

Allelopathic Potential of Cassava (*Manihot esculenta* L.) Extracts on Germination and Seedling Growth of Selected Weeds and Aerobic Rice

(Potensi Alelopati Ekstrak Ubi Kayu (*Manihot esculenta* L.) terhadap Percambahan dan Pertumbuhan Anak Benih Rumpai Terpilih dan Padi Aerobik)

SITI AISYAH MOHAMMAD TAUPIK^{1,2,3,4}, SITI NUR ANISAH AANI³, CHIA POH WAI⁵ & CHUAH TSE SENG^{2,*}

¹*Institute of Graduate Studies, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan, Malaysia*

²*Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 02600 Arau, Perlis Indera Kayangan, Malaysia*

³*Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 77300 UiTM Merlimau, Melaka, Malaysia*

⁴*Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu, 21030 UMT Kuala Nerus, Terengganu Darul Iman, Malaysia*

⁵*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 UMT Kuala Nerus, Terengganu Darul Iman, Malaysia*

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ABSTRACT

Weed infestation is a major problem in the aerobic rice system due to the lack of standing water that could prevent the growth of weeds. To reduce heavy reliance on herbicide, this research aims to determine the potential of cassava allelopathy for inhibition of weeds in aerobic rice. The allelopathic potential of cassava extracts on the germination and growth of tested weed species (*Eleusine indica*, *Ageratum conyzoides*, and *Cyperus distans*) and aerobic rice (*Oryza sativa*) was conducted in the laboratory. The results showed that increasing the aqueous extract concentrations of cassava extracts inhibited the germination and seedling growth of tested weeds, suggesting the allelopathic effects of cassava extracts are concentration dependent. The degree of phytotoxicity of different vegetative parts of cassava can be classified in order of decreasing inhibition as follows: leaf, stem, tuber, and tuber peel. Aqueous leaf extract of cassava at a concentration of 0.5% (w/v) provided complete inhibition on *A. conyzoides*, *E. indica*, and *C. distans* germination whereas 25% to 100% inhibition on the shoot growth was recorded. By contrast, the shoot growth and germination of aerobic rice were not affected. These results suggest that the cassava leaf extracts contain water-soluble allelochemicals for inhibition on *A. conyzoides*, *E. indica*, and *C. distans* in aerobic rice.

Keywords: *Ageratum conyzoides*; aqueous leaf extract; *Cyperus distans*; *Eleusine indica*; *Manihot esculenta*

ABSTRAK

Serangan rumpai adalah masalah utama dalam sistem padi aerobik kerana kekurangan air bertakung dapat mengawal pertumbuhan rumpai. Bagi mengurangkan kebergantungan yang tinggi pada racun rumpai, kajian ini bertujuan untuk menentukan potensi alelopati ubi kayu untuk perencatan rumpai dalam padi aerobik. Potensi alelopati ekstrak ubi kayu terhadap percambahan dan pertumbuhan rumpai (*Eleusine indica*, *Ageratum conyzoides* dan *Cyperus distans*) dan padi aerobik (*Oryza sativa*) dijalankan dalam pengasaian makmal. Hasil kajian menunjukkan bahawa peningkatan kepekatan ekstrak akues ubi kayu telah merencatkan percambahan, pertumbuhan anak benih dan pertumbuhan akar rumpai yang diuji dan ini mencadangkan kesan alelopati ekstrak ubi kayu bergantung kepada kepekatan. Tahap kefitotoksikan daripada bahagian vegetatif ubi kayu yang berbeza dapat dikelaskan dalam urutan penurunan perencatan seperti berikut: daun, batang, ubi dan kulit. Ekstrak daun ubi kayu pada kepekatan 0.5% (w/v) memberi perencatan sepenuhnya terhadap percambahan rumpai manakala perencatan sebanyak 25% hingga 100% ke atas pertumbuhan pucuk *A. conyzoides*, *E. indica* dan *C. distans* dicatatkan. Sebaliknya, pertumbuhan pucuk dan percambahan padi aerobik tidak terjejas. Hasil ini menunjukkan bahawa ekstrak daun ubi kayu mengandungi alelokimia yang larut dalam air untuk merencat *A. conyzoides*, *E. indica* dan *C. distans* dalam padi aerobik.

Kata kunci: *Ageratum conyzoides*; *Cyperus distans*; ekstrak daun ubi; *Eleusine indica*; *Manihot esculenta*

INTRODUCTION

Aerobic rice system is a potential new technology of rice production to address water scarcity problem. Aerobic rice production systems require less water than conventional systems, where rice is grown without flooding as a direct crop. However, a major problem that affects aerobic rice greatly is the rapid growth of weeds compared to flood-irrigated rice, due to the lack of standing water that could prevent the growth of weeds (Zhao et al. 2006). Previous study showed that 90 weed species compete with rice and cause a decrease in grain yields of 23%-100% under aerobic systems (Jabran & Chauhan 2015). Most of the weed community in aerobic rice was dominated by broadleaf weeds, followed by sedges and grasses, which are quite different from a typical anaerobic rice field. Herbicide remains to be the major strategy in the aerobic rice system because it is the most realistic, efficient, and cost-effective method for weed control (Jaya Suria et al. 2011). However, the excessive use of herbicide could lead to evolution of herbicide-resistant weeds (Peterson et al. 2018), environmental pollution (Ferreira-Junior et al. 2017; Moreira et al. 2019), and human health concern (Cowie et al. 2020).

Allelopathy is one of environmental friendly approaches to reduce the reliance on herbicides for sustainable weed management (Ghersa et al. 2000). Some crops produce toxic allelochemicals that could suppress the growth and reduce survival of weeds (Nakamaru & Iwasa 2000). There are studies that showed crop allelopathy including sorghum (Moosavi et al. 2011), corn (Ahmed 2018), and rice (Jafari et al. 2011), and this could be applied as water extract to inhibit weeds. Different vegetative parts such as leaf, root, stem, litter, twig produced different secondary compounds with varying amounts in terms of composition and concentration and physicochemical properties (Altemimi et al. 2017). Previous study by Sodaeizadeh et al. (2009) documented that aqueous leaf extracts of *Peganum harmala* caused the greatest phytotoxic effect on seedling growth of *Avena fatua* and *Convolvulus arvensis* as compared to stem and root extracts.

Cassava (*Manihot esculenta* L.) is a major world crop and source of carbohydrate for over 800 million throughout the humid tropics (Liu et al. 2017). It is one of the famous crops produced in Malaysia because of the ability of cassava plants to grow under low fertilizers, agrochemical inputs and water supply, thus making it easier to cultivate and manage. Unfortunately, manufacturing of cassava may result in agricultural

waste generation where leaves and stems of cassava are produced during the post-harvest activity. These wastes which represent 50% of the production are discarded or burned (Idris et al. 2020). As a result, they could lead to environmental pollution.

A study on screening for allelopathic potential of higher plants from Southeast Asia has shown that cassava is the second most suppressive plant for radish germination and growth (Hong et al. 2003). It is hypothesized that cassava has herbicidal activity for weed control in aerobic rice. Three groups of weed species, *Eleusine indica* (Take-Tsaba et al. 2018), *Ageratum conyzoides* (Sunil et al. 2011) and *Cyperus distans* (Anwar et al. 2012), representing grass, broadleaf weed, and sedge were examined as bioassay species due to their invasiveness and high abundance in aerobic rice. Aerobic rice was also included as bioassay species to examine its tolerance level towards cassava extracts. This research aimed to evaluate the allelopathic potential of aqueous extracts of cassava plant on germination and seedling growth of selected weeds and aerobic rice.

MATERIALS AND METHODS

Seeds of *E. indica* (goosegrass), *A. conyzoides* (billy goat weed), and *C. distans* (slender) were collected from Bukit Kor, Marang (5°12'39.9"N 103°09'57.1"E), Terengganu, Malaysia. Rice *Oryza sativa* (AERON) seeds were obtained from Mardi Seberang Perai, Pulau Pinang, Malaysia (5°32'37"N 100°28'3"E). Seed viability was examined to ensure that the seeds have a germination percentage of higher than 90%. Each seed was soaked in 0.2% potassium nitrate solution for 24 h to break seed dormancy. Only seed coats of *E. indica* were detached by using sandpaper. Then, each seed was rinsed with distilled water and dried on tissue before being subjected to treatments. Mature plants of cassava (*Manihot esculenta* var. kuning) were collected from Tumpat, Kelantan, Malaysia (6°08'52.7"N 102°10'39.4"E) in June 2017. Different vegetative parts of cassava plant including tuber, leaf, stem, and tuber peel were cleaned with running tap water, air dried and kept in an universal oven (MEMMERT) at 50 °C for one week. After drying, the cassava parts (tuber, leaf, stem, and tuber peel) were cut into small pieces with length ranging from 2 to 4 cm using a chopper machine and separately ground using a mill grinder (COLE PARMER Model P-04301-35) to produce powder form (<2 mm). The samples were packed, labeled and stored in a freezer at -20 °C before use.

PREPARATION OF CASSAVA EXTRACTS

Each 30 g vegetative part of cassava powders was transferred into respective conical flask containing 200 mL distilled water and agitated using an orbital shaker (Tech-lab T 05-100) for 48 h at a speed of 200 rpm at 25 °C, and then sieved with muslin cloth, followed by centrifugation (HERMLE Z 36 HK) at a speed of 9000 rpm for 15 min at 4 °C. The extracts were filtered through two layers of cheesecloth and Whatman #1 filter paper (Whatman International Ltd., England) using a vacuum pump. The extracts were transferred into vials and kept in a freezer -80 °C for 24 h before being freeze-dried to yield the crudes in crystalline form. The resulting yields were weighted and stored in a freezer at -40 °C and wrapped with aluminum foil until later use, according to the modified method of Chuah and Lim (2015).

LABORATORY BIOASSAY

The experiment was conducted using aqueous extracts of cassava tuber, leaf, stem, and tuber peel at six concentrations, 0.0%, 0.06%, 0.125%, 0.25%, 0.5%, and 1.0% (w/v), against *E. indica*, *A. conyzoides*, *C. distans*, and *O. sativa*. Each treatment was replicated six times and arranged as factorial in a complete randomized design where factor one was bioassay species whereas factor two was concentration of cassava extracts. The aqueous extracts and distilled water were autoclaved at 121 °C for 15 min prior to use. Each 9 cm diameter glass Petri dish was lined with 2 pieces of 9 cm diameter Whatman #1 filter paper, and 5.0 mL of the extract was added. Distilled water was used to serve as a control. A total of 20 *E. indica*, *A. conyzoides*, *C. distans* seeds, and 10 *O. sativa* seeds were placed onto the filter papers, respectively. Petri dishes were sealed with parafilm and incubated in a seed germinator GC-1050 (PROTECH, Malaysia) at 30/20 °C (day/night) with a 12-hour photoperiod. At 7 days after incubation, the germination, root, and shoot lengths of seedlings were recorded and expressed as percentages of their respective control seedlings. Seeds are considered germinated when the root, length is >2 mm (Gulden et al. 2003).

STATISTICAL ANALYSIS

The inhibitory effect of aqueous extracts on germination shoot and root lengths of bioassay species was calculated (Kordali et al. 2009) as follows:

$$I = 100(C-A)/C$$

where I is the percentage of inhibition; C is the mean germination, shoot, and root length of the control; and A is the mean germination, shoot, and root length of the aqueous extracts. The data were plotted using Sigma plot to show the most phytotoxic part of cassava extract against each bioassay species. The percentage data were tested for the normality and homogeneity of variance before being subjected to two-way ANOVA at 5% significance level.

RESULTS

SEED GERMINATION

The phytotoxic effects of cassava tuber, leaf, stem, and tuber peel extracts on germination of selected weeds and aerobic rice are shown in Figure 1. There were significant ($p < 0.05$) interactions between germination of bioassay species and concentration of cassava extracts. At concentration of 0.5% (w/v) aqueous extracts, cassava tuber gave complete inhibition on *E. indica* germination, 33% inhibition on *A. conyzoides* germination, but no effect was observed on seeds of *C. distans*. At the same concentration, cassava stem extracts provided 100% inhibition on *A. conyzoides* and *C. distans* germinations as well as 37% inhibition on *E. indica*. In contrast, the aqueous extracts of cassava tuber peel at 0.5% concentration was less phytotoxic where *E. indica*, *A. conyzoides*, and *C. distans* were inhibited by 71%, 48%, and 33%, respectively. Interestingly, the cassava leaf extracts at 0.5% concentration showed 100% inhibition on seed germination regardless of any weed bioassay species. However, when the concentration of cassava extract was increased to 1.0%, none of *C. distans*, *A. conyzoides*, and *E. indica* seeds could germinate irrespective of any vegetative parts of cassava extracts. It is surprising to note that seeds of aerobic *O. sativa* were tolerant to aqueous extracts of tuber, stem, and tuber peel except the leaf extracts which gave 68% of inhibition at 1.0% concentration.

SHOOT GROWTH

The phytotoxic effects of cassava tuber, leaf, stem, and tuber peel extracts on shoot growth of selected weeds and aerobic rice are shown in Figure 2. There were significant ($p < 0.05$) interactions between shoot growth of bioassay species and concentration of cassava extracts. At the concentration of 0.5% (w/v), the aqueous extracts of cassava tuber inhibited shoot growth of *E. indica* by 44%,

but stimulatory effects were evident for *A. conyzoides* and *C. distans*. At the same concentration, cassava stem

extracts stimulated shoot growth of *E. indica* by 56%, but the shoot growth of *A. conyzoides* and *C. distans*

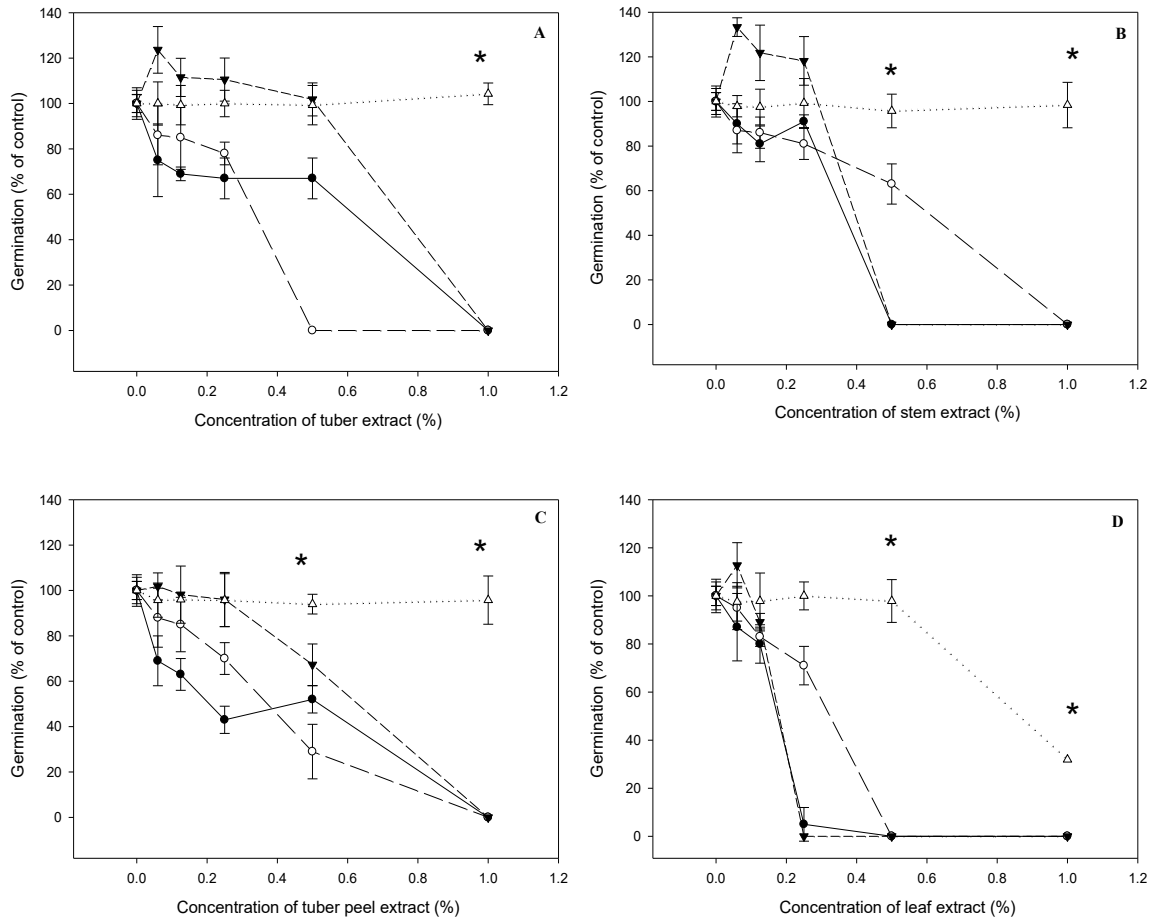


FIGURE 1. Allelopathic effects of aqueous extracts of cassava on seed germination of *Ageratum conyzoides* (—), *Eleusine indica* (---), *Cyperus distans* (···), and aerobic *Oryza sativa* (····). Each bar represents standard deviations of mean. (A) tuber, (B) stem, (C) tuber peel, (D) leaf extracts of cassava. * denotes significant difference between aerobic rice and weed species within the same extract concentration at 5% of significant level

were inhibited by 2% and 19%, respectively. Cassava tuber peel extracts inhibited the shoot growth of *E. indica* and *A. conyzoides* by 38% and 10%, respectively, but stimulation was observed for shoot growth of *C. distans*. It is interesting to note that cassava leaf extracts provided complete inhibition of *E. indica* and *A. conyzoides* shoot growth. However, only 39% inhibition was recorded for *C. distans*.

As the concentration of cassava extracts was increased to 1.0%, each vegetative part of cassava

extracts gave 100% inhibitory effect on *E. indica* shoot growth. Cassava tuber and tuber peel extracts at 1% concentration inhibited the shoot growth of *A. conyzoides* by less than 5% while 20% to 28% for *C. distans*. At the same concentration, cassava stem extracts could inhibit shoot growth of *E. indica* and *A. conyzoides* completely, while 39% of inhibition was observed for *C. distans*. Surprisingly, *C. distans*, *A. conyzoides*, and *E. indica* failed to produce shoots in response to the cassava leaf extracts. In contrast, aerobic *O. sativa* was tolerant to cassava tuber,

leaf, and stem extracts but inhibitory effect was evident when treated with tuber peel extract, which could reduce the shoot length by 39% at 1% concentration. On the other hand, the cassava tuber, leaf, and stem extracts exhibited stimulatory effects on the shoot growth of bioassay species at low concentration of 0.06%.

ROOT GROWTH

The phytotoxic effects of cassava tuber, leaf, stem, and tuber peel extracts on root growth of selected weeds and aerobic rice are shown in Figure 3. There were significant ($p < 0.05$) interactions between root growth of bioassay species and concentration of cassava extracts.

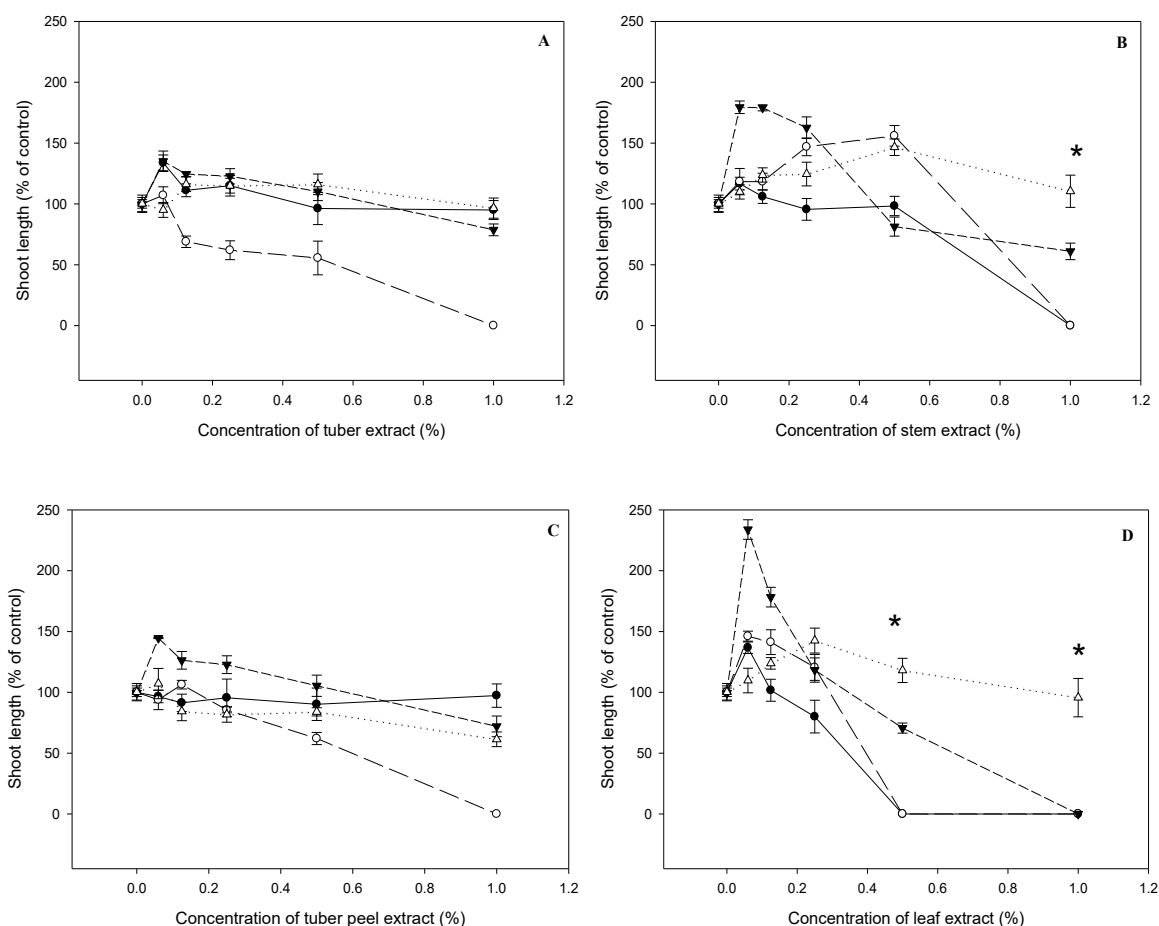


FIGURE 2. Allelopathic effects of aqueous extracts of cassava on shoot growth of *Ageratum conyzoides* (—), *Eleusine indica* (---), *Cyperus distans* (---), and aerobic *Oryza sativa* (---). Each bar represents standard deviations of mean. (A) tuber, (B) stem, (C) tuber peel, (D) leaf extracts of cassava. * denotes significant difference between aerobic rice and weed species within the same extract concentration at 5% of significant level

At a concentration of 0.5% (w/v), the aqueous extracts of cassava tuber inhibited root growth of *E. indica*, *A. conyzoides*, and *C. distans* by 100%, 65%, and 33%, respectively. Cassava stem extract exhibited complete inhibition on *A. conyzoides* and *C. distans* root growth,

while 81% inhibition on root growth of *E. indica*. Tuber peel extracts inhibited root growth of *E. indica*, *A. conyzoides*, and *C. distans* by 51%, 63%, and 33%, respectively. It is interesting to note that cassava leaf

extracts inhibited root growth of each weed bioassay species completely at the same concentration.

When the concentration of cassava extracts was increased to 1.0%, all vegetative parts of cassava extracts gave almost 100% inhibition on the root growth of *E. indica*, *A. conyzoides*, and *C. distans* except the cassava tuber extracts inhibited 86% of *A. conyzoides* root growth. Conversely, the root growth of aerobic *O. sativa* was less affected by the cassava extracts at 1.0% concentration. Meanwhile, root growths of *E. indica* and *C. distans* were stimulated when subjected to cassava extracts at a concentration of as low as 0.06%.

Figure 4 presents the response of *E. indica* to cassava leaf extracts 7 days after treatment. It is clearly shown that *E. indica* seedlings were initially inhibited at 0.25% extract concentration (Figure 4(D)). Almost all *E. indica* seedlings were stunted markedly and died 7 days after treatment at 0.5% concentration extracts (Figure 4(E)). It has been documented that aqueous extracts of

alfalfa leaf provided complete inhibition of barnyard grass at 0.4% concentration. Similar to the alfalfa leaf extracts, it is suggested that cassava leaf extracts contain allelochemicals which could induce the appearance of abnormal seedlings (Chon et al. 2002). In contrast, the shoot growth of *E. indica* seedlings was stimulated after treatment of leaf extracts at 0.06% and 0.125% concentrations (Figure 4(B)-4(C)). On the other hand, zero germination was evident where the seeds died and were infected with fungus at 1% concentration of leaf extract (Figure 4(F)). Similar trend was observed for other weed species in the present study. Aqueous extracts of cassava leaf may contain water-soluble carbohydrates which are easily available sources of energy for heterotrophic microorganisms. It is most likely that leaf extracts of cassava at 1% concentration consist of sufficient water-soluble carbohydrates which could serve as external sources of energy and thus stimulate fungal growth (Mao et al. 2006).

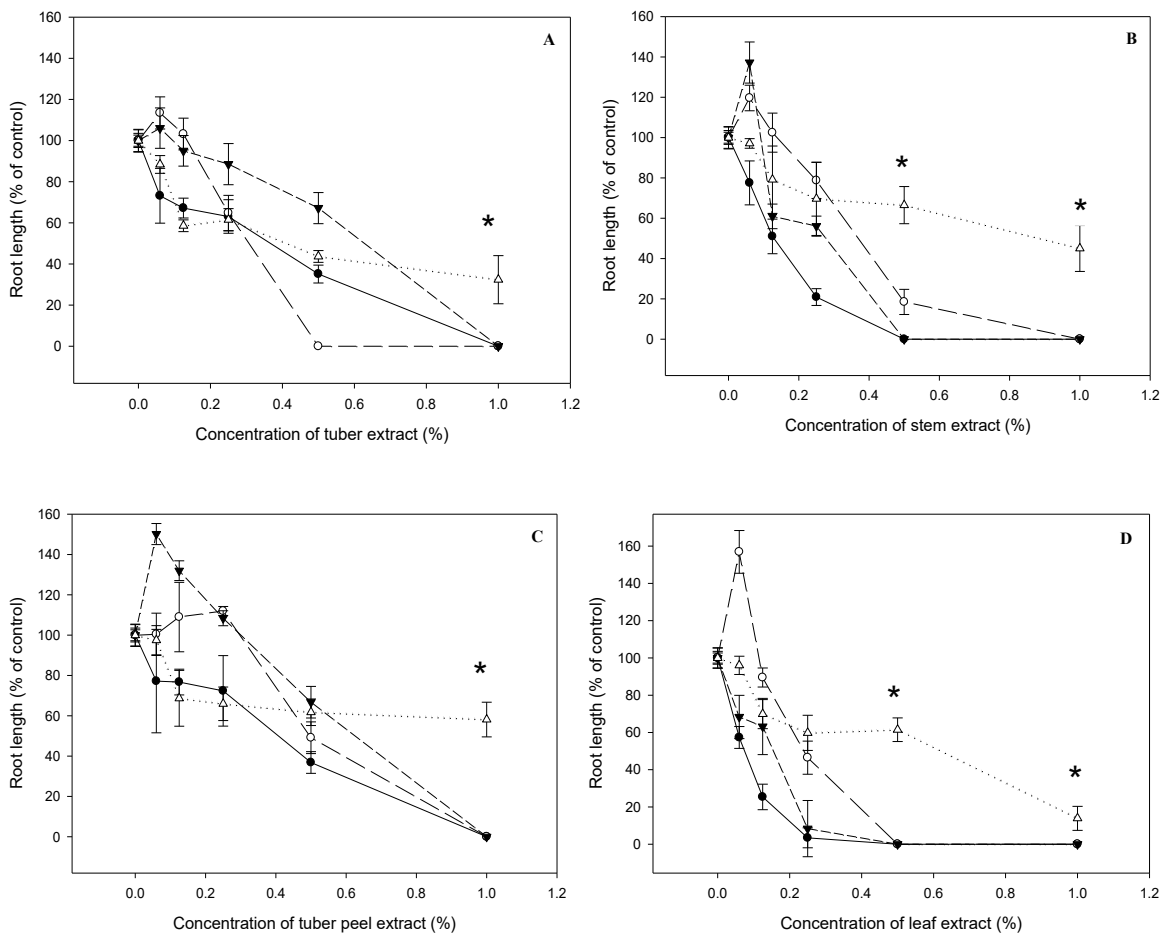


FIGURE 3. Allelopathic effects of aqueous extracts of cassava on root growth of *Ageratum conyzoides* (—), *Eleusine indica* (---) *Cyperus distans* (---), and aerobic *Oryza sativa* (— · —). Each bar represents standard deviations of mean (A) tuber, (B) stem, (C) tuber peel, (D) leaf extracts of cassava. * denotes significant difference between aerobic rice and weed species within the same extract concentration at 5% of significant level

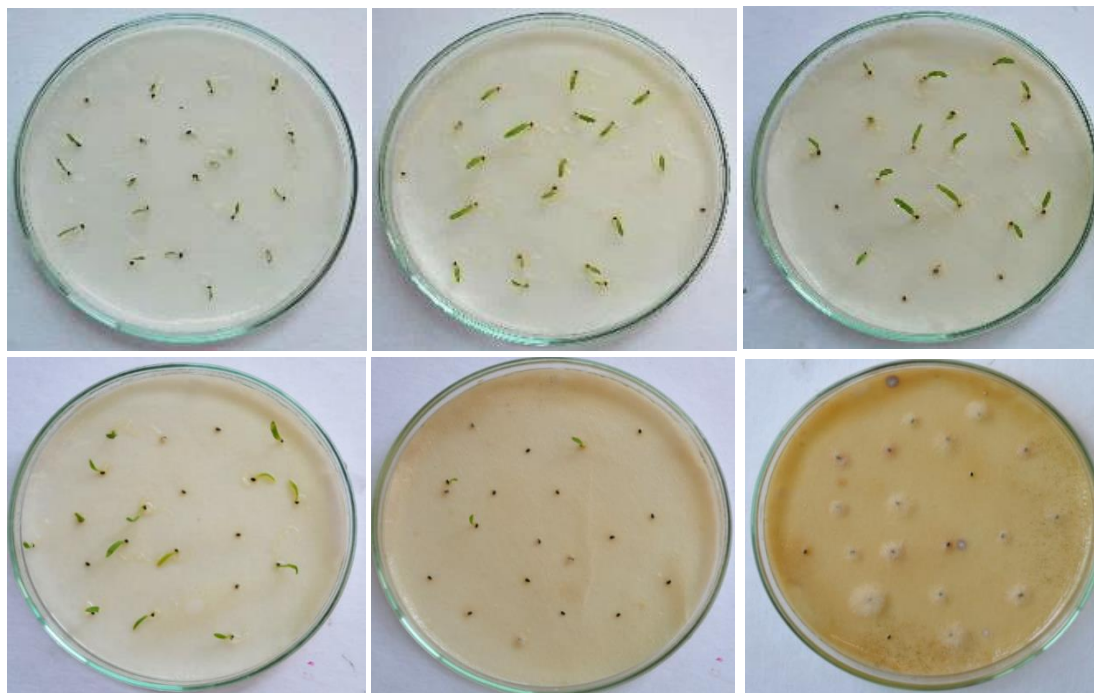


FIGURE 4. Responses of *E. indica* seeds 7 days after treated with different concentrations of cassava leaf extracts in a seed germinator (A) Control, (B) 0.06%, (C) 0.125%, (D) 0.25%, (E) 0.5%, and (F) 1% extract concentration

DISCUSSION

Abundant studies have demonstrated that aqueous extracts of several plant species possess allelopathic effects, including *Ludwigia hyssopifolia* (Mangao et al. 2020), *Sorghum bicolor* (Kamran et al. 2019), *Pennisetum purpureum* (Ismail et al. 2018), *Oryza sativa* (Alam et al. 2015), and *Cannabis sativa* (Pudelko et al. 2014). Water is a hydrophilic solvent that could extract water-soluble allelochemicals occurring naturally in the environment. In the present study, active water-soluble allelochemicals contained in aqueous extracts of cassava at concentrations ranging from 0.5 to 1.0% might be present and lead to zero germination of all tested weed species in the laboratory bioassay.

It is found that the highest inhibitory effect was exhibited by cassava leaf extracts, followed by stem, tuber, and tuber peel extracts of cassava against three bioassay weed species in the present study. These results imply that the presence of higher water-soluble compounds active substances may be contained in the leaves and stems than the part roots of cassava to affect radicle germination and seedling growth of bioassay species as shown by Turk and Tawaha (2003). The finding

of current study is in agreement with Ladhari et al. (2020) who reported that *Ficus carica* leaf extracts at 40 g/L concentration was more phytotoxic than twig extracts in which the leaf extracts provided 100% inhibition of radish, lettuce, peganum and thistle seedling elongation.

In addition, the results from the current study indicated that different vegetative parts of cassava contain different amounts of allelochemicals and the allelopathic effect is concentration dependent. Similarly, Laosinwattana et al. (2010) documented that the potential and magnitude of inhibitory effects of allelochemicals differed among plant parts. Marked allelopathic effects of different organs and concentrations of *Chromolaena odorata* on photosynthetic pigments and stomata of *A. conyzoides* leaves have been reported by Yuliyani et al. (2019).

Phenolic compounds have been detected in stem (Yi et al. 2010) and leaf (Gazola et al. 2019) of cassava as secondary metabolites. It is generally accepted in the literature that phenolic compounds at low concentrations could stimulate plant growth (Ghareib et al. 2010). Stimulatory effects were evident on shoot and root growth of bioassay species at low concentration of 0.06%

regardless of any vegetative parts of cassava extracts, pointing the hormesis effect of cassava extract with the biphasic dose-response and a low-dose stimulatory (Abbas et al. 2017), but inhibitory effects were apparent at high concentration of as low as 0.5%. The inhibition may be due to synergistic or additive effects of the extract constituents rather than a single compound when the inhibitory effects increase with extract concentration (Al-Shatti et al. 2014). On the other hand, previous study by Li et al. (2016) showed that aqueous leachates of cassava (*Manihot esculenta* variety SC5) root, stem, and leaf at 5% and 10% (w/v) concentrations inhibited the seed germination and seedling growth of all tested weed species but stimulatory effect was evident at 2.5% concentration. These findings suggest that the allelopathic potential of cassava may be variety dependent.

It is noted that root growth of bioassay species is very sensitive to allelochemicals of cassava extracts as compared to shoot growth and germination of bioassay species. Previous studies have revealed that root was more sensitive than shoot (Adhikary 2019; Jafariehyazdi & Javidfar 2011) in response to the allelopathic effect of plant extract. This may be due to the fact that root is the first organ where the extract comes into direct contact with phytotoxic compounds (Qasem 1995).

In the present study, the target species of weeds representing different morphological characteristics have different degrees of susceptibility to cassava extracts, where broadleaf weed *A. conyzoides* is the most sensitive, followed by grassy weed *E. indica* and sedge *C. distans*. Likewise, the study conducted by Uddin et al. (2014) showed a higher sensitivity of broadleaf weeds compared to grass weeds in response to sorgoleone extract. There are several reasons for the different sensitivity of various plant species to inhibitory compounds. These include differential penetrability of the active compounds into seeds (Hanley & Whiting 2005), seed structure (Hodgson & Mackey 1986), and the physiological and biochemical characteristics of each plant species (Kobayashi 2004).

The aqueous leaf extracts of cassava were found to be the most phytotoxic as compared to other vegetative parts of cassava extracts when the extract was increased to 0.5% concentration. However, germination of aerobic rice was not affected when treated with the aqueous leaf extracts at the same concentrations whereas germinations of other bioassay species were completely inhibited.

This result clearly indicates that the aerobic rice is more tolerant or less affected than other all tested weed

species in response to cassava leaf extracts. This result is in line with the findings of Dilipkumar and Chuah (2013) who reported that rice was more tolerant than barnyard grass when treated with sunflower leaf extracts. The sunflower leaf extract at 10%-15% concentration inhibited shoot emergence and seedling growth of barnyard grass by 80%-100% whereas inhibition on aerobic rice ranged at 10%-65% was evident. Therefore, the leaf extracts of cassava contain potential water-soluble allelochemicals for weed control in aerobic rice as supported by Xuan et al. (2005).

CONCLUSION

In summary, the inhibitory effects of cassava leaf extracts were higher than cassava stem, tuber peel, and tuber extracts against all tested weed bioassay. The aqueous extracts of cassava leaf have promising water-soluble allelochemicals for inhibitions on *A. conyzoides*, *E. indica*, and *C. distans* without affecting germination and shoot growth of the aerobic rice depending on the concentration of cassava extract. Further studies are needed to identify the allelochemicals of cassava leaf which are responsible for inhibition of weeds in aerobic rice.

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*Corresponding author; email: chuahts@uitm.edu.my