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# LABORATORY EFFICACY OF NATURAL SUBSTANCES ON *PLANOCOCCUS FICUS* (SIGN.) AND THEIR IMPACT ON ITS TWO NATURAL ENEMIES

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## Abstract

Management of *Planococcus ficus* infestations in viticulture is a challenge for organic producers. Lack of information regarding the efficacy of natural insecticides used against vine mealybugs make its control more difficult. Principal aim of this study was to validate the efficacy of some natural substances used in organic vineyards, on *P. ficus.* The impact on parasitoid *Anagyrus* sp. near *pseudococci* (Girault) and predator *Cryptolaemus montrouzieri* (Mulsant) was also evaluated in laboratory bioassays. Natural insecticides that contain potassium salts of fatty acids (a.i. 49%), paraffinic oil (a.i. 98,8%), powder sulphur (a.i. 95%), spinosad (a.i. 11,6 % and 44,2 %) and pyrethrin (a.i. 1,4%) were tested by using maximum dose on label. Moreover, two commercial formulations containing seaweed and plant extracts were also tested. Mortality of *P. ficus*, at 24 hours, caused by potassium salts of fatty acids (69%) and paraffinic oil (42%) was significantly higher($p \leq 0.05$) than other substances. Other tested substances were not significantly different than each other. Potassium salts of fatty acids and paraffinic oil showed similar impacts on *A. pseudococci* and both were categorized as harmless. Substances categorized as harmful for parasitoids were Spinosad followed by sulphur and pyrethrin. Predator *C. montrouzieri* resulted sensible to potassium salts of fatty acids and pyrethrin. This two products were categorized as harmful for predators.

**Keywords:** *Organic Agriculture, Pest Management, Natural Insecticide, Bioassay, Mealybug.*

## Introduction

Vine mealybug, *Planococcus ficus* Signoret (Hemiptera:Pseudococcidae) is among the list of common pests of vineyards and increased vine mealybug infestations are considered to be related to increased economic losses in vineyards during the last decade (Daane *et al.*, 2012). Most of the problems related to vine mealybugs are originated from large amount of excreted honeydew as a result of its intensive feeding behavior. Honeydew is considered as a substrate for many fungi, especially for black sooty mold which grows easily on it (Godfrey *et al.*, 2005). *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae), is a generalist predator and important biological control agent used for regulation of mealybugs and soft scale insects in warm climates and greenhouses (Kairo *et al.*, 2013). *Anagyrus* sp. near *pseudococci* (Hymenoptera: Encrytidae) on the other hand is a commonly used parasitoid for management of *P. ficus* (Triapitsyn *et al.*, 2007). Despite the fact that parasioid was reported to be the destroyer of 75% of host populations, insecticides are still utilized by intention of taking mealybug populations to lower densities (Flaherty and Wilson, 1999). Furthermore, treatment on immature stages of *P. ficus* are usually recommended while information related to efficacy on pest and side effects on natural enemies are insufficient (Landers *et al.*, 2012). Aim of this study was to contribute the sustainability improvement of *P. ficus* management, by providing information concerning efficacy of several natural substances on pest and their impact on its natural enemies.

## Materials and Methods

### Insects

All insects used on this study was reared prior to bioassays in insectary of Mediterranean Agronomic Institute of Bari (MAIB). Continuous supply of pest population was obtained by rearing *P. ficus* on squashes *(Cucurbita moschata* cv.Butternut). Infested squashes were also used for the rearing of natural enemies. Parasitoid and predator were collected from experimental vineyard of MAIB and introduced to plexiglass cages (40x40x50 cm) where squashes infested with *P. ficus* were placed. A circular hole was opened on shorter side panel for cage access while top of the cage covered with fine gauze was left for air circulation. A cotton soaked into honey: water (2:1) solution was left for parasitoids as a food and water source and a cotton soaked into water was left for predators as a water source. Emerged natural enemies were transferred into new cages regularly and same procedure was repeated for continuum of rearing.

### Bioassays

Lethal activity bioassays were conducted on *P.ficus, A.pseudococci* and *C. montrouzieri* using products listed on Table 1. Products were chosen by mentioned/recommended uses from literatures reviewed (Baldacchino *et al.*, 2010; Landers *et al.*, 2012; Kahramanoglu and Usanmaz, 2013; Guario *et al.*, 2014). Seaweed extract was tested only on *P. ficus.*

### Lethal Activity Bioassays on *P. ficus*

Bioassays were conducted using methodology of Karamaouna *et al.* (2013) with slight modifications. Bioassays were carried out only on immature stages of vine mealybugs. Squashes were cut into pieces and placed on a 5% agar solution inside 5cm petri dish. Number of vine mealybug crawlerson each squash piece were counted under stereoscope prior to sprayment and petri dishes were placed into plastic cups. Insecticides were applied with an hand sprayer on rate of approximately 28µl/cm$^2$. Treatments were replicated 5 times and control was treated with distilled water only. After each treatment, top of the plastic cups were closed by using fine gauze and rubber bands. Cups were kept in controlled laboratory conditions at25˚C, 50-60% RH and 16:8 L:D. Mortality was controlled by nudging vine mealybugs using a fine paint brush, at 24 hours after treatment. Vine mealybugs without any responses to nudging were recorded as dead.

Table 1. Detailed list of substances used in laboratory bioassays.

| Active Ingredient / Use | Commercial Name | Producer Company | Dose Used |
|---|---|---|---|
| Potassium Salts of Fatty Acids (49%) *Insecticide/Acaricide* | Ciopper | EuroAgro | 2 l / hl |
| Paraffinic Oil (98,8%) *Insecticide* | UFO | Biogard | 2 l / hl |
| Powder Sulphur (95%) Fungicide/Acaricide | Fiori di zolfo | Zolfindustria | 35 kg / ha |
| Spinosad Based (44,2%) *Insecticide* | Success | Bayer | 80 ml /hl |
| Spinosad Based (16%) *Insecticide* | Laser | Dow AgrSciences | 20 ml / hl |
| Pyrethrin (1,4%) Insecticide | PyGanic | Biogard | 250 ml / hl |
| Seaweed Extract *Growth Promoter* | Boundary SW | ICAS | 400 ml / hl |
| *Foliar fertilizer* | DuoLif | Triumph | 200g powder + 1 l oil / hl |

Lethal Activity Bioassays on *A.pseudococci*

Bioassays were conducted using methodology of Mead-Briggs *et al.* (2000) with some modifications. Prior to bioassays ten female parasitoids were taken from rearing cages using test tubes. Test cells, details of which are given below in Figure 1 were cleaned before each use and dried completely. Insecticides were applied on inner surface of glass panels located above and below the frame of cell, at rate of approximately 39µl/cm$^2$.Treatments were replicated 5 times and control was sprayed with distilled water only. Treated panels were left minimum two and a half hours until dried before assembly of the cell. Ten females were then introduced to cells assembled with dried glass panels and secured with rubber bands. Self made fan for aeration was connected to system immediately to provide air current and to prevent accumulation of volatiles inside the cells. Piece of cotton soaked into honey: water (2:1) solution was put to entrance hole as a food and water source for test insects. Cells were kept in controlled laboratory conditions at 25˚C, 50-60% RH and 16:8 L:D. Acute toxicity caused by residues were checked by counting dead insects inside the cells at 24 hours after treatment.

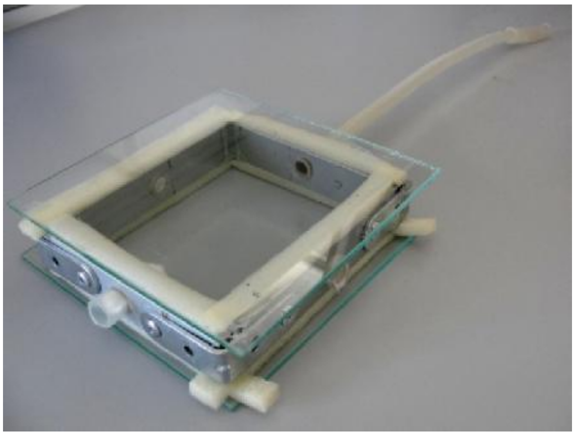

**Figure 4**. A cell used for *Anagyrus* sp.near. *pseudococci*

Lethal Activity Bioassays on *C. montrouzieri*

Bioassays were conducted using methodology of Babu and Azam (1987). Prior to experiments ten newly emerged predators (5 Males/5 Females) were taken from rearing cages by using test tubes and introduced into plastic cups (20cl). Insecticides were applied inside the plastic cups at rate of approximately 24µl/cm$^2$. Treatments were replicated 5 times and control was sprayed with distilled water only. After insecticide applications cotton soaked into water and 30 *P.ficus* individuals were left inside the test tube. Following the treatments top of the plastic cups were closed using fine gauze and rubber bands. Cups were kept in controlled laboratory conditions at 25˚C, 50-60% RH and 16:8 L:D. Acute toxicity caused by direct contact and indirect contamination by food and water source was controlled by counting dead insects inside the cups at 24 hours after treatments.

Statistical Analysis

Data obtained from bioassays were subjected to one way analysis of variance (ANOVA) and Tukey's HSD ($p \leq 0.05$). Prior to statistical analyses mortality rates were corrected using several formulas (Table 2). Impact of substances on natural enemies were also categorized according to the IOBC's toxicity ranking of pesticides on beneficial arthropods as harmless (<30%), slightly harmful (30-79%), moderately harmful (80-99%) and harmful (>99%) (Hassan *et al.*, 1994).

**Table 2.** Efficacy correction formulas used for lethal effect bioassays.

| Species | Reference and Equation |
|---|---|
| *P. ficus* | Schneider-Orelli (1947)<br>$\text{Corrected \%} = \left(\frac{\text{Mortality \% inTreatment} - \text{Mortality \% inControl}}{100 - \text{Mortality \% inControl}}\right) * 100$ |
| *A. pseudococci*<br>*C. montrouzieri* | Abbott (1925)<br>$Corrected\ \% = \left(1 - \frac{ninTreatmentaftertreatment}{ninControlaftertreatment}\right) * 100$ |

## Results and Discussion

*Lethal Activity Bioassays on P. ficus*

Results of laboratory bioassays conducted on *P. ficus* are presented in Figure 2. Highest mean mortality (69%) was caused by active ingredient potassium salts of fatty acids (PSFA) . It was significantly higher than paraffinic oil which caused 42 % mean mortality. Foliar fertilizer, pyrethrin, two formulations of spinosad, sulphur and seaweed extract caused mean mortalities less than 20%. Among tested substances only results of potassium salts of fatty acids and paraffinic oils were considered sufficient for the control of *P. ficus.*

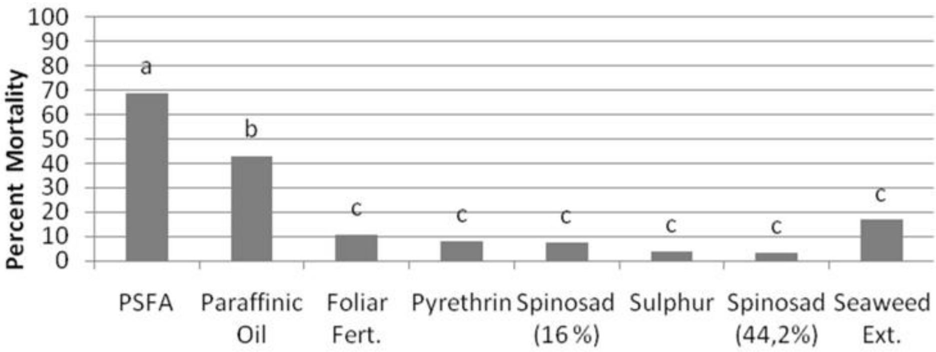


**Figure 5.** Mortality of *P. ficus* at 24h after treatments.

*Lethal Activity Bioassays on A. pseudococci*

Results of laboratory bioassays conducted on *A. pseudococci* are presented in Figure 3. Spinosad (a.i. 44,2%) caused 100% mean mortality of test insects and spinosad (16%) caused 97,5%. Sulphur and pyrethrin caused 77,5 % and 75,5 % mean mortalities, respectively. Paraffinic oil, PSFA and foliar fertilizer caused mean mortalities equal or less than 20%. According to results Spinosad (44,2%) was categorized as harmful while Spinosad (16%) was categorized as moderately harmful. Pyrethrin and sulphur were both categorized as slightly harmful and paraffinic oil, PSFA and foliar fertilizer were categorized as harmless.

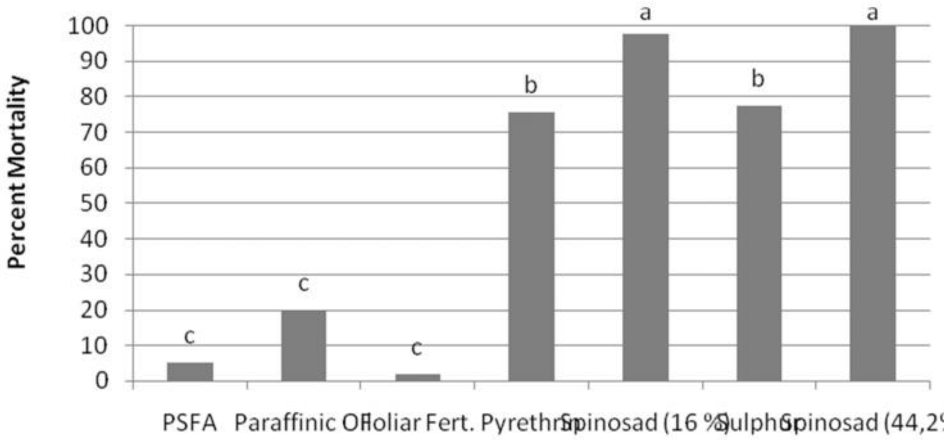


**Figure 6.** Mortality of *A. pseudococci* at 24h after treatments.

*Lethal Activity Bioassays on C. montrouzieri*

Results of laboratory bioassays on *C. montrouzieri* are presented on Figure 4. PSFA caused 90% mortality of test insects and phyrethrin caused 75% mean mortality. Paraffinic oil, foliar fertilizer and two formulation of spinosad caused mean mortality equal or less than 10%. According to results PSFA was categorized as harmful and pyrethrin was categorized as moderately harmful. Paraffinic oil, foliar fertilizer and two formulation of spinosad were all categorized as harmless.

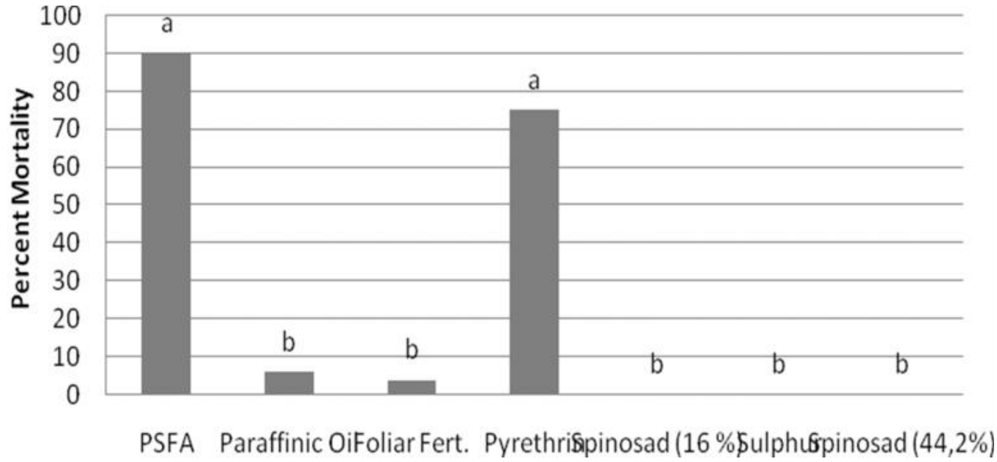


**Figure 7.** Mean mortality of *C. montrouzieri* at 24h after treatments.

*Lethal Activity Bioassays on P. ficus*

It was found that when used same doses and percentages of active ingredients potassium salts of fatty acids and paraffinic oil caused 22% and 12% mortalities, respectively (Hollingsworth, 2005). However, mentioned study was conducted under greenhouse conditions on later larval stages ($3^{rd}$ and $4^{th}$) while our study was conducted under laboratory conditions on crawlers. In field conditions, potassium salts of fatty acids and paraffinic oil caused 55% and 32% mortalities, respectively (Baldacchino *et al.*, 2010). In field conditions lower mortality than laboratory bioassays are generally expected as *P. ficus* individuals are generally located in hidden parts of plants and not easily reachable by the treatments.

*Lethal Activity Bioassays on A. sp.near. pseudococci*

Similar impact of spinosad on hymenopteran parasitoids was also confirmed by several other studies (Williams *et al.*, 2003; Newman *et al.*, 2004; Biondi *et al.*, 2012). Studies as early as 1975 reported that pyrethrin caused less mortality when applied in lower doses, recently in studies conducted with pyrethrin at similar doses also reported harmful impact on other hymenopteran parasitoids (Wilkinson *et al.*, 1975; Tunca *et al.*, 2012; Tunca *et al.*, 2014). Sulphur was also mostly categorized as harmful (De Courcy Williams and Gill, 1996; Martinson *et al.*, 2001; Thomson *et al.*, 2001; Jepsen *et al.*, 2007a). Sulphur residues was found to be toxic up to 21 days and was reported harmful even in lower percentages (Martinson *et al.*, 2001; Jepsen *et al.*, 2007a). Despite the fact that it is mostly reported as harmful, (Jepsen *et al.* (2007b)) also argued that no effect was found on reproductive success of sulphur treated hymenopteran parasitoids from *Anagrus* spp.

*Lethal Activity Bioassays on C. montrouzieri*

Pyrethrin and potassium salts of fatty acids were reported as harmless for adults of *Harmonia axyridis*, another member of Coccinellidae family, when same doses were applied under laboratory conditions (Kraiss and Cullen, 2008). In contrast, another study suggested potassium salts of fatty acids as an alternative to pyrethrin which was reported harmful also on larval stages of *Adalia bipunctata* (L.), another predator of Coccinellidae family (Jansen *et al.*, 2010). Our results were confirmed by the side of pyrethrins impact by both studies. Hovewer, potassium salts of fatty acids were reported as harmless in both studies in contrast to our results. Conflicting results can be related to our methodology that facilitated complete sprayment of the test arena and residual contamination of food and water source of predators.

## Conclusion

Laboratory bioassays on vine mealybugs and its two natural enemies resulted that only two substances (potassium salts of fatty acids and paraffinic oil) were efficient against pest and harmless for parasitoids while one of them (potassium salts of fatty acids) having harmful impact on predators. Efficacy on pest and potential impact on beneficial arthropods must be known for sustainability improvement of management by using natural substances. Results showed that even allowed natural substances in organic farming may have undesirable impacts on beneficial arthropods and they need to be carefully evaluated prior to use in pest management programs. Similar studies would be beneficial also for other natural enemies of vine mealybugs and our results should be confirmed by extended-laboratory and field trials in future.

## References

Abbott W. S. (1925). A method of computing the effectiveness of an insecticide. J Econ Entomol, 18: 265-267.

Babu T. R. and Azam K. M. (1987). Toxicity of different fungicides to adult Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae). Crop Protection, 6(3): 161-162.

Baldacchino F., Ambrico A., Trupo M., Colella T., Caponero A., Mennone G. and Quinto G. R. (2010). Prove di lotta contro cocciniglie farinose su vite da tavola coltivata in biologico. ATTI Giornate Fitopatologiche, 1: 151-158

Biondi A., Mommaerts V., Smagghe G., Vinuela E., Zappala L. and Desneux N. (2012). The non-target impact of spinosyns on beneficial arthropods. Pest Management Science, 68(12): 1523-1536.

Daane K. M., Almeida R. P. P., Bell V. A., Walker J. T. S., Botton M., Fallahzadeh M., Mani M., Miano J. L., Sforza R., Walton V. M. and Zaviezo T. (2012). Biology and Management of Mealybugs in Vineyards. In: Bostanian N. J., Vincent C. and Isaacs R. (ed). Arthropod Management in Vineyards: Pests, Approaches and Future Directions. Springer, pp.271-307.

De Courcy Williams M. and Gill G. (1996). Evaluation Of Pesticides For Side Effects On The Leafhopper Parasitoid Anagrus atomus With Particular Reference To Protected Crops. Annals of Applied Biology, 128: 98-99.

Flaherty D. L. and Wilson L. T. (1999). Biological Control of Insects and Mites on Grapes. In: Bellows T. S. and Fischer T. W. (ed). Handbook of Biological Control. Academic Press, California, USA, pp.853-869.

Godfrey K., Haviland D. R., Erwin J., Daane K. M. and Bentley W. J. (2005). Vine Mealybug: What You Should Know. USA, Division of Agriculture and Natiral Resources, University of California.

Guario A., Cavicchi V., Lasorella V., Antonino N., Grande O. and Convertini S. (2014). La Confusione Sessuale Per Il Controllo Di Planococcus Ficus Su Vite Da Tavola, In Puglia: Primi Approcci Di Un Biennio Di Sperimentazione.In: Brunelli A. and Collina M. (eds). Giornate Fitopatologiche, Chianciano Terme (Siena). CLUEB.

Hassan S. A., Bigler F., Bogenschütz H., Boller E., Brun J., Calis J. N. M., Coremans-Pelseneer J., Duso C., Grove A., Heimbach U., Helyer N., Hokkanen H., Lewis G. B., Mansour F., Moreth L., Polgar L., Samsøe-Petersen L., Sauphanor B., Stäubli A., Sterk G., Vainio A., Veire M., Viggiani G. and Vogt H. (1994). Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group «pesticides and beneficial organisms». Entomophaga, 39(1): 107-119.

Hollingsworth R. G. (2005). Limonene, a Citrus Extract, for Control of Mealybugs and Scale Insects. J Econ Entomol, 98(3): 772-779.

Jansen J. P., Defrance T. and Warnier A. M. (2010). Effects of organic-farming-compatible insecticides on four aphid natural enemy species. Pest Management Science, 66(6): 650-656.

Jepsen S. J., Rosenheim J. A. and Bench M. E. (2007a). The effect of sulfur on biological control of the grape leafhopper, Erythroneura elegantula, by the egg parasitoid Anagrus erythroneurae. BioControl, 52(721-732).

Jepsen S. J., Rosenheim J. A. and Matthews C. E. (2007b). The impact of sulfur on the reproductive success of Anagrus spp. parasitoids in the fiel. BioControl, 52: 599-612.

Kahramanoglu I. and Usanmaz S. (2013). Management strategies of fruit damaging pests of pomegranates: Planococcus citri, Ceratitis capitata and Deudorix (Virachola) livia. African Journal of Agricultural Research, 8(49): 6563-6568.

Kairo M. T. K., Paraiso O., Gautam R. D. and Peterkin D. D. (2013). Cryptolaemus montrouzieri (Mulsant) (Coccinellidae: Scymninae): a review of biology, ecology, and use in biological control with particular reference to potential impact on non-target organisms. CAB Reviews, 8(005).

Karamaouna F., Kimbaris A., Michaelakis A., Papachristos D. P., Polissiou M., Papatsakona P. and Tsora E. (2013). Insecticidal activity of plant essential oils against the vine mealybug, Planococcus ficus. Journal of Insect Science, 13.

Kraiss H. and Cullen E. M. (2008). Efficacy and Nontarget Effects of Reduced-Risk Insecticides on Aphis glycines (Hemiptera: Aphididae) and Its Biological Control Agent Harmonia axyridis (Coleoptera: Coccinellidae). J Econ Entomol, 101(2): 391-398.

Landers A., Muza A., Bates T., Cousins P., Curtis P., Dunst R., Helms M., Loeb G., Hed B., Martinson T., Reisch B., Senesac A., Timer J., Vanden Heuvel J., Walter Peterson H., Wilcox W. and Wise A. (2012). Production Guide for Organic Grapes. New York State Integrated Pest Management Program, New York. New York State IPM Publications

Martinson T., Williams III L. and English-Loeb G. (2001). Compatibility of Chemical Disease and Insect Management Practices Used in New York Vineyards with Biological Control by Anagrus spp. (Hymenoptera: Mymaridae), Parasitoids of Erythroneura Leafhoppers. Biological Control, 22(3): 227-234.

Mead-Briggs M. A., Brown K., Candolfi M. P., Coulson M. J. M., Miles M., Moll M., Nienstedt K., Schuld M., Ufer A. and McIndoe E. (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (DeStephani-Perez) (Hymenoptera: Braconidae). In: Candolfi M. P., Blümel S., Forster R., Bakker F. M., Grimm C., Hassan S. A., Heimbach U., Mead-Briggs M. A., Reber B., Schmuck R. and Vogt H. (eds). Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, Gent, Belgium, pp.13-27.

Newman I. C., Walker J. T. S. and Rogers D. J. (2004). Mortality Of The Leafroller Parasitoid Dolichogenidea Tasmanica (Hym: Braconidae) Exposed To Orchard Pesticide Residues. New Zealand Plant Protection, 57: 8-12.

Schneider-Orelli O. (1947). Entomologisches Praktikum: Einführung in die land- und forstwirtschaftliche Insektenkunde. Sauerländer.

Thomson L. J., Glenn D. C. and Hoffmann A. A. (2001). Effects of sulfur on Trichogramma egg parasitoids in vineyards: measuring toxic effects and establishing release windows Australian Journal of Experimental Agriculture, 40(8): 1165-1171.

Triapitsyn S. V., González D., Vickerman D. B., Noyes J. S. and White E. B. (2007). Morphological, biological, and molecular comparisons among the different geographical populations of Anagyrus pseudococci (Hymenoptera: Encyrtidae), parasitoids of Planococcus spp. (Hemiptera: Pseudococcidae), with notes on Anagyrus dactylopii.Biological Control, 41(1): 14-24.

Tunca H., Kilinçer A. N. and Ozkan C. (2014). Toxicity and repellent effects of some botanical insecticides on the egg-larval parasitoid Chelonus oculator Panzer (Hymenoptera: Braconidae). Scientific Research and Essays, 9(5): 106-113.

Tunca H., Kilinçer N. and Ozkan C. (2012). Side-effects of some botanical insecticides and extracts on the parasitoid, Venturia canescens (Grav.) (Hymenoptera: Ichneumonidae). Turkiye Entomoloji Dergisi, 36(2): 205-214.

Wilkinson J. D., Biever K. D. and Ignoffo C. M. (1975). Contact toxicity of some chemical and biological pesticides to several insect parasitoids and predators. Entomophaga, 20(1): 113-120.

Williams T., Valle J. and Vinuela E. (2003). Is the Naturally Derived Insecticide Spinosad Compatible With Insect Natural Enemies? Biocontrol Science and Technology, 13(5): 459-475.