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ALLELOPATHIC SUPPRESSION OF WEEDS IN RICE FIELD BY Tinospora tuberculata Beumee

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ALLELOPATHIC SUPPRESSION OF WEEDS IN RICE FIELD BY *Tinospora tuberculata* Beumee

By

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ALLELOPATHIC SUPPRESSION OF WEEDS IN RICE FIELD BY *Tinospora tuberculata* Beumee

By

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August 2015

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Faculty: Agriculture

Herbicides produce a wide range of toxic side-effects that pose a potential hazard to the environment. The development of natural herbicide is one method of addressing these issues. *Tinospora tuberculata* is an aromatic shrub with pharmacological properties and belongs to the Menispermaceae family. Herbicidal potential of aerial parts of *T. tuberculata* on germination and seedling growth of rice (*Oryza sativa* L.) and two rice weeds, barnyardgrass (*Echinochloa crus-galli* L.) and weedy rice (*Oryza sativa* L. complex) were evaluated. Six concentrations of methanol and water extract (3.12, 6.25, 12.5, 25, 50, and 100 g L^{-1}) were compared with the control (distilled water). The magnitude of the phytotoxicity effects at the same concentration levels in the test plants was dependent on the source of the extract (either leaf or stem) and types of solvent (water or methanol). Leaf extract was found to have greater suppressive property than the stem extract and the methanol extracts of leaf and stem were present in higher phytotoxic compared with respective water extracts. The gas chromatography–mass spectrometry (GC-MS) and ultra-fast liquid chromatography (UFLC) analysis confirmed methanol extracts and leaf extracts contained higher number and amount of chemical compounds than did those of the water extracts and stem extracts, respectively. Benzoic acid (highest allelopathic activity) was found in the highest concentration in the methanol leaf extract, while this compound was not identified in the water leaf extract. On the other hand, the predominant compound was orientin (lowest allelopathic activity) from stem extracts. Therefore, the herbicidal activity of each extract (leaf methanol extract, leaf water extract, stem methanol extract, and stem water extract) was dependent on their number, concentration, combination and inhibitory activity of the compounds. In the glasshouse experiments, allelopathic potential of powder and methanol extract of *T. tuberculata* leaf as pre emergence and post emergence (foliar spray at 14 days after sowing) applications on the growth and development of transplanted rice (*Oryza sativa* L. Var. MR219) and three common rice field weeds (barnyardgrass, smallflower umbrella sedge and weedy rice) were evaluated. The magnitude of the allelopathic effects at the same concentration levels in the test plants was dependent on the application methods (powder and extract), time of application (pre and post emergence), the concentration and the recipient species. The leaf powder was found to have greater suppressive property than the leaf extract.

The findings also recorded much more inhibition in application of leaf extract as pre-emergence relative to the post emergence application (foliar spray). Field experiment which involves comparison between *T. tuberculata* leaf powder, commercial herbicide (Solito® (pretilachlor + pyribenzoxim) at 1 L ha^{-1}) as positive control and no treatment (negative control) was done. The germination and biomass of weed plants were remarkably reduced in the plots receiving leaf powder treatment. Nevertheless, there was no significant difference between the leaf powder applied plots (at 2 and 4 t ha^{-1} doses) and those plots that received commercial herbicidal treatment in terms of reduction percentage of both parameters (the emergence and dry matter of weeds). There was a significant promotion on grain yield, straw dry weight and number of seed per panicle of rice, when treated with leaf powders and commercial herbicide compared with negative control. These results suggest that *T. tuberculata* contains a significant source of plant growth inhibitors. Hence, it can be used as a natural pre-emergent herbicide to suppress weeds in rice fields and for developing new herbicide based on phytotoxic compounds released by this plant.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERENCATANALELOPATI KE ATAS RUMPAI DI SAWAH PADI OLEH *Tinospora tuberculata* Beumee

Oleh

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Racun herba menghasilkan pelbagai toksik kesan sampingan yang menimbulkan potensi bahaya kepada alam sekitar. Pembangunan racun herba semula jadi adalah satu kaedah untuk menangani isu-isu ini. *Tinospora tuberculata* adalah pokok renek aromatik dengan sifat-sifat farmakologi dan tergolong dalam keluarga Menispermaceae. Potensi herbisid bahagian-bahagian *T. tuberculata* ke atas pada percambahan dan pertumbuhan anak benih padi (*Oryza sativa* L.) dan dua rumpai padi, barnyardgrass (*Echinochloa crus-galli* L.) dan padi angin (*Oryza sativa* L. complex) telah dinilai. Enam kepekatan metanol dan ekstrak air (3.12, 6.25, 12.5, 25, 50, dan 100 g L-1) telah dibandingkan dengan kawalan (air suling). Magnitud kesan fitotoksik pada kepekatan sama terhadap tumbuh-tumbuhan yang diuji bergantung kepada sumber ekstrak (sama ada daun atau batang) dan jenis pelarut (air atau metanol). Ekstrak daun didapati mempunyai sifat menindas lebih tinggi daripada ekstrak batang, dan ekstrak metanol daun serta batang mempunyaifitotoksik lebih tinggi berbanding dengan ekstrak air masing-masing. Analisis kromatografi-spektrometri jisim gas (GC-MS) dan kromatografi cecair ultra-cepat (UFLC) mengesahkan ekstrak metanol dan ekstrak daun mengandungi bilangan dan kandungan bahan kimia yang lebih tinggi daripada ekstrak air dan ekstrak batang masing-masing. Asid benzoik (aktiviti alelopati tertinggi) didapati dalam kepekatan yang paling tinggi dalam ekstrak metanol daun, manakala sebatian ini tidak dijumpai dalam ekstrak air daun. Sebaliknya, kompaun yang utama adalah orientin (aktiviti alelopati terendah) yang dijumpai dalam ekstrak stem. Oleh itu, aktiviti herbisid setiap ekstrak (ekstrak metanol daun, ekstrak air daun, ekstrak metanol batang,, ekstrak air batang) bergantung kepada bilangan, kepekatan, gabungan dan aktiviti perencatan di dalam sebatian. Dalam uji kaji rumah kaca, potensi alelopati serbuk dan ekstrak metanol daun *T. tuberculata* sebagai aplikasi pra dan pasca cambah (semburan pada daun pada 14 hari selepas menyemai) ke atas pertumbuhan dan pembesaran padi dalam penanaman secara kaedah mengubah (Oryza sativa L. Var. MR219) dan tiga rumpai sawah (barnyardgrass, rusiga “smallflower umbrella” dan padi angin) telah dinilai. Magnitud kesan alelopati pada tahap kepekatan sama pada tumbuh-tumbuhan yang diuji adalah bergantung kepada kaedah aplikasi (serbuk dan ekstrak), masa aplikasi (sebelum dan selepas percambahan), kepekatan dan spesies.

Serbuk daun didapati mempunyai sifat menindas lebih tinggi daripada ekstrak daun. Hasil kajian juga mencatatkan lebih perencatan dalam aplikasi ekstrak daun sebagai pra-cambah berbanding dengan permohonan lepas cambah (semburan daun). Kajian di lapangan yang melibatkan perbandingan antara serbuk daun *T. tuberculata*, racun komersil (Solito® (pretilachlor + pyribenzoxim) pada 1 L ha-1) sebagai kawalan positif dan tiada rawatan (kawalan negatif) telah dilakukan. Percambahan dan biomas pokok-pokok rumpai didapati berkurangan dengan ketara dalam plot-plot yang menerima rawatan serbuk daun. Walaubagaimanapun, tidak terdapat perbezaan yang signifikan di antara plot serbuk daun (pada 2 dan 4 t ha-1 dos) dan plot yang menerima rawatan herbisid dari segi peratusan pengurangan kedua-dua parameter (kemunculan dan berat kering rumpai). Terdapat peningkatan yang tinggi ke atas hasil bijirin, berat kering jerami dan bilangan biji bagi setiap tangkai beras, apabila dirawat dengan serbuk daun dan racun komersil berbanding dengan kawalan negatif. Keputusan ini menunjukkan bahawa *T. tuberculata* mengandungi sumber penting perencat pertumbuhan tumbuhan. Oleh itu, ia boleh digunakan sebagai racun herba semula jadi pra-cambah untuk menyekat rumpai di sawah padi dan untuk membangunkan racun herba baru berdasarkan sebatian fitotoksik yang dibebaskan oleh tumbuhan ini.

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I certify that a Thesis Examination Committee has met on 25/8/2015 to conduct the final examination of Farzad Aslani on his thesis entitled " Allelopathic Suppression of Weeds in Rice Field by Tinospora tuberculata Beumee" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the most widely consumed food for more than 557 million people in Southeast Asia (IRRI, 2009), and represents 30-75% of the total daily calories to more than 2 billion Asian people (Haefele and Hijmans, 2007). It is predicted that rice global demand will be doubled by 2050 (Van Nguyen, 2009). Weed infestations are recognized as a serious biological constraint to rice production in both lowland and upland ecosystems at all seasons (Ni et al., 2000). The weed reduces yields by around 10% of total rice production (Bastiaans and Kropff, 2003) which is equivalent to US$30 billion annually (Beltran et al., 2012). Barnyardgrass (*Echinochloa crus-galli*), weedy rice (*Oryza sativa*) and smallflower umbrella (*Cyperus difformis*) are the most troublesome weeds occurring in rice cultivation in Malaysia (Hakim et al., 2010).

The reduction in manual weeding because of the high labor costs has been accompanied globally by intensive use of synthetic herbicides. Approximately, 7% of the total expenditure on herbicides which were equivalent to about US$ 4.10 million is spent on herbicides per year for application in Malaysian rice production systems (Karim et al., 2004). It is expected that the global cost of herbicides in rice cultivation systems could reach US$3 billion per year by 2025 (Zhang et al., 2004). The constant use of chemical herbicides leads to an increased risk of herbicide-resistant, environmental pollution, unsafe agricultural products and negative effects on human health (Vyvyan, 2002). Thus, these problems have caused an interest among researchers to replace synthesis herbicides with alternatives.

The application of strong allelopathic plants as a natural herbicide for weed management can reduce the reliance on chemical herbicide (Khanh et al., 2006a).
There are many higher plants in Southeast Asia and numerous species among them may possess strong allelopathic properties (Hong et al., 2004a). Malaysia has been classified as one of the top rich countries in terms of biodiversity (Eswani et al., 2010).There are 15,000 plant species, of which about 10% are known to be medicinal (Hanum et al., 2001). Nevertheless, only a few studies have been reported regarding the identification of allelopathic plants and associated allelochemicals in this country (Sahid and Sugau, 1993; Ismail and Chong, 2009; Toosi and Bakar, 2012).

Tinospora tuberculata (*Tinospora rumphii* Boerl or *Tinospora crispa*), Malay name: Batawali, belongs to the family Menispermaceae, and is an aromatic shrub with pharmacological properties (Forman, 1981). It is a wild plant that grows in primary rainforests in all parts of Malaysia, Thailand and Indonesia. It is a large, glabrous, deciduous, climbing and a perennial medicinal shrub, originating from the south-western part of China to Southeast Asia. Batawali has been of great interest to Malaysian folk medicine. This galenical has antipyretic, abdominal pain-relieving, antidiarrheal, detoxifying, anthelmintic and tonic properties. Some studies on application of medicinal plants as a natural herbicide for weed management under sustainable agriculture have been widely noticed and confirmed that it is easier to screen allelopathic plants from medicinal plants than other plants (Fujii et al., 2003;

Nazir et al., 2007; Batish et al., 2007a; Islam and Kato-Noguchi, 2013a; Nesrine et al., 2011; Sodaeizadeh et al., 2010; Khanh et al., 2005; Lin et al., 2006).

Therefore, we hypothesized that *Tinospora tuberculata* may produce and release several types of secondary metabolites and could exhibit phytotoxic effects on the growth of rice weeds.

Allelopathic effects of the aerial organs of this plant is not known, except for a single study that dealt with the herbicidal activity of the aerial part of *T. tuberculata*, in which Pukclai and Kato-Noguchi (2012) noted that *T. tuberculata* inhibited hypocotyl and radicle growth of a range of plant species. To date, no experiments have been conducted to assess the allelopathic potential of *T. tuberculata* for weed suppression in rice field soils.

Therefore, a combination of the laboratory, glasshouse and field trials were performed to determine the possibility of using *Tinospora tuberculata* to control rice weeds and it can be achieved by following objectives:

1. To evaluate allelopathic activity of methanol and water extracts derived from aerial parts of *T. tuberculata* and to identify and quantify causative phytotoxic components.

2. To determine effects of leaf powder and leaf methanol extract of *T. tuberculata* as pre-emergence and post-emergence natural herbicide on the growth and development of rice and associated weeds in glasshouse condition.

3. To assess possible utilization of *T. tuberculata* leaf powder as a natural herbicide in rice fields.

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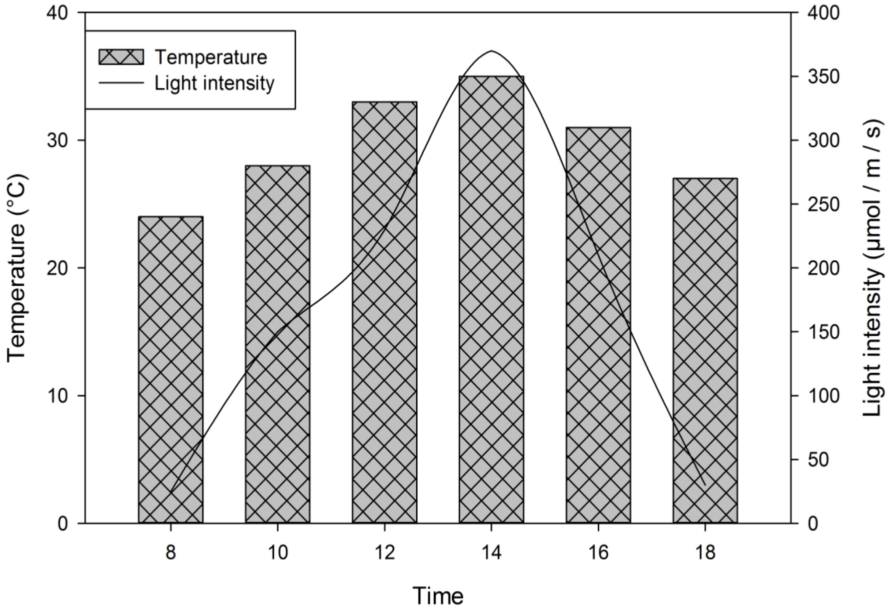
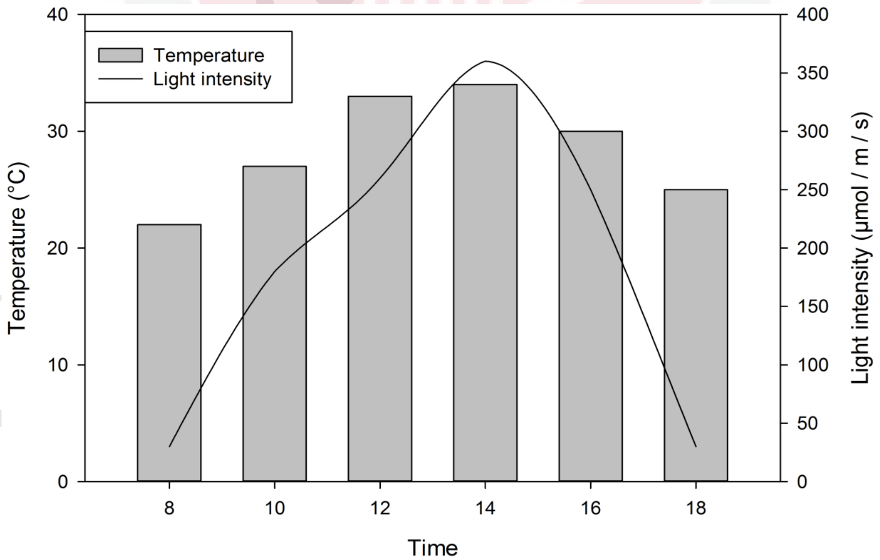
APPENDICES

Appendix A1 Temperature and light intensity fluctuation in the glass house



Appendix A2 Temperature and light intensity fluctuation in the field



Appendix B1 Physico-chemical properties of soil of glass house experiments

Soil properties	Analytical value
Sand (%)	23.1
Silt (%)	45.3
Clay (%)	31.6
pH	6.1
Electrical conductivity (EC dSm^{-1})	1.36
Organic carbon (%)	1.06
Total N (%)	0.13
Available P (ppm)	13.12
Exchangeable Na (m eq/100 g soil)	0.30
Exchangeable K (m eq/100 g soil)	0.27
Exchangeable Ca (m eq/100 g soil)	0.72
Exchangeable Mg (m eq/100 g soil)	0.67
Available S (ppm)	7.01
Available Zn (ppm)	0.82

Appendix B2 Physico-chemical properties of soil of field experiment

Soil properties	Analytical value
Sand (%)	18.1
Silt (%)	37.5
Clay (%)	44.4
pH	6.2
Electrical conductivity (EC dSm^{-1})	1.51
Organic carbon (%)	1.19
Total N (%)	0.18
Available P (ppm)	14.42
Exchangeable Na (m eq/100 g soil)	0.41
Exchangeable K (m eq/100 g soil)	0.32
Exchangeable Ca (m eq/100 g soil)	0.76
Exchangeable Mg (m eq/100 g soil)	0.70
Available S (ppm)	7.04
Available Zn (ppm)	0.88

Appendix C1. ANOVA of emergence and early growth of barnyardgrass treated with leaf and stem methanol extracts

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Extracts	1	3230.7 (<.0001)	2953.3 (<.0001)	1593.0 (<.0001)
Concentration	6	2013.8 (<.0001)	1561.6 (<.0001)	182.5 (<.0001)
Extracts * Concentration	6	3090.8 (<.0001)	263.8 (<.0001)	287.5 (<.0001)
Replication	2	16.7 (0.7232)	7.1 (0.8016)	5.7 (0.3977)

Appendix C2. ANOVA of emergence and early growth of rice treated with leaf and stem methanol extracts

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Extracts	1	12.2 (0.70)	6564.1 (<.0001)	32.2 (0.0204)
Concentration	6	441.8 (0.0013)	3968.9 (<.0001)	615.4 (<.0001)
Extracts * Concentration	6	203.3 (0.0557)	1004.9 (<.0001)	325.8 (<.0001)
Replication	2	48.1 (0.5714)	65.6 (0.2487)	3.9 (0.4819)

Appendix C3. ANOVA of emergence and early growth of weedy rice treated with leaf and stem methanol extracts

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Extracts	1	307.2 (0.0057)	1373.3 (<.0001)	51.0 (0.0029)
Concentration	6	169.1 (0.0017)	2016.5 (<.0001)	431.6 (<.0001)
Extracts * Concentration	6	99.4 (0.0255)	761.8 (<.0001)	133.8 (<.0001)
Replication	2	79.3 (0.1153)	12.9 (0.7319)	26.6 (0.0092)

Appendix D1. ANOVA of emergence and early growth of barnyardgrass treated with leaf and stem water extracts

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Extracts	1	4757.3 (<.0001)	5.4 (0.6339)	1976.3 (<.0001)
Concentration	6	221.6 (0.0018)	744.6 (<.0001)	354.1 (<.0001)
Extracts * Concentration	6	121.3 (0.0356)	63.3 (0.0360)	78.2 (<.0001)
Replication	2	5.9 (0.8753)	32.7 (0.2642)	0.1 (0.9762)

Appendix D2. ANOVA of emergence and early growth of rice treated with leaf and stem water extracts

Source of variance	DF	Mean of squares (Pr>F)		
		Germination	Radicle length	Shoot length
Extracts	1	4.8 (0.6464)	6058.1 (<.0001)	59.0 (0.0078)
Concentration	6	39.9 (0.1484)	3181.5 (<.0001)	470.8 (<.0001)
Extracts * Concentration	6	2.4 (0.9945)	1120.4 (<.0001)	305.7 (<.0001)
Replication	2	7.7 (0.7131)	0.6 (0.9668)	0.5 (0.9204)

Appendix D3. ANOVA of emergence and early growth of weedy rice treated with leaf and stem water extracts

Source of variance	DF	Mean of squares (Pr>F)		
		Germination	Radicle length	Shoot length
Extracts	1	5.4 (0.3771)	2772.1 (<.0001)	294.5 (<.0001)
Concentration	6	13.1 (0.1090)	1983.3 (<.0001)	334.9 (<.0001)
Extracts * Concentration	6	2.5 (0.8817)	98.2 (0.0805)	45.5 (0.0005)
Replication	2	7.7 (0.3298)	3.4 (0.9264)	0.1 (0.9783)

Appendix E1. ANOVA of emergence and early growth of barnyardgrass treated with volatile compounds emission from dry and fresh leaves

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Volatiles	1	464.4 (<.0001)	583.2 (0.0113)	1024.5 (<.0001)
Concentration	3	38.1 (0.0777)	1458.0 (<.0001)	206.8 (<.0001)
Volatiles * Concentration	3	25.5 (0.1766)	366.5 (0.0119)	34.1 (0.2251)
Replication	2	1.5 (0.8902)	38.0 (0.5807)	11.8 (0.5771)

Appendix E2. ANOVA of emergence and early growth of rice treated with volatile compounds emission from dry and fresh leaves

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Volatiles	1	45432.0 (<.0001)	74.3 (0.0224)	184.6 (0.0098)
Concentration	3	0.04 (0.4157)	1019.2 (<.0001)	185.6 (0.0016)
Volatiles * Concentration	3	0.02 (0.6424)	29.5 (0.0912)	7.2 (0.7853)
Replication	2	0.04 (0.3672)	11.5 (0.3807)	6.0 (0.7452)

Appendix E3. ANOVA of emergence and early growth of weedy rice treated with volatile compounds emission from dry and fresh leaves

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Volatiles	1	8.01 (0.0023)	2956.9 (<.0001)	29.1 (0.2880)
Concentration	3	3.1 (0.0107)	3245.8 (<.0001)	272.5 (0.0006)
Volatiles * Concentration	3	2.1 (0.0369)	20.0 (0.6670)	240.3 (0.0010)
Replication	2	0.9 (0.2102)	6.6 (0.8392)	8.0 (0.7181)

Appendix F1. ANOVA of growth of barnyardgrass treated with leaf methanol extract at two weeks old stage, under hydroponic condition

		Mean of squares (Pr>F)						
Source of variance	**DF**	**Root length**	**Shoot length**	**Dry Weight**	**Transpiration**	**Chl a**	**Chl b**	**Carotenoids**
Concentration	4	970.8 (<.0001)	1428.6 (<.0001)	0.6 (0.0004)	718.5 (<.0001)	157.1 (<.0001)	36.0 (<.0001)	4.0 (<.0001)
Replication	2	6.0 (0.3068)	5.9 (0.4222)	0.0 (0.9996)	52.0 (0.0596)	0.01 (0.1991)	0.0 (0.0043)	0.0 (0.1244)

Appendix F2. ANOVA of growth of rice treated with leaf methanol extract at two weeks old stage, under hydroponic condition

		Mean of squares (Pr>F)						
Source of variance	**DF**	**Root length**	**Shoot length**	**Dry Weight**	**Transpiration**	**Chl a**	**Chl b**	**Carotenoids**
Concentration	4	261.6 (0.0003)	175.5 (0.0002)	0.4 (0.0074)	364.5 (0.0005)	0.1 (<.0001)	0.1 (0.0119)	0.7 (<.0001)
Replication	2	4.2 (0.5789)	4.8 (0.2393)	0.0 (0.9110)	4.0 (0.7280)	0.04 (0.0016)	0.02 (0.2612)	0.0 ( )

Appendix F3. ANOVA of growth of weedy rice treated with leaf methanol extract at two weeks old stage, under hydroponic condition

		Mean of squares (Pr>F)						
Source of variance	**DF**	**Root length**	**Shoot length**	**Dry Weight**	**Transpiration**	**Chl a**	**Chl b**	**Carotenoids**
Concentration	4	514.5 (0.0001)	412.9 (<.0001)	1.0 (0.0792)	2509.1 (<.0001)	7.0 (<.0001)	3.0 (<.0001)	0.1 (<.0001)
Replication	2	1.3 (0.8789)	0.8 (0.8734)	0.3 (0.3285)	12.2 (0.6525)	0.0 (0.8901)	0.04 (0.0003)	0.0 (0.5637)

Appendix G. ANOVA of emergence and early growth of barnyardgrass treated with detected compounds

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Germination**	**Radicle length**	**Shoot length**
Compounds	11	15.7 (<.0001)	1062.1 (<.0001)	65.7 (<.0001)
Concentration	4	132.4 (<.0001)	7092.0 (<.0001)	584.2 (<.0001)
Compounds * Concentration	44	7.1 (<.0001)	82.9 (<.0001)	15.7 (<.0001)
Replication	2	5.2 (0.1555)	13.2 (0.0948)	42.3 (0.0006)

Appendix H1. ANOVA of emergence and growth of barnyardgrass treated with powder and extract of leaf under glasshouse condition

		Mean of squares (Pr>F)			
Source of variance	**DF**	**Germination**	**Plant height**	**Shoot dry weight**	**Root dry weight**
Powder and extract application	1	1.0 (0.1264)	24.6 (0.5992)	20.5 (<.0001)	0.6 (0.0146)
Concentration	3	21.0 (<.0001)	4155.8 (<.0001)	185.7 (<.0001)	10.3 (<.0001)
Powder and extract application * Concentration	3	0.08 (0.8957)	6.0 (0.9753)	3.4 (<.0001)	0.09 (0.3841)
Block	3	0.3 (0.4581)	56.6 (0.5885)	0.7 (0.0441)	0.09 (0.3714)

Appendix H2. ANOVA ANOVA of emergence and growth of smallflower umbrella treated with powder and extract of leaf under glasshouse condition

		Mean of squares (Pr>F)			
Source of variance	**DF**	**Germination**	**Plant height**	**Shoot dry weight**	**Root dry weight**
Powder and extract application	1	12.2 (0.1077)	197.3 (0.0003)	7.3 (0.0213)	10.7 (0.0020)
Concentration	3	314.2 (<.0001)	1173.1 (<.0001)	173.6 (<.0001)	136.9 (<.0001)
Powder and extract application * Concentration	3	7.8 (0.1778)	97.5 (0.0005)	1.4 (0.3230)	2.6 (0.0478)
Block	3	2.3 (0.6563)	14.61 (0.2793)	0.4 (0.7386)	1.6 (0.1626)

Appendix H3. ANOVA of emergence and growth of weedy rice treated with powder and extract of leaf under glasshouse condition

		Mean of squares (Pr>F)			
Source of variance	**DF**	**Germination**	**Plant height**	**Shoot dry weight**	**Root dry weight**
Powder and extract application	1	3.7 (0.0233)	11.3 (0.3045)	86.2 (<.0001)	17.4 (0.0006)
Concentration	3	16.5 (<.0001)	107.8 (0.0002)	123.6 (<.0001)	126.5 (<.0001)
Powder and extract application * Concentration	3	0.5 (0.4885)	23.5 (0.1086)	22.8 (<.0001)	7.6 (0.0017)
Block	3	2.1 (0.0349)	2.3 (0.8736)	1.7 (0.2286)	1.6 (0.2315)

Appendix J1. ANOVA of growth of barnyardgrass sprayed with leaf extract under glasshouse condition

		Mean of squares (Pr>F)	
Source of variance	**DF**	**Plant height**	**Biomass**
Concentration	3	12955.8 (<.0001)	0.7 (0.0001)
Block	3	351.9 (0.1337)	0.1 (0.0331)

Appendix J2. ANOVA of growth of smallflower sprayed with leaf extract under glasshouse condition

		Mean of squares (Pr>F)	
Source of variance	**DF**	**Plant height**	**Biomass**
Concentration	3	264.2 (<.0001)	0.1 (0.0016)
Block	3	27.5 (0.0271)	0.01 (0.2646)

Appendix J3. ANOVA of growth of weedy rice sprayed with leaf extract under glasshouse condition

		Mean of squares (Pr>F)	
Source	**DF**	**Plant height**	**Biomass**
Concentration	3	1.5 (0.7591)	0.007 (0.8874)
blk	3	6.8 (0.2345)	0.01 (0.6408)

Appendix K. ANOVA of emergence and growth of natural weed growth in rice field soil treated with leaf powder under glasshouse condition

		Mean of squares (Pr>F)	
Source of variance	**DF**	**Dry weight of weed**	**Number of weed**
Concentration	3	1817.0 (0.0006)	1399.6 (0.0006)
Block	3	26.7 (0.8677)	81.8 (0.4205)

Appendix L. ANOVA of growth of rice treated with leaf powder and leaf extract under glasshouse condition

		Mean of squares (Pr>F)		
Source of variance	**DF**	**Yield**	**Straw**	**Plant height**
Powder and extract application	2	0.09 (0.3966)	0.1 (0.2634)	6.5 (0.4880)
Concentration	3	0.5 (0.0053)	0.1 (0.1968)	3.8 (0.7294)
Powder and extract application * Concentration	6	0.1 (0.2721)	0.03 (0.8376)	0.8 (0.9965)
Block	2	0.1 (0.2494)	0.01 (0.8075)	20.0 (0.1295)

Appendix M. ANOVA of emergence and growth of weeds and rice treated with leaf powder under field condition

		Mean of squares (Pr>F)						
Source	**DF**	**Yield**	**Straw**	**100 seed weight**	**Number of seed per panicle**	**Plant height**	**No. weed**	**Dry weight weed**
Leaf powder	4	0.7 (0.0010)	0.8 (<.0001)	0.004 (0.8775)	342.5 (0.0055)	3.1 (0.9359)	1517.4 (<.0001)	8988.6 (<.0001)
Block	2	0.01 (0.7834)	0.05 (0.1088)	0.001 (0.9020)	11.6 (0.7546)	4.0 (0.7833)	23.4 (0.4131)	86.4 (0.2626)

BIODATA OF STUDENT

Farzad Aslani is from Tehran, the capital of Iran. He was born into a Kurdish family in Tehran, on 12 March 1984. He is the first child of Mohammadsedigh Aslani and Khadijeh Mohammadi. He graduated from High School in 2001 and then earned a 4-year BSc degree in Agricultural engineering in 2007 from Lorestan University, Khoramabad, Iran and his MS (Agronomy) in 2009 from Islamic Azad University Science and Research Branch. In April 2012, he enrolled in Faculty of Agriculture at Universiti Putra Malaysia (UPM), Malaysia as a Ph. D student in the field of Weed Science, and was awarded Special Graduate Research Allowance Scheme (S-GRA) and Graduate Research Fellowship (GRF) by UPM for pursuing his Ph. D study. During his Ph. D candidature, he has published several articles in different journals as you can see his curriculum vitae through following accounts:

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LIST OF PUBLICATIONS

A. Journal articles

1. **Aslani**, **F**., Juraimi, A. S., Ahmad-Hamdani, M. S., Dzolkhifli, O., Alam, M. A., Hakim, M. A., and Uddin, M. K. (2013). Allelopathic effects of Batawali (*Tinospora tuberculata*) on germination and seedling growth of plants. *Res. on Crops*, 14 (4), 1222-1231. (IF=0.10; Q4)
2. **Aslani**, **F**., Juraimi, A. S., Ahmad-Hamdani, M. S., Dzolkhifli, O., Alam, M. A., Hashemi, F. S. G., Hakim, M. A., and Uddin, M. K. (2014). Allelopathic effect of methanol extracts from *Tinospora tuberculata* on selected crops and rice weeds. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science*, 64, (2), 165–177. (IF=0.64; Q3)
3. **Aslani**, **F**., Juraimi, A. S., Ahmad-Hamdani, M. S., Alam, M. A., and Dzolkhifli, O. (2015). Phytotoxic interference of volatile organic compounds and water extracts of *Tinospora tuberculata* on growth of rice weeds. *South African Journal of Botany,* 100, 132–140. (IF=1.34; Q2)
4. **Aslani**, **F**., Juraimi, A. S., Ahmad-Hamdani, M. S., Dzolkhifli, O., Alam, M. A., Hashemi, F. S. G., Hakim, M. A., and Uddin, M. K. (2015). Variation in the phytotoxic activity of Tinospora tuberculata extracts as influenced by solvent type and chemical profile. *Current Science.* Accepted. (IF=0.94; Q2)
5. **Aslani**, **F**., Juraimi, A. S., Ahmad-Hamdani, M. S., Alam, M. A., Golestan Hashemi, F. S., Hakim M. A., and Md. Kamal Uddin. (2015). Factors influencing the natural herbicides of plant origin. *Scientia Agricola*. Accepted. (IF= 0.80; Q2)
6. **Aslani**, **F**., Juraimi, A. S., Ahmad-Hamdani, M. S., Alam, M. A., Golestan Hashemi, F. S., Hakim M. A., and Md. Kamal Uddin. (2015). Phytotoxic influences of leaf methanol extract of Tinospora tuberculata on seedling growth of rice and associated weeds in hydroponic culture. *Journal of Integrative Agriculture*. Accepted. (IF= 0.83; Q2)
7. **Aslani**, **F**., Juraimi, A. S., Ahmad-Hamdani, M. S., Alam, M. A., Golestan Hashemi, F. S., Hakim M. A., and Md. Kamal Uddin. (2015). Allelopathic suppression of weeds in rice field by Tinospora tuberculata leaves. *Journal of Agricultural Science*. Under review. (IF= 1.15; Q2)

B. Proceedings paper

1. **Aslani**, **F**., and Juraimi, A. S. (2014). Phytotoxic behavior of *Tinospora tuberculata* extracts against crops and paddy weeds. 8th International conference on plant protection in the tropics. 8-10 April, Berjaya Times Square Hotel, Kuala Lampur, Malaysia, 78-80.