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Research Article

Effect of Crude Plant Extracts on Mushroom Mite, *Luciaphorus* sp. (Acari: Pygmephoridae)

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The use of plant extracts for controlling agricultural pests has become increasingly popular in the recent years. Mushroom mite, *Luciaphorus* sp., is a destructive pest of several mushroom species and has been reported to cause severe loss of mushroom cultivation in many settings. The efficacies of 23 rhizome and leaf extracts were evaluated against female adults of *Luciaphorus* sp. At 3 days after treatment, the rhizome extracts derived from *Curcuma xanthorrhiza* Roxb. and *Zingiber montanum* (Koenig) Link ex Dietr. were found to have very strong acaricidal activities, resulting in 100% mite mortality, followed by *Curcuma longa* Linn. (98.89%), *Zingiber zerumbet* (L.) Smith. (97.78%), *Kaempferia parviflora* Wall. Ex Baker (88.89%), and *Zingiber officinale* Roscoe. (84.44%). The leaf extracts of *Ocimum sanctum* Linn. and *Melissa officinalis* L. also resulted in 100% mite mortality 3 days after treatment, while the other leaf extracts induced mite mortality only below 70%. The results suggested that rhizome extracts of *C. xanthorrhiza* and *Z. montanum* and leaf extracts of *O. sanctum* and *M. officinalis* have a great potential for future development as natural acaricides for controlling *Luciaphorus* sp.

1. Introduction

Luciaphorus sp. (Acari: Pygmephoridae) is considered as one of the most destructive pests of mushroom cultivation in Thailand. This pygmephorid mite is responsible for the severe production losses of *Lentinus squarrosulus* (Mont.) Singer, *L. polychrous* Lev., *Auricularia auricula-judae* (Bull.:Fr.) Wettst. and *Flammulina velutipes* (Curt.:Fr.) Karst. mushrooms in the Northeast of Thailand [1]. Despite that, little is known about the effective measures for controlling this mite and routine horticultural hygiene is the only procedure to alleviate the problem. To make the situation worse, desperate mushroom growers in Thailand use a large amount of carbamate and organophosphate insecticides and even some harmful solvents to manage this mite; however, this results in very limited success [2].

As a consequence, this mite becomes rapidly resistant and more harmful miticides have to be applied. The use of toxic miticides raises the concerns because of their effects on environments, human safety, and nontarget organisms. Hence, the use of nontoxic natural products for controlling this agricultural pest has been proposed. There are several higher plants that are rich in natural substances, especially the secondary metabolites, such as terpenes, steroids, alkaloids, phenolics, and cardiac glycosides, and can be used as nonharmful, environmentally friendly agents for insect control. Indeed, the use of natural compounds derived from plant extracts has been suggested as alternative treatments for insect and mite controls due to their multiple modes of action, including repellence, feeding and oviposition deterrence, toxicity, and growth regulatory activity [3–6]. Moreover, plant-based pesticides are often found to contain a mixture of active substances which can delay or prevent resistance development [7]. Therefore, in this study, the acaricidal activities of 23 plant extracts were determined against the mushroom mite, *Luciaphorus* sp.

2. Materials and Methods

2.1. Mushroom and Mite Culture. Lentinus squarrosulus Mont. mushroom culture was obtained from the Mushroom Growers Society of Thailand. The mycelium was freshly sub cultured on 90 mm plastic Petri dish plates containing potato dextrose agar (PDA, Sigma) and grown at 25°C.

Luciaphorus sp. mites were collected from infested *L. squarrosulus* composts obtained from Rapeephan mushroom farm in Khon Kaen province in the Northeast of Thailand. A pair of male and female mites was maintained at 28°C using fresh *L. squarrosulus* spawn that was grown on sawdust and sorghum grains in a glass bottle. The offspring that were in-house bred inside this glass bottle were used for all the experiments.

2.2. Preparation of Plant Extracts. Leaves and rhizomes of 23 plants were collected locally from Mahasarakham province in the Northeast of Thailand (Table 1). Plant materials were cut into small pieces and dried in hot air oven at 45°C for 3 days.

The dried plants were separately ground into powders using a small grinder and stored at 4°C in polypropylene bags. For extraction, 100 g of each powdered plant materials and 300 mL of 80% ethyl alcohol were added into sterile 2L Erlenmeyer flask, and the flask was agitated at 100 rpm for 24 h. After filtering through a Buchner funnel and Whatman No. 1 filter paper, the extracts were concentrated under low pressure using rotary evaporator. The crude extracts were reconstituted to have the concentration of 20% (w/v) using 80% ethyl alcohol (v/v, in distilled water) and stored at 4°C in glass vials to be used as stock plant extracts. For the tests, these stock plant extracts were dissolved in distilled water containing 0.05% Tween 80 to have the concentration of 5% (w/v).

2.3. Bioassay. For evaluation of each plant extract, 100 adult female mites were transferred to a 50 mm Petri dish plate containing mushroom mycelial culture grown on PDA medium, and the plate was then sprayed with 500 μL of each plant extracts prepared at the concentration of 5% (w/v). The same volumes of the sterilized distilled water (DW) and 0.005% Omite (commercial miticide) were used as control groups. The experiments for each plant extracts were performed in triplicates. All plates were incubated in the growth chamber at 28°C and 85% RH in the dark. The mortality of mites was recorded every day for 5 days after application with plant extracts.

2.4. Statistical Analysis. Data on the percentages of mite mortality due to application with plant extracts were arcsine-transformed and subjected to analysis of variance using the general linear models procedure (SAS Institute, Cary, NC, USA). Significant differences between the treatments were determined using the LSD test at $P < 0.05$.

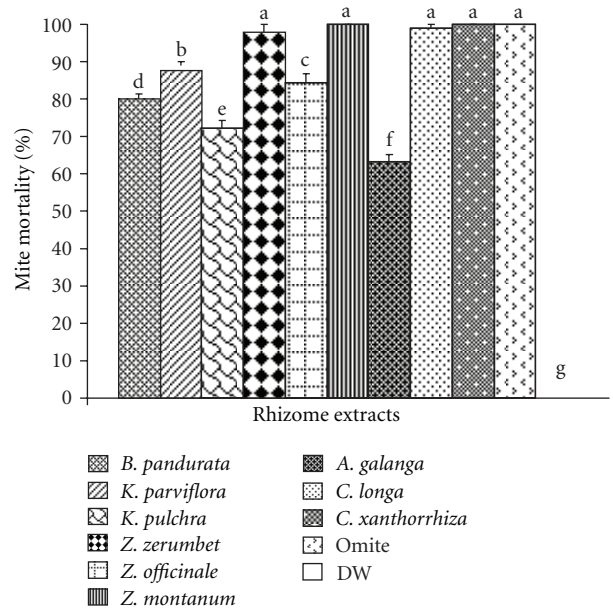

Figure 1: The mortality rates of adult female *Luciaphorus* sp. after being treated with 5% rhizome extracts at 3 days after application. Bars (mean ± SE) with same letter(s) are not significantly different as determined by LSD test at $P < 0.05$.

3. Results

3.1. Acaricidal Activities of Rhizome Extracts. In this study, all rhizome extracts were shown to have acaricidal activities against *Luciaphorus* sp., and the percentages of mite mortality increased progressively and reached the plateau after 3 days of applications (Figure 1). On day 3, the significantly high levels of mortality rates were caused by the rhizome extracts of *C. xanthorrhiza* (100%), *Z. montanum* (100%), *C. longa* (98.89%), and *Z. zerumbet* (97.78%), followed by *K. parviflora* (88.89%), *Z. officinale* (84.44%), *B. pandurata* (80.00%), *K. pulchra* (72.22%), and *A. galanga* (63.33%) (Figure 1). Interestingly, on day 1, *K. parviflora*, *Z. officinale, C. longa*, and *C. xanthorrhiza* extracts resulted in mortality rates at over 70% which were significantly higher than the other treatments (data not shown). However, on day 2, mite mortality rates in almost all treatments were over 70% with the exception of *A. galanga* (56.67%) and *K. pulchra* (67.78%) (data not shown).

3.2. Acaricidal Activity of Leaf Extracts. The levels of mite mortality after applications with leaf extracts also reached maximum on day 3 (Figure 2). On day 3, the leaf extracts of *O. sanctum* and *M. officinalis* resulted in maximum mortality (100%), but the other treatments were shown to result in mortality at levels below 70% (Figure 2). This was not unexpected because only the applications with the leaf extracts of *O. sanctum* and *M. officinalis* caused over 70% of mortality on day 1 (data not shown). Also, on day 2, mortality rates in all treatments increased and the leaf extracts of *O. sanctum* and *M. officinalis* still resulted in mite mortality at the levels significantly higher than the rest,

Table 1: Plants and their parts used for evaluation of acaricidal activities against *Luciaphorus* sp.

Scientific name	Family	Common name	Parts
Boesenbergia pandurata (Roxb) Schltr.	Zingiberaceae	Fingerroot	Rhizome
Kaempferia parviflora Wall. Ex Baker	Zingiberaceae	Belamcanda chinensis	Rhizome
Kaempferia pulchra (Ridl.) Ridl.	Zingiberaceae	Peacock ginger, resurrection lily	Rhizome
Zingiber zerumbet (L.) Smith.	Zingiberaceae	Wild ginger, Martinique ginger	Rhizome
Zingiber officinale Roscoe.	Zingiberaceae	Ginger	Rhizome
Zingiber montanum (Koenig) Link ex Dietr.	Zingiberaceae	Phlai, cassumunar	Rhizome
Alpinia galanga (L.) Swartz.	Zingiberaceae	Kha, galingale, galanga	Rhizome
Curcuma longa Linn.	Zingiberaceae	Turmeric	Rhizome
Curcuma xanthorrhiza Roxb.	Zingiberaceae	Curcuma	Rhizome
Cymbopogon citratus Stapf.	Gramineae	Takhrai, lemongrass	Leaf
Citrus hystrix DC.	Rutaceae	Leech lime	Leaf
Ocimum basilicum Linn.	Labiatae	Ho-ra-pa, sweet-basil, common basil	Leaf
Ocimum canum Linn.	Labiatae	Hairy basil	Leaf
Ocimum sanctum Linn.	Malvaceae	Holy basil, sacred basil	Leaf
Moringa oleifera Lam.	Moringaceae	Horse radish tree	Leaf
Annona squamosa Linn.	Annonaceae	Sugar apple	Leaf
Psidium guajava Linn.	Myrtaceae	Guava	Leaf
Eucalyptus camaldulensis Dehnh.	Myrtaceae	Red river gum, Murray red gum, red gum	Leaf
Artocarpus heterophyllus Lam.	Moraceae	Jackfruit tree	Leaf
Piper sarmentosum Roxb. Ex Hunter.	Piperaceae	Cha-plu	Leaf
Murraya paniculata (L.) Jack.	Rutaceae	Orange jessamine, satin-wood,	Leaf
Melissa officinalis L.	Lamiaceae	Kitchen mint, marsh mint	Leaf
Cassia siamea (Lam.) Irwin et Barnaby	Fabaceae	Kassod tree, siamese senna, Thai copperpod, siamese cassia	Leaf

accounting for 97.78% and 94.44%, respectively (data not shown).

4. Discussion

Several plants have been found to contain bioactive compounds with a variety of biological actions against insects and mites, including repellent, antifeedant, anti-ovipositional, toxic, chemosterilant, and growth regulatory activities [4, 8]. Therefore, botanical insecticides have long been recommended as attractive alternatives to synthetic chemical insecticides for pest management because these chemicals pose little threat to the environment or to human health [9]. For example, the crude foliar extracts of five subfamilies of Australian Lamiaceae, including Ajugoideae, Scutellarioideae, Chloanthoideae, Viticoideae, and Nepetoideae, were found to have contact toxicity against the polyphagous mite (*Tetranychus urticae* Koch) [10]. This *T. urticae* could also be inhibited by the essential oil in crude foliar extract of sandalwood (*Santalum austrocaledonicum*), resulting in 87.2 ± 2.9% mortality and 89.3% reduction of the total number of eggs on leaf disks treated with this oil [11]. Piperoctadecalidine, which is the alkaloid isolated from *Piper longum* Linn., was also found to have activities against *T. urticae* at LD_{50} of 246 ppm [12]. Moreover, Aslan et al. [13] reported that essential oil vapours from *Satureja hortensis* Linn., *Ocimum basilicum* Linn, and *Thymus vulgaris* Linn. had potential against *T. urticae*, but the essential oil obtained from *S. hortensis* was the most effective at 1.563 μL/L air dose by causing 100% mortality of *T. urticae* after 4 days of treatment.

In recent years, many studies have also been conducted to investigate the activities of plant extracts or essential oils against carmine spider mite (*Tetranychus cinnabarinus* Boisd. Tunc) and Hawthorn red spider mite (*Tetranychus viennensis* Zacher). The chloroform extract of *Kochia scoparia* Linn. was shown to have rapid acaricidal activities against *T. urticae*, *T. cinnabarinus,* and *T. viennensis*, resulting in the highest mortality at 92.58, 88.88, and 84.47%, respectively, within 24 h after treatment [14]. Also, toxicity against *T. cinnabarinus* and *T. viennensis* could be quickly induced by the petroleum ether extract of *Juglans regia* Linn., resulting in mortality rates at 81.58 and 78.58%, respectively, within 24 h [7].

Furthermore, the complete 100% mortality of *T. cinnabarinus* was found to be induced by the essential oils of *Cuminum cyminum* Linn., *Pimpinella anisum* Linn., and *Origanum syriacum* var. *bevaii* (Holmes) as fumigants in greenhouse experiments [15]. This complete mortality could also be produced by using the acetone parallel extract of *Artemisia annua* Linn. leaves collected in July [16]. In addition, Zhang et al. [17] reported that benzene extracts derived from *C. longa* Linn. had LC_{50} against *T. cinnabarinus* at 99.3 ppm after 72 h. The high mortality rates of *T. cinnabarinus* could be induced by methanol extracts of *Gliricidia sepium* (Jacq) Kunth ex Steud. (100%) and *Lippia origanoides* Kunth (96.6%) when used at the concentration of 20% [18]. Additionally, Sertkaya et al. [8] evaluated the

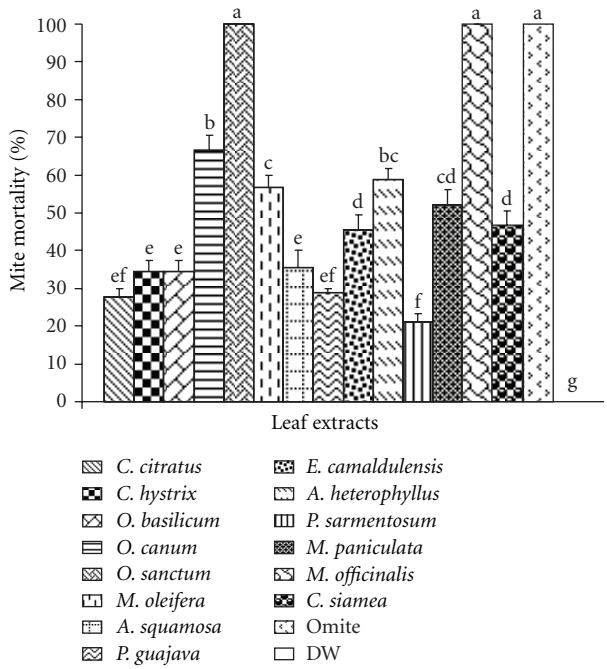

FIGURE 2: The mortality rates of adult female *Luciaphorus* sp. after being treated with 5% leaf extracts at 3 days after application. Bars (mean ± SE) with same letter(s) are not significantly different as determined by LSD test at $P < 0.05$.

efficacy of essential oils derived from medicinal plants against *T. cinnabarinus* and showed that thyme (*Thymbra spicata* Linn. subsp. *spicata*), oregano (*Origanum onites* Linn.), mint (*Mentha spicata* Linn.), and lavender (*Lavandula stoechas* Linn. subsp. *stoechas*) essential oils had LC_{50} values of 0.53, 0.69, 1.83, and 2.92 ppm, respectively. Moreover, the acetone extract of *Aloe vera* Linn. leaves was shown to have acaricidal activity against female *T. cinnabarinus* at 3 days after treatment with LC_{50} value of 90 ppm [6].

Other insect pests were also found to be inhibited by plant extracts. According to the results of Liu et al. [19], the ethanol extracts of *Eupatorium adenophorum* Spreng. (0.1% w/v) could cause mortality of citrus red mite (*Panonychus citri* (McGregor)) at 71.10 and 73.53% after 12 and 24 h, respectively. Also, the activities against *P. citri* of the ethanol extracts derived from *Boenninghausenia sessilicarpa* H. Lev., *Laggera pterodonta* (DC.) Benth., *Humulus scandens* (Lour) Merr., and *Rabdosia* were reported with LC_{50} values of 0.9241, 0.9827, 0.9905, and 1.0196 mg/mL, respectively [20]. In addition, applications with aqueous extracts of *Acorus calamus* Linn., *Xanthium strumarium* Linn., *Polygonum hydropiper* Linn., and *Clerodendron infortunatum* (Gaertn.) could lead to more than 50% mortality of *Oligonychus coffeae* (Nietner) [21]. Moreover, 3% methanolic extracts of *Ocimum tenuiflorum* Linn. and *Cassia alata* Linn. exhibited acaricidal activities against *Tetranychus neocaledonicus* Andre. and resulted in the mortality at 93.3 and 97.0%, respectively [22]. On the other hand, 3% aqueous extracts of *C. alata* and *O. tenuiflorum* could lead to mortality of *T. neocaledonicus* at 75% and 82.2%, respectively, after exposure for 3 days. In addition, the volatile oils of *Citrus reticulata* Blanco. and *C. longa* Linn. could cause mortality of *Sitophilus oryzae* Linn. as high as 100 and 90%, respectively [23]. The essential oils of *Ocimum basilicum* Linn., *Coriandrum sativum* Linn., *Eucalyptus globulus* Labill, *Mentha piperita* Linn. and *Satureja hortensis* Linn. were toxic against poultry red mite (*Dermanyssus gallinae* (De Geer)), and, when using the *in vitro* direct contact method, these essential oils at the dose of 0.6 mg/cm could result in mortality rates over 80% after 24 h of contract [24]. Furthermore, *Eucalyptus citriodora* Hook extract was found to be effective against *D. gallinae*, resulting in 85% mortality over a 24 h exposure period in contact toxicity tests [25].

In this study, the rhizome extracts of *C. xanthorrhiza* and *Z. montanum* and the leaf extracts of *O. sanctum* and *M. officinalis* at the dose of 5% (w/v) were found to be highly effective against female adults of *Luciaphorus* sp. The results revealed that the rhizome extracts were likely to have more potent acaricidal activities than those derived from leaves. The acaricidal activities of plant extracts against *Luciaphorus* sp. mites have been previously described. The essential oils derived from lemon grass (*Cymbopogon citratus* Stapf.) and citronella grass (*Cymbopogon nardus* Rendle) were shown to be effective against *Luciaphorus perniciosus* Rack., and the median effective concentration (EC_{50}) was 18.15 and 19.66 ppm, respectively [26]. In addition, the essential oils of *Litsea cubeba* Pers. were effective against *L. perniciosus* by contact and fumigation methods with LD_{50} values equivalent to 0.932 and 0.166 ppm, respectively [27].

In conclusion, the results in this study suggest the possibility of developing plant extracts derived from the rhizomes of *C. xanthorrhiza* and *Z. montanum* and the leaves of *O. sanctum* and *M. officinalis* for controlling *Luciaphorus* mites. The effective concentration and mode of action of these plant extracts against *Luciaphorus* sp. remain to be determined for the future development of highly potent products to be used in the real settings.

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