Allelopathic effect of aqueous extract of *Vignaradiataat* three growth stages on seed germination and seedling growth of *Vignaradiata, Zea mays* and *Abelmoschusesculentus*

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ABSTRACT:
Allelopathic substances from plant affect seed germinationand seedling growth at different growth stages. *Vignaradiata* contains allelochemicals that inhibit growth of many agricultural crops. A study was conducted at the experimental farm of Institute of Sustainable Agrotechnology, University Malaysia Perlis, Padang Besar, Perlis, Malaysia, to investigate the effects of *Vignaradiata* on the germination of seeds and seedling growth of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*. *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus* seeds and seedlings were subjected to 10% concentration of the fresh plant aqueous extracts at different stages (vegetative, flowering and maturity and distilledwater as control). Ten seeds of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus* were germinated in petri dishes lined with a layer of filter paper moistened with 8ml of each extract. Germination percentage, number of root, total root length and shoot length were determined at the end of the study. The results revealed that plant extract at maturity stage significantly reduced germination of *Vignaradiata* and *Abelmoschusesculentus* and hypocotyl length of *Abelmoschusesculentus* while flowering stage significantly reduced total root length of *Zea mays*. Extracts of all threegrowth stages significantly reduced shoot length of *Zea mays*. The study indicated that aqueous fresh shoot extracts of *Vignaradiata* have both stimulatory and inhibitory effects on *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus* germination and seedling growth.

KEYWORDS: Allelochemical, Fresh extract, Vegetative, Flowering, Maturity, growth stages.

1. INTRODUCTION

The direct or indirect, negative or positive effects of plants on another plant in the environment is called allelopathy[1]. Plants release various compounds into their surroundings from many ways namely: plant exudates, volatile compounds released from leaves, decomposing plant residues and leaf leachates [2]. The plants vary in the amount of allechemicalsreleased

according to plant age or growth stage. Aqueous extracts of different plants affected germination and seedling growth of other plants that lived close together[3],[4],[5], [6], [7], and [8].

*Vignaradiata*is one of the most important legume crops, short-season and grow widely throughout the tropics and subtropics.

*Vignaradiata*hasallelochemicals that can lead to inhibitionof plant growth byas much as 10–25%, especially when mungbeanwas planted in the same land for many years [9].Study by [10]revealed that the population of lowland weeds, like *Cyperusdifformis*was reduced by the introduction of a relay crop of mungbean in the sequence.

Therefore, this study was conducted to evaluate the allelopathiceffect of *Vignaradiata* at different growth stages on germination and seedling growth of*Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*.

2. MATERIALS AND METHODS

2.1 Planting and collection of plant materials

Planting of *Vignaradiata* were carried out on 14/6/2015 and collection of plants from the field were done at three stages, vegetative stage at 37 days, flowering stage at 44 days and maturity stage at 75 days after planting. All plants were grown at the experimental farm of Institute of Sustainable Agrotechnology, University Malaysia Perlis, Padang Besar, Perlis, Malaysia.

2.2 Preparation of aqueous extracts of *V. radiata*

Fresh plants of *V. radiata* harvested at the different growth stages were cut into small pieces of about 2cm length. Twenty grams was placed in 250 mL conical flask and distilled water was added till the volume became 200 mL (giving concentration of 10%, or 100 g L^{-1}, as recommended by [11]. The mixture was stirred for ten minutes and left in 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.

2.3 Germination test

The seeds of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus* 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. Then 8 mL of extract treatments was applied to each petri dish and distilled 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/seedling, total root length and shoot length of *Zea mays* and hypocotyl length of *Vignaradiata* and*Abelmoschusesculentus*were measured from four randomly selected seedlings. The percentage of inhibition was calculated following the formula by [12],

% inhibition = [(control-extract)/control] x100, by which negative sign shows stimulation and positive sign showsinhibition.

2.4 Statistical analysis

The experimental layout was a complete randomised design replicated four times. Data were subjected to one way analysis of variance. Statistical analyses were performed using SAS statistical software (Version 9.0). Data were analysed by analyses of variance (ANOVA) and treatment means were compared using Duncan's at $P \leq 0.05$.

3. Results and Discussion

3.1 Germination percentage and Inhibition

The aqueous extracts of*Vignaradiata* at maturity stage inhibited the germination of the seeds of *Vignaradiata* and *Abelmoschusesculentus*, 8.69% and 13.95%, respectively. The inhibition of

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the germination of seeds of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*at different growth stages by the *Vignaradiata* extract varied in a wide range between - 46.66% and 13.95%, compared to the control sample (Table 1). It was found that with increasing age of *Vignaradiata*, the percentage of germinated seeds decreased on average in all test plants,

except*Zea mays* (Table 1). Aqueous extract at vegetative stage gave increment in germination from -4.65% to -46.66%on average in all test plants.Although there were reports of the negative effect of *Vignaradiata* extracts on germination [9], the effect was dependent on the concentration, type of the material, plant age and type of the extract (fresh, dry). It was also reported that seed characteristics such as seed size and seed coat permeability can affect the uptake and effects of allelochemicals in seeds and interference of the allelochemicals varied accordingly [13].Results of present study agreed with [14] that reported that the leaves fromthreeout of four cultivars of *Vignaradiata* significantly increased seed germination of *Echinochloa crus-galli*. [15]reported that extract of *Belericmyrobalan* and *Arjuna*significantly increased the germination percentage of turnip and ladies finger compared with control treatment. However, [16] found there was no negative eaffect by the extract of Mesquite on germination percentage of rice. These results showed that maturity stage extract significantly reduced the germination percentage of *Vignaradiata* and *Abelmoschusesculentus*, but in contrast it significantly increased the germination percentage of *Zea mays*, probably due to the species with small seeds were more sensitive, and hence were inhibited more than larger seeded species when exposed to similar treatment of the allelochemicals[17][18] and [19].

Table 1. Effect of differentgrowth stages of *Vignaradiata* on germination percentage of *Vignaradiata*, Zea *mays* and *Abelmoschusesculentus*.

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Treatments	*Vignaradiata*		*Zea mays*		*Abelmoschusesculentus*	
	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)	Germination (%)	Inhibition (%)
Control	92 ab		50.00 b		86 ab	
Vegetative	98 a	- 6.12	73.33 a	- 46.66	90 a	- 4.65
Flowering	94 a	- 2.12	66.66 a	- 33.32	92 a	- 6.97
Maturity	84 b	8.69	73.33 a	- 46.66	74 b	13.95

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

3.2 Number of roots and total root length

Aqueous extracts at all stages indicated the presence of stimulatory substances as indicated by increased number and total root length of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*, respectively (Table 2). However, control treatment showed variable effects on *Vignaradiata*, *Zea mays*and *Abelmoschusesculentus*. Aqueous extracts of vegetative, flowering and maturity stages significantly reduced the total root length of *Zea mays* by 6.79, 5.53 and 6.92 cm, respectively. There was no significant difference in number of roots of *Zea mays* and *Abelmoschusesculentus*.The reason for the reduction in total root length of *Zea mays* could be due to the roots were in continuous contact with the extracts. As seedling root growth is sensitive to the allelochemicals, longer contactwith the extract led to inhibition of cell division and cell elongation in the root apical meristems [20].

Table 2. Effect of extract from different growthstages of *Vignaradiata* on number of roots of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*.

Treatments	*Vignaradiata*		*Zea mays*		*Abelmoschusesculentus*	
	Number of roots/seedling	Total root length/seedling (cm)	Number of roots/seedling	Total root length/seedling (cm)	Number of roots/seedling	Total root length/seedling (cm)
Control	9.68 b	3.43 b	5.13 a	8.14 a	11.10 a	3.22 b
Vegetative	12.26 a	4.96 a	5.20 a	6.79 b	11.08 a	3.66 ab
Flowering	13.82 a	4.60 ab	5.23 a	5.53 c	11.25 a	4.02 a
Maturity	12.73 a	5.61 a	5.27 a	6.92 ab	11.04 a	4.07 a

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

3.3 Shoot length of *Zea mays*and hypocotyl length of *Vignaradiata* and *Abelmoschusesculentus*

From Table 3, aqueous extracts at all stages showed significant reduction on hypocotyl shoot length of *Zea mays*. But vegetative extract significantly increased the hypocotyl length of *Abelmoschusesculentus*, while extract maturity stagefrom significantly reduced the hypocotyl length of *Abelmoschusesculentus*. There was no significant difference among the treatmentsfor hypocotyl length of *Vignaradiata*. This could be due to differences among species in their susceptibility towards the effects of allelochemicals[21]. The results agree with [7],[22]and [23]. The effect of maturity stage on hypocotyl length of *Abelmoschusesculentus*was probably due to increase accumulation of phytochemicals in old plant more than young plant [24].

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Table 3. Effect of extract at different growth stages of *Vignaradiata* on shoot length of *Zea mays* and hypocotyl length of *Vignaradiata* and *Abelmoschusesculentus*.

Treatments	*Vignaradiata*	*Zea mays*	*Abelmoschusesculentus*
	Hypocotyl length/ seedling (cm)	Shoot length/ seedling (cm)	Hypocotyl length/ seedling (cm)
Control	8.07 a	6.25 a	4.60 ab
Vegetative	8.70 a	4.52 b	5.58 a
Flowering	8.35 a	4.23 b	4.46 ab
Maturity	8.20 a	4.44 b	3.56 b

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

Aqueous extract from all three growth stages of *Vignaradiata* (Table 1, 2 and 3) affected germination and seedling growth of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*. These might be due to differential behaviour of crops in response to the water extract. It has been observed that different crops

responded differently to the same type of allelochemicals[25] and[26]. Differential response of maize, wheat and rice to water extracts of *Ageratum conyzoides* and *Eupatorium adenophorum*were reported,where by germination and seedling growth of wheat and rice were affected more than maize. Similarly, water extract of *Hyptissuaveolens*was moresusceptibleto*P. setosum* than *M. invisa*[27].Also these results were in agreement with published reports on *Viciasativa*allelopathy against mungbean and mashbean[28].

4. CONCLUSIONS

The results indicated that fresh aqueous extractsof *Vignaradiata* have both inhibitory and stimulatory effects on *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*. Fresh aqueous extracts of *Vignaradiata* at vegetative and flowering stages stimulated *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*seed germination percentage, while fresh aqueous extracts of *Vignaradiata* at maturity stage stimulated *Zea mays*seed germination percentage. This was evidenced by the increasing percentage of seed germination.The extracts also stimulated number of root of *Vignaradiata* and total root length of *Vignaradiata* and *Abelmoschusesculentus*. The total root length and shoot length of *Zea mays*were inhibited by the extracts of *Vignaradiata*.These results confirmed both inhibitory and beneficial functions of the allelochemicals in *Vignaradiata* fresh extracts at different growth stages. The study clearly demonstrated that water soluble inhibitory and promotory substances were present within the fresh extracts of *Vignaradiata*.The soluble substances have the potential to significantly affect germination and growth of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*. The results suggest that*Vignaradiata* fresh biomass could to be used in vegetable field as green manure, which could be employed to achieve an increase in the germination

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and seedling growth of the plant. Further studies,however, should be conducted to identify the exact allelochemicals in *Vignaradiata* which inhibit or promote the growth of *Vignaradiata*, *Zea mays* and *Abelmoschusesculentus*.

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