Acaricidal activities of algal extracts against the house dust mite, *Dermatophagoides pteronyssinus* (Trouessart)

Suneerat RUANGSOMBOON* and Jarongsak PUMNUAN

Faculty of Agricultural Technology, King Mongkut’s Institute of Technology Ladkrabang, Bangkok 10520, Thailand

**ABSTRACT**

The acaricidal activities of seventeen crude algal extracts were tested against the house dust mite *Dermatophagoides pteronyssinus* (Trouessart) by the contact method at concentrations of 0 (95% ethanol as a control), 0.32, 3.2, and 32 µg/cm². The crude extracts were isolated from cyanobacteria (*Oscillatoria* sp., *Phormidium* sp., *Spirulina platensis*, *S. maxima*, *Fischerella* sp., *Hapalosiphon* sp., *Mastigocladopsis* sp.), red algae (*Acanthophora* sp., *Gracilaria* sp.), green algae (*Cladophora* sp., *Caulerpa lentillifera*, *Ulva intestinalis*, *U. rigida*), and brown algae (*Turbinaria* sp., *Dictyota* sp., *Padina* sp., *Sargassum* sp.) by means of serial extraction with methanol, dichloromethane, and hexane. Mite mortality was measured after 24 h of exposure to the extracts under laboratory conditions at 25±1°C and 86±1% relative humidity. The methanolic crude extracts caused higher mortality than the dichloromethane and hexane extracts. The methanolic crude extracts of *Oscillatoria* sp., *Phormidium* sp., *S. platensis*, *S. maxima*, *U. intestinalis*, *Sargassum* sp., and *Dictyota* sp. caused 72.5-99.3% mortality at a concentration of 32 µg/cm². These seven extracts were then reassessed at concentrations of 0, 0.16, 0.32, 1.6, 3.2, 16, 32, 48, and 64 µg/cm². The *Oscillatoria* sp. and *Phormidium* sp. extracts showed the highest acaricidal activity, with LC₅₀ values of 0.39 and 0.40 µg/cm², respectively. These results suggest that algal extracts may have potent acaricidal activity.

**Key words:** contact method, acaricidal, algal extracts

**INTRODUCTION**

The house dust mite *Dermatophagoides pteronyssinus* (Trouessart), which is abundant in homes in Thailand, is a major source of allergens and is also associated with sudden infant death syndrome (Helson 1971). Several synthetic acaricides such as γ-benzene hexachloride, pirimiphos-methyl, benzyl benzoate, *N,N*-diethyl-m-toluamide, and dibutyl phthalate effectively control house dust mites (Pollart et al. 1987). However, these agents must be employed carefully because repeated use can cause acaricidal resistance, posing risks to the environment and human health.

* Corresponding author: e-mail: krsuneer@kmitl.ac.th; phone-fax: (66) 2-3298517

DOI: 10.2300/acari.25.Suppl_169
This problem has necessitated a search for safe and effective alternatives for controlling house dust mites, but few such alternatives have been discovered. Natural resources, such as plant extracts, are potential sources of new acaricides. Plant extracts contain many bioactive chemicals, such as terpenes, terpenoids, phenol-derived aromatic components, and aliphatic components (Bakkali et al. 2008), and as a result, the use of plant extracts for house dust mite control has been considered (Bakkali et al. 2008; Rim and Jee 2006; Saad et al. 2006). In particular, algae have attracted much attention as a valuable source of chemically diverse bioactive compounds with numerous health benefits (Chamorro and Barron 2002; Jensen et al. 2001; Pugh and Pasco 2001; Tuney et al. 2006; Vo et al. 2012). Thailand is home to abundant freshwater and marine algae; most of the freshwater algae have been collected from ponds, and most of the marine algae have been collected from beaches. Many of these algae are treated as waste materials; only certain species are consumed as food or used as fertilizers and in the pharmaceutical industry.

The present study focused on extracts of algae widely encountered in Thailand; some of the studied species do not presently have any known uses. Algae have higher photosynthetic activity than do terrestrial plants, are adaptable to different growing conditions, and can be grown in either fresh water or salt water and thus do not require the use of land. We have already reported preliminary data on the effectiveness of crude extracts of six genera of cyanobacteria against house dust mites (Samosorn et al. 2010). In this study, we conducted more-detailed and comprehensive experiments.

The aim of this study was to screen the extracts of 15 genera of algae: cyanobacteria/blue-green algae ( Oscillatoria sp., Phormidium sp., Spirulina platensis [Norst.] Geitler, Spirulina maxima [Setch. et Gard.] Geitler, Fischerella sp., Hapalosiphon sp., Mastigocladopsis sp.), red algae ( Acanthophora sp., Gracilaria sp.), green algae ( Cladophora sp., Ca. lentillifera J. Agardh, Ulva intestinalis Linnaeus, Ulva rigida C. Agardh), and brown algae ( Turbinaria sp., Dictyota sp., Padina sp., Sargassum sp.) for acaricidal activity against the most common dust mite found in Thailand, Dermatophagoides pteronyssinus, under laboratory conditions.

MATERIALS AND METHODS

House dust mite culture

House dust mites ( D. pteronyssinus) were isolated from a private house in the Ladkrabang District of Bangkok, Thailand. They were reared in mite bottles kept in a mite chamber (1.2×10⁴ cm³) at 25.0±1.0°C and a relative humidity of 86±1% and were fed a laboratory diet (powdered rat feed, wheat grain, and yeast, 1:1:0.25 by weight).

Algal extracts

1. Algal materials

Cyanobacteria ( Oscillatoria sp., Phormidium sp., Sp. platensis, Sp. maxima, Fischerella sp., Hapalosiphon sp., Mastigocladopsis sp.) were isolated from a fish pond in the Ladkrabang District (13°41' N, 100°50' E) of Bangkok Province in April, 2009 and were cultured in BG-11 medium, the optimum medium for blue-green algae cultivation (Stanier et al. 1971), at 25±0.5°C for 14 days. Cyanobacterial cells were harvested during the exponential phase, dried in a
hot air oven at 60 °C, ground, and stored at 4 °C until use, as described in Samosorn et al. (2010). Red algae (Acanthophora sp., Gracilaria sp.), green algae (Cladophora sp., Ca. lentillifera, U. intestinalis, U. rigida), and brown algae (Turbinaria sp., Dictyota sp., Padina sp., Sargassum sp.) were collected from the Ao-Cho District (12° 3’ N, 102° 31’ E) of Trat Province in May, 2009. The algae were washed with tap water to remove surface-adhered particles, dried in a hot air oven at 60 °C, powdered, and stored at 4 °C until use.

2. Extraction
Algal cells (200 g) were subjected to three sequential extractions—with 1000 mL of methanol, 1000 mL of hexane, and 1000 mL of dichloromethane—by heating on a stirring hot plate at 60 °C for 24 h. The solution was then filtered under a vacuum through filter paper (Whatman No. 1), and the filtrate was evaporated to dryness under reduced pressure.

Toxicity testing
As described in Samosorn et al. (2010), toxicity experiments were performed in glass tubes measuring 0.4 cm in diameter and 3 cm in length with fine nylon mesh on both ends. Each tube was added 24 µl of a 0, 0.01, 0.1, or 1% (0, 0.32, 3.2, and 32 µg/cm², respectively) solution of crude algal extract in 95% ethanol. The solution was then distributed evenly around the inner wall of the test tube and allowed to air dry. Ten adult mites were placed in the glass tube. Control and treatment experiments (n = 10 each) were carried out at room temperature (25 ± 1 °C) and 86 ± 1% relative humidity. Twenty-four hours after treatment, mortalities were determined with a binocular microscope. Mites were considered dead if morphological changes were apparent or if appendages did not move when the mites were prodded with a pointed brush.

LC₅₀
Algal extracts that caused more than 70% mortality were reassessed at 0 (95% ethanol as a negative control), 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, and 2.0% (corresponding to concentrations of 0, 0.16, 0.32, 1.6, 3.2, 16, 32, 48, and 64 µg/cm²). LC₅₀ values were calculated by the probit analysis method.

Statistical analysis
Average values of mortality (n = 10) and their standard deviations are presented in the figures and tables. The significance of difference was tested using analysis of variance and the Tukey-Kramer honest significant difference test.

RESULTS
The masses of the crude cell extracts obtained by means of serial extraction with methanol, hexane, and dichloromethane are shown in Table 1. In all cases, the mass of the methanol extract was the largest. The total mass of crude extract was highest for Dictyota sp. (19.65 g), followed by Padina sp. (16.46 g) and Sp. platensis (15.22 g); and the total mass of crude extract was the lowest for Phormidium sp. (4.69 g).
Acaricidal activity of crude extracts

The results of direct contact testing on house dust mites after 24 h of exposure at three different algal extract concentrations are shown in Figs. 1-3. Extracts of the cyanobacteria Oscillatoria sp., Phormidium sp., and Sp. maxima showed high acaricidal activity at all tested concentrations. Increasing the concentration from 0.32 to 32 µg/cm² increased mite mortality. At the 32 µg/cm² concentration, the methanolic crude extract of the Oscillatoria sp. had the highest mortality (99.0 ± 1.0%), followed by Phormidium sp. (98.0 ± 1.3%), Sp. maxima (90.3 ± 3.5%), Sargassum sp. (89.4 ± 2.4%), U. intestinalis (86.1 ± 6.8%), Sp. platensis (85.8 ± 4.0%), and Dictyota sp. (70.0 ± 2.2%). We obtained LC₅₀ values for these seven algal extracts at various concentrations (Table 2). The negative control showed no mortality (data not shown), and at concentrations of 48 and 64 µg/cm², the crude methanolic extracts effectively killed the mites. Oscillatoria sp. showed the lowest LC₅₀ (0.39 µg/cm²), followed by Phormidium sp. (0.40 µg/cm²), and Sp. maxima (0.71 µg/cm²).

DISCUSSION

Herein we report the acaricidal activities of algal extracts against the house dust mite D. pteronyssinus. Previously, we reported the acaricidal activity of six cyanobacterial extracts at 32 µg/cm² against house dust mites (Samosorn et al. 2010) and suggested that crude methanol extracts obtained from the cyanobacteria Oscillatoria sp., Phormidium sp., and Sp. maxima had high potential to control dust mites. In this study, we confirmed the effectiveness of these

| Table 1. Masses of crude extracts (g) obtained by methanol, hexane, and dichloromethane extraction of 17 algae (200 g of raw material). |
|-------------------------------------------|
| **Solvent** | **Methanol** | **Hexane** | **Dichloromethane** |
|-------------------------------------------|
| **Cyanobacteria/blue-green algae**        |
| 1 Oscillatoria sp. | 8.25 | 0.91 | 0.41 |
| 2 Phormidium sp. | 3.28 | 0.74 | 0.67 |
| 3 Sp. platensis | 12.11 | 2.04 | 1.07 |
| 4 Sp. maxima | 10.87 | 1.25 | 1.11 |
| 5 Mastigocladopsis sp. | 6.12 | 1.11 | 1.02 |
| 6 Hapalosiphon sp. | 8.17 | 2.11 | 1.00 |
| 7 Fischerella sp. | 8.64 | 2.18 | 1.86 |
| **Red algae**        |
| 8 Gracilaria sp. | 4.75 | 0.63 | 0.92 |
| 9 Acanthophora sp. | 4.99 | 1.20 | 1.12 |
| **Green algae**      |
| 10 Ca. lentillifera | 8.23 | 2.41 | 0.89 |
| 11 U. intestinalis | 8.57 | 1.68 | 1.24 |
| 12 U. rigida | 6.64 | 0.81 | 0.84 |
| 13 Cladophora sp. | 4.01 | 2.63 | 2.54 |
| **Brown algae**      |
| 14 Sargassum sp. | 6.99 | 1.00 | 0.90 |
| 15 Turbinaria sp. | 2.80 | 1.30 | 0.77 |
| 16 Padina sp. | 10.41 | 1.84 | 4.21 |
| 17 Dictyota sp. | 17.68 | 1.04 | 0.93 |
Fig. 1 Percentage mortality of *D. pteronyssinus* caused by algal extracts at a concentration of 0.32 μg/cm² by the direct contact method. Error bars represent±SD (n=10).
Fig. 2 Percentage mortality of *D. pteronyssinus* caused by algal extracts at a concentration of 3.2 μg/cm² by the direct contact method. Error bars represent±SD (n = 10).
Fig. 3  Percentage mortality of *D. pteronyssinus* caused by algal extracts at a concentration of 32 μg/cm² by a direct contact method. Error bars represent±SD (n=10). Data for *Oscillatoria* sp., *Phormidium* sp., *Sp. maxima*, *Hapalosiphon* sp., *Mastigocladopsis* sp., and *Fischerella* sp. are from Samosorn et al. (2010).
We compared the acaricidal activities from the previous study and from this study with those of extracts from other plants and with the activities of several synthetic acaricides (Table 3). Overall, the acaricidal activities of the algal extracts, especially those of the cyanobacteria extracts, exceeded the activities of the other plant extracts and the synthetic acaricides.

Cyanobacteria are an important source of new bioactive natural compounds and are exploited in biotechnology developed for use in humans (Sielaff et al. 2006; Spolaore et al. 2006; Thajuddin and Subramanian 2005). Numerous metabolites from cyanobacteria may function ecologically as allelochemicals. The allelochemical roles of cyanobacterial toxins and their...
applications as algaecides, herbicides, and insecticides have been investigated (Berry et al. 2008). Allelochemicals (e.g., microcystin, lyngbyatoxin A, and cyanobacterin) may also play a defensive role against potential predators and grazers (Berry et al. 2008; Jang et al. 2007). It has been suggested that the use of such compounds as biocides in place of synthetic biocides would be beneficial from an environmental standpoint (Rastogi and Sinha 2009).

Bioactive algal compounds are present intracellularly (Teneva et al. 2005). *Sp. platensis* has been reported to have antimicrobial activity (Ozdemir et al. 2004) and to inhibit the replication of several viruses, such as herpes simplex and HIV-1 (Chamorro and Barron 2002). Moreover, *Oscillatoria* sp. has antibacterial and antifungal activities, and the extracts may be much more effective than currently available antibiotics and fungicides (Katircioglu et al. 2005).

Our current results indicate that among the tested algae groups, cyanobacteria had the highest acaricidal activity, and they were easy to cultivate under both indoor and outdoor conditions. The green alga *U. intestinalis* had lower acaricidal activity than *Cymbopogon nardus* and *C. citratus*, but its activity was nevertheless higher than the activities of other plant extracts and synthetic acaricides; whereas the brown algae *Sargassum* sp. and *Dictyota* sp. had higher acaricidal acidity than *Taiwania cryptomerioides* and *Cnidium officinale* and the synthetic acaricides (Table 3). The green alga *U. intestinalis*, and brown algae *Sargassum* sp., and *Dictyota* sp. are abundant in most areas and thus could be harvested from natural sources. For all the algal species, the methanol extract had the highest mass and the highest acaricidal activity. Therefore, our results could be used as the basis for further evaluation of these agents as natural alternatives for killing house dust mites.

**CONCLUSION**

Extracts of the algae *Oscillatoria* sp., *Phormidium* sp., and *Sp. maximam* may be useful as natural products to control house dust mites. The methanol extracts of these algae caused more than 90% mortality to mites. The *Oscillatoria* sp. extract, which showed the highest acaricidal activity, is promising for use as an acaricidal natural product.

**ACKNOWLEDGEMENTS**

This research was supported by grant fund from National Research Council of Thailand (NRTC).

**REFERENCES**

Bakkali, F., S. Averbeck, D. Averbeck and M. Idamor (2008) Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46: 446–475.

Berry, J. P., M. Gantar, M. H. Perez, G. Berry and F. G. Noriega (2008) Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. *Marine Drugs*, 6: 117–146.

Chamorro, M. G. and B. L. Barron (2002) Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. *Antiviral Research*, 56: 279–285.

Chang, S. T., P. F. Chen, S. Y. Wang and H. H. Wu. (2001) Antimite activity of essential oils and their constituents from
Taiwania cryptomerioides. Journal of Medical Entomology, 38: 455–457.

Helson, G. A. H. (1971) House-dust mites and possible connection with sudden infant death syndrome. The New Zealand Medical Journal, 74: 209.

Insung, A. and J. Pumnuan (2009) Acaricidal activity of essential oil of medicinal plants against house dust mite, Dermatophagoides pteronyssinus (Trouessart). KKU Science Journal, 37: 183–191. (In Thai with English abstract).

Jang, M. H., H. Kyong and N. Takamura (2007) Reciprocal allelopathic responses between toxic cyanobacteria (Microcystis aeruginosa) and duckweed (Lemma japonica). Toxicon, 49: 727–733.

Jensen, G. S., D. I. Ginsberg and C. Drapeau (2001) Blue-green algae as an immuno-enhancer and biomodulator. Journal of the American Nutraceutical Association, 3: 24–30.

Katircioglu, H., Y. Beyatli, B. Aslim, Z. Yüksel and T. Atici (2005) Screening for antimicrobial agent production of some microalgae in freshwater. The Internet Journal of Microbiology, Volume 2 Number 2. https://ispub.com/IJMB/2/2/9098

Kim, E. H., H. K. Kim and Y. J. Ahn (2003) Acaricidal activity of clove bud oil compounds against Dermatophagoides farina and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry, 51: 885–889.

Kwon, J. H. and Y. J. Ahn (2002) Acaricidal activity of butylidenephthalide identified in Cnidium officinale rhizome against Dermatophagoides farinae and D. pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry, 50: 4479–4483.

Ozdemir, G., N. U. Karabay, M. C. Dalay and B. Pazarbasi (2004) Antibacterial activity of volatile components and various extracts of Spirulina platensis. Phytotherapy Research, 18: 754–757.

Pollart, S. M., G. W. Jr. Ward and T. A. E. Platts-Mills (1987) House dust sensitivity and environmental control. Immunology and Allergy Clinics of North America, 7: 447–461.

Pugh, N. and D. S. Pasco (2001) Characterization of human monocyte activation by a water soluble preparation of Aphanizomenon flos-aquae. Phytomedicine, 8: 445–453.

Rastogi, R. P. and R. P. Sinha (2009) Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnology Advances, 27: 521–539.

Rim, I. S. and Jee C. H. (2006) Acaricidal effects of herb essential oils against Dermatophagoides farinae and D. pteronyssinus (Acari: Pyroglyphidae) and qualitative analysis of a herb Mentha pulegium (pennyroyal), Korean Journal of Parasitology, 44: 133–138.

Saad, E.-Z., R. Hussien, F. Saher and Z. Ahmed (2006) Acaricidal activities of some essential oils and their monoterpenoidal constituents against house dust mite, Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Zhejiang University SCIENCE B, 7: 957–962.

Samosorn, A., J. Pumnuan, A., Insung and S. Ruangsomboon (2010) Effectiveness of Cyanobacteria Extracts on the House Dust Mite, Dermatophagoides pteronyssinus (Trouessart) by Contact Method. Proceedings of 16th Asian Agricultural Symposium and 1st International Symposium on Agricultural Technology "Sufficiency AgricultureTokai University and Faculty of Agricultural Technology KMITL, Bangkok, Thailand. 25–27 Aug. 2010: 700–703.

Sielaff, H., G. Christiansen and T. Schwecke (2006) Natural products from cyanobacteria: exploiting a new source for drug discovery. IdDrugs, 9: 119–27.

Spolaore, P., C. Joannis-Cassan, E. Duran and A. Isambert (2006) Commercial applications of microalgae. Journal of Bioscience Engineering, 101: 87–96.

Stanier, R. Y., R. Kunisawa, M. Mandel and G. CohenBazire (1971) Purification and properties of unicellular bluegreen algae (Order Chroococcales). Bacteriological Reviews, 35: 171–205.

Teneva, I., D. Balik, K. Lyubka, M. Rumen and M. Rumen (2005) Toxic potential of five freshwater Phormidium species (Cyanoprokaryota). Toxicon, 45: 711–725.

Thajuddin, N, and G. Subramanian (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Current Science, 89: 47–57.

Tuney, I., B. H. Cadirci, D. Unal and A. Sukatar (2006) Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). Turkish Journal of Biology, 30: 171–175.

Vo, T. S., D. H. Ngo and S. K. Kim (2012) Marine algae as a potential pharmaceutical source for anti-allergic therapeutics. Process Biochemistry, 47: 386–394.