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# Acaricidal Toxicity of Nano Essential Oil of Black Pepper against African Red Mite (*Eutetranychus africanus* (Tucker))

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Acaricidal property of nano essential oil of black pepper, *Piper nigrum* Linn against the African red mite, *Eutetranychus africanus* (Tucker) was investigated by using contact methode. Test in terms of mortality and repellency effects was made by dipping the mulberry leaf cut in to circle, 2.7 cm diameter in nano-essential oils at 0 (water), 0.2 0.4 0.6, 0.8 and 1% concentrations for 1 minute. The mite mortality was checked at 24 hrs and compared with various concentrations of surfactant (Tween60+PEG). As for repellent test, the choice test was performed by dipping an half cut leaf in the nano-essential oil at 0 (surfactant), 0.02, 0.06 and 0.1% concentrations, whereas, the other side of cut leaf was dipped with water. The mite repellency was checked at 24 hrs. The results presented that by contact method, both nano-essential oil of black pepper and surfactants showed high effectiveness against African red mite. Therefore, at 24 hrs, both with the concentration of 1% caused the mite mortalities of 96 and 92% and gave $LC_{50}$ at 0.34 and 0.54%, respectively. Nano-essential oil of black pepper presented moderately repellency effect, with 42.39-51.67 %RI at 24 hrs. However, nano-essential oil of black pepper at all concentrations showed higher mortality and repellency effects when compared to the surfactants.

**Keywords:** nano, essential oil, black pepper, African red mite

## Introduction

The African red mite (*Eutetranychus africanus* (Tucker)) is a pest of many economic crops such as durian, pomelo, tangerine, mandarin orange and papaya. It is remarkably an important pest of durian in Thailand, especially during the cool season. It suck leaves juice at the upper surface of leaves. The pest multiplies rapidly during hot and dry weather conditions. The infested leaves show whitish spot where the mites were feeding and the leaves become pale and do not have a glossy green like normal leaves. The trees can tolerate quite a number of mites, but heavy infestations may result in durian leaves falling down, which will effect the development of flowers and fruit.

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Mites are small and with the naked eye, they are hardly visible as small reddish or brown dote on the leave surface. The body appears oval shape of the female mites is 417.67 micron long and 350.33 micron wide. The male is light brown and slightly smaller than the female. Mating starts as the male finished molting and becomes an adult. It starts looking for a third instar larva female and when it finds one, then waits for the final molting of the female. Mating then takes place immediately and 1-2 days after mating the female will start to produce eggs. The life cycle of both males and females takes about 9 days. The developmental stages including larva, 1st nymph and 2nd nymph with 4-5 days of developmental period, whereas adult live for around 6-8 days. (Mite and Spider Research Section, 2013).

Using chemical method is widely used to control mite pests it because of convenient application and high efficacy. But also brought harm on users, consumers, and the environment as well as the occurrence of mite resistance. Kulpiyawat *et al*., (2002) reported the resistance of African red mite to many insecticides such as bromopropylate, propargite, amitraz and dicofol. They revealed that all African red mite species in some northern area resistant to all chemicals. The use of chemicals to control mites also resulted in both increment of the production cost and the risk to the environment. The use of medicinal plants to control mite pest in one of alternative methods. It is definitely safe to users, consumers and environment.

Black pepper (*Piper nigrum*; Family: Piperaceae) is an important spice containing valuable medicinal and aromatic properties (Shanmugapriya *et al*., 2012) Black pepper having multifunctional properties which is utilized as medicine, spice, human dietary, preservative, and also as a biocontrol agent against various pathogens and pests (Hussain *et al*., 2011) The secondary metabolites isolated from *P. nigrum* play a defensive role against infections caused by different pests, mammals, microbes, and insects (Ahmad *et al*., 2012) The principle compound called piperamides from *P. nigrum* had various biological activities including antimicrobial, antioxidant and insecticidal activities (Scott *et al*., 2005) George *et al*. (2009) also reported that essential oil of black pepper at 0.14 mg/cm3 could be used to repel the red mite *Dermanyssus gallinae* (De Geer) during the first 2 days. Insung and Pumnuan (2008) stated that the essential oil of citronella grass (*Cymbopogon nardus*) was remarkably toxic to *D. pteronyssinus* with $LD_{50}$ value at 0.935 µg/$cm^3$ 24 hrs after fumigation. Moreover, essential oil of *P. nigrum* showed acaricidal effect on the spider mite, *Eutetranychus cendanai* Rimando with $LC_{50}$ at 23.6 ml/l (Sornlek, 2001).

Nano-emulsion techniques play a lot of roles during the past 3-4 years, particularity in the section of medications. Nano-emulsion techniques would increase stability of active ingredients during storage (Satingam, 2012).

Nano-emulsion technique is appropriate in order to keep the active substance quality that is volatile and soluble in oil (lipophilic action) (Shafiq-un-Nabi, 2007) It would be promising if essential oil of black pepper diluted in water as nano particle and may have more effectiveness then usual condition.

Objective of this study was to evaluate the efficacy of nano essential oil from black pepper to control the African red mite in both mortality and repellent activities.

## Materials and Methods

### *Insect preparation*

A culture of the African red mite, *Eutetranychus africanus* (Tucker) was reared on mulberry leaves at temperature 28±2 ℃ in the laboratory of Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand. The mulberry leaves were put on a cotton swab soaked in a tray to serve as a food source. Identified mite were released to the leaves when new leave was replaced every 4-5 days.

### *Nano essential oil of black pepper preparation*

Essential oil (EO) of black pepper used in the experiment was purchased from Thailand – China Flavours and Fragrances Industry Co., Ltd. To prepare nano plant essential oils, each EO was diluted in water when primary surfactant (surfactant) was Tween60 (HLB = 14.9), and co-surfactant was ethylene glycol 400 (PEG400) (HLB = 13). The above matches with the ingredients called Smix. Each couple had a ratio of 1:1, 1:1.5 to 1:2, 1:2.5, 1:3, 1:3.5, 1:4 and 1:4.5 and after that filled distilled water for 10 ml, then essential oils of black pepper, was mixed with Smix at ratios of 1:1, 1:1.5 to 1:2, 1.2.5, 1:3, 1:3.5, 1:4 and 1:4.5, set aside at room temperature. Reduction of particle size of formulas plant essential oils by High Pressure Homogenizer. Stability of nano-essential oil formulas was measured to obtain zeta Potential Charge by using Nano plus Zeta / Nano Particle Analyze.

### *Bioassay*

Efficiency test in term of acaricidal and repellent activities of nano-essential oil of black pepper against the African red mite, *Eutetranychus africanus* (Tucker) was performed by using contact method.

The bioassay of acaricidal property was adapted from Charoensak *et al*. (2009) when the mulberry leaf was cut in to circle, 2.7 cm diameter and it was dipped in nano-essential oils at different concentrations; 0.0 (water), 0.2 0.4 0.6, 0.8 and 1% for 1 minute. Amount of 10 African red mite adult females were released to treated mulberry leaves. The mite mortality was checked at 24 hrs and compared with various concentrations of surfactant (Tween60+PEG).

As for repellent test, mulberry leaf was cut in to circle, 2.7 cm diameter. An half cut mulberry leaf was dipped in the nano-essential oil at various concentrations as 0.0 (surfactant), 0.02, 0.06 and 0.1% for 1 minute whereas, the other side was dipped with water (control) and placed close to the treated one. A circle paper size 0.6 cm was laid between those cut mulberry leaves. Ten African red mite adult females were released to the paper. The number of mite found on each side of leave was checked and calculated to obtain %RI according to $[(C-T)/C+T)] \times 100$ (Pascual-Villalobos and Robledo, 1998) at 24 hrs.

### *Statistical analysis*

The experiment was designed in five completely randomized replicates. The data obtained was statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). Median lethal concentration ($LC_{50}$) was calculated by the probit method.

## Results

The result showed that both nano-essential oils of black pepper and surfactant gave high effectiveness against the African red mites at 24 hrs particularly at the concentration of 1%. The mortality of African red mite caused by nano-essential oil of black pepper was higher than that of surfactant when 1% concentration caused the mortality of 96 and 92% and showed the median lethal concentration ($LC_{50}$) at 0.34 and 0.54%, respectively at 0.4 and 0.6% concentration but was not significantly different (Table 1, Figure 1) Nano-essential oil of black pepper presented moderate repellency effect, with 42.39 - 51.67 %RI at 24 hrs. Surfactant alone presented lower repellency effect,

with 26.85 – 40 %RI (Figure 2) Control treatment presented 5.19% mortality with the lowest repellency at 26.85 %RI.

**Table 1.** Mortality percentage of the African red mite (*Eutetranychus africanus* (Tucker)) treated with nano-essential oil of black pepper after 24 hrs by contact method.

| Nano-essential oil | % Mortality[1/] | | | | | | LC 50 | LC 90 | Slope | SE |
|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration (%) | | | | | | | | | |
| | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1 | | | | |
| **surfactant** | 5.19±5.69$^{Da}$ | 37.1±12.1$^{Ca}$ | 37.0±5.6$^{Cb}$ | 42.6±6.3$^{Cb}$ | 67.8±7.3$^{Bb}$ | 92.0±4.5$^{Aa}$ | 0.54 | 1.11 | 2.265 | 0.184 |
| **surfactant+oil black pepper** | 5.19±5.69$^{Da}$ | 44.8±17.7$^{Ca}$ | 67.3±8.3$^{Ba}$ | 75.4±5.8$^{Ba}$ | 88.0±8.4$^{Aa}$ | 96.0±5.5$^{Aa}$ | 0.34 | 0.78 | 2.889 | 0.213 |
| **%CV** | - | 37.11 | 13.59 | 10.23 | 9.50 | 5.32 | - | - | - | - |

[1]/Means in row with the same capital letter and means in column followed by the same common letter are not significantly different at the 5**%** level as determined by DMRT **(**P < 0**.**05**).**

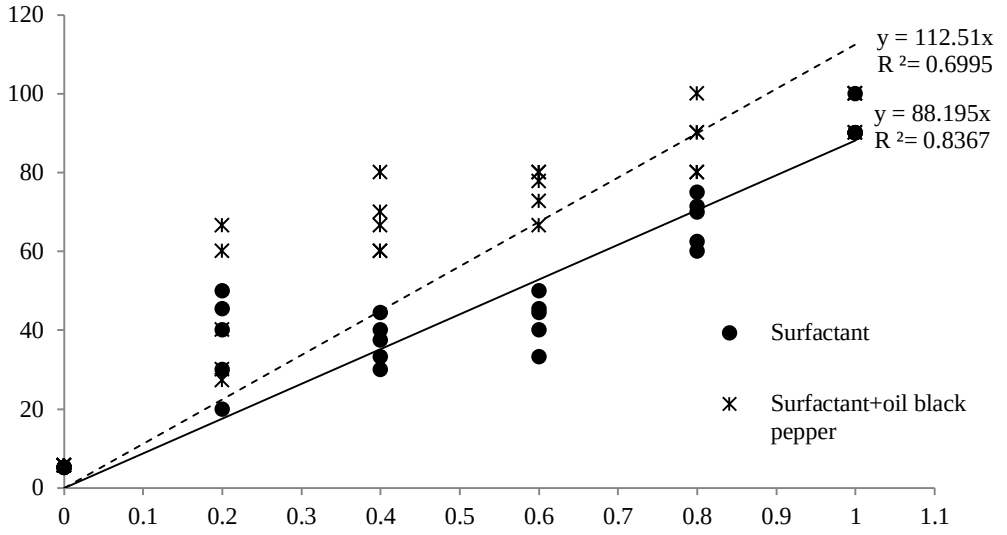


**Figure 1.** Mortality percentage of the African red mite (*Eutetranychus africanus* (Tucker)) treated with nano-essential oil of black pepper after.

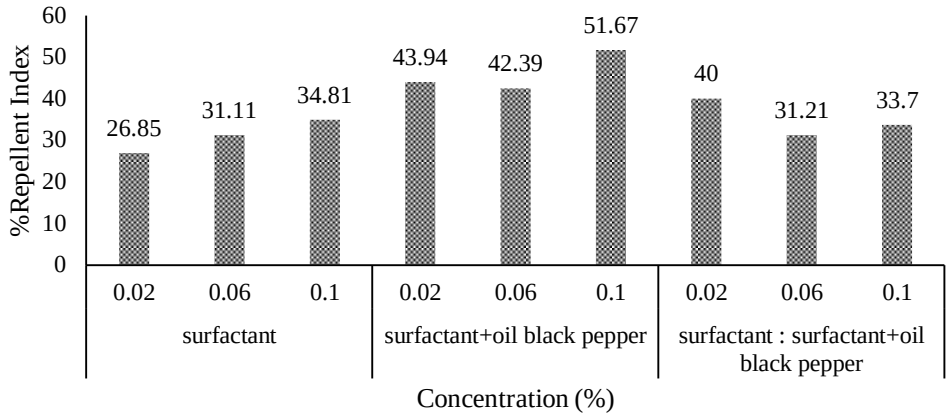


**Figure 2.** Repellent percentage of nano-essential oil of black pepper against the African red mite *(Eutetranychus africanus* (Tucker)) at 24 hrs by contact method.

## Discussion

The percentage of mortality of African red mite caused by nano-essential oil of black pepper was higher than that of surfactant with $LC_{50}$ at 0.34 and 0.54%, respectively. However, it showed moderate repellency whit 51.67 %RI at 0.1% concentration. According to Jarongsak (2009) studied the effect of essential oils obtained from seed coat and seed kernel of black pepper, (*Piper nigrum* Linn.) against the African red mite. They found that the essential oil obtained from seed kernel of black pepper was effective in killing the African red mite. Whereas the essential oil obtained from seed coat was effective in repellency. Scott *et al.*, (2004) found that extract of black pepper contained repellency effect to beetles *Liliocerislilii* (Scopoli). Helen (1997) revealed that ground black pepper and its 95% ethanol crude extract were highly toxic to the rice weevil, *Sitophilus oryzae* (L.), when they were used to surface treat wheat subsequently infested with the insect. This toxicity was attributable to the presence of piperine in black pepper. Il-Kwon *et al*., (2002) studied insecticidal activity of materials derived from the fruits of *Piper nigrum* against third instar larvae of *Culex pipiens pallens*, *Aedes aegypti*, and *Aedes togoi* resulted retrofractamide A was isolated from *P. nigrum* fruits as a new insecticidal principle. On the basis of 48 hrs $LC_{50}$ values, the compound most toxic to *C. pipiens pallens* larvae. Scott *et al*., (2003) presented the efficacy of extracts from two Piperaceae species, *Piper nigrum* Linn. and *P. tuberculatum* Jacq. against larvae and adults of the Colorado potatobeetle *Leptinotarsa*

*decemlineata* (Say). *P. nigrum* reduced larval survival up to 70%. Sighamony *et al*., (1984) tested oil of clove, cedarwood (*Juniperus virginiana*), karanja (*Pongamia glabra*) and an acetone extract of black pepper (*Piper nigrum*) in India by a choice method to determine their repellent effects on adults of *Tribolium castaneum*. All oils or extract were rather highly toxic.

## Conclusion

Nano-essential oil of black pepper showed high toxic activity against the African red mite by contact method. At 1% concentration caused 96% mortality of mite, however the surfactant was also highly toxic. Surfactant mixed with oil black pepper at 0.1% concentration has moderate of repellent gave 51.67 %RI.

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