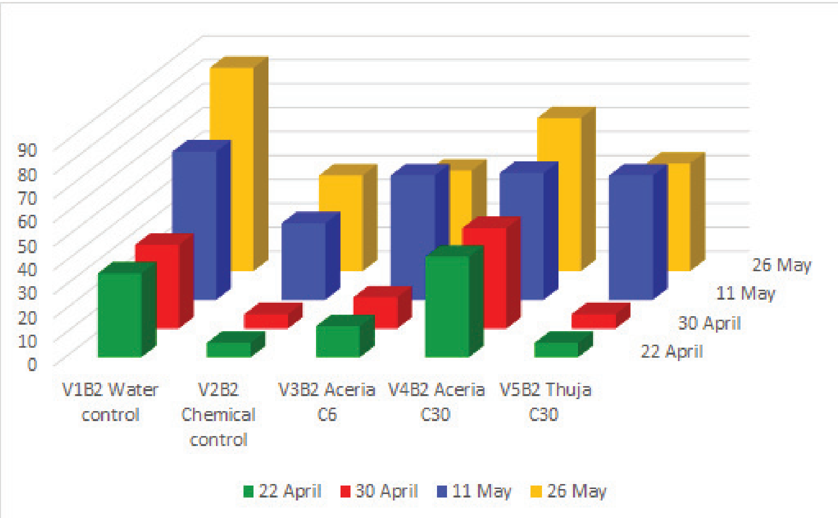

Figure 6. Attack rate of *Aceria kuko* mite on leaves of *Lycium barbarum* biotype **B2** - spring 2018

Using t-test for paired two means, we observe that overall, during May 11-26, we found favourable results for *Thuja CH 30* treatments, which had better effects than the chemical control at B1 (p values=0.03<0.05) and equal to the chemical control at B2 biotypes (p value=0.28>0.05).

At the end of observation period, while the leaves and young shoots of biotype B2 still had many new formed galls, the biotype B1 had healthy new leaves and shoots, only with few new formed galls (figure 7).

Over the same period, treatments with *Aceria CH 30* were also superior to the control, but not statistically significant (p value for B1 equal 0.19 > 0,05 and p value for B2 equal 0.60>0.05).

Figure 7. *Lycium barbarum* leaves of biotypes B1 and B2 treated with homeopathic biopreparation *Thuja* CH 30 at the end of observation period. **A**. biotypes B2; **B**. biotypes B1

CONCLUSIONS

The homeopathic treatments against *Aceria kuko* mites on goji berry showed promising results during the preliminary studies.
The results obtained using homeopathic dilutions from the leaves of the plant attacked by the mites and also with homeopathic preparations of *Thuja* CH 30 were favourable, even showing similar results with the chemical control in some growth stages.
The treatments made at the end of vegetation season, which were made in the idea to strengthen the plants over the winter and beginning of the next season gave promising results, but have to be confirmed in the next years.
Further studies regarding the plant protection efficacy of homeopathic treatments and the effects of these treatments on the goji plants and goji fruits quality on long term are required, before making pest control recommendations for goji farmers.

AKNOLEDGMENTS

The research was carried out on the goji berry plants belonging to the Faculty of Horticulture, Fruit growing department, of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.
Many thanks to assoc. prof. dr. Adrian Asănică, for his willingness and continuous support and patience and to the students from Horticulture Faculty who took good care of the plants, during the experiment.

REFERENCES

Asănică, A., Manole, C., Tudor, V., Dobre, A., Teodorescu, R.I. (2016). *Lycium barbarum* L. juice - Natural source of biologically active compounds. *AgroLife Scientific Journal*, Volume 5, Number 1, 15-20.

Chireceanu, C, Chiriloaie, A, Teodoru, A, Sivu C (2015). Contribution to knowledge of the gall insects and mites associated with plants in southern Romania. *Scientific Papers. Series B, Horticulture*. Vol. LIX:27-36,

Dzhugalov, H., Lichev V., Yordanov A., Kaymakanov P., Dimitrova V., Kutoranov G. (2015). First results of testing goji berry (*Lycium barbarum* L.) in Plovdiv region, Bulgaria. S*cientific Papers. Series B, Horticulture*, Volume LIX, 47-50.

EPPO/OEEP, (2017). *Aceria kuko* reported from several European countries, EPPO Reporting Service no. 05 - 2017 Num. article: 2017/098.

Georgiev, K.D., Slavov, I. J., Iliev, I. A. (2019). Antioxidant activity and antiproliferative effects of *Lycium barbarum*'s (Goji berry) fractions on breast cancer cell lines. *Folia Med* (Plovdiv), 61(1), 96-103. doi: 10.3897/folmed.61.e34919.

Hrudová, E., Šafránková, I. (2018): Goji berry gall mite Aceria kuko occurrence in the Czech Republic – short communication. *Plant Protect*. Sci., 54: 39–42.

Kaviraj, V. (2012). *Homeopathy for farm and garden*, Kandern, Narayana Verlag.

Maute, C. (2019). *Homeopathy for Plants*, Kandern, Narayana Verlag.

Mencinicopschi, I. C., Balan, V. (2013a). Scientific substatiation for the introduction, on Romanian territory, of *Lycium barbarum* L., a species with sanogene properties, *AgroLife Scientific Journal* 2(1) 95–102.

Mencinicopschi, I. C., Bălan, V. (2013b). Growth and development characteristics of plant individuals from two Lycium barbarum L. varieties. *Scientific Papers. Series A. Agonomy*, Vol. LVI, ISSN 2285-5785, 490-497.

Ostoja-Starzewski, J. (2008) Plant Pest Notice: Goji gall mite, Aceria kuko (Kishida). Central Science Laboratory, Defra.

Pawa, K.S., Archana, S. (2014). Homeopathy in agriculture. *Rahmann G. & Aksoy U. (Eds),* *Proceedings of the 4th ISOFAR Scientific Conference. 'Building Organic Bridges', at the Organic World Congress 2014, 13-15 Oct., Istanbul, Turkey (eprint ID* 23727), 667-670.

Rajendran, E.S. (2015). *Nanodynamics*, Kerala, India, Mohna Publications.

Rajendran, E.S. (2017). Homeopathy a material science! Nanoparticle characterization of and 100000c, *International Journal of Current Research*. 9, (04), 48923-48927.

Rajendran, E.S. (2019). Homeopathy seen as Personalised Nanomedicine, Homeopathy. 2019 Feb;108(1):66-70. doi: 10.1055/s-0038-1669988.

Scofield, A.M. (1984). Experimental Research in homeopathy - a critical review. *British Homeopathic Journal*; 73 (3), 161-180.

Skenderidis, P., Lampakis, D., Giavasis, I., Leontopoulos, S, Petrotos, K., Hadjichristodoulou, C, Tsakalof, A. (2019). Chemical Properties, Fatty-Acid Composition, and Antioxidant Activity of Goji Berry (*Lycium barbarum* L. and *Lycium chinense* Mill.) Fruits. *Antioxidants*, 8, 60, 1-13; doi:10.3390/antiox8030060

Tatiani, A. M., Pedro, B., Mari, I. C. B., David, J. M. (2012). Homeopathic and high dilution preparations for pest management to tomato crop under organic production system. *Horticultura Brasileira* 30, 51-57.

Trajčevski, T. (2018). *Aceria kuko* (Kishida, 1927), (Acari: Eriophyidae) new type of pest in the fauna of Macedonia and the success in chemical measurments of protection, *Glasnik Zaštite Bilja*, Vol. 41 No. 3.

Tudor, V., Asănică, A, Teodorescu, R.I., Gîdea, M., Tănăsescu, C., Tudor, A. D., Țiu, J.V. (2017). Germination capacity of some *Lycium barbarum* L. and *Lycium chinense* Mill. biotypes seeds, *Romanian Biotechnological Letters*, Vol. 22, No. 1, 12191-12196.

Wassenhoven, M.V. (2018). A Comprehensive Approach of Homeopathic Medicine, Nanoparticles Search, Solvent, and Electron Behavior using a Metal and a Plant Model to Answer the Question: What is the Signature of a Homeopathic Medicine? *Homeopathy* 107(S 01), 55-78.

Zovko, M., Ostojić, I., Bošnjak, L. (2018). The prevalence of the goji gall mite, *Aceria kuko* (Acari: Eriophyidae), in Bosnia and Herzegovina. Works of the Faculty of Agriculture University of Sarajevo 2018 Vol.63 No. 68 (1), 49-57.

EX·TERRA·AVRVM