



Special Issue: Agricultural Productivity and Sustainability Improvement in Tropical Region

# Assessment of Three Natural Pesticide Concentration on the Imago Phase Red Mites Persistency

Dyah Roeswitawati<sup>1\*</sup>, Iva Kristova<sup>1</sup>, Muhidin Muhidin<sup>1</sup>, Otto Endarto<sup>2</sup>, Manar Fayiz Mousa Atoum<sup>3,4</sup>, Irum Iqar<sup>5,6</sup> and Luqman Ali Shah<sup>7</sup>

<sup>1</sup>Department of Agrotechnology, Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang, Jl. Raya Tlogomas 246 Malang, 65144 Indonesia; <sup>2</sup>Indonesian Citrus and Tropical Fruits Research Institute, Jl. Raya Tlekung 1 Batu, 65327 Indonesia; <sup>3</sup>Molecular Biology and Genetics, The Hashemite University, PO Box 330127, 13133 Zarqa, Jordan; <sup>4</sup>Department of Medical Laboratory Sciences, The Hashemite University; <sup>5</sup>Department of Biotechnology, Quaid-i-Azam University, NCB Building Islamabad 45320, Pakistan; <sup>6</sup>Pakistan Academy of Sciences, 3 Constitution Ave, G-5/2, Islamabad Capital Territory, Pakistan; <sup>7</sup>National Center of Excellence in Physical Chemistry (NCEPC), University of Peshawar 25120, Pakistan.

**Abstract** | Red mites [*Panonychus citri* (McGregor, 1916)] is one of the pests of the *Citrus* L. That causes yellow/brown spots on *Citrus* leaves. So far, farmers have dealt with chemical pesticides that contain compounds with high toxicity. The purpose of the study was to assess the effect of type and concentration of natural pesticides soursop (*Annona muricata* L.) leaves, papaya (*Carica papaya* L.) leaves, chrysanth (*Chrysanthemum morefolium* Ramat.) leaves on the persistence of red mites. This research was conducted at the Research Institute for Citrus and Tropical Fruit Plants in Batu City, East Java, Indonesia from July to September 2017. The study was a factorial experiment, the 1<sup>st</sup> factor is the type of natural pesticides: A1 = soursop leaves, A2 = papaya leaves, A3 = chrysanth leaves. The 2<sup>nd</sup> factor is the concentration of natural pesticides: B1 = 5 %, B2 = 10 %, B3 = 15 %. Pesticide persistence was observed based on the mortality rate of the red mite phase of the imago. The results showed that the pesticide persistence test of imago had the highest mortality rate for the treatment 2 × 24 h after pesticide spraying. Each pesticide treatment showed significant differences in the persistence of imago red mite. Papaya leaf pesticide treatment of a concentration of 15 % was effective in suppressing imago red mite 80 %. The 10 % concentration of chrysanth leaf pesticide was effectively suppressing the imago red mite 80 %. Soursop leaf pesticide treatment of a concentration of 5 % was effective in suppressing imago red mite 82 %.

**Received** | September 23, 2021; **Accepted** | December 25, 2021; **Published** | December 30, 2021

**\*Correspondence** | Dyah Roeswitawati, Department of Agrotechnology, Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang, Raya Tlogomas 246, Malang 65144, Indonesia; **Email:** dyahwati@umm.ac.id

**Citation** | Roeswitawati, D., I. Kristova, M. Muhidin, O. Endarto, M.F.M. Atoum, I. Iqar and L.A. Shah. 2021. Assessment of three natural pesticide concentration on the imago phase red mites persistency. *Sarhad Journal of Agriculture*, 37(Special issue 1): 153-158.

**DOI** | <https://dx.doi.org/10.17582/journal.sja/2021.37.s1.153.158>

**Keywords** | Citrus pest, Environmental friendly, Organic pest control, *Panonychus citri* (McGregor, 1916), Reduce chemical pesticide

## Introduction

Red mites [*Panonychus citri* (McGregor, 1916)] are one of the pests of *Citrus* L. that cause symptoms: brown spots on the fruit and yellow/brown spots on *Citrus* leaves (Auger et al., 2013; NSW, 2017; Riahi et al., 2013). So far, farmers have dealt with chemical pesticides that contain compounds with high toxicity

(Childers and Fasulo, 2009; UC-IPM, 2017). Due to the persistent nature of pesticides, it resulted in increased pest resistance to pesticides, increased maintenance costs, and resulted in poisoning for humans and the environment (Novizan, 2002). Persistent chemical pesticides left residues that are difficult to be cleaned on plants. The high negative impact of the use of synthetic pesticides encourages

various efforts to pursue the utilization of natural pesticides as an alternative substitute for synthetic pesticides (Zusfahair *et al.*, 2014). Some of the natural pesticides that can be used are papaya (*Carica papaya* L.) leaf extract, chrysanth (*Chrysanthemum morefolium* Ramat) leaf extract, and soursop (*Annona muricata* L.) leaf extract (Hermawan and Laksono, 2013). Besides being environmental friendly, natural pesticides are pesticides that are relatively safe in their use and economical (Kardinan, 2005).

The prospect of developing natural pesticides in Indonesia is still very wide open because the existing biodiversity is very potential to be utilized (Wiratno, 2010). Because of the nature of pesticides which are rapidly decomposed by natural components such as sunlight, humidity, air temperature, it would not cause soil and water pollution (UC.IPM, 2017). Natural pesticides that can be used include papaya (*C. papaya*) leaf extract, chrysanth [*Chrysanthemum cinerariaefolium* (Trevir.) Vis] leaf extract, and soursop (*Annona muricata* L.) leaf extract. The purpose of the study was to examine the effect of type and concentration of natural pesticides soursop leaves, papaya leaves, chrysanth leaves on the persistence of red mites (*P. citri*).

## Materials and Methods

This research was conducted at the Research Institute for Citrus and Tropical Fruit Plants in Batu City, East Java, from July to September 2017.

### Experimental design

The experiment was arranged in a randomized complete block design (RCBD) with a factorial design. The 1st factor is the type of natural pesticides: A1 = Soursop leaves, A2 = Papaya leaves, A3 = Chrysanth leaves. The 2nd factor is the concentration of vegetable pesticides: B1 = 5 %, B2 = 10 %, B3 = 15 %, each combination treatment was repeated three times. Each replication of two samples and each were given three control treatments, so the number of Petri dishes used was 57 Petri dishes in each treatment phase. Imago persistence test, 57 Petri dishes × 7 DAS (days after spraying): 399 Petri dishes. Imago resistance to natural pesticides was observed based on the imago phase red mite mortality rate.

### Making papaya leaf extract, soursop leaves, chrysanth leaves

The process of making leaves extract uses the

maceration extraction method. The total weight of each leaf, namely papaya leaf, soursop leaf, and chrysanth leaf, was 635 g, 500 g, and 635 g (Azwanida 2015; Gusthinnadura *et al.*, 2017).

All three ingredients are extracted in the same process. Leaves were heated in the "National" microwave oven at 45 °C for 6 sec and then pounded using Bamix at 200 rpm (1 rpm = 1/60 Hz). Amount 100 g dried leaves macerated with 350 mL methanol solvent 0.762 polarities for 24 h, then filtered with Whatman filter paper no 2 to separate the filtrate and supernatant. The supernatant was concentrated using a vacuum rotary evaporator R 3001 CE at 30 °C for 75 min, and 9 g of thick extract was obtained (Azwanida 2015; Gusthinnadura *et al.*, 2017). The extract is diluted with aqua dest with concentration according to treatment (5 %, 10 %, 15 %).

### Test of persistence of natural pesticides

The persistence test of natural pesticides was carried out by spraying the leaves of citrus plants with natural pesticide extract according to the treatment. Imago phase red mite pests were maintained in a petri dish (30 red mites/petri dish) and fed with citrus leaves that had been sprayed with natural pesticide extracts (soursop leaves, papaya leaves, chrysanth leaves). According to the observations were made on the mortality of mites after 24 h eating the treated citrus leaves.

### Statistical analysis

The observation data were analyzed using variance analysis to determine whether there were interactions between factors. To find out the difference in treatment, a comparative test of Tukey's honestly significance difference (HSD) 5 % was carried out if the treatment proved to be influential (Adinurani, 2016).

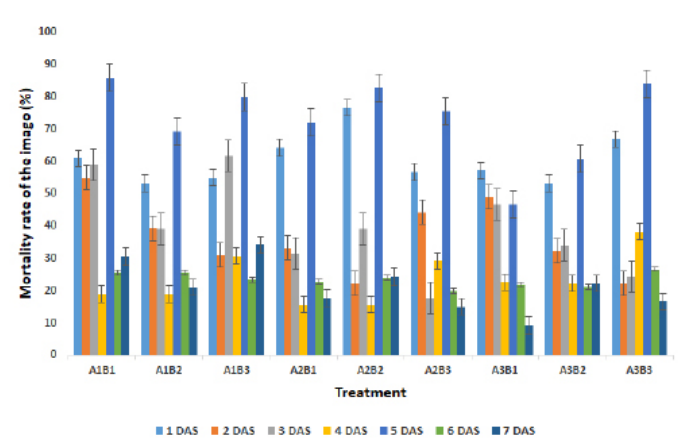
## Results and Discussion

### Persistence of natural pesticides on Red Mites mortality first observation imago phase

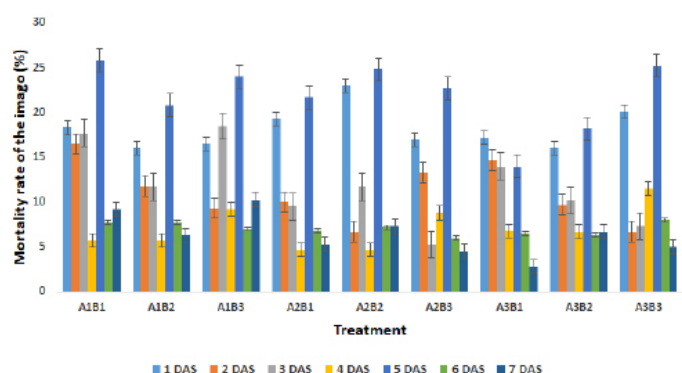
Based on the results of variance analysis, there was no real interaction between the type treatment and the concentration of natural pesticides on the persistence (red mite mortality) at 1 DAS, 6 DAS, and 7 DAS, but there are real interactions between the type treatment and the concentration of natural pesticides on the persistence (red mite mortality) at 2 DAS, 3 DAS,

4 DAS, and 5 DAS. The mean mortality rate of the imago phase in the 1<sup>st</sup> d observation was presented in Figure 1.

In Figure 1 it appears that at 1 DAS and 2 DAS each treatment showed mortality that was not significantly different. But at 3 DAS, 4 DAS and 5 DAS for each treatment showed significantly different mortality, the highest mortality rate was 15 % soursop leaf treatment. Similarly, at 6 DAS and 7 DAS, the highest mortality rate in chrysanth leaves pesticides at a concentration of 15 %. Overall, in Figure 1, the mortality of imago decreases over time. This shows that the persistence of natural pesticides decreases over time, which is indicated by the decreasing mortality of the imago phase.



**Figure 1:** Persistence of three natural pesticides on red mite mortality to imago phase at 1<sup>st</sup> observation.



**Figure 2:** Persistence of three natural pesticides on red mite mortality to imago phase at 4<sup>th</sup> d observation.

#### *Persistence of natural pesticides on Red Mites mortality 4<sup>th</sup> observation imago phase*

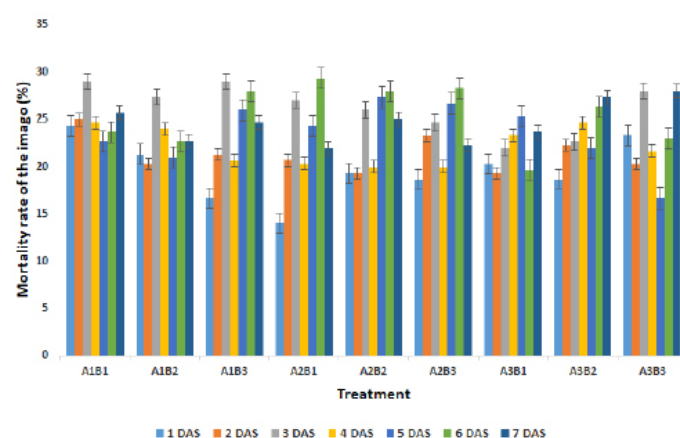
The based on the results of variance analysis, there was no real interaction between the type treatment and the concentration of natural pesticides on the persistence (red mite mortality) at 1 DAS, 2 DAS, 4 DAS, 5 DAS, 6 DAS, and 7 DAS, but there are real interactions between the type treatment and the

concentration of natural pesticides on the persistence (mortality of red mites) in 3 DAS. The mean mortality rate of the imago phase in the 4<sup>th</sup> d observation is presented in Figure 2.

Figure 2 it appears that at 1 DAS, 2 DAS, 4 DAS, 5 DAS, 6 DAS, and 7 DAS for each treatment showed mortality that was not significantly different. However, at 3 DAS each treatment showed significantly different mortality, the highest mortality rate was 5 % soursop leaves and not significantly different from the 15 % concentration of the chrysanth leaf treatment.

#### *Persistence of natural pesticides on Red Mites mortality 7<sup>th</sup> observation imago phase*

Based on the results of variance analysis, there was no real interaction between the type treatment and the concentration of natural pesticides on the persistence of natural pesticides (red mite mortality) at 1 DAS, 2 DAS, 3 DAS, 6 DAS, and 7 DAS, but there is a real interaction between the type treatment and the concentration of natural pesticides on the persistence of natural pesticides (red mite mortality) at 4 DAS and 5 DAS. The imago phase mortality rate on the 1<sup>st</sup> d of observation is presented in Figure 3.



**Figure 3:** Persistence of three natural pesticides on red mite mortality to imago phase at 7<sup>th</sup> d observation.

Figure 3 it appears that at 1 DAS, 2 DAS, 3 DAS, 6 DAS and 7 DAS for each treatment showed mortality that was not significantly different. However, at 4 DAS and 5 DAS, each treatment showed significantly different mortality, the highest mortality rate was a 5 % concentration of soursop leaves and not significantly different from the 15 % concentration of papaya leaf treatment.

The most mortality test in the imago phase and the most effective pesticide was the soursop leaf pesticide

with a concentration of 5 %. In the persistence test of the first observation imago the highest mortality rate in 5 DAS pesticide leaf extract treatment concentrations of 5 %, while the 4<sup>th</sup> observation of the highest mortality rate in 3 DAS pesticide leaf extract treatment of soursop concentration of 5 %, and the observation of the 7 DAS highest mortality rates in 2 DAS pesticide leaf extract treatment with a concentration of 5 %. It means that overall shows that red mite pests are persistent with natural soursop leaf extract pesticides with a concentration of 5 % which is indicated by high imago phase mortality. Overall shows that red mite pests have been tested for persistence on natural pesticides of soursop leaf extract, papaya leaf extract, and chrysanth leaf extract. The results of the soursop leaf extract were 5 % more persistent than papaya leaf extract and chrysanth leaf extract. The persistence was indicated by the mortality rate of red mites in the imago phase at 3 DAS, and the mortality rate of the instar phase 1 red mite pest at 6 DAS.

Soursop leaves, papaya leaves, and chrysanth leaves that have been destroyed by adding a solvent, after spraying on insects can disrupt the nervous system in insects. Based on the results of the study stated that among tropical plants, members of *Annonaceae* have great potential as a source of natural pesticides (Neela and Alexander, 2010; Revathy *et al.*, 2016). The results of research that have been developed that the genus extract *Annonaceae* contains ethanolic and acetogenin which are used as pest control *Helicoverpa armigera* Hubner, 1808. (Camila *et al.*, 2016). The insect nervous system consists of functional units in the form of sensory, internal (sensory connectors), and motors. Insects also have a central nervous system found in the brain which acts as a coordination center for insect activity that covers the entire body (Siswarni *et al.*, 2016). Soursop leaves contain acetogenin compounds, among others, asimisin, bulatacin, and squamosin. At high concentrations, the acetogenin compound has an anti-feedent feature (Hermawan and Laksono, 2013; Nadia *et al.*, 2019). In this case, the insect is no longer excited to devour the plant parts he likes (Klashoven, 1981). Whereas at low concentrations, it is a poisonous stomach that can cause dead insects (Kardinan, 2005; Murray, 2020). That soursop leaf extract can be used to overcome grasshopper pests and other pests (Camila *et al.*, 2016; Hermawan and Laksono, 2013). Other research results show that the use of papaya leaf extract can decide or

thwart the metamorphosis of pests that have perfect metamorphosis. Papaya leaves contain the enzyme papain which when it enters the trachea of insects, then spreads and can interfere with the performance of the nervous system of these insect pests (Camila, 2016; Ezekiel and Mamboya, 2012; Zufahair *et al.*, 2014). Papain is a proteolytic enzyme found naturally in papaya, this enzyme can cut organic molecules made by amino acids and are known as polypeptides, and play a role in biological processes, physiology, and pathology phases. Papain enzyme is made from the latex of unripe papaya fruit (Ezekiel and Mamboya, 2012).

In general, synthetic pesticides have high toxicity, are persistent and dangerous because they cause contaminant residues in plants, food commodities, soil pollution, and groundwater. Synthetic pesticides kill non-target organisms such as pollinators, fish, birds, other animals and if used excessively can increase the resistance of the pest to these drugs. While phytochemical biopesticides, in other words, are less toxic, less persistent, safe for the environment, humans, and non-target organisms (Suresh *et al.*, 2017).

The results of the study showed that red lice pests showed persistence in chrysanth leaf pesticides which were not significantly different compared to pesticides of papaya leaves and soursop leaves. Some phytochemicals that act as biopesticides that are successfully commercialized include azadirachtin, nicotine, pyrethrin, rotenone, veratrum, rocaglamides, isobutyl amides. These natural insecticides can be derivatized to be used as resistance to insect pests (Suresh *et al.*, 2017). Chrysanth leaf extract contains several types of active substances that are insecticidal (Novizan, 2002; NPIC, 2014). One of the active substances whose levels are large is pyrethrin, it is also stated that the pyrethrin levels contained in chrysanth plants reach 0.9 % to 1.3 %. Pyrethrum is a botanic insecticide that is commercialized globally and is produced for non-agriculture but is dominated by insecticides (Murray, 2020). According to Wiratno (2010), states that chrysanth plants contain pyrethrin compounds that can be used to repel insects, which pyrethrin works by disrupting the insect's nerve tissue. Pyrethrin can work quickly and can instantly make insects faint. Pyrethrin obtained from chrysanth leaf extract is a contact poison that does not leave a residue and is safe for the environment (ATSDR,



2003; Murray, 2020).

## Conclusions and Recommendations

It can be concluded from this study that there is an interaction between the types of natural pesticides and the concentration of natural pesticides on the mortality of red mites imago phase. The persistence test on the imago highest mortality rate in the imago phase of the 7<sup>th</sup> highest mortality rate in 2 DAS with pesticide treatments soursop leaves with a concentration of 5 % and observation to 7<sup>th</sup> the highest mortality rate at 6 DAS (days after spraying) with pesticide treatment of papaya leaves with a concentration of 15 %.

## Novelty Statemnet

The use of plants to control plant pests known as natural pesticides is safe from the environment. The mechanism of action of natural pesticides is the role of secondary metabolites contained in these plants. These secondary metabolites are able to control insect pests through nervous disorders and stomach poisons.

The novelty of this article is that it was found that the persistence of natural pesticide solutions (soursop, papaya, and chrysanth leave) suppressed the red mite pest in the imago phase. The natural pesticide of soursop leaf with a concentration of 5 % was effective in suppressing red mite imago up to 82 %. Papaya leaf pesticides with a concentration of 15 % were effective in suppressing red mite pests up to 80 %.

## Author's Contribution

**DR:** Conceptualized and designed the study, elaborated the intellectual content performed the literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, and manuscript revision.

**IK:** Carried out experimental studies, data analysis, statistical analysis, performed literature.

**MM:** Carried out data analysis and manuscript review.

**OE:** Carried out an experimental study.

**MFMA:** Elaborated the intellectual content, explored literature search, data acquisition, manuscript revision and guarantor.

**II and LAS:** Elaborated the intellectual content, explored literature search, data acquisition, and manuscript review.

All authors read and approved the final manuscript.

## Conflict of interest

The authors have declared no conflict of interest.

## Reference

- Adinurani, P.G. 2016. Design and analysis of agro trial data: Manual and SPSS. Plantaxia, Yogyakarta, Indonesia.
- ATSDR - Agency for Toxic Substances and Disease Registry. 2003. Public health statement. Pyrethrins and pyrethroids. (<https://www.atsdr.cdc.gov/ToxProfiles/tp155-c1-b.pdf>)
- Auger, P., A. Migeon, E.A. Ueckermann, L. Tiedl and M. Navajas. 2013. Evidence for synonyme between *Tetranychus urticae* and *Tetranychus cinnabarinus* (Acari, Prostigmata, Tetranychidae): Review and new data. *Acarologia*, 53(4): 383–415. <https://doi.org/10.1051/acarologia/20132102>
- Azwanida, N.N. 2015. A review on the extraction method used in medicinal plants, principle, strength and limitation. *Med. Aromat. Plants*, 4(3) 1000196: 1–6.
- Camila, M.S., L.I.B. Edson, P.R. Leandro, F.S. Ivana, M. Rafaela, U.B. Keylla, D.V. Jase and B.F. Joao. 2016. Lethal and growth inhibitory activities of Neotropical *Annonaceae* – derived extracts, commercial formulation and an isolated acetogenin against *Helicoverpa aemygera*. *J. Pest. Sci.*, 90 (2): 701–709. <https://doi.org/10.1007/s10340-016-0817-9>
- Childers, C.C. and T.R. Fasulo. 2009. Citrus red Mite, IFAS extension, ENY817 University of Florida. (<https://ufdcimages.uflib.ufl.edu/IR/00/00/46/19/00001/CH02100.pdf>)
- Gusthinnadura, O.D.S., A.T. Obeyesundara and M.M.W. Aponso. 2017. Extraction method, qualitative and quantitative techniques for screening of phytochemicals from plants. *Am. J. Essent. Oil Nat. Prod.*, 5(2): 29–32.
- Enyiukwu, D.N. and C.C. Ononuju. 2016. Comparative Priming effects of Phytochemicals from *Alchornea cordifolia* and piper guineese on Cowpea (*Vigna unguiculata* L. Walp) Seeds. *J. Plant Pest. Sci.*, 3 (1): 01-04.
- Ezekiel, A. and F. Mamboya. 2012. Papain, a plant enzyme of biological importance: A review. *Am. J. Biochem. Biotechnol.*, 8(2): 99–104. <https://doi.org/10.3844/ajbbsp.2012.99.104>

- Hermawan, P.G. and H. Laksono. 2013. Extraction of soursop leaves (*Annona muricata* L.) using ethanol, Jurnal Teknologi Kimia dan Industri, 2: 98–105.
- Kardinan A. 2005. Natural pesticides: Capabilities and applications. Penebar Swadaya, Jakarta, Indonesia.
- Klashoven, L.G.E. 1981. The pest of crops in Indonesia. PT Ichtiar Baru. Jakarta, Indonesia
- Murray, B.I. 2020. Botanical insecticide in the twenty-first century- fulfilling their promise. Annu.Rev.Entomol.,(65):233–249.<https://doi.org/10.1146/annurev-ento-011019-025010>
- Nadia J.H., C. Pérez-Plasencia, V.A. Castro-Torres, M. Martínez-Vázquez, A.R. González-Esquinca and A. Zentella-Dehesa. 2019. Selective acetogenins and their potential as anticancer agents. Front. Pharmacol., 10 (Article 783):1–12. <https://doi.org/10.3389/fphar.2019.00783>
- NPIC- National Pesticide Information Center. 2014. Pyrethrins, general fact sheet. (<http://npic.orst.edu/factsheets/pyrethrins.html>)
- Neela, B. and G.S. Alexander. 2010. Soursop (*Annona muricata* L.): Composition, Nutritional Value, Medicinal Uses and Toxicology. p. 621–635. In: Watson RR and V. Preedy (Eds.). Bioactive Foods in Promoting Health: Fruits and Vegetables. Publisher: Academic Press: Oxford. <https://doi.org/10.1016/B978-0-12-374628-3.00039-6>
- Novizan. 2002. Making and using environmentally friendly pesticides PT. AgroMedia Pustaka, Jakarta, Indonesia.
- NSW Department of Primary Industries. 2017. Citrus Red Mite (*Panonychus citri*) McGregor <http://www.dpi.nsw.gov.au>
- Riahi, E., P. Shishehbor, A.R. Nemati and Z. Saeidi. 2013. Temperature effects on development and life table parameters of *Tetranychus urticae* (Acari: Tetranychidae). J. Agric. Sci. Technol., 15(4): 661–672.
- Revathy B., R. Ramasamy and S. Rajarathnan. 2016. Thermal processing alters the chemical quality and sensory characteristics of Sweetsop (*Annona squamosa* L.) and Soursop (*Annona muricata* L.) pulp and nectar. J. Food Sci., 81 (1) ID 26642109: S182-S188. <https://doi.org/10.1111/1750-3841.13165>
- Siswarni, M.Z., Nurhayani, S.D. Sinaga. 2016. Extraction of acetogenin from leaves and seeds of Sirsak (*Annona muricata* L.) with acetone solvent. Jurnal Teknik Kimia USU, 5 (2):1–4. <https://doi.org/10.32734/jtk.v5i2.1533>
- Suresh, W., S. Saha, V. Tripathi and K.K. Sharma. 2017. Phytochemical biopesticides: some recent developments. Phytochem. Rev., (16): 989–1007. <https://doi.org/10.1007/s11101-017-9512-6>
- UC – IPM. University of California, Integrated Pest Management. 2017. UC Pest management guidelines, for citrus Red Mite (*Panonychus citri*) on citrus, Agriculture and Natural Resources, University of California. (<http://www.ipm.ucdavis.edu>)
- Wiratno. 2010. Some vegetable pesticide formulas from cloves. Jurnal Agritek, 13 (1): 6–12
- Zusfahair, D.R. Ningsih and F.N. Habibah. 2014. Characterization of papain from *Carica papaya* L. Leaves. Jurnal Molekul, 9(1): 44–55. <https://doi.org/10.20884/1.jm.2014.9.1.149>