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Allelopathic effects of Arabian jasmine (*Jasminum sambac* Ait.) and preliminary test for estimation of its natural herbicide activity

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Leaves of *Jasminum sambac* were extracted by various percentage of ethanol and water for the highest yield of crude extracts. The highest yield of crude extract was achieved by using 50% ethanol with two time extractions. Each crude extract was tested to inhibit the germination and seedling growth of *Echinochloa crus-galli* and *Sesbania aculeate*. The results showed that crude extract from 50% ethanol gave the highest inhibitory activity. The crude extract from 50% ethanol was further separated into acidic fraction (AE), neutral fraction (NE) and aqueous fraction (AQ). AE fraction was greater inhibited plant than other fractions, and it was selected to formulate as natural herbicide in wettable powder (30% ai.). This natural herbicide was completely inhibited the germination of *S. aculeate* and slightly inhibited *E. crus-galli* by applying onto the soil surface at rate of 200 kg a.i. ha^{-1}.

Key words: allelopathy, Arabian jasmine, bioassay, *Jasminum sambac*, *Echinochloa crus-galli*, *Sesbania aculeate*

Introduction

The success of modern agricultural practices is due to the discovery and adoption of chemicals for weed and pest control (Dayan *et al.*, 2009). Recently, synthetic herbicides have been used increasing day by day to promote productivity. The overuse of synthetic herbicides has caused environmental pollution and human health. Moreover, the potential impact of pesticides on the environment has become much pressing and stringent pesticide registration procedures such as the Food Quality Protection Act in the United States. Efforts have begun towards searching for alternative weed management tools for

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sustainable crop production. Recently, the allelopathy has been introduced as a viable option for alternative weed management under sustainable agriculture (Fujii, 2001; Singh *et al*., 2003; Hong *et al*., 2003). Allelopathy can play a beneficial role in various sustainable weed management tools such as cover cropping (Isik *et al*., 2009; Campiglia *et al*., 2010), soil surface mulching (Aladesanwa and Adigun, 2008) and soil incorporation (Chon *et al*., 2005; Kobayashi *et al*., 2008). However, there are many limitations for using plant residues such as mulch or incorporating them due to heavy fieldwork for applying large amount of plant residues, which is often cost prohibitive. There are several natural products or mixtures of natural products mainly extracts from plant origins such as essential oil that are commercialized as crop protection products for use in organic agriculture (Hüter, 2010). For example, Matran® contains up to 50% clove oil and Burnout II® consists of a mixture of 12% clove oil with acetic acid (Dayan *et al*., 2009). Other natural products produced from allelopathic plant residues showed a potential as natural herbicides, such as corn gluten (Dayan *et al*., 2009), and organic herbicides from *Aglaia odorata* Lour. in pellet form (Laosinwattana *et al*., 2009). The natural products have been used as active ingredients in weed control but the market is relatively small because many natural products have resulted insufficient biological activity, low persistence under field conditions, or supplied in industrial quantities and consistent quality cannot be assured (Hüter, 2010). These occurrences are agreed by previous reports (Inderjit *et al*., 2002; Teerarak *et al*., 2010). Another challenge is the products formulated from crude extract to joint action of allelochemicals mixtures from strong allelopathic plants might successfully produce effective natural herbicide products.

The maximum yield of bioactive compounds from natural plant materials would be investigated to find the high quality of solvents for those active substances. The different solvent systems have been used for the extraction of secondary metabolites from plant materials because their extraction efficiency depends on their chemical's nature. Extracted yield is depended on the solvents and the method of extractions (Goli *et al*., 2004.). The extraction method would perform in completely extraction of the compounds. Water and aqueous mixtures of ethanol, methanol and acetone are commonly used in plant extraction (Sun and Ho, 2005). Aqueous ethanol was reported as a superior to methanol and acetone for extracting flavonoilds from tea (Wang and Helliwell, 2001). However, water was reported to be a better solvent for extracting tea catechins than 80% methanol or 70% ethanol (Khokhar and Magnusdotti, 2002). Alcoholic solvents have been commonly employed to extract bioactive compounds from plant materials, particularly mixtures of alcohol and water (Spigno *et al*., 2007). However, methanol is a toxic and harsh organic solvent,

whereas ethanol is more acceptable for use in the extraction process. Thus, ethanol was used as the solvent in all subsequent studies.

The present study was designed to evaluate the herbicidal effects of crude extracts from Arabian jasmine (*Jasminum sambac* Ait.) on germination and seedling growth of *Echinochloa crus-galli* and *Sesbania* . The optimum organic solvent system and fractionation procedure were determined to enhance extracted yield and activity.

Materials and methods

Plant materials

Two years-old Arabian jasmine (*Jasminum sambac* Ait.) plants growing around the experimental field at the King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand were collected. The plant was identified and authenticated by Bangkok Herbarium, Division of Plant Varieties Protection, Department of Agriculture, Thailand, where a voucher specimen (No. BK/61394) of the plant has been kept in the herbarium. The mature and healthy leaves were harvested, cleaned with running tap water, dried in a hot-air oven at 45°C for 3 days and ground to powder (100 mesh) in an electrical blender. Seeds of barnyardgrass (*Echinochloa crus-galli* L. Beauv.) and sesbania (*Sesbania aculeate* L.) collected from paddy fields in the Ladkrabang district, Thailand. Seeds of *E. crus-galli* were placed in the shade at room temperature for 3 months and then incubated at 60°C in a hot-air oven for 48 hours to break dormancy. Hard seed coats of *S. aculeate* were scrubbed with No.0 sandpaper to break their dormancy. These species were selected for the experiment due to big seeds and tolerate allelochemicals.

Effect of solvent extraction on the crude extracts yield and bioassay

Twenty gram of 100 mesh *J. sambac* leaf powder was extracted (ratio 20g: 200 ml), with a different solvent system at 25°C constant temperature for 24 hours. Solvent systems were absolute ethanol containing different volumes of distilled water (75%, 50%, 25%, and 0%), and distilled water. After 24 hours of extraction, the brown supernatants were filtered through four layers of cheesecloth and re-filtered through Whatman no. 1 filter paper (Whatman Inc. Clifton, NI, USA.). Following filtration, the brown supernatants were evaporated using a rotary evaporator (BUCHI Rotavapor R255), BUCHI, Lausanne, Switzerland), under a partial vacuum at 45° C to get crude extract, then weighted. Each residue was re-extracted 2 times with the same condition

as the first extraction procedure, and then crude extract number 1, 2 and 3. Stock solution of each crude extract was performed by ethanol in water at 100%, 75%, 50%, 25% and 0% (v/v) and was prepared by dissolving each sticky crude extract with acetone in a motar jar and wettable powder (bentonite:anionic surfactant; 95:5 (w/v)), then placed into motar jar at a 3:7 ratio (crude fraction:wettable powder). The mixture was slowly pulverized until completely dried; acetone was added three times and kept in the dark at a low temperature until used. Each concentration of crude extract (100%, 75%, 50%, 25% and 0% ethanol in water was performed in wettable powder by dissolved in distilled water to contain four concentrations ranging from 1000 to 8000 ppm. Five milliliters of each treatment was added to the germination paper, and placed in each 9 cm diameter glass Petri dish. Twenty healthy seeds of *E. cruss-galli* and *S. aculeate* were placed in each Petri dish and maintain in a growth chamber with a temperature of 25–32°C, a 12/12 hour dark/light photoperiod, with light intensity (cool White 840) of 100 μmol m^{-2} s^{-1} and relative humidity of 80%. Treatments with distilled water were served as the control. Germination was determined only after the radicle protruded beyond the seed coat for seven days after treatment. Seedling growth was measured as the root and shoot lengths at seven days after treatment.
The experiment was done by using completely randomized design with four replications.

Solvent partitioning of active compounds and bioassay

The crude extracts were prepared from *J. sambac* leaf powder by extraction with 50% ethanol in distilled water. After filtration using Whatman No. 1 filter paper, the residue was repeatedly extracted 2 times with 50% ethanol for 24 hours at 25°C and filtered. The filtrates were combined and evaporated in the rotary evaporator at 45°C, leaving a sticky residue (original crude fraction; OR fraction). The sticky residue was diluted with 0.5 L of distilled water and stirred vigorously on a magnet at 45°C for 20 minutes, resulting in aqueous solution and acidified to a pH 3 by 6 N HCl. The filtrate was extracted with ethyl acetate for three times (0.5 L×3). The ethyl acetate solution was mixed with anhydrous magnesium sulfate, concentrated to 0.5 L and then extracted for three times with saturated aqueous $NaHCO_3$ (0.5 L×3). The ethyl acetate phase was dried with anhydrous magnesium sulfate and concentrated by reducing pressure and an ethyl acetate-soluble neutral fraction (NE fraction) was obtained. The combined sodium bicarbonate phase was evaporated to 0.5 L, adjusted to a pH 3 by 6 N HCl, and then extracted with ethyl acetate (0.5 L×3). The ethyl acetate solution was combined, dried with

$MgSO_4$, and then evaporated to obtain the ethyl acetate-soluble acidic fraction (AE fraction), and the remains of the aqueous phase were discarded (Fig. 1). The inhibitory activities of each fractions (OR, AQ, NE and AE fractions), were prepared as same as previously described. Each fraction of OR, AQ, NE and AE fractions in wettable powder form was prepared to contain four concentrations of each fraction from 1000 to 8000 ppm. Bioassays on seed germination and seedling growth were tested as previously described.

Efficacy of the high activity potential fraction in soil bioassay

From previous studies, the selected fractions showed the most allelochemical compounds that produced by Arabian jasmine. Wettable powder product from the most active fraction was prepared by dissolving sticky crude with acetone in a motar jar and mixed wettable powder (bentonite: anionic surfactant; 95:5 (w/v)) at ration of 3:7. This product was namely as NHJ 30%ai, and kept in the dark and at low temperature until used. The efficacy of NHJ on germination and seedling growth of *E. crus-galli* (L.) Beauv. and *S. aculeate* L. was performed in pot experiment. Soil used in this experiment was sandy loam soil with a pH of 6.5. Ten kilograms of soil, prepared by mixing soil with farmyard manure (2:1 ratio), was poured into plastic pots (15 cm diameter). Twenty seeds of each bioassay species were placed in separated pots at 0.5 cm deep from soil surface. The NHJ was applied on soil surface at 50, 100, and 200 kg ai/ha and non-treated pot was served as a control. Seed germination were counted at 7 days after treatments, and plant height was determined at 7, 14, 21 and 28 days after treatments. The upper and lower ground parts of survival seedlings were recorded separately at 28 days after treatments.
The experiment was done by using completely randomized design with six replications.

Data analysis

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

Results and Discussion

Effect of solvents on the yield of the crude extract

The effect of different solvent systems and extraction numbers on the yield of the crude extract was done. The results showed that the extracted yield

from each solvent was increased when repeating the number of extractions from 1 to 2. However, extracted yield was no longer significantly changed as the extracted number that repeated from 2 to 3. It is suggested that dried leaves powder of *J. sambac* was extracted with a mixture of ethanol in water with various ethanol percentages from 0-100% for 24 hours; the optimal value for extraction number was 2. The optimal percentage of ethanol in water was examined. There was a markedly significant difference in the extracted yield of total crude extracts from dried leaves, which repeated with increased ethanol concentration until the concentration reached 50%. The greatest yield was achieved when using ethanol at 50%. Many authors reported that an extracted yield is dependent on the solvents (Goli *et al*., 2004; Li *et al*., 2009). The differences in the extracted yields were obtained using various ethanol-water ratios due to several factors such as composition of each particular plant differences in the solubility of extractive and their polarity as also stated Zuo *et al*., 2002).

Bioassay of solvent extraction

The dried leaves of *J. sambac* was extracted by various solvent systems showed different inhibition of seed germination and seedling growth of *E. crus-galli* and *S. aculeate*. The inhibitory effect was different depending on bioassay species, source of crude extract and tested concentrations. There were no significantly differences among crude extract obtained from five solvent system extractions on germination and seedling growth of *E. crus-galli*. However, there were significantly differences on germination and seedling growth of *S. aculeate*. The degree of inhibition was dependent on tested concentrations obtained from different solvent systems. Crude extract of *J. sambac* at concentration of 4,000 ppm obtained from 100%, 75%, 50%, 25% and 0% ethanol in water reduced seed germination of *S. aculeate* by 57%, 73%, 78%, 56%, and 42% over control (distilled water), respectively. Thus, it proved that extracts prepared by different solvents resulted to varying degrees of inhibitory activity. These results indicated that extraction of natural sources by appropriate solvent systems would important to obtain fractions with high allelopathic potential and high crude extracted yield. This finding is supported by Li *et al*. (2006); Luthria *et al*. (2007); Garcia *et al*. (2010), who reported that different solvent systems used for the extraction of secondary metabolites from plant materials resulted to extraction efficacy and their chemicals.

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Inhibition of various fractions after solvent partitioning

The 50%ethanol in water demonstrated the highest potential extraction activity in the bioassay which fractionated by a simple partitioning procedure (Fig. 1), A 50% ethanol crude extract (OR) from *J. sambac* Ait was partitioned into aqueous (AQ) fraction, acidic (AE) and neutral (NE) fractions. The growth inhibition activities of AQ, NE, and AE fractions were compared with the original extract (OR). Although, the concentrations for bioassays varied from 1000 to 8000 ppm, resulted that the most fractions inhibited germination and seedling growth of *E. crus-galli* and *S. aculeate* (Fig. 4). The OR at 4,000 ppm showed strong inhibition of 35 and 87% seed germination of *E. crus-galli* and *S. aculeate*, respectively. After solvent partitioning, the inhibition increased as compared to the OR fraction. The AE fraction at 8,000 ppm showed the greatest activity fraction with complete inhibition of germination, shoot length and root length of both bioassay species. The NE and AQ fractions showed a weak inhibitory effect on germination, shoot length and root length of *S. aculeate* when compared with the OR fraction while NE fraction showed strongly inhibition on shoot and root length of *E. crus-galli*. These results indicated that most allelochemical compounds produced by *J. sambac* could be presented in AE fraction. It was similar results to Teerarak *et al*. (2010), who reported that a secoiridoid glucoside named oleuropine which identified as an allelopathic compound from AE fraction of a related *Jasminum officinale* var. *grandiforum*. The importance of allelochemicals mixtures is recognized both in herbicide research and exploring plant allelochemicals (Inderjit and Olofsdotter, 2002). It is suggested that the mixture compound in AE fractions gave significantly inhibited the tested weed species.

Soil application bioassay

Emergence and seedling growth (plant height and dry weight) of *E. crus-galli* and *S. aculeate* was significantly suppressed by soil surface application of AE fraction in wettable powder formulation from *J. sambac* leaves extract (30%aiNHJ) when compared with the control (Fig. 5). However, the lowest dose (50 kg ai.ha^{-1}) of NHJ did not reduce emergence and growth of both bioassay species. Plant dry weight of *E. crus-galli* was a slightly increased by 12.8%. The application 100 kg ai ha^{-1} weed emergence of *S. aculeate* was reduced by 72.5%, whereas seed germination of *E. crus-galli* reduced by 3.75%. With increasing doses of application, the weed emergence as well as the plant height and dry biomass declined. The growth of *S. aculeate* was completely inhibited at 200 kg ai.ha^{-1} of NHJ, whereas plant height and dry

weight of *E. crus-galli* were reduced. These results are congruent with Batish *et al*. (2007), who reported that allelochemicals from allelopathic plants on bioassay weed species gave a greater negative effect on dry biomass than emergence and plant height. The effect of NHJ to the treated weed species were similar to several previous reports, which noted that *E. crus-galli* possessed stronger resistance against allelochemicals from other plants than other bioassay seeds (Seal, *et al*., 2004; Khanh, *et al*., 2005; Laosinwattana *et al*., 2010). The sensitivity of weed species to allelochemicals depends on the physiological and biochemical characteristics of each species. The activity of allelochemicals was greatly influenced by the soil physicochemical properties such as organic mater content (Kobayashi, 2004).

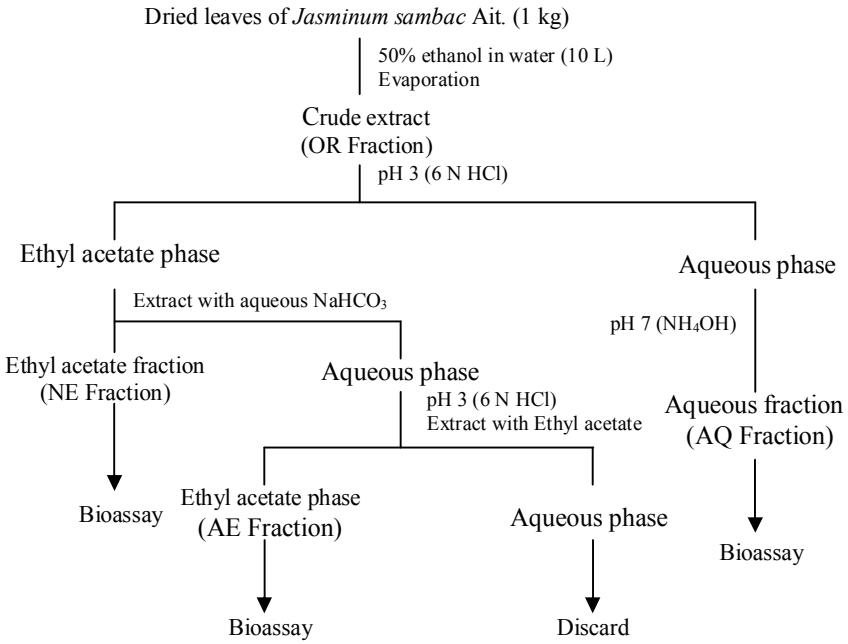

Fig. 1. Flow chart for extraction and partial separation of active compounds from *J. sambac* Ait. dried leaves.

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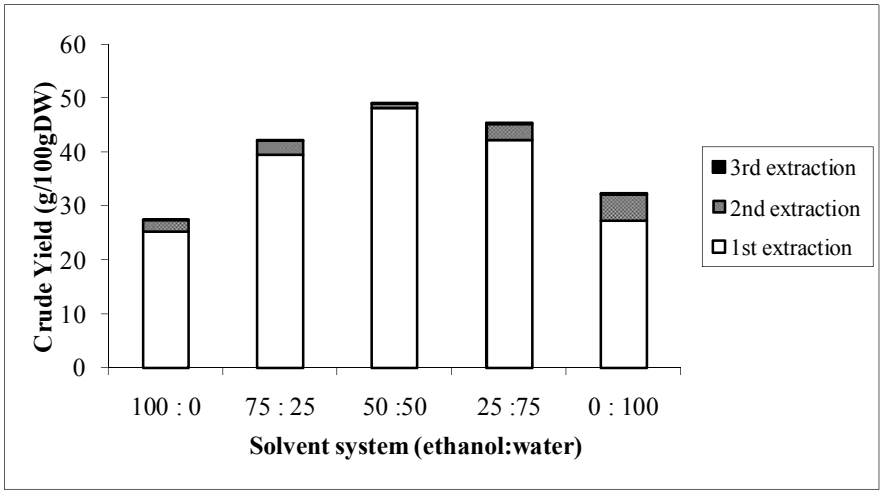
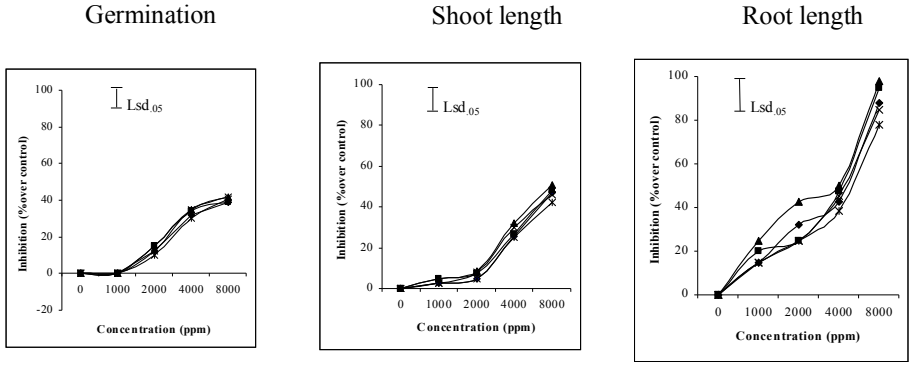


Fig. 2. The effect of different ethanol percentage in water and extraction number on crude extraction yield from *J. sambac* Ait. dried leaves. Note:1st extraction (□), 2nd extraction (▩), 3rd extraction (■).

Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.



Sesbania (*Sesbania aculeate* L.)

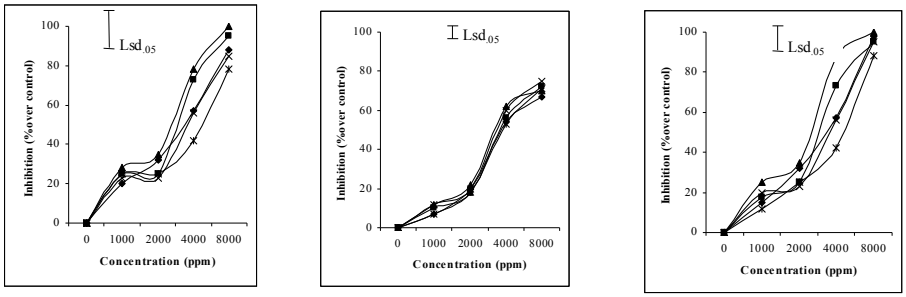


Fig. 3. The inhibitory effect of crude extraction obtained by different ethanol percentages in water from *J. sambac* on germination, shoot length and root length of barnyardgrass (*E. crus-galli* (L.) Beauv.) and sesbania (*S. aculeate* L.). 100% (●); 75% (■); 50% (▲); 25% (×); 0% (✱) (ethanol in water)

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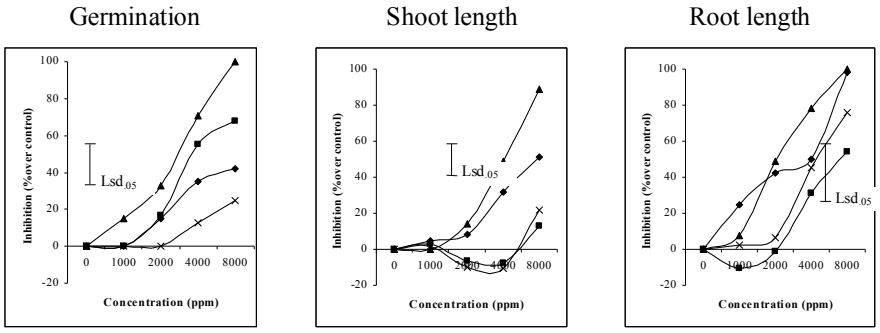
Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.)



Sesbania (*Sesbania aculeate* L.)

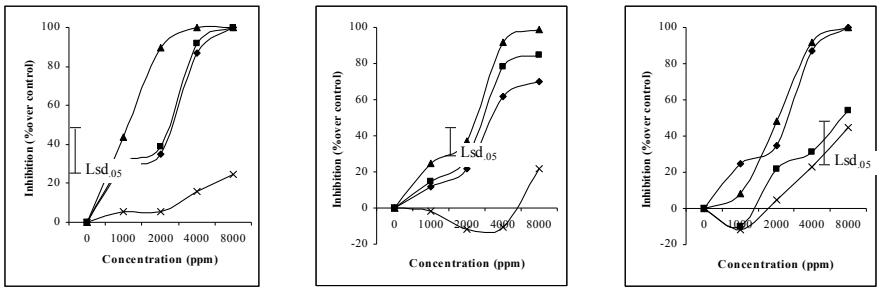
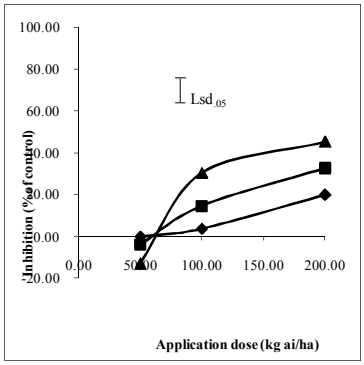


Fig. 4. The inhibitory effect of crude extraction yield obtained by different solvents systems from *J. sambac* on germination, shoot length and root length of barnyardgrass (*E. crus-galli* (L.) Beauv.) and sesbania (*S. aculeate* L.). Original fraction (◆); neutral fraction (■); acidic fraction (▲); aqueous fraction (×).

barnyardgrass



sesbania

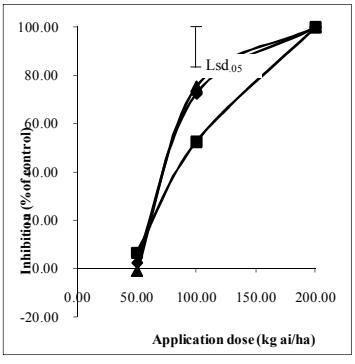


Fig. 5. Effect of application of natural herbicide product from AE fraction of *J. sambac* Ait. (NHJ) in wettable powder form on emergence, plant height and dry weight of barnyardgrass (*E. crus-galli* (L.) Beauv.) and sesbania (*S. aculeate* L.). Emergence (◆); Plant height (■); Dry weight (▲)

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