The Stimulatory and Inhibitory Effects of Mungbean Extract on Germination and Seedling Growth of Three Crop Species

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ABSTRACT: *The experiment was conducted at the experimental farm and laboratory of Institute of Sustainable Agrotechnology, University Malaysia Perlis, Padang Besar, Perlis, Malaysia, with the objective toinvestigate the inhibitory and stimulatory effects of aqueous extract of mungbean on seed germination and seedling growth of three crop species, mungbean, sweet corn and okra. Different treatments of mungbean aqueousextracts (vegetative fresh, vegetative after 2 weeks drying, vegetative after 4 weeks drying, flowering fresh, flowering after 2 weeks drying, flowering after 4 weeks drying, flowering fresh, flowering after 2 weeks drying, flowering after 4 weeks drying, maturity fresh, maturity after 2 weeks drying, maturity after 4 weeks drying and water as control) were used to test their effect on the test species. The experiment was randomly distributed and according to Completely Randomized Design(CRD) with five replicates. The results showed the fresh vegetative aqueous extract of mungbean had a significant effect (stimulatory) on germination percent and growth parameters such as number of root, root length and shoot height, of the three crop species. The study revealed that the aqueous extract of mungbean have different effects (inhibitory and stimulatory) on the seedlings and the mode of action depends on the associated plant species.Our results suggest that the aqueous extract of mungbean from the different growth stages and drying periods have an allelopathic effect.*
KEYWORDS**:** *Allelochemicals, fresh aqueous extract, total root length, allelopathic effect, mungbean*

I. INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek) is seen as a vital food source in Asia, particularly in India and in South, Southeast and East Asia, all of which are places where tropical and subtropical conditions persist (Chankaew et al., 2011, Lambrides and Godwin, 2007). Mungbean can be cultivated as an additional crop after harvesting winter crops, such as winter wheat, winter legumes and oilseeds and before planting main season rice. More high quality food can be provided by replacing short fallows during spring, which will increase the yield of the following crops (Joshi et al., 2014). Also it could be used in the intercropping system in order to increment the crop production.

Intercropping is the practice of growing two or more crops together in the same piece of land in stipulated time, particularly in the tropics. Intercropping can be seen as the practical application of diversity, competition and facilitation in arable cropping systems. The majority of the agriculture for developing countries in the tropics is done on small acreages by farmers growing food mainly for their families and perhaps some for the local markets. This is survival agriculture in that there may be no other source of food for the farmer and the entire family may be involved in the production of food. Intercropping systems over the centuries have demonstrated that the probability of crop failures is much lower for these systems than it is for sole cropping systems (Pearce and Edmondson, 1982).Grain leguminous-cereal mixed intercrops are better at exploiting natural resources as compared to the sole crops of different plant species (Hauggaard-Nielsen et al., 2006). Leguminous-crop intercropping may produce higher yields as compared to monoculture in various regions, including African semi-arid regions such as Eastern Africa (Fisher, 1977a; b; 1979; Pilbeam et al., 1994; Alemseged et al., 1996a; b), Southern Africa (Rees, 1986a; b; c; Austin and Marais, 1987; Lightfoot and Tayler, 1987a; b and Tsubo et al., 2003) and Brazil (Carlos et al., 2014).

Generally, yield production of any crop grown under intercropping depends on the crops selected as well as allelopathic matters which was produced from it.The term “allelopathy” refersto the effectsthat can be positive and negative, according to organism affected (Einhellig, 1995). The plants affect the growth and productivity of neighboring plants directly through secondary metabolites. Allelochemicals are found in living plants, either released as volatile compounds from leaves, decomposing plant residues or from leaf leacheates. In field condition, the allelochemicals are released mostly in the form of leacheates from plant residues. Allelopathic interactions are primarily based on the synthesis and release of secondary metabolites by higher plants that initiate a wide array of biochemical reactions, which induce several biological changes. In nature, many plant species grow together and interact with each other by inhibiting or stimulating the growth and development through allelopathic interactions.

Therefore, the aims of the study were to determine the effect of dry and fresh extract at different growth stages of mungbean on seed germination and seedling growth of three different species.

II. II. MATERIAL AND METHODS

The experiment was conducted at the experimental farm and laboratory of Institute of Sustainable Agrotechnology, University Malaysia Perlis, Padang Besar, Perlis, Malaysia.

II. 1.SAMPLE PREPARATION

Mungbean seeds were sown on 14th June in the field and the plants were then harvested at vegetative stage (37 days), flowering stage (44 days) and maturity stage (75 days) after planting. Mungbean plants were dried at 40°C for 2 and 4 weeks in the oven. Twenty grams was placed in 250 mL conical flask and distilled water was added till the volume became 200 mL [giving concentration of 1:10, w/v, or 100 g L^{-1}, as recommended byWardle et al., (1992)]. The mixture was stirred for ten minutes and left at room temperature for 48 h. The extracts were filtered with two layers of cheese cloth followed by Whatman Number1 filter paper. Extracts were kept at 5°C in the refrigerator till use. The filtrates were taken out of the refrigerator 24 h before being used experimentally, in order to achieve room temperature.

II. 2.EXTRACTS FROM FRESH PLANTS

Mungbean plants harvested at the different growth stages were cut into small pieces. Twenty grams of plant was added to 200 mL of distilled water (giving the ratio of 1:10, w/v).The mixture was shaken for 10 min and then left at room temperature for 48 h. Filtration was like that of oven dried samples.

II. 3.SEED GERMINATION TEST

Mungbean, sweet corn and okra were used as test crops. Ten seeds per treatment were placed evenly in sterile 9 cm petri dishes lined with two layers of filter papers in five replicates. Then 8 mL of extract treatments was applied to each petri dish and water was used as control. The experiment was conducted under dark condition with minimum exposure to light during data collection.Four days after treatment application, germination was determined by counting the number of seed germinated in each petri dish and expressed in percentage. On the 5th day, number of roots per seedling, total root length and shoot length of sweet corn and hypocotyl length of mungbean and okra were measured from four randomly selected seedlings.

II. 4.GERMINATION INHIBITION OR STIMULATION

The percentage of inhibition or stimulation was calculated following the formula bySingh and Chaudhary (2011).

Inhibition (-) or stimulation (+) = [(Germinated seeds in extracts - Germinated seed in control)/Geminated seeds in control] x 100.

II. 5.DATA ANALYSIS

The bioassay experiment was designed in factorial CRD with two factors and replicated five times. One factor was extract: vegetative fresh, vegetative after 2 weeks drying, vegetative after 4 weeks drying, flowering fresh, flowering after 2 weeks drying, flowering after 4 weeks drying, flowering fresh, flowering after 2 weeks drying, flowering after 4 weeks drying, maturity fresh, maturity after 2 weeks drying, maturity after 4 weeks drying and water as control.The other factor was the three plant species: mungbean, sweet corn and okra. The analysis of variance was carried out using SAS program (version 9). Mean values were separated based on Duncan's at 0.05 probability levels.

III. RESULTS AND DISCUSSION

III.1.GERMINATION PERCENTAGE AND INHIBITION OR STIMULATION

Considering the main treatments, extracts of mungbean decreased germination of all species significantly except fresh extract at vegetative and flowering stages, with germination of all species being most severely affected in presence of dry extracts compared to the control (Table 1). Contrary to the present results, Williamson et al.(1989) and Assaeed and Al-Doss(1997) noted that the inhibition effect of fresh leaves was less than dry leaves. Results from different localities and different allelopathic plants were reported to affect germination. May and Ash (1990) reported less suppressive effect of leachate from intact fresh leaves of *Eucalyptus spp* than dry foliage on tested plants. For extract of 4 weeks drying at flowering stage gave lowest value for seed germination (54%) compared to fresh extracts at vegetative and flowering stages and control treatment that gave highest values in seed germination (87.33%, 82.66% and 80% respectively),probably due to higher metabolic activities in flowering stage resulting in accumulation of potential phytochemicals(Narsingh et al., 2013).

There were significant differences among species over the three treatments. Germination varied between 81.6% in mungbean and 62.4% in sweet corn (Table 1) and there was a significant interaction between treatments and species. The differences in germination response of tested species were expected and are in agreement with those of Patil (1994) who reported variations in germination response of various field crops in the presence of *Glyricidia maculata* leaf extract.

As seen from Table 1, the interaction among extract treatments for mungbean with plant species, vegetative fresh extract with mungbean gave the highest average of 98%,while, extract of maturity after2 weeks drying with okra gave lowest value of 44%.

Table 1. Effect of extract from different treatmentson germination percentage of mungbean, sweet corn and okra

Treatments	Mungbean	Sweet corn	Okra	Mean
Control	92 abc	62 hij	86 abcdef	80 abc
Vegetative fresh	98 a	74 cdefgh	90 abcd	87.33 a
Vegetative dry 2wk	62 hij	68 fghi	74 cdefgh	68 de
Vegetative dry 4wk	90 abcd	50 ij	72 defgh	70.66 cde
Flowering fresh	94 ab	62 hij	92 abc	82.66 ab
Flowering dry 2wk	74 cdefgh	62 hij	76 bcdefgh	70.66 cde
Flowering dry 4wk	50 ij	46 j	66 ghi	54 f
Maturity fresh	84 abcdefg	70 cdefgh	74 cdefgh	76 bcd
Maturity dry 2wk	88 abcde	62 hij	44 j	64.66 e
Maturity dry wk	84 abcdefg	68 fghi	50 ij	67.33 de
Mean	81.6 a	62.4 c	72.4 b	

*Different alphabets in the same column show significant difference using Duncan's Multiple Range test (P≤ 0.05)

Duncan multiple comparison results for mean germination percentage showedthat the highest germination percentage of both seed crops occurred in the treatments of fresh at vegetative and flowering stage (Fig. 1).

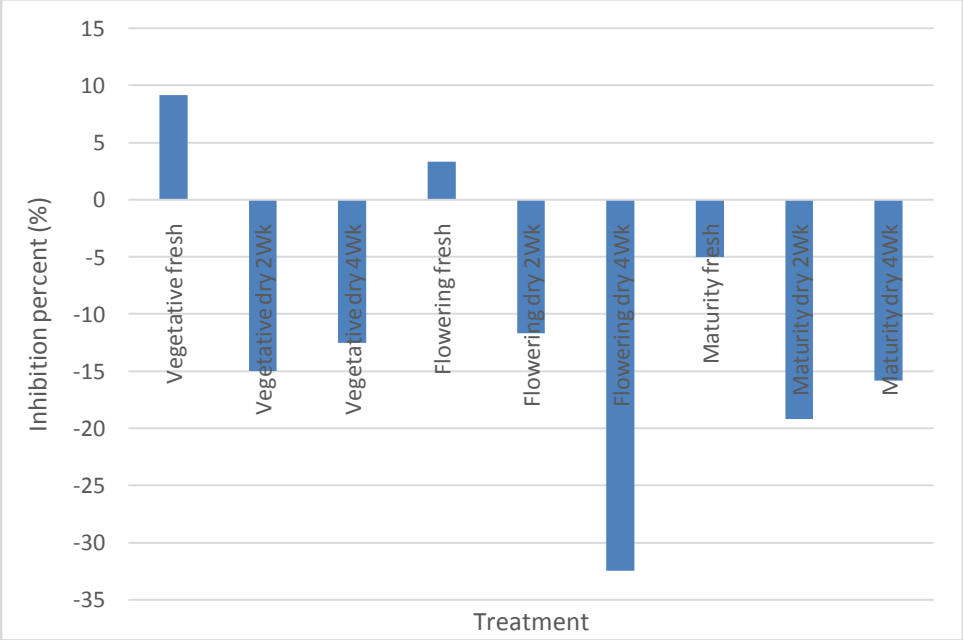

Fig 1. Stimulatory and inhibitoryeffect of extracts from different treatments on seed germination

Results from this study showed that the dry plant extracts were more phytotoxic in comparison to the fresh extract and this probably was due to synthesis and accumulation of more potential phytochemicals in plants after drying.Also the effect is dependent on the concentration, type of the material being used, plant age and type of the extract (fresh or dry). It was reported that seed characteristics such as seed size and seed coat permeability can also affect the uptake and effects of allelochemicals in seeds and interference of the allelochemicals varied accordingly (Marianne et al., 2000; Riti, 2012). Results of the present study agreed with Sukumarn et al. (2011), Talukder et al. (2015) and Gowsiya and Santosh (2016) that there was inhibitory and stimulatory effect by the extracts on germination percentage of the species tested.

III.2. NUMBER OF ROOTS

Not only percentage of germination was affected but also the number of roots was affected significantly by mungbean extracts where, the effect was positive (stimulatory) by the fresh extracts while the effect was negative (inhibitory) by the dry extracts (Table 2). Highest values was in fresh extract at all stages (vegetative, flowering and maturity), 9.51, 10.10 and 9.68 root seedling $^{-1}$, respectively, whereas, lowest value was extract of 4 weeks after drying at flowering stage, 1.42 root seedling $^{-1}$. The reason for the reduction in number of roots at dry extracts could be due to the dry extracts of mungbean may have released more toxic compounds and the roots were in continuous contact with the extracts. As seedling root growth is sensitive to the allelochemicals, longer contact with the extract led to inhibition of cell division and cell elongation in the root apical meristems (Zhang and Fu, 2009).

Mungbean had the highest average of root numbers and was significantly different from any other species, while okra was the lowest,at 4.44 root seedling^{-1}.These might be due to differential behavior of crops in response to water extract. It has been observed that different crops responded differently to the same type of allelochemicals (Pukclai and Kato-Noguchi, 2012).

Complete inhibition of root growth occurred in both mungbean and okra in extract after 2 weeks of drying at vegetative stage, 2 and 4 weeks after drying at flowering stage and also in okra extract after 4 weeks of drying at vegetative stage, and 2 and 4 weeks at maturity stage (germination of seed in this treatments wereabnormal, Fig. 2). Mungbean in fresh extract at flowering stage gave significantly highest value 13.82 root seedling^{-1} (Table 2).

Table 2. Effect of extract from different treatmentson number of roots of mungbean, sweet corn and okra

Treatments	Mungbean	Sweet corn	Okra	Mean
Control	9.68 e	5.13 fg	11.10 cd	8.63 b
Vegetative fresh	12.26 bc	5.20 fg	11.08 cd	9.51 a
Vegetative dry 2Wk	0 h	5.65 fg	0 h	1.88 d
Vegetative dry 4Wk	11.96 bc	5.60 fg	0 h	5.85 c
Flowering fresh	13.82 a	5.23 fg	11.25 cd	10.10 a
Flowering dry 2Wk	0 h	5.45 fg	0 h	1.81 d
Flowering dry 4Wk	0 h	4.27 g	0 h	1.42 e
Maturity fresh	12.73 ab	5.27 fg	11.04 cd	9.68 a
Maturity dry 2Wk	10.35 de	5.80 f	0 h	5.38 c
Maturity dry 4 Wk	11.26 cd	5.06 fg	0 h	5.44 c
Mean	8.20 a	5.26 b	4.44 c	

*Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

Dry extract indicated the presence of inhibitory level of phytochemicals as seen in reduced number and total root length of plants. But for fresh extracts the variable effect could be due to the phytochemical effects probably depended upon the freshness and dryness of the material. It was obvious that dried extract were more inhibitor, than fresh and that dried extracts were more toxic than fresh (Fazal et al., 2013). Our findings agree with many other similar studies (Hussain et al., 2004; Hamayun et al., 2005; Carmo et al., 2007 and Pereira et al., 2008).

Okra (Normal)

Okra (Abnormal)

Mungbean (Normal)

Mungbean (Abnormal)

Fig. 2. Seed germination (normal and abnormal) of okra and mungbean as affected by different extracts

III. 3. TOTAL ROOT LENGTH

Data of the test species demonstrated significant degrees of suppression and a negative response to the dry extract of different extracts. There were significant differences among the different dry periods and control especially at increment of dry periods. Dry mungbean extract at all stages caused a significant decrease in total root lengths (Table 3). Fresh extract at vegetative and maturity stages, there were a significant increase in the total root length (5.13 and 5.53 cm, respectively.), compared with control (4.93 cm) whereas extract of dry 4 weeks at flowering stage gave lowest value of 0.98 cm compared to control treatment.Apparently, dry extract of mungbean may have released more toxic compound.

Considering the variation in total root length among species, the percentage reduction in total root length was calculated. Okra was the first in sensitivity to both extracts then mungbean and sweet corn ranked the third in sensitivity to both extracts (Table 3). The variable effect could be due to difference in genetics and resistance to the extracts.

There were very significant differences of extraction treatments × crop species interaction effect for total root length (Table 3). Vegetative after 2 weeks drying and flowering after 2 and 4 weeks drying extracts showed more reduction effects on total root length in mungbean and okra respectively. Control treatment in sweet corn gave highest value 8.14 cm.

Table 3. Effect of extract from different treatmenton total root length of mungbean, sweet corn and okra

Treatments	Mungbean	Sweet corn	Okra	Mean
Control	3.43 hij	8.14 a	3.22 hij	4.93 b
Vegetative fresh	4.96 cde	6.79 b	3.66 ghi	5.13 ab
Vegetative dry 2Wk	0 k	4.57 defg	0 k	1.52 f
Vegetative dry 4Wk	3.85 fgh	4.71 cdef	0 k	2.85 c
Flowering fresh	4.60 defg	5.53 cd	4.02 efgh	4.71 b
Flowering dry 2 Wk	0 k	4.94 cde	0 k	1.64 ef
Flowering dry 4 Wk	0 k	2.80 ij	0 k	0.93 g
Maturity fresh	5.61 c	6.92 b	4.07 efgh	5.53 a
Maturity dry 2 Wk	3.39 hij	3.85 fgh	0 k	2.41 cd
Maturity dry 4 Wk	3.64 ghi	2.66 j	0 k	2.10 de
Mean	2.94 b	5.09a	1.49 c	

*Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

This present study showed that fresh aqueous extract of mungbean may contain phytochemicals that performed stimulatory function. The stimulatory functions of these chemicals were evident in the significant enhancement of the growth parameters (number of root and total root length). These results were in agreement with published reports on sunflower allelopathy against wheat and maize (Zahir and Majeed., 2014) and a similar growth promoting effect on wheat seedlings was reported where application of senna mulching was the phytochemical source (Hussain et al., 2007).

III.4. SHOOT LENGTH OF SWEET CORN AND HYPOCOTYLE LENGTH OF MUNGBEAN AND OKRA

From Table 4, extract of all treatments showed significantly reduced shoot length of plants compared with control treatment except vegetative fresh and flowering fresh that were not significant with control treatment.Flowering stage after 4 weeks drying treatment gave lowest value of 0.40 cm compared to control.The effect of allelopathic compounds on cell division, cell elongation, cell wall structure and permeability of the membrane were the probably reasons for the reduced growth observed (Zhang and Mu, 2008). Jafariehyazdi and Javidfar (2011) reported that full flowering stage extract of brassica showed more reducing effects on hypocotyl length of sun flower. Yarnia et al., (2009) also noticed that alfalfa extracts from the vegetative stage had more effect than reproductive stage. However, mungbean was the least affected bydry and fresh extracts.
Thus,overall species ranking in extracts sensitivity was as follows: mungbean > sweet corn > okra.

The mean shoot length of three crop species were compared to determine which extract treatments inhibited shoot growth of mungbean, sweet corn and okra. The result showed that the extractshad an extreme inhibitory effect on the shoot length of sweet corn and okra. The difference was significant between control and extract but was not different among control and the fresh extracts at all stages on shoot lengthin mungbean(Table 4). This could be due to marked differences among species in their susceptibility towards the effects of allelochemicals (An et al., 2005). This results agree with Otusanya et al. (2007), Abu Roman et al. (2010) and Musyimi et al. (2012).

Table 4. Effect of extract from different treatmenton shoot length and hypocotyl length of mungbean, sweet corn and okra

Treatments	Mungbean	Sweet corn	Okra	Mean
Control	8.07 a	6.25 b	4.83 cd	6.38 a
Vegetative fresh	8.70 a	4.52 cd	5.68 bc	6.30 a
Vegetative dry 2Wk	0 h	1.68 g	0 h	0.56 de
Vegetative dry 4Wk	2.00 fg	0.81 gh	0 h	0.94 cde
Flowering fresh	8.35 a	4.23 d	4.88 cd	5.82 ab
Flowering dry 2Wk	0 h	1.77 fg	0 h	0.59 de
Flowering dry 4Wk	0 h	1.20 g	0 h	0.40 e
Maturity fresh	8.20 a	4.49 cd	3.80 de	5.49 b
Maturity dry 2Wk	3 ef	1.67 g	0 h	1.55 c
Maturity dry 4Wk	2.44 fg	1.14 gh	0 h	1.19 cd
Mean	4.08 a	2.87 b	1.92 c	

*Different alphabets in the same column show significant difference using Duncan's Multiple Range test ($P \leq 0.05$)

IV. CONCLUSION

The results indicated that aqueous extracts of mungbean have both inhibitory and stimulatory effects on seed germination and seedling growth of different plants. Fresh aqueous extracts of mungbean at vegetative and flowering stages stimulated seed germination percentage; this was evidenced by the increasing percentage of seed germination. The fresh extracts also stimulated number of roots of plants. The shoot length was inhibited by the extracts of mungbean. These results confirmed both inhibitory and beneficial functions of the allelochemicals in mungbean extracts. The study clearly demonstrated that water soluble inhibitory and promo substances were present within the extracts of mungbean. We therefore suggest mungbean fresh biomass especially in vegetative stage to be used in vegetable planting, which can be employed to achieve an increase in the germination and seedling growth of sweet corn and okra. Further studies should be conducted to identify the allelochemicals present in mungbean extract which inhibit or promote the growth of plants.

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