Research article

Allelochemical of *Archidendron jiringa* Pods and *Sorghum bicolor* Biomass Extracts Combined with a Premixed Formulation of Atrazine and Mesotrione

Marulak Simarmata*, Wendy Yosevin Siahaan, Prasetyo Prasetyo and Marlin Marlin

Department of Crop Production, University of Bengkulu Jl. WR Supratman Kandang Limun, Bengkulu 38121, Indonesia

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Abstract

keywords

allelochemicals; herbicide combination; jering pods; sorghum biomass; weed control The aim of this research was to evaluate the allelochemicals in jering pods and sorghum biomass extracts combined with premixed formulations of the herbicides, atrazine and mesotrione. The research was conducted in the laboratory and experimental field of the Department of Agronomy, University of Bengkulu, Indonesia, from December 2019 to July 2020. The allelochemicals in jering pods and sorghum biomass extracts and their combination with the herbicides were tested on seed germination, and weed control was tested on corn grown in polybags. The results showed that aqueous extracts of jering pods and sorghum biomass combined with the herbicide mixtures of atrazine and mesotrione did not inhibit the seed germination of corn but suppressed the length and the weight of the radicles and plumules. The study of the efficacy of jering pods and sorghum biomass extracts on the weeds (total of grasses and broadleaves) revealed that the two agents, when applied separately, suppressed weed biomass by 27 and 32%, respectively. When the two extracts were combined, their combined efficacy increased to 56%. Furthermore, the plant height, stem diameter, and corn stover weight increased with the allelochemicals and herbicide treatments. The cobs yield per plant increased from 19 to 22% with the separate application of the allelochemicals found in the jering pods and sorghum biomass, respectively, and from 27 to 34% when the allelochemicals were combined with the herbicides. This research provided a novel approach using the allelochemical extracts from jering pods and sorghum biomass as natural herbicides for weed control.

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^{*}Corresponding author: E-mail: marulak_simarmata@yahoo.com

1. Introduction

Herbicide use for weed control continues to be a common practice [1]. Using synthetic herbicides for a long time can have a negative impact on the environment, and the emergence of herbicideresistant weeds is an ongoing concern [2, 3]. Therefore, nowadays the agricultural practices are turning to alternative natural control methods such as the use of allelochemical compounds [4, 5]. Exploration of allelochemicals from local promising plants as methods of weed control that are sustainable and safe for the environment is being carried out [6, 7].

Allelopathy is an ecological event where a plant indirectly has a negative or positive influence on other plants around it through the release of chemical compounds [8, 9]. Allelopathy from plants can occur in several ways including evaporation, leaching, the release of exudate through roots, and decomposition of plant residues [10]. The allelochemical compounds of plants can be found in various plant organs: the leaves, stems, roots, rhizomes, flowers, fruits, and seeds [8, 11]. Allelochemicals are not evenly distributed in all plant organs, so their working power depends on the amount of allelochemicals contained in the organs used [12]. Currently, the potential of allelopathic compounds as a natural alternative herbicides for weed control are attracting broad research interest [13]. Previous studies reported that jering fruit pods and sorghum biomass showed a potential as allelochemicals on various plants tested [12].

Archidendron jiringa (Jack) I.C. Nielson, Pithecellobium jiringa (Jack) Prain, Pithecellobium lobatum Benth. or Zygia jiringa (Jack) Kosterm, are the botanical names of jering [14]. Since jering beans are consumed in some countries such as Indonesia, their pods are remaining in an abundant amount and are usually disposed of as waste [15]. It was reported that freshly jering pods applied onto paddy fields functioned not only as soil amendments but also as weed inhibitors. This evidence indicated that jering pods showed potential as an organic herbicides [12]. Some chemical compounds that are contained in the pods can be extracted aqueously for use as allelochemical compounds in agriculture. Sitanggang et al. [16] showed that a concentration of 10% sorghum extract was able to suppress the seed vigor index of mustard and cucumber.

Sorghum is a cereal plant that is cultivated to produce food or feed. Several countries such as the United States, China, and India, also cultivate sorghum as a raw material for the ethanol industry [17]. Sorghum has a strong adaptability, especially in drought-stressed areas because sorghum plants are very efficient in using water and can grow well in dry marginal soil conditions like those found in the coastal areas of Indonesia [18]. Sorghum contains allelochemical compounds that can suppress weed growth, so sorghum biomass is a very promising natural herbicide [19-21]. Sorghum biomass has abundant allelochemicals, such as benzoquinone (sorgoleone), phenolics, and acyanogenic glycoside (dhurrin) [4]. Sorghum plant extracts and residues can suppress weed development due to the presence of allelochemical compounds produced from the secondary metabolite processes. Molecules of cyanogenic glycosides (dhurrin) are toxic to plants because they are able to release hydrogen cyanide (HCN) through a process catalyzed by hydroxynitrile lyase (HNL). Farooq *et al.* [22] showed that the extracts and residues of sorghum biomass were effective in reducing weed biomass weight by up to 23%, and were very effective in increasing maize yields.

A premixed formulation of atrazine and mesotrione is a relatively new herbicide that has been marketed since 2001 [23, 24]. The ratio of the mixture is (500:50) per liter. It is recommended as an early post-emergence herbicide that is highly effective for controlling some broadleaf weeds, grasses, and sedges [25]. This formulation is widely used as a selective herbicide for maize, wheat, sorghum, and sugarcane, and it efectively controls atrazine resistent weeds [26]. Atrazine is one of the herbicides in the triazine group which is effective in controlling grass and broadleaf weeds in corn [27]. The mechanism of action of atrazine is to inhibit electron transport in photosystem II [28]. Mesotrione is a new organic herbicide in the triketone group [29]. The mechanism of action of mesotrione is to inhibit the enzyme of *p-hydroxy-phenylpyruvate dehydrogenase* (HPPD) which

functions in the biosynthesis of carotenoid pigments that cause symptoms of bleaching in the leaves [30]. The objectives of the study were to investigate the allelochemical potential of aqueous extracts of jering pods and sorghum biomass as a natural herbicide and to study their use in combination with a premixed formulation of atrazine and mesotrione for corn plants grown in polybags.

2. Materials and Methods

2.1 Research location and experimental design

The research was carried out in the Agronomy Laboratory and experimental field of the Faculty of Agriculture, Bengkulu University, Indonesia, over the period of December 2019 to July 2020. Eight treatments were combination of aqueous extract of jering pods, sorghum biomass and herbicide mixture of atrazine and mesotrione (Table 1), arranged in a completely randomized design (CRD). The laboratory experiments and the polybag experiments consisted of 5 and 3 replications.

Table 1. Treatment of allelochemical extracts of jering pods and sorghum biomass in combination with the premixed formulation of atrazine and mesotrione

No.	Treatment Combination	Concentration (%)	
		(/0)	
1	Control	0	
2	Extract of jering pods	10	
3	Extract of sorgum biomass	10	
4	Extract of jering pods+Extract of sorgum biomass	10+10	
5	Premixed formulation of atrazine and mesotrione (Calaris)	0.5	
6	Calaris+Extract of jering pods	0.5 + 10	
7	Calaris+Extract of sorgum biomass	0.5 + 10	
8	Calaris+Extract of jering pods+Extract of sorgum biomass	0.5+10+10	

2.2 Allelochemical preparations

The experiment began with the preparation of aqueous allelochemical extracts of jering pods and sorghum biomass following the protocol of Sitanggang *et al.* [16]. The pods of fresh jering fruit were cleaned and chopped to the sizes of 1 cm, and then blended with sterile water at a ratio of 1:1 (w/v). The blended materials were squeezed into a beaker and shaken for 24 h, and then filtered using Whatman #1 filter paper. The filtrate was then considered to be the 100% stock solution for the jering pod alleochemicals, and was stored at 5°C. Similar protocols modified from Murimwa *et al.* [21] were carried out to extract the allelochemicals from the sorghum. The biomass used was sorghum plant stems and leaves that had been chopped and dried in the sun to a constant weight. The dried biomass was blended with sterile water at a ratio of 1:1 (w/v). The next processes were squeezing, shaking, and filtering. The filtered extract was then taken to be the sorghum biomass extract allelochemical stock solution of 100%, and was stored at 5°C. A herbicide combined with allelochemicals is Calaris, a premixed formulation of atrazine and mesotrione. The final solutions of the treatments were prepared according to the concentrations shown in Table 1.

2.3 Bioassay on seed germination

The efficacy of the allelochemical extracts combined with the herbicide Calaris was tested on the germination of corn seeds. The procedures were followed from Murimwa *et al.* [21]. The experiment was carried out in the germination room. Five corn seeds were planted in a 10 cm diameter petri dish, which was replicated 5 times. The planting medium used was straw paper soaked with 5-ml of treatment solution (according to the concentration of the treatment) while the control was soaked with the same volume of water (Table 1). The petri dishes were closed and placed in the germination chamber at room temperature. Maintenance of germination was done by adding sufficient treatment solution every day to maintain the humidity of the germination media. Sprouts were observed at 2 weeks after planting (WAP). The variables observed included percentage of germination, length of radicle and plumule, dry weight of radicle and plumule.

2.4 Study on the effect of allelochemicals on corn and weeds

The efficacy of allelochemical extracts combined with Calaris herbicide was tested on corn planted in polybags. The procedures were modified from Cheema et al. [19] and Faroog et al. [22]. Ten kg of planting media mixed with ultisol soil and manure (1:1) were filled into each polybag. As many as 3 corn seeds were planted per polybag at a depth of 2 cm. The polybags were arranged with a completely randomized design (CRD) by placing in an open area with a distance of 0.5 m x 1.0 m with sunlight. The maintenance carried out included watering, thinning, fertilizing, and pest and disease control. The plants were watered every day (morning) when there was no rain. Thinning was done one week after planting. The plants were fertilized with N, P₂O₅, and K₂O fertilizers at doses of 200, 150, and 150 kg/ha, respectively. Fertilizers were spreading evenly on the surface of the planting medium. Pest and disease control were carried out if there were real symptoms using insecticides and fungicides. Weeds were not controlled in any other way than by the treatment being tested. The application of Calaris herbicide and allelochemical extract at the concentrations described in Table 1 was carried out at 2 WAP following James et al. [24]. The treatment solutions were sprayed directly onto the soil surface using a hand sprayer with a spray volume of 400 L/ha. Harvesting was carried out at 14 WAP or when the cobs had met the harvest criteria. Data observed included plant height, number of leaves, stem diameter, dry weight of the corn biomass, length of cob, diameter of cob, and weight of cob. The weeds were harvested by cutting them at the soil surface and separating them by group into grasses and broadleaves weeds. The weeds were dried in an oven at 72°C for 3 x 24 h. The dry weight of weeds was measured after reaching constant weight.

2.5 Data analysis

Statistical analysis was performed by analysis of variance (ANOVA) at the 5% level. Means of significant data were separated by Duncan's Multiple Range Test (DMRT) at 5%.

3. Results and Discussion

3.1 Effect of allelochemical extracts on seed germination

The results showed that corn seed reached 100% of germination. However, there was discoloration of the sprouts from the treatment of premixed atrazine and mesotrione herbicides and their mixtures with allelochemical extracts (Figure 1). The symptoms of discoloration or bleaching were caused



Figure 1. Germination seeds on control (1); treated with allelochemicals of jering pod extract (2); treated with sorghum biomass extract (3); treated with jering pod + sorgum biomass extracts (4); treated with Calaris herbicide (5); treated with Calaris + extract of jering pods (6); treated with Calaris + extract of jering pods + extract of sorghum biomass (7); treated with Calaris + extract of jering pods + extract of sorghum biomass (8)

by the mechanism of action of mesotrione, which inhibits the formation of the carotenoid enzymes that function in photosynthesis. According to Chahal *et al.* [25] and Székács [27], abnormality of sprout color also occurs during early growth in the field, but sprout color becomes normal after 4 weeks of herbicide application.

Analysis of variance of germination parameters showed that the treatments tested had a significant effect on the length and weight of the radicles and plumules. Table 2 shows that the longest radicle was found on the control (without allelochemical and without herbicide) at 16.80 cm. Radicle length decreased significantly with the treatments of allelochemical extracts of jering pods and sorghum biomass, and the shortest radicles were seen in the herbicide and herbicide mixtures with allelochemical treatments. A similar indication was observed on the plumules where the longest one was seen on the control and the plumules significantly decreased in all treatments. The radicle and plumule weights also decreased due to the treatment with allelochemical extracts and the combination of allelochemicals with herbicides. Germination is the initial phase of plant growth and is very sensitive to external chemical compounds such as allelochemical compounds and herbicides [21]. The initial process of germination is imbibition of water which degrades the endosperm to become a source of energy, so plumules and radicles are very susceptible to chemical compounds in the medium [16].

According to Nurjanah *et al.* [12], jering pods contain chemical compounds like alkaloids, steroids, triterpenoids, saponins, flavonoids, and tannins. Sorghum biomass extract contains allelochemical compounds such as phenolics, quinones (sorgoleone), and dhurrin which are toxic to several weed species [4]. All of these allelochemicals, as well as the herbicides of atrazine and mesotrione, can inhibit the germination process of plant seeds. Some processes that may occur after water absorption into the seeds are cell division, respiration, and the activity of various germination enzymes such as amylases, proteases, and lipases which function to remodel the food reserves of the seed endosperm [30]. Efficacy or growth retardation of sprouts at the radicle is significant because allelochemical compounds and toxic herbicides come into direct contact with the radicle. The retardation efficacy of allelochemicals of jering peel extracts has also been reported to suppress germination of weeds in rice plants [12]. An aqueous extract of sorghum biomass significantly inhibited the germination of mustard and cucumber seeds, reduced the vigor-index of the germination of rice, mustard, and cucumber seeds, and suppressed the growth of the radicle length of mustard sprouts [16].

Table 2. The effects of allelochemical extracts of jering pods and sorghum biomass combined with Calaris on seed germination variables of corn

Treatment Combination	Percent of Seed Germination (%)	Length of Radicle (cm)	Length of Plumule (cm)	Weight of Radicle (g)	Weight of Plumule (g)
Control	100	16.80a	14.58ª	2.08ª	2.10 ^a
Extract of jering pods	100	15.08^{b}	12.56 ^b	1.53 ^b	1.79 ^b
Extract of sorgum biomass	100	12.55°	12.16 ^b	1.35^{b}	1.96ª
Extract of jering pods+Extract of sorgum biomass	100	12.82°	10.51°	1.44 ^b	1.33°
Calaris herbicide	100	8.88^{d}	11.22 ^{bc}	1.35^{b}	1.41°
Calaris+Extract of jering pods	100	9.83^{d}	11.11°	1.42^{b}	1.73 ^b
Calaris+Extract of sorgum biomass	100	9.02 ^d	10.42°	1.56 ^b	1.67 ^b
Calaris+Extract of jering pods+Extract of sorgum biomass	100	9.96 ^d	10.88°	1.44 ^b	1.56 ^{bc}
ANOVA (5%)	ns	*	*	*	*

Notes: Means of data in a column were separated by DMRT at 5%; ns = non-significant effect, *= significant effect of the treatment.

3.2 Efficacy of allelochemical extracts on weeds

The efficacy allelochemical of jering pods and sorghum biomass combined with Calaris herbicide on weeds of corn grown in polybags was conducted after harvesting corn. Weeds were harvested and separated into broad leaves, grasses, and sedges. Analysis of variance was carried out after data transformation because the data obtained did not show statistical normality. The premixed formulation of atrazine and mesotrione inhibited the emergence of weeds completely (100% efficacy), while the allelochemical extracts showed partial inhibition of weed emergence. Overall, the sedges weeds were not found in the plant polybags (Table 3). The allelochemicals from jering pod extract, sorghum biomass extract, and the mixtures of both showed efficacies on grass weeds observed as the suppression of weeds biomasses of 26, 34, and 65%, respectively, and efficacies against broadleaf weeds of 28, 25 and 18%, respectively. The data indicated that allelochemical compounds were more effective at inhibiting the broadleaf weeds [16]. Previous study reported that allelochemicals in jering pod extracts suppressed the dry weight of narrow-leaf weeds to a higher degree than the broad-leaf weeds, the results being 74.4 and 37.5%, respectively [12]. Data in Table 3 showed that the efficacies on total weeds (average of the efficacy of grasses and broadleaf weeds) were 27, 32, and 56%, respectively. These data suggest an additive interaction of the mixture of jering pods and sorghum biomass extracts on grass weeds [28].

The application of a premixed formulation of atrazine with mesotrione was able to prevent the emergence of weeds. The addition of allelochemical compounds did not raise the level of efficacy; or the addition of allelochemical compounds did not increase the herbicide efficacy anymore as it had already reached 100% efficacy. This was because the dose of herbicide mixture applied at the early post-emergence was sufficient to inhibit the emergence of weeds, and the level

Table 3. Allelochemical efficacy of jering pods and sorghum biomass extracts combined with Calaris herbicide on weeds of corn grown in polybags

	Grass Weeds		Broad-leaves weeds		Total Weeds	
Treatment Combination	Dry Weight (g/m²)	Efficacy (%)	Dry Weight (g/m²)	Efficacy (%)	Dry Weight (g/m²)	Efficacy (%)
Control	75.2ª	-	16.6ª	-	91.8ª	-
Extract of jering pods	55.4^{b}	26	12.0^{b}	28	67.4 ^b	27
Extract of sorgum biomass	49.8^{b}	34	12.5 ^b	25	62.3 ^b	32
Extract of jering pods+Extract of sorgum biomass	26.4°	65	13.6 ^b	18	40.0°	56
Calaris herbicide	$0.0^{\rm d}$	100	$0.0^{\rm c}$	100	0.0^{d}	100
Calaris+Extract of jering pods	$0.0^{\rm d}$	100	$0.0^{\rm c}$	100	$0.0^{\rm d}$	100
Calaris+Extract of sorgum biomass	0.0^{d}	100	$0.0^{\rm c}$	100	$0.0^{\rm d}$	100
Calaris+Extract of jering pods+Extract of sorgum biomass	0.0^{d}	100	0.0°	100	0.0^{d}	100
ANOVA 5%	*	-	*	-	*	-

Notes: Means of data in a column were separated by DMRT 5 %; *=significant effect of the treatment; Efficacy was calculated from the decrease of weed dry weight of a treatment compared to control.

persisted for one season [24, 31]. To evaluate the mixing effects, it is necessary to test the combination of herbicide at a lower dose combined with allelochemicals so that the synergistic efficacy of the mixed compounds can be observed [25]. On the other hand, the additive effect of mixing allelochemicals of jering pods with sorghum biomass extracts was more significant in grass weeds compared with the broadleaf weeds. This result was different from the previous research which stated that the allelochemical efficacy of jering pods on broadleaf weeds and narrow leaf weeds was similar [12].

3.3 Growth and yield of corn

The combination of herbicide mixtures with jering pod and sorghum biomass extracts showed a significant effect only on corn stover weight, but not on plant height, number of leaves, and stem diameter (Table 4). However, the plant height and stem diameter showed a tendency to increase in all treatments, while the number of leaves did not respond to the treatments (Table 4). The lowest plant heights and stem diameters (169.16 cm and 2.78 mm) were found in the control (without allelochemicals and herbicide treatment). These then increased in all treatments, and the maximum was observed in the combination treatment of herbicides with allelochemicals of jering pods and sorghum biomass extract, which were 182.52 cm and 3.14 mm, respectively. Corn stover weight experienced an insignificant increase in all treatments, except in the combination of herbicide with the allelochemicals of jering pods and sorghum biomass extracts, where it was 70.2 gram per plant. The weight of this stover was significantly different from all other treatments and controls. The results for corn stover were in line with the increases in plant height and stem diameter.

Table 4. The effect of allelochemical extracts of jering pods and sorghum biomass combined with Calaris herbicide on the growth of corn grown in polybags

Treatment	Plant Height (cm)	Number of Leaves (sheets)	Stem Diameter(cm)	Corn Stover Weight (g/plant)
Control	169.16	11.6	2.78	54.8 ^b
Extract of jering pods	184.38	11.4	2.74	56.4 ^b
Extract of sorgum biomass	178.98	11.0	2.77	57.8 ^b
Extract of jering pods+Extract of sorgum biomass	178.32	11.2	3.01	56.2 ^b
Calaris herbicide	188.14	11.4	2.93	$55.0^{\rm b}$
Calaris+Extract of jering pods	175.34	11.0	2.94	$59.0^{\rm b}$
Calaris+Extract of sorgum biomass	180.42	11.2	3.04	60.8 ^b
Calaris+Extract of jering pods+Extract of sorgum biomass	181.52	12.2	3.14	70.2ª
ANOVA 5%	ns	ns	ns	*

Notes: Means of data in a column were separated by DMRT at 5%; ns=non-significant effect, *=significant effect of the treatment.

The yield and yield components were not significantly affected by the treatments applied, but descriptively there were increases in cob length, diameter of cob, and weight of cob (Table 5). The three variables were observed to be the lowest in the control at 20.42 cm, 41.87 mm, and 175 grams per plant, respectively, while for the allelopathic treatments, the yield and yield components increased and the maximum values were obtained for a combination of herbicides with allelochemicals from jering pods and sorghum biomass extracts, and were 25.12 cm, 44.16 mm, and 234.6 grams per plant, respectively. Moreover, it appears that corn yield was observed to have increased by 19, 22, and 22%, respectively, as the cob weight per plant increased in all treatments starting with jering pod extract, and sorghum biomass extract and the mixture of jering pods and sorghum biomass extracts. The increase in yield with herbicide treatment was 27%, while with a mixture of herbicides and allelochemical compounds, the yield increased to 33-34% (Table 5). The increase in yields indicates that there was a synergy when the allelochemical compounds were mixed with premixed formulation herbicides [28].

The increase in growth and yield is an indicator of better metabolic processes in plants. Plant growth as observed as increase in corn stover weight, and corn yield in terms of increase in cob weight per plant was caused by the effectiveness of weed control on the planting environment. The herbicide mixture of atrazine and mesotrione was able to prevent the emergence of weeds while the allelochemical treatments were able to control the emergence and growth of weeds partially [7]. Weeds are strong competitors for crops, occupying the growing space, and competing for nutrients, CO₂, water, and sunlight [8]. Inhibition of the early emergence and suppression of weed growth that decreases the weed weight of grasses and broad leaves can be seen in Table 3. As a consequence, the plants did not experience significant disturbances from weeds, or there was minimal competition [26, 28]. Crop growth process that proceeds at maximum metabolism can produce optimal organic carbon levels which accumulate vegetatively and increase crop yields.

Table 5. The effect of allelochemical extracts of jering pods and sorghum biomass combined with Calaris herbicide on the yield variables of corn grown in polybags

Treatment	Cob Length (cm)	Cob Diameter (mm)	Cob Weight (g/plant)	Yield Increased (%)
Control	20.42	41.87	175.0	-
Extract of jering pods	23.38	43.45	208.8	19
Extract of sorgum biomass	23.26	46.52	213.8	22
Extract of jering pods+Extract of sorgum biomass	22.38	43.91	214.0	22
Calaris herbicide	23.46	44.61	222.8	27
Calaris+Extract of jering pods	22.22	43.46	235.0	34
Calaris+Extract of sorgum biomass	23.60	45.31	234.2	33
Calaris+Extract of jering pods+Extract of sorgum biomass	25.12	44.16	234.6	34
ANOVA 5%	ns	ns	ns	-

Notes: Yield increase was calculated from the increase in cob weight of a treatment compared to control; ns=non-significant effect of the treatment.

4. Conclusions

Aqueous extracts of the allelochemicals from jering pods and sorghum biomass, as well as those extracts in combination with a herbicide mixture of atrazine and mesotrione, did not inhibit corn seed germination but suppressed the growth of radicles and plumules. Water extract of the jering pods and sorghum biomass, and a mixture of both, were able to suppress grass more significantly than broad leaf weeds. The efficacy of allelochemicals from jering pods, sorghum biomass, and their mixture against weeds reached 27, 32, and 56%, respectively. Mixing the allelochemicals of jering pods and sorghum biomass showed an additional level of efficacy. Premixed formulations of atrazine and mesotrione and their combination with allelochemicals controlled weeds, while allelochemical compounds had only partial control. The effect of partial weed control and total weed control improved the yield of cob weight by 19-22% and 27-34%, respectively.

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