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Primary Selection of Essential Oil from Some Medicinal Plants to Inhibit *Didymella bryoniae*, Causal Agent of Gummy Stem Blight Disease of Watermelon

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Abstract

The objective of this study was conducted to select of an essential oil from some medicinal plant for inhibiting *Didymella bryoniae*, causal agent of Gummy Stem Blight of watermelon. The essential oils from 9 species of tested plants were extracted by using water distillation method. The essential oils from tested plants were mixed to PDA medium at 0.6% final concentration for fungal inhibition test. The results showed that the essential oils from 7 species consists of, wild ginger: *Boesenbergia pandurata* Holtr., ginger: *Zingiber officinale* Roscoe, Siamese ginger: *Alpinia galanga* (L.) Willd., lemon grass: *Cymbopogon citratus* Stapf., leech lime: *Citrus hystrix* DC., hairy basil: *Ocimum americanum* L. and sweet basil: *O. basilicum* Linn. could inhibit the mycelial growth of *D. bryoniae* up to 100% on PDA medium. In addition, there was no significantly different when compared with using benomyl fungicidal. Whereas an essential oil from curcuma: *Curcuma longa* Linn. showed partial inhibition to the mycelial growth of *D. bryoniae* on PDA, and tangerine: *C. reticulata* Blanco. did not inhibit this fungal.

Keywords: *Didymella bryoniae*, essential oil, medicinal plant, watermelon

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Introduction

The Gummy Stem Blight, caused by *Didymella bryoniae* (Auersw), is one of the most important diseases that caused economic loss in watermelon seed production areas such as Khon Kaen and Loei province. The disease symptom found on all plant parts except roots. Leaf symptoms appear as dark yellow or reddish–brown lesions in various shapes. Lesions begin at leaf margins and extend rapidly back into the leaf blade, the pycnidia may be found in leaf lesions.

The control procedure of Gummy Stem Blight of watermelon known as chemical control is responsible for increase in the productivity and quality of the crop. But this method is inappropriate and nondiscrminatory use has put human and animal health at risk. In addition, its can be contaminated in the environment. One of methods could be developed to solve the chemical problem, the utilization of an essential oil from some medicinal plants, which are natural sources of antimicrobial substances and whose fungitoxic potentials have been referred in previously studies. Such as, an essential oil of *Ocimum adscendes* at a concentration of 0.1% can protect the seeds of *Capsicum annuum* against storage fungi and its activity more effective than treatment with fungicides (Asthana et al., 1989). The extract of *Cymbopogon citrates* leaves can completely inhibit the mycelial growth of *Fusarium solani* f. sp. *phaseoli, Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Valarini et al., 1994). The crude extracts and essential oils of *Achillea millefoium, C. citrates, Eucalyptus citriodora* and *Ageratum conyzoides* could inhibit spore germination and mycelial growth of D. bryoniae in vitro (Fiori et al., 2000).

Thus the antifungal effects of medicinal essential oils are of interest regarding their possible use as alternatives to *D. bryoniae* prevention. The objective of this work was evaluated to primary selection of essential oils from 9 Thai medicinal plants that can inhibit *D. bryoniae* mycelial growth *in vitro.*

Materials and Methods

Fungal strain and pathogenicity test

The *D. bryoniae* was supported by Department of Plant Pathology, Khon Kaen University, Thailand. The *D. bryoniae* was cultured in PDA medium for 48 hours and used as pathogenicity test on watermelon fruit. This result was obtained in 7–14 days after inoculation.

Plant material and the essential oil extraction

The medicinal plant from 9 species consisted of wild ginger: *Boesenbergia pandurata* Holtr. (rhizome), ginger: *Zingiber officinale* Roscoe (rhizome), Siamese ginger: *Alpinia galanga* (L.) Willd. (rhizome), curcuma:

Curcuma longa Linn. (rhizome), lemon grass: *C. citratus* Stapf. (stem), leech lime: *Citrus hystrix* DC. (leaf), hairy basil: *Ocimum americanum* L. (leaf), sweet basil: *O. basilicum* Linn. (leaf), and tangerine: *C. reticulata* Blanco. (peel fruit) were collected from Sakon Nakhon province. The 300 g fresh leaves of all tested plants were chopped into small pieces and air-dried under the shade (30 °C) until the leaves became crispy dry after 3 days. The dried leaves were grinder with 700 ml distilled water. The essential oils were extracted by water distillation method by evaporate and condense 2-3 hours. All separated essential oils were used for fungal mycelium growth inhibition test.

Assessment of inhibition of fungal mycelium growth

All 0.6% final concentration of an essential oil samples were mixed into PDA medium for antifungal activity. The fungal hypha tip of *D. bryoniae* on PDA were cut by 0.9 cm diameter cork borror and placed on PDA medium contained of essential oils at 0.6% final concentration that previously preparation. The antifungal activity of all essential oils were measured by the diameter of fungal hypha growth compared with the fungal growth on PDA medium (control) and PDA contained of 750 ppm benomyl fungicide. The inhibition growth data were analyzed by using Duncan's New Multiple Range Test.

Results

Fungal strain and pathogenicity test

Three days after inoculation of *D. bryoniae* onto watermelon fruit, watermelon fruit showing water soak symptom around the inoculation site (Fig. 1A). And this disease symptom could increase and obtained fungal mycelial in water soak area at 5 days after inoculation. After that the diseases symptom came to tissue macerations and then hypha and pycnidia were developed (Fig. 1B). Whereas the negative control (PDA inoculation) could not induce disease symptom (Fig. 1C).

Assessment of inhibition of fungal mycelium growth

The 0.6% essential oils from 7 species of medicinal plants did responsible to the greatest inhibition of the mycelial growth of *D. bryoniae* are consisted of wild ginger, ginger, Siamese ginger, lemon grass, leech lime, hairy basil and sweet basil. These essential oils were completely inhibit *D. bryoniae* on PDA medium (100% inhibition growth). This inhibition efficiency were equal to 750 ppm benomyl fungicidal (Fig. 2 and Table 1). Whereas an essential oil from curcuma was partial inhibition the mycelial growth of *D. bryoniae.* On the other hand, an essential oil from tangerine could not effectively to control of this pathogen.

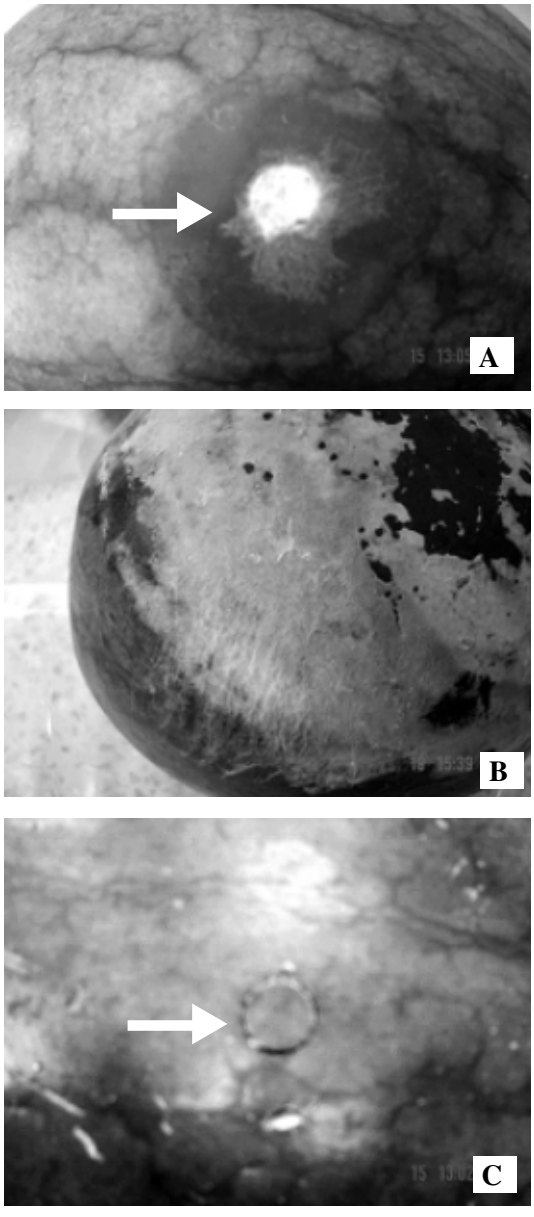

Fig. 1 Gummy Stem Blight symptoms after inoculation with *D. bryoniae* at 3 days (A), 5 days after inoculated (B) and the negative control (C)

Conclusion and Discussion

This study showed that essential oil extracts from 7 medicinal plants consists of wild ginger, ginger, Siamese ginger, lemon grass, leech lime, hairy basil and sweet basil could inhibit *D. bryoniae* mycelial growth *in vitro* up to 100%. Whereas curcuma was partial inhibit mycelial growth of *D. bryoniae* and tangerine did not observe *D. bryoniae* mycelial growth inhibition activity.

For these inhibitions activity, we observed that fungal inhibition activity was differently effective depending on kinds of medicinal plant because the various medicinal plants are produce various active chemical. Such as, lemon grass can produce neral, gernial and limonene, to inhibit *Fusarium graminearum* (Velluti *et al.*, 2004), cinnamon (*Cinnamomum zeylanicum* (L.) can produce caryophyllene, linalool and other terpenes (monoterpenes and sesquiterpenes) to inhibit *Colletotrichum musae* (Ranasinghe *et al.*, 2002) and *Aspergillus flavus* (Paranagama et al., 2003) whereas basil can produce chavicol and aromatic compound to inhibit *Uromyces fabae* and *Botrytis fabae*. Fieker *et al.* (2003) reported that ginger had a gingerols and gingerdiol as active compound to against 13 human fungal. Moreover, Singh *et al.* (2004) found fresh rhizome essential oil of *Zingiber officinale* showed 69% components, accounting for 96.93% of the total oil after analyzed, the major component was zingiberene. The Siamese

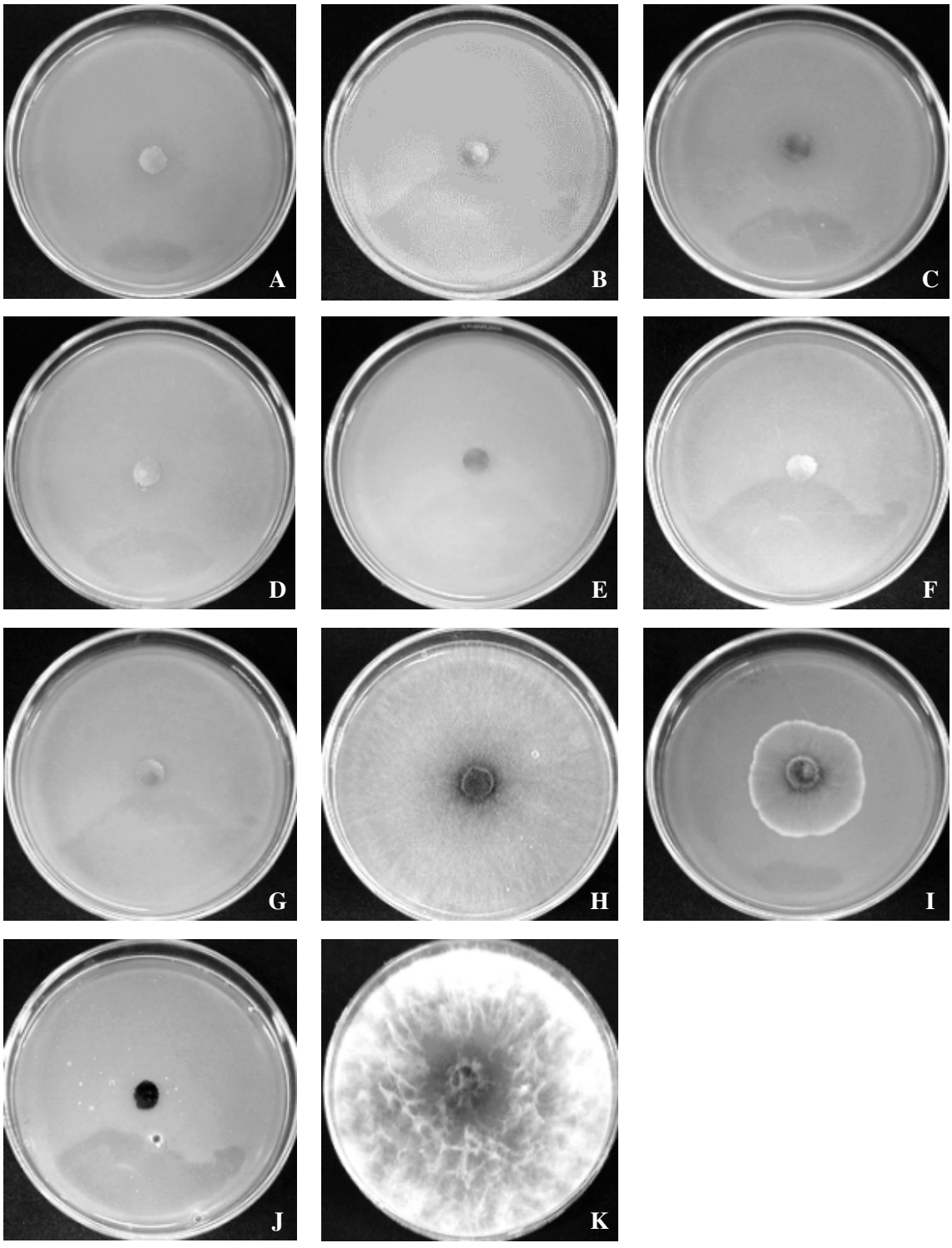

Fig. 2 The colony of *D. bryoniae* growing on PDA medium contained of different essential oils at 0.6% final concentration; wild ginger (A), ginger (B), Siamese ginger (C), lemon grass (D), hairy basil (E), sweet basil (F), leech lime (G), tangerine (H), curcuma (I), 750 ppm benomyl (J) and sterilized water (K) used as control

Table 1 Colony diameter of *D. bryoniae* grown on PDA medium contained of different essential oils at 0.6% final concentration

Treatment	Colony diameter of *D. bryoniae* (cm)				Mean[A]
	1	2	3	4	
wild ginger: *Boesenbergia pandurata* Holtr.	0	0	0	0	0a
ginger: *Zingiber officinale* Roscoe	0	0	0	0	0a
Siamese ginger: *Alpinia galanga* (L.) Willd.	0	0	0	0	0a
lemon grass: *Cymbopogon citrates* Stapf.	0	0	0	0	0a
leech lime: *Citrus hystrix* DC.	0	0	0	0	0a
hairy basil: *Ocimum americanum* L.	0	0	0	0	0a
sweet basil: *O. basilicum* Linn.	0	0	0	0	0a
curcuma: *Curcuma longa* Linn.	3	2.8	2.6	3.4	2.95b
tangerine: *C. reticulata* Blanco.	7.8	8	8.3	8.3	8.1c
750 ppm benomyl	0	0	0	0	0a
sterilized water (control)	8.3	8.3	8.3	8.3	8.3c

[A] Mean of colony diameter in the last column followed by different letters are significantly different referring to Duncan's New Multiple Range Test ($P<0.01$)

ginger can produce diterpene component to enhance the antifungal activity of quercetin and chalcone against *Candida albicans* (Haraguchi et al., 1996).

The formulation and concentration in used, is a one reason to support the antimicrobial activity of essential oils. For example, Bankole and Joda (2004) found that dry ground leaves of lemongrass showed higher effective inhibition of *Aspergillus* spp. in melon seed than essential oil after 3 month infestation because dry ground leaves have long sustainable activity. In addition, they found that the 0.5-1 percentage of essential oil content are the best condition to inhibit the mycelial growth and alflatoxin production of *Aspergillus* spp. in melon seed.

Moreover, the inhibition activity might be depending on the solvent. Vudhivanich and Supanuntorn (2002) suggested that the active ingredient might dissolve in the different kinds and percentages of solvent. However, the difference of extraction method would cause the different in experimental results and conclusions (Vudhivanich, 2003). Vudhivanich and Supanuntorn (2002) reported that each kind of herbal active ingredient was dissolved by different percentages of ethyl alcohol. Thus, an essential oil from tangerine plant may be highly effective to *D. bryoniae* when the optimum solvent and extraction method were investigated. Therefore, we suggest that the suitable solvent and extraction method for

kind of medicinal plants were needed to investigate.

In this study some of essential oils showed similarity effective to previously studies. Such as, essential oil of lemon grass provided 100% inhibit of the mycelium growth and spore germination of *D. bryoniae* (Fiori *et al.*, 2000). Moreover, the lemon grass's essential oil could inhibit some food borne bacterial growth and *Candida albicans* (Hammer et al., 1999). Shin and Lim (2004) reported that lemon grass's essential oil had completely inhibited *Trichophyton* spp. For an essential oil of sweet basil was studied by Hammer et al. (1999) showed highly effective to inhibit *Acinetobacter baumanii, Aeromonas veronii, C. albicans* and seven food borne pathogenic bacteria. Oxenham et al. (2005) observed that the methyl chavicol and linalool chemotype oil produced from basil could inhibit the mycelial growth of plant pathogenic fungus *Botrytis fabae.* Thus, the fungal inhibition from hairy basil and sweet basil oil in this experiment might be responsible by both chemotype oils as same as previously reported.

As a conclusion, an essential oil from wild ginger, ginger, Siamese ginger, lemon grass, leech lime, hairy basil and sweet basil had a potential antifungal activity against *D. bryoniae.* And the further studies; effective chemical, percentage or concentration of their effective chemical and formulation in used are needed before their application in watermelon production to prevent this fungal.

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References

Asthana, A., K. Dixit, N.N. tripathi, and S.N. Dixit. 1989. Efficacy of ocimum oil against fungi attacking chili seed during storage. Tropical Science 49: 15-20.

Bankole, S.A. and A.O. Joda. 2004. Effect of lemon grass (*Cymbopogon citrates* Stapf) powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (*Colocynthis citrullus* L.). African Journal of Biotechnology 3: 52-59.

Ficker, C., M.L. Smith, K. Akpagana, M. Gbeassor, J. Zhang, T. Durst, R. Assabgui, and J.T. Arnason. 2003. Bioassay-guided isolation and identification of antifungal compounds from ginger. Phytotherapy Research 17: 897-902.

Fiori, A.C.G., K.R.F. Schwan-Fstrada, J.R. Stangrlin, J.B. Vida, C.A. Scapim, M.E.S. Cruz, and S.F. Pascholati. 2000. Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae.* Journal of Phytopathology 148: 483-487.

Haraguchi, H., Y. Kuwata, K. Inada, K. Shingu, K. Miyahara, M. Nagao, and A. Yaji. 1996. Antifungal activity from *Alpinia galangal* and the competition for incorporation of unsaturated fatty acids in cell growth. Plant Medicine 62: 308-13.

Hammer, K.A., C.F. Carson, and T.V. Riley. 1999. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology 86: 985–990.

Oxenham, S.K., K.P. Svoboda, and D.R. Walters. 2005. Antifungal activity of the essential oil of basil (*Ocimum basilicum*). Journal of Phytopathology 153: 174–180.

Paranagama, P.A., K.H.T. Abeysekera, K. Abeywickrama, and L. Nugaliyadde. 2003. Fungicidal and anti–alflatoxigenic effects of the essential oil of *Cymbopogon citrates* (DC.) Stapf. (lemongrass) against Aspergillus flavus Link. Isolated from stored rice. Letters in Applied Microbiology 37: 86–90.

Ranasinghe, L., B. Jayawardena, and K. Abeywickrama. 2002. Fungicididal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M. Perry against crown rot and anthracnose pathogens isolated from banana. Letters in Applied Microbiology 35: 208–211.

Singh, G., S. Maurya, C. Catalan, and M.P. de Lampasona. 2004. Studies on essential oils, Part 42: chemical, antifungal, antioxidant and sprout suppressant studies on ginger essential oil and its oleoresin. Flavour and Fragrance Journal 20: 1–6.

Shin, S. and S. Lim. 2004. Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. Journal of Applied Microbiology 97: 1289–1296.

Valarini, P.J., R.T.S. Friguetto, and I.S. de Melo. 1994. Potencial da erva medicinal (*Cymbopogon citratus*) no controle de fitopatÓgenos do feijoeiro. Revista Agriculture 69: 139–150.

Velluti, A., V. Sanchis, A.J. Ramos, C. Turon, and S Marin. 2004. Impact of essential oils on growth rate, zearalenone and deoxynivalenol production by *Fusarium graminearum* under different temperature and water activity conditions in maize grain. Journal of Applied Microbiology 96: 716–724.

Vudhivanich, S. 2003. Efficacy of Thai herbal extract for growth inhibition of *Xanthomonas axonopodis* pv. citri, the bacterial canker of citrus. Kasetsart Journal (National Science) 37: 445–452.

Vudhivanich, S. and S. Supanuntorn. 2002. Potential of Thai herbal extract for growth inhibition of *Ralstonia solanacearum,* the causal agent of bacterial wilt of tomato, p161. In The First International Conference on Tropical and Subtropical Plant Diseases. November 5–8, 2002. Chiang Mai, Thailand