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Allelopathic effects of tamarind husk, lemongrass and citronella residues to suppress emergence and early growth of some weeds

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Abstract

The allelopathic effects were evaluated of water extracts from tamarind (*Tamarindus indica* L.) husk and from lemongrass (*Cymbopogon citratus* Stapf.) or citronella (*Cymbopogon nardus* Rendle.) leaves on 2 test plants: ruzi grass (*Brachiaria ruziziensis* Germ. & C.M. Evrard) and popping pod (*Ruellia tuberosa* L.). In the laboratory, the seeds were subjected to the different extracts at concentrations of 0, 1.25, 2.5, 5 and 10 % (w/v). The number of germinated seeds was counted daily for 6 days. The shoot/root length was measured 5 days after sowing (DAS). The results showed that the aqueous extract of tamarind husk did not inhibit the germination of either ruzi grass or popping pod. The lemongrass and citronella extracts had higher inhibition effects at high concentrations and clearly delayed seed germination at 10 % (w/v). With the seedling growth bioassays of the 3 extracts, only the citronella extract at the highest concentration had any effect on the shoot/root lengths of ruzi grass. Popping pod was susceptible to all 3 extracts, especially the popping pod roots treated with lemongrass and citronella extracts. The lemongrass and citronella extracts caused cell death and induced lipid peroxidation in both plants. Therefore, the primary action of these extracts on seedling growth inhibition might have been related to the loss of cell viability and triggering lipid peroxidation in affected tissues. The residue incorporation bioassay determined the effects of residue mixed in soil at concentrations of 0, 2.5, 5 and 10 % (w/w) on the growth of ruzi grass and popping pod at 30 DAS. Both plants showed an adverse effect than for the control, based on reduced leaf numbers and greater chlorosis, especially with soil incorporation of the lemongrass and citronella residues at 10 % (w/w). Mixing soil with lemongrass and citronella leaves at 2.5 % (w/w) and above resulted in reductions in the shoot/root lengths and shoot/root biomass values. Catechin, gentisic acid, syringic acids, *p*-coumaric acid and ferulic acid were the dominant allelochemicals found in the lemongrass and citronella residues.

Keywords: allelopathy; bioherbicide; *Cymbopogon*; phenolic compounds; *Tamarindus.*
Abbreviations: GSI_germination speed index, DAS_days after sowing, DW_dry weight, MDA_malondialdehyde, ROS_reactive oxygen species, TH_tamarind husk, LG_lemongrass, CN_citronella

Introduction

Weeds are one of the most important problems influencing the reduction of crop yield and causing economic losses (Abouziena and Haggag, 2016). Synthetic herbicides have been widely used in crop protection systems against weeds; in their review, Soltys et al. (2013) noted that inappropriate application of herbicides, such as overuse in the quantity and application at the incorrect developmental stage, have led to the accumulation of active compounds in the environment. They reported adverse effects on human health and an increase in resistant weed species, stating that 211 species and 393 biotypes of resistant weed species had been identified at that time. Allelopathy—a natural, ecological phenomenon where secondary metabolites produced by microorganisms and plants influence

Table 1. pH and total phenolic content of different aqueous extracts at concentration of 10% (w/v).

Residue extract (10% (w/v))	pH	Total phenolic content (mg gallic equivalent/g DW)
Tamarind husk	3.37 ± 0.03	2.89 ± 0.20
Lemongrass leaf	5.70 ± 0.02	8.10 ± 0.10
Citronella leaf	5.02 ± 0.04	6.27 ± 0.06

Note: Values represent mean ± standard deviation of three replications.

biological and agricultural systems—has become an alternative way for managing weeds (Weston and Duke, 2003; Farooq et al., 2011). Weed management using allelopathic species can be applied using different methods, such as a cover crop, mulching and allelopathic water extracts (Farooq et al., 2011). Water-soluble secondary metabolites or allelochemicals have been studied by several researchers based on weed suppression in the laboratory greenhouse and under field conditions (Jamil et al., 2009; Javaid et al., 2010; Khaliq et al., 2013; Safdar et al., 2016).

Numerous chemical groups have been isolated from over 30 families of plants as allelochemicals, including water-soluble organic acids, unsaturated lactones, long-chain fatty acids, phenolics, cinnamic acid, coumarins, flavonoids, tannins, steroids and terpenoids. Among these, phenolic compounds are the most important and are common in plant decomposition products and have produced adverse effects on plant metabolic processes (Putnam, 1988; Li et al., 2010). Allelopathic effects of tamarind (*Tamarindus indica* L.) residues were reported from the leaves, bark and seeds (Parvez et al., 2004; Syed et al., 2014). The addition of tamarind husk can control rice weeds, especially nut sedge, which was reduced by 61 % (Kathiresan, 2012). The essential oil of lemongrass (*Cymbopogon citratus*) had phytotoxic effects on barnyardgrass (*Echinochloa crus-galli*) (Poonpaiboonpipat et al., 2013). Fujii et al. (2004) revealed that the leaf material of lemongrass showed the strongest inhibitory activity determined using the sandwich method. The aqueous methanol extracts from the leaves, stalks and roots of citronella (*Cymbopogon nardus*), another species in the same genus, had highly inhibitory activity against some plants (Suwitchayanon and Kato-Noguchi, 2014). Tamarind husk, lemongrass and citronella leaves are all agricultural wastes that could be made useful as a source of allelochemicals.

The current experiment focused on testing the allelopathic effects of various water extracts on the germination and growth of ruzi grass (*Brachiaria ruziziensis*) and popping pod (*Ruellia tuberosa*), which are representative of monocotyledonous and dicotyledonous plants, respectively. In addition, the effects of soil incorporation with plant residues on the germination and growth of weeds were evaluated to provide a guideline for further weed control methods. The causative allelopathic compounds from the three plant residues were identified.

Results

Characterization of aqueous extracts

The aqueous extract of tamarind husk had a relatively low pH and low total phenolic content, while the aqueous extracts of lemongrass and citronella had similar pH values (5.02–5.70). The highest content of phenolic compounds was in the lemongrass extract (about 8.10 mg gallic acid equivalent/ g DW), as shown in Table 1.

Germination and seedling growth bioassay in laboratory

The aqueous extract of tamarind husk had no effect on the germination percentage of either ruzi grass or popping pod. The germination percentages of both test plants were progressively inhibited with increasing concentrations of lemongrass and citronella aqueous extracts; however, the lemongrass extract seemed to be the most effective residue as it caused the greatest reduction in germination percentage (Table 2). All three extracts delayed seed germination by decreasing the GSI evaluated at 6 DAS. The lemongrass extract tended to inhibit the germination of ruzi grass more than for popping pod, with the 10% concentration strongly inhibiting the percentage of germination and delaying seed germination (Table 2).

The effects of the three extracts on seedling growth are shown in Fig 1. The citronella extract at the highest concentration (10%) was the only extract that inhibited the growth of ruzi grass, though at low concentrations, it promoted the growth of ruzi grass. In contrast, all three extracts inhibited the root growth of popping pod, especially the extracts of lemongrass and citronella at concentrations of 1.25% and above. None of the extracts affected shoot growth inhibition, whereas shoot length was promoted at low concentrations.

Effect of root cell death and lipid peroxidation

The aqueous extract of lemongrass at 10 % (w/v) significantly increased the root cell death of ruzi grass and popping pod compared to the control, based on the increase in Evan's blue uptake by 48.72 and

Table 2. Germination percentage at 6 DAS (%Germination$_{6DAS}$) and germination speed index (GSI) of ruzi grass and popping pod seeds after exposure to different aqueous extracts.

Residue extract	Concentration (%)	Ruzi grass		Popping pod	
		%Germination$_{6DAS}$	GSI	%Germination$_{6DAS}$	GSI
Tamarind husk	0	88 ^{ns}	9.53 a	74 ^{ns}	8.22 ^{ns}
	1.25	84	8.18 a	78	8.93
	2.5	80	7.72 a	72	7.81
	5	68	5.08 b	66	7.43
	10	70	5.00 b	58	5.60
Lemongrass	0	94 a	8.49 a	82 a	9.43 a
	1.25	82 ab	4.48 b	74 a	8.40 a
	2.5	70 b	4.16 b	74 a	7.90 a
	5	40 c	2.02 c	66 a	6.00 b
	10	12 d	0.40 c	14 b	0.50 c
Citronella	0	96 a	8.09 a	94 a	8.49 a
	1.25	86 a	7.95 a	90 a	6.83 b
	2.5	82 a	7.29 a	94 a	4.63 c
	5	82 a	7.07 a	86 a	2.38 d
	10	56 b	3.49 b	52 b	1.24 d

Note: Data are mean values of five replications. Identical lowercase letters in each column of each group are not significantly ($p \leq 0.05$) different based on Duncan's multiple range test. ^{ns} is not significantly different.

241.67%, respectively. Extracts from the tamarind husk and citronella leaves did not cause cell death of ruzi grass root tips (Fig 2).

None of the extracts increased malondialdehyde (MDA) in the ruzi grass shoots but did increase MDA in the roots from the control (Fig 3). The extract of tamarind husk did not increase MDA in the shoots and roots of popping pod. The lemongrass and citronella leaf extracts at 10% (w/v) had a greater effect on roots than shoots by increasing MDA in the roots of popping pod by 1.66 and 1.35 times, respectively, compared to the control (Fig 4).

Effect of residues mixed in soil (pot trial)

Incorporation of the three plant residues had no effect on germination (data not shown). The tamarind husk residue at 15 g per 150 g soil (10% w/w) had no effect on the shoot and root lengths of ruzi grass nor on the root length of popping pod (Fig 6A, B and F). In contrast, it inhibited the shoot length of popping pod and caused a reduction in the shoot and root biomass of the two test plants (Fig 6E, C, D, G and H). Soil mixed with lemongrass and citronella residues significantly reduced the shoot and root lengths and biomass in both ruzi grass and popping pod (Fig 6). At high concentration (10% w/w), the lemongrass and citronella residues had similar inhibitory effects on the test plants, causing approximately 67.37% and 39.42% reductions in the shoot and root lengths of ruzi grass, respectively, and 62.20% and 42.98% reductions in the shoot and root lengths of popping pod, respectively. They also caused approximately 82.15% and 82.99% reductions in the shoot and root dry weights of ruzi grass, respectively, and 71.33% and 60.37% reductions in the shoot and root dry weights of popping pod, respectively. In addition, incorporation with lemongrass and citronella residues at 10% w/w caused abnormal characteristics by slowing the growth and inducing short, yellow leaves for the ruzi grass. The leaves of popping pod grew very slowly and were yellow, especially when receiving lemongrass residue (Fig 5).

Quantification of allelopathic compounds based on HPLC

After extraction and acid hydrolysis, the contents of phenolic compounds were determined using HPLC. Typical HPLC chromatograms of tamarind husk (TH), lemongrass leaf (LG) and citronella leaf (CN) are presented in Fig S1. The amounts of phenolic compounds detected in the samples are presented in Table 3. The tamarind husk residue had a slightly lower phenolic acid content, while (+)-catechin and quercetin were the main flavonoids. The most abundant phenolic acids in the lemongrass and citronella residues were gentisic acid, syringic acid, *p*-coumaric acid and ferulic acid, while (+)-catechin was the main flavonoid identified in citronella.

Discussion

In the laboratory bioassays, the aqueous extracts of lemongrass and citronella seemed to have higher inhibitory effects on the germination and seedling

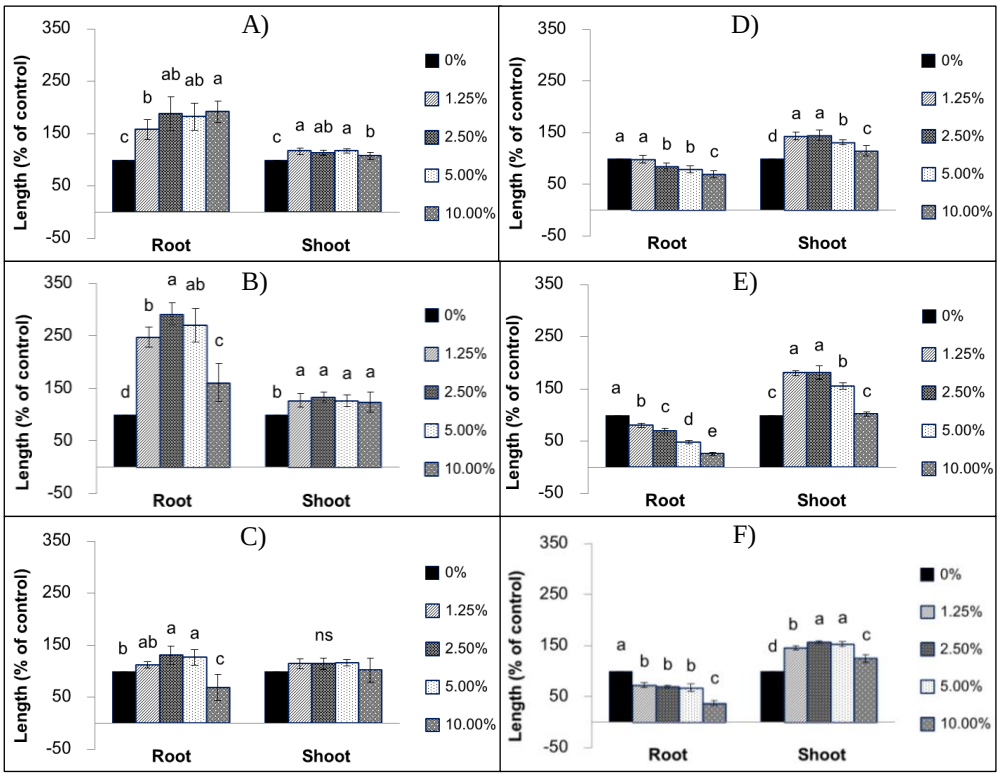

Fig 1. Root and shoot length as percentage of control for ruzi grass (A–C) and popping pod (D–F) after exposure to different aqueous extracts (tamarind husk: A and D; lemongrass leaf: B and E; citronella leaf: C and F) for 5 days. Data are mean values of three replications and standard deviation are indicated by vertical bars. Different lowercase letters above columns indicate significant ($p \leq 0.05$) differences based on Duncan's multiple range test and ns indicates not significant.

growth of test plants than the extract of tamarind husk. These results were related to the characterization of the aqueous extracts, with the total phenolic content being extremely high in the lemongrass and citronella extracts. It can be said that the dissolved phenolic compounds from the aqueous extract of lemongrass and citronella are active substances. Published reports have stated that phenolic compounds are most important and common in plant decomposition products, which showed an adverse effect on plant metabolic processes (Putnam, 1988; Li et al., 2010). This was supported by the study of Javaid et al. (2010) on the effect of aqueous methanol and n-hexane extracts from *Datura metel* against parthenium weed, with only the polar compounds being toxic to the germination and growth of parthenium. Iqbal et al. (2004) identified allelochemicals from the methanolic extract of *Ophiopogon japonicus* as salicylic acid and *p*-hydroxybenzoic acid and the extract inhibited the roots and shoots of lettuce by 50%, even at less than 3 ppm. Some researchers have concluded that high levels of polyphenols in extracts were correlated with the mechanism of plant growth inhibition (Reigosa et al, 1999; Koodkaew et al, 2018). In the current study, all three extracts delayed seed germination by decreasing the GSI. This was evident when the extract concentration was increased, especially for the lemongrass and citronella extracts at a concentration of 10%. This was consistent with the reported efficacy of aqueous lemongrass extract that resulted in decreased germination and germination speed of beggarticks (*Bidens pilosa*) seeds (Krenchinski et al., 2017). Furthermore, Suwitchayanon and Kato-Noguchi (2014) reported that citronella leaf and root extracts using 70% methanol had inhibitory effects on the seedling growth of lettuce, alfalfa, cress, jungle rice, barnyard grass and Italian ryegrass at a concentration of 3 g/100 ml.

Cell death and lipid peroxidation were analyzed to help understand the mechanism of seedling growth inhibition. MDA is used as an indicator for lipid peroxidation, the process by which lipids are destroyed; lipid peroxidation occurs when plants are under oxidative stress, which produces large amounts of reactive oxygen species (ROS) molecules (Halliwell and Gutteridge, 1999; Hodge et al., 1999). The lemongrass extracts significantly increased the MDA content, corresponding to severe root tip cell death. It was possible that the extract induced ROS overproduction; then the excessive ROS would lead to membrane disruption, loss of cell viability and the initiation of cell death. This phenomenon may indicate inhibition in the root growth of the two tested plant species due to enhanced membrane deterioration. The current study correlated with the herbicidal activity of other phytotoxic compounds, with their ability to induce lipid peroxidation leading to root cell

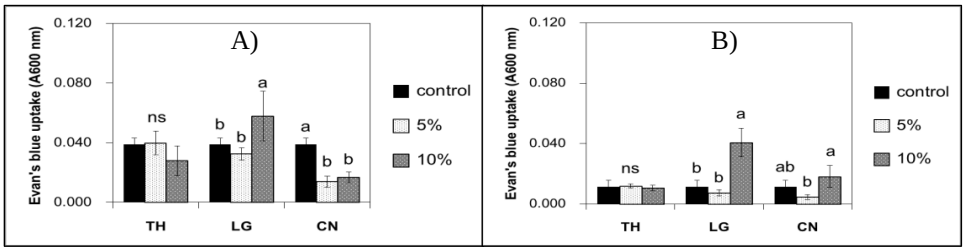

Fig 2. Loss of root tip cell viability of ruzi grass (A) and popping pod (B) after exposure to different aqueous extracts (tamarind husk: TH; lemongrass leaf: LG; citronella leaf: CN) for 5 days expressed as absorbance of Evan's blue uptake. Data are mean values of three replications and standard deviation are indicated by vertical bars. Different lowercase letters above columns indicate significant ($p \leq 0.05$) differences based on Duncan's multiple range test and ns indicates not significant.

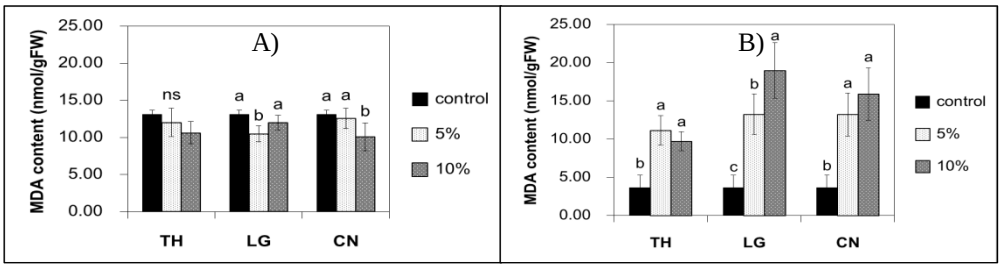

Fig 3. Malondialdehyde (MDA) content in shoot (A) and root (B) of ruzi grass after exposure to different aqueous extracts (tamarind husk: TH; lemongrass leaf: LG; citronella leaf: CN) for 5 days. Data are mean values of three replications and standard deviation are indicated by vertical bars. Different lowercase letters above columns indicate significant ($p \leq 0.05$) differences based on Duncan's multiple range test and ns indicates not significant.

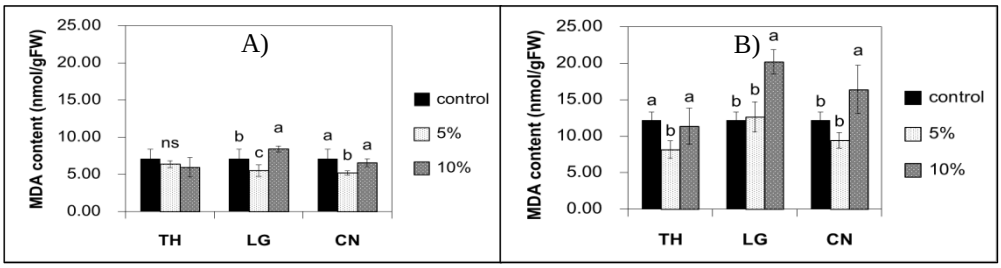

Fig 4. Malondialdehyde (MDA) content in shoot (A) and root (B) of popping pod after exposure to different aqueous extracts (tamarind husk: TH; lemongrass leaf: LG; citronella leaf: CN) for 5 days. Data are mean values of three replications and standard deviation are indicated by vertical bars. Different lowercase letters above columns indicate significant ($p \leq 0.05$) differences based on Duncan's multiple range test and ns indicates not significant.

death. Yan et al. (2015) reported that an isolated compound, artemisinin, could induce ROS overproduction that caused membrane lipid peroxidation and cell death, resulting in growth inhibition of lettuce seedlings. Likewise, the extract from the cyanobacterium *Nostoc* sp. enhanced oxidative stress by ROS production, resulting in lipid peroxidation of cell membranes and cell death that was confirmed by the destruction of root tip cells and aberrant mitochondria and ultimately root growth inhibition in *Mimosa pigra* (Sukkhaeng et al., 2015). The extracts from the citronella leaf and tamarind husk had inhibitory effects of the second and third order, respectively, as they were able to induce lipid peroxidation but did not increase root tip cell death. It was possible that these two extracts were less virulent than the lemongrass extract. Therefore, to clearly see death may require a longer exposure time. In other words, the decrease in root length after exposure to the citronella leaf and tamarind husk extracts may have been due to a combination of effects on other mechanisms, such as cell division or cell elongation.

Soil mixed with the lemongrass and citronella residues significantly reduced shoot length, root length and biomass in both ruzi grass and popping pod, while the tamarind husk residue had a slight effect. The adverse effect of the residues of lemongrass and citronella was supported by Fujii et al. (2004) who reported that leaf materials of lemongrass had the strongest inhibitory activity determined by the sandwich method. Suwitchayanon and Kato-Noguchi (2014) reported that citronella leaf extract had a 100% inhibitory effect on the germination and seedling growth of lettuce. It was possible that the lemongrass and citronella residues, when mixed in the soil, released some allelochemicals, including phenolic compounds, during the decomposition of plant material and this contributed to their inhibitory effects on weeds. In general, leaf litter leachate and the decomposition products of lignin-rich plant residues are the primary sources of

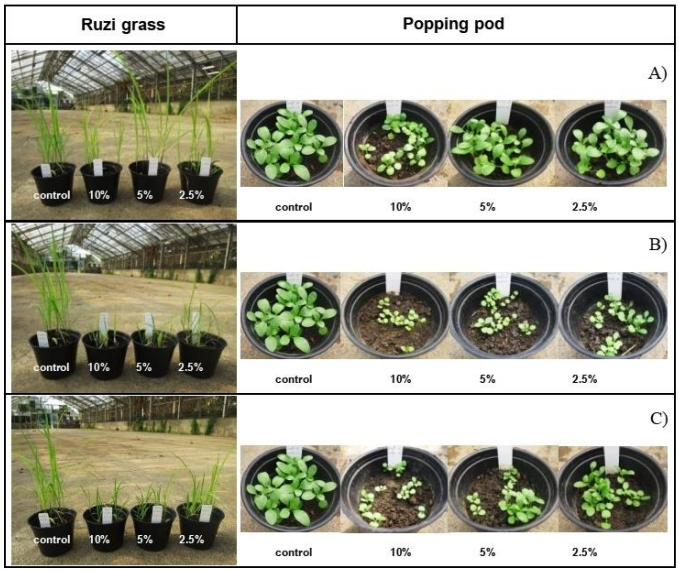

Fig 5. Effect of soil incorporation with tamarind husk (A), lemongrass leaf (B) and citronella leaf (C) at different concentrations on growth of ruzi grass and popping pod observed at 30 DAS.

phenolic acids in the soil (Wilhelm et al., 2021). In the present study, analysis was undertaken of possible phenolic compounds that might be involved in the phytotoxicity of 3 plant residues. Catechin, gentisic acid, syringic acids, *p*-coumaric acid and ferulic acid were the most abundant phenolics found in the lemongrass and citronella residues. The herbicidal potential of the phenolic compounds is recognized because they can lead to increased cell membrane permeability, lipid peroxidation and cell death. In addition, they can inhibit cell division and change the cell ultra-structure, which subsequently interferes with the normal growth and development of the whole plant (Li et al., 2010). Perhaps soil incorporation with the lemongrass and citronella residues could release some phenolic compounds during decomposition and then these allelochemicals interfered with the normal growth and development of ruzi grass and popping pod by the reduction of growth and biomass. These observations were in line with the study of some plant residue incorporation where inhibition in emergence and seedling growth of all test species was associated with an increase in phenolic contents in the soil (Sodaeizadeh et al., 2010; Khaliq et al., 2015). Zohaib et al. (2014) studied soil incorporation with leguminous weeds and showed the presence of phenolic compounds, such as caffeic acid, gallic acid, chlorogenic acid, vanillic acid, 4-hydroxy-3-methoxybenzoic acid, ferulic acid, *p*-coumaric acid, *m*-coumaric acid and syringic acid, which seemed to cause reductions in the shoot and root lengths and seedling dry weight of rice seedlings.

Materials and methods

Plant materials and extraction

Dried tamarind husk was collected from a local farmer in Nakhon Pathom province, Thailand. Dried leaf residues of lemongrass and citronella were purchased from local farmer in Sa Kaeo province, Thailand. These dried residues were ground into powder and passed through a 250 µm sieve. Aqueous extracts were prepared by soaking 10 g of residue powder in 100 ml of distilled water (10% w/v) for 48 h at room temperature and then filtering. The extract of each residue was measured for its pH and total phenolic content (Mohd-Esa et al., 2010).

Germination and seedling growth bioassay in laboratory

The test plants were ruzi grass (*B. ruziziensis*) and popping pod (*R. tuberosa*). Before germination bioassay, the seed dormancy of ruzi grass was broken by soaking the seed in sulfuric acid for 1 min, washing the seed and then immersing it in distilled water for 24 h. The popping pod seeds were immersed in distilled water for 24 h. The aqueous extracts (10% w/v) were diluted to 5, 2.5 and 1.25% w/v. An aliquot of 1.5 ml of various concentrations of the extracts was applied on a double layer of germination paper in a 5 cm diameter Petri dish. The papers were dried in a fume hood and 1.5 ml distilled water was added to each Petri dish. Ten seeds were placed on the germination paper and kept in the dark at 25°C. The number of germinated seeds was evaluated daily for 6 days after sowing (DAS). The germination percentage and germination speed index (GSI) were calculated according to the formulae of Pinheiro et al. (2015).

Seedling growth bioassay was carried out using a similar procedure to the germination bioassay but the seeds were pre-germinated to have root lengths of approximately 5 mm before being subjected to the extract. Root and shoot lengths were measured at 5 DAS. In both the germination and seedling growth

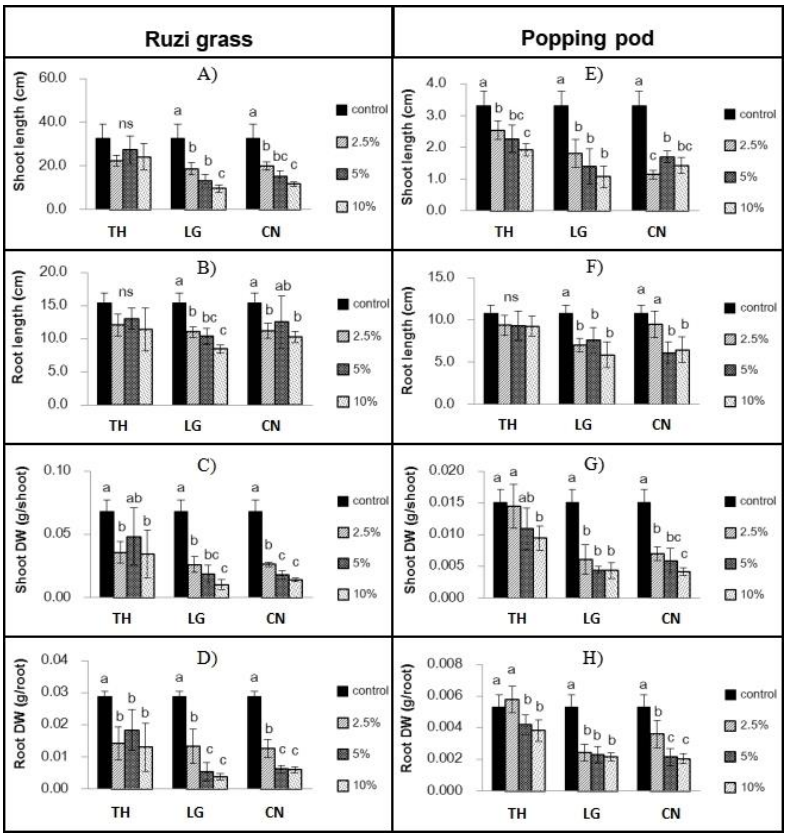

Fig 6. Effect of soil incorporation with tamarind husk (TH), lemongrass leaf (LG) and citronella leaf (CN) at different concentrations on shoot length (A, E), root length (B, F), shoot dry weight (C, G) and root dry weight (D, H) of ruzi grass and popping pod observed at 30 DAS. Data are mean values of three replications and standard deviation are indicated by vertical bars. Different lowercase letters above columns indicate significant ($p \leq 0.05$) differences based on Duncan's multiple range test.

bioassays, the experiment was performed with five replicates. After measuring the root and shoot lengths, the cell death and malondialdehyde content were determined.

Determination of root cell death

Treated roots were separated and stained with Evans blue solution (0.025% (w/v) in 100 µM $CaCl_2$, pH 5.6) for 30 min. Stained roots were washed 3 times with 100 µM $CaCl_2$ (pH 5.6), until there was no further dye elution from the roots (Yamamoto et al., 2001). Ten root tips were excised (3 mm) and allowed to soak in 200 µl of N,N-dimethylformamide for 24 h. The absorbance of released Evans blue was measured at 600 nm.

Determination of lipid peroxidation

The thiobarbituric acid test was used, which determines MDA as an end product of lipid peroxidetion (Velikova et al., 2000). The amount of thiobarbituric acid-reactive substances (red pigments) was calculated from the extinction coefficient 155 $mM^{-1}cm^{-1}$.

Effect of residues mixed in soil (pot trials)

Dried powder residues of tamarind husk and lemongrass and citronella leaves were mixed with 150 g of dried clay soil at 2.5, 5 and 10% w/w. The residue mixed soil was transferred to pots 10 cm in diameter and 8 cm deep. Seeds of ruzi grass and popping pod were sown in each pot (15 seeds per pot). Pots were placed in sunlight and irrigated with tap water daily at field capacity. Plants were observed and harvested at 30 DAS. The shoot/root length and dry biomass of shoots/roots were examined.

Identification and quantification of allelopathic compounds

The residues of tamarind husk and lemongrass and citronella leaves (0.1 g) were each extracted with 16 ml of 80% methanol and 4 ml of 6M HCl and refluxed in a water bath at 90 °C for 1 h. Then, the mixture was kept in a refrigerator for 16 h, filtered and made up to 20 ml with 80% methanol. The extract was filtered through a 0.45 µm membrane filter and investigated using high-performance liquid chromatography (HPLC). The analytical HPLC system consisted of a high-performance liquid chromatograph coupled with a photodiode array detector from Waters Corporation. Separation was achieved on a 5 µm ODS 4.6 × 250 mm column at 35 °C. The mobile phase consisted of water with 0.1% formic acid (solvent A) and methanol (solvent B). The isocratic phase was run at 60:40 (A:B) for 0–42 min.

Table 3. Content of phenolic compounds in examined plant residues.

Peak no.	Compound	Concentration (mg/g)			Rt (min)
		Tamarind husk	Lemongrass leaf	Citronella leaf	
1	Gallic acid	ND	ND	ND	6.881
2	Protocatechuic acid	0.03 ± 0.00	ND	ND	15.139
3	Catechin	10.82 ± 3.11	0.93 ± 0.28	10.40 ± 0.14	19.823
4	*p*-Hydroxybenzoic acid	ND	0.08 ± 0.02	0.14 ± 0.01	21.104
5	Chlorogenic acid	ND	0.38 ± 0.20	0.33 ± 0.01	22.318
6	Gentisic acid	ND	0.83 ± 0.09	0.70 ± 0.03	22.833
7	Vanillic acid	0.06 ± 0.00	ND	ND	23.369
8	Caffeic acid	ND	0.15 ± 0.00	0.24 ± 0.00	23.717
9	Syringic acid	0.01 ± 0.00	0.89 ± 0.15	0.75 ± 0.02	24.527
10	*p*-Coumaric acid	0.01 ± 0.00	0.80 ± 0.04	0.82 ± 0.03	27.756
11	Ferulic acid	0.03 ± 0.00	1.06 ± 0.13	1.29 ± 0.04	28.597
12	Quercetin	0.17 ± 0.03	ND	ND	35.104

Note: Rt = retention time, ND = Not detected. Values represent mean ± standard deviation of three replications.

The flow rate was 1 ml/min and the injection volume was 20 µl. The monitoring wavelength was 280 nm for the phenolic acids and 320 nm for quercetin. Identification of the phenolic compounds in the sample was determined by comparing the retention times of standard peaks.

Experimental design and statistical analysis

A completely randomized design (CRD) was used with five replications for the germination and seedling growth bioassays. In the pot trials, the CRD was used with three replications. Data regarding seed germination, germination speed index, root and shoot length percentage and root and shoot dry weight, Evan's blue uptake and MDA content were subjected to analysis of variance and means were compared using Duncan's multiple range test at $p \leq 0.05$.

Conclusion

The present study demonstrated for the first time that aqueous extracts of lemongrass and citronella had strong allelopathic effects on seed germination, delayed seed germination and inhibited root growth of ruzi grass and popping pod. The mechanism of these extracts on the retardation of seedling growth may have been related to an increase in lipid peroxidation and triggering cell death in root tissues. Incorporation of the lemongrass and citronella residues into soil at 10% (w/w) confirmed their weed-suppressing abilities by the reduction of growth and biomass. Some phenolic compounds identified in the lemongrass and citronella residues seemed to adversely affect weed growth.

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