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Allelopathic effects of *Bidens pilosa* var. *radiata* and its preliminary utilization to control weeds in rice

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Bidens pilosa L. var. *radiata* Sch.Biq. is an invasive weed generally distributed in Thailand. This study was carried out to investigate the phytotoxic effects of *B. pilosa* and its utilization on controlling paddy weeds, along with reports on allelopathic activities for the use of a weed control tool. A comparison between fresh and dry plants of *B. pilosa* were assessed by germination tests of *Echinochloa crus-galli* (L.) Beauv. 70% ethanol extract of the fresh whole plant showed to be more phytotoxic than dry plants. Different stages at 30, 45, 60 and 75 days after planting (DAP) process were tested against phytotoxic effects on *E. crus-galli*. The results showed that at 60 DAP had the highest effects followed by 45, 30 and 75 DAP, respectively. Soil mulching and soil incorporation with fresh *B. pilosa* were preliminarily tested on natural paddy soil in pot scale. Both mulching and incorporation had significantly reduced weed density. However, there was no-significance between the mulching and incorporation stage. The percentage of weed control was about 45%, 60%, 70% and 85% with the rate of 20, 30, 40 and 50 t fresh weight (f.w.)/ha, respectively. Another experiment was utilized the *B. pilosa* by a directed cut fresh plant integrated with irrigation at an early stage of rice and paddy weeds in pot conditions. *B. pilosa* applied within this method could reduce total weeds at all rates. The total weed density were inhibited by 39.57%, 43.60%, 79.44% and 100% with the rates of 1, 2, 4 and 6 t f.w./ha, respectively, while the rice seedling had no toxicity. This study demonstrated that the application of *B. pilosa* integrated with irrigation at an early growth stage of rice was highly possible to utilize the *B. pilosa* on the weed control tool with non-chemical methods. Future studies should focus on field trials on weed control and rice yields.

Keywords: Allelopathy, *Bidens pilosa* L. var. *radiata* Sch.Biq., Weed control, Paddy weed

Introduction

Weed infestation is a major problem of yield loss in paddy fields. Famers have widely used herbicides to control weeds because it's quick, reduces costs

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and convenient to use. However, the continuous use of herbicides have caused a negative impact on humans, pests, and contamination in the environment such as soil sickness, water resources and natural enemies (Poonpaiboonpipat *et al*., 2013, Laosinwattana *et al*., 2009). It also leads to an increase of herbicidal resistance in many weed species (Vyvyan, 2002). Nowadays, an eco-friendly agricultural production is essential in today's world because non-toxic food is required by consumers. The utilization of allelopathic plants for weed control is vital. Allelopathy, involves metabolites that are produced by plants and released to the environment. These compounds have an influence on growth and the development of surrounding organisms. Allelopathy can improve weed control in several such examples such as 1) use of natural or modified allelochemicals as herbicides 2) transfer of traits into commercial crop cultivars 3) use of allelopathic plants in a crop rotation, companion planting, and smother crops and 4) use of phytotoxins mulches and cover-crop management (Macias *et al*., 1999). The use of allelopathic plants on soil incorporation and soil mulching can suppress weed competition and also provide better soil properties. For example, soil incorporated with green manure of aromatic plants including *Pimpinella anisum, Foeniculum vulgare, Ocimum basilicum, Anethum graveolens, Coriandrum sativum, Petroselinum crispum, Phacelia tanacetifolia, Mentha Xverticillata, Origanum vulgare and Melissa officinalis* before maize cultivation, reduced weed emergence by 11–50%, 12–59%, 26–79% or 58–83% in order to aromatic species, while maize grain yields were increased by 27–43% (Dhima *et al*., 2009). The use of *Aglaia odorata* Lour granules as soil mulching at 1 t/ha reduced weed density of *Digitaria adscendens*, *Trianthema portulacastrum* and *Amaranthus gracilis* by 96.7%, 47.2% and 56.2%, respectively, while the emergence and growth of maize was not affected (Laosinwattana *et al*., 2012).

Bidens pilosa L. var. *radiata* Sch.Biq. is an annual weed in the Asteraceae family. It was introduced from Taiwan by bee-farmers. Recently, *B. pilosa* was an invasive weed that established and dominated other plants in generally areas of Northern Thailand. *B. pilosa* has been reported with allelopathic effects. Aqueous extract from leaves, roots and stems by *B. pilosa*, inhibited germination and seedling growth of *B. bipinanta* and *Ageratum conyzoides*. The leaves showed more phytotoxicity than others. (Hsu and Kao, 2009). The application of *B. pilosa* as pellets could reduce weed density and dry weight in transplanting rice in Vietnam. (Hong *et al*., 2004, Khanh *et al*., 2009). However, it was not known that phytotoxicity between fresh and dry materials of *B. pilosa*, and also with other applications of *B. pilosa*.

Thus, the objectives of this research is 1) to compare the phytotoxicity between fresh and dry of *B. pilosa*, 2) to compare the phytotoxicity of different stages, 3) to test weed control by soil incorporation and soil mulching by fresh materials of *B. pilosa*, and 4) to test weed control by fresh material integrating with water irrigation.

Materials and methods

Comparison of phytotoxic effect between fresh and dry plants of B. pilosa

B. pilosa was planted by seeds in plastic pots (30 cm diameter). Whole plant at the flowering stage (about 60 days after emergence), was harvested in the morning. It was cut into 1 - 2 cm and divided to two parts. The first was extracted by 70% ethanol giving stock of 670 g fresh weight per litre (670 g f.w./L). Another part was dried under 40 degrees ^{o}C giving the dry weight of about 15% of fresh weight. Then, dry material was extracted by 70% ethanol giving stock by 100 g dry weight per litre (100 g d.w./L) that was equal with 670 g f.w./L). The stock of fresh extract was diluted to give 502.5, 335 and 167.5 g f.w./L. The stock of dry extract was diluted to give 75, 50 and 25 g d.w./L. The 5-mL of aliquot concentration was added to 9-cm petri dish lined with germinated paper test. The papers were dried in a fume hood to evaporate the solvent. After drying, 5-mL of distilled water was added to the dish and then 20 seeds of each weed were placed in the petri dish. The bioassay weeds included barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) that was surface sterilized with 10% sodium hypochlorite for 5 min and was washed several times in distilled water. The dish that had only distilled water was used as a control. The experiment was repeated four times in a completely randomized design. The dishes were placed in a laboratory room that had a temperature of around 25 – 30 degrees ^{o}C. After 7 days, the germination, hypocotyls length, and roots length was measured by a ruler.

Phytotoxicity of different stages of B. pilosa

B. pilosa was planted similarly as the previous experiment. The whole plants were harvested at 30, 45, 60 and 75 days after planting (DAP). The materials were rapidly cleaned and chopped into 1-cm pieces. Then, the materials were extracted by 70% ethanol at the ratio of 200:1000 giving 200 g f.w./L of stocks. The stocks were diluted by 70% ethanol given 50, 100, 150 and 200 g f.w./L. The phytotoxic test was similar to previous experiments.

Soil mulching and soil incorporation with fresh B. pilosa on weed control of paddy soil

Allelopathic plants can reduce weed density by soil incorporation and mulching. This experiment was utilized to test the effect of fresh material of *B. pilosa* with soil incorporation and soil mulching on weed control in paddy soil.

A glasshouse condition was conducted in this experiment. The natural paddy soil was collected by 30-cm depth. The soil was dried under sunlight. After that, it was saturated in water for several days. The *B. pilosa* at the 60 days stage was harvested and regularly cut into small pieces. The saturated soil was incorporated with fresh *B. pilosa* in 30-cm plastic pots at rates of 20, 30, 40 and 50 tons fresh weight per hectares. Another set of pots was mulched with fresh material at the same rate as soil incorporation. The untreated pot was used as a control. Experiments were carried out in four replications for each pot in a completely randomized design manner. Total weed emergence was counted at 7, 14, 21 and 28 days after applications. Shoot biomass of total weeds were collected at the 28 day period.

Application of fresh B. pilosa integrated with water irrigation on weed control

This method was observed by farmers that always irrigated at 10 – 15 days after directed germinated rice seeds. At this stage, weeds are always smaller rice seedlings. The water level is about 5 – 10 cm above soil level. Our preliminary study was shown that direct application of plant material integrated with water irrigation could reduce or kill paddy weeds while rice seedling was not infected with toxicity. This outcome was the background of this experiment.

The preparation of soil and *B. pilosa* materials were similar to the previous experiment. The saturated soil was added to 30-cm plastic pots by 75% of pot volume, and then 3 germinated rice seeds were sowed into the pot. Water irrigation was added to the pot at 10 days after rice sowing together with B. pilosa materials. The water level was levelled off by 5 cm throughout the experiment. The treatments were fresh *B. pilosa* materials by 0 (control non-weeded), 1, 2, 4, 6 and 8 t f.w./ha. Another control was hand-weeded. Experiments were carried out in four replications for each pot in a completely randomized design manner. Total weed density was counted at 7, 14, 21 and 28 days after applications. Shoot biomass of total weeds was collected at 28 days after sowing.

Data analysis

All the data were analyzed by one-way ANOVA and the means were separated by Least Significant Difference (LSD) at P = 0.05.

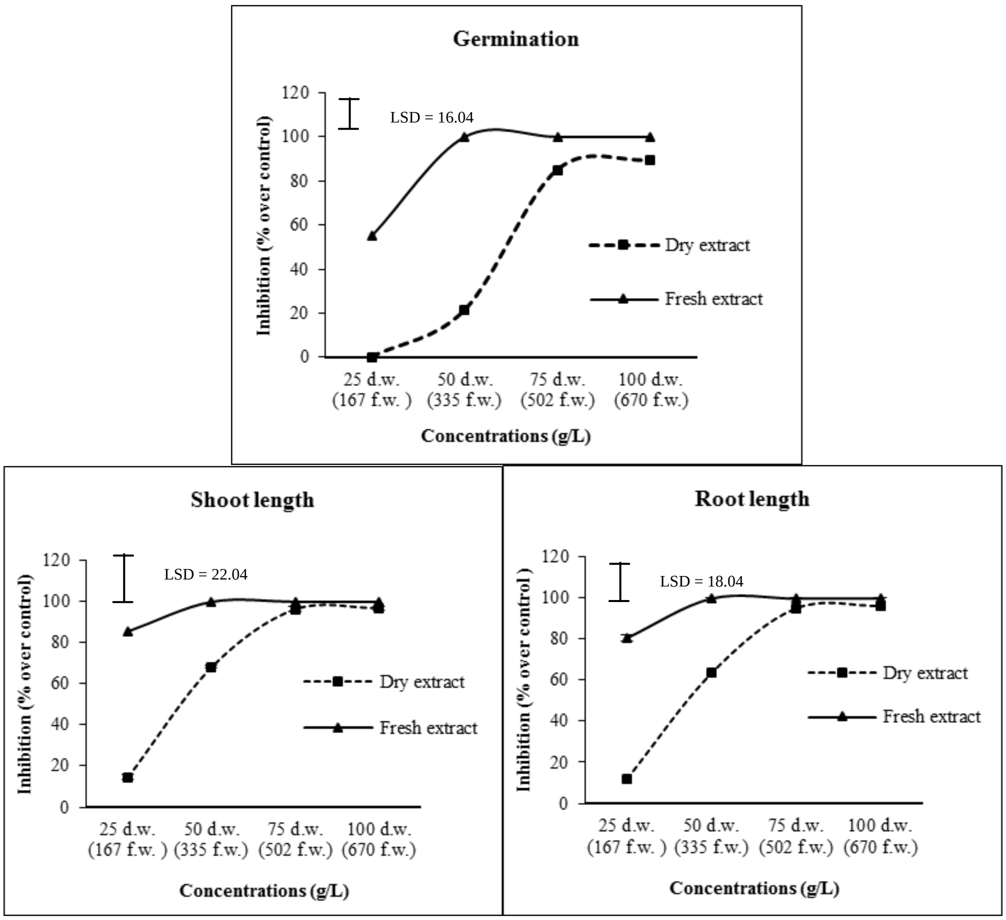


Fig. 1 Effects of fresh extract and dry extract of *B. pilosa* on germination, shoot and root length of barnyard grass (*E. crus-galli*).

Results

Comparison of phytotoxic effect between fresh and dry plants of B. pilosa

Phytotoxicity of B. pilosa between fresh and dry conditions were tested by germination and growth and *E. crus-galli*. Previous observations found the dry biomass of *B. pilosa* was about 15% of fresh weight. Thus, the concentrations of dry extract by 25, 50, 75 and 100 g d.w./L were equal with 167, 335, 502 and 670 g f.w./L. The results showed that the phytotoxicity of fresh extract was higher than dry extract. For example, the inhibition of *E. crus-galli* germination at the concentrations of 50, 75 and 100 g d.w./L was 21%, 85% and 89% over control respectively, while the concentrations of 335, 502 and 670 g f.w./L was completely inhibited. Effects on *B. pilosa* both fresh and dry conditions on seedling growth of test weeds had the similar result with the inhibition on germination. The fresh extract showed more inhibitory effects than dry extract.

Phytotoxicity of different stages of B. pilosa

The result showed that phytotoxicity of different stages of *B. pilosa* was significant. The extract from 60 DAP seemed to the most inhibitory effect on *E. crus-galli*, however it was no-significance with 45 DAP. For example, at 200 g/L the germination of *E. crus-galli* was inhibited by 33%, 50%, 57% and 36% with 30, 45, 60 and 75 DAP, respectively, The shoot length was inhibited by 70%, 72%, 80% and 50%, while the root length was inhibited by 65%, 70%, 80% and 54%, respectively. The degree of inhibition was increased with increasing on the concentrations.

Soil mulching and soil incorporation with fresh B. pilosa on weed control of paddy soil

In a natural field, *B. pilosa* had aerial shoots biomass about 20-30 t f.w./ha depending on areas. Thus, the treatments were distributed by 20, 30, 40 and 50 t f.w./ha. The result showed that both application reduced weed emergence depending on the rates. The significant rates of over 50% weed reduction was 40 and 50 tons t f.w./ha, while 20 and 30 ton t f.w./ha were lower than 50%. The control pot showed a continuous increase on weed emergence 1 week after treatments (WAT), while the soil mulching and incorporation produced no weeds from emergence at 1 WAT but they initially emerged 10 – 14 days after treatments. The total weed densities at 4 weeks in control was 92 plants/pot. Weed densities at 4 WAT with soil mulching were 51, 35, 31 and 9 plants/pot at the rates of 20, 30, 40 and 50 t f.w./ha respectively, while soil incorporation by 61, 41, 30, and 17 plants/pot.

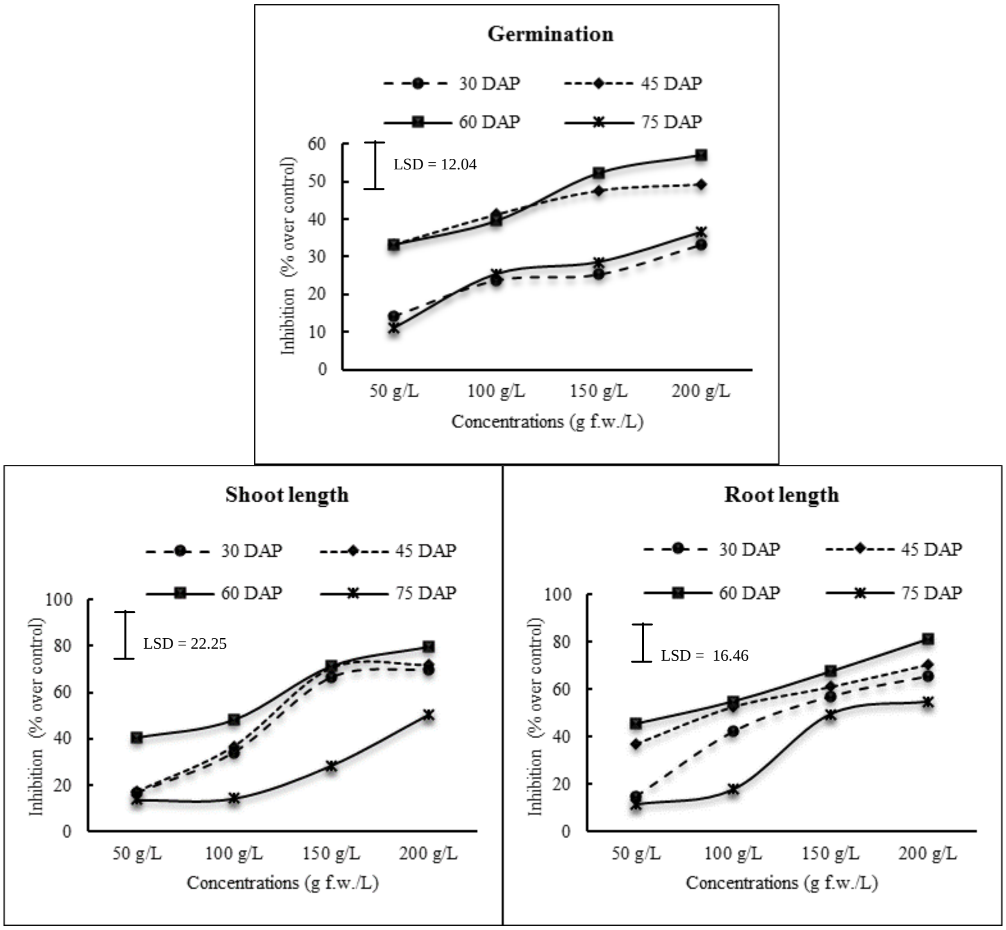
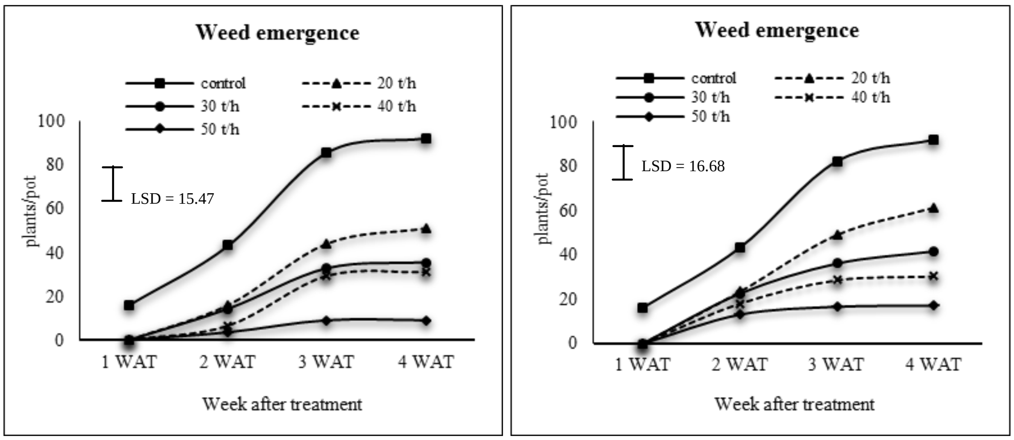


Fig.2 Effects of fresh extract of *B. pilosa* at different stages on germination, shoot and root length of barnyard grass (*E. crus-galli*). DAP = days after planting.

Application of fresh B. pilosa integrated with water irrigation on weed control

This experiment was adapted from the practice by farmers with directed germinated seeds as mentioned above. Average total weed density was about 145 - 152 plants/pot before treatments (Table 1). The types of weed species in this soil included *Fimbristylis miliacea, E. crus-galli, and Ludwigia hyssopifolia*. The results showed that weed density was significantly decreased after fresh material integrated with water irrigation at all application rates. Total weed density in a control pot was continuously increased every week, while the treatment pots were decreased after fresh material were applied. Increased application rates showed continuously reduced weed density until complete control at 6 and 8 t f.w./ha within 2 weeks after treatment. At 4 WAT, total weed density was 200 plants in control pot, while the treatment pots were 95.25, 103.75, and 26.25 plants/pot with 1, 2 and 4 t f.w./ha, respectively, while 6 and 8 t f.w./ha were completely control.



Soil mulching   Soil incorporation

Fig. 3 Effect of fresh material of *B.pilosa* with soil mulching and soil incorporation on total paddy weeds emergence

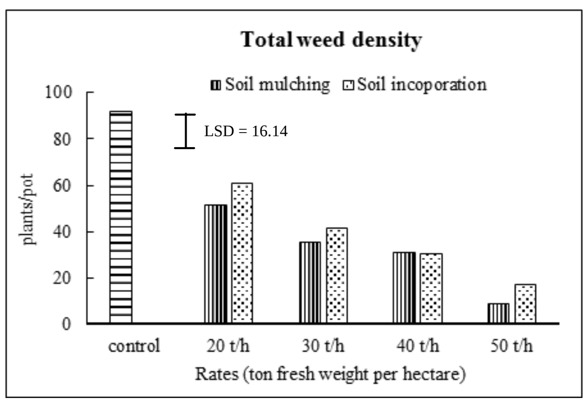


Fig. 4 Comparison of soil mulching and soil incorporation by fresh material of *B. pilosa* on weed density at 28 days after treatments.

Table 1. Effect of fresh material of *B. pilosa* integrated with water irrigation on total weeds density and dry weight at 1, 2, 3 and 4 weeks after treatment (WAT).

Application rates (tons fresh weight per hectare)	Total weeds density (plants per pot)					Total weeds dry weight
	Before treatments	1 WAT	2 WAT	3 WAT	4 WAT	
Control	152.47+18.4 ^{ns}	162.25+17.51 a	182.75+20.12 a	199.14+17.07 a	200.25+25.79 a	27.47+5.07 a
1 t/ha	148.05+19.6	103.15+15.23 b	95.25+14.52 b	95.53+15.29 b	95.25+16.24 b	25.60+7.06 a
2 t/ha	151.62+17.6	105.50+10.84 b	101.75+11.87 b	103.25+19.58 b	103.75+17.91 b	18.40+3.94 b
4 t/ha	145.91+18.8	58.75+14.17 c	28.5+10.37 c	28.25+8.08 c	26.25+8.73 c	1.06+2.46 c
6 t/ha	148.85+20.1	19.00+0.95 d	0.00 d	0.00 d	0.00 d	0.00 d
8 t/ha	149.6+19.23	0.00 e	0.00 d	0.00 d	0.00 d	0.00 d
LSD 0.05	21.8	18.4	18.3	18.2	18.2	6.8

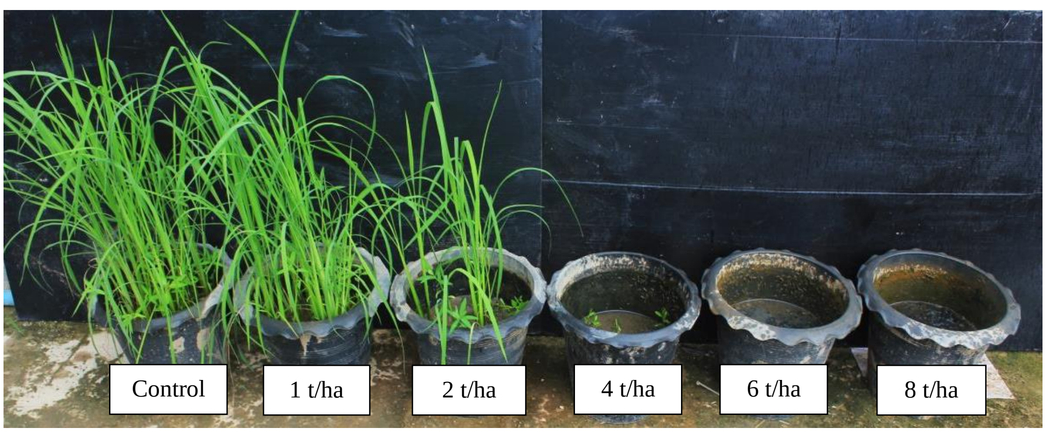


Fig. 5 Effect of fresh material of *B. pilosa* integrated with water irrigation on

paddy weeds at 4 weeks after treatment.

Discussions

This result agreed with Lertmongkol (2015) who reported that the fresh extract of *Cleome rutidosperma* was more of an inhibitory effect on germination and growth of *E. crus-galli* and *Amaranthus spinosus* than dry extract. However, the effect was depended on plant species.

Phytotoxicity of plant extract had a difference depending on several factors such as conditions, parts, stage, varieties, habitats, etc. The extract from fresh whole plant of *B. pilosa* had more phytotoxic on *E. crus-galli* than dry extract. This indicated that the phytotoxic compounds were possibly aromatic compounds. The plant in the Asteraceae family was always found with aromatic compounds. *β*–caryophyllene and ᴛ-cadinene were the main compounds found in the essential oils from leaf and flower of *B. pilosa* (Deba *et al*., 2008)

Otherwise, a stage of plant was once a factor. *B. pilosa* is an annual plant. The extract showed more inhibitory effects at the stage of 60 DAP, in which it is initially at the flowering stage. This indicated that the phytotoxic compounds were the most accumulated at this stage. However, it differed with tree plants for example, leaf extract of *Melia azedarach* L. showed the most phytotoxic *E. crus-galli* and *Phaseolus lathyroides* L. at young stage. (Phuwiwat *et al*., 2012).

Soil mulching and soil incorporation were the methods of allelopathy utilization for weed control. This result was not a surprise. Several reports accomplished on the decreasing weed density by soil incorporation and mulching such as ground dried residues of *Crotalaria juncea* L. and *Mucuna deeringiana* (Bort) inhibited germination, plant height and dry weight of *Eluesine indica* and *Amaranthus rividus* L. (Adler and Chase, 2007) Dry leaves powder of *Murraya paniculata* L. at 8 t/ha reduced weed emergence of *E. crus-galli*, *Chloris barbata* Sw., *Bidens pilosa* L. and *Amaranthus viridis* L. by 51.77%, 92.71%, 37.88% and 53.39% respectively. (Poonpaiboonpipat *et al*., 2013) Soil incorporation with Origanum vulgare reduced the emergence of *E. crus-galli*, *Setaria verticillata* and *Portulaca oleracea* by 0–55%, 38–52%, and 43–86%. (Vasilakoglou *et al*., 2011) Crop residues can provide selective weed control through their physical presence on the soil surface and through the release of allelochemicals (Bhowmik and Inderjit, 2003).

Similar observations as regards to Hong *et al*. (2004) reported that dry material of *B. pilosa* at 2 t/ha that applied at 15 days after transplanting rice could control paddy weeds by 80% and increase rice yield by 20%. However this research was different from their report. This research was essential in controlling weeds for directed germinated seeds of rice production system in Thailand. This system has been admired by farmers because of its convenience, cost savings and a reduction in man hours. However, this method always produces weed competition, especially *E. crus-galli*, *L. chinensis, F. miliacea*, *F. dichotoma*, *S. zeylanica* and *L. hyssopifolia*, that greatly completed rice cultivation. In this method, farmers always irrigate water to the paddy field for

10 – 15 days after seeding when the weeds are at the 2-3 leaves stage. Thus, we pointed to apply the fresh material of *B. pilosa* together with water irrigation in this method. The water level was about 5 -cm above soil level where almost all weeds sank into the water, while the rice seedlings did not. The materials were degraded for 2 – 3 days after application and the weeds slowly died 7 days after application.

As regards with weed control in this condition, nothing is known as to whether it is due to flooding stress, shading effect or allelochemicals, as true or functional allelopathy. However, it is likely to affect by allelochemicals on weeds. The answers why this condition can control weeds will be investigated in the future.

The benefit of this application was that farmers can plant and easily cut materials to paddy fields with irrigation time, especially at the early growth stage of rice seedlings about 10 – 15 days after seeding because the weeds were very small at this stage. However, a limitation of this application was water supply and the level must be necessary for weed control. In a natural condition, it was observed that B. pilosa had a biomass about 20 – 30 t f.w./ha, while the complete weed control was at 6 t f.w./ha.

From the present study, it could therefore be concluded that the phytototoxic of *B. pilosa* by fresh extract of a whole plant had more inhibitory effects on germination and growth of barnyard grass and white head than dry extract. The soil mulching and soil incorporation by fresh material from *B. pilosa* could reduce weed emergence at the rate of 40 and 50 t f.w./ha. The application of fresh *B. pilosa* that integrated with water irrigation could surprisingly control paddy weeds. The weeds were initially controlled over 50% at the rate of 4 t f.w./ha which was less than soil incorporation and mulching by 10 times. This method was very interesting for field conditions. However, it must be tested under the field trial. The site effect on rice injury will be investigated and also the answers why it killed weeds will be required in the future.

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