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Efficacy of weed extract as a bioherbicide in rice (*Oryza sativa* L.) cultivation

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ABSTRACT

Weeds around rice crops can cause competition and then must be controlled. Pre-emergence weed control by utilizing weed extract as herbicides is the alternative solution for substituting synthetic herbicides. In addition, allelopathic compounds of weed can inhibit the seed germination of other species. This study aims to know the efficacy of weed extract types as bioherbicides to inhibit weed growth and increase rice yields. The study was conducted from 2021-2022 in the greenhouse, Ngestiharjo, Yogyakarta, Indonesia. The Padjajaran Agritan variety was used. This study was a pot experiment arranged in a complete randomized design (CRD) with three replications. The weed extract treatment consisted of four types: goat weed, nut grass, siam weed, and cogon grass, which was applied in two ways: seven days before and when planting seedlings. In addition, one control was added, namely without weed extract application. In this study, 81 polybags were used. Each replication consisted of three plant samples. The results showed that the application of weed extract could inhibit weed growth, including the individual weed number and weed dry weight. In addition, it can also increase tiller number, shoot dry weight, panicle number, and grain dry weight of rice. The nut grass and cogon grass were given panicle number and grain dry weight higher than goat weed and siam weed. The right time for weed extract application was seven days before planting for nut grass and when planting for cogon grass to increase the rice yield. Nut grass and cogon grass extracts have efficacy higher than nut grass and siam weed in inhibiting weed growth and increasing rice yield. Therefore, we suggest that nut grass and cogon grass extract can be used for pre-emergence weed control in rice cultivation.

Key words : Allelopathic, bioherbicide, pre-emergence, rice, weed extract

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of most people in Asia, especially in Indonesia. Weeds in the rice environment cause competition and must be controlled (Mutakin *et al.*, 2021). Using excessive synthetic herbicides can harm farmers' health, consumers, and the environment (Selvaraj and Hussainy, 2020). The best solution is using natural herbicides from weed extracts called bioherbicides. Bioherbicides can be used instead of synthetic herbicides. Some weeds have a high potential as bioherbicides and are very easy to find in the farmers' environment. The allelopathic compound in weed extract can inhibit the germination of the weed seed banks in the soil.

Allelopathy can be utilized for sustainable weed management by using allelochemicals as natural herbicides (Li *et al.*, 2020; Sooman *et al.*, 2020). Some weeds have allelopathic activity in the form of secondary metabolite compounds, namely phenolics, terpenoids, tannins, alkaloids, steroids, polyacetylene, and essential oils. Therefore, certain types of weed extracts can be used as bioherbicides. However, the degree of inhibitory for each type of weed extract was not the same for weed germination. Therefore, it is necessary to know the weed extract types that have a high potential as bioherbicides. In addition, the right application of weed extract to increase rice growth and yield needs to be known.

Weed seed banks are stored in the soil in large quantities under dormant conditions. The weed species that germinate and grow on the surface of the soil depend on the type of crop cultivated. Weed species composition in rice crop cultivation was different from the others. Kurniadie *et al.* (2019) stated that the weed species with the highest summed dominance ratio (SDR) in conventional and organic rice cultivation was *Fimbristylis miliace*.

The results of other studies showed that *Oryza sativa* (rice weed), *Leptochloa chinensis, Echinochloa crus-galli, Ischaemum rugosum, Ludwigia hyssopifolia,* and *Fimbristylis miliace* were found more dominant in paddy rice (Begum *et al.*, 2005). The weeds with the highest importance value index were *Echinochloa colonum* (L.) Link, then decreases on *Cyperus cephalotes* Vahl., *Euphorbia hypericifolia* L., *Eclipta prostrata* (L.) and *Digitaria ciliaris* (Retz.) Koel. in paddy rice (Haris *et al.*, 2019).

So far, pre-emergence weed control in rice cultivation has always relied on synthetic herbicides. Weed extract bioherbicides have a high potential for substituting synthetic herbicides. According to Kumar and Kumar (Kumar and Kumar, 2018), bioherbicides are products of a new approach derived from living organisms or natural metabolites of plants to control weeds. Bioherbicides can be made from natural products, including plant extracts and essential oils. Ferguson *et al.* (2014) stated that secondary metabolite compounds are not necessary for a weed species' metabolism (growth and development). The secondary metabolite compound is called allelopathy. Weeds released into the surrounding environment.

Allelopathic compounds can inhibit the growth and development of neighbouring weeds of other species. Allelopathic compounds can be released through leaves, flowers, seeds, stems and roots (Binumol and Santhoshima, 2019; Sathishkumar *et al.*, 2020), leaching, root exudation, evaporation, residues decomposition, and other natural processes in agricultural systems. Allelopathic compounds produced by plants can be used as natural herbicides for weed control.

Bioherbicides can be prepared from weed extracts containing phytotoxic allelopathies or microbes carrying certain diseases (Hasan *et al.*, 2021). Allelopathy is important for agricultural practices and has gained the attention of sustainable agricultural management (Muzzo *et al.*, 2018). The phytotoxic impact on weed growth is reflected in the low rate of root cell division, absorption of nutrients, growth hormone, and pigment synthesis. Also, the development of reactive oxygen species (ROS), stress-related hormones, and abnormal antioxidant activity was low (Hasan *et al.*, 2021). Some weed species identified had the potential to produce allelopathic compounds, including *Ageratum conyzoides* L., *Cyperus rotundus* L., *Chromolaena odorata* (L.) R. M. King & H. Rob. and *Imperata cylindrica* (L.) Beauv.

Goat weed (*Ageratum conyzoides* L.) is one of the weed species that has the potential to be a bioherbicide because it has allelopathic compounds. The chemical content in goat weed leaves are saponins, flavonoids, polyphenols, eugenols, and roots containing essential oils. According to Agbafor *et al.* (2015), leaves and roots of goat weed contained alkaloids (26.80 mg/100 g), flavonoids (21.24 mg/100 g), tannins (4.78 mg/100 g), saponins (3.16 mg/100 g), cardiac glycosides (3.05 mg/100 g), and anthraquinones (3.09 mg/100 g). In comparison, terpenoids (0.84 mg/100 g) were only present in leaves.

Concentrations of more than 10% positively affected the percentage of seed germination, root growth, budding, nodulation, and biomass production of *Pisum sativum* than control (Kumar *et al.*, 2018; Singh, 2021). The inhibitory effect of goat weed leaf extract was greater than root extract. Dried leaf and root extracts were more inhibiting than fresh leaf extracts (Negi *et al.*, 2020). The concentration of 20% goat weed leaf extract showed the greatest inhibition to *Amaranthus spinosus* growth (equal to 100% control), followed by *Cyperus rotundus* (86% control), *Chromolaena odorata* (78% control), *Imperata cylindrica* (24% control), and lowest in *Axonopus compressus* (13% control) (Erida *et al.*, 2019). In addition to goat weed, nut grass weed has a high potential as a bioherbicide.

Nut grass *(Cyperus rotundus* L.) is a weed that can grow naturally in tropical areas (Tania *et al.*, 2021). The main compounds of essential oils from the rhizomes of nut grass were cyperone, myrtenol, caryophyllene oxide, and pinene (Lawal and Oyedeji, 2009). The total phenolics from the rhizomes of nut grass with extract samples of 10 and 25 mg/ml were 1.176 and 2.096 mg/ml. Pure flavonoids of 10 and 25 mg/ml were 1.016 and 1.186 mg/ml, respectively (Al-Jumaily and Al-Isawi, 2014).

Most inhibition of the growth of tomato seedlings occurs at a concentration of 30-40% nut grass tuber extract (Dadar *et al.*, 2014). At a concentration of 5% nut grass tuber extract, it lowered the growth tolerance index, dry weight, and root length of soybean seedlings (Darmanti *et al.*, 2015). The application of nut grass extract caused chlorosis on the leaves of *Mimosa invisa* and *Melochia corchorifolia*, but did not affect seed germination and growth inhibition against both weed species (Setyowati and Suprijono, 2001). The concentration of 25% nut grass tuber extract had a noticeable effect on the inhibition of seed germination of tomato seeds (Sardoei *et al.*, 2013). Using nut grass extract at a concentration of 9% had no significant effect on seed germination and early growth of arrears bean seedlings (Pereira *et al.*, 2018). In addition to weed, siam weed also had the potential to be bioherbicides.

Siam weed (*Chromolaena odorata* (L.) R. M. King & H. Robinson), commonly known as siamese weed, was derived from neotropics (Muniappan and Bamba, 2000). Siam weed can potentially be a bioherbicide for weed control (Yuliyani *et al.*, 2019). Siam weed leaves contained secondary metabolite compounds, including alkaloids (38%), flavonoids (23%), carotenoids (5%), benzoic acid derivatives (4%), lignin (7%), hydroxycinnamic derivatives (2%), saponins (4%), terpenoids (5%), and tannic acid (10%) (Muzaiyanah, 2020). The presence of tannin compounds, flavonoids, alkaloids, and terpenoids in siam weed inhibited the seed germination and growth of mung beans (Hamidi *et al.*, 2014), and such compounds can be allelopathic. Tannins inhibited the function of gibberellin, thereby inhibiting plant growth. Terpenoids can inhibit the function of auxin hormones that can inhibit the occurrence of etiolation in plant coleoptile (Muzaiyanah, 2020). Releasing phenols from siam weed leaf extract into the soil will inhibit the growth of surrounding plants (Laxman *et al.*, 2019).

Increased concentration of cogon grass extract leads to a decrease in the growth of Basil and Purslane seedlings (Golparvar *et al.*, 2015). In addition, it can suppress the percentage of germination, germination rate, and length of sprouting of weed seeds of *Amaranthus spinosus*, *Bidens biternata*, and *Tridax procumbens*. The highest level of weed seed sensitivity occurred in *Tridax procumbens*, decreased *Bidens biternata*, and the lowest in *Amaranthus spinosus*.

Weed extract of *Imperata cylindrica*, *Cyperus rotundus*, *Chromolaena odorata*, *Ageratum conyzoides*, and *Axonopus compressus* contained terpenoid, phenolic, and steroid compounds, except *Cyperus rotundus*. All weeds contained flavonoids, except *Ageratum conyzoides*. Based on its application, weed extract as a bioherbicide can be applied before and when planting rice seedlings. However, there was no clear information about the right time application of weed extract types as bioherbicides.

Some weed species have been known to produce metabolite compounds or allelopathies. The results of previous studies showed that weed extracts concentration of goat weed, nut grass, siam weed, or cogon grass varying between 1-35% were recommended. Weed extract can inhibit the growth of weed species and increase crop growth, including rice plants. In this study, a concentration of 30% of each type of weed extract was used. However, these weed extracts have never been compared to their potential as bioherbicides in rice cultivation. In addition, it has also never been tested for application before or when planting rice seedlings. Nevertheless, the application of weed extract types at the right time can increase the growth and yield of rice.

Based on the background and literature review above, this study aims to know the efficacy of weed extract types as bioherbicides to inhibit weed growth and increase rice yields.

MATERIALS AND METHODS

Study Site

The study was conducted from December 2021 to April 2022. The experiment location was conducted in the greenhouse, Ngestiharjo, Yogyakarta, Indonesia. The height of the study site was 118 m above sea level (m ASL) and located at 8°30'-7°20' South Latitude and 109°40'-111°0' East Longitude.

Experimental Design

This study was a pot experiment

arranged in a complete randomized design with three replications. Bioherbicide treatment consisted of four types of weed extracts: goat weed, nut grass, siam weed, and cogon grass. The application of weed extract consisted of two ways: seven days before and when planting rice seedlings. In this research, one control was added, namely, without weed extract application. Each replication consisted of three plant samples. In this study, 81 polybags were needed.

Research Procedures

This study used four weed extracts: goat weed, nut grass, siam weed, and cogon grass. Each weed extract was made in a solution with a concentration of 30%. Extract material for goat weed and siam weed were made from fresh leaves, while nut grass and cogon grass were made from tubers and rhizomes. A total of 150 g of weed organs were put into the blender, 200 mL of water was added and then extracted. Weed extract was fed into an Erlenmeyer tube for a centrifuge for 10 minutes at a speed of 500 rpm. The resulting weed extract was poured into a measuring cup and added water up to a volume of 500 mL. After that, the weed extract was filtered with filter paper. The liquid escaped to the sieve's bottom and was used as a bioherbicide. Next, the extracted liquid was fermented for seven days. This method was carried out on all four types of weeds.

Latosol soil was taken from the top-soil layer at 0-20 cm depth. The soil was dredged, then crushed with a hoe to a uniform grain, and filtered with a soil sieve of 2 cm × 2 cm. Furthermore, as many as 81 polybags measuring 40 cm × 35 cm, each filled with 10 kg of soil. The weight of the soil was measured by manual scales of a capacity of 30 kg. Finally, polybags were placed on the research table inside the greenhouse building.

The Padjajaran Agritan variety was used in this study. The nursery was carried out on wooden bath germination in the size of 50 cm × 80 cm. The seedling medium was a mixture of soil and manure in a ratio of 1:1. Seeds were stocked over the soil medium in wooden bath germination. Four days after stocking (DAS), the seeds were already germinated. Then, seedlings were planted in polybags at 18 DAS.

Randomization was carried out at once on all replications. Furthermore, according to the randomization results, the treatment label (mica plastic) with bamboo sticks was plugged into the planting media in polybags. Then, weed extract was applied to soil media based on the treatment code listed on the label.

One rice seedling was planted in the middle of the soil surface in polybag. The planting spacing between the middle points of the soil surface in the polybag was 25 cm × 25 cm. The seedlings were planted at a depth of 2 cm. In this experiment, 81 seedlings were needed. Water was given to polybags every two days at the height of 2 cm from the soil surface at the age of 1-50 days after planting (DAP). However, after this, watering was carried out every afternoon because evapotranspiration was high.

The recommended fertilizer dose was 225 kg/ha urea and 225 kg/ha NPK Phonska 15-15-15. Fertilizer application was carried out in two stages, 40% of the recommended dose, and was given at 14 DAP. The second stage was applying as much as 60% of the recommended dose and was given at 35 DAP.

Weeds were not controlled because they were used as objects of study. Pest control of grasshoppers was carried out when flowering plants using Dursban 200 EC pesticide. Control was carried out twice during flowering. Rice harvesting was carried out at the age of 105 DAP when the grains had physiologically ripe (95% turn yellow).

Observation Variables

Individual weed numbers per polybag and weed dry weight (g/polybag) were observed at 35 DAP. In addition, the tiller number (stem/clump) was measured at 80 DAP, while shoot dry weight (g/clump), panicle number per clump, and grain dry weight (g/clump) were observed at 105 DAP.

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) at P=0.05 level of probability with IBM SPSS Statistic 23. The treatment means were compared using Duncan's new multiple range test (DMRT) at P=0.05 level of probability.

RESULTS AND DISCUSSION

Weed Growth

In this study, there was no difference between the number of weed species. Based on summed dominance ratio (SDR), one of the dominant weed species was *Echinochloa crus-galli* (L.) Beauv. Several weed species were also found but not dominant, namely *Cyperus esculentus* (L.), *Alternanthera philoxeroides* (Mart.) Griseb, *Lindernia procumbens* (Krock.) Philcox, *Fimbrystilis miliacea* (L.) Vahl, *Ludwigia ortovalvis* (Jacq.) P. H. Raven, and *Phyllanthus urinaria* (L.).

Treatment of weed extract type and application time significantly affected the individual weed number and the weed dry weight/polybag. The DMRT at P=0.05 level of probability on the individual weed number and weed dry weight can be seen in Table 1.

Table 1 shows that the lowest individual weed number occurs in cogon grass extract, increases in goat weed, nut grass, siam weed, and highest in control. The application of weed extract could inhibit the individual weed number. Applying goat and siam weed extract when planting can inhibit weed dry weight, likewise the cogon grass on seven days before planting and when planting. Inhibition of weed dry weight lower occurs nut grass applied seven days before and when planting or siam weed seven days after planting. The highest weed dry weight occurred in control.

Therefore, weed extract was effective for weed control and can be levied as a bioherbicide. The efficacy of weed extract types on weed growth can be seen in Fig. 1.

The mechanism of action of allelopathic compounds from weed extract was more effective in field capacity. Furthermore, weed seeds absorbed allelopathic compounds and inhibited the activity of enzymes degrading the food reserves of seeds. As a result, the activity of enzymes decreased, and the energy produced was so low. Therefore, it caused the germination potential of weed seeds to be low.

Nut grass tubers contained phenolic

Table 1. Efficacy of weed extract type and application time on individual weed number and weed dry weight.

Weed extract (application time)	Observation variables	
	Individual weed number/polybag	Weed dry weight (g/polybag)
Control	12.2 a	12.11 a
Goat weed (7 days before planting)	5.2 bc	6.16 de
Goat weed (at planting time)	5.1 bc	4.92 e
Nut grass (7 days before planting)	7.2 bc	8.44 bc
Nut grass (at planting time)	5.3 bc	6.95 cd
Siam weed (7 days before planting)	8.3 b	9.03 b
Siam weed (at planting time)	5.6 bc	4.83 e
Cogon grass (7 days before planting)	4.8 c	5.06 e
Cogon grass (at planting time)	4.6 c	4.69 e

Remarks: The figures in the same column having the same letters are not significantly different based on Duncan's new multiple range test (DMRT) at P=0.05 level of probability.

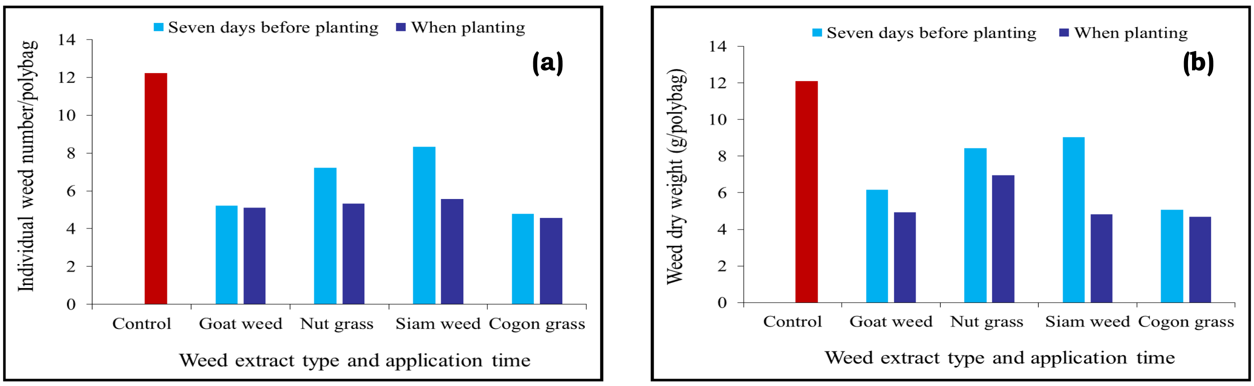

Fig. 1. Efficacy of weed extracts types and application time on the individual weed number/polybag (a) and weed dry weight (b).

compounds. Such compounds can serve as molecules for synthesizing bioherbicides (Ameena *et al.*, 2015). In addition, goat weed contained secondary metabolites such as flavonoids, alkaloids, terpenes, chromates, chromones, benzofurans, coumarins, essential oils, sterols, and tannins (Kamboj and Saluja, 2008), which may inhibit the weed growth. Siam weed leaf extract also controlled weed growth (Muzaiyanah, 2020). In addition, Siam weed leaf extract contains inhibitory compounds such as herbicide activity and can be used as a post-growing herbicide for weed control (Poonpaiboonpipat *et al.*, 2021).

Allelopathic compounds of weeds can be used to control other weeds and are more environmentally friendly (Srikrishnah and Begam, 2019). Extracts of goat weed, nut grass, siam weed, and cogon grass had high potential as bioherbicides as a substitute for the role of synthetic herbicides. The ability level of weed extracts to inhibit weed growth from high to low was cogon grass, goat weed, nut grass, and siam weed.

Growth and Yield of Rice

Treatment of weed extract type and application time significantly affected tiller number, shoot dry weight, panicle number, and grain dry weight/clump. The DMRT at P=0.05 level of probability on tiller number, shoot dry weight, panicle number, and grain dry weight per clump can be seen in Table 2.

Table 2 shows that there tended to be a decrease in the tiller number in the application of goat weed when planting and siam weed at seven days before planting. However, it was not significantly different from the control. There was an increase in the tiller number with the application of cogon grass when planting, but there was no significant difference from the control. The siam weed extract was applied seven days before planting and had a negative effect, while cogon grass positively affected the shoot dry weight when planting. However, it was not significantly different from the control. Siam weed extract applied at seven days before planting could inhibit the growth and yield of rice. However, the application of nut grass extract when planting can inhibit the growth and yield of rice, but it has a positive effect if applied seven days before planting. The efficacy of weed extract types on the growth and yield of rice can be seen in Fig. 2.

Weeds produced secondary metabolite compounds. The mechanism of secondary metabolites of weed was not found in synthetic herbicides. Bioherbicides had more than one secondary metabolite compound and did not cause the poisoning of plants (Sihombing *et al.*, 2018). The application time of weed extract will correlate with the degree of plant intoxication. Applying goat weed and nut grass seven days before planting did not inhibit the growth and yield of rice. But it would inhibit if it was applied when planting. Goat weed extract was applied when planting, or siam weed was applied seven days before planting, which caused the tiller number to be lower. Negi *et al.* (2020) showed that goat weed allelopathic

Table 2. Effect of weed extract type and application time on tiller number, shoot dry weight, panicle number and grain dry weight.

Weed extract (application time)	Observation variables			
	Tiller number (stem/clump)	Shoot dry weight (g/clump)	Panicle number/ clump	Grain dry weight (g/clump)
Control	19.8 abc	46.20 abc	13.2 abc	83.5 bc
Goat weed (7 days before planting)	24.2 abc	52.89 ab	15.1 abc	108.8 ab
Goat weed (at planting time)	18.0 c	41.05 bc	9.1 c	57.2 c
Nut grass (7 days before planting)	23.0 abc	57.02 ab	17.0 a	133.9 a
Nut grass (at planting time)	19.2 abc	45.29 abc	12.2 abc	88.1 bc
Siam weed (7 days before planting)	17.6 c	31.87 c	11.1 bc	80.9 bc
Siam weed (at planting time)	22.7 abc	52.49 abc	12.8 abc	97.7 bc
Cogon grass (7 days before planting)	25.2 ab	49.30 abc	12.2 abc	76.8 bc
Cogon grass (at planting time)	28.8 a	62.32 a	16.2 ab	133.8 a

Remarks: The figures in the same column having the same letters are not significantly different based on Duncan's new multiple range test (DMRT) at P=0.05 level of probability.

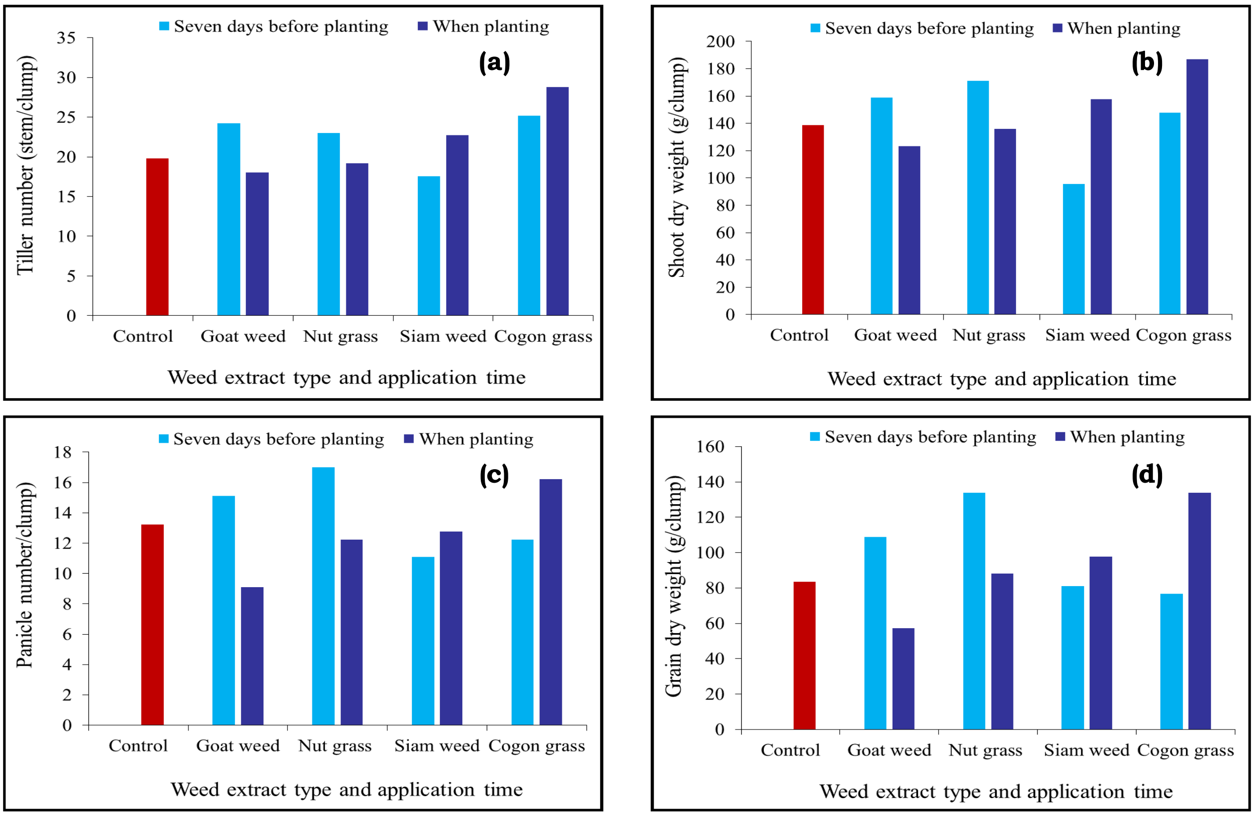

Fig. 2. Efficacy of weed extract types and application time on the tiller number/clump (a), shoot dry weight (b), panicle number/clump (c), and brain dry weight (d).

compounds negatively affected the growth of rice crops by releasing water-soluble phytochemicals. Research results by Kaur and Sharma (2016) indicated that goat weed leaf extract could cause a decrease in chlorophyll content in plants by inhibiting biosynthesis.

According to Ameena *et al.* (2015), nut grass tuber extract contains phenolic compounds that can inhibit weed seed germination by reducing competition with rice crops. In addition, such phenolic compounds can serve as molecules for synthesizing bioherbicides. As a result, cogon grass extract can suppress the weed growth around rice crops so that competition can be avoided. Low competition with weeds caused the shoot dry weight and grain dry weight to be higher than control. In addition, cogon grass extract contained allelopathic compounds in the form of flavonoids and inhibited the growth of weed seeds in the soil.

Weed extract was safe to use as a bioherbicide to control weeds and increase rice yields (Sadiq *et al.*, 2011). The study results showed that the application of the cogon grass extract had a positive effect on increasing the growth and yield of rice. Therefore, roots and rhizomes of cogon grass extract were high potential as bioherbicides. On the contrary, applying goat weed extracts harmed panicle number and grain dry weight when planting.

CONCLUSIONS

Based on research results and discussion, the application of weed extract could inhibit weed growth, including the individual weed number and weed dry weight. In addition, it can also increase tiller number, shoot dry weight, panicle number, and grain dry weight of rice. However, the efficacy of weed extract types was different in the growth and yield of rice. The nut grass and cogon grass were given panicle number and grain dry weight higher than goat weed and siam weed. The right time for weed extract application was seven days before planting for nut grass and when planting for cogon grass to increase the rice yield. Research findings show that Nut grass or cogon grass extracts have efficacy higher than nut grass and siam weed in inhibiting weed growth and increasing rice yield. Therefore, we recommend that pre-emergence weed control in rice cultivation can use nut grass or cogon grass extract.

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