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Allelopathic effects of *Xylocarpus gangeticus* Parkins on germination and growth of weed in rice fields

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ABSTRACT

The leaves of *Xylocarpus gangeticus* Parkins were extracted with solvents polarity sequential extraction for maceration technique with hexane, ethylacetate and methanol, respectively. Evaporation of the filtrate under reduced pressure gave crude extracts of hexane, ethylacetate and methanol. The ethylacetate extracts were separated by Column chromatography to gave ethylacetate 9 fractions (F1-F9). Ethylacetate extracts and ethylacetate fractions tested to inhibited seed germination and growth of *Echinochloa crus-galli* , a weeds in rice fields. Analysis of data to compare the percentage inhibition of germination, percentage inhibition of root length, percentage inhibition of stem length and percentage inhibition of dry weight of weeds. The results showed that, At a concentration of 3,000 ppm, ethylacetate extract showed a optimally inhibitory effect on seed germination of *Echinochloa crus-galli* by 69.98% and inhibited root length, stem length, and dry weight by 66.01%, 65.15% and 60.39%, respectively. Fraction F1 of the ethyl acetate extract showed a significantly inhibitory effect on seed germination of *Echinochloa crus-galli* by 67.91% and inhibited root length, stem length and dry weight by 64.42% 61.08%, 70.15% and 57.39%, respectively. Thus it can be concluded that the *X.gangeticus*, can be used to control weeds in rice fields led to develop further research to commercialization.

Key words: Allelopathic effects, *Xylocarpus gangeticus* Parkins, weeds

INTRODUCTION

Allelopathy is defined as the direct or indirectly harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment [1]. Plants are known to produce certain substants which react with other organisms in the environment [2]. Allelopathy of plants is strongly coupled with external biotic and abiotic stress factors [3]. Allelopathy is an interference mechanism in which living or dead plants release allelochemicals exerting an negative effect on the associated plants, and can play an important role in ecosystems [4]. Since crop plants were cultivated, weed control has been an important aspect of their management practices [5]. Therefore, various other methods of weed control have been studied, and exploitation of allelopathic properties in plants may give promising results [6].

Xylocarpus gangeticus Parkins. is a plant family Meliaceae that been found evergreen forest drought in throughout Southeast Asia, In Thailand found in western, which know the local name of Tabun [7].

There are very few publications concerning of the Allelopathic effects of *Xylocarpus gangeticus* Parkins. It was found that the plant in the same family study to Allelopathic effects such as [8]. showed that the effect of organic solvent extracts, after hexane ethyl acetate and methanol, from the stem bark of *W. Trichostemon.* a plant family Meliaceae. The allelopathic effect was tested against *Brassica campestris* var. *chinensis* L. (Chinese cabbage) seed germination and seedling growth, the crude ethyl acetate extract caused a decreased in the percentage of germination and shoot and root growth. [9]. showed that the effects of dry leaves organic solvent extracts from *W. trichostemon.* a plant family Meliaceae by solvent partitioning extract on germination and seedling growth of *Brassica campestris* var. *chinensis* L. was investigated. The results found that the acidic fraction had high inhibitory effect which completely inhibited *B. campestris* var. *chinensis* seed germination at concentration 4,000 ppm.

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Fig. 1: *Xylocarpus gangeticus* Parkins

Fig. 2: *Echinochloa crus-galli* , a weed in rice fields

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chinochloa crus-galli is a plant family Poaceae, is a type of wild grass originating from tropical Asia that was formerly classified as a type of panicum grass,. It is commonly known as barnyard grass. This plant can grow to 60" (1.5 m) in height and has long, flat leaves which are often purplish at the base. Most stems are upright, but some will spread out over the ground. Stems are flattened at the base. The seed heads are a distinctive feature, often purplish, with large millet-like seeds in crowded spikelets. Considered one of the world's worst weeds, it reduces crop yields and causes forage crops to fail by removing up to 80% of the available soil nitrogen (http://en.wikipedia.org). Echinochloa crus-galli, a C4 grass, is one of the world's most serious weeds [10]. *Echinochloa crus-galli* is problematic weed species in direct-seeded rice systems in Asia. Because of concern about the continuous use of single herbicides, cultural weed management strategies need to be developed to maintain the sustainability of direct-seeded rice systems [11]. Allelopathic activity of *Echinochloa crus-galli* on rice seedlings exhibited 5.3–6.3-fold increases when rice and barnyard grass seedlings were grown together, where there may be the competitive interference between rice and barnyard grass for nutrients. Barnyard grass is one of the most noxious weeds in rice cultivation. The momilactone B concentration in rice seedlings incubated with barnyard grass seedlings was 6.9-fold greater than that in rice seedlings incubated independently [12].

This paper describes the extractions of crude ethylacetate extract from the leaves of *Xylocarpus gangeticus* Parkins, and the allelopathic effects of *Xylocarpus gangeticus* on the inhibition of germination and growth of *Echinochloa crus-galli* , a weeds in rice fields.

Materials and Methods

Extractions of bioactive constituents from the leaves of *Xylocarpus gangeticus* Parkins with a polarity sequential extraction and maceration techniques with hexane, ethyl acetate and methanol, respectively. Crude ethyl acetate extracts were tested for inhibition on germination and growth of weeds

(*Echinochloa crus-galli*) each class of compounds and separated by chromatographic techniques.

Plant materials:

The leaves of *Xylocarpus gangeticus* Parkins were collected from natural populations in Samutsongkhram Province, Thailand and identified at the Department of biology, Faculty of science and technology, Rajamangala University of Technology Phra Nakhon,(RMUTP) Bangkok. Seeds of *Echinochloa crus-galli* were collected from paddy fields in November 2012. Empty and undeveloped seeds were discarded by floating in tap water. The remaining seeds were air-dried and hermetically stored at 25–30 ◦C. In germination and growth tests, these seeds was randomly checked and was found to be >95%.

Extraction:

The leaves of *Xylocarpus gangeticus* Parkins were extracted with solvents polarity sequential extraction for maceration technique with hexane, ethylacetate and methanol, respectively. Fresh leaves of *X. gangeticus* were cleaned and then chopped into small pieces. The aerial parts were dried in an oven at 40 ◦C for 12 h. The aerial parts 1kg were macerated overnight for 7 days with hexane 5 liters at room temperature. The crude hexane extract was filtered and evaporated under reduced pressure at 45 ◦C to give a crude hexane. The marc was then similarly extracted with ethyl acetate and methanol to obtain corresponding crude ethyl acetate and methanol extracts, respectively.

Seed germination and growth test:

The dried samples concentrated from crude ethyl acetate extract were again dissolved in the ethyl acetate to compare their inhibitory effect. Five milliliters of extract solutions at concentrations 5,000 ppm, 4.000 ppm, 3,000 ppm, 2,000 ppm and 1,000 ppm was added to Petri dishes with germination paper and distilled water was used as a control, evaporated to dryness for 12 hrs., at room temperature. After evaporation, 5 mL of distilled water was added to the germination paper to give the final concentration at 5,000 ppm, 4.000 ppm, 3,000 ppm, 2,000 ppm and 1,000 ppm. Then twenty seeds of test plants were placed on the germination paper and grown. After 7 days, germination percentage was determined by counting the number of germinated seeds. Root lengths and stem lengths were determined after 10 days. After measuring root lengths and stem lengths the plants were then dried and their respective dry weights recorded. The treatments were arranged in a completely randomized design with three replications.

Analytical TLC thin layer chromatography (TLC):

The ethylacetate extracts of *Xylocarpus gangeticus* Parkins were analyzed through thin layer chromatography (TLC) using the method of Wagner and Bladt, 1996. TLC was performed was performed on silica gel 60 aluminum plates (Merck, 8 ×10 cm^2). About 10 µl of ethylacetate extracts were loaded on TLC plates. The plate was air dried and developed in Hexane: Diethyl ether (2:3). The plate was dried in a hot oven (at 105 ºC for 5 min) and spots were visualized by UV light (254 and 366 nm) .For spraying anisaldehyde reagent (0.5ml p-anisaldehyde in 50 ml glacial acetic acid and 1 ml conc. sulfuric acid) (http://delloyd.50megs.com/) were used in the experiment.

Fractions Separation Column chromatography:

Column chromatography was performed on silica gel 60. Ethyl acetate extract 5 g were loaded to silica gel column chromatography, eluted with hexane and hexane with increasing amounts of ethyl acetate, to give nine fractions (F1–F9) on the basis of TLC analysis. These fractions were again evaluated on Seed germination and growth. Analysis of data to compare the percentage inhibition of germination, percent inhibition of root length, percent inhibition of stem length and percent inhibition of dry weight of weeds.

Results and Discussion

Ethylacetate extracts on germination and growth percentage:

The results of ethylacetate extracts on germination and growth percentage showed that the percentage of optimally inhibitory effect of ethyl acetate extract on seed germination of *Echinochloa crus-galli*, with increasing of concentrations of ethylacetate extracts, 1,000 ppm, 2000, ppm, 3,000 ppm, 4,000 ppm, to 5,000 ppm), fund that inhibition on germination and growth percentage has increased and there is a significant difference between the concentrations of 1,000 ppm, 2,000 ppm and concentrations of 3,000 ppm, 4,000 ppm, 5,000 ppm. But the inhibition percentage at concentrations 3,000 ppm, 4,000 ppm, 5,000 ppm is no different. That is the appropriate concentration to inhibit the germination, root length stem length and dry weigt concentration is 3,000 ppm. At a concentration of 3,000 ppm inhibited on seeding germination by 69.98% and inhibited root length, stem length, and dry weight by 66.01%, 65.15% and 60.39%, respectively. (Tables 1.and fig 3). Therefore this research, It points out the use of ethylacetate extracts of *Xylocarpus gangeticus* Parkins to inhibition germination and growth *of Echinochloa crus-galli*

concentrations at 3,000 ppm, which was that the ethyl acetate extract was effective as in the case of [8] showed that the allelopathic effect was tested against *Brassica campestris* var. *chinensis L.* (Chinese cabbage) seed germination and seedling growth, the crude ethyl acetate extract caused a decreased in the percentage of germination and shoot and root growth.

It also shows that extracts of Echinochloa crus-galli using ethylacetate containing 3,000 ppm concentration effective inhibition germination and growth over the aqueous methanol extracts of duckweed (*Lemna minor* L.) and water lettuce (*Pistia. stratiotes* L.), on inhibited root growth of Echinochloa crus-galli [14]. Explained by allelopathic effects not only lead to reduced germination but also delayed in germination that can impact a lot the result of competition and seedling plants which may have acquired larger size under incompatible conditions such as low soil moisture or food restriction may compete better with adjacent plant. Delayed in germination of seeds can have osmotic effects on the rate of water absorption, delayed at the beginning of germination and especially cell elongation [15].

Table 1: Influence of concentration of ethylacetate extracts on inhibition germination and growth percentage

Inhibition percentage(%)	Concentration of ethylacetate extracts(ppm)				
	1,000 ppm	2,000 ppm	3,000 ppm	4,000 ppm	5,000 ppm
Germination	51.23[a]	60.59[b]	69.98[c]	70.01[c]	71.22[c]
Root length	46.99[a]	61.36[b]	66.01[c]	67.29[c]	66.43[c]
Stem length	49.47[a]	57.95[b]	65.15[c]	64.02[c]	65.91[c]
Dry weight	46.78[a]	56.81[b]	60.39[c]	59.94[c]	60.08[c]

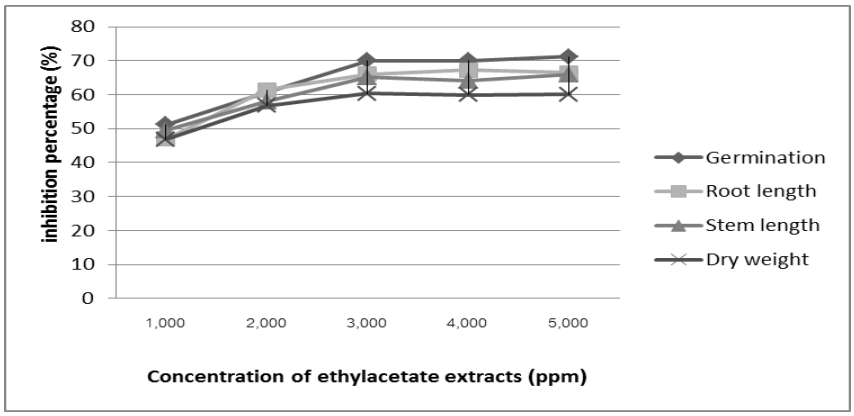

Fig. 3: Effect of different concentrations on germination and growth percentage

Ethylacetate fractions on germination and growth percentage:

When ethylacetate extracts was separated by chromatographic techniques, to gave ethylacetate 9 fractions (F1-F9). These fractions were evaluated in the Seed germination and growth inhibitory effect.

The results of fractions of ethylacetate extracts on germination and growth percentage, fraction F1 showed that significantly inhibitory effect on seed germination of Echinochloa crus-galli by 67.91% and inhibited root length, stem length. and dry weight by 64.42% 61.08%, and 57.39%, respectively. (Tables 2 and fig 4).

The research involved Charoenying, P et al, 2008. Showed that the acidic fraction had high inhibitory effect which completely inhibited B. campestris var. Chinensis seed germination at concentration 4,000 ppm.

Table 2: Influence of fractions of ethylacetate extracts on inhibition germination and growth percentage

Inhibition percentage (%)	fractions of Ethylacetate extracts								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Germination	67.91	55.34	55.29	25.29	46.67	58.88	28.14	37.56	51.84
Root length	64.42	54.99	51.74	27.53	47.91	58.19	24.69	36.54	53.66
Stem length	61.08	50.71	42.61	24.76	42.55	55.23	25.37	29.58	49.91
Dry weight	57.39	48.93	45.32	22.94	40.19	51.41	20.44	27.36	47.23

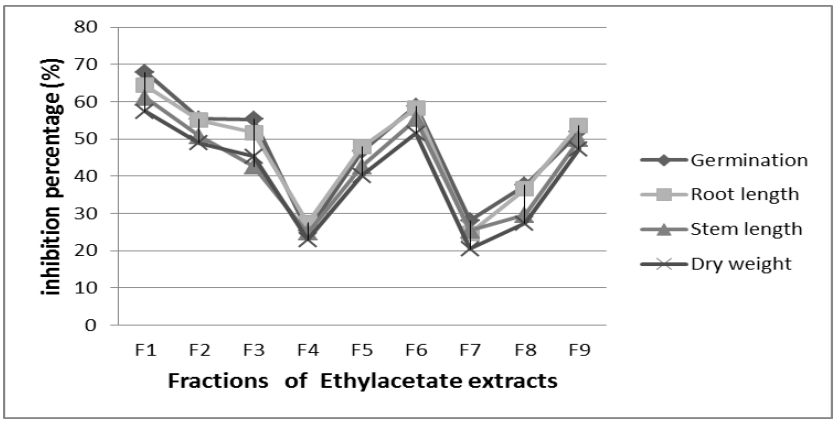

Fig. 4: Effect of different concentrations on germination and growth percentage

Conclusions:

In summary, it can be concluded that the *Xylocarpus gangeticus* Parkins, can be used to control germination and growth of *Echinochloa crus-galli* ,weeds in rice fields led to develop further research to commercialization.

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