ALLELOPATHIC POTENTIAL OF BANGLADESH INDIGENOUS RICE VARIETIES

(バングラデシュの在来イネのアレロパシーについて)

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March 2018

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The dissertation submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (Ph.D.)

in Agriculture

The United Graduate School of Agricultural Sciences
Kagoshima University

BY

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Science of Bioresource Production

The United Graduate School of Agricultural Sciences

Kagoshima University, Japan

March 2018

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To my parents, Your indefinite LOVE

To Professor Dr. Md. Hazrat Ali, for His affectionate guidance

"As you go the way of life, you will see a great chasm. Jump. It is not as wide as you think".

Robert L. Zimdahl

ALLELOPATHIC POTENTIAL OF BANGLADESH INDIGENOUS RICE VARIETIES

ENGLISH ABSTRACT

Rice (*Oryza sativa* L.) is among the most focused on food crops worldwide and its production is a crucial part of the national economy of Bangladesh. Weeds are a major constraint to rice production globally. Concern over environmental and human health impacts from herbicidal weed management practices have researchers seeking innovative strategies for weed control. This Ph.D. project uses an application of the knowledge regarding the recent inclinations in ecological management of the weeds by using Bangladeshi indigenous rice (*O. sativa* L. spp. *indica*) allelopathy and their allelochemicals. A series of experiments was conducted in the laboratory, glasshouse, and field of the Subtropical Field Science Centre, University of the Ryukyus, Japan from April 2015 to November 2017 to assess the allelopathic potential of 50 Bangladeshi indigenous rice.

Initially 'Boterswar', 'Goria', 'Biron' and 'Kartiksail' varieties were screened out as the most allelopathic by donor-receiver bioassay and equal compartment agar method (ECAM) tests, where *Lactuca sativa* L., *Lepidium sativum* L., *Raphanus sativus* L., *Echinochloa crus-galli* L. Beauv. and *E. colona* L. were used as test species. Among these selected four varieties 'Boterswar' gave the strongest inhibitory effect on the growth of *E. crus-galli* seedlings in both aqueous methanol extract and aqueous extract tests in laboratory and glasshouse condition, respectively.

Four biologically active compounds, syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), (-)-loliolide, 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one and 3-hydroxy-*β*–ionone, were isolated and identified from the ethyl acetate phase of 'Boterswar' plant extract. The concentration of the compounds as low as 10 µM significantly inhibited the root and shoot growth of *E. crus-galli* seedlings.

In the field study, the infestation levels of weeds were estimated using Simpson's Diversity Index (*SDI*) which ranged from 0.2 to 0.56, in which a significant correlation coefficient (0.87, P <0.001) was obtained by comparing with the root inhibition (%) from the in vitro bioassay. The variety 'Boterswar' was found as the most allelopathic among six tested varieties including weakly allelopathic 'Hashikolmi' and non-allelopathic 'Holoi'.

An experiment was conducted to separate and assess the extent of allelopathic interference relative to resource competition by the interactions between the varieties 'Boterswar', 'Hashikolmi', *E. crus-galli* var. *oryzicola* via a target (rice) – adjacent (*E. oryzicola*) mixed culture in a hydroponic system. The results showed that the allelopathic effects of 'Boterswar' were much higher than the resource competition in rice-*E. oryzicola* mixed-cultures and verified that the allelopathic effect of 'Boterswar' was leading in rice-*E. oryzicola* interactions.

Allelopathic rice 'Goria' straw incorporation into the soil gave inhibitory effects on the growth and dry weight of *E. oryzicola* in a pot study but had no autotoxicity on the growth of rice variety. Aqueous methanol extracts of 'Goria' straw inhibited the seedling growth of *L. sativum* and *E. oryzicola*, and two biologically active compounds (-)-loliolide and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one were isolated and identified. The inhibitory activity of 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one on the seedling growth of *L. sativum* and *E. oryzicola* was more than (-)-loliolide as demonstrated by comparison of the I_{50} values. However, a strong synergistic inhibitory activity of both compounds was observed on the growth of test species.

Among identified compounds, syringaldehyde was compared to another allelochemical (*trans*-cinnamic acid) and one herbicide 'Nominee' (i.e., 100 g/L bispyribac-sodium) to develop an understanding of rice allelopathy and the phytotoxicity of the allelochemicals. Syringaldehyde inhibited seed germination of *E. crus-galli* completely at the concentration of

1000 µM, and delayed seed germination and significantly affected the germination indices from 100 µM. In general, with the increasing concentration from 100 to 1000 µM, the inhibitory effects on seedlings growth of test species increased and leaf blade wilting, chlorosis and necrosis occurred. Roots of *E. crus-galli* treated with 1000 µM syringaldehyde had black points on root nodes but had no root hairs, root pith cells contracted or reduced, and had fewer and larger vacuoles compared to the control. The syringaldehyde also showed remarkable effects on the growth, physiology and biochemical content of *E. crus-galli* seedlings, supporting the hypothesis that the allelochemicals caused a chemical interference.

Considering the results of all the experiments among 50 Bangladeshi indigenous rice variety 'Boterswar' was found to be the most promising allelopathic variety. Thus, the allelopathic potential of Bangladesh indigenous rice raises the opportunity to be utilized for weed control in the form of allelopathic rice variety in crop rotation, use in allelopathic variety development, mulching or incorporation, and/or synthetization of possible natural herbicides to achieve sustainable weed management.

バングラデシュ在来イネのアレロパシーに関する研

学位論文要旨

バングラデシュ人民共和国は、耕地面積の 75%を稲作が占めており、稲作における雑草管理は、収量の損失を避ける重要な作業となっている。

本研究では除草剤に頼らない稲作体系にアレロパシー作用の活用を検討するため、2015 年 4 月から 2017 年 11 月まで琉球大学農学部附属亜熱帯フィールド科学教育研究センターの施設、ガラスハウスおよび圃場において、バングラデシュ在来イネ（*Oryza sativa* L. spp. *indica*）を用いて一連の実験を行った。

実験では *Lactuca sativa*, *Lepidium sativum*, *Raphanus sativus*, *Echinochloa crus-galli* および *E. colona* の 5 種を検定植物として用い、バングラデシュ在来イネ 50 品種の中からアレロパシーによる生育抑制作用が高い Boterswar、Goria、Biron および Kartiksail を選抜した。選抜した 4 品種のうち Boterswar は、メタノール抽出物および水性抽出物を用いた実験において、*E. crus-galli* 実生の成長に最も強い抑制効果を与えた。

Boterswar を酢酸エチルで抽出した相から、syringaldehyde (4-hydroxy-3, 5-dimethoxybenzaldehyde), (-)-loliolide, 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one and 3-hydroxy-*β* –ionone の 4 種の生物活性物質を単離し同定した。これらの物質はそれぞれ 10μM で、*E. crus-galli* 実生の根および芽の成長を有意に抑制した。

圃場実験において、雑草の侵入レベルを推定するシンプソンの多様度指数（SDI）が Boterswar は、0.2～0.56 となり最も高いアレロパシー作用を示した。

アレロパシーによる抑制効果と養水分競合による効果を分けるため、Boterswar と *E. oryzicola* を水耕栽培で実験を行った。この実験により、Boterswar のアレロパシー効果は、養水分競合よりもはるかに高かった。

次に、アレロパシー作用を持つ Goria のわらを土壌へすき込んだポット実験を行ったところ、*E. oryzicola* の生育と乾物重に抑制効果をもたらしたが、イネの生育には影響は見られなかった。Goria のわらのメタノール抽出物は *L. sativum* と *E. oryzicola* の実生成長を阻害し、生物学的に活性のある 2 つの化合物 (-)-loliolide と 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one が単離および同定された。*L. sativum* および *E. oryzicola* の幼植物の成長に対する 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen -9-one の阻害活性は (-)-loliolide よりも高かった。

同定された syringaldehyde の植物に対するアレロパシー効果を検討するため、代表的なアレロケミカル・*trans*-cinnamic acid と除草剤「ノミニー液剤」を用いて比較実験を行った。Syringaldehyde は *E. crus-galli* の種子発芽を 1000 μM の濃度で完全に阻害し、100 μM の濃度で遅延させ、発芽指数の急激な低下に有意に影響した。syringaldehyde 1000 μM で処置した *E. crus-galli* の根は、対照区と比較すると、根節に黒点を生じ根毛がなく、根髄細胞が収縮または縮小し、空隙の数は少ないがサイズは大きかった。100～1000 μM の濃度増加に伴い、検定植物 *E. crus-galli* の苗生育に対する阻害効果が増大し、葉身のクロロシスおよび壊死が生じた。

以上の研究成果は、バングラデシュ在来イネ 50 品種中から雑草生育に抑制的に作用するアレロパシーの高い品種を選抜し、また、4 種の生物活性物質を単離・同定するとともにその作用を検証したものであり、イネが持つアレロパシーによる雑草

防除の可能性について新たな知見をもたらしたもので、特にバングラデシュの稲作における雑草防除に有益な情報もたらす研究成果である。

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LIST OF ABBREVIATIONS

%: Percent

@: At the rate of

°C: Degree Centigrade

a.i: Active ingredient

$CDCl_3$: Chloroform D

CD_3OD: Methanol-d4

COSY: Correlation spectroscopy

DMSO: Anhydrous dimethyl sulfoxide

EDTA: Disodium ethylenediamine tetra acetic acid

ESI: Electrospray ionization

et al.: And others

etc.: Etcetera

EtOAc: Ethyl acetate

G: Gram

GC: Gas chromatography

HMBC: Heteronuclear multiple bond correlation

HMQC: Heteronuclear multiple-quantum coherence experiment

HPLC: High performance liquid chromatography

i.e.: That is

I_{50}: Concentration of approximately 50%

IR: Infrared

kg: Kilograms

km: Kilometers

LC: Liquid chromatography

LSD: Least significant difference

m^2: Meter square

MHz: Megahertz

MOP: Murate of Potash

MS: Mass spectrometry

NADPH: Nicotinamide adenine dinucleotide phosphate

NMR: Nuclear magnetic resonance

PAL: Phenylalanine Ammonia-lyase

ppm: Parts per million

SDI: Simpson’s Diversity Index

t ha^{-1}: Tons per hectare

TMS: Tetramethylsilane

TSP: Triple Super Phosphate

viz.: Namely

µM: Micro molar

CHAPTER I

GENERAL INTRODUCTION

Rice (*Oryza sativa* L.) is a semi-aquatic cereal favored by the hot and humid climate (Mikkelsen et al. 1995). It is among the most interesting three food crops of the world from both social and economic point of views. Rice is grown more than one hundred countries in nearly 1.5 million square kilometers of land producing more than 700 million tons every year and is the staple food for nearly half (3.5 billion people) of the world's population (Prasad et al. 2017). Rice has now become a foreign exchange earner in many countries and playing a big role in the economy and it is the crucial economy part of Bangladesh (Workman 2017). Bangladesh with its flat topography, abundant water, and humid tropical climate constitutes an excellent habitat for the rice plant (Haque et al. 2016), and the world's fourth-largest rice producer (53.1 million MT, FAOSTAT 2016). There are eight thousand indigenous rice varieties in Bangladesh, and more than one thousand rice varieties are being grown by farmers due to their wide adaptability in saline, flood or drought-prone areas, superior grain quality and resistance against biotic stress (Hussain et al. 2013). Regrettably, in Bangladesh as well as in the world after the introduction of high yielding varieties research interest shifted from indigenous varieties to modern varieties of rice and thus the indigenous resources remained neglected (Masum et al. 2008). The ability to control weeds efficiently has been one of the key components, along with the use of fertilizers, of the 'green' revolution that resulted in formidable increases in crop yield over the past 50 years. Whereas, modern rice weed management practices rely significantly on the use of herbicides. However, with the current trends of increasing agricultural activities worldwide, it becomes crucial to protect our remaining natural ecosystems from non-sustainable forms of human use.

Weeds are the biggest barrier to rice production around the globe (Adkins 2017). While many factors cause losses in rice production, there is little doubt that weeds are a major concern. Weeds compete with rice plants and reduce yield through competition for water, nutrients, light, and spaces. The annual rice yield loss due to weed infestation is about 60–80% (Dass et al. 2017). It has been reported China losses 10 million metric tons of rice production yearly due to weed competition (Zhang 2001). Weed infestation also reduces the grain quality (Kwon et al. 1991). However, in many situations, the negative impacts of weeds are not perceived to be as dramatic, as those caused by pests and diseases. It appears, historically that farmers were most concerned by the losses from pests, other than weeds. Estimates indicate that about 30% of rice farmers are losing about 500 kilograms per hectare of rice, due to poor weed control. As in other countries, weeds cause a serious problem for rice production in Bangladesh. Both the low and upland rice fields in Bangladesh are generally infested with many tropical and subtropical weeds such as *Echinochloa cruss-galli*, *Echinochloa colona, Leersia hexandra*, *O. rufipogon*, *Cyperus esculentus*, *C. diformis, Fimbristylis miliacea*, *Monochoria vaginalis*, *Spilanthes acmella*, *Eclipta prostrata* etc., and weed management strategies in Bangladesh have shifted from non-chemical to the intense use of herbicides (Ali 2017).

In the past 50 years, researchers reported weed control as an essential part of cropping. Many scientists think that a basic understanding of science is necessary before a practical application is developed as the present challenge that is causing the most concern to agriculture and is compromising the environment. Therefore, it’s important to consider possible new improvements to rice production and environmental protection through better Weed Science.

Weed Science is a scientific discipline that deals with weeds which is a serious biotic threat capable of causing heavy economic loss to the farmer (Monaco et al. 2002; Fernandez-Quintanilla et al. 2008). However, Weed Science research mostly observant on weed control (physical, chemical and biological). While less care is paid on basic principles, such as ecology and weed biology. Weeds are difficult to control for a number of reasons. Some of the important biological characteristics shared by weeds include large numbers of seed production, persistent of seeds in soil seed banks, seasonal dormancy, more than one mode reproduction (sexual and vegetative). Therefore, without identifying the weakness of the problematic weeds, it would be difficult to develop effective and new control technique.

Among 250 *Echinochloa* plants species, most of them are considered as weed (Bajwa et al. 2015) which vary in their growth habit, distribution, and morphology (Barret & Wilson 1983). Among them *E. crus-galli* spp. is one of the most noxious weeds in rice (Michael 2003), produces large number seeds, has competitive and adaptive features which are required for survival and successful competition under a wide range of geographical and climatic conditions (Marambe & Amarsinghe 2002). It may limit 21-79% rice yield, depending on the variation of cropping and management (Ottis & Talbert 2007; Wilson et al. 2014). Even at a ratio of 100 rice plants to 10 *E. crus-galli* plants, rice biomass is reduced by 75% and yield is narrowed by about 50% (Graf 1992). The persistent use of herbicides in herbicide-tolerant rice varieties has encouraged weeds to evolve herbicide resistance especially *E. crus-galli* evolve very quickly more than any other weeds in worldwide (Heap 2017) and this is the case the world's main weed of rice is *E. crus-galli* (Macias et al 2006). Therefore, understanding the crop - weed interactions is a way to control this noxious weed. Since plant interactions such as resource competition or allelopathy play an important role in the species performance and their establishment in the field (Muller 1966).

Allelopathy arises from the chemical interactions by one plant species that affect other species in its vicinity where some plants influence the growth and development of other plants through the release of allelochemicals (Molisch 1937; Rice 1984). It has been demonstrated a factor of ecological implication by manipulating plant succession, dominance, climax formation, species diversity, the structure of plant communities and productivity (Chou 1999). In agroecosystems, allelopathic effects between living weeds and crops, crops in mixtures, plant residues and succeeding crops during decomposition of residue are also well documented (Putnam & Tang 1986; Rice 1984).

This phenomenon has been observed for over 2000 years. Reports as early as 300 BC documented that many crop plants (eg., *Cicer arietinaum*, *Hordeum vulgare*, *Lathyrus linifolius*) demolished weeds and inhibited the growth of other crop plants. However, intensive scientific research on this phenomenon only started in the 20th century. The term allelopathy was first introduced by a German scientist Molisch in 1937 to include both inhibitory and stimulatory biochemical interactions between all types of plants including microorganisms. Muller (1969) somewhat used plant interference, including both competition and allelopathy, and defined competition to mean that one plant takes up necessary substances form a habitat so as to have a harmful effect on the growth of other plants that required the same substances. On the other hand, allelopathy is the process that plant releases phytotoxic compounds into the environment to inhibit the growth of plant sharing the same habitat. However, many plant studies recommended that allelopathy and resource competition could play synergically (Rasmussen & Einhellig

1977; Einhellig 1986; Mallik 1998). Whittaker and Feeny (1971) published a classic monograph entitled “Allelochemics: chemical interaction between species”, and stated that “chemical agents are of major consequence in the adaptation of species and organization of communities.” Allelopathy thus plays a significant role in plant dominance, succession, the formation of plant communities and climax vegetation, and crop productivity (Muller 1969; Rice 1984; Rizvi & Rizvi 1992; Chou 1999). Initially, Chou and Waller (1983) used the widespread term “allelochemical” by coining from allelochemics, which deals with the mechanism of chemical interactions among organisms, such as crop-weed. Rice (1984) reinforced this definition in the first review on allelopathy. This definition considers all biochemical interactions between living systems - where plants, algae, bacteria, fungi and their environments are included. Modern researchers have broadened the context of allelopathy to include interactions between plants and higher animals, and have suggested that allelopathy is a sub-discipline of a whole network of chemical ecology between plants, and between plants and other organisms, including bacteria, yeasts, insects and mammals, and that such communication may contribute to plant defense (Harborne 1987; Lovett & Ryuntyu 1992; Einhellig 1995; Siemens et al. 2002). Finally, the definition of allelopathy accepted by the International Allelopathy Society (IAS) and according to the definition, it involves interaction between plants and other organisms excluding animals (Torres et al. 1996). Although the clarification of allelopathy includes both inhibitory and stimulatory feature of allelochemical action, most explanations suggest rather mostly harmful (negative) effect of the allelopathic compound on receiver plant. Allelochemicals are secondary metabolites, biosynthesized from the carbohydrates, fats and amino acids metabolism and arise from acetate or the shikimic acid pathway (Seigler 2006). Although these are biosynthesized and stored in the plant cells, do not affect the cell activities. However, after their release from the plant cells through volatilization, leaching root exudates and decomposition of biomass, these allelochemicals start influencing the organisms (plants, pathogens, insect pests, etc.), when they come in contact. Allelochemicals may regulate plant growth and development, including seed germination, photosynthesis, respiration, transpiration, biochemical metabolism and even in the molecular basis of protein and nucleic acid synthesis (Chou 2006). Different plant parts contain allelochemicals, but also leachates and root exudates may be vital sources of these compounds and are therefore taken into consideration (Ebana et al. 2001; Kong et al. 2004a). Each year allelochemists isolate and identify several classes of compounds including phenolics, alkaloids, terpenoids, polyacetylenes, fatty acids, and steroids from higher plants and microbes as allelochemicals (Inderjit et al. 2008).

Rice is known to have allelopathic potential crop and rice allelopathy has been studied for more than 20 years when Dilday et al. (1994) examined about 10,000 accessions from rice germplasm collections for allelopathic effects on ducksalad [*Heteranthera limosa* (Sw.) Willd]. Now allelopathic rice and its role in weed suppression have been reported from worldwide. However, a few rice varieties or straw left in the fields after harvesting can produce and release allelochemicals into the environment to inhibit the adjacent and succeeding plants (Olofsdotter et al. 1999; Gealy et al. 2003; Kong et al. 2008). Olofsdotter et al. (1997) reported that 45 out of 1000 screened rice varieties contained promising allelopathic activity against one or more weeds. About 20-40% of 1000 rice varieties in Egypt have shown strong allelopathic activity against indicator plants (Hassan et al. 1998). In Japan, 24 out of 189 rice strains belonging to Japonica (72), Indica (18), tropical Japonica (32), Chinese (29) and African rice types (4), and 34 unknown strains, showed strong allelopathic activity (about 75% inhibition against indicator plant growth). Tropical Japonica and African rice (*O. glaberrima*) possessed greater allelopathic potential than other types, especially the improved types, exhibited the least allelopathic activity (Fujii 1992; Fuji & Shibuya 1992). Among Japanese rice varieties 'Koshihikari' marked the greatest inhibitory activity on several paddy weeds including *E. crus-galli* (Kato-Noguchi et al. 2002). In India, 12 rice varieties were evaluated for their allelopathic activity against *Phalaris minor* Retz.; only three varieties inhibit the germination of *P. minor* by more than 50% (Om et al. 2002). 'Taichung'-an indigenous variety of the Republic of Korea was found allelopathic potential against *Triantema portulacastrum* L., *Echinochloa* spp., and *L. sativa* (Kim et al. 2005). In China allelopathic rice 'PI312777' reported for many weeds suppressions including *E. crus-galli*, *E. colona*, *C. difformis*, *C. irria*, *E. prostrata* and *M. vaginalis* (Kong et al. 2008). Fifteen Iranian rice varieties were evaluated for their allelopathic activity and phenolic contents, only three varieties 'Dinorado' 'Domsorkh' and 'Dular' were found to be possessed highest allelopathic (Berendji et al. 2008). In Australia, Seal and Pratley (2010) found seven rice genotypes out of 27 rice genotypes expressed a greatly variable allelopathic activity against weeds such as *E. crus-galli, Sagittaria graminea* Michx., and *Alisma lanceolatum.* Among 40 rice varieties from Sri Lanka four were found most allelopathic potential against *E. crus-galli* in pot, tray, and field experiments (Ranagalage & Wathugala 2015). Similarly, allelopathic activity of rice varieties on several weeds reported from various parts of the world (Mattice et al. 1998; Ahn & Chung 2000; Rimando et al. 2001; Jung et al. 2004; Chung et al. 2006; Khanh et al. 2007a; Mennan et al. 2012; Gealy et al. 2013; Ma et al. 2014; Le Thi et al. 2014; El Shamey et al. 2015).

It is thought that the indigenous rice varieties contain different allelochemicals which could suppress the population and growth of weeds, and many allelochemicals remain unidentified (Einhellig 1995). Dilday et al. (1998) reported from their field experiment in the USA during 1989, Bangladeshi rice variety 'Mala' inhibited 80% of the growth of *H. limosa*. Hassan et al. (1998) found an allelopathic activity of Bangladeshi rice 'BR 4608-R1-R2' 70-90 % around *E. crus-galli* in the field of Egypt. Main et al. (2007) observed that in Bangladesh most of the common weed species are dominant in semi-dwarf modern variety than in indigenous tall varieties. Salam and Kato-Noguchi (2009) compared allelopathic activity among 102 modern and indigenous varieties and they reported that modern variety 'BR17' and indigenous variety 'Kartiksail' are the most allelopathic. Karim et al. (2014) found that the highly allelopathic varieties were 'Kataribhog', 'WooCo', 'WITA12', 'Dular', 'Lalpaika', 'BRRI dhan27', 'WITA3', 'FARO8', 'BR26', 'BRRI dhan39', 'IR64', 'WITA8', 'Dharial' and 'Nizersail' among 120 modern and indigenous rice varieties in Bangladesh.

Allelopathy research is not completed until the fundamental part allelochemicals present in the materials are isolated, identified and characterized. Several allelochemicals have been documented from rice in different parts of the world, many were found in aqueous extracts, rice root exudates, and decomposing residues. Chung *et al.* (2001) identified some secondary metabolites including ferulic acid as allelopathic compounds from straw extracts of rice varieties. The labdane-related diterpenoid momilactones are the most significant rice allelochemicals, with momilactone B playing the main role (Kato-Noguchi 2017). Kato-Noguchi et al. (2002) identified momilactone B from root exudates of Japanese rice variety 'Koshihikari'. Similarly, many researchers around the world identified momilactones A and B from rice plant extracts and root exudates (Chung et al. 2006; Mennan et al. 2012; Xu et al. 2012 Schmelz et al. 2014; El Shamey et al. 2015). Kong et al. (2004a) isolated and identified 3-isopropyl-5-acetoxycyclohexene-2-one-1 and 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone from allelopathic rice variety ‘PI312777’. Seal et al. (2004a) isolated and identified as allelopathic compounds from phenolics, phenylalkanoic acids, and indoles classes including caffeic acid, *p*-hydroxybenzoic acid, *trans*-ferulic acid. Macias et al. (2006) fruitfully isolated and identified bioactive ergosterol peroxide and 7-oxo-stigmasterol steroids from plant parts of a rice variety which exhibited high suppression on the growth of *E. crus-galli*. Similarly, many isolation and identification of rice allelochemicals studies have obtained a number of chemicals from several classes (Chon & Kim 2004; Bi et al. 2007; Berendji et al.2008; Jeong et al. 2006; Le Thi et al. 2014; Schmelz et al. 2014). Among the Bangladeshi rice varieties 2,9-dihydroxy-4-megastigmen-3-one from ‘BR17’ (Salam et al. 2009) and 9-hydroxy-*β*-ionone and 9-hydroxy-4-megastigmen-3-one from ‘Kartiksail’ (Kato-Noguchi et al. 2011) were identified as allelochemicals.

As reported by Rice (1984), one of the essential features of allelopathic interactions between plants is that phytotoxins are released into the soil by one plant and absorbed by a second plant. Furthermore, the ecological relevance of such phytotoxic root exudates also depends on the susceptibility of the plants with which the allelopathic plants coexist. In addition, allelopathic rice plants are able to detect the coexistence of inter-specific adjacent plants and respond by increasing certain allelochemicals (Kong et al. 2006). The concentrations of the allelochemicals released from the allelopathic rice seedlings in soil increased vividly when they were enclosed with *E. crus-galli*. Kato-Noguchi (2017) reported that allelopathic activity of rice seedlings increased by 6-fold when rice and *E. crus-galli* were grown in a mixed culture.

A cumulative number of studies have clearly documented that allelopathic rice might demonstrate useful in controlling paddy weeds and increasing grain yields. Kong (2007a) found that allelopathic rice with integrated cultural management options completely controlled the emergence and growth of most weeds without losing the grain yield. Besides, several studies showed that a traditional breeding method could be realistic to develop of commercially satisfactory allelopathic rice varieties. In USA, Dilday et al. (2000) made a cross between allelopathic donors ('PI312777' and 'PI338046') and commercial US cultivars ('Katy' and 'Lemont') which yielded progenies with good agronomical features and weed suppression. In Korea, a cross between variety 'Donginbyeo' (non-allelopathic high yielding and good quality) and variety 'Kouketsumochi' (indigenous allelopathic rice) was made and advanced by the single-seed descent method (Kim & Shin 2003). Another successful breeding was Huagan-3 from allelopathic 'PI312777' with high yield and strong weed suppression (Chen et al. 2008).

Allelopathic rice could be used in different ways also such as mulching or incorporation into the soil which provides sustainable weed management (Jabran et al. 2015), and reduces the negative effect on agroecosystem (Cheema et al. 2004). Besides, straw incorporation can increase soil fertility and improve the soil organic matter content which may influence the growth of crops in both nutritional and physiological terms (Dobermann & Fairhurst 2002). Therefore, incorporation of allelopathic rice straw could decrease weed, and boost rice yield (Xuan et al. 2005). Moreover, rice and weeds always simultaneously grow in the field. Thus, the possibility of allelopathic variety into developed variety, which would show suppressive effects on natural growth of paddy weeds, would considerably reduce herbicide use (Kong 2007b).

The ecological role of allelochemicals as herbicides has recently drawn great attention, due to increasing public concern against the use of synthetic. Current researches on allelopathy emphasize not only the need for structural elucidation of the isolated compounds, but also for their natural biological function. Many secondary metabolites have the potential to induce a wide array of biological effects and can provide great benefits to agriculture and weed management (Macias et al. 2006). Allelopathy offers the source for sustainable agriculture, hence, presently allelopathy research is being done in most countries worldwide and is receiving more attention from bioscientists to synthesize new effective and environment-friendly bioherbicide (Chou 2006).

Rice holds a strong allelopathic activity owing to allelochemicals. Integrating allelopathic rice variety with other weed management options may provide sustainable weed management in rice. A lot of breeding efforts are already in a process aiming at improving the allelopathic activity of rice. Allelopathic rice residues incorporation or mulching also hold the potential to suppress the weeds in rice or other field crops. Future research may contain synthesizing the rice allelochemicals in the form of a bioherbicide.

Bangladesh is a small (1,47,570 square kilometers) development country in the world. However, Bangladesh has a wide range of rice landraces and has a long history of rice cultivation. Rice is grown in both lowland and upland areas and can be planted all year round, with two or three crops per year. Both the low and upland rice fields in Bangladesh are generally infested with many tropical and subtropical weeds such as *E. crus-galli*, *E. colona*, *Leersia hexandra*, *Oryza rufipogon*, *Fimbristylis miliacea*, *C. esculentus*, *C. diformis*, *Monochoria vaginalis*, *Spilanthes acmella*, *E. prostrata* etc. Hence, it is unavoidable to apply miscellaneous weed control method in order to reach sustainable weed control in rice. Although the role of rice allelopathy is significant in the agricultural sector, the information of allelopathic potential of Bangladesh rice on weed management is intermittent due to lack of infrastructures, advanced technology, and laboratory facilities, still, a little work has been done on Bangladesh rice allelopathy potential whereas indigenous varieties are remained totally neglected.

Since there is no advanced research was done on Bangladesh indigenous rice allelopathy, the research on identifying of allelopathic potential Bangladesh indigenous rice variety will be very decisive for the development of allelopathy based sustainable weed management. Therefore, the main objective of the Ph.D. research project was to find out the allelopathic potential of Bangladeshi indigenous rice varieties.

The specific objectives of the research were:

i. Screen out allelopathic potential of Bangladesh indigenous rice
ii. Isolate and identify the allelochemical(s)
iii. Determine the biological activity of identified substance(s) on the growth of weed
iv. Correlate the field performance of rice in terms of weed control with in vitro screening
v. Distinguish the resource competition and allelopathic effect
vi. Evaluate allelopathic potential rice straw incorporation on the growth of weed and rice
vii. Identify potential phytotoxic substance(s) from allelopathic rice straw and their biological activities on the growth of weed, and
viii. Justify isolated allelochemical(s) as bioherbicide

CHAPTER II

SCREENING OF ALLELOPATHIC POTENTIAL BANGLADESH INDIGENOUS RICE

ABSTRACT

A series of experiments was conducted in the laboratory and glasshouse of the Subtropical Field Science Centre, University of the Ryukyus, Japan from April to October, 2015 to assess the allelopathic potential of 50 indigenous Bangladesh rice varieties by using donor-receiver bioassay, Equal Compartment Agar Method (ECAM), plant residue extracts method and pot culture method. Lettuce (*Lactuca sativa* L.), cress (*Lepidium sativum* L.), radish (*Raphanus sativus* L.), barnyardgrass (*Echinochloa crus-galli* L. Beauv.) and jungle rice (*Echinochloa colona* L.) were used as test plants. The highest inhibition effect was given by Boterswar (46%) while the stimulating effect was given by Kartikbalam and Panbira in donor-receiver bioassay and ECAM tests. Boterswar, Goria, Biron and Kartiksail were selected as the highest allelopathic potential varieties by donor-receiver bioassay and ECAM. In the methanol extracts test Boterswar gave the strongest inhibitory effect on both barnyardgrass (66% root and 49% shoot) and jungle rice (27% root), while Kartiksail gave the highest (16%) inhibitory effect on jungle rice shoot. Growth parameters and total dry matter of barnyardgrass in the glasshouse pot-experiment were significantly reduced due to the application of aqueous extracts of the selected rice varieties, which was similar to the results of the laboratory experiments. The varieties of Boterswar, Goria, Biron and Kartiksail were selected as the most allelopathic among 50 indigenous Bangladesh rice varieties. These rice varieties could be used for isolation and identification of allelochemicals and further to develop new varieties tolerant to weeds.

2.1 Introduction

Rice (*Oryza sativa* L.), one of the most important food crops, provides 21% of the world's food calories (Pacanoski & Glatkova 2009). Worldwide, 480.71 million metric tons of paddy rice are produced annually, of which 90.9% in Asian countries such as China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Japan, etc. (FAOSTAT 2014). On the contrary, weeds pose an important biological constraint to rice productivity (Zimdhal 1999; Rao 2000) and result in a 30-100% loss in upland rice yield (Hassan et al.1994). Weeds compete seriously with crops for resources, especially during establishment and early growth stages (Zimdahl 1980). Both *Echinochloa crus-galli* (barnyardgrass) and *Echinochloa colona* (jungle rice) are among the top ten most troublesome rice weeds (Smith 1983). Rice production has been now characterized by the heavy use of herbicides which cause environmental and health problems. The prolonged and widespread use of herbicides in rice growing regions increases the threat of herbicide resistant weeds. Therefore, non-chemical tactics need to be included in rice weed management systems.

Allelopathy, as first described by Molisch (1937), is the stimulatory or inhibitory impact of any biochemical interaction between plants (Rice 1984). Such phenomenon occurs widely among natural plant communities and is postulated to be one mechanism by which weeds interfere with crop growth (Rice 1984; Smith & Martin 1994). Since Dilday and his coworkers (1989; 1991; 1994) have reported some rice accessions possessing allelopathic activity in weed suppression, rice allelopathy has received a great deal of attention. The allelopathic effect of rice itself on weeds could be applied to reduce the use of herbicides which might result in less environmental contamination (Kong 2008). Therefore, one option could be for rice allelopathy to solve dependency on herbicides (Olofsdotter et al. 1999). The potential use of allelochemicals in controlling weeds in rice fields has also been explored by several researchers worldwide (Fujii 1992; Hassan et al. 1998; Kim et al. 1999; Olofsdotter et al. 1999; Azmi et al. 2000; Chau et al. 2008; Kato-Noguchi et al. 2008; Khanh et al. 2009; Salam & Kato-Noguchi 2010). Accessions with high inhibitory activity were found among wild, traditional, and red rice species (Fujii 1994). Besides this, the genetic variability in weed control among allelopathic rice varieties shows that breeding is a possible strategy to improve the capacity for self–defense against paddy weeds. Thus, research on the development of commercially acceptable allelopathic rice has been carried out throughout the world (Kong et al. 2011).

Bangladesh, a small country, is 4th in rice production in the world (FAOSTAT 2014). Both the low and upland rice fields in Bangladesh are generally infested with many tropical and subtropical weeds such as *Echinochloa cruss-galli*, *Echinochloa colona, Leersia hexandra*, *Oryza rufipogon*, *Fimbristylis miliacea*, *Cyperus esculentus*, *Cyperus diformis, Monochoria vaginalis*, *Spilanthes acmella*, *Eclipta prostrata* etc. Farmers in Bangladesh usually control weeds by mechanical, cultural and chemical methods. It is reported that the IRRI Gene Bank contains more than 8,000 traditional rice varieties collected from Bangladesh (Hossain et al. 2013). It is thought that the indigenous rice varieties contain different allelochemicals which could suppress the population and growth of weeds. Main et al. (2007) observed that in Bangladesh most of the common weed species are dominant in semi-dwarf modern variety than in traditional tall cultivars. Salam and Kato-Noguchi (2009) compared allelopathic activity among 102 modern and traditional varieties and they reported that BR17 (modern variety) is the most allelopathic. They also reported that Kartiksail (indigenous variety) might have great inhibitory activity against barnyardgrass. Karim et al. (2014) found that the highly allelopathic varieties were Kataribhog, WooCo, WITA12, Dular, Lalpaika, BRRI dhan27, WITA3, FARO8, BR26, BRRI dhan39, IR64, WITA8, Dharial and Nizersail among 120 modern and traditional rice varieties in Bangladesh. However, limited information exists on weeds suppression indigenous rice varieties, despite more than 60 indigenous rice varieties being cultivated regularly in Bangladesh, and no bioactive chemical identification study has yet been done. Therefore, the present research was undertaken in order to screen out the allelopathic potential of indigenous rice varieties of Bangladesh which can suppress paddy weeds and could be used for commercial cultivation as well as a genetic source for rice breeding.

2.2 Materials and Methods

2.2.1 Seed Collection and Experimental Materials

The research was conducted in the laboratory and glasshouse of the Subtropical Field Science Centre, Faculty of Agriculture, University of the Ryukyus, Japan during the period from April to October, 2015. Fifty indigenous Bangladesh rice varieties (Table 1) were collected from the Bangladesh Rice Research Institute, Bangladesh Geetanjoly Agro Society, and farmers of Barisal and Bandarban districts of Bangladesh. These collected seeds were brought into Japan by maintaining official procedures. All the rice verities are non sticky and *indica* rice. Lettuce (*Lactuca sativa*), cress (*Lepidum sativum*), radish (*Raphanus sativus*), barnyardgrass (*Echinochloa crus-galli*) and jungle rice (*Echinochloa colona*) were used as receiver plants since cress, lettuce and radish are usually used as model plants for bioassay while barnyard grass and jungle rice are important rice weeds. Barnyardgrass seeds were collected from the rice field of the Okinawa Agricultural Research Centre, Nago, Okinawa, Japan and jungle rice seeds were collected from the research field of the University of the Ryukyus. In our experiments, we used two screening methods as described by Kato-Noguchi et al. (2002) and Wu et al. (2000a) to select some possible allelopathic varieties. We also selected potential allelopathic varieties by using aqueous methanol extracts, including the effects of the aqueous extracts on weeds in laboratory and glasshouse experiments, respectively.

Table 1. List of indigenous rice varieties of Bangladesh used for study

Sl. No.	Name	Growing season	Plant height (cm)	Life span (days)	Sl No.	Name	Growing season	Plant height (cm)	Life span (days)
1	Baila Bokri	*Aus*	130	115	26	Kartik Balam	*Aman*	157	150
2	Bashful Chikon	*Aman*	126	145	27	Kartik Sail	*Aman*	146	150
3	Basmoti/ Sakkorkhana	*Aman*	120	150	28	Kataktara	*Aus*	116	115
4	Begun Bahar	*Aus*	100	110	29	Kataribhog	*Aman*	149	145
5	Bini Dhan	*Aman*	131	150	30	Kazliboro	*Boro*	106	167
6	Biron	*Aman*	177	167	31	Khaia Boro	*Boro*	102	165
7	Bolo Rum	*Aus*	135	107	32	Kushiara	*Aus*	115	115
8	Bonjira	*Aus*	100	100	33	Kilong	*Aman*	136	150
9	Boterswar	*Aus*	146	110	34	Lal Bini Monoching	*Aman*	157	150
10	Cahngsai	*Aman*	116	150	35	Lal Muta	*Aman*	168	150
11	Chini gura	*Aman*	100	145	36	Langda	*Aman*	151	150
12	Cockrro	*Aman*	146	145	37	Lekuch	*Aman*	160	150
13	Dharial	*Aus*	115	115	38	Marich Bate	*Boro*	101	152
14	Dholi Boro	*Boro*	120	170	39	Mohonbhog	*Aman*	125	145
15	Dholi Chikon	*Boro*	105	152	40	Moulata	*Aman*	129	145
16	Dudh Kolom	*Aman*	126	150	41	Naizersail	*Aman*	151	150
17	Dular	*Aman*	127	145	42	Nakhusimuta	*Aman*	148	150
18	Goria	*Aus*	131	108	43	Nayan tara	*Aus*	101	103
19	Hasha Kumira	*Aus*	127	110	44	Panbira	*Aus*	137	108
20	Hashikolmi	*Aus/Boro*	106	105	45	Panki Raj	*Aus*	106	110
21	Holoi	*Aus*	100	100	46	Rani Salute	*Aman*	150	150
22	Kalizira	*Aman*	146	150	47	Rata Boro	*Boro*	121	170
23	Kalo Bini	*Boro*	151	150	48	Surjamukhi	*Aus*	110	108
24	Kala Boro	*Boro*	111	170	49	Tongvoga, Lal Bini	*Aman*	167	150
25	Kalo khoia	*Boro*	105	145	50	Tupa Boro	*Boro*	121	170

Note: *Aus* = March –July; *Aman* = July- December; *Boro* = November - May

2.2.2 Donor-receiver Bioassay

In order to break rice dormancy, the seeds were incubated at 45–48°C for 7 days. Then the seeds were soaked in distilled water for 24 h and transferred onto moistened filter paper (no. 2; Toyo Roshi Kaisha, Tokyo, Japan) in Petri dishes (9 cm, Fisher Company, Hanover Park, IL, USA). Following dark incubation at 25°C for 48 h, the seeds were transferred to a growth chamber (Versatile Environmental Test Chamber MLR-351**,** SANYO Electric Co., Ltd.) with a 12 h photoperiod for another 48 h (25°C, 80–100 μE $m^{-2}s^{-1}$). The uniform germinating rice seedlings were transferred to Petri dishes (six rice seedlings per Petri dish) that contained a sheet of filter paper moistened with 2.5 ml of 1 mM phosphate buffer (pH), and grown for an additional 48 h. Then 10 seeds of cress, lettuce or radish were placed onto the filter paper with the growing rice seedlings. In the case of barnyardgrass or jungle rice, the seeds were pre-germinated by soaking in distilled water for 36 h, transferred onto a Petri dish with a sheet of moistened filter paper, as described above, and then dark-incubated at 25°C for 48 h. Finally, the germinating barnyardgrass seeds were placed onto the filter paper with the growing rice seedlings. Rice and the receiver species were allowed to grow in the growth chamber (conditions as described above) for 48 h prior to the growth measurements. The shoot (hypocotyls and/or coleoptiles) and root lengths of cress, lettuce, radish, barnyardgrass and jungle rice were measured. Controls were established by treating and incubating the receiver species, as described above, in the absence of the rice seedlings (Kato-Noguchi et al. 2002). Each experimental unit contained six donor (rice) seedlings and/or 10 receiver (cress, lettuce, radish, barnyardgrass and jungle rice) seedlings. The experimental design was completely randomized with four replications.

2.2.3 ECAM Bioassay

The Equal Compartment Agar Method (ECAM) developed by Wu et al. (2000a) and modified by Seal et al. (2004a) was used for the screening of rice accessions. Glass beakers (500 ml, 12 cm depth, 9 cm diameter) containing 30 ml of 0.3% water agar (no-nutrients, 1.3 cm depth) were autoclaved (HMC EUROPE HG-50/HG-80). Six pre-germinated rice seeds of each accession were uniformly selected and aseptically sown on one half of the agar surface with the embryo up. The beaker was wrapped with parafilm to prevent contamination and evaporation from the agar surface and placed in the controlled growth incubator with daily light/dark cycle of 12/12 hr and a temperature cycle of 25°C/25°C. The fluorescent light intensity in the cabinet was $3.56 \pm 0.16 \times 10^3$ lux. Seven days later 10 pre-germinated seeds of barnyardgrass and jungle rice were aseptically sown on the other half of the agar surface. A piece of pre-autoclaved white paperboard was inserted across the center and down the middle of the beaker with the lower edge of the paperboard kept one cm above the agar surface. After the sowing of the receiver seeds the beaker was again wrapped with parafilm and placed back in the growth incubator for further 7 days of co-growth before parameter measurements. The growth of the receiver species alone was considered as a control. The experimental design was completely randomized with four replications.

2.2.4 Plant Extract Bioassay: Aqueous and Methanol Extracts

Based on the donor-receiver bioassay and ECAM Bioassay, the four highest inhibition capability rice varieties were selected. Rice plants (20 days old) from each variety (100 g fresh) were extracted with 500 ml of 80% (v/v) aqueous methanol for 2 days. After filtration using filter paper (No. 2; Toyo Roshi, Tokyo, Japan), the residue was extracted again with 500 ml of methanol for 2 days and filtered, and the two filtrates were combined. An aliquot of the extract (final assay concentration was 0.3 g fresh rice plant equivalent extract ml^{-1}) was evaporated to dryness, dissolved in a 0.2 ml of methanol and moistened a sheet of filter paper (No. 2; Toyo) in a Petri dish (9 cm). The methanol was evaporated in a draft chamber. Then, the filter paper in the Petri dishes was moistened with 0.8 ml of a 0.05% (v/v) aqueous solution of Tween®20 (Polyoxyethylene Sorbitan Monolaurate). After germination in the darkness at 25^0C for 16–120 h, 10 seeds of lettuce, cress, radish, barnyardgrass or jungle rice were sown on the Petri dishes. The length of their shoots and roots were measured after 48 h of incubation in the darkness at 25^0C. For control treatments, methanol (0.2 ml) was added to a sheet of filter paper in the Petri dish and evaporated as described above. After germination, control seedlings were then placed on the filter paper moistened with the aqueous solution of Tween®20 without the methanol extract. The bioassay was repeated four times using a completely randomized design with 10 plants for each replication.

2.2.5 Pot Culture Bioassay: Glasshouse

A glasshouse pot (Wagner pot, 0.02 m^2) experiment was conducted to evaluate the effect of residue extracts of the four highest allelopathy capability rice varieties on barnyardgrass. Each pot was filled with 4 kg of gray soil (coarse sand 3.61%, fine sand 30.94%, silt 24.32%, clay 32.84%, apparent density 0.90 g cm^{-3}, pH 7.43, C 0.96%, N 0.12%, P 4.60 µ g^{-1} soil, K 42.89 µ g^{-1} soil, Ca 2604.15 µ g^{-1} soil, Mg 279.30 µ g^{-1} soil, S 2765.07 µ g^{-1} soil, Fe 0.16 µ g^{-1} soil, Na 102.36 µ g^{-1} soil, and Al 5.42 µ g^{-1} soil). One hundred ml of distilled water was added to 10 g of ground fresh plants (20 days old). Each sample was stirred on a rotary shaker at 160 rpm for 24 h (NEO shaker, AS ONE) and centrifuged (KUBOTA) at 3000 rpm for 15 min. At three-leaf stage of barnyardgrass (12 DAS), pots were irrigated with 250 ml of aqueous extracts or with distilled water (control treatment). A factorial experiment based on a completely randomized design with four replications was used. Ten days after the addition of extracts to the pots, barnyardgrass seedlings (22 days old) were harvested and their height (from the basal node to the end of leaf), tiller number, leaf number, largest leaf area and total leaf area were measured. Then, the plants were dried in the oven at 70ºC for 48 h and dry weights were recorded.

2.2.6 Statistical analysis

All experiments were repeated twice by using a completely randomized design with four replications and percentage inhibition was then determined by the following formula (Lin et al. 2004).

$$\text{Inhibition (\%)} = \left(\frac{\text{Control plant length} - \text{Plant length infested with rice}}{\text{Control plant length}}\right) \times 100$$

The treatment means were separated using Fisher's Protected Least Significant Difference test. The Type I error was set at 0.01 for all statistical comparison.

2.3 Results

2.3.1 Donor–receiver Bioassay

Significant differences in growth inhibition were observed among rice varieties in donor-receiver bioassay test on test plants (Table 2). Out of 50 Bangladesh indigenous rice varieties seven varieties, Bailabokri (50.60%), Biron (58.33%), Boterswar (73.00%), Goria (69.88%), Hashikalmi (50.03%), Kartiksail (58.70%) and Kataktara (50.45%) demonstrated more than 50% growth inhibition of lettuce roots. A good number (10) of varieties exhibited 40 to 50% inhibition of lettuce roots. Interestingly, some varieties like Kalokhoia (-30.97% inhibition), Kartikbalam (-39.77% inhibition) and Panbira (-34.23% inhibition) stimulated the root growth of lettuce. Growth inhibition of lettuce shoot was relatively lower than lettuce root. The highest (28.83%) lettuce shoot inhibition was observed from Rataboro. On the contrary, the variety Dharial (-2.53% inhibition) and Kartikbalam (-4.53% inhibition) stimulated the growth of lettuce shoot.

In response to cress weed, variety Boterswar gave the highest inhibitory effect (61.62% in root and 20.85% in shoot) followed by Goria (57.23% in root and 26.36% in shoot). However, Kalizira (-8.82% inhibition in root), Kartikbalam (-29.88% inhibition in root and -4.03% inhibition in shoot) and Panbira (-10.14% inhibition) gave the stimulating effect.

Among the tested varieties, Boterswar (81.96%) demonstrated the highest inhibitory effect on radish root but the shoot growth was inhibited by Goria (83.66%). The variety Biron (79.66% in root and 78.92% in shoot) gave the second highest inhibitory effect on both root and shoot of radish while Kalizira (-6.89% inhibition in root) and Kartikbalam (-19.63% inhibition in root and -1.64% inhibition in shoot) gave the stimulating effect on radish.

The highest level of inhibition by the Boterswar variety resulted in maximum inhibition of barnyardgrass root and shoot growth (71.50% and 30.45%, respectively), followed by Goria (69.32% in root and 25.19% in shoot), Kartiksail (64.55% in root and 23.26% in shoot) and

Biron (62.67% in root and 24.02% in shoot). However, Bashful chikon (-1.17% inhibition in shoot), Khaiaboro (-1.60% inhibition) and Panbira (-7.30% inhibition in root and -1.49% inhibition in shoot) stimulated growth of barnyardgrass.

Out of the test varieties, only Kartiksail (59.73%) showed more than 50% growth inhibition on jungle rice root, however, Boterswar (46.69%), Goria (40.77%) and Biron (35.80%) showed a promising effect on the root of jungle rice. The highest (21.11%) level of shoot inhibition in jungle rice was observed from Marichbate.

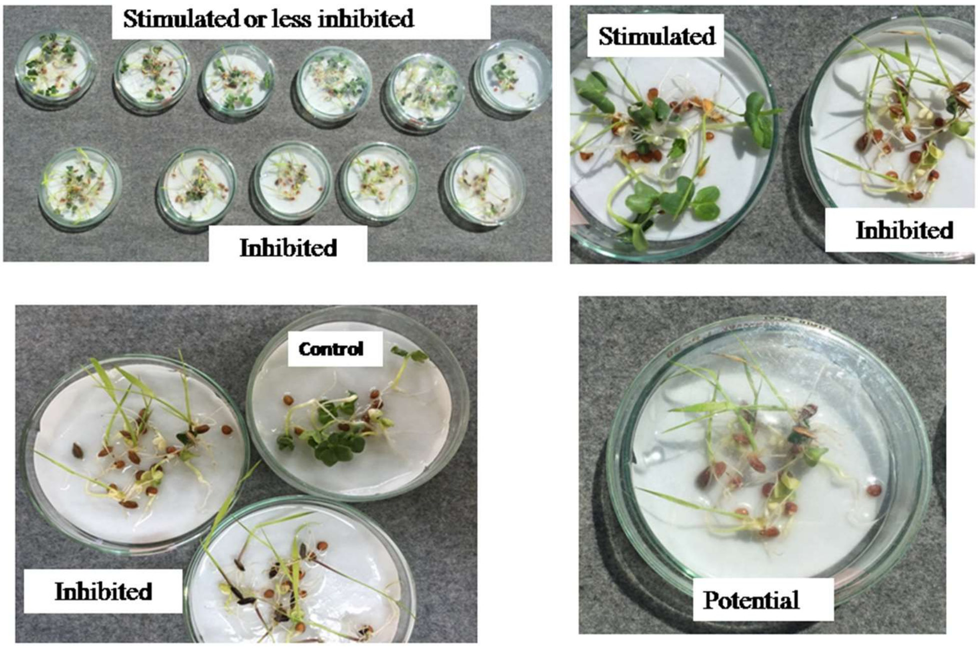

Figure 1. Observations of allelopathic potentiality of collected indigenous rice varieties

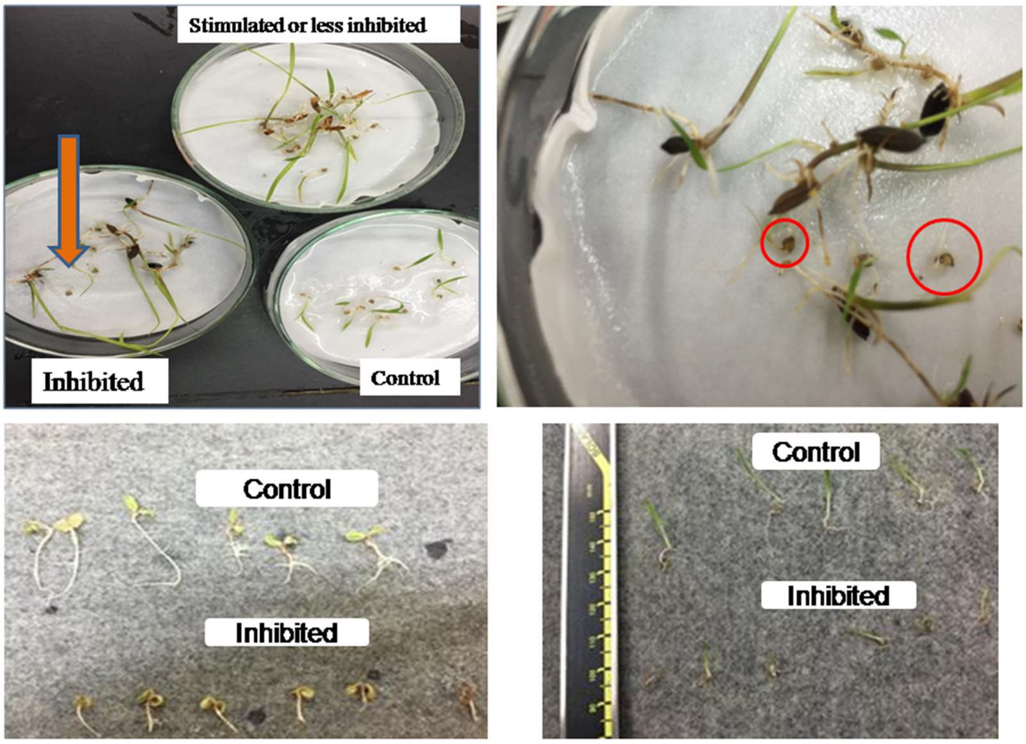

Figure 2. Allelopathic effect on test plants and weeds by collected indigenous varieties of Bangladesh

Figure 3. Observations of allelopathic effect of Bangladesh indigenous rice on weeds

Table 2. Allelopathic potential of indigenous rice varieties in donor-receiver bioassay under laboratory condition

Variety	Inhibition (%)									
	Lettuce		Cress		Radish		Barnyardgrass		Jungle Rice	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Bailabokri	50.60±2.94 c	16.26 ± 1.66ef	42.27 ± 0.49 de	10.38 ± 0.55gh	49.75 ± 0.89 d	39.49 ± 0.78c	52.15 ± 0.74 d	24.29 ± 3.87b	22.36 ± 1.19 i	3.33 ± 0.42 q-s
BashfulChikon	42.73 ± 2.37ef	16.37 ± 0.80ef	45.61 ± 1.23 c	5.19 ± 0.61 k	31.67 ± 1.37i	7.94 ± 1.51 m-o	18.34 ± 1.52 k-m	-1.17 ± 0.15rs	12.33 ± 1.11 kl	20.05 ± 0.15bc
Basmoti /Sakk.	47.93 ± 1.52 cd	21.60 ± 1.11bc	31.10 ± 0.64i-k	2.40 ± 1.04 m-q	20.04 ± 0.15 kl	1.41 ± 0.17pq	12.92 ± 0.52 n	8.65 ± 1.21gh	7.01 ± 0.49 n-p	2.597 ± 0.34 s-u
Begunbahar	15.70 ± 3.62 lm	3.47 ± 0.12 l-p	8.84 ± 0.95 n-q	2.09 ± 0.75 m-q	8.51 ± 1.52 o	3.08 ± 0.55pq	1.64 ± 0.61st	1.28 ± 0.17 o-q	6.14 ± 0.64 op	3.96 ± 0.45pq
Bini Dhan	29.40 ± 0.96 h	6.10 ± 0.44i-k	18.97 ± 3.20 m	4.36 ± 0.33 kl	22.39 ± 1.29 k	7.57 ± 0.61 no	14.29 ± 0.75 n	3.49 ± 0.46 l-n	6.61 ± 0.47 n-p	6.69 ± 0.25 o
Biron	58.33 ± 2.06 b	11.93 ± 2.17gh	19.03 ± 1.61m	8.79 ± 0.65 h-j	79.66 ± 1.72 a	78.92 ± 2.38 b	62.67 ± 2.19 b	24.02 ± 1.00 b	35.80 ± 0.30 d	19.17 ± 0.61 c
Bolorum	18.06 ± 1.11 kl	11.83 ± 0.23gh	31.67 ± 1.50i-k	17.06 ± 0.71 d	49.88 ± 1.00 d	20.34 ± 1.33 g	21.25 ± 1.33 j	12.24 ± 0.91 e	33.38 ± 0.13 e	13.52 ± 0.72 g
Bonjira	8.70 ± 1.70 o-q	5.53 ± 0.55i-l	9.13 ± 0.60 n-q	4.50 ± 0.53 kl	12.45 ± 1.12 n	7.85 ± 1.06 m-o	1.22 ± 1.06 t	1.80 ± 0.11 n-q	2.41 ± 0.13 q-t	1.48 ± 0.35 v
Boterswar	73.00 ± 1.61 a	22.20 ± 1.63bc	61.62 ± 1.64 a	20.85 ± 0.62bc	81.96 ± 2.42 a	79.51 ± 1.08 b	71.50 ± 1.08 a	30.45 ± 2.12 a	46.69 ± 1.12 b	16.16 ± 0.71 e
Cahngsai	22.47 ± 0.71ij	3.30 ± 0.36 m-p	17.83 ± 1.09 m	1.57 ± 0.35 n-q	19.19 ± 1.50 l	3.41 ± 0.23pq	5.71 ± 0.23 p-r	2.52 ± 1.13 m-q	3.71 ± 0.17qr	2.23 ± 0.10 t-v
Chinigura	9.83 ± 0.81n-p	5.30 ± 0.61 j-m	41.47 ±1.15 e	16.86 ± 0.75 d	50.35 ± 0.45 d	25.09 ± 1.57ef	20.67 ± 1.57jk	5.66 ± 0.19i-k	-5.39 ± 0.06 u	12.69 ± 0.73gh
Cockrro	49.43 ± 1.50 c	16.00 ± 1.59ef	32.97± 1.91i	8.55 ± 0.59ij	34.96 ± 2.41 h	13.37 ± 1.40i	17.55 ± 1.39 lm	10.77 ± 0.50ef	22.47 ± 0.57i	10.15 ± 0.23jk
Dharial	47.54 ± 1.35 cd	-2.53 ± 0.88 r	44.77 ± 2.43 cd	3.26 ± 0.31 l-p	48.37 ± 2.24 d	33.68 ± 1.61 d	20.05 ± 1.26 j-l	9.96 ± 0.25fg	13.18 ± 1.02k	8.08 ± 0.17mn
DholiBoro	37.47 ± 1.12 g	22.91 ± 0.54 b	11.70 ± 1.06 n	3.87 ± 0.70 k-m	15.65 ± 1.19 m	6.83 ± 1.00 no	6.44 ± 0.68 o-q	4.88 ± 0.68 j-l	-16.95 ± 0.18 x	3.34 ± 0.48 q-s
DholiChikon	6.27 ± 1.27 p-r	2.50 ± 0.30 o-q	6.92 ± 0.77pq	1.37 ± 0.38pq	5.55 ± 0.28pq	3.15 ± 0.16pq	6.07 ± 0.18 o-q	2.59 ± 0.46 m-q	32.04 ± 3.58ef	9.92 ± 0.07jk
Dudhkolom	15.33 ± 1.42 lm	10.10 ± 0.27 h	29.20 ± 0.80 kl	13.26 ± 0.73ef	34.98 ± 2.35 h	10.16 ± 0.95 l	24.90 ± 1.35i	2.91 ± 0.49 l-p	12.99 ± 0.41 k	4.58 ± 0.52 p
Dular	23.27 ± 4.22ij	6.40 ± 0.99i-k	40.52 ± 2.18ef	19.64 ± 0.87 c	49.75 ± 0.84 d	26.48 ± 1.28 e	40.04 ± 1.24 f	20.55 ± 1.24 c	30.42 ± 0.71fg	9.27 ± 0.28 kl
Goria	69.88 ± 4.33 a	27.08 ± 0.20 a	57.23 ± 1.85 b	26.36 ± 1.00 a	69.48 ± 0.89 b	83.66 ± 1.28 a	69.32 ± 1.47 a	25.19 ± 0.82 b	40.77 ± 0.41 c	12.65 ± 0.50gh
Hashakumira	48.80 ± 2.69 c	10.36 ± 0.74 h	39.74 ± 1.36ef	17.27 ± 1.26 d	1.62 ± 0.08 s	7.87 ± 0.83 m-o	25.54 ± 2.43i	2.30 ± 0.06 m-q	2.67 ± 0.48 q-t	8.46 ± 0.10 lm
Hashikalmi	50.03 ± 1.76 c	26.97 ± 1.44 a	40.01 ± 1.23ef	9.25 ± 0.57 hi	49.44 ± 0.96 d	23.12 ± 0.87 f	34.75 ± 1.41 g	9.09 ± 0.68 f-h	-11.56 ± 0.29 w	-2.52 ± 0.17 y
Holoi	3.80 ± 0.40 r-t	0.93 ± 0.12 q	2.02 ± 0.84 r	1.27 ± 0.40 q	2.03 ± 0.76 s	1.26 ± 0.07pq	1.33 ± 0.10 t	1.18 ± 0.23 pq	0.90 ± 0.03st	1.81 ± 0.16t-v

Table 2 (Continued)

Variety	Inhibition (%)									
	Lettuce		Cress		Radish		Barnyardgrass		Jungle Rice	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Kalizira	5.63 ± 0.21 q-s	12.43 ± 2.15 g	-8.82 ± 0.98 s	2.01 ± 0.55 m-q	-6.89 ± 0.82 t	1.08 ± 0.23 q	17.31 ± 0.53 m	5.70 ± 0.07 i-k	29.97 ± 1.07fg	10.90 ± 0.87ij
Kalobini	6.60 ± 0.90 p-r	3.53 ± 0.15 l-p	6.86 ± 0.81pq	2.67 ± 0.29 l-q	8.42 ± 0.58 o	7.31 ± 1.18 no	2.67 ± 0.66st	1.52 ± 0.34 n-q	7.58 ± 0.25 no	2.26 ± 0.20 t-v
Kala Boro	43.34 ± 1.05ef	15.08 ± 0.35 f	39.84 ± 0.96ef	12.63 ± 2.10 f	36.96 ± 0.79gh	10.71 ± 0.57 j-l	14.63 ± 0.51 n	3.30 ± 0.13 l-p	18.42 ± 0.38 j	9.32 ± 0.48 kl
Kalokhoia	-30.97 ± 1.42 u	3.53 ± 0.15 l-p	1.64 ± 0.20 r	1.00 ± 0.10 q	2.41 ± 0.06rs	2.15 ± 0.18pq	8.58 ± 0.60 o	4.15 ± 0.62 k-m	23.95 ± 0.73i	20.49 ± 0.74 ab
Kartikbalam	-39.77 ± 1.27 v	-4.53 ± 0.31 s	-29.88 ± 1.01 t	-4.03 ± 0.55 r	-19.63 ± 0.75 u	-1.64 ± 0.61 r	4.15 ± 0.61 q-s	1.29 ± 0.16 o-q	-3.77 0.20 u	-1.38 ± 0.17 x
Kartiksail	58.70 ± 1.10 b	21.93 ± 1.46bc	26.84 ± 1.01 l	19.74 ± 0.78 c	66.69 ± 2.86 c	26.59 ± 1.02 e	64.55 ± 1.76 b	23.26 ± 0.72 b	59.73 ± 1.00 a	17.51 ± 0.63 d
Kataktara	50.45 ± 0.18 c	11.53 ± 0.05gh	38.16 ± 1.51fg	11.89 ± 1.00fg	40.12 ± 1.24 f	16.90 ± 0.80 h	38.55 ± 1.02 f	7.34 ± 0.51 hi	22.91 ± 0.67i	3.41 ± 0.17 q-s
Kataribhog	30.07 ± 0.35 h	11.63 ± 0.06gh	40.67 ± 1.17ef	21.63 ± 1.02 b	50.07 ± 1.24 d	17.70 ± 0.92 h	57.68 ± 1.31 c	2.37 ± 0.52 m-q	28.55 ± 0.59gh	14.99 ± 0.76 f
Kazliboro	24.33 ± 1.11i	7.37 ± 0.87 e	46.46 ± 0.61 c	7.25 ± 0.73 j	48.59 ± 2.44 d	6.63 ± 0.34 no	43.34 ± 1.85 e	17.69 ± 0.86d	18.43 ± 0.61 j	10.61 ± 0.42 j
KhaiaBoro	38.34 ± 0.63 g	17.16 ± 0.31 e	40.46 ± 0.73ef	11.51 ± 0.56fg	39.13 ± 0.35fg	10.07 ± 0.18 lm	19.79 ± 1.04 j-m	-1.60 ± 0.36 s	22.80 ± 0.58 i	16.78 ± 0.11 de
Kushiara	12.17 ± 1.00 m-o	3.47 ± 0.31 l-p	9.31 ± 0.51 n-q	3.18 ± 0.07 l-p	10.14 ± 0.24 no	2.52 ± 0.53 pq	1.53 ± 0.24st	0.67 ± 0.12qr	8.60 ± 0.93mn	8.32 ± 0.58 l-n
Kilong	19.97 ± 1.31jk	4.87 ± 0.31 k-n	10.33 ± 0.22 no	3.23 ± 0.10 l-p	8.51 ± 0.62 o	3.51 ± 0.33 p	4.22 ±0.67 q-s	2.28 ± 0.16 m-q	3.59 ± 0.27qr	1.370 ± 0.17vw
Lal Bini Mo.	8.57 ± 1.08 o-q	6.10 ± 0.44i-k	8.69 ± 0.12 n-q	4.45 ± 0.14 kl	4.76 ± 0.21qr	1.25 ± 0.08pq	7.07 ± 1.57 op	2.30 ± 0.17 m-q	3.60 ± 0.17 qr	2.773 ± 0.12 r-t
Lal muta	13.07 ± 1.24mn	4.47 ± 1.15 k-o	9.86 ± 0.95 n-p	3.29 ± 0.14 l-n	12.55 ± 0.59 n	2.41 ± 0.06pq	8.03 ± 0.79 op	4.04 ± 0.52 k-m	4.63 ± 0.16pq	1.59 ± 0.22uv
Langda	11.10 ± 1.25 no	4.60 ± 0.36pq	7.55 ± 0.50o-q	3.70 ± 0.34 k-m	2.35 ± 0.03rs	1.46 ± 0.08pq	4.21 ± 0.24 q-s	2.50 ± 0.32 m-q	3.21 ± 0.11 q-s	1.66 ± 0.33uv
Lekuch	11.00 ± 1.15 no	2.30 ± 0.00pq	7.44 ± 1.53 o-q	2.24 ± 0.13 m-q	12.78 ± 0.62 n	1.22 ± 0.02pq	4.18 ± 0.67 f	3.45 ± 0.22 l-o	3.37 ± 0.34 q-s	4.48 ± 0.27 p
Marichbate	6.59 ± 1.43 p-r	5.41 ± 0.47i-m	32.30 ± 2.23ij	11.71 ± 0.65fg	28.84 ± 0.99 j	10.33 ± 0.50 kl	39.99 ± 1.62 r-t	8.78 ± 0.90gh	27.47 ± 0.69 h	21.107 ± 1.00 a
Mohonbhog	1.03 ± 0.21 t	3.53 ± 1.30 l-p	6.96 ± 0.65pq	7.58 ± 0.28ij	7.92 ± 0.18 op	5.92 ± 0.17 o	3.38 ± 0.23 t	3.01 ± 0.62 l-p	-4.66 ± 0.10 u	3.34 ± 0.11 q-s
Moulata	8.37± 0.29 o-q	3.50 ± 0.56 l-p	6.49 ± 0.19 q	2.50 ± 0.23 m-q	1.28 ± 0.10 s	1.30 ± 0.24pq	1.44 ± 0.22 op	1.26 ± 0.07 o-q	1.30 ± 0.15 r-t	1.29 ± 0.07vw
Naizersail	18.67 ± 2.83 kl	7.33 ± 0.51ij	29.52 ± 0.93 j-l	17.52 ± 0.61 d	43.86 ± 0.64 e	40.16 ± 2.67 c	7.37 ± 0.46 op	11.91 ± 0.59 e	10.19 ± 2.06 lm	-1.25 ± 0.23 x
Nakhusimuta	12.23 ± 0.67 m-o	4.40 ± 0.46 k-p	9.29 ± 0.79 n-q	3.21 ± 0.09 l-p	8.41 ± 1.13 o	3.40 ± 0.31pq	2.19 ± 0.13st	1.26 ± 0.07 o-q	-7.87 ± 0.20 v	-8.32 ± 0.87 [
Nayantara	10.69 ± 0.50 no	2.49 ± 0.24 o-q	3.18 ± 0.96 r	1.43 ± 0.11nq	12.12 ± 1.20 n	2.23 ± 0.10pq	1.18 ± 0.17 t	0.70 ± 0.17qr	0.59 ± 0.08 t	0.41 ± 0.06 w

Table 2 (Continued)

Variety	Inhibition (%)									
	Lettuce		Cress		Radish		Barnyardgrass		Jungle Rice	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Panbira	-34.23 ± 1.31 u	3.33 ± 0.32 m-p	-10.14 ± 0.24 s	1.40 ± 0.41 o-q	18.64 ± 0.46 l	17.37 ± 0.89 h	-7.30 ± 0.78 u	-1.49 ± 0.28 s	-6.07 ± 0.55uv	-3.51 ± 0.34 z
Pankiraj	40.23 ± 0.44fg	19.23 ± 0.59 d	33.74 ± 2.56 hi	12.42 ± 2.21 f	40.11 ± 0.23 f	12.37 ± 0.45i-k	40.53 ± 2.32 f	12.66 ± 1.07 e	27.17 ± 0.64 h	7.44 ± 0.23 no
Ranisalute	31.53 ± 0.49 h	2.77 ± 0.58 n-q	19.82 ± 0.83 m	1.80 ± 0.08 n-q	37.13 ± 0.65gh	12.56 ± 0.59ij	19.24 ± 0.53 j-m	1.62 ± 0.27 n-q	1.40 ± 0.44 r-t	1.56 ± 0.36uv
Rata Boro	40.94 ± 1.70 e-g	28.83 ± 0.96 a	37.80 ± 2.69fg	12.01 ± 1.50fg	30.11 ± 1.24ij	8.51 ± 0.47 l-n	29.03 ± 0.56 h	4.36 ± 0.44 k-m	4.66 ± 0.47pq	3.74 ± 0.27 p-r
Surjamukhi	44.28 ± 1.03 de	11.51 ± 0.51gh	32.26 ± 0.91ij	8.22 ± 0.49ij	30.40 ± 0.73ij	34.62 ± 1.26 d	45.66 ± 1.24 e	3.31 ± 0.12 l-p	17.47 ± 0.37 j	11.77 ± 0.19 hi
Tongvoga,Lal Bini	2.30 ± 0.92st	1.23 ± 0.15 q	1.19 ± 0.07 r	1.59 ± 0.29 n-q	1.10 ± 0.11 s	1.14 ± 0.08pq	2.56 ± 0.81st	1.34 ± 0.40 n-q	3.58 ± 0.45qr	2.59 ± 0.49 s-u
TupaBoro	40.46 ± 2.43 e-g	20.84 ± 0.57 cd	36.44 ± 1.00gh	14.82 ± 1.51 e	40.15 ± 1.24 f	9.99 ± 1.07 lm	18.55 ± 1.52 k-m	6.73 ± 1.19ij	18.49 ± 3.17 j	1.87 ± 0.11 t-v

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

It is also observed that across all the rice varieties, radish (21.09%) was the most inhibited when grown with rice, followed by lettuce (16.99%), cress (15.28%), barnyardgrass (13.71%) and jungle rice (9.82%) (Figure 4).

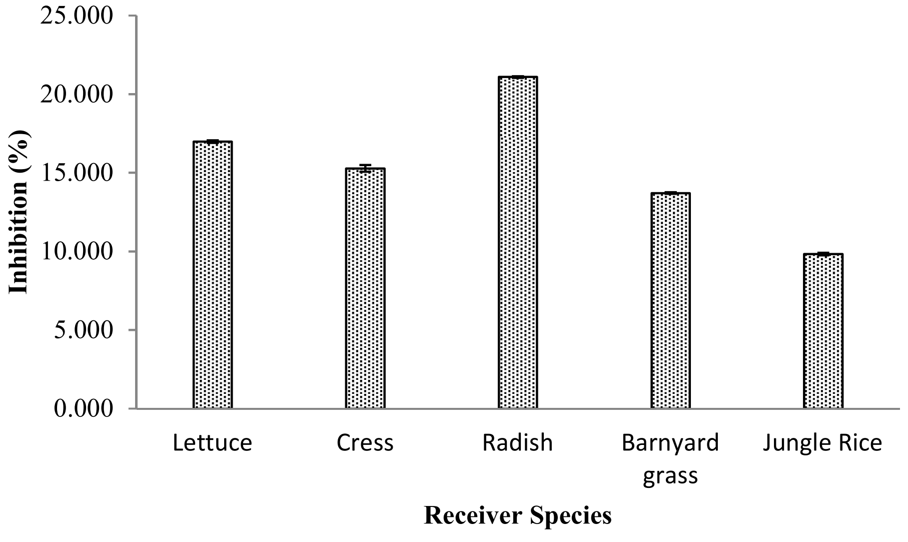

Figure 4. Average inhibition (%) on receiver species due to infested with irrespective of rice varieties

2.3.2 ECAM Bioassay

Significant differences existed among the rice varieties in their ability to suppress the root and shoot of each weed species studied (Table 3). The highest (62.06%) root inhibition of barnyardgrass was recorded from Boterswar. In contrast, the shoot growth of barnyardgrass was not considerably reduced. The highest shoot (26.58%) inhibition was observed from Boterswar. The stimulating effect was observed by Bashful chikon (-2.20% in shoot), Khaiaboro (-1.10% in shoot) and Panbira (-8.63% in root and -1.50% in shoot). In the case of jungle rice, the highest (40.06% in root and 16.38% in shoot) inhibition was observed by the infestation of Kartiksail while the stimulating effect on jungle rice was given by Chinigura (-4.39% in root), Dholiboro (-8.29% in root), Hashikalmi (-8.39% in root and -2.06% in shoot), Kartikbalam (-3.07% in root and -1.27% in shoot), Mohonbhog (-4.06% in root), Nizersail (-1.12% in shoot), Nakhusimuta (-6.54% in root and -8.06% in shoot) and Panbira (-10.73% in root and -10.51% in shoot).

Table 3. Allelopathic potential of indigenous rice varieties in ECAM bioassay under laboratory condition

Variety	Inhibition (%)			
	Barnyardgrass		Jungle Rice	
	Root	Shoot	Root	Shoot
Bailabokri	46.52 ± 2.18 c	20.26 ± 1.05c	15.42 ± 0.70 k	4.66 ± 0.80mn
BashfulChikon	18.37 ± 0.52 n	-2.20 ± 0.10 q	17.06 ± 0.55ij	10.75 ± 0.99ef
Basmoti /Sakk.	14.25 ± 0.12 o	8.50 ± 0.62 f-h	3.34 ± 0.01 q-s	1.60 ± 0.25rs
Begunbahar	0.91 ± 0.06 z	1.32 ± 0.42 no	4.21 ± 0.23 o-r	3.12 ± 0.16 o-q
Bini Dhan	11.62 ± 1.06 p	2.42 ± 0.10 l-o	6.28 ± 0.33 n	4.86 ± 0.49mn
Biron	48.04 ± 0.71 c	16.41 ± 0.74 d	34.91 ± 0.50 b	14.40 ± 0.69 c
Bolorum	23.24 ± 0.79 j	15.33 ± 0.89 d	17.34 ± 0.89ij	11.52 ± 2.63 de
Bonjira	6.37 ± 1.01 s-u	1.86 ± 0.06 m-o	1.05 ± 0.03uv	1.14 ± 0.14rs
Boterswar	62.06 ± 1.28 a	26.58 ± 0.86 a	35.96 ± 0.71 b	16.42 ± 0.34 a
Cahngsai	3.77 ± 0.22 v-x	4.22 ± 0.11 j-l	1.32 ± 0.24 t-v	1.19 ± 0.15rs
Chinigura	28.30 ± 0.93 h	8.33 ± 0.76gh	-4.39 ± 0.06 w	8.36 ± 0.80 h-j
Cockrro	10.85 ± 0.95 p	6.77 ± 0.95 hi	23.81 ± 0.59 g	7.15 ± 0.23jk
Dharial	22.75 ± 0.59jk	11.29 ± 0.91 e	13.52 ± 0.53 l	8.08 ± 0.17ij
DholiBoro	3.70 ± 0.25 v-x	1.48 ± 0.32 m-o	-8.29 ± 0.61 y	2.11 ± 0.07qr
DholiChikon	8.48 ± 0.34 q-s	3.35 ± 0.21 k-m	20.91 ± 0.51 h	8.43 ± 0.63 h-j
Dudhkolom	26.24 ± 1.01i	8.24 ± 0.91gh	10.56 ± 0.76 m	4.61 ± 0.46mn
Dular	30.37 ± 1.69 g	23.22 ± 2.96 b	30.76 ± 0.57 d	9.30 ± 0.78 g-i
Goria	60.32 ± 0.90 a	23.53 ± 2.37 b	32.77 ± 0.41 c	14.65 ± 0.58bc
Hashakumira	20.54 ± 0.53 lm	3.33 ± 0.01 k-m	2.33 ± 0.57 s-u	5.49 ± 0.17 lm
Hashikalmi	25.42 ± 1.80i	11.39 ± 0.66 e	-8.39 ± 0.06 y	-2.06 ± 0.02 t
Holoi	1.37 ± 0.06yz	1.04 ± 0.05 no	0.73 ± 0.14uv	1.21 ± 0.22rs
Kalizira	17.67 ± 0.95 n	5.70 ± 0.95ij	25.30 ± 2.25 f	6.66 ± 0.23 kl
Kalobini	2.30 ± 0.06 x-z	1.54 ± 0.30 m-o	6.54 ± 0.18 n	1.81 ± 0.54 q-s
Kala Boro	22.99 ± 0.75 j	5.64 ± 0.53ij	16.42 ± 0.72jk	9.05 ± 0.05 g-i
Kalokhoia	10.65 ± 0.99 p	4.35 ± 0.86jk	20.28 ± 0.80 h	12.53 ± 0.60 d
Kartikbalam	3.48 ± 0.07 v-y	1.15 ± 0.22 no	-3.07 ± 0.03 w	-1.27 ± 0.07 t
Kartiksail	50.05 ± 0.67 b	11.59 ± 1.21 e	40.06 ± 0.88 a	16.38 ± 0.46 a
Kataktara	36.22 ± 0.67 e	6.73 ± 0.35 hi	20.91 ± 1.10 h	3.64 ± 0.64 no
Kataribhog	39.14 ± 0.54 d	1.37 ± 0.07 no	27.21 ± 0.57 e	10.65 ± 0.48ef
Kazliboro	32.64 ± 1.57 f	18.63 ± 0.33 c	13.59 ± 0.75 l	9.94 ± 0.12fg
KhaiaBoro	18.79 ± 1.08mn	-1.10 ± 0.09pq	20.23 ± 0.96 h	15.81 ± 0.84 ab
Kushiara	1.32 ± 0.45 z	0.47 ± 0.06 op	5.60 ± 0.25 no	6.42 ± 0.41 kl
Kilong	3.38 ± 0.06v-z	2.05 ± 0.05 m-o	2.76 ± 0.37 r-t	1.14 ± 0.16rs
Lal Bini Mo.	5.30 ± 0.40 t-v	2.17 ± 0.25 m-o	3.13 ± 0.10 q-s	2.07 ± 0.02qr
Lal Muta	7.33 ± 0.47 r-t	2.67 ± 0.68 k-n	4.63 ± 0.16 o-q	1.59 ± 0.22rs
Langda	4.38 ± 0.13 u-x	2.14 ± 0.14 m-n	3.88 ± 0.47 p-s	1.16 ± 0.14rs
Lekuch	8.56 ± 0.91 r	4.52 ± 0.23jk	5.37 ± 0.34 n-p	4.35 ± 0.37 m-o
Marichbate	18.59 ± 1.25mn	6.78 ± 1.05 hi	20.40 ± 1.25 h	14.77 ± 0.56bc
Mohonbhog	2.78 ± 0.34 w-z	2.17 ± 0.15 m-o	-4.06 ± 0.01 w	3.11 ± 0.11 o-q
Moulata	1.04 ± 0.02 [	1.39 ± 0.32 no	1.26 ± 0.15 t-v	1.19 ± 0.14rs
Naizersail	6.71 ± 0.23 r-t	10.14 ± 1.05ef	10.39 ± 0.85 m	-1.12 ± 0.16 t
Nakhusimuta	7.19 ± 0.13 r-t	4.26 ± 0.07 j-l	-6.54 ± 0.43 x	-8.06 ± 0.97 u
Nayantara	1.02 ± 0.02 [	1.06 ± 0.03 no	0.63 ± 0.08 v	0.44 ± 0.01 s
Panbira	-8.63 ± 0.21 \	-1.50 ± 0.40 q	-10.73 ± 0.95 z	-10.51 ± 0.34 v
Panki raj	32.86 ± 1.50 f	8.66 ± 0.71fg	22.53 ± 1.87 g	9.52 ± 0.24 f-h
Ranisalute	10.51 ± 2.72pq	1.05 ± 0.02 no	1.04 ± 0.04uv	1.26 ± 0.16rs
Rataboro	19.69 ± 0.06 l-n	4.03 ± 0.03 j-l	4.10 ± 0.10 o-r	3.61 ± 0.49 n-p
Surjamukhi	26.89 ± 0.54 hi	6.31 ± 0.12i	18.14 ± 0.22i	10.77 ± 0.19ef
Tongvoga,Lal Bini	4.52 ± 0.23 u-w	1.10 ± 0.12 no	3.38 ± 0.35 q-s	2.29 ± 0.41 p-r
TupaBoro	20.95 ± 0.96 kl	6.40 ± 0.35	16.55 ± 0.85jk	1.30 ± 0.40rs

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

On the basis of donor–receiver bioassay and ECAM bioassay results, the highest average inhibition on test plants and weeds was from Boterswar (46.07%) followed by Goria (43.78%), Biron (36.58%) and Kartiksail (35.97%) (Figure 5).

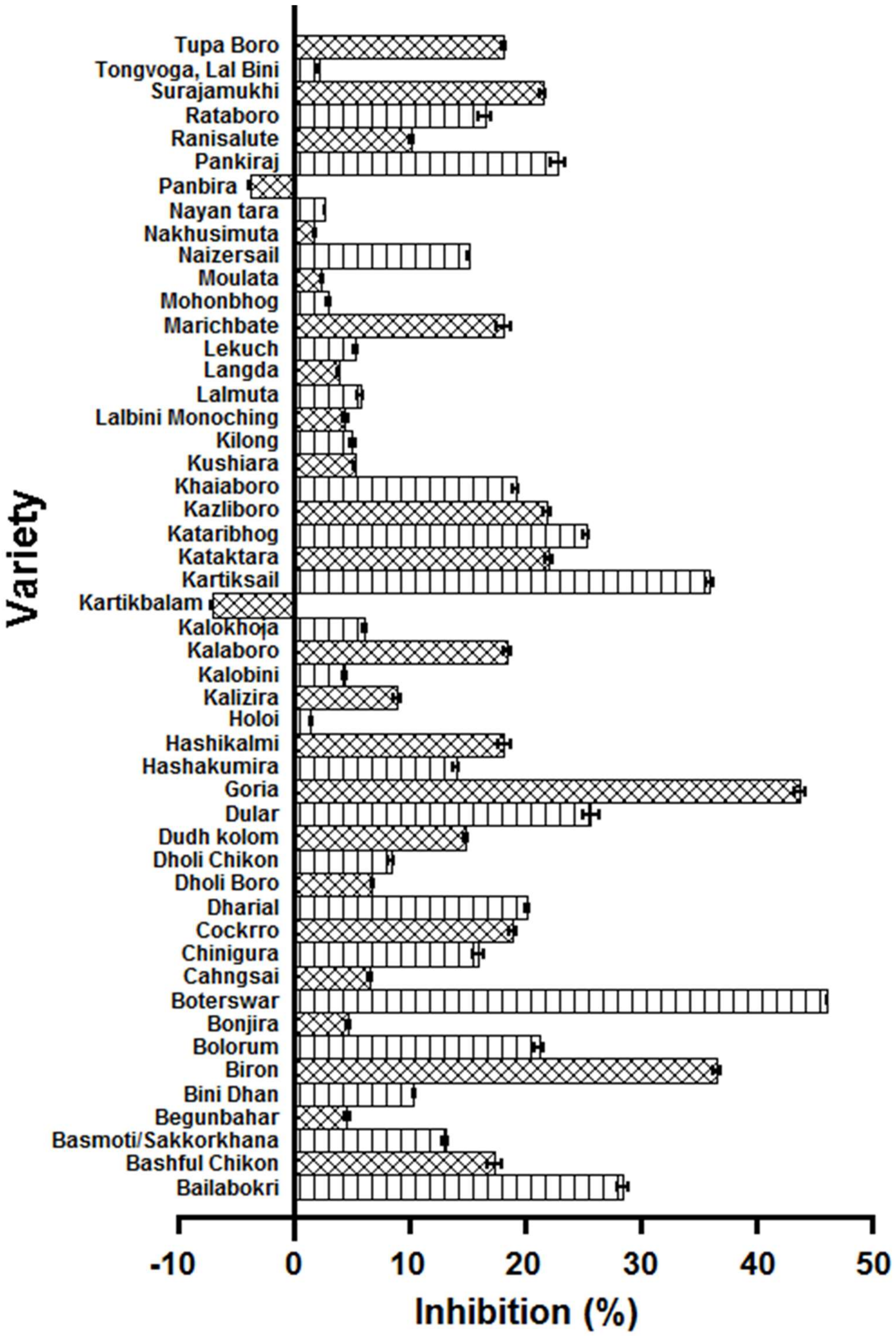

Figure 5. Average inhibition (%) on irrespective of receiver species by tested rice varieties from donor-receiver bioassay and ECAM bioassay screening test

2.3.3 Plant Extract Bioassay

Activity of the aqueous methanol extracts of Boterswar, Goria, Biron and Kartiksail on cress, lettuce, radish, barnyardgrass and jungle rice

The aqueous MeOH extract of Boterswar, Goria, Biron and Kartiksail rice tissue inhibited the shoot and root growth of all the test species at concentrations that were 0.3 g fresh rice plant equivalent extract ml^{-1} (Table 4). The extract from Boterswar inhibited the root growth of lettuce, cress, radish, barnyardgrass and jungle rice by 65.37, 60.40, 84.10, 65.50 and 26.67%, respectively, and the shoot growth by 43.50, 38.41, 52.84, 49.44 and 7.66%, respectively. The extract from Goria inhibited the root growth of lettuce, cress, radish, barnyardgrass and jungle rice by 53.02, 57.29, 71.42, 62.14 and 22.77%, respectively, and the shoot growth by 39.38, 26.99, 55.13, 46.88 and 6.30%, respectively. The extract from Biron inhibited the root growth of lettuce, cress, radish, barnyardgrass and jungle rice by 60.24, 36.94, 75.82, 44.51 and 20.01%, respectively, and the shoot growth by 40.79, 13.22, 58.40, 43.32 and 6.54%, respectively. The extract from Kartiksail inhibited the root growth of lettuce, cress, radish, barnyardgrass and jungle rice by 35.33, 47.14, 49.89, 37.02 and 25.34%, and the shoot growth by 21.67, 48.97, 38.99, 40.59 and 16.38%, respectively. The extracts from all rice varieties resulted in a greater inhibition of the root growth than the shoot growth of receiver plants. However, the shoot growth of cress was more sensitive than the root growth to the extracts from Kartiksail.

Table 4. Comparison of allelopathic potential of indigenous rice varieties in aqueous methanol extracts bioassay under laboratory condition

Variety	Inhibition (%)									
	Lettuce		Cress		Radish		Barnyardgrass		Jungle Rice	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Boterswar	65.37 ± 1.32 a	43.50 ± 0.58 a	60.40 ± 1.19 a	38.41 ± 1.56 b	84.10 ± 1.07 a	52.84 ± 1.78 b	65.50 ± 1.65 a	49.44 ± 1.19 a	26.68 ± 1.50 a	7.66 ± 1.34 b
Goria	53.02 ± 1.51 c	39.38 ± 0.84 b	57.29 ± 1.23 a	26.99 ± 1.09 c	71.42 ± 1.13 c	55.13 ± 1.18 ab	62.14 ± 1.58 a	46.88 ± 2.93 ab	22.77 ± 1.16bc	6.30 ± 0.30 b
Biron	60.24 ± 1.93 b	40.79 ± 1.68 ab	36.94 ± 1.35 c	13.22 ± 0.84 d	75.82 ± 0.91 b	58.40 ± 1.03 a	44.51 ± 1.68 b	43.32 ± 0.85 ab	20.01 ± 0.85 c	6.54 ± 0.33 b
Kartiksail	35.33 ± 0.64 d	21.67 ± 1.14 c	47.14 ± 1.33 b	48.97 ± 0.89 a	49.89 ± 1.01 d	38.99 ± 1.45 c	37.02 ± 2.10 c	40.59 ± 1.76 b	25.34 ± 0.86 ab	16.38 ± 0.46 a

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

2.3.4 Pot Culture Bioassay: Allelopathic Potential of Rice Varieties in Glasshouse Bioassay

The response of growth parameters of barnyardgrass to rice extracts indicated significant allelopathy potential of tested rice varieties (Figure 7). Results of the most inhibitory effects from Boterswar variety extract on different growth parameters of barnyardgrass were, 41.73, 64.43, 74.72, 72.04, 92.15 and 50.41% in plant height, number of total tillers, number of leaves, largest leaf area, total leaf area and shoot dry matter, respectively.

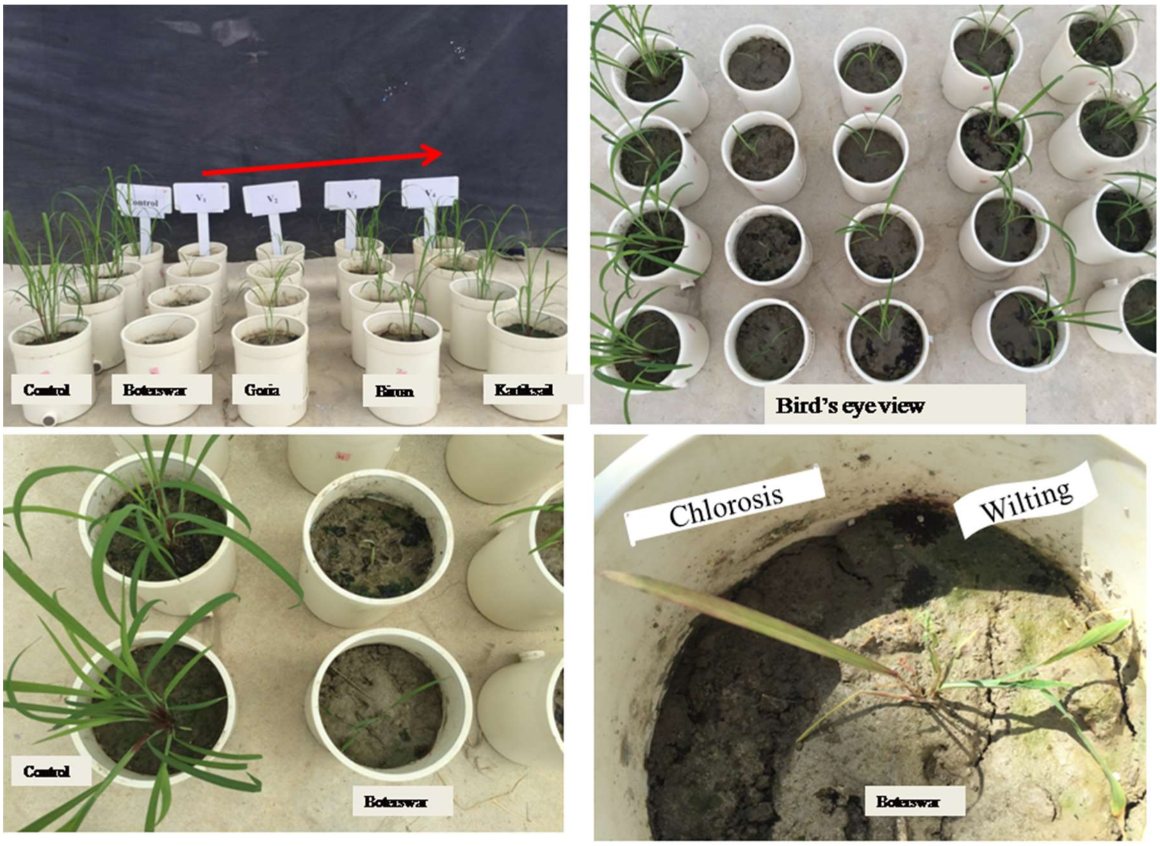

Figure 6. Effects of aqueous extracts of selective varieties on barnyardgrass growth

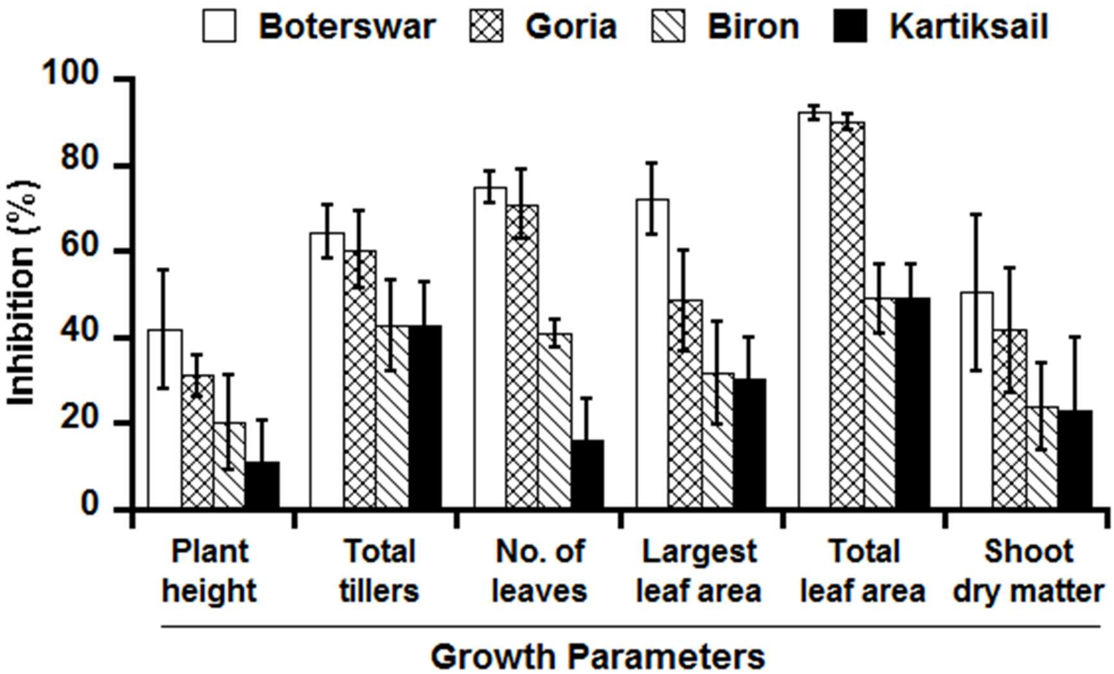

Figure 7. Effect of extracts of selected rice varieties on different growth parameters of barnyardgrass (22 days old)

2.4 Discussion

To establish an alternative strategy for weed management in rice, the phenomenon of allelopathy has been a subject of continued research for a long time. In this experiment, rice has been extensively studied with respect to its allelopathy as part of a strategy for sustainable weed management. Some rice varieties were found to have allelopathic activity against lettuce, cress, radish, barnyardgrass and jungle rice. Rice has been extensively studied with respect to its allelopathy, and a large number of rice varieties were found to inhibit the growth of several plant species when they were grown together (Dilday et al. 1989; Olofsdotter et. al. 1999; Azmi et al. 2000; Khanh et al. 2007a; Salam & Kato-Noguchi, 2009; Thi et al. 2014). When the short-term co-cultivation of rice varieties with test species and weeds was conducted the highest inhibited growth was recorded with Boterswar (46.07%) followed by Goria (43.78%), Biron (36.58%) and Kartiksail (35.97%) among 50 indigenous rice varieties of Bangladesh. Previously, Salam and Kato-Noguchi (2009) compared the allelopathic traits among 102 varieties and found the greatest inhibitory activity of BR17 rice on cress, lettuce, barnyardgrass and jungle rice; the average growth of the shoots and roots was inhibited by 39.5%. Inhibitions on root growth were greater than those on shoot growth in all receiver species. This result was consistent with the report of Olofsdotter and Navarez (1996), and Kim and Shin (1998) who reported that allelopathic rice varieties strongly inhibited the root growth rather than the shoot of paddy weeds.

More inhibitory effective was found on root and shoot growth in dicotyledonous test plants (lettuce, cress and radish) than that of monocotyledonous (barnyardgrass and jungle rice) in our study. Khanh et al. (2006) also found that *Passiflora edulis* aqueous extracts strongly suppressed the growth of lettuce and radish, whereas the growth of barnyardgrass was less affected. These results are consistent with other findings, which found different allelopathic responses for different test plants to have asymmetrical selectivity of allelopathic substances

(Inderjit and Duke, 2003). In addition, allelopathic activity of rice was variety and origin dependent (Khanh et al. 2007a). All variability of effects of rice varieties on root/shoot length of the receiver species also support the fact that rice varieties possess different genotypic characteristics in respect to their allelopathic effects. In this study, the stimulating effect of rice varieties was found. Karim et al. (2006) observed increased root and shoot length of barnyardgrass in some rice accession cases. Rice (1984) stated that the stimulatory effects could occur at a lower concentration of allelopathic substances, while a higher concentration may cause inhibitory effects.

The reduction of receiver growth in laboratory experiments by aqueous methanol extracts indicates allelopathic potential of the four selected varieties. The sensitivity of roots of all target plant species against the rice extracts was greater than that of shoots (Table 2-4). These results are in agreement with the studies of Zimdahl and Stachon (1980) who reported that the extracts of allelopathic plants had a more inhibitory effect on root growth than shoot growth. It might be due to the fact that roots are the first to absorb the allelochemicals or autotoxic compounds from the environment. In addition, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue (Nishida et al. 2005). The similar pattern of inhibition in growth and development was reported by Escudero et al. (2000). The reasons of the inhibitory effects caused by allelopathic substances could be i) disruption and impairment of mitochondrial respiration, ii) breakdown of the activity of metabolic enzymes (Weir et al. 2004), iii) breakage of cells leading to cell death (Lin et al. 2000) and iv) the correlation with increased cell membrane degradation (Bogatek et al. 2006). Besides these, plant physiological activities such as respiration, photosynthesis, cell division and structure, ion uptake and membrane permeability could be affected by the actions of allelopathic substances and thus growth and development might be consequently arrested (Gniazdowska & Bogatek 2005).

Barnyardgrass is one of the most noxious paddy weeds in the world since this weed competes with rice for nutrients, light and water, and reduces the yield of rice. The reduction of barnyardgrass height and biomass in the glasshouse experiment indicates allelopathic potential of the rice varieties (Figure 2). Inhibitory effects of rice varieties have also been reported by other researchers (Chung et al. 2001; Jung et al. 2004; Asghari et al. 2006; Pheng et al. 2009). Weir et al. (2004) declared that the inhibition of photosynthetic rate, interruption of respiration, ATP synthesis and amino acids metabolism were major physiological and biochemical mechanisms that might be mediated by allelochemicals. Selection of rice cultivars with greater allelopathic potential can be used as a tool in sustainable weed management and might be a way to minimize herbicide use (Asghari et al. 2006). Allelopathic and competitive rice lines could be particularly useful in subsistence farming systems where the cost of selective herbicides is prohibited or when the organic production of rice is the objective (Pheng et al. 2009).

As allelopathy in rice was polygenic and quantitatively inherited and thus allelopathic activity may be a polygenic trait slightly correlated with yield or other agronomic features (Khanh et al. 2007b). Application of allelopathy through genetic manipulation by using molecular genetics and biotechnology or conventional breeding in rice varieties can be considered as a successful tool for weed management, insect pests and disease pathogens (Amb & Ahluwalia 2016). Jensen et al. (2001) performed gene mapping and epistatic QTLs associated with allelopathic activity by using DNA markers and indicated that allelopathy in rice is a quantitative trait involving several loci and probably some levels of epistasis. Varietal improvement is an essential prerequisite for the practical application of rice allelopathy for paddy weed management and thus much efforts have been done to develop commercially acceptable allelopathic rice cultivars with high grain quality, high yield, labour saving, low cost and safe grain production (Khanh et al. 2007a; Chen et al. 2008). Selected elite allelopathic rice genotypes have enabled breeding efforts to improve weed suppressive traits in modern cultivars (Belz 2007). Several studied (Dilday et al 2000; Lin et al. 2000; Kim & Shin 2003; Chen et al. 2008) showed that a traditional breeding method can be reasonable to develop of commercially acceptable allelopathic rice cultivars. In this study we have evaluated 50 indigenous rice varieties, of which some varieties showed high allelopathic effect. In addition, some varieties show vigorous growth with higher number of effective tillers under drought and high temperature in Bangladesh. The selected elite varieties could be used in variety development using molecular genetics (QTLs) and biotechnology or conventional breeding techniques. Success in breeding allelopathic rice varieties of Bangladesh would make a great contribution to sustainable rice production.

CONCLUSION

The short-term co-cultivation of rice varieties with test species and weeds, the highest inhibition was found by Boterswar followed by Goria, Biron and Kartiksail among 50 indigenous rice varieties of Bangladesh. All variability in effects of rice varieties on root/shoot length of the receiver species also support the fact that rice varieties possess differential genotypic characteristics in respect of their allelopathic effects. The reduction in receiver species initial growth in laboratory experiments by aqueous methanol extracts indicates allelopathic potential of the selected four varieties. The reduction of barnyardgrass biomass and height in glasshouse experiment indicates allelopathic potential of the selected four rice cultivars. Therefore, the present research suggests that Boterswar, Goria, Biron and Kartiksail are the most allelopathic among 50 Bangladesh indigenous rice varieties. Therefore, additional research is necessary in order to isolate and identify of allelochemical(s), as well as to characterize its production and release from rice plants. Moreover, these allelopathic rice varieties may be used for breeding to develop a new variety with good weed-suppressing ability that would be beneficial for farmers.

CHAPTER III

ISOLATION AND CHARACTERIZATION OF ALLELOPATHIC COMPOUNDS FROM THE INDIGENOUS RICE VARIETY 'BOTERSWAR' AND THEIR BIOLOGICAL ACTIVITY AGAINST *Echinochloa crus-galli* L

ABSTRACT

Aqueous methanol extracts of the Bangladesh indigenous rice (*Oryza sativa* L. ssp. *indica*) variety 'Boterswar' inhibited the germination and seedling growth of *Lepidium sativum* L. and *Echinochloa crus-galli* L. Beauv which suggested that this variety may contain phytotoxic substance(s). Four biologically active compounds, syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), (-)-loliolide, 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one and 3-hydroxy-β-ionone, were isolated from the ethyl acetate phase using several chromatographic steps. The chemical structures of the compounds were determined through electrospray ionization and spectroscopic analyses. The biological activity of these compounds showed that concentration > 10 μM significantly inhibited the root and shoot growth of *E. crus-galli* seedlings, and the I_{50} (50% growth inhibition) values ranged from 16.03 to 27.23 μM and 23.94 to 75.49 μM for root and shoot growth, respectively. The four compounds synergistically suppressed the growth of *E. crus-galli* more strongly than the individual compounds. Thus, the indigenous rice 'Boterswar' has potential use for weed management and this indigenous variety could be used to develop a new commercial rice variety that may suppress weeds.

3.1 Introduction

Rice (*Oryza sativa* L.) is a major staple crop worldwide particularly in Bangladesh. Weeds are the key biotic threat to rice productivity hence, in rice production herbicides are used (Kong et al. 2008), but their harmful impacts make it desirable to search for other eco-friendly weed management options such as allelopathy (Bhowmik & Inderjit 2003; Nirmal et al. 2010; Tesio & Ferrero 2010). Allelopathy refers to the direct or indirect harmful or beneficial effects of one plant to another plant from the release of biochemicals, known as allelochemicals into the environment (Rice 1984). Thus, allelopathy is a phytotoxic interference in most circumstances (Romeo 2000). Allelochemicals are present in different plant parts such as the root, stem, leaf, bud and flower of many plants (Inderjit 1996). Under certain conditions, allelochemicals are released into the environment as exudates from living plants and from the decomposition of plant residues in abundant quantities to inhibit the growth of adjacent and successive plants (Seigler 1996; Einhellig 1999). Herbicide use rates in rice can be minimized by exploiting weed suppressive allelopathic rice varieties (Gealy et al. 2003) which may not add any extra cost (Jabran et al. 2015). Many rice varieties have been studied (Olofsdotter 1998; Dilday et al. 2001; Kong et al. 2002) and it has been found that some are weed suppressive (Jabran et al. 2015). An allelopathic rice variety could release water-soluble chemicals which can suppress the growth of adjacent and successive weeds (Zhou et al. 2009). Many researchers have endeavored to recognize that allelochemicals are released from rice (Rimando et al. 2001; Kong et al. 2004b). Among the Bangladeshi rice varieties 2,9-dihydroxy-4-megastigmen-3-one from ‘BR17’ (Salam et al. 2009) and 9-hydroxy-β-ionone and 9-hydroxy-4-megastigmen-3-one from ‘Kartiksail’ (Kato-Noguchi et al. 2011) were identified as allelochemicals. Several studies showed that a conventional breeding method could possibly be used to develop commercially cultivable allelopathic rice varieties (Chen et al. 2008; Kong et al. 2011). The discovery of the allelopathic compounds will allow the efficient production of more allelopathic rice varieties through conventional breeding or biological-based genetic modifications, which may be less dependent on herbicides (Bhowmik & Inderjit 2003; Fageria & Baligar 2003). Another approach involves the isolation, characterization, and elucidation of the specific mode of action of phytotoxic natural products from allelopathic rice to develop eco-friendly herbicides (Iqbal et al. 2003; Chung et 2017). In addition, allelopathic rice can initiate its weed resistance mechanism through the production and release of allelochemicals (Kong 2008). On the other hand, a specific plant response to allelochemicals is to trigger a cell death cascade in susceptible plants, while these allelochemicals are not very toxic themselves and they induce a toxic response (Weir et al. 2004).

Kong (2007a) reported that a few rice varieties produce and release allelochemicals into the paddy fields and suppresses the growth of adjacent or successive weeds. There are eight thousand indigenous rice varieties in Bangladesh, and farmers still cultivate more than one thousand rice varieties (Hossain et al. 2013). Determination of allelochemicals in Bangladesh rice varieties and their use for weed control could be advantageous for those who primarily depend on human labour or herbicide uses. We previously reported that the Bangladesh indigenous rice variety ‘Boterswar’ had the highest allelopathic potentiality out of several test species and weeds (Masum et al. 2016), which suggested that this variety has a higher concentration of allelochemicals. Our objectives in current research were to isolate and identify the allelopathic compound(s) present in ‘Boterswar’ indigenous rice variety.

3.2 Materials and Methods

The Bangladesh indigenous rice (*Oryza sativa* L. ssp. *indica*) variety 'Boterswar' was grown hydroponically (Rimando et al. 2001) in glasshouse at the University of Ryukyus for 55 days. At this stage, the average tiller per hill was 16, and the plants developed an extensive and strong root system and obtained an average of 143 g of fresh biomass per hill. After harvesting the rice plants were stored at -20°C until use. Seeds of *L. sativum* L. were purchased from the Green Field Project (Kumamoto, Japan) and seeds of *E. crus-galli* L. were collected from the rice field of the Okinawa Agricultural Research Centre, Nago, Okinawa, Japan. Because of its known germination behavior, *L. sativum* was used as a model test plant for the bioassay (Xuan et al. 2005), and *E. crus-galli*, which has developed resistance to many herbicides (Heap 2016), is considered one of the worst weeds in rice production in 61 countries, including Bangladesh (Holm et al. 1991).

3.2.1 Aqueous methanol extraction

Extracts were prepared using the method described by Salam et al. (2009) for isolating allelochemicals. A total of 3 kg of fresh rice plants (roots, stems and leaves) were blended and extracted with 15 L of 80% (v/v) aqueous methanol for 48 h. The extract was filtered through one layer of filter paper (no. 2; Toyo Roshi Kaisha Ltd., Tokyo, Japan), and the filtrate was extracted again with the same volume of methanol for another 48 h and filtered then, both filtrates were stirred and concentrated at 40ºC *in-vacuo* to prepare the aqueous concentrate (100 mL).

3.2.2 Plant extract bioassay

Rice plants (100 g fresh weight) were extracted and concentrated as described above for bioassay experiment. An aliquot of the aqueous concentrate (1, 3, 10, 50 and 100 mg fresh weight [FW] equivalent extract per mL final assay concentration) was evaporated on an evaporator at 40°C until dry. Then, the dried sample was dissolved in cold methanol (0.2 mL) placed on a sheet of filter paper (no. 2) in a 3 cm Petri-dish, desiccated in a draft chamber and then soaked in 0.8 mL of 0.05% (v/v) an aqueous solution of Tween20 (polyoxyethylene sorbitan monolaurate, Nacalai, Tesque, Inc., Kyoto, Japan) as a surfactant. For the control treatment, methanol (0.2 mL) was added to a sheet of filter paper in the Petri-dish and evaporated, as described above. Ten seeds of *L. sativum* or *E. crus-galli* were placed on the filter paper and then incubated at 25ºC in a dark incubator. Germination was assessed every 12 h with a magnifying glass by counting the germinating seeds, searching for the rupture of the seed coats and the emergence of a radicle ≥ 1 mm (Mayer & Poljakoff-mayber 1963), until no further seeds germinated (48 h for *L. sativum* and 72 h for *E. crus-galli*). The germination (%) was determined for the control (without extracts) according to methods by Salam et al. (2009). For the seedling growth bioassay, ten uniform germinated seedlings of *L. sativum* and *E. crus-galli* were placed in the Petri-dishes and then incubated using the aforementioned procedure. The root and shoot lengths of the test species were determined after 48 h of incubation. The growth inhibition (%) was calculated with respect to control (without extracts) seedlings.

3.2.3 Purification of active substances in the ethyl acetate fraction

According to Salam et al. (2009), the aqueous concentrate was adjusted to pH 7.0 with 1 M phosphate buffer, separated five times against the same volume of ethyl acetate to obtain aqueous and ethyl acetate fractions. The biological activity of the aqueous and ethyl acetate fractions was determined by germination and seedling growth bioassays using *L. sativum* and *E. crus-galli*. The active ethyl acetate fraction was evaporated until dryness after standing with added anhydrous Na_2SO_4 overnight and then chromatographed on a column of silica gel (70 g, silica gel 60N, 70-230 mesh; ASTM, Kanto Chemical Co., Inc., Tokyo, Japan), eluting with a stepwise gradient of ethyl acetate (10% per step, v/v; 150 mL per step) and methanol (300 mL) in *n*-hexane, affording 11 fractions. The biological activity of the collected fractions was determined using the *L. sativum* germination bioassay according to the above procedure, and complete inhibition was found in fractions obtained by elution with 70-80% ethyl acetate in *n*-hexane. After evaporation, the concentrate was filtered through a column of Sephadex LH-20 (60 g, GE Healthcare Bio-Sciences AB SE-75184 Uppsala, Sweden), eluting with 20, 40, 60, and 80% (v/v) aqueous methanol (150 mL per step) and methanol (300 mL). The most active fraction was eluted with 60% aqueous

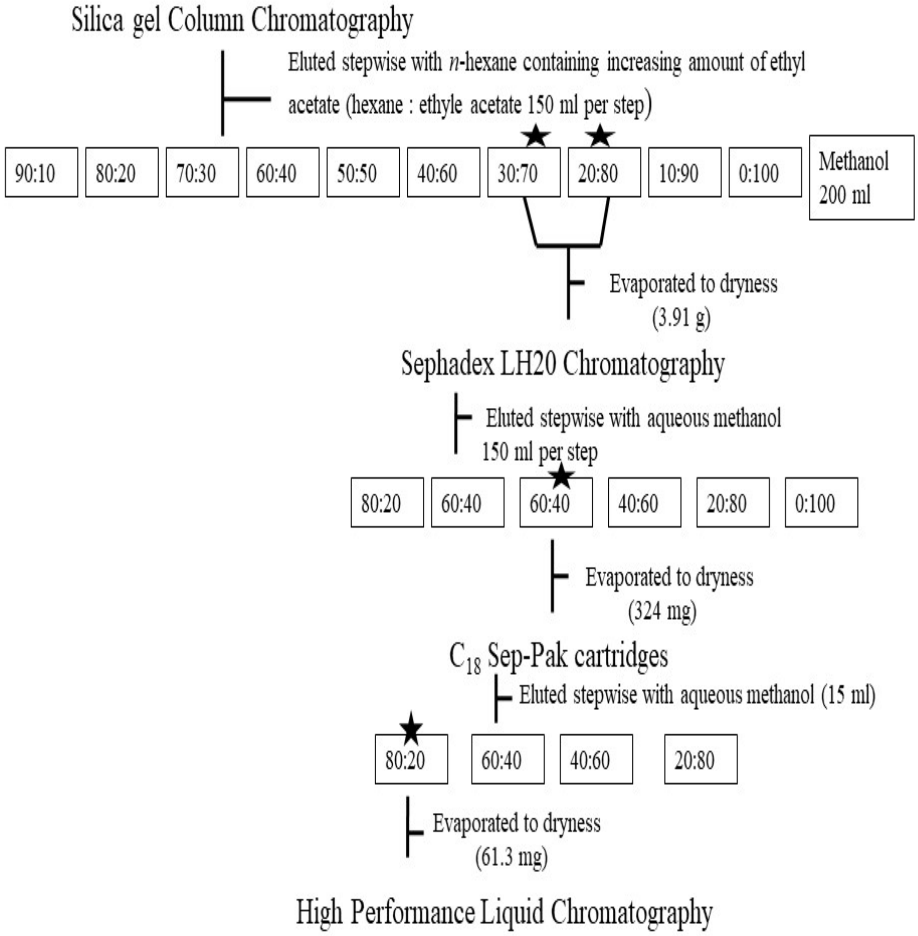

Figure 8. Flow diagram of chromatographic steps for isolation allelopathic compounds from ethyl acetate phase of variety 'Boterswar' rice plant extracts

***indicates active fractions**

methanol and subsequently evaporated until dryness. The concentrate was dissolved in 20% (v/v) aqueous methanol (2 mL) and loaded onto a reverse-phase C_{18} Sep-Pak cartridges (Waters Corporation, Milford, Massachusetts, USA) and purified with 20, 40, 60, 80% (v/v) aqueous methanol and methanol (15 mL per step). The most active fraction was eluted with 20% aqueous methanol and evaporated until dryness. The concentrate was finally purified by C_{18} reversed-phase HPLC (COSMOSIL 5 C_{18}- AR-II; Nacalai Tesque, Inc., Kyoto, Japan), eluting at a flow rate of 3 mL/min with 50% aqueous methanol and detecting at 220 nm. Complete inhibition was detected for four peaks that eluted at 16.0, 19.0, 20.0 and 24.0 min as colorless substances. Mass Spectrometry with electro-spray ionization (ESI-MS) analysis was carried out on a Waters mass specttrometer. NMR spectra were measured in $CDCl_3$ on Bruker NMR spectrometers (500 MHz for ^1H and 125 MHz for ^{13}C). All chemical shifts were reported relative to tetramethylsilane (TMS). Optical rotation was measured in chloroform on a JASCO P-1010 polarimeter.

3.2.4 Bioassay of the isolated compounds

The isolated compounds were dissolved in methanol to prepare the concentrations of 1, 3, 5, 10, 30, 50, 100, 300, 500 and 1000 μM for each compound and 0.1, 0.3, 1, 3, 5, 10, 30, 50, 100 and 300 μM for a mixture of the compounds at a ratio of 1:1:1:1. The biological activity against *E. crus-galli* seedlings was examined using the above procedure.

3.2.5 Statistical analysis

To compare the results, the bioassays were carried out twice using a completely randomized design with three replicates. Significant differences between the treatments and controls were analyzed by Fisher's Protected Least Significant Difference test for each *L. sativum* and *E. crus-galli* species. The Type I error was set at 0.01 for all the statistical comparisons. The I_{50} (concentration of approximate 50% inhibition of the growth rate) value in the assay was analyzed from the regression equation of the concentration curves.

3.3 Results

3.3.1 Phytotoxic effects of aqueous methanol and ethyl acetate extracts

Increased concentrations of the aqueous methanol extracts inhibited the germination and growth of the test species with very low (1 mg DW equivalent per mL) caused stimulation of test species (Figs. 9A, 9B, 10A, 10B). At 100 mg DW equivalent extract per mL *L. sativum* germination was completely inhibited, whereas *E. crus-galli* germination was 16.39**%** with respect to control (Fig. 9A, 9B). At the same concentration, the root growth of *L. sativum* and *E. crus-galli* was 5.90 and 4.10% that of the control, respectively, while the shoot growth of both species was completely inhibited (Fig. 10A, 10B). The fractionated ethyl acetate extract showed significant activity and the ethyl acetate extract treated plants showed severe root browning (data not shown).

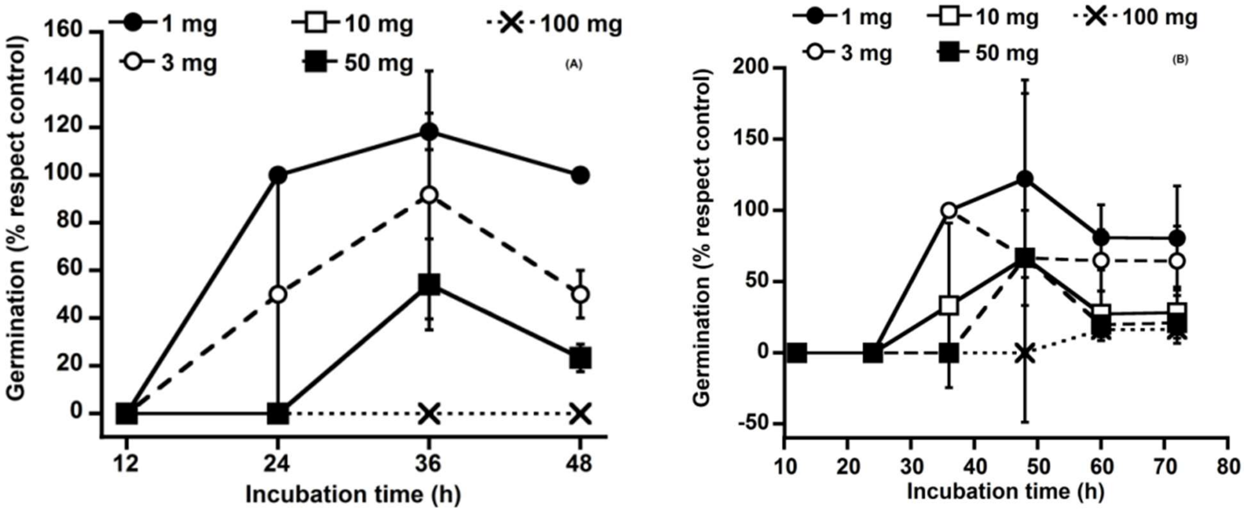

Figure 9. Effects of aqueous methanol extract of ʻBoterswarʾ rice plants on the germination of *L. sativum* (A) and *E. crus-galli* (B) at different concentration. *Bars* represent ±SD of values obtained from three biological replicates

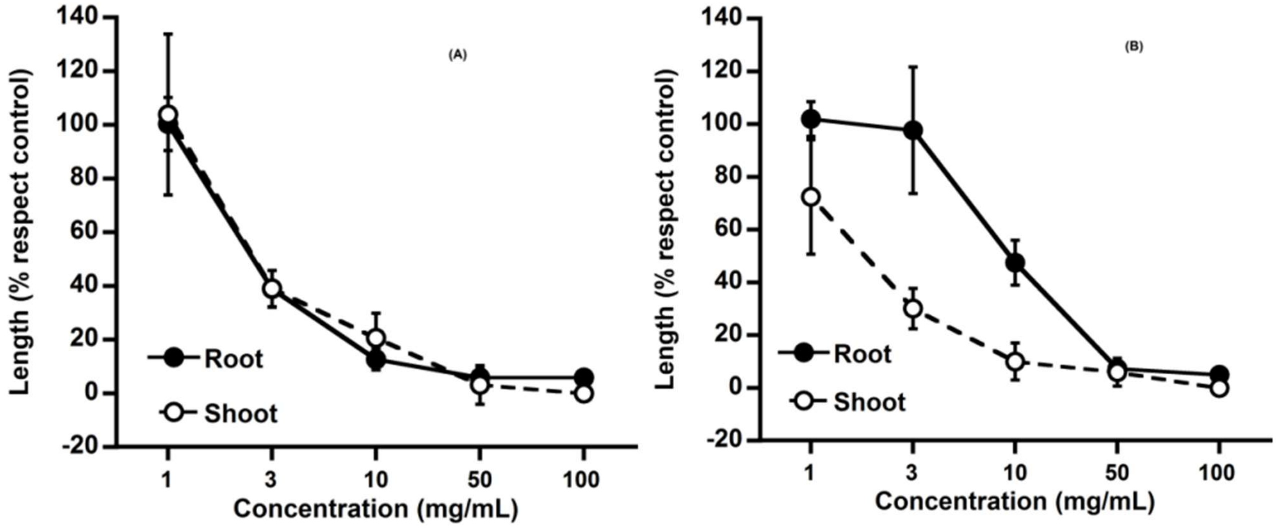

Figure 10. Effects of the aqueous methanol extracts of 'Boterswar' rice plants on the shoot and root growth of *L. sativum* (A) and *E. crus-galli* (B) at different concentration. *Bars* represent ± SD of values obtained from three biological replicates

3.3.2 Structural elucidation of isolated compounds

Four biologically active compounds were obtained from the repeated column chromatography of the aqueous methanol extracts of 'Boterswar' indigenous rice.

Compound 1: It had a molecular formula of $C_9H_{10}O_4$ (LR-ESI-MS *m/z*183 $[M+H]^+$) and a specific rotation of $[\alpha]_D^{23}$+0.04 (*c* 0.01, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$), δ 9.82 (^{1}H, s, H-7), 7.15 (2H, s, H-2 and 6), 3.97 (6H, s, 2×OCH_3); ^{13}C-NMR (125 MHz $CDCl_3$), δ 128.6 (s, C-1), 140.9 (s, C-2 and 4), 147.4 (s, C-3 and 5), 106.8 (d, C-2 and 6), 190.7 (d, C-7), 56.9 (OCH_3). The NMR data are consistent with those reported in Bo Yi et al. (2010), and we identified the substance as 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde) (Fig. 11A).

Compound 2: It had a molecular formula of $C_{11}H_{16}O_3$ (LR-ESI-MS *m/z*197 $[M+H]^+$) and a specific rotation of $[\alpha]_D^{20}$ -65.7 (*c* 0.01, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.27 (3H, s, H-9), 1.47 (3H, s, H-8), 1.52 (dd, J = 13.7 and 3.9 Hz, H-7), 1.76 (dd, J = 13.7 and 4.1 Hz, H-5), 1.78 (3H, s, H-10), 1.98 (dt, J = 2.4 and 14.0 Hz, H-7), 2.54 (dt, J = 2.8 and 14.0 Hz, H-5), 4.30 (m, H-6), 5.69 (s, H-3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 26.9 (C-10), 26.4 (C-9), 30.6 (C-8), 36.0 (C-4), 45.4 (C-7), 47.0 (C-5), 66.3 (C-6), 87.1 (C-7a), 112.4 (C-3), 172.2 (C-2), 183.0 (C-3a). The data were compared with the data reported by Park et al. (2004), and the substance was identified as (-)-loliolide (Fig. 11B).

Compound 3: It had a molecular formula of $C_{13}H_{20}O_3$ (LR-ESI-MS *m/z* 225 $[M+H]^+$ and 247 $[M+Na]^+$) and a specific rotation of $[\alpha]_D^{25}$ -10.6 (*c* 0.01, $CHCl_3$). The ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum of the compound showed two coupled olefinic protons [δ_H 7.03 and 6.29 (each 1H, d, *J*=15.6 Hz), an oxygenated methane proton [δ_H 3.91(1H, m)], and four methyl groups [δ_H 2.28, 0.98 (each 3H, s) and 1.20 (6H, s)]. Its ^{13}C NMR spectrum revealed 13 carbon signals: a conjugated ketone (δ_C 197.5), one double bond [δ_C 142.4 and 132.7] and an oxygenated methine (δ_C 64.1). An extra four methyl groups, two methylenes, and three quaternary carbons were also found. Except for one double bond, a conjugated ketone, and four methyl groups, the isolated compound had a six-membered ring and was presumed to be a megastigmen derivative. In the HMBC spectrum, the proton signal at δ_H 6.29 (H-8) correlated with the carbon signals at δ_C 69.6 (C-6) and 197.5 (C-9), while the methyl proton signals at δ_H 0.98 (H_3-11) and 1.20 (H_3-13) could be correlated with the carbon signal at δ_C 69.6 (C-6). Thus, the 3-oxobutenyl group was located position C-6. Likewise, based on the HMBC and ^{1}H-^{1}H COSY spectra, the hydroxyl group was attached at C-3. Thus, the hydroxyl group was assigned to the C-3 position. Therefore, the structure was 3*β*-hydroxy-5*α*, 6*α*-epoxy-7-megastigmen-9-one (Fig. 11C) and this corresponds to the data reported by Duan et al. (2002).

Compound 4: It had the molecular formula of $C_{13}H_{20}O_2$ (LR-ESI-MS *m/z* 209 $[M+H]^+$) and a specific rotation of $[\alpha]_D^{25}$ -5.4 (*c* 0.01, $CHCl_3$). The ^{1}H NMR spectrum of the substance (500 MHz, $CDCl_3$, TMS as the internal standard) revealed δ values of 7.32 (1H, d, *J* = 16.2 Hz, H-7), 6.13 (1H, d, *J* = 16.2 Hz, H-8), 3.92 (1H, m, H-3), 2.40 (1H, dd, *J* = 17.4 and 5.4 Hz, H-4a), 2.30 (3H, s, H-10), 2.06 (1H, dd, *J* = 17.4 and 9.6 Hz, H-4b), 1.79 (3H, s, H-13), 1.77 (1H, dd, *J* = 12.6 and 2.4 Hz, H-2a), 1.46 (1H, dd, *J* = 12.6 and 12.0 Hz, H-2b), 1.14 (3H, s, H-11), and 1.11 (3H, s, H-12). The ^{13}C NMR spectrum of the substance (125 MHz, $CDCl_3$, TMS as the internal standard) contained δ values of 201.3 (C, C-9), 144.5 (CH, C-7), 136.9 (C, C-6), 134.3 (C, C-5), 133.3 (CH, C-8), 64.9

(CH, C-3), 49.6 (CH_2, C-2), 43.5 (CH_2, C-4), 37.8 (C, C-1), 30.8 (CH_3, C-11), 28.9 (CH_3, C-12), 27.3 (CH_3, C-13), and 21.8 (CH_3, C-10). These data are consistent with previous studies (Güldner & Winterhalter 1991; Kato-Noguchi *et al.* 1993; Dietz & Winterhalter 1996) and the substance was identified as 3-hydroxy-*β*-ionone (Fig. 11D).

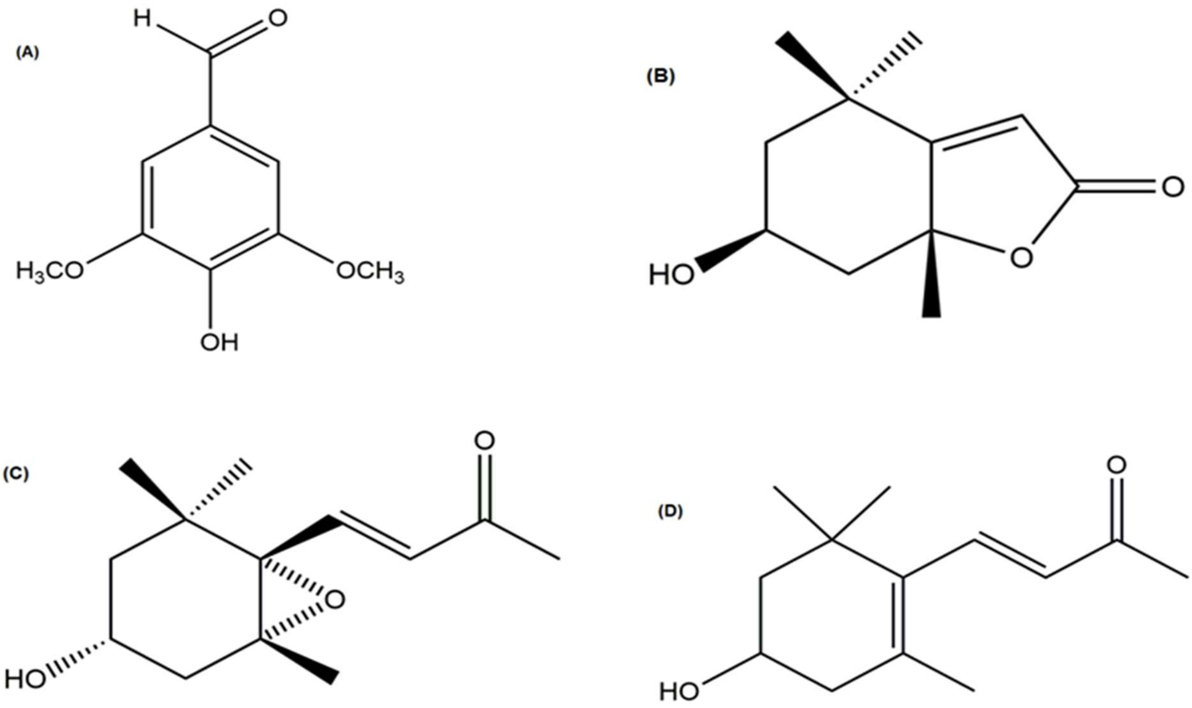

Figure 11. Structures of isolated allelochemicals viz., 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde) (A), (-)-loliolide (B), 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one (C) and 3-hydroxy-*β*-ionone (D) from the Bangladesh indigenous rice var. 'Boterswar'

The endogenous concentrations of syringaldehyde, (-)-loliolide, 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one, and 3-hydroxy-*β*-ionone were at least 5.86, 8.16, 4.16 and 1.28 μmol/kg, respectively because 3.2, 4.8, 2.8 and 0.8 mg of the respective substances (MW 182, 196, 224 and 208, respectively) were isolated from 3 kg of fresh rice plants.

3.3.3 Biological activity of compounds

In the *E. crus-galli* seedling growth bioassay, the effects of four compounds were evaluated on the same basis but different indices were used for the same compounds. The results verified that the four compounds had diverse inhibitory effects on the root and shoot growth of *E. crus-galli* seedlings at the concentrations as low as 10 μM, and the effects increased with increasing concentrations of compounds (Fig. 12.A, B, C, D). The combined effects of the four compounds at concentrations as low as 3 μM showed significant inhibition of the root and shoot growth of *E. crus-galli* seedlings (Fig. 13).

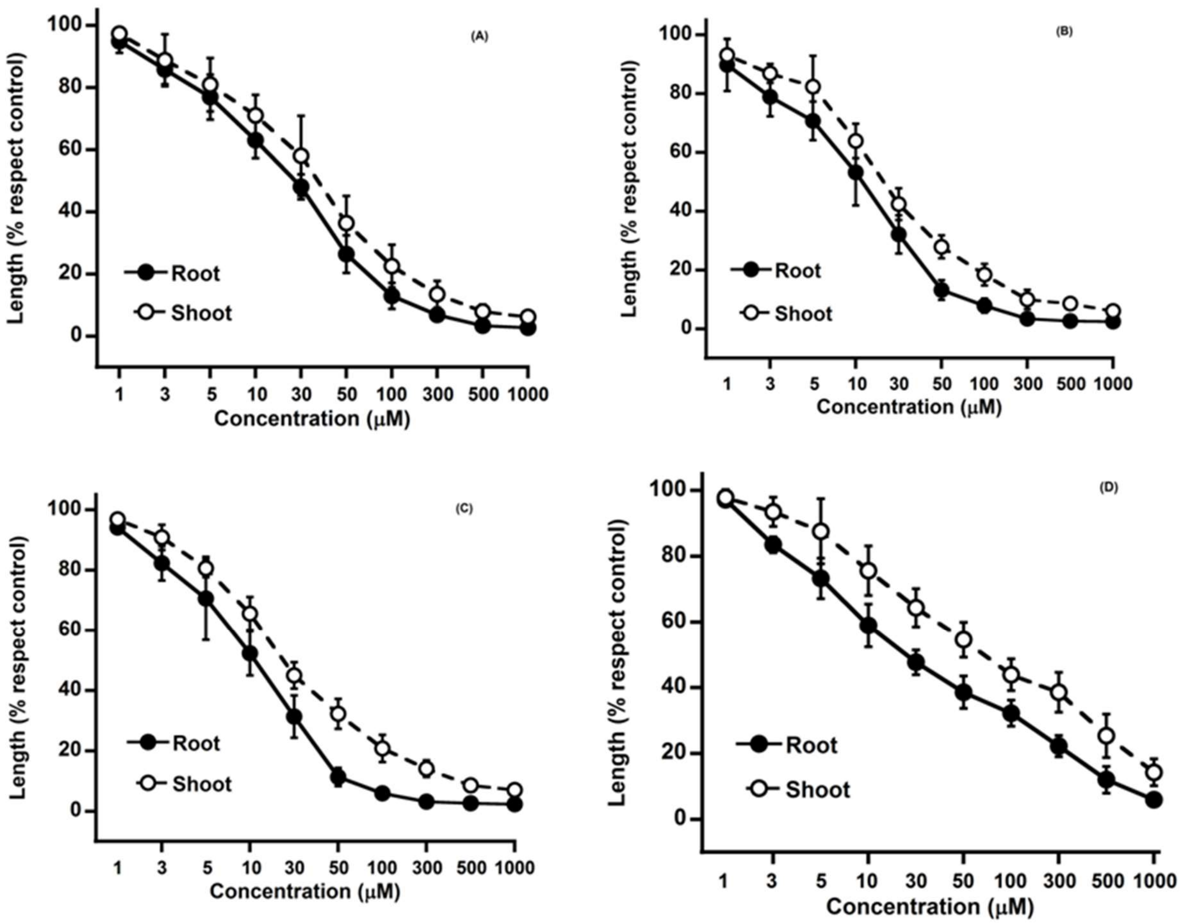

Figure 12. Inhibition of root and shoot growth of *E. crus-galli* s at different concentrations of 1 (A), 2 (B), 3 (C) and 4(D) isolated compounds. *Bars* represent ±SD of values obtained from three biological replicates

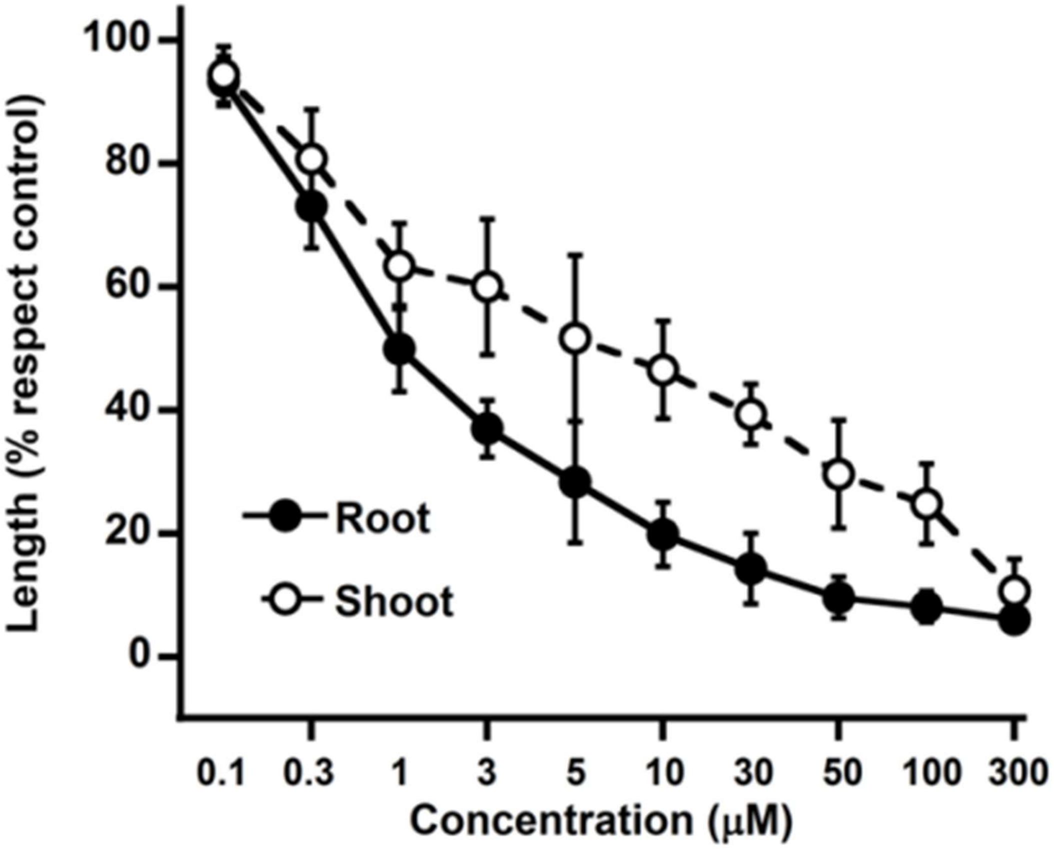

Figure 13. Effects of the mixture of four compounds on the root and shoot growth of *E. crus-galli*. The concentration of 100 µM represents 25 µM syringaldehyde, 25 µM (-)-loliolide, 25 µM 3*β*-hydroxy-5*α*, 6*α*-epoxy-7-megastigmen-9-one, and 25 µM 3-hydroxy-*β*–ionone. *Bars* represent ±SD of values obtained from three biological replicates

The concentrations causing approximately 50% growth inhibition in the assay (defined as I_{50}) were 27.23 and 35.99, 16.46 and 23.94, 16.03 and 25.50, 26.23 and 75.49 µM for compounds **1**, **2**, **3** and **4** for *E. crus-galli* roots and shoots, respectively. The I_{50} values of the compound mixture were 0.97 and 7.58 µM for *E. crus-galli* roots and shoots, respectively (Table 5).

Table 5. Regression analyses of dose response curves for effects on *E. crus-galli* growth of different concentrations of the isolated compounds and their mixture

Compound	on root			on shoot		
	Regression Equation	r^2	I_{50} (µM)	Regression Equation	r^2	I_{50} (µM)
1	y = 1.039x + 21.71	0.906	27.23	y = 0.866x + 18.83	0.979	35.99
2	y = 1.403x + 26.91	0.924	16.46	y = 1.446x + 15.38	0.918	23.94
3	y = 1.420x + 27.24	0.916	16.03	y = 1.311x + 16.57	0.942	25.50
4	y = 0.978x + 24.35	0.806	26.23	y = 0.277x + 29.09	0.954	75.49
Mixture	y = 44.23x+ 7.200	0.927	0.97	y = 1.754x + 36.71	0.864	7.58

r^2 = Determination coefficient, I_{50}= concentration required to obtain 50% growth inhibition

3.4 Discussion

The shoots of rice are the major site of the synthesis or accumulation of allelochemicals, while roots are a pathway of release (Kong et al. 2004a); alternatively, allelochemicals are leached directly from leaves (Ebana et al. 2001). Therefore, the whole rice plant was chosen as the source of allelochemicals in these experiments. Germination and growth bioassays are primary tools for assessing phytotoxic activity (Inderjit and Dakshini 1995; Szabo 2000) in which allelopathic effects can be observed under controlled laboratory conditions (Reigosa & Pazos-Malvido 2007). In these studies, both stimulatory and inhibitory effects were found. Rice (1984) reported how allelopathic activity that has a stimulatory effect at a lower concentration and an inhibitory effect at a higher concentration can occur due to allelopathic compounds. Inderjit and Duke (2003) also found different allelopathic responses for asymmetrical test plants due to the different selectivity of allelopathic substances. These isolated and identified compounds were phenolic compounds which are one of the most common forms of allelochemicals (Liu et al. 2013) and have the potential to inhibit seed germination and seedling root and shoot elongation (Kushima et al. 1998). Lin et al. (2004) isolated syringaldehyde from *Ophiopogon japonicus* K**.** and described its allelopathic effect on *E. crus-galli*. Reigosa and Pazos-Malvido (2007) also observed that the radicle length of *Arabidopsis thaliana* was inhibited by syringaldehyde both with and without nutrient bioassays. Grabarczyk et al. (2015) noted that (-) loliolide provides defense against insect herbivory and affects the development of certain plants (allelopathic activity). Islam et al. (2017) also found that loliolide inhibited the seedling growth of *E. crus-galli*. Duan et al. (2002) isolated 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one from *Saussurea medusa* as an immunosuppressive constituent. Kato-Noguchi et al. (2010) isolated 3-hydroxy-*β*-ionone from the moss *Rhynchostegium pallidifolium* and described it as an allelochemical. Concentration dependent inhibitory activities of the compounds were found on the seedling growth of *E. crus-galli*, which could be attributed to the allelopathic effects. Several studies also showed that growth inhibitory compounds released from rice inhibited the growth of *Cyperus difformis*, *C. iria*, *E. crusgalli, Eclipta prostrata* and

Leptochloa chinensis weeds associated with rice (Kong et al. 2004a; Macias et al. 2006; Kong 2007b; Yang et al. 2017) The mixture of the four compounds enhanced inhibition greatly than the individual of the four compounds, which implies that the four compounds may exert synergistic activity to strongly reduce the growth of *E. crus-galli*. The assumptions of Einhellig (1995) appear to be realistic in that allelopathic growth inhibition is associated with the combined effects of several compounds. Similar consequences were also stated by many researchers (Gealy et al. 2000; Chung et al. 2001; Kato-Noguchi et al. 2011).

CONCLUSION

The evidence from this study suggested that the syringaldehyde, (-)-loliolide, 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one, and 3-hydroxy-*β*-ionone are allelopathic compounds isolated and identified initially in rice. With respect to the endogenous levels and inhibitory activity, these compounds may impart a competitive benefit to rice plants in the rhizosphere and may inhibit the growth of adjacent and successive weed species. The findings of this research explored the phytotoxic activity of the Bangladesh indigenous rice variety 'Boterswar' which may be useful for weed management under field conditions. Moreover, this variety may be used for the development of a commercially acceptable allelopathic rice variety. Further studies need to assess the dynamics of these compounds, in addition to evaluating their fate and activity under field conditions.

CHAPTER IV

BANGLADESH INDIGENOUS RICE VARIETIES PERFORMANCE IN WEED INFESTED FIELD

ABSTRACT

This research aimed to correlate with the screening results of allelopathic potential of Bangladesh indigenous allelopathic rice (*Oryza sativa* L. spp. *indica)* varieties in laboratory and glasshouse experiments through a field study. Six varieties namely, "Boterswar', 'Goria', "Biron' and 'Kartiksail' as most allelopathic, 'Hashikolmi' as the tallest and weakly allelopathic, and 'Holoi' as non-allelopathic were transplanted and raised by following no weed control method. There were seven weeds *viz*., *Echinochloa crus-galli* var. *oryzicola*, *Brachiara syzigachne*, *Scirpus fluviatilis* A. Gray, *Cyperus difformis*, *Eclipta prostrata* L., *Lindernia dubia* Philcox and *Dopatrium junceum* Hamilt from four different families infested rice field. The infestation levels of these seven weeds species were calculated using Simpson's Diversity Index (*SDI*) which, ranged from 0.2 to 0.56, where, *S. fluviatilis* had the highest frequency and infested irrespective of varieties. However, a significant correlation coefficient (0.87, $P < 0.001$) was obtained from these field data by comparing with the root inhibition (%) from the in vitro bioassay and varieties 'Boterswar' and 'Biron' were found as the most allelopathic. Allelopathic 'Boterswar' and 'Biron' also significantly reduced the vegetative growth and delayed to initiate the reproductive organ of *S. fluviatilis*. Among *E. crus-galli* species only *E. crusgalli* var. *oryzicola* infested in weakly allelopathic 'Hashikolmi' and non-allelopathic 'Holoi' growing plots. Results also showed that the growth and yield contributing parameters of rice varied with a varietal difference, however, an irregular crop growth and high tiller mortality rate were observed in case of 'Hashikolmi' and 'Holoi' rice varieties. The allelopathic 'Boterswar' and 'Biron gave significant yield over other varieties in weed infested field condition. This allelopathic potential of rice varieties might be useful for the development of the weed-suppressing capacity of rice which will likely have a great influence on paddy weed control.

4.1 Introduction

Rice (*Oryza sativa* L.) is among the most important grain crops in the world. Rice is grown in all parts of the world, whereas its cultivation is highly intense in Asia. Bangladesh is famous for extensive rice biodiversity. The farmers have long been growing ample number of indigenous land races with various quality of grains, resistance to number of diseases and insects and with erratic growing conditions, due to its varied agroecological conditions. However, weeds remain a barrier to rice production due to the cost of herbicides and the risk of resistance, and yield and quality losses where weeds are ineffectively controlled. Weeds compete seriously with crops for resources, especially during establishment and early growth stages (Zimdahl 1980). Rice is grown in both lowland and upland areas and can be planted all year round, with two or three crops per year. Both the low and upland rice fields in Bangladesh are generally infested with many tropical and subtropical weeds such as *E. crus-galli*, *E. colona*, *Leersia hexandra*, *Oryza rufipogon*, *Fimbristylis miliacea*, *C. esculentus*, *C. diformis*, *Monochoria vaginalis*, *Spilanthes acmella*, *E. prostrata* etc. Hence, it is unavoidable to apply miscellaneous weed control method in order to reach sustainable weed control in rice. Recently, researchers have paid more attention to the utilizing of allelopathic potential variety of rice for weed management because it is a cost-effective, safe measure that can reduce weeding inputs (Khanh et al. 2007a). This entails increasing rice varieties that express an allelopathic activity to overwhelm weeds (Jabran et al. 2015). Furthermore, the allelopathic rice straw residues can also be mulched in rice or other crop fields for controlling weeds (Jabran & Farooq 2013).

The evolution of herbicide-resistant (HR) weed populations is a natural response to selection pressure imposed by modern agricultural management activities (Norsworthy et al. 2012). Thus, the insistent use of herbicides in herbicide-tolerant rice varieties has fortified weeds to evolve herbicide resistance (Heap 2017). For example, nineteen weeds have evolved resistance to glyphosate; about half grew in glyphosate-resistant (GR) crops including rice (Heap 2017). Such weeds and glyphosate-resistant varieties have greater potential to become snags as volunteer crops than do straight crops (Cerdeira & Duke 2006). Under such situations, the control of both weeds and weedy rice (*Oryza sativa*) may become more worrying. These encounters have focused consideration on the opportunity of using non-chemical control strategies in weed management.

Suppression of weeds by a crop is an important approach for weed management. Allelopathy can be a component of plant/plant interference, the other component being competition. Plant interference can be parted into competition and allelopathy. Ideally, allelopathy studies should eliminate or minimize competition from the interaction between species. However, the next Chapter V will be on the separation of competition and allelopathy. It has been known that the allelopathic potentialities in rice vary between varieties or genotype (Dilday et al. 1989, 1994, 2000; Olofsdotter et al. 1997; Lee et al. 2004; Zhou et al. 2011). Dilday et al. (1994) examined about 10,000 accessions from rice germplasm collections for allelopathic effects on ducksalad [*Heteranthera limosa* (Sw.) Willd]. Olofsdotter et al. (1997) reported that 45 out of 1000 screened rice varieties contained hopeful allelopathic activity against one or more weeds. About 20-40% of 1000 rice varieties in Egypt have shown strong allelopathic activity against indicator plants (Hassan et al. 1998). A few allelopathic rice varieties produce and release allelochemicals to inhibit the germination and growth of *E. crus-galli* into the paddy field (Olofsdotter et al. 1999; Gealy et al. 2003; Kong et al. 2008). Also, Garrity et al. (1992) explored the differential weed suppression capability of upland rice varieties and reported rice with strong allelopathic constituents often attains high yield, and sufficient plant characters (plant height and leaf area). These studies imply that it might be possible to develop and use an allelopathic component to control weeds via plant breeding. The impact of genetic differences of

Bangladeshi rice allelopathy against weeds has been assessed under laboratory conditions by Salam and Kato-Noguchi (2009), Karim et al. (2014) and in recent Masum et al. (2016). These methodologies have led to the isolation of a number of phytotoxins that are secreted by Bangladeshi rice plants (Salam et al. 2009; Kato-Noguchi et al. 2011; Masum et al. 2018).

Laboratory screening is useful and necessary for initial allelopathic investigation in rice varieties, but it is important to demonstrate that such observed in vitro allelopathy effects also occur under field conditions. As the full complexity of interactions that occur in the natural rhizosphere is eliminated in such a system, and so the obtained results should be analyzed with caution before any conclusion can be made. Indeed, studies conducted without using soil might not reproduce the conditions that are needed for the expression of such allelochemicals in nature (Inderjit & Callaway 2003). Beside this, in the field, phytotoxins produced by an allelopathic rice can be rendered harmless by the mutual interactions of soil texture, organic matter, temperature, irradiance and microbial breakdown (Bais et al. 2006; Goodall et al. 2010). Therefore, laboratory bioassay alone does not sufficiently prove that allelopathy is effective in the field due to the intricacy of field interactions and retorts (Inderjit &Weston 2000). Few studies have attempted to correlate laboratory and field results and for Bangladeshi rice varieties there is no. Regrettably, linking laboratory and field results goes some way to elucidate the possible allelopathic performance of rice varieties. Importantly, the use of allelopathic rice varieties can be integrated with other weed control methods in order to practice integrated weed management and attain sustainable weed control. Allelochemicals exuding from rice roots are likely to be received and absorbed by the roots of weeds in the vicinity; which will give allelopathic inhibition of weeds (Gealy & Moldenhauer 2012). As a result, weeds will receive a suppressive effect if growing along a rice variety with allelopathic possessions rather than a non-allelopathic rice variety (Gealy & Fischer 2010; Gealy et al. 2013).

In light of these, it becomes clear that a greater understanding of crop–weed interactions is essential in order to develop cost-effective and sustainable weed management practices. Therefore, the overall aim of this study was to identify superior competitive and/or allelopathic rice genotypes which can suppress weeds under field conditions; and to relate field performance to published rankings of allelopathy in Bangladesh indigenous rice (Masum et al. 2016) from an in vitro bioassay.

4.2 Materials and Methods

4.2.1 Experimental site

The field experiments was conducted at the research filed of Subtropical Field Science Center, University of the Ryukyus (26˚14′59″ north latitude and 127˚45′59″ east longitude) in a gray soil (coarse sand 3.61%, fine sand 30.94%, silt 24.32%, clay 32.84%, apparent density 0.90 g cm^{-3}, pH 7.43, C 1.83%, N 0.14%, HPO_4^{2-} 0.44 mg g^{-1} soil, K^+ 0.75 mg g^{-1} soil, Ca^{2+} 4.99 mg g^{-1} soil, Mg^{2+} 0.70 mg g^{-1} soil, SO_4^{2-} 1.39 mg g^{-1} soil, Fe^{3+} 0.64 mg g^{-1} soil, Mn^{2+} 0.41 mg g^{-1} soil, Zn^{2+} 0.47 mg g^{-1} soil, Na^{2+} 1.01 mg g^{-1} soil, Cu^{2+} 0.41 mg g^{-1} soil, and Al^{2+} 0.81 mg g^{-1} soil) in the summer season from April to July, 2017.

4.2.2 Climate

The experimental area was under the subtropical climate and was characterized by high humidity and heavy precipitation with occasional gusty winds during the hot summer period from April to September, and during the moderately low temperature prevailed period from October to March. The average meteorological data in respect of air temperature and rainfall recorded by the Okinawa Regional Headquarters, JMA Naha, Okinawa, Japan for the period of experimentation have been presented in Fig. 14.

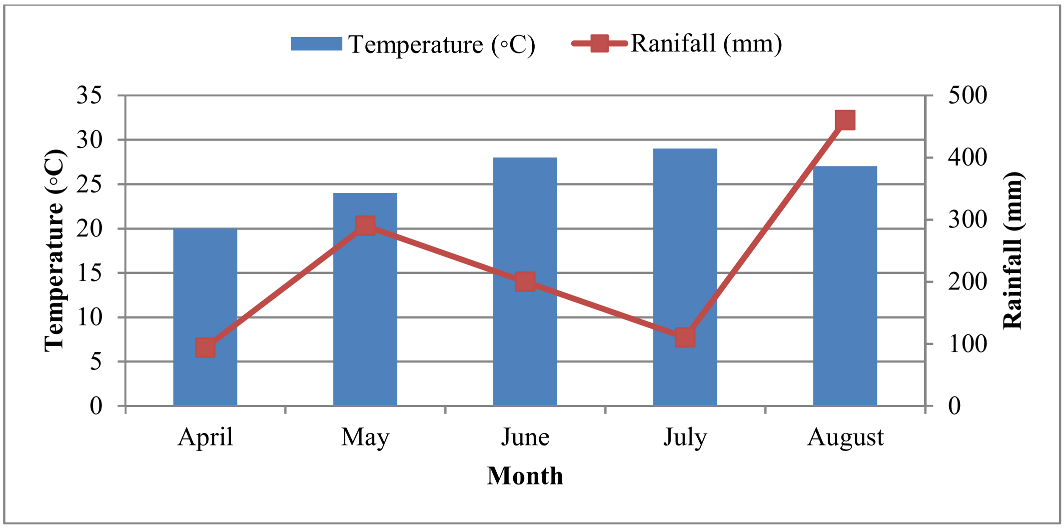

Figure 14. Monthly average temperature and rainfall during experimental period

Source: http://www.jma-net.go.jp/okinawa/

4.2.3 Plant materials and features

Initially, fifty indigenous Bangladesh rice varieties were collected from the Bangladesh Rice Research Institute, Bangladesh Geetanjoly Agro Society, and farmers of Barisal and Bandarban districts of Bangladesh. These collected seeds were brought into Japan by maintaining official procedures. All the rice verities are non-sticky and *indica* rice. The features of these fifty varieties are presented below (Table 6) which was collected from the pot studies during the period from November 2014 to December 2015 in the glasshouse of Field Science Center, University of the Ryukyus, Japan.

Table 6. Growth dynamics of Bangladesh Indigenous rice in pot study at glasshouse of University of the Ryukyus

Sl. No.	Name	Growing Season	Plant height (cm)	Total tillers $Plant^{-1}$ (Nos.)	Effective tillers $Plant^{-1}$ (Nos.)	Life span (Days)
1.	Baila Bokri	*Aus*	130.33	34.33	5.67	115
2.	Bashful Chikon	*Aman*	125.67	18.88	4.33	145
3.	Basmoti/ Sakkorkhana	*Aman*	120.00	26.34	4.67	150
4.	Begun Bahar	*Aus*	100.67	19.34	3.67	110
5.	Bini Dhan	*Aman*	130.78	34.45	5.78	150
6.	Biron	*Aman*	175.45	46.76	8.11	167
7.	Bolo Rum	*Aus*	135.22	24.45	5.23	107
8.	Bonjira	*Aus*	100.00	10.45	3.33	100
9.	Boterswar	*Aus*	146.12	26.16	7.45	110
10.	Cahngsai	*Aman*	115.67	14.45	5.78	150
11.	Chini gura	*Aman*	100.00	43.23	8.45	145
12.	Cockrro	*Aman*	145.67	34.34	5.34	145
13.	Dharial	*Aus*	115.00	24.46	4.76	115
14.	Dholi Boro	*Boro*	120.23	18.76	5.13	170
15.	Dholi Chikon	*Boro*	105.34	22.14	4.67	152
16.	Dudh Kolom	*Aman*	125.78	34.12	5.00	150
17.	Dular	*Aman*	126.67	16.12	4.13	145
18.	Goria	*Aus*	130.56	29.16	7.67	108
19.	Hasha Kumira	*Aus*	126.76	35.34	9.12	110
20.	Hashikalmi	*Aus*	175.45	35.12	7.45	125
21.	Holoi	*Aus*	120.00	23.13	6.35	120
22.	Kalizira	*Aman*	145.67	14.12	3.23	150
26.	Kartik Balam	*Aman*	156.78	40.11	6.87	150
27.	Kartik Sail	*Aman*	145.67	35.34	6.67	150
28.	Kataktara	*Aus*	115.67	10.33	3.67	115
29.	Kataribhog	*Aman*	149.26	16.76	4.34	145
30.	Kazliboro	*Boro*	105.45	19.13	5.67	167

Table 6. Continue

Sl. No.	Name	Growing Season	Plant height (cm)	Total tillers Plant^{-1} (Nos.)	Effective tillers Plant^{-1} (Nos.)	Life span (Days)
31.	Khaia Boro	*Boro*	102.23	34.16	6.67	165
32.	Kushiara	*Aus*	115.00	20.12	3.45	115
33.	Kilong	*Aman*	135.67	14.24	4.45	150
34.	Lalbinimonoching	*Aman*	156.67	15.12	4.33	150
35.	Lal Muta	*Aman*	167.45	25.26	5.34	150
36.	Langda	*Aman*	150.67	18.13	4.34	150
37.	Lekuch	*Aman*	160.33	13.16	4.00	150
38.	Marich Bate	*Boro*	100.45	20.11	7.45	152
39.	Mohonbhog	*Aman*	125.33	30.33	6.00	145
40.	Moulata	*Aman*	129.00	20.23	5.11	145
41.	Naizersail	*Aman*	150.67	45.67	7.32	150
42.	Nakhusimuta	*Aman*	147.78	15.34	4.14	150
43.	Nayan tara	*Aus*	100.67	12.13	3.33	103
44.	Panbira	*Aus*	136.67	26.34	6.67	108
45.	Panki Raj	*Aus*	105.45	44.23	7.33	110
46.	Rani Salute	*Aman*	150.23	34.21	6.67	150
47.	Rata Boro	*Boro*	120.76	18.00	5.67	170
48.	Surjamukhi	*Aus*	110.34	25.34	8.34	108
49.	Tongvoga, Lal Bini	*Aman*	167.00	42.32	6.23	150
50.	Tupa Boro	*Boro*	120.91	39.23	8.67	170

Note: *Aus* - March –July; *Aman* -July- December;, *Boro* -November - May

The Bangladesh indigenous rice (*Oryza sativa* L. spp. *indica*) varieties ‘Boterswar’, ‘Goria’ ‘Biron’, ‘Kartiksail’, ‘Hashikolmi’ and ‘Holoi’ were selected for field study as the varieties of ‘Boterswar’, ‘Goria’, ‘Biron’ and ‘Kartiksail’ the most allelopathic, ‘Hashikolmi’ as tallest and weakly allelopathic, and ‘Holoi’ as non-allelopathic among 50 indigenous Bangladesh rice varieties (Masum et al. 2016).

4.2.4 Seed sprouting and sowing

Initially, seed soaking was done in water for 24 hours. These were then taken out of water and kept in gunny bags. The seeds started sprouting after 48 hrs which were suitable for sowing in 72 hrs. The sprouted seeds were then placed in seedling trays (25-by-25-by-5cm; one seed per hole) filled with commercial potting mixture. The seedlings were watered with tap water daily for 25 days.

4.2.5 Fertilizer Management

The experimental plots were fertilized with @ 100, 50, 62.5, 10 kg ha^{-1} in the form of triple superphosphate (TSP), muriate of potash (MOP), gypsum and zinc sulphate, respectively one day before transplanting. Urea was top dressed @ 125 kg ha^{-1} in three equal splits at as basal dose, 20 and 40 DAT (days after transplanting). The entire amounts of TSP, MOP, gypsum and zinc sulphate were applied at final land preparation as basal dose.

4.2.6 Uprooting and Transplanting of seedlings

Twenty-five days old seedlings were uprooted carefully and were kept in soft mud in shade. The seedling trays were made wet by application of water in the previous day before uprooting the seedlings to minimize mechanical injury of roots. Seedlings were then transplanted @ 2 seedlings per hill on the well-puddled plots on 3 May 2017. In each plot, there were 7 rows, each row containing 7 hills of rice seedlings. There were in total 49 hills in each plot. To maximize experimental precision, there were three replications.

4.2.7 Application of irrigation water

Irrigation water was added to each plot according to the critical stage. Irrigation was done up to 5 cm.

4.2.8 General measurement of weed abundance and growth stage:

The time of 50% rice flowering of each variety was recorded (late June to mid-July) for each genotype. After 50% flowering and during harvest, abundances of targeted weed species were measured by counting against each variety. Each species of weeds also evaluated on the basis of their stage of development as juvenile, vegetative, flowering and mature stage and categorized by 1, 2, 3 and 4, respectively (Fig. 15B). Just before harvest, average rice plant heights to the top of the panicle, total tillers $hill^{-1}$ and effective tillers $hill^{-1}$ were also measured.

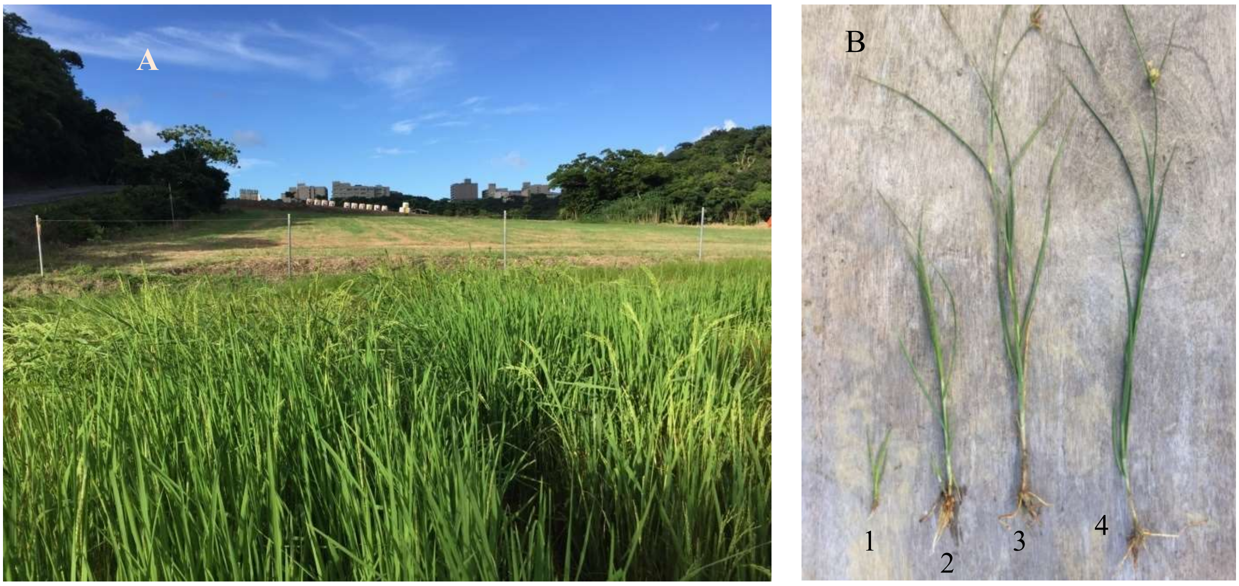

Figure 15. (A) Field view during 50% flowering of Bangladesh indigenous rice; (B) Category of weed development measurement where 1 - juvenile; 2 - vegetative; 3 – flowering; 4 – mature stage

4.2.9 Detailed measurements

Based on previous laboratory studies (Masum et al. 2016), six rice varieties were selected for more detailed observations. The weed suppressive performance of the selected six genotypes was assessed against seven different weed species. Rice density (average, 36 plants m^{-2}), weed diversity and frequency of each weed species were counted in each plot using a random quadrate of 1 m^2 over three days, with one replicate completed per day. Weed diversity and frequency were summarized using Simpson's Diversity Index (*SDI*; Simpson 1949). *SDI* is used to quantify biodiversity in ecological studies.

It takes into account the number of species present, as well as the abundance of each species:

$$SDI = 1 - \sum n\frac{(n-1)}{N(N-1)}$$

Where n is the total number of plants of a particular species and N is the total number of all weed species.

SDI values (%) for the six rice varieties were correlated with the inhibition index from the laboratory bioassay (Masum et al. 2016), which used barnyardgrass in equal compartment agar method (ECAM) bioassay as the target weed species. The % root inhibition values for the respective varieties are provided in Table 7.

Table 7. Inhibition (%) of selected Bangladesh indigenous rice varieties on root growth *E. crus-galli* in ECAM bioassay under laboratory condition

Sl. No.	Variety	Root Inhibition (%)
1.	Biron	48.04 ± 0.71^{c}
2.	Boterswar	62.06 ± 1.28^{a}
3.	Goria	60.32 ± 0.90^{a}
4.	Hashikolmi	25.42 ± 1.80^{d}
5.	Holoi	1.37 ± 0.06^{e}
6.	Kartiksail	50.05 ± 0.67^{b}

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability

4.2.10 Harvesting and postharvest operation

Maturity of crop was determined when 90% of the grains become golden yellow in color. The harvesting was done for yield contributing characters on three pre-selected hills from which growth parameters were collected and mid 1 m^2 from each plot was separately harvested for yield, then bundled, properly tagged and brought to the glasshouse. Threshing was done by pedal thresher. The grains were cleaned and sun dried to moisture content of 12%. Finally grain yields $plot^{-1}$ were recorded and converted to t ha^{-1}.

4.2.11 Statistical Analysis

4.2.11. 1 Weed parameters

The data variance was visually inspected by plotting the residuals to confirm homogeneity of variance before statistical analysis. Data normality and distribution was verified by Q-Q plot and Shapiro-Wilk normality test. Different statistical models were compared to get best fitted model. The models were compared with extracted AIC values and finally data were fitted into GLM model for weed counting data. Model convergence status is: glm(weed~variety*species,poisson). Extensive use was made of several R packages including ggplot2 (Wickham 2009), lattice (Sarkar 2008).

4.2.11.2 Crop parameters

Crop parameters analyses were performed using the general linear model of the statistical analysis program of Fisher's Protected Least Significant Difference test. The experimental designs were completely randomized design with three replications. The Type I error was set at 0.05 and 0.01 for all the statistical comparisons.

4.3 Results

4.3.1 Weed parameters

There are seven weed species belonging to four families were found to infest the experimental rice field. These are given in Table 8.

Table 8. List of infesting weeds in experimental field of Bangladesh Indigenous rice at University of the Ryukyus, Japan

Sl. No.	Name	Type	Family	Infested variety	Occurrence
1.	*Echinochloa crus-galli. var. oryzicola*	Grass	Poaceace	V_5, V_6	Throughout the season
2.	*Brachiara syzigachne*	Grass	Poaceace	V_2	After 50% flowering of rice
3	*Scirpus fluviatilis* A. Gray	Sedge	Cyperaceae	V_1, V_2, V_3, V_4, V_5, V_6	Throughout the season
4	*Cyperus difformis*	Sedge	Cyperaceae	V_6	Throughout the season
5	*Eclipta prostrata* L.	Broad leaf	Compositae	V_4 , V_5 , V_6	After 50% flowering of rice
6	*Lindernia dubia* Philcox	Broad leaf	Scrophulariaceae	V_4, V_6	At maturity stage
7.	*Dopatrium junceum* Hamilt	Broad leaf	Scrophulariaceae	V_1, V_2 , V_3	At maturity stage

Here, V_1- 'Boterswar'; V_2 – Goria; V_3- Biron; V_4 – Kartiksail; V_5 – Hashikolmi; V_6- Holoi

The most abundant weed species was *S. fluviatilis* under both allelopathic and non-allopathic rice varieties (Fig. 16). This sedge species was found throughout the experimental periods and on an average 15 plants were counted. Another sedge weed *C. difformis* were found on an average 3 plants only in non-allelopathic 'Holoi' raised plots. Among *E. crus-galli* (barnyardgrass) species only *E. oryzicola* was found on an average 1 and 2 plants in the weakly allelopathic 'Hashikolmi' and non-allelopathic 'Holoi' raised plots, respectively. This observation showed that all screened allelopathic rice varieties from in vitro bioassay inhibited growth of *E. crusgalli* in the field. Another grass weed (*B. syzigachne*) was found infesting on average one plant in allelopathic 'Goria' variety. Among broad leaf weed *E.*

prostrata, *L. dubia* and *D. junceum* infested at later stage of rice growth on an average 1, 3 and 2 plants, respectively (Table 8; Fig. 16).

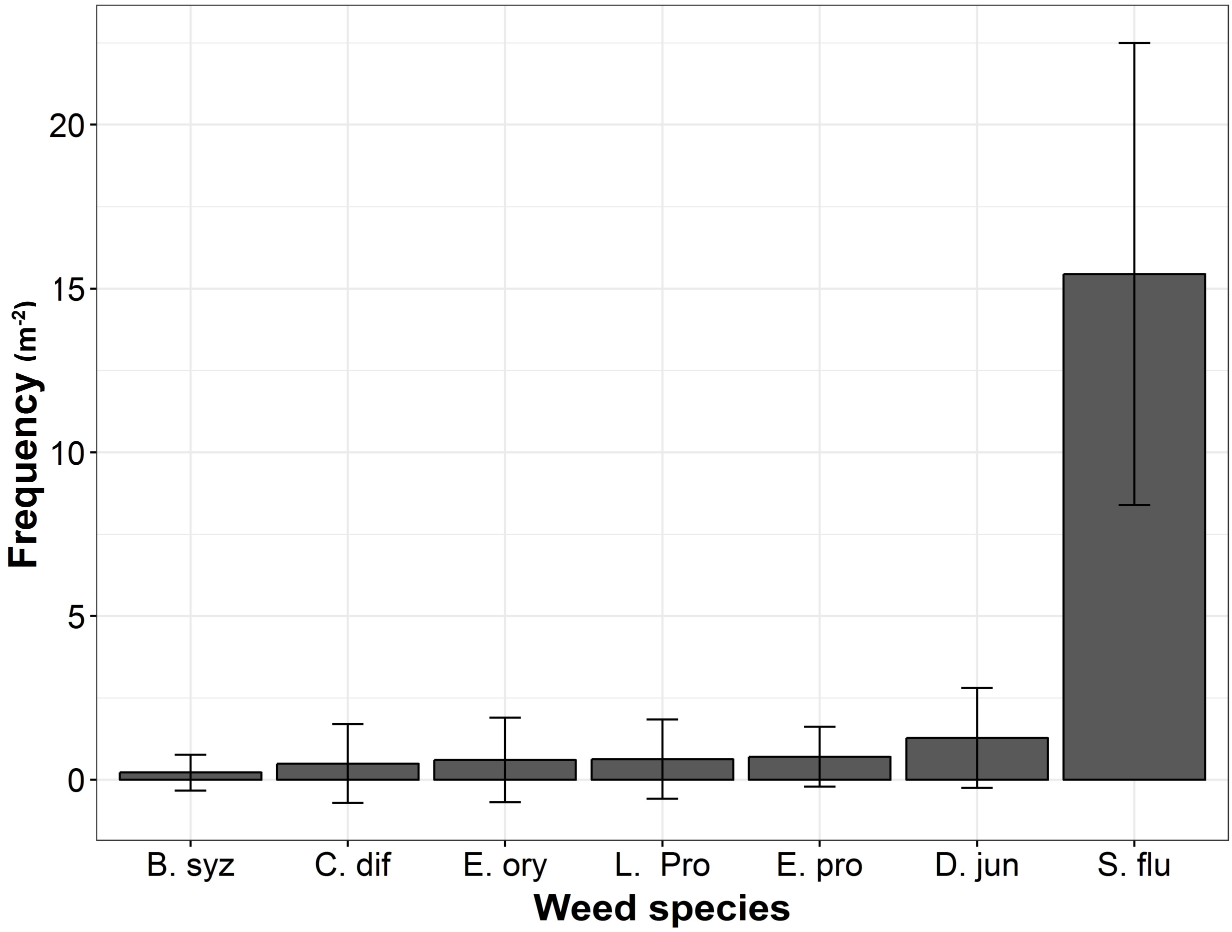

Figure 16. Frequency of seven tested weeds species against allelopathic and non-allelopathic rice varieties. Mean data were pooled form three experimental units

Here, B. syz- *Brachiara syzigachne*; C. dif- *Cyperus difformis*; E. ory- *Echinochloa crus-galli. var. oryzicola*; L. pro-*Lindernia dubia* Philcox; E. pro – *Eclipta prostrata* L.; D. jun- *Dopatrium junceum* Hamilt; S. flu- *Scirpus fluviatilis* A. Gray

The infestation levels of the seven weeds species were reflected in the calculated values of SDI, which ranged from 0.2 to 0.56. (Fig. 17). A significant correlation co-efficient of 0.87 (P < 0.001) was obtained when the SDI value from these field data by comparing with the root inhibition (%) from the in vitro bioassay (Table 7). Variety 'Boterswar' and 'Biron' identified as the most allelopathic in the bioassay, also performed well in terms of weed suppression under field conditions, while 'Holoi', was consistently poor in both field and laboratory experiment.

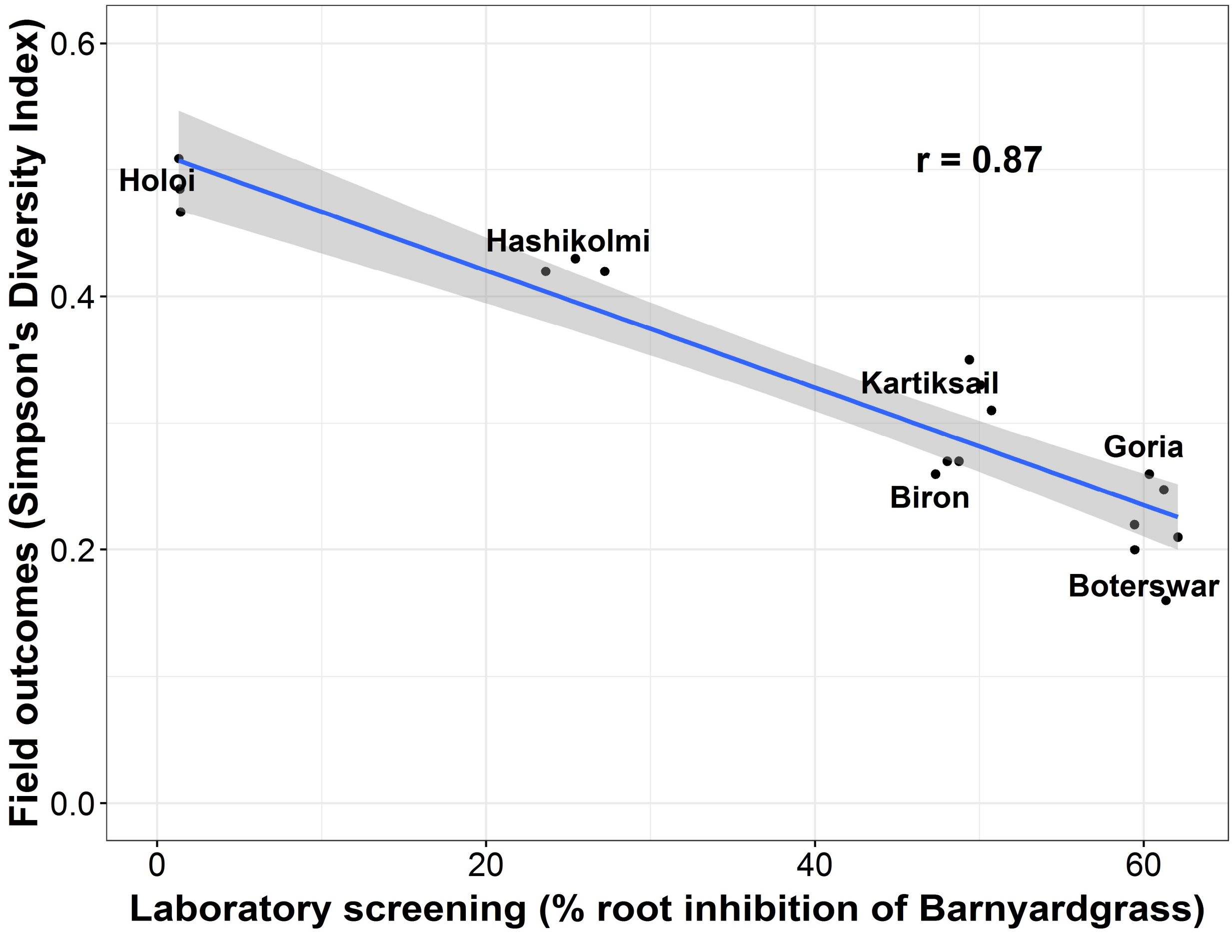

Figure 17. Correlation between in vitro laboratory screening (% root inhibition) using barnyardgrass (*E. crus-galli*) as the weed species (Masum et al. 2016), and weed infestation under field conditions (Simpson Diversity Index) at the University of the Ryukyus in 2017

The shade representing the 95% confidence interval

Rice variety differentially reduced weeds vegetative growth (Fig. 18). Strong interference by some rice varieties such as 'Boterswar' and 'Biron', significantly reduced the vegetative growth and delayed the reproductive organs development of *S. fluviatilis*. So, it can be said that despite high frequency of *S. fluviatilis* under both strong and weak allelopathic rice cultivars, it has loss his fitness under strong allelopathic rice variety.

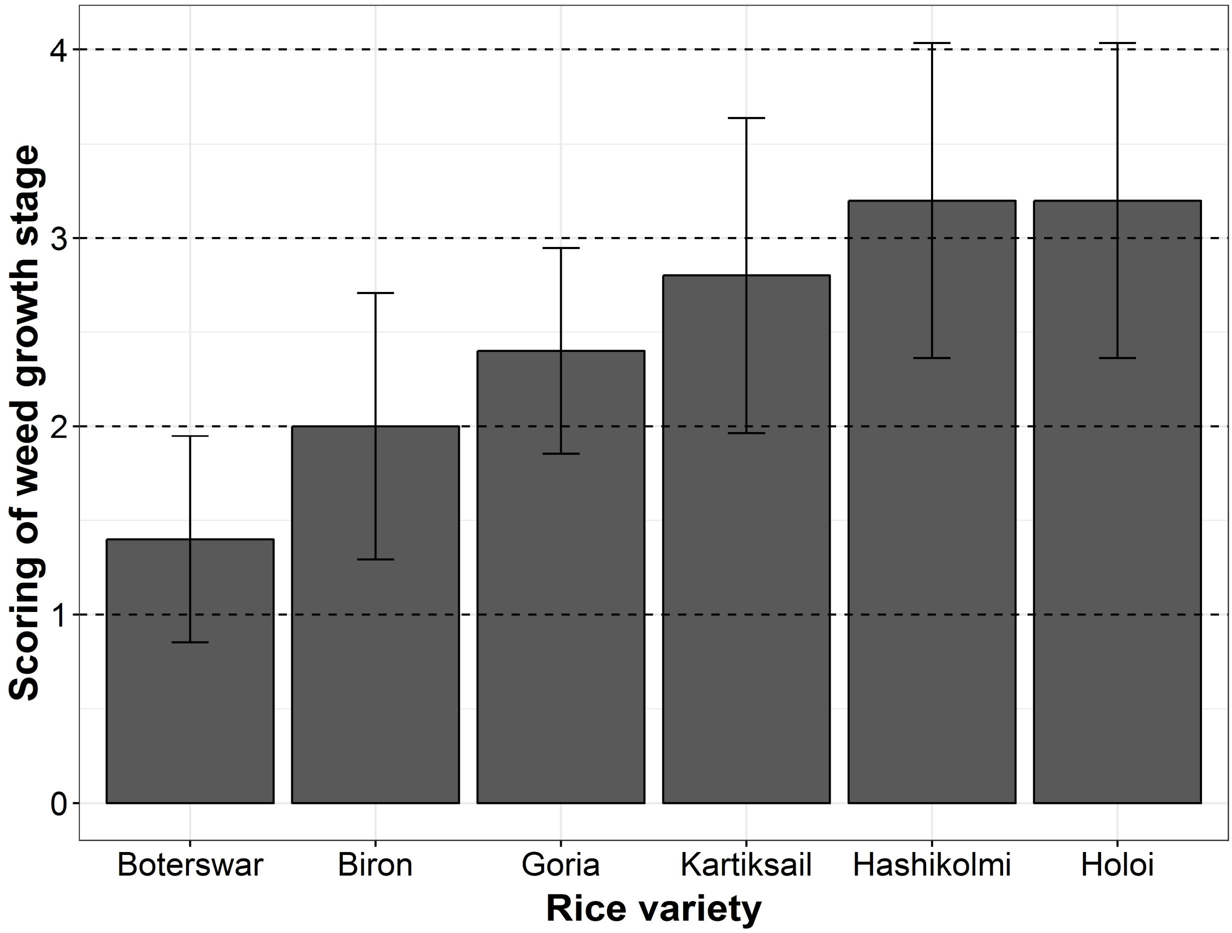

Figure 18. Growth stage of *S. fluviatilis* under allelopathic and non-allelopathic rice varieties. Mean data were pooled form 5 replications of test species

The dash lines on y axis at 1, 2, 3 and 4 points are representing values for juvenile, vegetative, flowering and mature stage of tested weed species

4.3.2 Crop parameters

Plant height varied significantly for varietal variation throughout the growing period (Fig. 19. A). 'Hashikolmi' was the tallest variety (151 cm) and 'Holoi' was the shortest variety (86 cm) at harvest. However, even though crop vigour was not measured, we observed that allelopathic 'Boterswar or 'Biron' tended to be more vigorous at the vegetative stage. The tallest 'Hashikolmi' was not always the most weed-suppressive and observed an irregular growth, however, sever irregular growth was observed in case of non-allelopathic 'Holoi' variety (Fig. 20). Varietal variation had also a significant effect on tillers hill^{-1} over time (Fig. 19. B). Most of the allelopathic rice varieties observed with high tiller capacity showed stronger suppression on the growth of weeds. Although, variety ‘Hashikolmi’ produced significantly higher tillers, the mortality of tillers also higher as at harvest the least number of tillers were found whereas mortality rate of tillers in allelopathic varieties was less than weakly allelopathic ‘Hashikolmi’ and non-allelopathic ‘Holoi’.

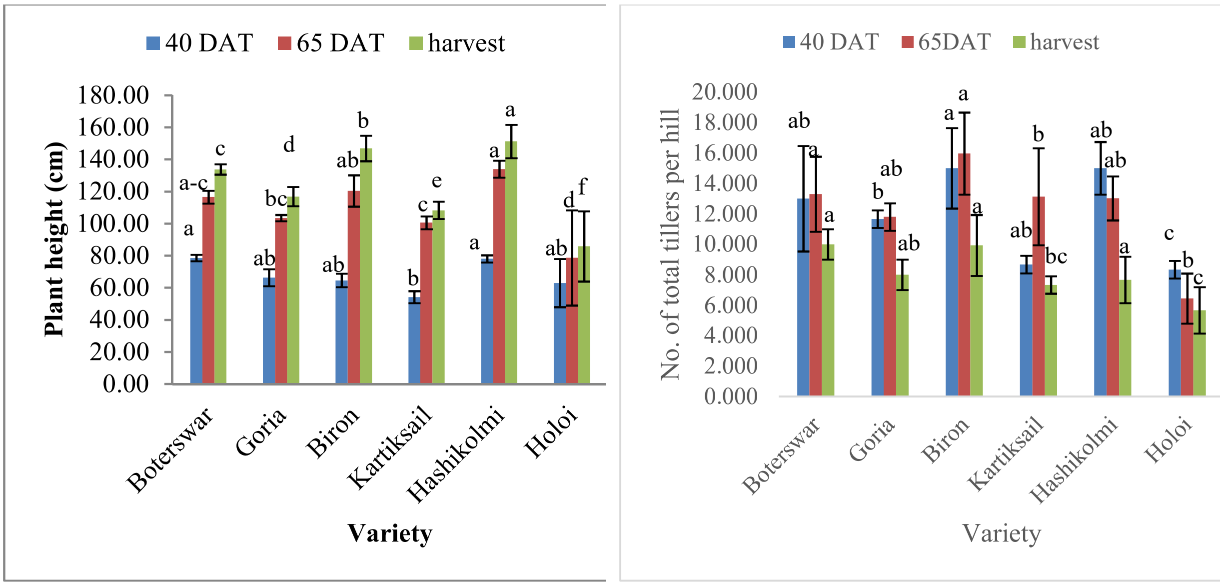

Figure 19. Plant height (A) and total tillers (B) of Bangladesh indigenous rice as influenced by various naturally growing weeds

In a bar means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

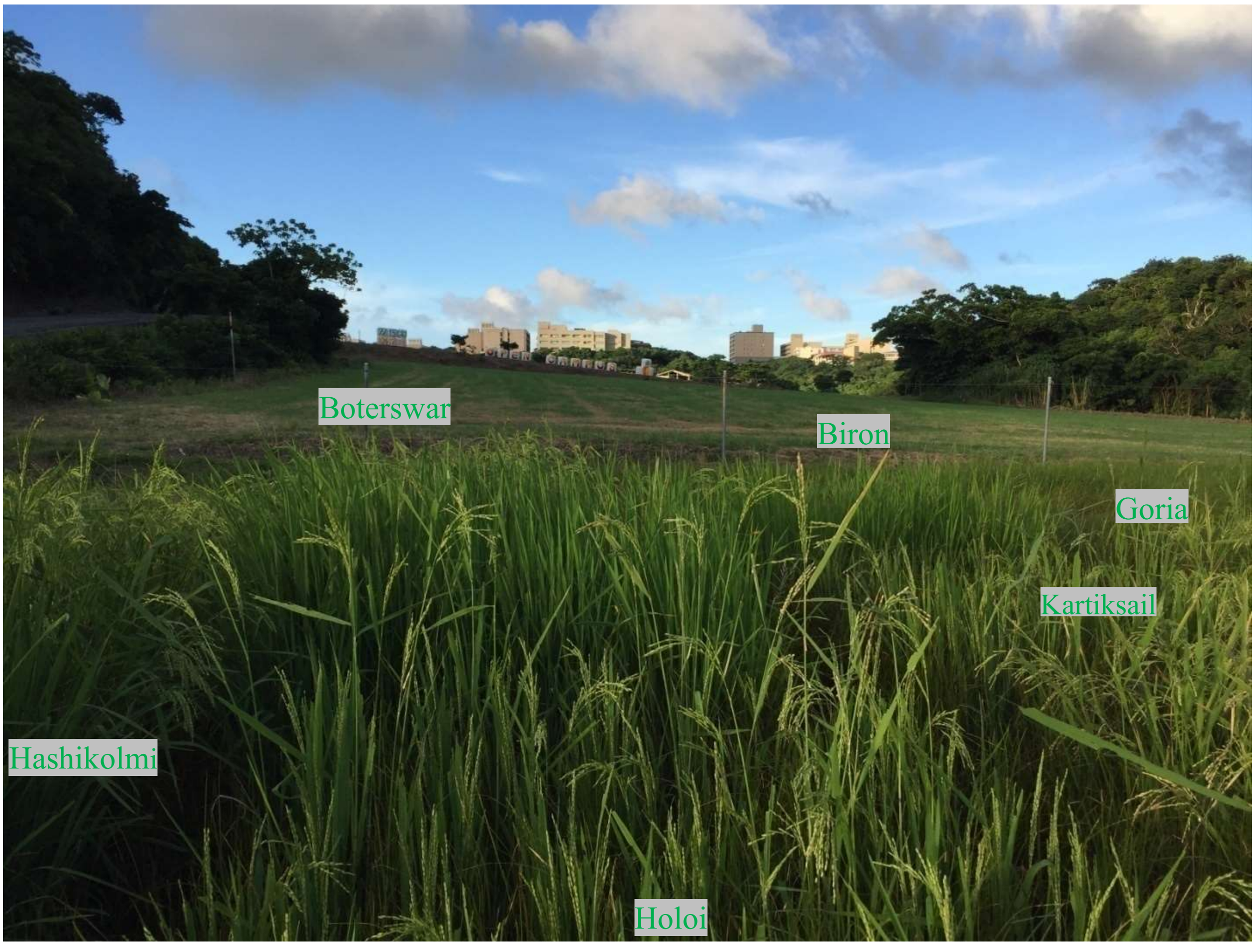

Figure 20. Field view at 50% flowering stage of Bangladesh indigenous rice varieties at the research field of University of the Ryukyus

Total tillers determine the amount of dry matter production unit area^{-1} while effective tillers unit area^{-1} determined the final yield of rice. This is why it is said that the higher the effective tillers, the higher the yield. It is evident from the Table 9 that variety had a significant effect on number of effective tillers. Other yield contributing parameters also significantly varied due to the varietal difference in weed infested field (Table 9). Grain yield is a function of interplay of various yield components such as number of productive tillers, grains panicle^{-1} and thousand-grain weight. Allelopathic 'Boterswar' and 'Biron' gave significant yield to others. However, panicle length, total grain, and thousand-grain weight mostly depend on genotype.

Table 9. Yield parameters and yield of Bangladesh indigenous rice as influenced by various naturally growing weeds

Variety	Effective tillers m^{-2}	Panicle length (cm)	Total grain $panicle^{-1}$	Filled grain (%)	Thousand-grain weight (g)	Yield (t ha^{-1})
Boterswar	5.93 ± 0.90^{a}	22.54 ± 0.06^{ab}	82.67 ± 3.21^{b}	95.54 ± 1.87^{ab}	27.58 ± 0.53^{a}	4.22 ± 0.89^{ab}
Goria	4.52 ± 0.90^{ab}	23.17 ± 1.76^{ab}	78.00 ± 12.49^{b}	97.36 ± 1.33^{a}	21.34 ± 0.41^{c}	2.20 ± 0.70^{bc}
Biron	5.93 ± 1.79^{a}	23.97 ± 1.38^{a}	136.00 ± 23.07^{a}	84.86 ± 2.93^{bc}	24.00 ± 0.21^{b}	5.58 ± 2.57^{a}
Kartiksail	3.83 ± 0.84^{bc}	21.83 ± 1.16^{ab}	72.67 ± 5.03^{b}	77.63 ± 4.41^{c}	23.78 ± 0.89^{b}	1.41 ± 0.49^{c}
Hashikolmi	5.87 ± 0.85^{a}	21.70 ± 1.056^{bc}	51.33 ± 6.66^{c}	79.23 ± 12.71^{c}	21.51 ± 0.56^{c}	1.54 ± 0.72^{c}
Holoi	2.70 ± 0.61^{c}	19.60 ± 0.52^{c}	83.67 ± 2.52^{b}	74.74 ± 1.91^{c}	26.99 ± 0.49^{a}	1.27 ± 0.48^{c}

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

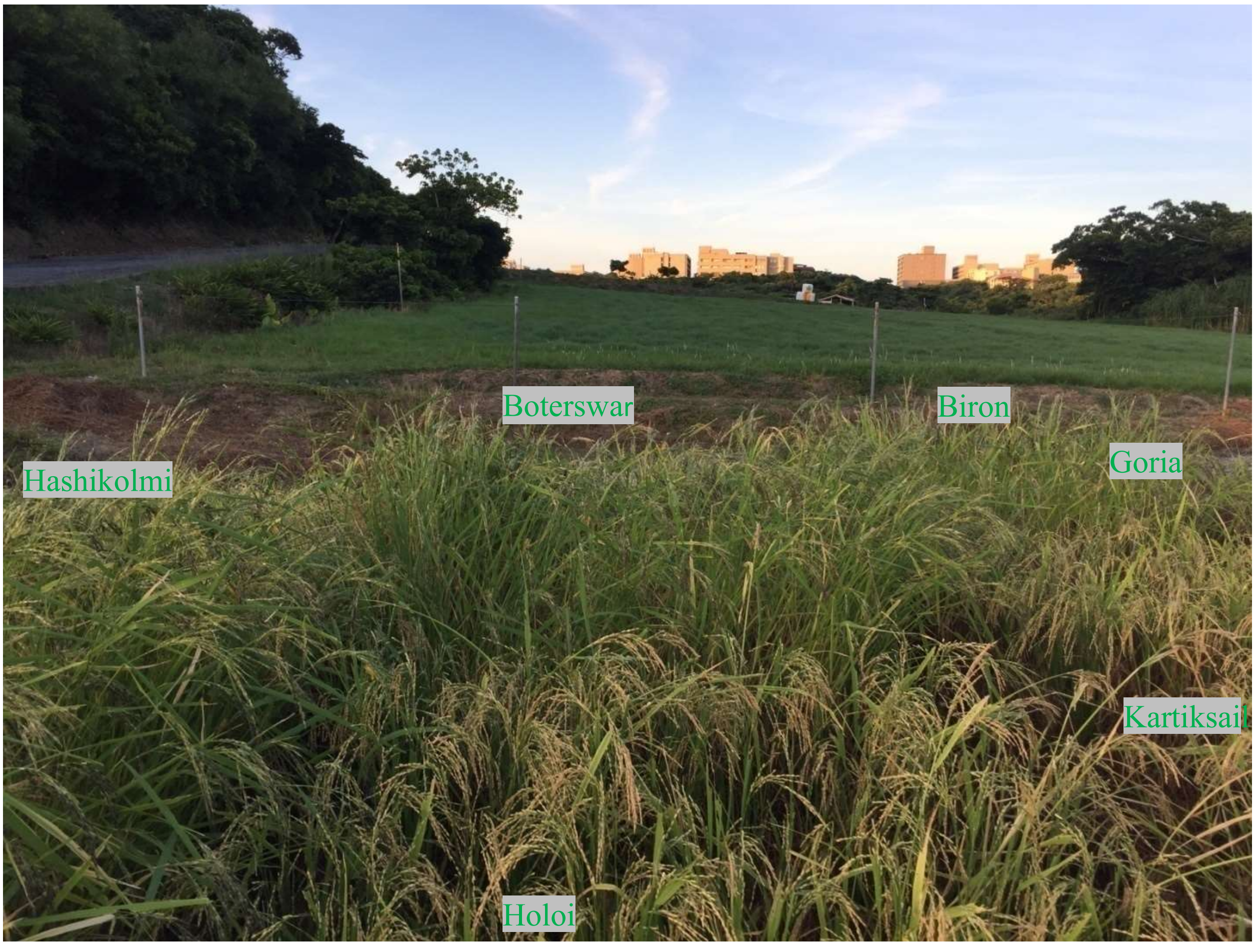

Figure 21. Field view at ripening stage of Bangladesh indigenous rice at the research field of University of the Ryukyus

4.4 Discussion

Allelopathy arises from the release of chemicals by one plant species that affect other species in its vicinity, usually to their detriment. It has been demonstrated, in plant communities, to be a factor of ecological significance by influencing plant succession, dominance, climax formation, species diversity, the structure of plant communities and productivity (Whittaker & Feeney 1971; Rice 1984; Chou 1989). Therefore, this study was done by following no weed control method. The results revealed a significant differentiation between the Bangladesh indigenous rice varieties regarding their weed competitive ability. Allelopathic activity of Bangladeshi indigenous rice varieties showed variety dependence on the growth of weeds in the field experiment. The results showed, in general, rice varieties exhibited higher weed suppression in the field than that observed in laboratory and greenhouse screenings (Masum et al. 2016). This result was consistent with many allelopathy researches conducted in the controlled and natural conditions (Stowe 1979; Rice 1984; Leather & Einhellig 1988; Inderjit & Weston 2000; Olofsdotter 2001; Inderjit et al. 2005). Khanh et al. (2009) also found that growth of barnyardgrass was much more suppressed in fields than in the laboratory and greenhouse. Previously a good linking between lab and field allelopathic outcomes of same canola genotypes was observed by Asaduzzaman et al. (2014a). Moreover, allelopathic activity of rice varieties was reported to be significantly different by releasing phytotoxic substances at different times of the growing stages (Lee et al. 1999; Dilday et al. 2001; Jung et al. 2004; Kato-Noguchi et al. 2008). Weed seedling frequency is the second most important variable, as clearly there is a relationship between weed frequency and duration of interference. The length of a critical period for weed control varies with frequency just as frequency thresholds vary with time of weed emergence relative to the crop (Dunan et al. 1995). However, the sedge species (*S. fluviatilis*) was found with high frequency throughout the experimental periods which may have tolerance capacity against inhibitory chemical effect of neighboring rice variety. It can also presume that it might uptake the bioactive compounds produced by rice and later on detoxify it by its internal mechanism. Thirdly, weed species may differ in competitive ability based on such traits as rapid leaf area development, high-density root systems, plant height, etc. Life cycles, reproductive strategies, and morphological features are important traits in determining the competitive ability of individual weed species. Rice variety differentially reduced weed growth. Strong interference by variety 'Boterswar' and 'Biron', significantly reduced the vegetative growth and delayed the reproductive organs development of *S. fluviatilis*. So, we can say that despite the high frequency of *S. fluviatilis* under both strong and weak allelopathic rice cultivars, it has loss his fitness under strong allelopathic rice variety. It can be argued that impact of rice interference on *S. fluviatilis* growth stage may not influence the weed competitive ability. However, it must reduce seed production and any reduction in weed vigour is an advantage (Cousens and Mortimer 1995). The use of high interference rice variety on reproductive growth may, therefore, have an important long-term effect on the *S. fluviatilis* weed population in a rice rotation. A similar result was also demonstrated in canola (Asaduzzaman et al. 2014b), where weed's rosette diameter and reproductive development was delayed by both strong allelopathic variety and early sowing technique. In this study, rice variety responded differently to the same weed species, presumably due to selective allelopathic action from a specific chemical mode-of-action which may not act on the other weeds. In rhizospheric research, it has been reported that root-secreted chemicals and their quantity may deter one species while attracting another (Bais et al. 2006; Pierik et al. 2013). Furthermore, often plants do not secrete just one substance but a mixture of chemicals which is highly species-specific or even ecotype-specific (Pierik et al. 2013). The yield reduction due to weed infestation is very common in field crop studies. Maintaining crop yield under weed pressure and weed suppression are two different mechanisms of crop competition

(Lemerle et al. 2001). The Bangladesh indigenous varieties 'Boterswar' and 'Biron' showed best performance in terms of yield in the weed infested field. Therefore, weed suppression by these varieties under field conditions was due to negative interference either via competition or allelopathy or both. However, sparse or no vegetation patterning around a particular species can indicate that it is allelopathic (Duke 2015) which was observed in these in vitro screened varieties raised plots. Bertholdsson (2010) found mean early weed biomass was significantly lower in the highly allelopathic wheat lines compared with the non-allelopathic lines. On the contrary, weakly allelopathic 'Hashikolmi' was also found weed suppressive, as there was less irregular growth observed in this variety which is a very common phenomenon in a field crop growth due to high weed infestation (Fig. 21). These differences can be due to the combination of a variety competitive and allelopathic mechanism as, in the field, both phenomena occur together but are difficult to identify and quantify separately (Olofsdotter et al. 1999). Therefore, it should be needed further clarification whether these allelopathic varieties are weed competitive or allelopathic as on the issue of demonstrating allelopathy in natural systems, there are intense and potentially fruitful conflicts between contesting views. Furthermore, information on the mechanisms of interference (resource competition and allelopathy) is required, if we hope to develop a logical scenario to explain interference as it occurs in nature.

CONCLUSION

In conclusion, our findings showed a wide variation of allelopathic activity based on variety-dependence which confirms the allelopathy ranking from laboratory and glasshouse screening results. These selected Bangladeshi indigenous allelopathic rice varieties may be grown for an effective and natural weed control. Therefore, it is feasible to reduce herbicide input in paddies if allelopathic rice is grown under integrated cultural management practices. These rice varieties can also be used as a source of genes which can be used in rice breeding for further developing high yielding rice varieties with better and acceptable weed suppressive allelopathic traits as well as utilizing rice allelopathy for weed control in Bangladesh. However, rice varieties with potent weed suppressing ability can be re-examined in the glasshouse to evaluate the weed suppression in conditions that are close to natural settings, which include competitive and interferential factors and finally need to be reconfirmed in the field.

CHAPTER V

SEPARATION OF ALLELOPATHY FROM RESOURCE COMPETITION USING BANGLADESH INDIGENOUS RICE-*Echinochloa oryzicola* MIXED CULTURE

ABSTRACT

Allelopathy is not acknowledged among ecologists and many have claimed that its effects cannot be separated from other mechanisms of plant interference mainly competition. An experimental technique was used to separate and assess the extent of allelopathic interference relative to resource competition by the interactions between Bangladeshi indigenous rice (*Oryza sativa* L. spp. *indica*) allelopathic variety 'Boterswar', weakly allelopathic variety 'Hashikolmi' and *Echinochloa crus-galli* var. *oryzicola* via a target (rice)-adjacent (*E. oryzicola*) mixed culture in a hydroponic system in glasshouse of University of the Ryukyus. In the beginning competition index was estimated between each of two rice varieties and *E. oryzicola* by using the relative competitive intensity (RCI), the relative neighbor effect (RNE) and the competitive ratio (CR). The RCI and RNE values showed that the crop-weed interaction was facilitation for 'Boterswar' and was competition for 'Hashikolmi' and *E. oryzicola* in rice/*E. oryzicola* mixed-cultures. The allelopathic effects of 'Boterswar' were much higher than the resource competition in rice/*E. oryzicola* mixed-cultures. The converse was factual for 'Hashikolmi'. Moreover, the mineral content *E. oryzicola* severely affected by 'Boterswar'/*E. oryzicola* mixed cultures exudates solution. These results demonstrated that the allelopathic effect of 'Boterswar' was leading in rice/*E. oryzicola* interactions. Thus, the study showed that two different interference mechanisms of plant interference can be separated and further verified the allelopathic potential of 'Boterswar'.

5.1 Introduction

Weeds are one of the greatest biological limits in rice (*Oryza sativa* L.) production. Even at a proportion of 100 rice plants to 10 *E. crus-galli* plants, rice biomass is reduced by 75% and yield is diminished by about 50% (Graf 1992). Recently, researchers have paid more attention to the utilization of allelopathic potential rice varieties for weed management in rice because it is a cost-effective, safe measure that can reduce weeding inputs. As conveyed by Rice (1984), one of the essential features of allelopathic interactions between plants is that phytotoxins are released into the soil by one plant and absorbed by a second plant. The sources of allelochemicals released into the rhizosphere include leaching from leaves and other aerial parts, volatilization, root exudation (Weir et al. 2004; Hussain & Reigosa 2012; Uddin et al. 2012a), the ecological significance of such phytotoxic also depends on the vulnerability of the plants with which the allelopathic plants coexist. According to Olofsdotter et al. (2002), precisions in the competitive ability of crops might reduce dependency on herbicides, making use instead of the chemical defense mechanisms that are part of the natural competition between plants. It might, therefore, be possible to manipulate this phenomenon of allelopathy to either increase the toxicity of a crop plant towards its weeds or to increase the tolerance of a crop plant to its weeds. Either approach requires a variation in the toxicity or tolerance, respectively, within the crop species. The existence of genetic variation for allelopathic potential has been shown in different crop species (Fay & Duke 1977; Lockerman & Putnam 1979; Wu et al. 2000b; Olofsdotter et al. 2002). However, several ecologists have criticized this phenomenon due to the lack of ecologically realistic bioassays that include dynamic soil system to provide information about its existence and relevance under field conditions (Harper 1977; Conway et al. 2002). Nevertheless, a few studies have provided convincing evidence regarding its existence under field conditions.

Plant species that are particularly aggressive in their interactions with other species may be allelopathic. A potential complication is that the plant making the putative allelochemical (the donor plant) may only make sufficient amounts of allelochemicals for an allelopathic effect when in the presence of a targeted plant species (receiving plants). This is similar to the case of induction of phytoalexin production in the presence of plant pathogens (Kato-Noguchi 2011; Duke 2015). Kong et al. (2006), and Gealy and Fischer (2010) reported that an allelopathic rice variety exuded significantly more phytotoxins from its roots when grown with the rice weed *E. crus-galli* than when grown in monoculture. There is an affluence of information on the influence of rice allelochemicals by competing *E. crus-galli* and other stress factors (Koeduka et al. 2005; Zhao et al. 2005; Kato-Noguchi et al. 2007).

Plant interference can be separated into competition and allelopathy. Competition occurs in live plant communities when two or more plants seek a common resource within limited space, such as nutrients, light, and water (Harper 1977; Aldrich 1987; Zimdahl 1993). Allelopathy results when plants release into the environment chemicals that usually inhibit the growth of another plant. Competition creates only adverse effects due to a paucity of resources for growth and establishment of crops (Zimdahl 1993). Allelopathic interference includes inhibitory and promoting effects (Rice 1986), but inhibitory effects are the focus of plant interactions. As indicated by Wu et al. (2000a) in conniving of a suitable screening technique, several necessities are essential: (i) the allelopathic component must be dispersed from any interplant race; (ii) a procedure needs to be developed to pretend the usual release of allelochemicals from living donor plants into the growth medium; (iii) the crop should be directly tested as a ‘receiver’ species; and (iv) priority should be placed on allelopathic activity during the initial seedling stage, when critical stage of weed. Indeed, competitive hierarchies often form during these early stages of plant growth (Hoffman et al. 1996). The results of Masum et al. (2016 & 2018) and field study (Chapter III) have clearly shown the allelopathic potential of Bangladesh indigenous rice variety ‘Boterswar’, however, the effects should be considered in more ecologically realistic ways by differentiating allelopathic interactions from resource competition because allelopathy is not an isolated phenomenon in natural ecosystems. It works with resource competition and many other ecophysiological processes interacting simultaneously. Therefore, the allelopathic effects might be concealed by resource competition among target plants (Weidenhamer et al. 1989; Barto & Cipollini 2009). Inderjit and del Moral (1997) suggested that separating allelopathy from resource competition is almost impossible in natural systems but the relative contribution of the two mechanisms on plant interference is possible to determine and important to do so.

Therefore, the aim of the present investigation was to separate allelopathy from resource competition by quantifying the intensity of competition between rice and *E. crus-galli* by target-neighbor mixed-culture. Several experimental designs had been devised to study the separation of allelopathy from resource competition (Weidenhamer 1989; Nilsson 1994; Kong et al. 2006; Weidenhamer 2006). Each design deals with plant density, plant spatial arrangement, and proportion of competing plants in different ways. Our experimental design has been done according to He et al. (2012) by adding an observation on the changes of mineral composition of receiver plants due to the allelopathic effect.

5.2 Materials and Methods

The Bangladesh indigenous rice (*Oryza sativa* L. spp. *indica*) variety 'Boterswar' as allelopathic and 'Hashikolmi' as weakly allelopathic (Masum et al. 2016) as target plants, and *E. crus-galli* L. var. *oryzicola* as adjacent plants were chosen. The experiment was conducted in a glasshouse (12 h photoperiods; light intensity 20.5 ±1 $MJm^{-2}d^{-1}$; 25 to 40°C temperature and 70-80 % relative humidity) of the University of the Ryukyus. Seeds of *E. oryzicola* were collected from the rice field of the research field of Subtropical Field Science Center, University of the Ryukyus, Japan.

5.2.1 Evaluation of competition intensity

According to He et al. (2012), the first experiment was designed to investigate the competition intensity of each of the two different allelopathic rice accessions and *E. oryzicola* using rice/ *E. oryzicola* mixed-cultures in hydroponic solutions. Initially, rice seed soaking was done in water for 24 hours. These were then taken out of the water and kept in gunny bags. The seeds started sprouting after 48 hrs which were suitable for sowing in 72 hrs. The sprouted seeds were then placed in seedling trays (25-by-25-by-5cm; one seed per hole) filled with commercial potting mixture. The seedlings were watered with tap water daily for 25 days (3 leaf stage). In case of BYG, after 36 h soaking uniformly pre-germinated seeds were placed in seedling trays (25-by-25-by-5 cm; two seeds per hole) filled with the commercial potting mixture and raised until use (12DAS- two leaf stage) by watering tap water daily. The uniform seedlings of rice and *E. oryzicola* were transplanted into Styrofoam plates (each size 45 cm × 17 cm) with 2 perforated holes (r = 7.5 cm) in each one. The seedlings were stabilized with pumice stone and by wrapping with aluminum foil. Four Styrofoam plates with seedlings were floated in a plastic basin (84 cm × 52 cm × 20 cm) containing 25-L Hoagland solution with pH 5.5 and EC 1.2 ms/cm. Seven days after recovery, uniform six seedlings of rice of each accession and two seedlings of *E. oryzicola* were chosen for mixed—culture in alternating rows within a basin. New 25-L Hoagland solution was supplied, and pH and EC were adjusted to 5.5 and 1.2 ms/cm, respectively. The controls were eight- seedling monocultures containing either of the two rice accessions or *E. oryzicola*. The treatments were performed in triplicate in completely randomized design. Additional water was added daily to each pot to maintain the 25-L volume of the culture solution, and pH and EC adjusted regularly. Seven days after treatment, growth parameters were measured and all plants were harvested. Then the plants were oven dried at 70ºC for 72 hrs. Plant dry weights were recorded. The root length, plant height, SPAD value and plant dry weight of the two rice accessions and *E. oryzicola* were used as indices of plant competition as follows.

Relative competition intensity (RCI) was used to evaluate the competition between the two rice accessions and *E. oryzicola*, respectively and was calculated according to Grime (1995) as follows:

$$RCI = (P_{mono} - P_{mix}) / P_{mono}$$

Here, where P_{mono} represents the performance (root length, plant height, SPAD value and plant dry weight) of a plant in monoculture for controls of two rice accessions and *E. oryzicola*, respectively. P mix represents the performance of a plant in the mixture (treatments). Positive RCI values indicate competitive inhibition and the negative values indicate competitive facilitation.

According to Willey and Rao (1979), the competitive ratio (CR) was calculated to compare the competitive abilities of rice and *E. oryzicola*. It was calculated as follows:

$$CR_{rb} = (P_{mix,\,r} / P_{mono,\,r})/(P_{mix,\,b} / P_{mono,\,b}) \text{ and}$$

$$CR_{br} = (P_{mix,\,b} / P_{mono,\,b})/(P_{mix,\,r} / P_{mono,\,r})$$

Here, CR_{rb} is the competitive ratio of rice on *E. oryzicola* and CR_{br} is the competitive ratio of *E. oryzicola* on rice. By definition, $CR_{rb} \times CR_{br} = 1$, so CR values indicate the ratio by which one plant is more competitive than the other.

According to Markaman and Chanway (1996), the relative neighbor effect (RNE) was calculated to indicate the inter-specific competitive effect on each of the two rice accessions and *E. oryzicola* for plant dry weight. It was calculated as follows:

RNE = (P_{mono}-P_{mix})/P mix

Here, P_{max} is the highest value of (P_{mono}, P_{mix}). RNE is a modified version of RCI because RCI is not symmetrical around zero. RNE ranges from -1 to +1, with negative values indicating facilitation and positive values indicating competition.

5.2.2 Separation of allelopathic effect and competition

The second experiment was designed to separate allelopathic effects (AE) from the total biointerference (TB) of each of the two rice varieties on the associated *E. oryzicola*. The culture mode and design of plants were the same as in the first experiment. After seven days the plants were harvested and data were measured as first experiment. The culture solutions (containing root exudates of both rice accessions in rice/*E. oryzicola* mixed-cultures, the supposed allelochemicals) of each of the basins described above were corrected for balancing of the levels of nitrogen (N), phosphate (P), and potassium (K) by using NH_4NO_3, $NaH_2PO_4 \cdot 2H_2O$ and K_2SO_4, and adjusted to normal level of 25-L Hoagland solution, and pH to 5.5 and EC to 1.2 ms/cm. Eight *E. oryzicola* seedlings (2-leaf stage) were transplanted into these solutions. The controls were eight *E. oryzicola* seedlings in 25-L Hoagland solution. The results of this step were defined as AE of each of the two rice varieties on the associated *E. oryzicola* because any actual competition between rice and *E. oryzicola* had been removed. The difference between this treatment and the control could only have come from the allelochemicals in the residual solutions. The treatments were performed in triplicate in completely randomized design. Additional distilled water was added daily to each pot to maintain the 25-L volume of the culture solution. Seven days after treatment, the root lengths, plant heights, SPAD value and plant dry weights of *E. oryzicola* seedlings were obtained as in the first experiment**.** Nutrient contents were also measured from these plants where N and C by NC-220F (SUMIGRAPH Model, Japan) and rest nutrients by ICPE-9000 (ICP-AES Multitype ICP Emission Spectrometer, SHIMADZU, Japan).

The allelopathic effects (AE) and total biointerference (TB) were calculated as follows. According He et al. (2012,) the inhibitory rate (IR) was used to assess the inhibition of each of the two rice varieties on the growth of *E. oryzicola*. The IR was calculated as follows:

IR = (control – treatment/control) × 100%.

IR > 0 and IR < 0 indicate inhibitory effects and stimulatory effects, respectively. The IRs from the mixed-cultures represent the TB of each of the two rice accessions on *E. oryzicola* and IRs from the monoculture represent the AE of each of the two rice accessions on *E. oryzicola*.

Therefore, resource competition (RC) = TB – AE.

5.2.3 Statistical Analysis

All experimental data are presented as mean ± standard deviation (SD). They were subjected to a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) at 5% level of probability. The statistical analysis was performed using the Fisher data processing system.

5.3 Results

5.3.1 Competition index of the two rice accessions and *E. oryzicola* in rice/*E. oryzicola* mixed-cultures

The root length, plant height, SPAD value and plant dry weight of *E. oryzicola* were significantly decreased in 'Boterswar' rice/*E. oryzicola* mixed-cultures relative to controls whereas root length and plant dry weight of *E. oryzicola* was significantly decreased other parameters remain unaffected in 'Hashikolmi' rice/*E. oryzicola* mixed-cultures relative to controls (Table 10). Although root length of *E. oryzicola* in both cultural modes affected, visual differences in root system were also observed (Fig. 22). The number of roots and their distribution area were severely affected than controls roots of *E. oryzicola* in 'Boterswar' rice/*E. oryzicola* mixed-cultures.

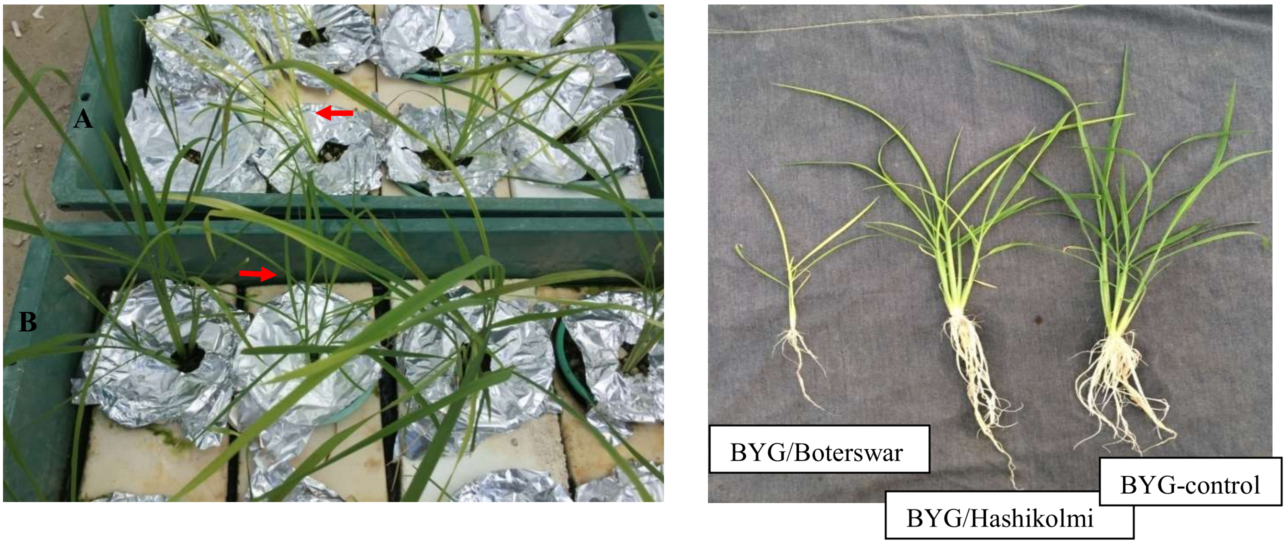

Figure 22. Effect on growth of *E. oryzicola* in rice/*E. oryzicola* mixed cultures. A- 'Boterswar'/*E. oryzicola*; B- 'Hashikolmi'/*E. oryzicola*; Red arrow indicates the *E. oryzicola* within mix culture; BYG- *E. oryzicola*

Table 10. Morphological attributes of rice and *E. oryzicola* in rice/*E. oryzicola* mixed-cultures

Plant	Culture mode	RL (cm)	PH (cm)	SPAD value	DW (g $plant^{-1}$)
BYG	Monoculture	23.00 ± 1.00	42.33 ± 2.52	30.57 ± 2.10	0.93 ± 0.13
	Mixed with V_1	15.00 ± 3.00*	31.00 ± 3.61*	7.57 ± 2.82*	0.14 ± 0.07*
	Mixed with V_2	20.27 ± 1.53*	39.00 ± 3.61	24.67 ± 2.25	0.29 ± 0.05*
V_1	Monoculture	25.80 ± 0.30	59.67 ± 1.53	35.60 ± 2.72	2.12 ± 0.22
	Mixed with BYG	26.38 ± 0.94	60.33 ± 0.58	36.07 ± 3.99	2.21 ± 0.07
V_2	Monoculture	25.11 ± 0.30	58.67 ± 3.06	35.97 ± 1.86	2.11 ± 0.20
	Mixed with BYG	22.847 ± 0.13	54.33 ± 4.04	25.80 ± 1.76*	1.92 ± 0.08

Note: BYG – *E. oryzicola*; V_1 – 'Boterswar'; V_2 = 'Hashikolmi'; RL - root length; PH - plant height; DW - dry weight
*Significantly different from control

The RCI values of root length, plant height, SPAD value and plant dry weight were negative for 'Boterswar', indicating facilitation in 'Boterswar'/*E. oryzicola* mixed-cultures. Whereas, the RCI values of root length, plant height, SPAD value and plant dry weight were positive for 'Hashikolmi', indicating competition in 'Hashikolmi'/*E. oryzicola* mixed-cultures (Table 11). These results showed that these two rice varieties have different responses to *E. oryzicola* stress. The RCI values for *E. oryzicola* were positive for all parameters of *E. oryzicola*/'Boterswar' mixed culture, and plant height and dry weight of *E. oryzicola*/'Hashikolmi' mixed culture, indicating competition in rice/*E. oryzicola* mixed-cultures. However, the RCI values for *E. oryzicola* in *E. oryzicola*/'Boterswar' mixed-cultures were much higher than those in 'Boterswar'/'Hashikolmi' mixed-cultures, indicating that 'Boterswar' was more competitive against *E. oryzicola* than 'Hashikolmi'. Whereas, The RCI values for *E. oryzicola* were negative for root length and SPAD value of *E. oryzicola*/'Hashikolmi' mixed culture, indicating facilitation in rice/*E. oryzicola* mixed-cultures which refers increase root length and SPAD value of *E. oryzicola* in *E. oryzicola*/'Hashikolmi' mixed culture.

Table 11. Competition indices of rice and *E. oryzicola* in rice/*E. oryzicola* mixed-cultures

Plant	RCI				CR			
	RL	PH	SPAD value	DW	RL	PH	SPAD value	DW
V_1 mixed with BYG	-0.022	-0.011	-0.013	-0.042	1.568	1.381	4.093	6.732
V_2 mixed with BYG	0.090	0.074	0.283	0.090	1.127	1.393	1.046	6.654
BYG mixed with V_1	0.348	0.268	0.753	0.845	0.638	0.724	0.244	0.149
BYG mixed with V_2	0.119	0.079	0.193	0.691	0.968	0.995	1.125	0.340

Note: RCI - Relative competition intensity; CR – Competitive Ratio; RL – root length; PH – plant height; DW – plant dry weight; V_1 – 'Boterswar'; V_2 - 'Hashikolmi'; BYG – *E. oryzicola*

CR value indicates the ratio by which one plant is more competitive than another. The CR values of 'Boterswar' showed that one individual 'Boterswar' plant was as competitive as 1.568 *E. oryzicola* plants with respect to root length, 1.381 *E. oryzicola* plants with respect to plant height, 4.093 *E. oryzicola* with respect to SPAD value and 6.732 *E. oryzicola* plants with respect to plant dry weight (Table 11). On the other component, the CR values showed that one 'Hashikolmi' individual was equal to about one *E. oryzicola* plant except dry weight. The CR value of root length and SPAD value of 'Boterswar' was more than twice of 'Hashikolmi'.

The RNE values for *E. oryzicola* were positive, indicating that the plant-plant interactions between *E. oryzicola* and the two rice accessions involved competition in rice/*E. oryzicola* mixed-cultures (Fig. 23. A). The RNE for *E. oryzicola* in 'Boterswar'/*E. oryzicola* mixed-cultures was higher than that in 'Hashikolmi'/*E. oryzicola* mixed-cultures, indicating inter-specific competition of greater intensity between *E. oryzicola* and 'Boterswar' than between *E. oryzicola* and 'Hashikolmi'. In rice/ *E. oryzicola* mixed-cultures, the RNE value was negative for 'Boterswar' but positive for 'Hashikolmi', indicating that the plant-plant interactions involved facilitation for 'Boterswar' and competition for 'Hashikolmi' (Fig. 23. B).

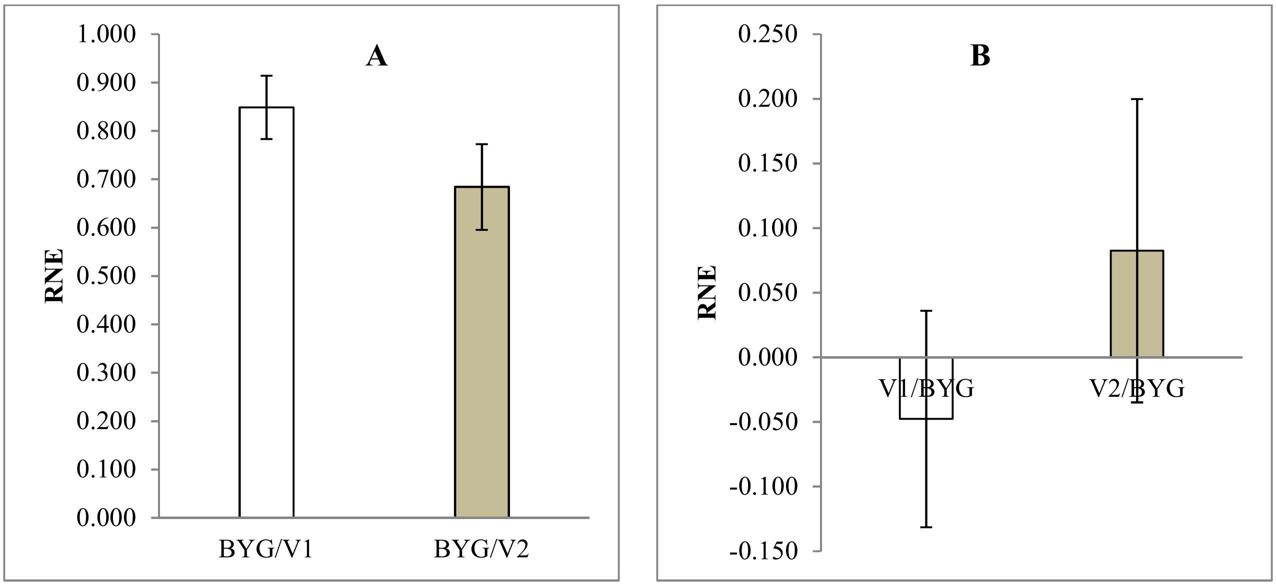

Figure 23. Relative neighbor effect (RNE) of each of the two rice varieties and *E. oryzicola* in rice/*E. oryzicola* mixed-cultures. A-RNE of *E. oryzicola* in mixed cultures with 'Boterswar' and with 'Hashikolmi'. B-RNE of 'Boterswar' and 'Hashikolmi' in mixed cultures with *E. oryzicola*.

Note: BYG - *E. oryzicola*; V_1 - Boterswar V_2 - Hashikolmi

5.3.2 Total biointerference, allelopathic effect and resource competition

The total biointerference (TB) of 'Boterswar' on root length, plant height, SPAD value, and dry weight of *E. oryzicola* was two times higher than that of 'Hashikolmi' in rice/*E. oryzicola* mixed-culture (Table 12). The allelopathic effect (AE) of 'Boterswar' on root length, plant height, SPAD value, and dry weight of *E. oryzicola* was greatly higher than that of 'Hashikolmi'.

Table 12. Inhibitory rate (%) of *E. oryzicola* in monoculture and Bangladesh indigenous rice/*E. oryzicola* mixed culture

Index	TB		AE		AE/TB (%)	
	Boterswar	Hashikolmi	Boterswar	Hashikolmi	Boterswar	Hashikolmi
RL	59.93±0.63	27.14 ±0.84	44.06±0.51	6.32±1.96	73.53	23.29
PH	42.31 ±1.23	15.39 ± 0.56	30.41 ±3.65	7.52±2.07	71.88	48.88
SPAD	75.98 ±1.34	31.48 ± 1.72	62.90±3.96	16.25±2.56	82.78	51.60
DW	54.11 ±1.45	26.67 ± 2.23	50.50±1.50	15.95±2.94	93.32	59.81

TB- total biointerference; AE- allelopathic effect; RL- root length; PH- plant height; DW- plant dry weight

The exudates solution from 'Boterswar'/*E. oryzicola* mixed cultures had great inhibition effects on *E. oryzicola* growth (Fig. 24). The AEs on root length, plant height, SPAD value, and dry weight of *E. oryzicola* elucidated 74, 72, 83 and 93%, respectively, of the TB on *E. oryzicola* (Table 12). Whereas, AEs of the TB on *E. oryzicola* from 'Hashikolmi'/*E. oryzicola* was only 23, 49, 52 and 60%, respectively.

Figure 24. Effect on the growth of *E. oryzicola* seedlings from exudates solution of 'Boterswar'/*E. oryzicola*' (B) and 'Hashikolmi'/*E. oryzicola* (C) mixed cultures, and control culture (A).

The allelopathic effect on root length, plant height, SPAD value, and dry weight of *E. oryzicola* of 'Boterswar' was much bigger than its resource competition in 'Boterswar'/*E. oryzicola* mixed cultures, which was dominant factor for *E. oryzicola* suppression (Fig. 25. A). The converse was found from 'Hashikolmi' (Fig. 25. B)

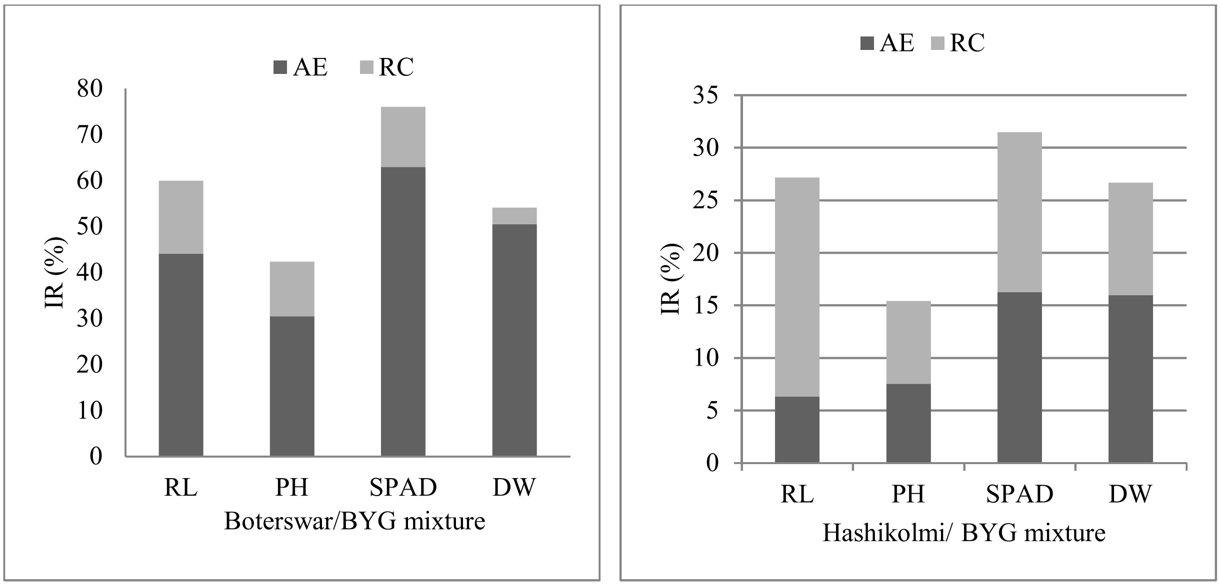

Figure 25. Separation of resource competition (RC) and allelopathic effect (AE) in rice/*E. oryzicola* mixed cultures. A- inhibitory rate (IR) of 'Boterswar' on *E. oryzicola* in 'Boterswar'/*E. oryzicola* mixed cultures. B- inhibitory rate (IR) of 'Hashikolmi' on *E. oryzicola* in 'Hashikolmi'/*E. oryzicola* mixed cultures.

Here, total bar represents total biointerference (TB); RL- root length; PH- plant height; DW- plant dry weight

5.3.3 Effects of exudates solution of rice/*E. oryzicola* mixed culture on nutrient content of *E. oryzicola*

Each of the exudates solution consistently affected the *E. oryzicola* mineral content (Table 13). However, CN ratio of the seedlings of *E. oryzicola* significantly decreased, and P, K, Mg and Mn were severely inhibited and S decreased by 'Boterswar'/*E. oryzicola* mixed cultures exudates solution whereas, the 'Hashikolmi'/*E. oryzicola* mixed cultures residual solution stimulated the uptake of Ca, **S**, Zn and Mn but CN ratio remain unaffected. Besides, Zn and Fe strongly enhanced compared to the control by the both of two cultural modes.

Table 13. Effects of exudates solution of 'Boterswar'/*E. oryzicola* and 'Hashikolmi'/*E. oryzicola* mixed culture on nutrient content of *E. oryzicola* seedlings

Culture mode		C:N		Nutrient concentration (μmol/g dry weight)								
				P	K	Ca	Mg	S	Cu	Zn	Mn	Fe
BYG Control		9.58 ± 0.03		137.76 ± 4.39	761.38 ± 1.33	71.84 ± 1.34	136.96 ± 3.38	101.86 ± 2.52	3.40 ± 0.15	5.53 ± 0.09	3.56 ± 0.29	10.70 ±1.60
BYG/Boterswar		8.59 ± 0.25**		100.37± 2.21**	647.08 ± 16.18**	61.49 ± 0.60**	102.14 ± 5.14**	99.33 ± 2.79	3.16 ± 0.05	6.99 ±** 0.08	1.74 ± 0.22**	40.70 ± 1.70**
BYG/Hashikolmi		9.50 ± 0.17		129.94 ± 2.61*	736.54 ± 10.61*	77.48 ± 3.82*	130.36 ± 2.51	122.88 ± 2.25**	3.58 ± 0.15	7.51 ± 0.29**	7.05 ± 0.17**	34.93 ± 1.473**

BYG- *E. oryzicola*; * $P < 0.5$; ** $P < 0.01$ levels of significance (compared to the untreated control)

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5.4 Discussion

The vast majority of published plant competition monographs have focused on resource-dependent processes such as competition for limiting resources such as water, nutrients, and light. The competitive ability of crops and weeds is regulated by physiological and morphological attributes that allow them to explore, capture, and exploit available resources. Competition is one of the main modes of interaction between cultivated crops and their adjacent plants (Inderjit & del Moral 1997; Xiong et al. 2005; He et al. 2012; An et al. 2013). Since rice and *E. oryzicola* are morphologically and phonologically similar is believed to occupy similar niches and make the same demands on the habitat and adjust themselves less readily to their mutual interactions. Competitive index analysis results showed that 'Boterswar' was a stronger competitive potential against *E. oryzicola* than 'Hashikolmi' which ultimately gave facilitation for 'Boterswar' but competition for 'Hashikolmi' in plant-plant interaction. There is strong evidence to support the hypothesis that allelopathy provides plants with an advantage for competing in plat-plant interaction (Singh et al. 1999; Bruin & Dicke 2001; Fitter 2003; He et al. 2012; Gioria & Osborne 2014). Thus, it is assumed that 'Boterswar' achieves its competitive advantages over *E. oryzicola*. While 'Boterswar' has clearly shown phytotoxic potential (Masum et al. 2018), the effects should be considered in more ecologically realistic ways by differentiating allelopathic interactions from resource competition as plant–plant interference is the combined effect of allelopathy and resource competition with many other factors (Uddin & Robinson 2017). In order to fix the incident of the differential competition between the two rice varieties, the total biointerference (TB) of each two rice varieties over *E. oryzicola* were divided into two components, allelopathic effect (AE) and resource competition (RC) as separating allelopathy from competition is essential to find out the ecological validation of allelopathy (Weidenhamer 2006). The results showed that the TB of 'Boterswar' on *E. oryzicola* was about twice than 'Hashikolmi', and the AE of 'Boterswar' on *E. oryzicola* was greatly higher than that of 'Hashikolmi'. Therefore, AE was absolutely leading factor in 'Boterswar'- *E. oryzicola* interactions and had more powerful interaction with *E. oryzicola* than 'Hashikolmi' which suggests that 'Boterswar' had strong allelopathic potential. This result was inconsistent with Dilday et al. (1991; 1998). He et al (2012) also concluded similar results. The reduction of plant parameters of *E. oryzicola* in rice/*E. oryzicola* mixed culture indicated that the allelopathic inhibition of a plant will likely reduce its effectiveness in competing for resources (Humphry et al. 2001). Many other such interactions are theoretically possible. The results of Nilsson (1994) are noteworthy in providing evidence of the extent of interaction between resource competition and allelopathy in one system. However, like other ecological interactions, allelopathy is a complex phenomenon (Trezzi et al. 2016). These complexities have been explored in several plant studies (Einhellig 1987; Williamson 1990; Weidenhamer 1996; Inderjit and del Moral, 1997; Blum et al. 1999), and have important ramifications for experimental design (Romeo & Weidenhamer 1998; Blum 1999). Therefore, it is impossible to demonstrate the allelopathy phenomenon as it is almost impossible to do so. Although some screening methods were developed with the use of either aqueous or chemical solvent extracts of rice leaves, stems, mixture rice residues and hulls (Fujii & Shibuya 1992; Ahn & Chung 2000; Lee et al. 2003; Jung et al. 2004; Chung et al. 2004), this technique is greatly criticized by scientific committee (Qasem 2017). As different other factors could be involved in the inhibition obtained among which is the osmotic potential of the extract solution, pH and even the duration of experiment (Duke 2015). Therefore, authors examined the allelopathic effects of rice from its root exudates by the hydroponic experimental system where pH and EC adjusted regularly and we found the chemicals in the exudates solution of 'Boterswar'/*E. oryzicola* interactions had a strong inhibitory effect on *E. oryzicola* growth which demonstrated allelopathy by separating from resource competition by excluding possible complexity in agroecosystem where the allelopathic effect of 'Boterswar' was much bigger than its resource competition on *E. oryzicola* growth. Each year natural product chemists isolate and identify a number of phenolics, alkaloids, terpenoids, polyacetylenes, fatty acids, and steroids from rice as allelochemicals. However, it is commonly accepted allelopathy is steady for a complex of chemicals as a result of interference (Einhellig 1999). We previously isolated four allelochemicals from variety 'Boterswar' and reported their synergistic inhibitory effect on *E. crus-galli* (Masum et al. 2018). Similar consequences were also stated by many researchers (Dilday et al. 1998; Mattice et al. 1998; Blum 1999; Inderjit et al. 2002; Seal et al. 2004b). On the other hand, Kato-Noguchi (2011) reported that the allelopathic activity of rice seedlings was significantly increased when rice and *E. crus-galli* were grown together. These results confirmed that rice allelopathy is an inductive responsible mechanism that is associated with chemical inference. Results from the plant nutrient content analysis of the receiver in the exudates solution of 'Boterswar'/*E. oryzicola* indicate another possible explanation for the inhibition of *E. oryzicola* growth by allelopathic effect. Barros de Morais et al. (2014) reported sunflower residues negatively affected nutrient accumulation in radish plants. Inhibitions of the same order of magnitude due to allelochemicals on nutrient content of receiver have been reported in plant studies (McClure et al. 1978; Kobza & Einhellig 1987; Baziramakenga et al. 1994; Yu & Matsui 1997; Lv et al. 2002).

CONCLUSION

Although various queries remain unanswered regarding the act of plant-plant interactions, the study marked the significance and the intricacy of interactions between rice - *E. oryzicola* in agroecosystems. Whereas conclusive proof of chemical interference may not be reachable, the challenge of gaining strong supportive indication leftovers. Advancement is desirable in bioassay methods that distinguish allelopathy from other interference mechanisms. However, these results are the evidence of the allelopathic potential of Bangladesh indigenous rice variety ‘Boterswar’.

CHAPTER VI

EVALUATION OF ALLELOPATHIC POTENTIAL AND IDENTIFICATION OF PHYTOTOXIC SUBSTANCES FROM BANGLADESHI INDIGENOUS RICE STRAW AND ASSESSMENT OF INHIBITORY BIOACTIVITY AGAINST *Echinochloa oryzicola*

ABSTRACT

Straw from two Bangladeshi indigenous rice (*Oryza sativa* L. spp. *indica*) varieties, namely, 'Goria' as allelopathic and 'Holoi' as non-allelopathic, was incorporated into a gray soil in a pot study to observe the phytotoxic effects on seedling growth of *Echinochloa crus-galli* L. Beauv. var. *oryzicola.* The results showed that the degree of weed-suppression did not vary significantly with the rice varieties tested. However, the residues of 'Goria' caused greater quantitative inhibition of the growth and dry weight of *E. oryzicola* than that of 'Holoi.' The amount of 'Goria' straw incorporation was sequentially evaluated to observe autotoxicity in the cultivation of the same rice variety in another pot study using the same type of soil. A positive improvement was found on the growth and yield parameters of rice due to the combination of commercial fertilizers and straw incorporation. Aqueous methanol extracts of 'Goria' straw inhibited the seedling growth of *Lepidium sativum* L. and *E. oryzicola*, which suggested that this straw variety might contain phytotoxic substance(s). Two biologically active compounds, (-)-loliolide and 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one, were isolated from the ethyl acetate phase using several chromatographic steps. The structures of these compounds were determined based on spectroscopic data. The phytotoxic potential of these two compounds was assayed in vitro on seedling growth of test species to validate their phytotoxic potential on weed species. The results clearly indicated that both compounds inhibited seedling growth of test species at high concentrations. The inhibitory activity of 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one was greater than that of (-)-loliolide, as demonstrated by comparison of the I_{50} values. However, the two compounds synergistically suppressed the growth of *L. sativum* and *E. oryzicola* more strongly than the individual compounds. The results suggest that incorporation of allelopathic rice straw could help suppress *E. oryzicola* in rice and in other crops to achieve non-herbicidal weed control.

6.1 Introduction

Rice (*Oryza sativa* L.) is the primary crop of Bangladesh, covering approximately 70% of the total crop area (BBS 2016). The dramatic increase in the use of agricultural chemicals has contributed considerably to increasing rice productivity worldwide and in Bangladesh in the last half century (Erisman et al. 2008). However, in Bangladesh, rice cultivation is seriously threatened by several factors and circumstances. Agricultural practices, including pesticide and fertilizer use, have caused water and soil pollution of the paddy ecosystem. The current trend in agriculture focuses on reducing the use of herbicides because of their adverse effects on the environment. Anxiety about the potential connections among human health, environmental quality, and agricultural productivity reflects the growing demand to address the long-term sustainability of existing agricultural practices and determine which agricultural technologies will be safe and economically productive.

Rice is heavily infested with several noxious weeds, including *E. crus-galli* (Kraehmer et al. 2016*).* In both irrigated and direct-sowing rice areas in Bangladesh, *E. oryzicola* is one of the primary weeds, which is difficult to control because the weed is a mimic of rice and easily adapts to the rice growing environment. Several methods are used to control weeds. Depending on the weed flora and critical period of weed competition, we must intelligently select and adopt different weed management techniques based on the available resources. One of the alternative methods for the control of weeds is the use of allelopathic residues as mulches or incorporated into the soil (Diaz et al. 2004). Allelopathy is a biological phenomenon in which a living or dead plant releases allelochemicals applying an effect (mostly negative) on the neighboring or successive plants (Inderjit & Duke 2003, Kong et al. 2006; Yang & Kong 2017). The application of allelopathic effects to agricultural production, reduction of the use of synthetics and ensuing environmental pollution, and demand for effective methods for sustainable agricultural production and ecological schemes are the primary motivations for research on allelopathy (Han et al. 2013).

To develop natural herbicides, allelochemicals have been on the research agenda of phytochemists for a decade (Chung et al. 2017). A huge opportunity is available to use rice residues as a source of bio-herbicides. Mulching or incorporation of allelopathic plant materials provides sustainable weed management (Jabran et al. 2015) and reduces the negative effects on an agroecosystem (Cheema et al. 2004). The adoption of mechanized agriculture has resulted in leaving a significant amount of rice straw that is available for most rice farmers as organic material. Thus, we can use this rice straw for recycling in agricultural practices (Mandal et al. 2004). Xuan et al. (2005) and Seal et al. (2005) describe inhibition activities of rice straw and leachates on several weeds. The content of phytotoxic chemicals in rice straw is large and diverse (Chung et al. 2001; Kong et al. 2004; Kato-Noguchi & Ino 2005; Kong et al. 2006; Macias et al. 2006; Kato-Noguchi et al. 2012). Significant amounts of rice straw left in the fields after harvest also produce and release allelochemicals (Kong et al. 2006; Cao et al. 2008) and reduce soil erosion and increase the biological activity of soil microorganisms, which efficiently inhibit the growth of weeds (Ramakrishna et al. 2006). Furthermore, removal of straw from the field diminishes reserves of soil K and Si, whereas incorporation of the remaining stubble and straw into the soil returns nutrients and assists in conserving soil nutrient reserves in the long-term (Dobermann & Fairhurst 2002). Thus, straw incorporation can increase soil fertility and improve soil organic matter content, which can influence the nutritional and physiological aspects of crop growth. Generally, rice straw is applied back into paddy and upland fields as an organic material, and particularly in greenhouse cropping. Mendoza and Samson (1999) indicated that the use of rice straw mulching or incorporation for weed control in different crops is possible. Rice straw mulching had a significant effect on controlling weed growth under no-till wheat (*Triticum* *aestivum* L.) in experimental field conditions following rice in Bangladesh (Rahman et al. 2005). Hence, weed control coupled with yield enhancements by rice straw mulching would benefit integrated pest management systems, while minimizing the effects of agrochemicals, which are an important concern in current agricultural activities. Regrettably, Schreiber (1992), Lund et al. (1993) and Inderjit et al. (2004) reported that the incorporation of straw into the soil was the primary cause for the suppression of crop growth in the next season. However, few studies have examined the autotoxicity of rice straw on the growth of rice.

Evolution of weed resistance against herbicides demands that new classes of herbicides are created with new modes of action that have not been previously exploited and that can be used in organic agriculture. Moreover, using synthetics for weed suppression also requires public acceptance (Dayan et al. 2009). Phytotoxic substances can be used as potential natural herbicides because of their inhibitory activities on the germination, growth, physiological response, and genetic factors of receivers (Weir et al. 2004; Khanh et al. 2009), which ultimately manipulate the dominance and succession of plants, their arrangement in communities, and agricultural production and management (Weidenhamer & Callaway 2010). In agriculture, allelochemicals are investigated as a supplement because they are less persistent and eco-friendly in nature (Weston & Duke 2003). Identification of allelopathic compounds, including momilactones A and B, from rice straw and their biological activities have been reported (Lee et al.1999; Chung 2001). However, for Bangladeshi rice straw, allelopathic compounds obtained by extraction and separation techniques and their identification by spectroscopic analysis have not been reported. Therefore, the current study was designed to investigate the use of Bangladeshi rice straw for the control of weeds, to observe autotoxicity in rice, and to isolate and identify potential phytotoxic substances and determine their inhibitory bioactivity on the growth of *E. oryzicola.*

6.2 Materials and Methods

6.2.1 Plant Materials

The Bangladesh indigenous rice (*Oryza sativa* L. spp. *indica*) varieties 'Goria' as allelopathic and 'Holoi' as non-allelopathic (Masum et al. 2016) were grown in pots (Wagner pot, 0.02 m^2) from April to July 2016 in a glasshouse of the University of the Ryukyus for 120 days and then allowed to sunder in the glasshouse for an additional period (21 days). Seeds of *L. sativum* L. were purchased from the Green Field Project (Kumamoto, Japan), and seeds of *E. crus-galli* L. var. *oryzicola* were collected from the rice field of the University of the Ryukyus, Japan.

6.2.2 Pot studies

Pot (Wagner pot, 0.02 m^2) studies were undertaken in a two-step procedure, with both steps conducted in a glasshouse at the University of the Ryukyus, Japan. The first step was to determine the effect of rice straw incorporation into the soil on *E. oryzicola* growth, and the second step was to observe rice growth. In the first step, each pot was filled with 4 kg of gray soil (coarse sand 3.61%, fine sand 30.94%, silt 24.32%, and clay 32.84%; apparent density 0.90 g cm^{-3}; pH 7.43; C 1.83% and N 0.14%; and in mg g^{-1} soil, HPO_4^{2-} 0.44, K^+ 0.75, Ca^{2+} 4.99, Mg^{2+} 0.70, SO_4^{2-} 1.39, Fe^{3+} 0.64, Mn^{2+} 0.41, Zn^{2+} 0.47, Na^{2+} 1.01, Cu^{2+} 0.41, and Al^{2+} 0.81). Soils with the shoot debris (1–2 cm) of 'Goria' or 'Holoi' straw @ 0.5, 1.0, 1.5 and 2.0 t ha^{-1} were fully mixed in pots. The control pots contained soil only. All pots were saturated with water and allowed to decompose for 30 days. Two pre-germinated *E. oryzicola* seeds were sown in each pot. Weed-suppression was obtained from the comparison of the weed growth parameters and dry weights between the treated and control pots.

In the second step, each pot was filled as in the first step, and four treatments were applied: control, 'Goria' straw @ 2.0 t ha^{-1}, commercial fertilizers, and 'Goria' straw @ 2.0 t ha^{-1} with commercial fertilizers. Residues were decomposed as previously described. Commercial fertilizers were applied @ 125, 100, 50, 62.5, and 10 kg ha^{-1} in the form of urea, triple superphosphate (TSP), muriate of potash (MOP), gypsum and zinc, respectively, one day before transplanting; urea was applied in three equal splits at one day before transplanting and at 20 and 40 DAT (days after transplanting). Twenty-five-day-old seedlings of Bangladesh indigenous rice variety 'Goria' were transplanted @ 1 seedling per pot. Growth and yield contributing parameters of rice were observed.

6.2.3 Straw extract bioassay

Dried straw (leaves and stems) of 'Goria' variety was blended and stored at -40°C until required for bioassays and analysis. Extraction was performed using a slight modification of the method developed by Kato-Noguchi et al. (2011). Forty (40) grams of straw from 'Goria' was finely ground, placed in a 1000-mL flask containing 500 mL of 80% methanol (distilled water:methanol, 20:80), and stirred for 48 h at room temperature. Extracts were filtered through Whatman No. 42 filter paper (Toyo, Tokyo, Japan). The residue was extracted again with 500 mL of methanol for 48 h and filtered. Then, the two filtrates were combined. The filtrate was evaporated to dryness on a rotary evaporator at 40°C.

An aliquot of the aqueous concentrate (0.001, 0.003, 0.01, 0.03, 0.1 and 0.3 g dry weight [DW] equivalent extract per mL final assay concentration) was evaporated to dryness on a rotary evaporator at 40°C. Then, the dried sample was re-dissolved in cold methanol (0.2 mL), placed on a sheet of filter paper (no. 2) in a 3 cm Petri dish, desiccated in a draft chamber and then soaked in 0.8 mL of 0.05% (v/v) aqueous solution of Tween 20 (polyoxyethylene sorbitan monolaurate; Nacalai Tesque Inc., Kyoto, Japan) as a surfactant. For the control treatments, methanol (0.2 mL) was added to a sheet of filter paper in a Petri dish and evaporated as described above. Ten uniform germinated seedlings of *L. sativum* or *E. oryzicola* were placed on the filter paper and then incubated at 25°C in a dark incubator. The root and shoot lengths of the test species were determined after 48 h of incubation. The percentage of growth inhibition was calculated with respect to the control (without extracts) seedlings.

6.2.4 Extraction of rice straw for purification of growth inhibitors

Extracts were prepared using the method described by Kato-Noguchi et al. (2011) for isolating allelochemicals. A total of 2.4 kg of finely ground dry straw was extracted as described above. Then, the supernatant was concentrated under vacuum to prepare the aqueous concentrate (100 mL). This material was suspended in water and extracted with ethyl acetate and evaporated to produce ethyl acetate extracts (12.4 g). The active ethyl acetate fraction was chromatographed on a column of silica gel (70 g, silica gel 60N, 70-230 mesh; ASTM, Kanto Chemical Co., Inc., Tokyo, Japan), eluting with a stepwise gradient of ethyl acetate (10% per step, v/v; 150 mL per step) and methanol (300 mL) in *n*-hexane, affording 11 fractions. The biological activity of the collected fractions was determined using the *L. sativum* growth bioassay according to the above procedure, and complete inhibition was found in fractions obtained by elution with 80% ethyl acetate in *n*-hexane. After evaporation, the concentrate (1.4 g) was filtered through a column of Sephadex LH-20 (60 g, GE Healthcare Bio-Sciences AB SE-75184; Uppsala, Sweden), eluting with 20, 40, 60, and 80% (v/v) aqueous methanol (150 mL per step) and methanol (300 mL). The most active fraction was eluted with 60% aqueous methanol and subsequently evaporated until dryness. The concentrate (254 mg) was dissolved in 20% (v/v) aqueous methanol (2 mL) and loaded onto a reverse-phase C_{18} Sep-Pak cartridge (Waters Corporation, Milford, Massachusetts, USA) and purified with 20, 40, 60, and 80% (v/v) aqueous methanol and methanol (15 mL per step). The most active fraction was eluted with 20% aqueous methanol and evaporated until dryness. The concentrate (71 mg) was finally purified by C_{18} reversed-phase HPLC (COSMOSIL 5C_{18}-AR-II; Nacalai Tesque, Inc., Kyoto, Japan), eluting at a flow rate of 3 mL/min with 50% aqueous methanol and detecting at 220 nm. Complete inhibition was detected for two peaks that eluted at 22.0 and 24.0 min as colorless substances. Mass spectrometry with electro-spray ionization (ESI-MS) analysis was conducted on a Waters mass spectrometer. NMR spectra were measured on a Bruker NMR spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C), available at the Central Instrumental Center, University of the Ryukyus. All chemical shifts were reported relative to tetramethylsilane (TMS). Optical rotation was measured in chloroform on a JASCO P-1010 polarimeter.

6.2.5 Inhibitory effects of pure compounds and aqueous extracts of rice straw on *L. sativum* and *E. oryzicola* seedling growth

The isolated compounds were dissolved in methanol to prepare the concentrations of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 μM for each compound, in addition to 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 and 300 μM for a mixture of the compounds at the ratio of 1:1, with the concentration the sum of the two compounds. The biological activity against *L. sativum* and *E. oryzicola* seedlings was examined using the above procedure.

6.2.6 Statistical analyses

Statistical analyses were performed using the general linear model of the statistical analysis program of Fisher's Protected Least Significant Difference test. The experimental designs were completely randomized with triplicates. The Type I error was set at 0.05 and 0.01 for all statistical comparisons. The I_{50} (concentration of approximately 50% inhibition of the growth rate) value in the assays was determined from the regression equation of the concentration curves.

6.3 Results

6.3.1 Pot studies

Residues of both varieties inhibited the growth and dry matter of *E. oryzicola* at the highest amounts of 1.5 and 2 t ha^{-1}, whereas less straw incorporation had no effect (Fig. 26). Although the degree of weed-suppression did not vary significantly for the two rice varieties tested, the residues of allelopathic 'Goria' caused greater inhibition of plant height, tillers per plant and dry matter per plant of *E. oryzicola* than those of the non-allelopathic 'Holoi'.

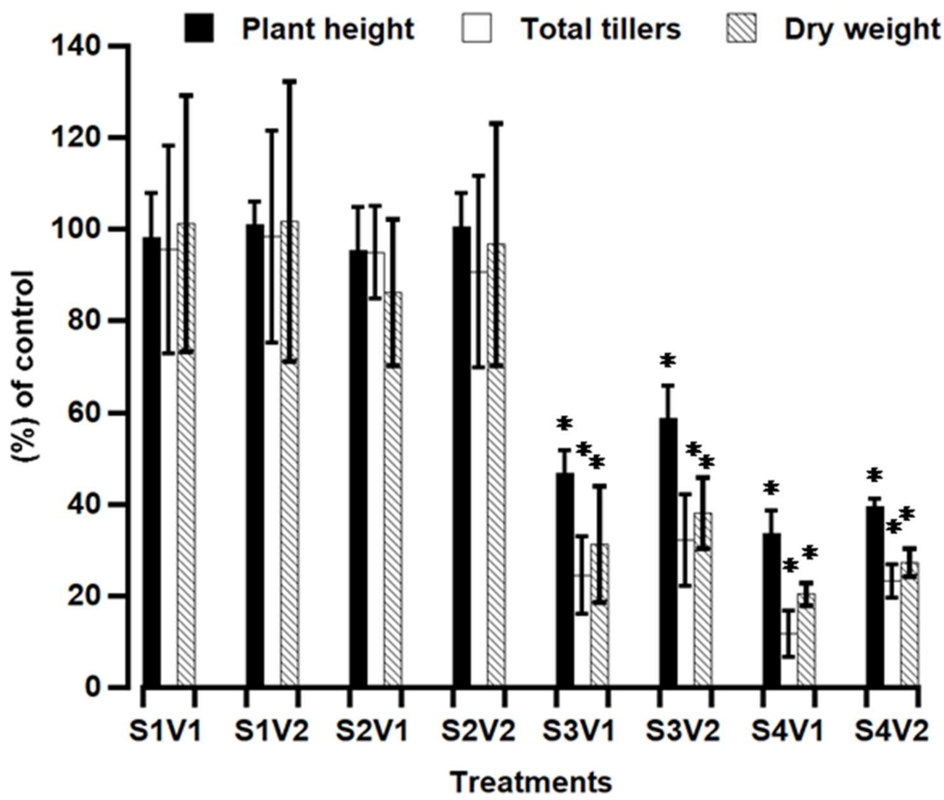


Figure 26. Effect of straw incorporation on growth and dry weight of *E. oryzicola*.

Here, S_1 = 0.5 t ha^{-1}, S_2 = 1 t ha^{-1}, S_3 = 1.5 t ha^{-1}, and S_4 = 2 t ha^{-1}; V_1 = allelopathic 'Goria' and V_2 = non-allelopathic 'Holoi'. Asterisks indicate significant differences between the control and treatment: *, $P < 0.01$.

In the second step of the pot studies, the growth and yield contributing parameters of 'Goria' rice were not significantly different between commercial fertilizer application and the combination of straw incorporation with commercial fertilizer (Table 14).

Table 14. Effect of allelopathic straw incorporation into the soil on growth and yield contributing parameters of Bangladesh indigenous rice variety 'Goria'

Treatment	Plant height (cm)	Total tillers $plant^{-1}$ (Nos.)	Effective tillers $plant^{-1}$ (Nos.)	Panicle length (cm)	Total grain $plant^{-1}$ (Nos.)	Filled grain percentage $plant^{-1}$	Thousand seed weight (g)
Control	87.67 ± 2.52^{c}	5.33 ± 0.58^{c}	2.67 ± 0.58^{c}	18.33 ± 1.53^{b}	49.26 ± 7.76^{c}	46.91 ± 9.74^{c}	17.98 ± 0.21^{b}
Straw 2 t ha^{-1}	109.00 ± 3.61^{b}	17.00 ± 1.00^{b}	4.00 ± 1.00^{b}	19.00 ± 1.00^{b}	72.83 ± 2.72^{b}	74.30 ± 5.88^{b}	18.26 ± 0.18^{b}
Chemical Fertilizer	117.67 ± 2.52^{a}	25.67 ± 1.53^{a}	8.33 ± 1.53^{a}	20.73 ± 0.64^{a}	89.60 ± 2.11^{a}	96.03 ± 2.36^{a}	20.09 ± 0.70^{a}
Chemical fertilizer + Straw 2 t ha^{-1}	120.67 ± 3.06^{a}	26.33 ± 2.08^{a}	9.33 ± 0.58^{a}	20.50 ± 0.50^{a}	90.55 ± 2.36^{a}	96.96 ± 2.14^{a}	20.21 ± 0.79^{a}

Means having similar letter(s) in a column are statistically similar and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

Table 15. Effect of straw incorporation into the soil on plant nutrients in soil after rice harvest

Treatment	Plant nutrients								
	C	N	HPO_4^{2-}	K^{+}	Ca^{2+}	SO_4^{2-}	Mg^{2+}	Fe^{3+}	Zn^{2+}
	%		mg g^{-1} soil						
Control	1.61 ± 0.02	0.11 ± 0.01	0.34 ± 0.01	0.63 ± 0.08	4.60 ± 0.33	1.25 ± 0.01	0.65 ± 0.06	0.47 ± 0.01	0.46 ± 0.01
Straw 2 t ha^{-1}	1.77 ± 0.12	0.13 ± 0.01	0.42 ± 0.13	0.74 ± 0.08	5.01 ± 0.34	1.38 ± 0.15	0.70 ± 0.06	0.64 ± 0.30	0.47 ± 0.03
Chemical Fertilizer	1.87 ± 0.06	0.14 ± 0.01	0.36 ± 0.02	0.67 ± 0.09	4.72 ± 0.19	1.40 ± 0.04	0.63 ± 0.07	0.47 ± 0.01	0.45 ± 0.01
Chemical fertilizer + Straw 2 t ha^{-1}	2.23 ± 0.01	0.16 ± 0.01	0.36 ± 0.02	0.73 ± 0.05	4.53 ± 0.30	1.47 ± 0.33	0.70 ± 0.06	0.48 ± 0.01	0.45 ±0.01

However, positive responses were observed in the parameters for the combination of straw incorporation and commercial fertilizers, except for panicle length. Based on the results of soil analysis after harvest, a significant amount of plant nutrients remained under the combination of straw incorporation and commercial fertilizers (Table 15).

6.3.2 Phytotoxic effects of aqueous methanol extracts

High concentrations of the aqueous methanol extracts inhibited the growth of the test species; whereas with very low concentrations (0.01 g DW equivalent per mL), stimulation or no effect on test species was observed (Figs. 27. A, B). At 0.3 g DW equivalent extract per mL of rice plants, the root growth of *L. sativum* and *E. oryzicola* was inhibited by 3 and 5% compared with that of the control root, respectively. At the same concentration, the shoot growth of *L. sativum* and *E. oryzicola* was 6 and 29% of control shoot growth, respectively. These results suggest that the Bangladeshi indigenous rice straw 'Goria' may contain growth inhibitory substances and possess allelopathic potential.

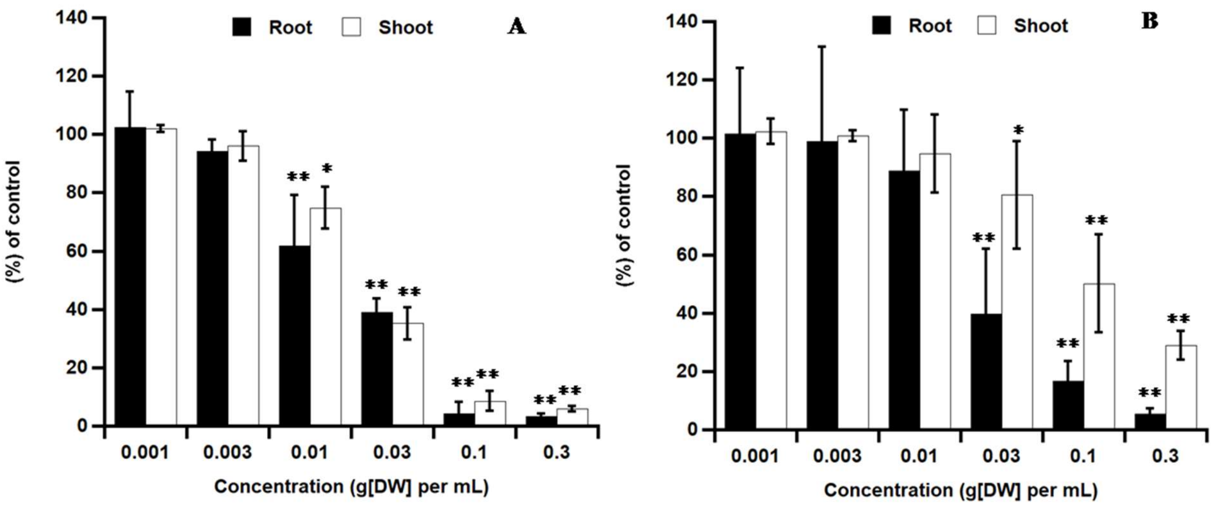


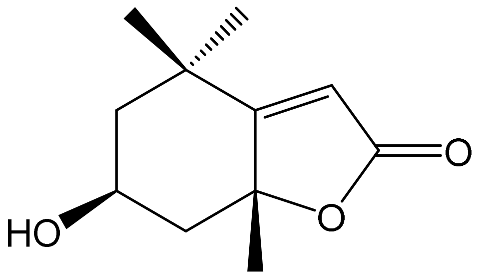
Figure 27. Effect of aqueous methanol-extract of rice straw on root and shoot growth of *L. sativum* (A) and *E. oryzicola* (B). *Bars* represent ± SD of values obtained from three biological replicates. Asterisks indicate significant differences between the control and treatment: *, *P* < 0.05; **, *P* < 0.01.

6.3.3 Structural identification of isolated compounds

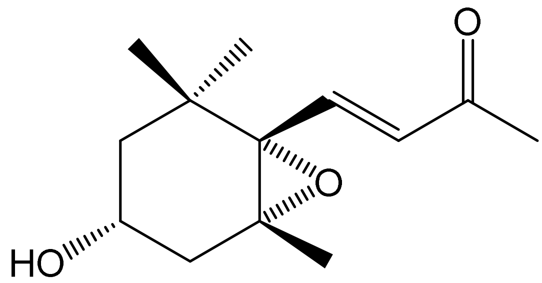
Two biologically active compounds were obtained from the repeated column chromatography of the aqueous methanol extracts of Bangladeshi rice straw variety 'Goria'.

Compound 1 (3.8 mg) had a molecular formula of $C_{11}H_{16}O_3$ (LR-ESI-MS *m/z* 197 $[M+H]^+$) and a specific rotation of $[\alpha]_D^{20}$ -68.8 (*c* 0.01, MeOH). The following were obtained for NMR spectra: ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.27 (3H, s, H-9), 1.47 (3H, s, H-8), 1.53 (dd, *J* = 14.6 and 3.7 Hz, H-7), 1.78 (3H, s, H-10), 1.79 (dd, *J* = 13.5 and 4.1 Hz, H-5), 1.98 (dt, *J* = 14.5 and 2.6 Hz, H-7), 2.46 (dt, *J* = 14.1 and 2.6 Hz, H-5), 4.33 (m, H-6), 5.70 (s, H-3); and ^{13}C-NMR (125 MHz, CD_3OD) δ: 27.0 (C-10), 26.5 (C-9), 30.6 (C-8), 35.9 (C-4), 45.6 (C-7), 47.3 (C-5), 66.8 (C-6), 86.7 (C-7a), 112.9 (C-3), 171.9 (C-2), 182.4 (C-3a). These data were compared with the data reported by Park et al. (2004), and the substance was identified as (-)-loliolide (Fig. 28 A).

Compound 2 (1.5 mg) had a molecular formula of $C_{13}H_{20}O_3$ (LR-ESI-MS *m/z* 225 $[M+H]^+$ and 247 $[M+Na]^+$) and a specific rotation of $[\alpha]_D^{25}$ -10.6 (*c* 0.01, $CHCl_3$). The following were obtained for NMR spectra: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.98 (s, H-11), 1.19 (s, H-12 and 13), 1.26 (m, H-2□), 1.64 (dd, *J* = 14.7 and 1.7 Hz, H-4□), 1.65 (dd, *J* = 14.4 and 8.6 Hz, H-2□), 2.28 (s, H-10), 2.39 (ddd, *J* = 14.4, 5.0 and 1.6 Hz, H-4β), 3.90 (m, H-3), 6.29 (d, *J* = 15.6 Hz, H-8), 7.03 (d, *J* = 15.6 Hz, H-7); and ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 19.9 (C-13), 25.0 (C-11), 28.3 (C-10), 29.3 (C-12), 35.1 (C-1), 40.6 (C-4), 46.7 (C-2), 64.0 (C-3), 67.2 (C-5), 69.5 (C-6), 132.6 (C-8), 142.3 (C-7), 197.4 (C-9). Therefore, the structure was 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one (Fig. 28 B), which corresponds with the data reported by Duan et al. (2002).



Compound **1**
(-)-Loliolide
Chemical Formula: $C_{11}H_{16}O_3$



Compound **2**
3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one
Chemical Formula: $C_{13}H_{20}O_3$

Figure 28. Structures of isolated allelochemicals (-)-loliolide (compound 1) and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one (compound 2) from the Bangladeshi indigenous rice straw variety 'Goria'

The endogenous concentrations of (-)-loliolide and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one were at least 8 and 3 µmol/kg, respectively, because 3.8 and 1.5 mg of the respective substances (MW 196 and 224, respectively) were isolated from 2.4 kg DW of rice straw. With decomposition of 1 kg of rice straw in 1 L of soil water, the concentration of (-)-loliolide and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one would be 8 and 3 µM, respectively.

6.3.4 Biological activity of compounds

Concentration and species-dependent inhibitory activities of (-)-loliolide and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one were determined using the seedling growth of test species (Figs. 29 A, 29B and 30A, 30B). The results demonstrated that significant inhibitory effects of (-)-loliolide began at concentrations as low as 10 µM on the root and shoot growth of *L. sativum* and at 10 and 30 µM on the root and shoot growth of *E. oryzicola* seedlings, respectively. However, significant inhibitory effects of 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one began at the concentrations 0.3 µM on the root and shoot growth of *L.*

sativum and 0.3 and 1 µM on the root and shoot growth of *E. oryzicola* seedlings, respectively.

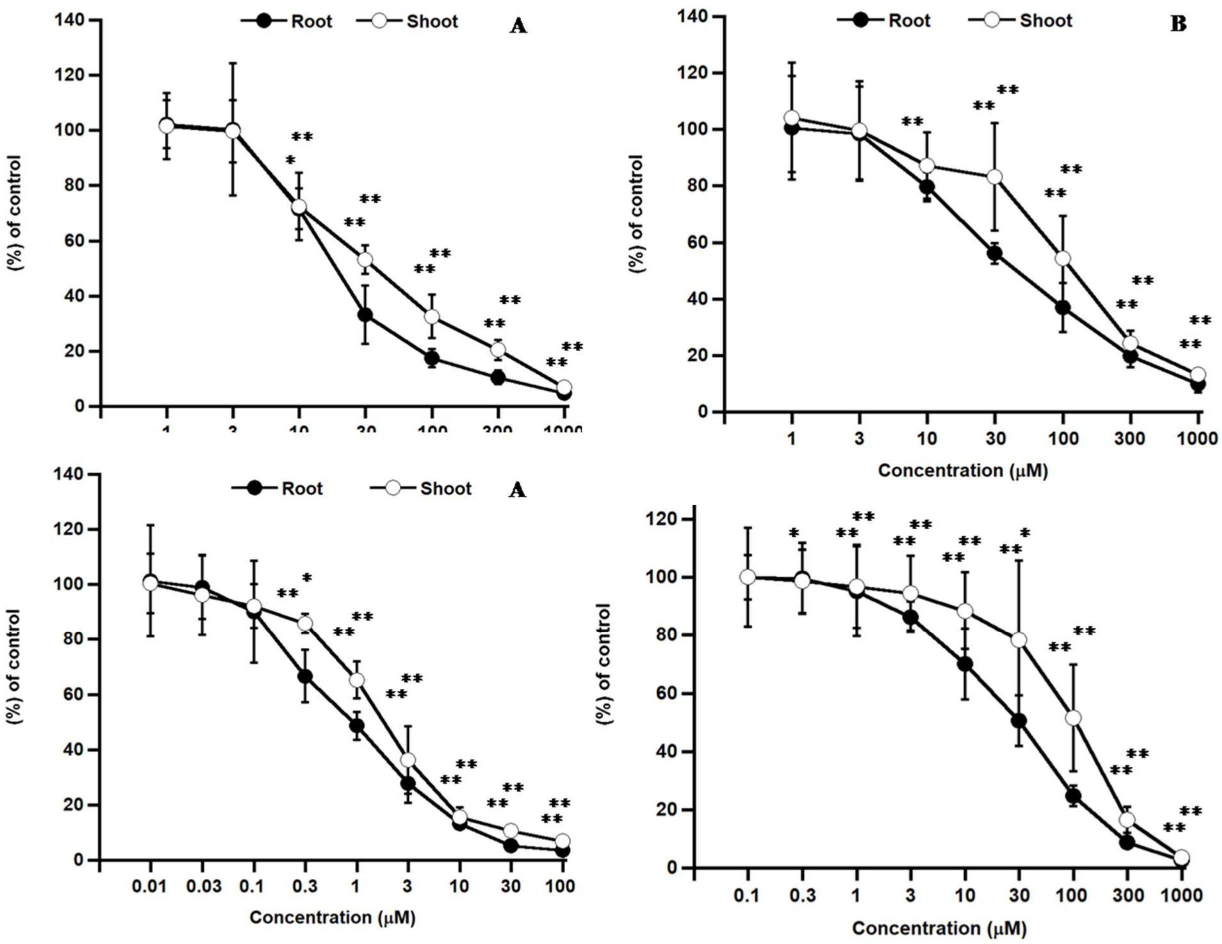


Figure 30. Inhibition of root and shoot growth of *L. sativum* (A) and *E. oryzicola* (B) at different concentrations of 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one

Bars represent ± SD of values obtained from three biological replicates. Asterisks indicate significant differences between the control and treatment: *, $P < 0.05$; **, $P < 0.01$.

The mixture effect of the two compounds at concentrations as low as 0.03 and 0.1, and 1 µM showed significant inhibition of the root and shoot growth of *L. sativum* and *E. oryzicola* seedlings (Fig. 31. A, B). Additionally, the inhibitory effects increased with increasing concentrations of the compounds. The concentrations causing approximately 50% growth inhibition in the assay (defined as I_{50}) for *L. sativum* roots and shoots were 28.23 and 53.4, 1.26 and 2.14, and 0.26 and 0.33 µM for (-)-loliolide, 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one and their mixture, respectively. For *E. oryzicola* roots and shoots, the I_{50} values were 64.62 and 162.92, 43.28 and 137.64, and 2.31 and 17.86 µM for (-)-loliolide, 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one and their mixture, respectively (Table 16).

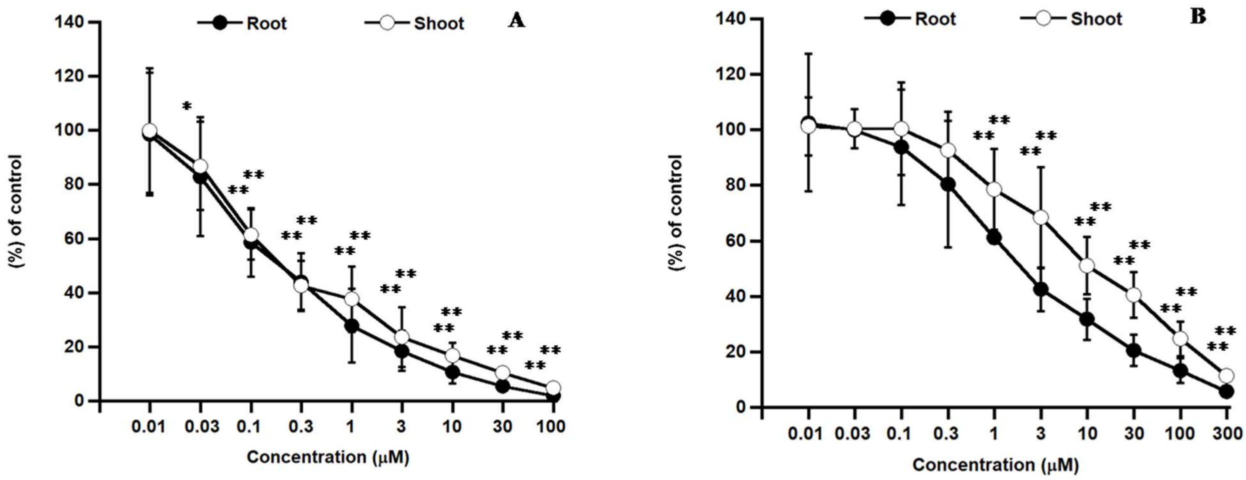


Figure 31. Inhibition of root and shoot growth of *L. sativum* (A) and *E. oryzicola* (B) at different concentrations of (-)-loliolide and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one. The concentration of 100 µM represents 25 µM (-)-loliolide and 25 µM 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one

Bars represent ± SD of values obtained from three biological replicates. Asterisks indicate significant differences between the control and treatment: *, $P < 0.05$; **, $P < 0.01$.

Table 16. Regression analyses of dose response curves for effects on *L. sativum* and *E. oryzicola* growth at different concentrations of the isolated compounds and their mixture

Test species	Compound	On root			On shoot		
		Regression equation	r^2	I_{50} (µM)	**Regression equation**	r^2	I_{50} (µM)
L. sativum	1	y = -0.502x + 64.17	0.729	28.23	y = -0.403x + 71.50	0.916	53.40
	2	y = -13.54x + 67.03	0.949	1.26	y = -17.49x + 87.46	0.970	2.14
	Mixture	y = -31.31x + 58.03	0.924	0.26	y = -21.41x + 57.14	0.644	0.33
E. oryzicola	1	y = -0.422x + 77.27	0.870	64.62	y = -0.202x + 82.91	0.932	162.92
	2	y = -0.467x + 70.21	0.946	43.28	y = -0.216x + 79.73	0.962	137.64
	Mixture	y = -12.96x + 79.89	0.922	2.31	y = -0.919x + 66.41	0.840	17.86

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6.4 Discussion

This study was conducted with the preliminary objective of determining rice straw toxicity based on testing different quantities of the residue and then using the most effective concentration in rice culture. The results reported here clearly indicated that both allelopathic and non-allelopathic rice straw was very toxic to *E. oryzicola* with significant reductions in seedling growth and dry weight. Kong et al. (2006) found that the weed-suppression of rice residues in soil depends on the decomposition period but is not related to allelopathic traits of rice varieties. A sharp increase of rice growth and yield parameters was observed in the rice culture in pot studies. Therefore, no autotoxicty effect on the growth and yield parameters of rice was found due to the incorporation of rice residues. The rice plant may develop adaptive mechanisms to avoid a severe autotoxic effect due to the residual effects of decomposing rice plant materials (Chou 1980). Xuan et al. (2005) reported that incorporation of allelopathic rice straw at 1–2 t ha^{-1} decreased weed biomass by approximately 70% and boosted rice yield by approximately 20% compared with the respective controls. In our study, a remarkable amount of plant nutrients was found in rice-raised soils. According to Dobermann and Fairhurst (2002), incorporation of rice straw by shallow tillage at the 5-10 cm depth has beneficial effects on soil fertility in intensive rice-rice systems, which include increased soil aeration during fallow periods, more complete carbon (C) turnover (approximately 50% of the C with 30-40 days), minimized negative effects (e.g., phytotoxicity) of the products of anaerobic decomposition on early rice growth, increased N mineralization and soil P release to the succeeding crop, and reduced weed growth. The aqueous methanol extract of Bangladeshi indigenous rice 'Goria' straw inhibited the root and shoot growth of the test plant species, and with increases in the concentration of the extract, the inhibition increased. Kawther et al. (2006) reported that rice straw extract potentially controlled *E. crus-galli* and *E. colona*. In our studies, both stimulatory and inhibitory effects were found. Rice (1984) reported that allelopathic activity could have stimulatory effects at a low concentration and an inhibitory effect at a high concentration depending on the allelopathic compounds. Inderjit and Duke (2003) also found different allelopathic responses for asymmetrical test plants due to the different selectivity of allelopathic substances. Grabarczyk et al. (2015) noted that (-)-loliolide inhibited the development of certain plants, and Islam et al. (2016) also found that loliolide inhibited the seedling growth of *E. crus-galli*. Duan et al. (2002) isolated 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one from *Saussurea medusa* as an immunosuppressive constituent. Lu et al. (2011) isolated (-)-loliolide and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one from the seaweed *Gracilaria lemaneiformis* and described their allelopathic potential on the alga *Skeletonema costatum*. Growth inhibition of test species increased significantly with the mixture of the two compounds compared with the inhibition of the individual compounds. Chung et al. (2001) reported that allelopathic effects are affected by the interactions of concentrations, combination of compounds and sensitivity of test species and suggested that allelopathic compound mixtures could collectively reach sufficiently high concentrations to be bioactive on weeds.

However, whether these compounds are directly phytotoxic or become phytotoxic after microbial transformation under field conditions is difficult to determine. Further studies are required to determine interactions among the types and roles of microorganisms and cultivation practices and their effects on the activities of these compounds in the field.

CONCLUSION

Bangladesh indigenous allelopathic rice ‘Goria’ straw incorporation into the soil gave inhibitory effects on the growth and dry weight of *E. oryzicola* but had no autotoxicity on the growth of rice variety. Isolated two biologically active compounds from straw extracts, (-)-loliolide and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one had a strong synergistic inhibitory effect on the growth of tested weed. Thus, our current work suggests that rice straw could be useful in multidisciplinary approaches for controlling weeds and/or minimizing the herbicidal doses and for soil nutrient improvement. Overall, based on such studies, the use of straw wastes for controlling high densities of weeds in many crops might be considered one of the remarkable achievements for the recycling of rice straw wastes in rice producing countries such as Bangladesh, which will be reflected in reductions of the effects of environmental pollution. These results may also be useful in providing basic information for developing natural herbicides through the extension of databases.

CHAPTER VII

COMPARISON STUDY OF ALLELOCHEMICALS AND A COMMERCIAL HERBICIDE ON THE GERMINATION AND GROWTH RESPONSE OF *Echinochloa crus-galli* L

ABSTRACT

The phytotoxic effects of two allelochemicals (*trans*-cinnamic acid and syringaldehyde) at different concentrations (1000, 100, 10 and 1 µM) on seed germination, seedling growth, and physiological and biochemical changes of *Echinochloa crus-galli* L. were tested by comparison to a commercial herbicide 'Nominee' (i.e. 100 g/L bispyribac-sodium). *trans*-Cinnamic acid and the herbicide inhibited seed germination completely at 100 µM, whereas for syringaldehyde, complete inhibition required 1000 µM. However, with 100 µM syringaldehyde, the seed germination of the test species was 53% of the control. Allelochemicals and the herbicide delayed seed germination and significantly affected the speed of germination index (S), speed of cumulative germination index (AS) and coefficient of germination rate (CRG). The roots were more affected when nutrients were not added to the growth bioassay. In general, with the increasing concentration of allelochemicals from 100 to 1000 µM, the inhibitory effects increased. Via microscopy analysis, we found leaf blade wilting and necrosis at concentrations above 100 µM in allelochemical-treated plants. Roots of *E. crus-galli* treated with 1000 µM allelochemicals had black points on root nodes but had no root hairs. The anatomy of roots treated with allelochemicals (1000 µM) showed contraction or reduction of root pith cells as well as fewer and larger vacuoles compared to the control. The allelochemicals also showed remarkable effects on seedling growth, SPAD index, chlorophyll content and free proline content in a pot culture bioassay, indicating that *trans*-cinnamic acid and syringaldehyde are potent inhibitors of *E. crus-galli* growth and can be developed as herbicides for future weed management strategies.

7.1 Introduction

Weeds have been a serious problem since the beginning of crop cultivation. In fact, in agricultural lands, weeds cause large reductions in crop yield and quality, increase the time and costs involved in crop production, interfere with harvesting, and create problems in animal feeding (including poisoning) and livestock management, among other issues (Kraehmer & Baur 2013). Many strategies have been developed for the purpose of controlling weeds, including hand or mechanical weeding, smothering with mulch, lethal wilting with high heat, burning, and the least expensive and most popular strategy, chemical attack with herbicides (weed killers). Unsurprisingly, weed management in current agriculture relies on herbicides because they are highly effective (Senseman 2007). However, the extensive use of herbicides to manage weeds has resulted in the emergence of herbicide resistance among target weeds as well as a host of health risks, particularly their ability to kill placental and umbilical cord cells. In addition, negative environmental and ecological effects occur from the use of herbicides. Therefore, in recent years, there has been considerable desire to reduce herbicide use and search for alternative ways to control weeds (Ackerman et al. 2014). Allelopathy, which studies biochemical plant-plant interaction based on secondary metabolites, including positive and negative effects of biological and agricultural systems (IAS 1996), is considered to be a promising option for weed management. Tens of thousands of secondary metabolites of plants have been identified, and some of these natural products show inhibitory activity on other plants (Macias et al. 2007). Thus, allelochemicals may help to overcome weed problems through the use of allelopathic crop varieties or use of natural (from plants or microbes) or synthetic-derivative phytotoxin plant growth inhibitors (Macias et al. 2000).

Considering both ecological and economic perspectives, natural products may provide clues to develop new herbicide chemistry through modifications that could be more active, selective, persistent or cost effective. Cinmethylin, a derivative of 1,4-cineole, an allelochemical of eucalyptus, is a good example of an herbicide that was developed using this approach (Hirai 2003). Another good example, leptospermone from *Callistemon citrinus*, which was initially found to be too weak to use as an herbicide, was transformed to be more effective via chemical synthesis into mesotrione (trade name Callisto), which is used as a commercial selective herbicide for maize (Bhowmik & Zhang 2003; Cornes 2005). Moreover, some allelochemical inhibitory activities are similar to herbicides, and their features allow them to be treated as bio-herbicides (Soltys et al. 2013). Therefore, there is a wide scope of use of plant-based herbicides in integrated management of weeds. Although naturally released allelochemicals have low bioactivity, less specificity and wide inconsistency compared to herbicides, they have different modes of action and have a short half-life as they are biodegradable. Hence, they perform better in ecosystem, and the receiver may not easily adapt or develop resistance against them. Therefore, allelochemicals are considered to be environmentally and toxicologically safer than synthetic compounds (Bhowmik & Inderjit 2003).

Each year natural product chemists isolate and identify hundreds of phenolics, alkaloids, terpenoids, polyacetylenes, fatty acids, and steroids from higher plants and microbes as allelochemicals (Inderjit et al. 2008). However, because a chemical can be extracted from a plant does not imply that it is released from the plant naturally, but rigorous proofs are required to demonstrate allelopathy (Duke 2015). Regrettably, often, only a minute amount of a potential compound can be purified from a natural source, and it is even more difficult to prove the incidence of allelopathy. Therefore, a synthetic source is essential for to sufficiently study the agent’s mode of action as an allelochemical (Vyvyan 2002).

We previously investigated the Bangladesh indigenous rice ‘Boterswar’ as an allelopathic variety and reported four biological active compounds along with syringaldehyde (4-

hydroxy-3,5-dimethoxybenzaldehyde) as an allelochemical (Masum et al. 2018). As the isolated amount was too low to identify the mode of action, commercially available syringaldehyde was compared to another allelochemical, *trans*-cinnamic acid, and one commercial herbicide (Nominee) to develop an understanding of rice allelopathy and the phytotoxicity of the allelochemicals. Seeds of *E. crus-galli* were used in a bioassay as *E. crus-galli* has superior biology and tremendous ecological adaptations and is the one of the top 15 herbicide-resistant weeds in the world. Its proliferation seriously impacts rice production and can result in major losses in rice yield (Khanh et al. 2008).

Thus, the present investigation was undertaken to further our knowledge of the allelochemical interactions involved in this rice, their modes of action and biochemical or physiological changes of the receptor. This will allow us to develop new strategies in developing natural herbicides.

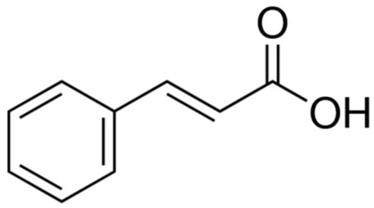
7.2 Materials and Methods

7.2.1 Plant Materials

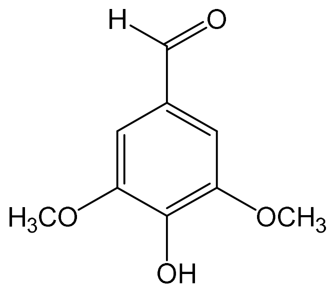
According to Xuan et al. (2016), unfilled and immature seeds of *E. crus-galli* were screened by suspension in tap water. The remaining seeds were hermetically stored (–20°C) after air-drying. Stored seeds were sterilized with 1% sodium hypochlorite for 30 min and rinsed with distilled water before use. The germination percentage was randomly checked and was found to be >80%.

7.2.2 General Procedure

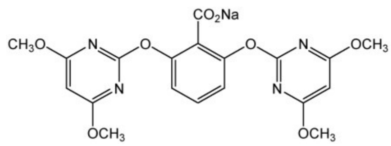
The experimental procedure involved testing the phytotoxic effects of *trans*-cinnamic acid, MW 148.161 g/mol (Wako Pure Chemical Industries, Ltd. Osaka, Japan); syringaldehyde, MW 182.175 g/mol (4-hydroxy-3,5-dimethoxybenzaldehyde) (Toronto Research Chemicals, Inc.); and a herbicide (Nominee a.i. 100 g/L bispyribac-sodium MW 452.355 g/mol) (Fig. 32) on the seed germination and seedling growth of *E. crus-galli*.



trans- Cinnamic acid
Chemical formula: $C_9H_8O_2$
Molecular weight: 148.161



Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde)
Chemical formula: $C_9H_{10}O_4$
Molecular weight: 182.175



Bispyribac-sodium
Chemical formula: $C_{19}H_{17}N_4NaO_8$
Molecular weight: 452.355

Figure 32. Chemical structures of *trans*-cinnamic acid, syringaldehyde and Nominee (bispyribac-sodium)

7.2.3 Chemical Solutions

To assess the effects of allelochemicals on *E. crus-galli*, stock solutions of test allelochemicals were prepared in a 1 M dimethyl sulfoxide solvent, from which different aqueous solutions of 1000, 100, 10 and 1 μM were prepared with the pH (6.0) adjusted by NaOH (Jose & Gillespie 1998). The herbicide solutions were prepared in distilled water at the same concentrations. These solutions were stored at 4°C until use. In all experiments, distilled water along with a 1 M dimethyl sulfoxide solvent was used as the control.

7.2.4 Germination bioassay

Fifty seeds of *E. crus-galli* were placed on Whatman 2 MM paper in a Petri-dish (9 cm), and 4 mL of a treatment solution or control was added. They were then placed in a thermostatically controlled incubator (total darkness at 25°C) to germinate. Every 48 h, one milliliter of each solution was added per Petri-dish. Germination was assessed (rupture of seed coats and the emergence of radicle, Mayer & Poljakoff-mayber 1963) every 12 h until no further seeds germinated. The total germinated seeds (%) were calculated from the cumulative germination data after one week (Weidenhamer et al. 1987). Treatments were replicated four times. The same data were then used to calculate and compare different indices. Four germination indices were selected because of their common use in germination studies and were calculated as proposed by Chiapusio et al. (1997). The four germination indices and their calculations were as follows:

Total germination (G_T) = (N_T – 100)/N

Here, N_T: Proportion of germinated seeds at each treatment for the last time measurement

N: Number of seeds used in the bioassay

Speed of germination (S) = $(N_1 - 1) + (N_2 - N_1) \times \frac{1}{2} + (N_3 - N_2) \times \frac{1}{3} + \ldots\ldots (N_n - N_n - 1) \times \frac{1}{n}$

Where, N_1, N_2, N_3, ………….., N_n: Proportion of germinated seeds obtained the first (1), second (2), third (3), ……., (n – 1), (n) hours

Speed of Accumulated germination (AS) = $\left[\frac{N1}{1} + \frac{N2}{2} + \frac{N3}{3} + \ldots\ldots\ldots \frac{Nn}{n} \right]$

Here, N_1, N_2, N_3, ……………., N_n : Cumulative number of seeds which germinate on time 1, 2, 3, …………, N following set up of the experiment

Coefficient of the rate of germination (CRG) = $\frac{[N1+N2+N3+\cdots+Nn]}{(N1^{\times}T1)+(N2^{\times}T2)+(N3^{\times}T3)+\cdots(Nn^{\times}Tn)}$ × 100

Where, N_1: Number of germinated seeds on time T_1; N_2: Number of germinated seeds on time T_2; N_3: Number of germinated seeds on time T_3; and, N_n: Number of germinated seeds on time T_n

7.2.5 Growth bioassay

Glass beakers (500 mL volume, 12 cm depth, 9 cm diameter) containing 30 mL of 0.3% water agar without nutrients and containing 0.5 × Murashige and Skoog salts, 1 × Gamborg's B5 vitamins, 1% sucrose (w/v), and 2% Gelrite (w/v) adjusted to pH 6 were autoclaved (HMC EUROPE HG-50/HG-80, Tuessling, Germany). Ten uniformly pre-germinated seeds of *E. crus-galli* were placed and watered with three mL of solution or control treatments. The beaker was enclosed with parafilm and kept in the growth chamber with a daily light/dark cycle of 12/12 h, $3.56 \pm 0.16 \times 10^3$ lux fluorescent light intensity and temperature cycle of 25°C/25°C. An additional one milliliter of each solution was added every 48 h. Root and shoot growth was measured 6 d after starting the test without nutrients and 14 d after starting the test with nutrients (Reigosa & Pazos-Malvido 2007).

7.2.6 Growth chamber culture bioassay

After 36 h of soaking, uniformly pre-germinated seeds were placed in seedling trays (25-by-25-by-5cm; two seeds per hole) filled with commercial potting mixture. All trays were placed in the glasshouse (12 h photoperiods; light intensity 21.5 ±1 $MJm^{-2}d^{-1}$; 20 to 35°C temperature and 70-80% relative humidity), and the seedlings were watered with tap water daily until use. After 12 DAS(days after sowing), uniform seedlings were transferred in conical flasks (200 mL, one seedling) containing Hoagland solution (250 mL; pH 5.5 and EC 1.2 ms/cm) and placed in a growth chamber under controlled conditions as previously described, and after 24 h, the allelochemicals, herbicide and control were added as per each treatment. The conical flasks were kept for another five days (18 DAS) in the same environment as previously described, and at 24 h intervals, the solution level of the conical flasks was maintained by adding Hoagland nutrient solution. At the end of the experiment, the morphology of the leaves and roots of the bioassayed species as well as the root tip excised were observed under a microscope (Leica Microsystems LAS X). Thin sections (18 μm), cut with a diamond knife on a Supernova microtome, were examined using a microscope (Leica Microsystems LAS X).

7.2.7 Glasshouse pot culture bioassay

A glasshouse pot (Wagner pot, 0.02 m^2) experiment was conducted to evaluate the effect of the allelochemicals and herbicide on *E. crus-galli*. Each pot was filled with 4 kg of gray soil (coarse sand 3.61%, fine sand 30.94%, silt 24.32%, clay 32.84%, apparent density 0.90 g cm^{-3}, pH 7.43, C 0.96%, N 0.12%, P 4.60 μ g^{-1} soil, K 42.89 μ g^{-1} soil, Ca 2604.15 μ g^{-1} soil, Mg 279.30 μ g^{-1} soil, S 2765.07 μ g^{-1} soil, Fe 0.16 μ g^{-1} soil, Na 102.36 μ g^{-1} soil, and Al 5.42 μ g^{-1} soil). *E. crus-galli* seedlings were raised as previously described, and at the three-leaf stage (12 DAS), one seedling per pot was transplanted. After five days (17 DAS), the pots were randomly divided into four groups as per the treatments and irrigation was stopped. Tween®20 (Polyoxyethylene Sorbitan Monolaurate) was mixed into the solutions and the control 0.01% to wet the leaves. Application (250 mL - 50 mL per day) of the treatment solutions and control was carried out using a hand sprayer**.** Six days after the pot treatments, *E. crus-galli* seedlings (23-d-old) were harvested and their height (from the basal node to the end of leaf), tiller number, SPAD index, chlorophyll content and free proline (Pro) were measured.

7.2.8 Chlorophyll content determination

Based on the absorbance value, calculations were made using Arnon's (1949) equation, and the amount of chlorophyll (Chl) *a* and Chl *b* were estimated. Fully expanded leaves (0.5 g) were removed and then homogenized with an ice cool mortar and pestle using 80% acetone as the extraction buffer. The samples were then centrifuged at 0-4°C using a rotor with a speed of 15,000 rpm for 10 min. The absorbance of the supernatant was measured at 480, 645 and 663 nm in a spectrophotometer-UV-1700 (Shimazdu, Japan).

The concentrations of chlorophyll *a* and chlorophyll *b* were calculated using the following equations:

$$\text{Chl } a = \frac{(12.7 \times A_{663} - 2.6 \times D_{645}) \times \text{Volume of 80\% acetone}}{1000 \times \text{Weight of leaf sample (g)}} \text{ mg g}^{-1} \text{ fresh weight}$$

$$\text{Chl } b = \frac{(22.9 \times D_{645} - 4.68 \times D_{663}) \times \text{Volume of 80\% acetone}}{1000 \times \text{Weight of leaf sample (g)}} \text{ mg g}^{-1} \text{ fresh weight}$$

7.2.9 Proline determination

The proline (Pro) content was appraised according to the method of Bates et al. (1973). A 0.5 g sample from an upper fully expanded fresh leaf was homogenized in 5 mL of 3% sulfosalicylic acid, and the homogenate was filtered for use as an extract solution for extermination of the Pro content. Two milliliters of the filtrate was mixed with 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent, and the solution was heated at 100 ºC for 1 h. After the solution cooled, 4 mL of toluene was added and it was then transferred to a separating funnel. Toluene containing chromophores was separated and adjusted to the absorbance value at 520 nm with a spectrophotometer (UV-1700; Shimadzu Co., Ltd.). The concentration of Pro was estimated using a standard curve from the known concentration of Pro.

7.2.10 Statistical analysis

Germination and growth bioassay experiments were repeated six times using a completely randomized design with four replications, and the data were compared with respect to the controls**.** Growth chamber and glasshouse pot culture bioassay experiments were repeated three times with three replications using a completely randomized design. All statistical comparisons were analyzed using Fisher's Protected Least Significant Difference test with the Type I error (0.05).

7.3 Results

In all bioassay experiments, the organic solvents used to dissolve the allelochemicals had no significant effects.

7.3.1 Effects of allelochemicals on germination at each exposure time

The two allelochemicals and herbicide used, *trans*-cinnamic acid, syringaldehyde and Nominee, acted differently (Table 17), and the allelochemicals showed significant inhibitory effects on seed germination. At concentrations of 1000 and 100 µM, *trans*-cinnamic acid and the herbicide induced complete inhibition of germination, whereas syringaldehyde induced complete inhibition at 1000 µM. However, at 100 µM, syringaldehyde induced delayed germination. At lower concentrations, the allelochemicals had no significant effect on the germination of *E. crus-galli*.

Table 17. Effects of different concentrations of *trans*-cinnamic acid, syringaldehyde and herbicide on the germination of barnyardgrass seeds every 12 hours for 84 hours[a]

Treatment and concentration (µM)	Exposure time (h)				
	36	48	60	72	84
trans-Cinnamic acid					
1000	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
100	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
10	40.00 ± 17.32[b]	97.26 ±14.80	75.86 ± 7.09	75.111 ± 19.69[b]	74.701 ±17.40[b]
1	71.67 ± 30.14[b]	103.45 ± 34.33	95.83 ± 31.46[b]	99.37 ± 6.23	97.79 ± 11.12
Syringaldehyde					
1000	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
100	0.00 ± 0.00[b]	0.00 ± 0.00	22.06 ± 7.61[b]	41.65 ± 7.26[b]	53.25 ± 6.36[b]
10	55.00 ± 18.03[b]	89.41 ± 21.49[b]	94.44 ± 9.62	84.14 ± 11.13[b]	82.01 ± 10.35[b]
1	100.00 ± 0.00	78.57 ± 25.75	93.80 ± 25.69	105.10 ± 15.04	102.79 ± 9.04
Herbicide					
1000	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
100	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
10	0.00 ± 0.00[b]	75.48 ± 4.31[b]	84.24 ± 13.42	76.58 ± 13.65[b]	73.62 ± 10.16[b]
1	85.00 ± 13.23	113.21 ± 43.58[b]	116.67 ± 28.89	92.02 ± 13.97	88.77 ± 13.27[b]

[a] Results are expressed as a percentage of the control

[b] Significant differences, compared to the control for $P < 0.05$ according to Fisher's LSD Test

7.3.2 Effects of the allelochemicals on germination based on the calculated indices

The calculated indices are provided in Table 18. Although the four indices were calculated using the same data, they provided different results with low variability. Total germination, G_T, is a commonly used index that is affected by treatments at the highest concentrations. *trans*-Cinnamic acid and the herbicide completely inhibited germination at a concentration greater than 100 µM, whereas for syringaldehyde, 53% of the control germinated and demonstrated delayed germination. The herbicide proved to be the most deleterious and strongly inhibited the G_T of *E. crus-galli*. At very low concentrations, the allelochemicals had no significant effect on G_T.

Table 18. Germination indices for allelochemical and herbicide concentrations, expressed as a percentage of the control index

Treatment and concentration (µM)	Index			
	G_T	S	AS	CRG
trans-Cinnamic acid				
1000	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
100	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
10	74.70 ±17.40[a]	74.38 ± 16.46[a]	74.76±15.18[a]	99.94 ± 0.82
1	97.79 ± 11.12	96.68 ± 12.17	96.08 ± 14.18	99.37 ± 1.41
Syringaldehyde				
1000	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
100	53.25 ± 6.36[a]	44.79 ± 5.56[a]	34.29 ± 4.86[a]	92.45 ± 0.29[a]
10	82.01 ± 10.35[a]	82.05 ± 7.94[a]	83.12 ± 4.96[a]	99.88 ± 1.26
1	102.80 ± 9.04	100.94 ± 8.11	98.85 ± 7.99	99.08 ± 0.73
Herbicide				
1000	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
100	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
10	73.62 ± 10.16[a]	71.29 ± 9.96[a]	70.60 ± 10.24[a]	98.34 ± 0.25[a]
1	88.77 ± 13.27[a]	91.61 ± 13.68[a]	95.88 ± 14.92	101.09 ± 1.04

[a] Significant difference, compared to control, for $P< 0.05$ according to Fisher's LSD Test
G_T = Total germination, S = speed of germination, AS = speed of accumulated germination, CRG = coefficient of rate of germination

The allelochemicals and herbicide delayed *E. crus-galli* germination at concentrations of 1000, 100 and 10 µM and significantly affected the speed of germination index (S), speed of cumulative germination index (AS) and coefficient of germination rate (CRG) (Table 18). At the lowest concentration(1 µM), only the herbicide controlled S significantly, whereas the two allelochemicals had no significant effects. Similar results were also found in the case of AS and CRG for the two allelochemicals.

7.3.3 Root and shoot elongation

Figure 33 presents the inhibition effects of the evaluated allelochemicals and herbicide on root and shoot growth of *E. crus-galli* in the absence and presence of nutrients, respectively. All of the chemicals tested in the experiment demonstrated stronger inhibition effects on root growth than on shoot growth at higher concentrations (> 100 μM) in both with- and without-nutrients media, and the effects were more apparent when grown in a without-nutrients condition. At a lower concentration(1 μM), *trans*-cinnamic acid and the herbicide had no significant inhibition effect on seedling growth of *E. crus-galli*, whereas syringaldehyde showed a stimulatory effect on both root and shoot growth.

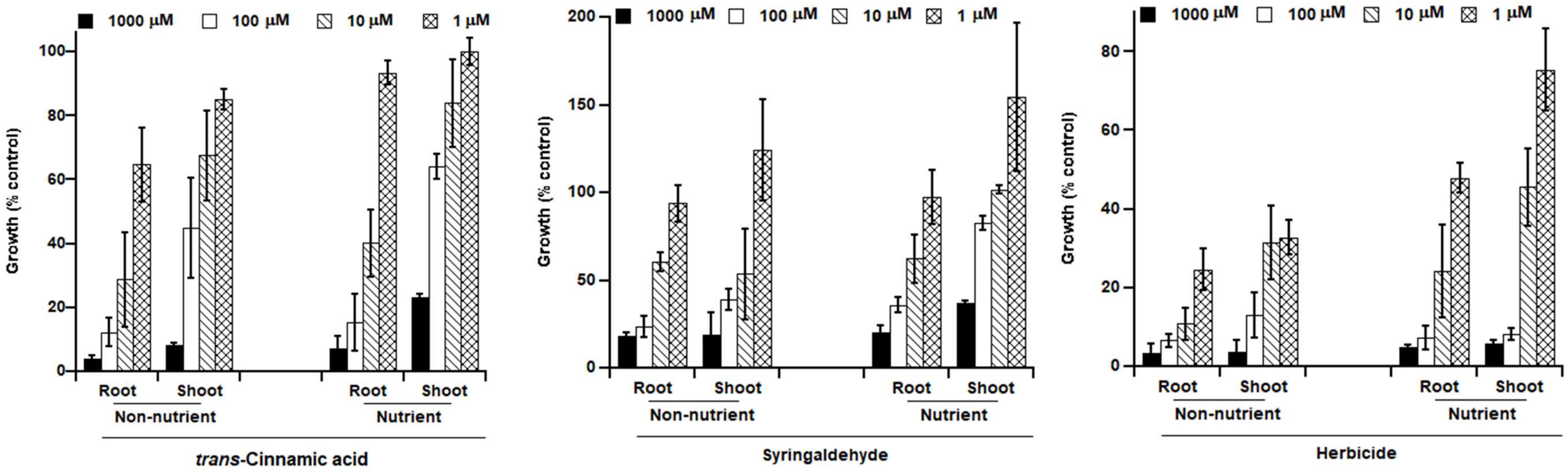


Figure 33. Effect on root and shoot growth (percentage with respect to control) of *E. crus-galli* treated with *trans*- cinnamic acid, syringaldehyde and herbicide at concentrations from 1000 to 1 µM under with and without nutrients conditions

7.3.4 Morphological attributes

The allelochemicals dose-dependently slowed or inhibited the growth of *E. crus-galli* seedlings. The bioassay species grown in a concentration of more than 100 µM allelochemicals were considerably smaller compared to the control plants and demonstrated leaf blade wilting, chlorosis and necrosis (Fig. 34) as well as inhibited root and root hair growth (Fig. 36). Visual differences in root systems were also observed (Fig. 35). The allelochemicals inhibited the growth and quantity of roots. It was also observed that the toxic effect of *trans*-cinnamic acid and syringaldehyde were manifested as a dark brown discoloration on the root tip and black points on root nodes. On the other hand, treatments with the herbicide at concentrations greater than 10 µM, lamina necrosis and a dark brown discoloration through the root pith and root tip were observed, but no root hair formation was observed. The lower concentration of allelochemicals either stimulated or did not affect the growth of the receiver species; however, herbicide at a 1 µM concentration showed leaf blade wilting. Microscopic images (Fig. 37) showed that root tip meristem cells treated with the allelochemicals at the highest concentration (1000 µM) demonstrated a significant contraction or reduction of root pith cells and fewer and larger vacuoles compared to the control root (Fig. 37. A-C). The herbicide at the same concentration showed similar symptoms along with a black discoloration (Fig. 37. D)

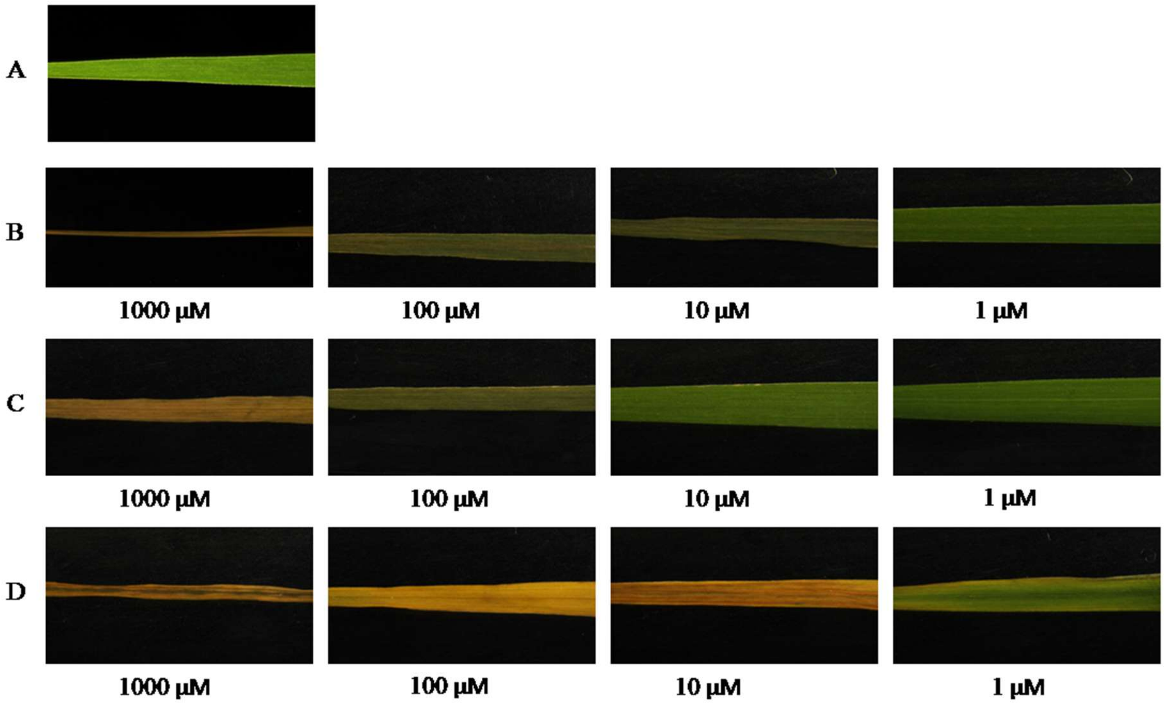


Figure 34. Effect of control (A), *trans*-cinnamic acid (B), syringaldehyde (C) and herbicide (D) at concentrations of from 1000 to1 µM on leaf growth of *E. crus-galli* in a growth chamber bioassay

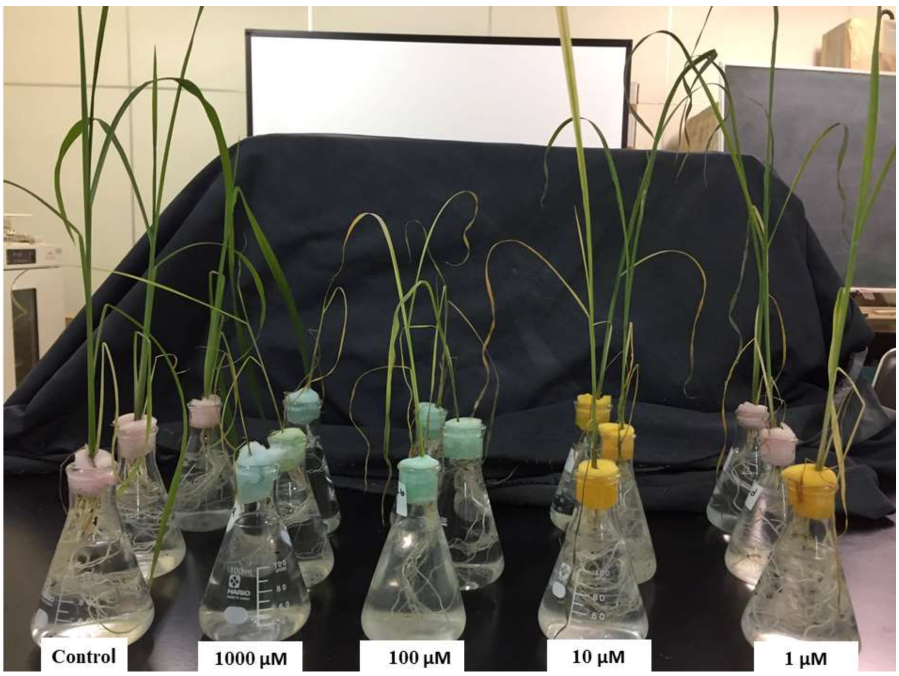


Figure 35. Effect of syringaldehyde at concentrations of from 1000 to 1 µM on rooting of *E. crus-galli* in a growth chamber bioassay

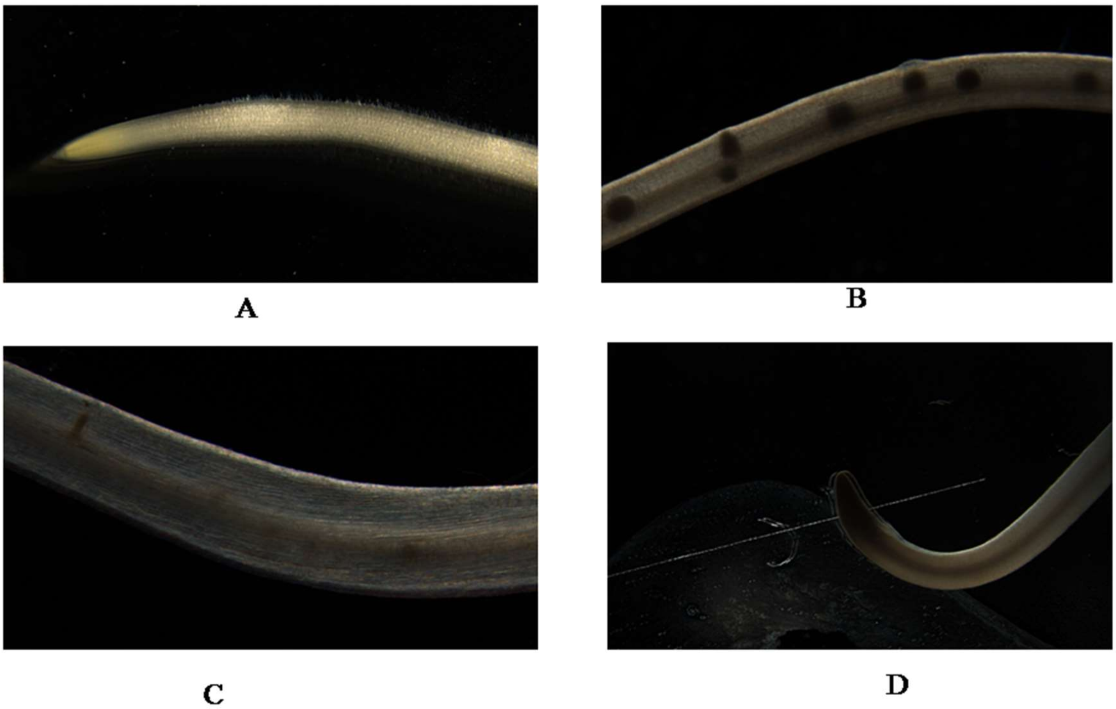


Figure 36. Effect of control (a), *trans*-cinnamic acid (b), syringaldehyde (c) and herbicide (d) at a concentration of 1000 µM on root growth of *E. crus-galli* in a growth chamber bioassay

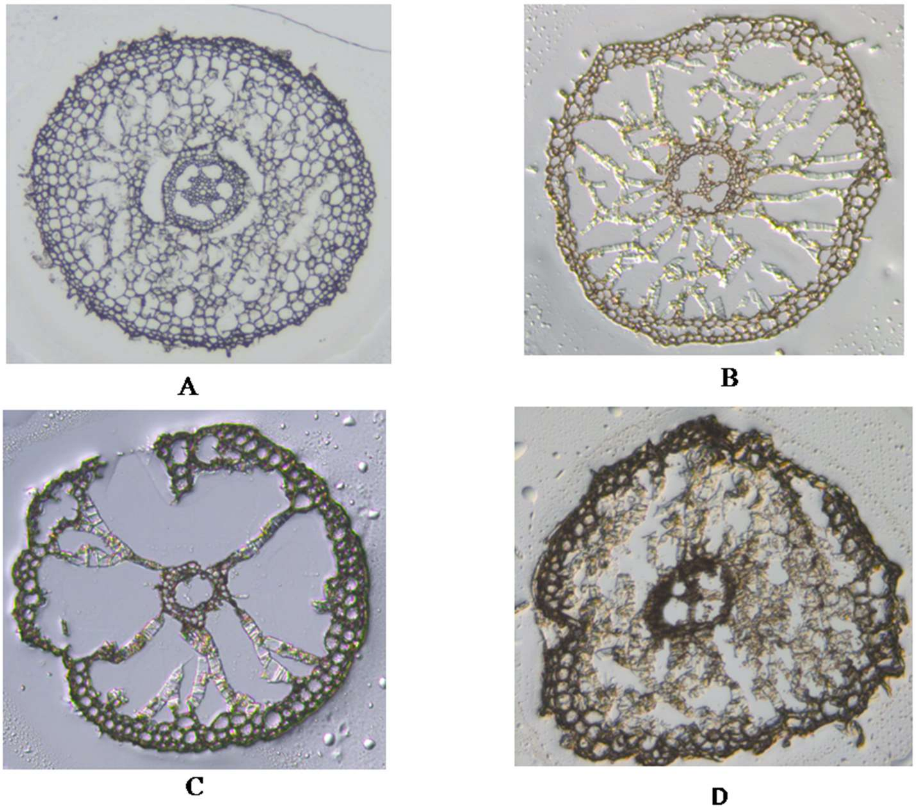


Figure 37. Effect of control (A), *trans*-cinnamic acid (B), syringaldehyde (C) and herbicide (D) at a concentration of 1000 µM on the root anatomy (18 µm) of *E. crus-galli* in a growth chamber bioassay

7.3.5 Effect on the seedling growth parameters in pot culture bioassay

The response of the growth parameters of *E. crus-galli* was significantly affected by different concentrations of the allelochemicals and herbicide (Fig. 38). The strongest inhibitory effects were found for the herbicide (> 10 µM concentration) on the plant height, number of total tillers and SPAD index of *E.crus-galli,* whereas both allelochemicals only showed significant inhibitory effects compared to the control at 1000 µM. At the lower concentration(1 µM), in the case of the allelochemicals, there was no effect or a stimulatory effect was observed.

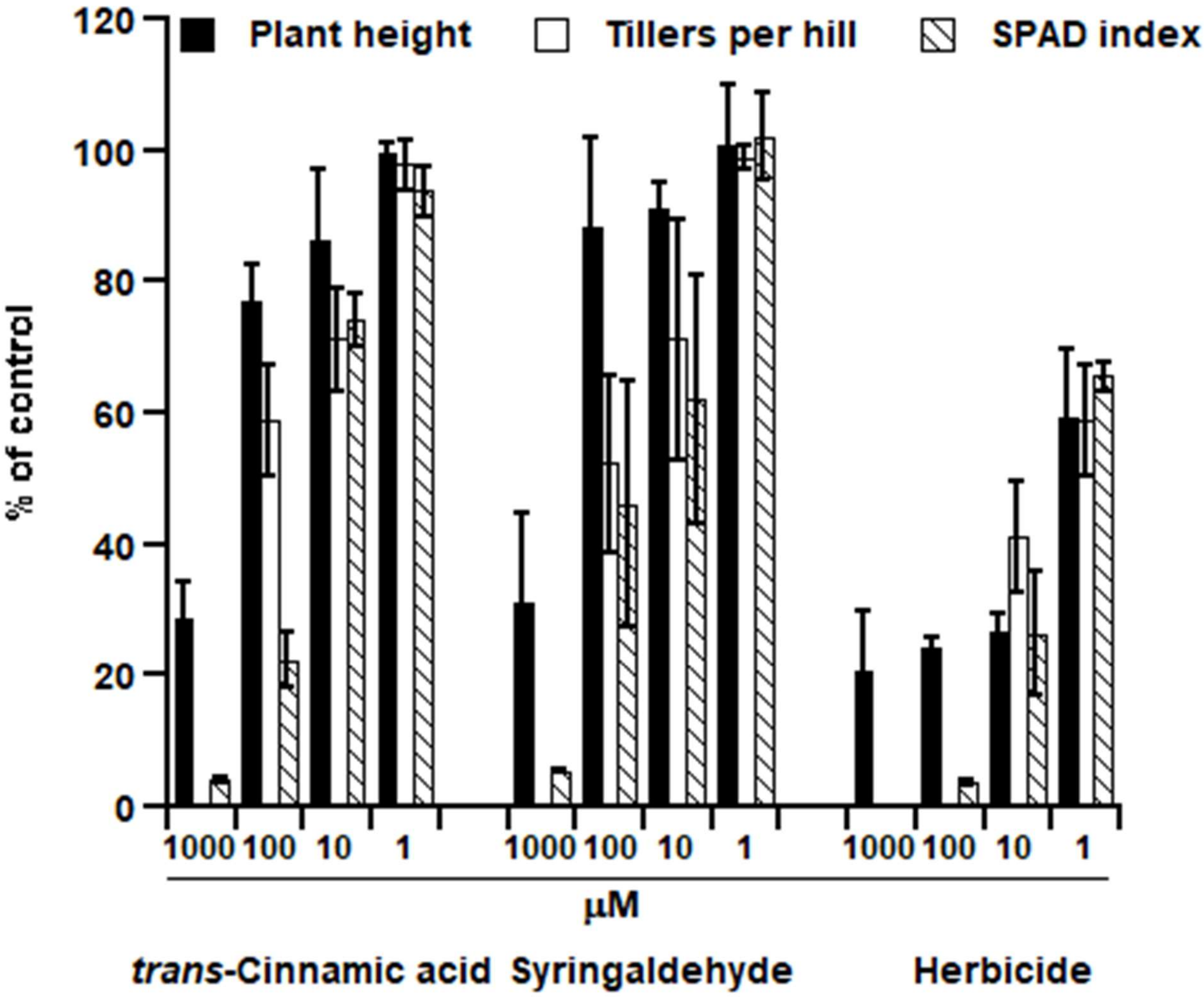


Figure 38. Effect on the growth parameters (percentage with respect to control) of *E. crus-galli* treated with *trans*-cinnamic acid, syringaldehyde and herbicide at concentrations from 1000 to1 µM in a pot culture bioassay

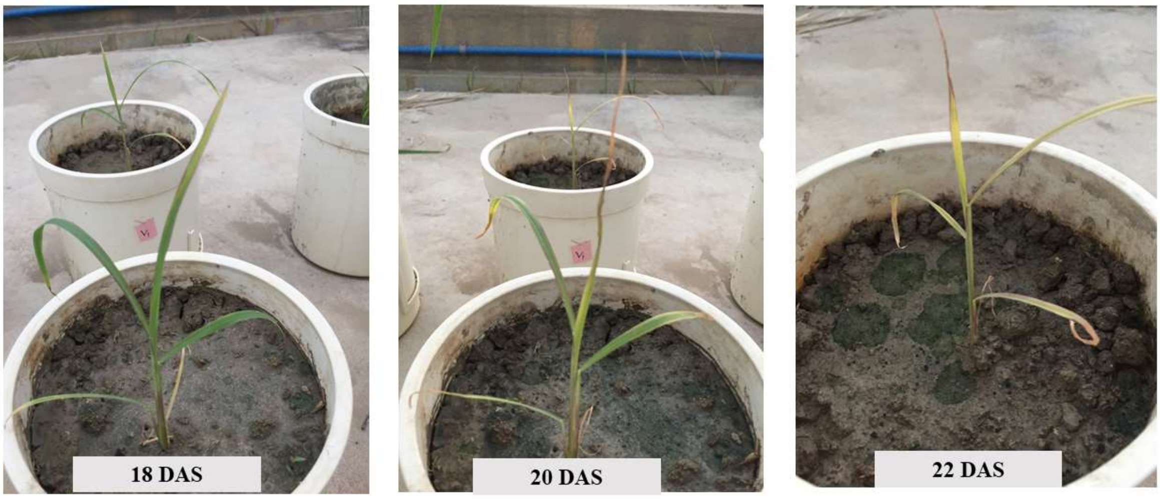


Figure 39. Enhancement phytotoxicity of syringaldehyde at concentrations 1000 µM on seedling growth of *E. crus-galli* in pot culture bioassay

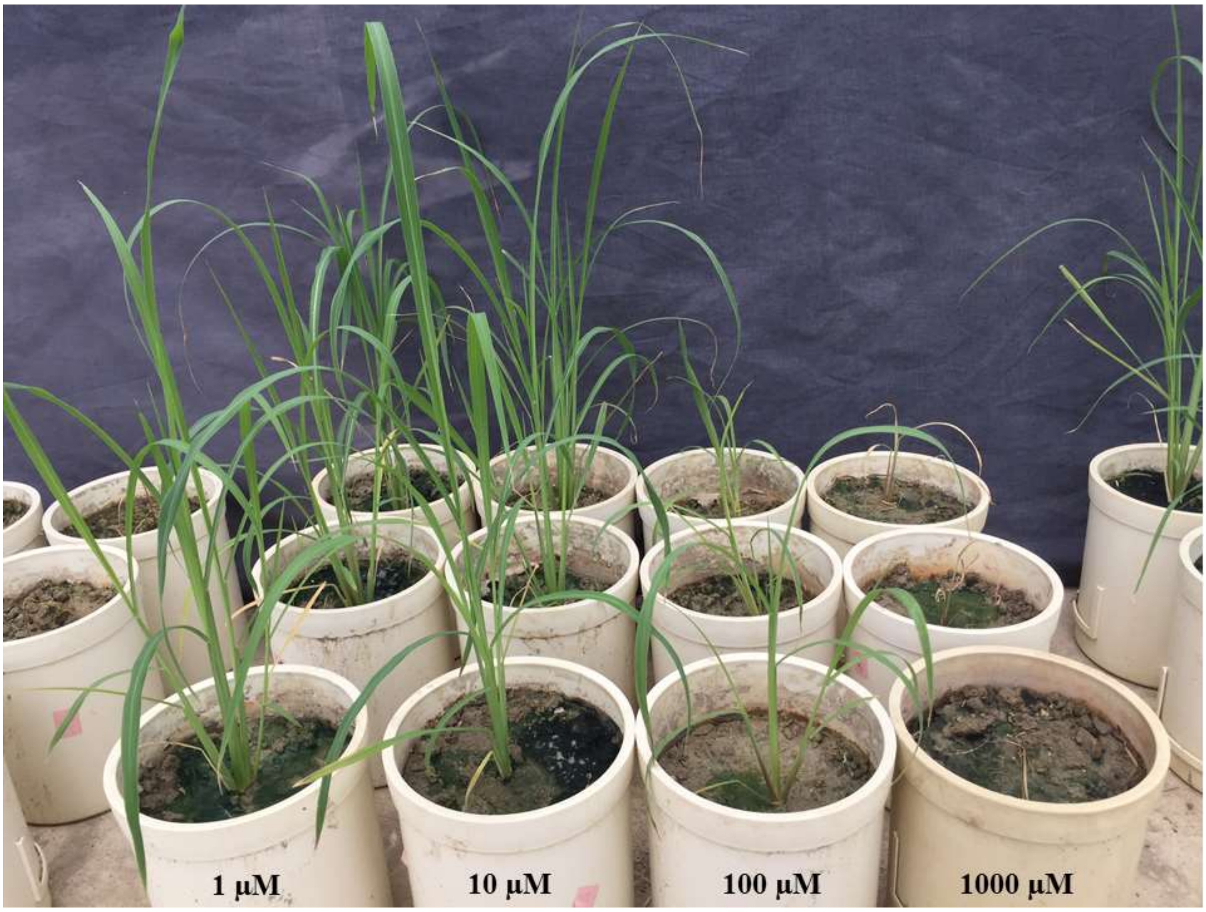


Figure 40. Effect of syringaldehyde at concentrations of from 1 to 1000 µM on seedling growth of *E. crus-galli* at 23 DAS in pot culture bioassay

7.3.6 Effect on chlorophyll content

Seedlings grown in the presence of the allelochemicals showed chlorosis when exposed to more than 100 µM, whereas the herbicide negatively affected the Chl content upon exposure to a concentration above 10 µM (Fig. 41). At the lower concentration (1 µM), syringaldehyde slightly enhanced the Chl content. At the 1000 µM concentration, Chl *a* declined by 56, 49 and 69% compared to the control using *trans*-cinnamic acid, syringaldehyde and herbicide, respectively, whereas the Chl *b* content decreased at a concentration of more than 10 µM for the herbicide treatments only.

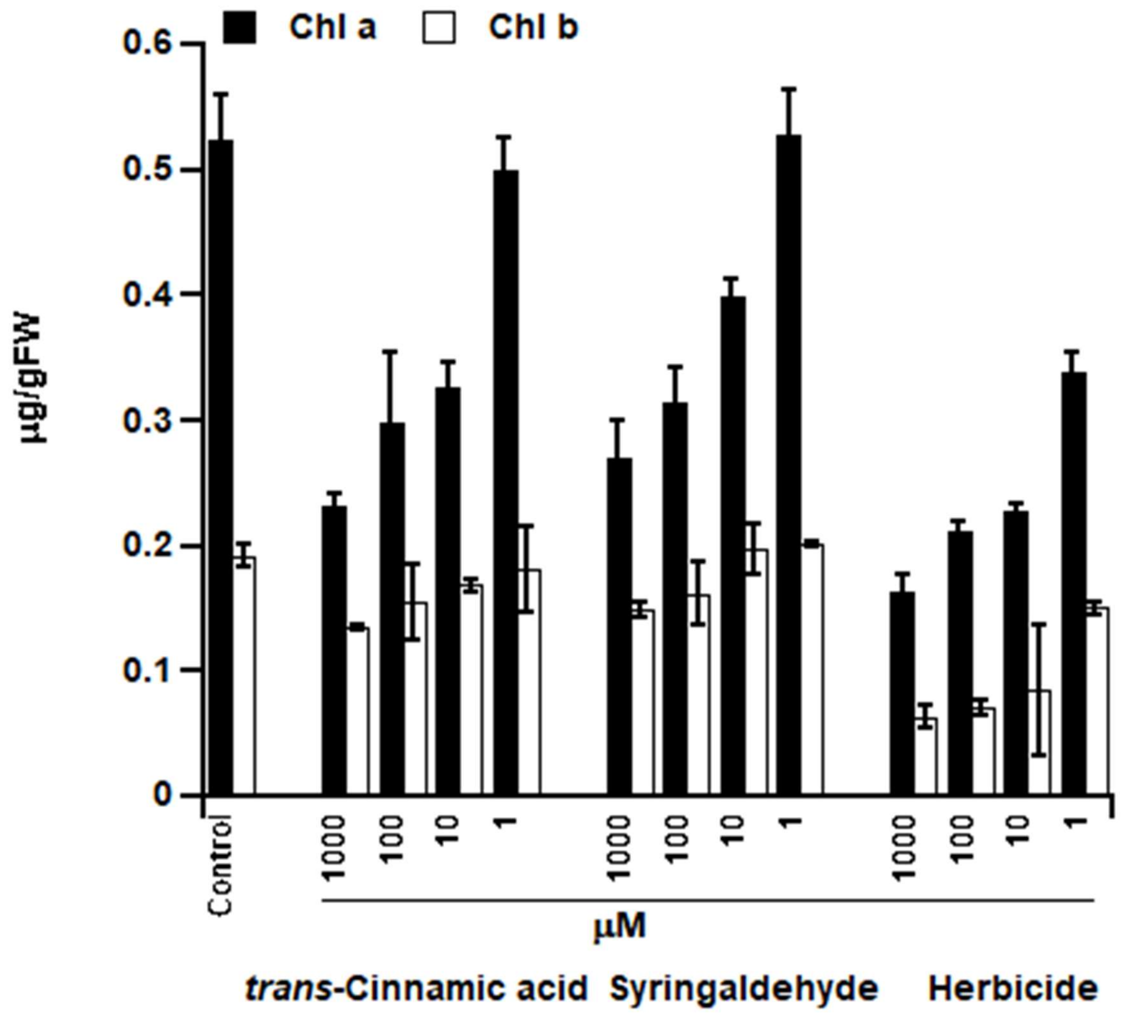


Figure 41. Effect of *trans*-cinnamic acid, syringaldehyde and herbicide at concentrations from 1000 to1 µM on the chlorophylls *a* and *b* contents of *E. crus-galli* in a pot culture bioassay

7.3.7 Effect on Pro content

The effects of the allelochemicals and herbicide on the changes in the Pro content are shown in Fig. 42. Application of the allelochemicals at concentrations of more than 100 µM ameliorated the Pro content compared to the control, and all of the herbicide treatments showed considerable variation compared to control plants.

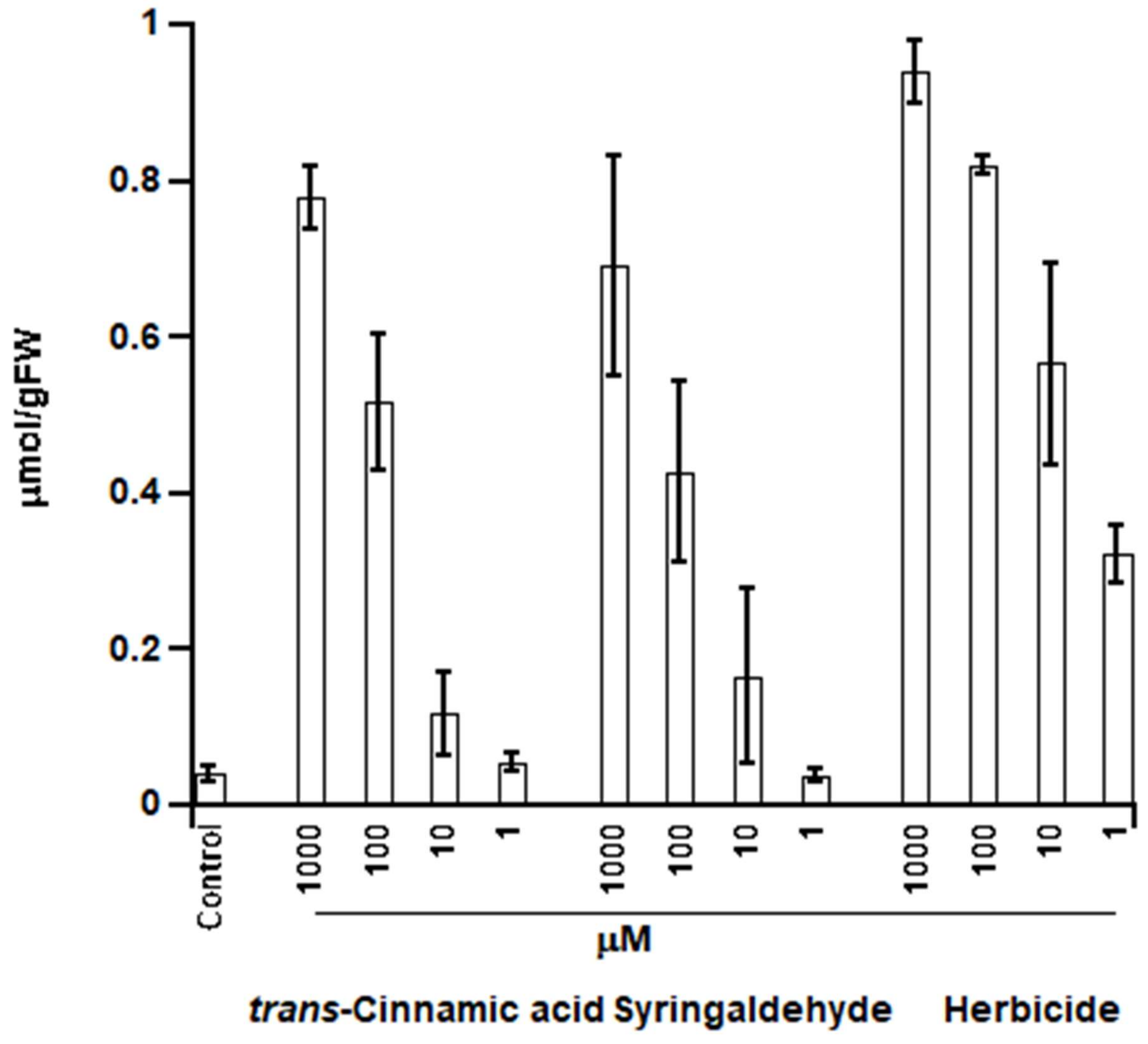


Figure 42. Effect of *trans*-cinnamic acid, syringaldehyde and herbicide at concentrations from 1000 to1 µM on the free proline content of *E. crus-galli* in a pot culture bioassay

7.4 Discussion

The allelopathic potentiality of compounds is often documented by examining their influence on seed germination, seed viability and seedling growth. The methods described in this paper are fast and reliable; hence, these methods may be able to be used in routine bioassays. A set of biochemical, physiological and morphological changes take place in a well-defined sequence during the seed germination process (Bentsinka & Koornneef 2008), which can be interrupted by a biochemical reaction or an excess or deficiency of a compound. Therefore, germination bioassays are effective tools to evaluate the effect of any exogenously applied compound from a natural or artificial source (Hoagland & Williams 2003). Germination of *E. crus-galli* was inhibited by test solutions in a dose-dependent approach. In general, increasing concentrations resulted in a greater reduction of the germination percentage and also influenced the average germination time. The germination pattern (speed and synchrony) was also modified by allelochemical activity. Seed germination inhibited or delayed by allelochemicals has been reported in many plant studies (Gniazdowska & Bogatek 2005; Santana et al. 2006; Reigosa & Pazos-Malvido 2007; Hussain at al. 2008; Grisi et al. 2015; Oliveira et al. 2016). Seedling growth of *E. crus-galli* was affected to a great extent by the allelochemicals compared to seed germination, and the sensitivity of the root was more susceptible compared to that of the shoot because the permeability of the allelochemicals in root tissue is greater than in shoot tissue and root absorbs the allelochemicals first (Nishida et al. 2005). A similar pattern of growth and development inhibition was also reported by Escudero et al. (2000). The without-nutrients condition inhibitory effects on seedling growth were very apparent, and the with-nutrients condition required a higher concentration to inhibit growth. Reigosa and Pazos-Malvido (2007) also found similar results and explained that this result may be due to the synergic effect of nutrient limitation with phytotoxicity. Belz and Hurle (2004) also observed that nutrient limitation increased the inhibition activities of allelochemicals. The phytotoxic effect of allelochemicals on the bioassay species was evident by lamina wilting, chlorosis and necrosis. *trans*-Cinnamic acid and syringaldehyde may inhibit Chl biosynthesis, thereby causing retardation of the growth of the weed. This finding was in agreement with the findings of Sanchez-Moreiras and Reigosa (2005), who reported inhibitory effects of BOA on *Lactuca sativa* L plants. In this study, the allelochemicals slowed or stopped the growth of *E. crus-galli* seedling roots and suppressed the growth of root hairs dose-dependently. The root anatomy study demonstrated that there was contraction or reduction of root pith cells as well as fewer and larger vacuoles of root meristem. Similarly, widened and shortened root cells, damaged cell walls, an increase in both the size and number of vacuoles, cell autophagy, disorganization of organelles, reduced intercellular communication and inhibited formation of root hairs by allelochemicals have been found in many plant studies (Liu & Lovett 1993; Kaur et al. 2005; Grana et al. 2013). It is often proposed that allelochemicals reduce cell division in the apical meristem (Sanchez-Moreiras et al. 2008) and strongly inhibit mitosis and/or disrupt organelle structure, e.g., of the nuclei and mitochondria (Gniazdowska & Bogatek 2005). The reduction of *E. crus-galli* height and biomass in the greenhouse experiment indicated the inhibition potentiality of the allelochemicals as these compounds directly affect many physiological and biochemical reactions and therefore influence growth (Weir et al. 2004; Gniazdowska & Bogatek 2005; Lara-Nunez et al. 2006). Uddin et al. (2012) also observed burning and growth inhibition at 2-3 days after treatment with sorgoleone in sensitive species (*Rumex japonicas* Houttuyn, *Galium spurium* L. and *Aeschynomene indica* L.). Among the physiological effects caused by the allelochemicals, disturbance of photosynthesis is frequently observed (Gniazdowska & Bogatek 2005). Chlorophylls are the base component of pigment protein complexes, which are essential for photosynthesis. Any changes in the chlorophyll content are expected to bring about changes in photosynthesis (Reigosa et al. 2006). Because plant dry matter production depends on the Chl content (Buttery & Buzzell 1977), any diminution of the leaf Chl content would limit net photosynthesis and thus reduce total plant growth. Therefore, precise determination of Chl *a* and Chl *b* can provide a scientific basis for the plant growth state as they play a significant role in the plant growth process and are the key points of implementing accurate agriculture (Dong et al. 2008). In allelochemical-treated plants allelochemicals may act in three ways: inhibit Chl synthesis, stimulate Chl degradation, or both (Zhou & Yu 2006). The Chl content of *E. crus-galli* was dependent and dramatically affected by the allelochemical concentration. In our study, we found that lower concentrations of *trans*-cinnamic acid and syringaldehyde stimulated the Chl content, whereas higher concentrations produced inverse effects. Baziramakenga et al. (1994) also reported that high concentrations of allelochemicals (benzoic acid and *trans*-cinnamic acid) caused a reduction in the leaf Chl content of soybean, whereas a lower concentration promoted it. Meazza et al. (2002) found that allelochemicals reduce the key enzyme of the receiver for plastoquinone synthesis of *p*-hydroxyphenylpyruvate dioxygenase (HPPD). Inhibition of this enzyme interrupts the biosynthesis of carotenoids and results in foliar bleaching. Phytotoxic effects of allelochemicals are termed 'allelochemical stress' (Lara-Nunez et al. 2006). Allelochemicals can induce accumulation and increase synthesis of compatible osmolytes as stress proteins, such as Pro (Durian-Serantes et al. 2002). Pro accumulation could be due to de novo synthesis, decreased degradation or both (Lattanzio et al. 2009). As our results were well-correlated with the results of growth and photosynthesis inhibition, we proposed to use Pro as a stress indicator to measure the effects produced by the allelochemicals and observed that Pro increased with the increasing concentration of allelochemicals, which may mitigate the deleterious effect of stress in *E. crus-galli* seedlings. This could be due to the generation of specific proteins in response to the oxidative damage caused by allelochemical stress (Mishra et al. 2006; Araniti et. al. 2017). Djanaguiraman et al. (2005) reported that allelochemicals from *Eucalyptus* sp. leaves have an increased Pro content in receivers. Thapar and Singh (2006) also noted an induction of the Pro content in the leaves of *Parthenium hysterophorus* treated by leachate leaves of *Cassia tora*. Similar findings were also reported by Reigosa et al. (2001) and Durian-Serantes et al. (2002).

CONCLUSION

The overall observation of the germination and growth reduction in the test weed species at high concentrations of allelochemicals was inconsistent with the control treatments and provides support for the hypothesis that there is the allelochemicals cause a chemical interference, and in most cases, the results demonstrate the concentration-dependent phytotoxicity concept. Therefore, these studies may provide an understanding of allelochemical interactions and may help to distinguish the mechanisms involved in plant interference. In general, allelochemicals are less active than commercial herbicides, but they can be naturally released in crop fields through the development of allelopathic varieties of crops for weed management. Our results also confirm the phytotoxicity of syringaldehyde. However, the suppressive ability of syringaldehyde should be tested in other weeds as well.

CHAPTER VIII

GENERAL DISCUSSION

Weeds are the biggest barrier to rice production worldwide. In traditional Bangladesh rice production, they are managed by hand weeding. But today's fast industrialization and outsource revenue income in Bangladesh offers the labor force an opportunity to earn more money outside the agricultural sector, and labor is in short supply for hand weeding. So the only existing weed management approach is herbicides and Bangladesh has promptly amplified their use as these are cheapest and most consistent weed management in rice. However, concerns about negative impacts on environmental contamination, development herbicide-resistant weeds, and human health problems, make it necessary to diversify weed management options. Allelopathy is an intricate biological phenomenon that is caused by the action of chemical compounds referred to as allelochemicals, determines the dynamics of plant species in different environments in which understanding could help to develop applications in both natural and agricultural systems (Rice 1984). Rice with an allelopathic potential has been reported from various parts of the world. Growing rice varieties possessing an allelopathic potential can help to suppress the weeds in rice. Similarly, the straw of rice can be applied in rice and other crops as well for achieving a non-herbicidal weed control. Allelochemicals derived from rice can be synthesized for use as natural herbicide. Therefore, the use of allelopathic comportment of the rice crop is one of the new options for sustainable options to achieve sustainable weed management.

Allelopathy governs the dynamics of plant species in different surroundings. Understanding this natural phenomenon could help to advance applications in agricultural systems. The main objectives of this research were to find out Bangladesh indigenous allelopathic rice varieties and their significance in weed management by cultivating as the main crop or applying as residues into the soil, and/or focusing the role of allelochemicals as natural herbicides. A set of outstanding and reliable methods (Wu et al. 2000b; Kato-Noguchi et al. 2002; Reigosa & Pazos-Malvido 2007; Salam et al. 2009; He et al. 2012; Asaduzzaman et al. 2014a;), and latest and advanced technologies (**S**ampietro et al. 2009) were used by maintaining scientific standards to solidify results.

In this research 50 Bangladeshi indigenous rice (Indica type) were collected from different districts of Bangladesh to assess the allelopathic potential in laboratory, glasshouse, and field experiments at Subtropical Field Science Center, University of the Ryukyus, Japan. Initially, four varieties *viz*., 'Boterswar', 'Goria', 'Biron' and 'Kartiksail' were selected as the highest allelopathic potential varieties by donor-receiver bioassay test against five receiver plant species namely, *Lactuca sativa* L., *Lepidium sativum* L., *Raphanus sativus* L., *Echinochloa crus-galli* L. Beauv. and *Echinochloa colona* L. The 'Boterswar' variety resulted in maximum inhibition of *E. crus-galli* root and shoot growth (72% and 31%, respectively), followed by 'Goria' (69% in root and 25% in shoot), 'Kartiksail' (65% in root and 23% in shoot) and Biron (63% in root and 24% in shoot). Regrettably, some varieties such as 'Bashful chikon', 'Khaiaboro', 'Panbira' etc. stimulated the growth of *E. crus-galli* seedlings. Similarly, in equal compartment agar method (ECAM) the highest root (62%) and shoot (27%) inhibition of *E. crus-galli* was recorded from 'Boterswar' and the stimulating effect was observed by 'Bashful chikon', 'Khaiaboro' and 'Panbira'. Therefore, these results support the plant physiologist Molisch's (1937) views on the allelopathic activity which cover both inhibitory and stimulatory interactions through chemical substances. The significant effects of varieties and test species interactions indicated that there were variations in allelopathic activity among rice varieties which imply that allelopathic activity of

Bangladesh indigenous rice was genotype or variety dependent. This result verifies the findings of Khan et al. (2007a). The short-term co-cultivation of rice varieties with test species and weeds in donor receiver bioassay and ECAM tests, the highest inhibition was found by ‛Boterswar’ (46%) followed by ‛Goria’ (44%), ‛Biron’ (37%) and ‛Kartiksail’ (36%) among 50 indigenous rice varieties of Bangladesh. We compared the previous investigation on the allelopathic activity of Bangladeshi rice by Salam and Kato-Noguchi (2009) who reported the highest inhibitory activity of ‛BR17” rice variety on test species and weeds was 40%. This result suggested that varieties ‛Boterswar’, ‛Goria’, ‛Biron’ and ‛Kartiksail’ may have strong allelopathic activity. Based on this result, 0.3 g fresh rice plant equivalent aqueous methanol extract ml^{-1} of selected varieties were tested on test species and weeds. The extract from ‛Boterswar’ strongly inhibited the root growth of *L. sativa*, *L. sativum*, *R. sativus*, *E. crus-galli*, and *E. colona* by 65, 60, 84, 66 and 27%, respectively, and the shoot growth by 44, 38, 53, 49 and 8%, respectively. The growth of roots of all target species against the rice extracts reduced greatly than that of shoots. Zimdahl and Stachon (1980) also reported that the extracts of allelopathic plants had a more inhibitory effect on root growth than shoot growth which might be due to the first absorb the allelochemicals or autotoxic compounds by roots from the environment. In addition, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue (Escudero et al. 2000; Nishida et al. 2005). Besides, more inhibitory actvity was found on root and shoot growth in dicotyledonous test plants which are consistent with many plant studies (Fujii1992; Hassan et al. 1998; Kim & Shin 1998; Olofsdotter et al. 2002; Weston & Duke 2003; Inderjit 2006; Khanh et al. 2006). The reduction of *E. crus-galli* height and biomass in the glasshouse experiment indicates the allelopathic potential of the rice varieties. The most inhibitory effect was found from

'Boterswar' variety aqueous extract on different growth parameters of *E. crus-galli*. Aliotta et al. (2006) reported the growth inhibition of several weed species due to the aqueous extracts of allelopathic plants. Likewise, inhibitory effects of rice varieties have also been reported by other researchers (Chung et al. 2001; Jung et al. 2004; Asghari et al. 2006; Pheng et al. 2009). Therefore, selection of rice varieties with greater allelopathic potential can be used as a tool in sustainable weed management and might be a way to minimize herbicide use.

Discovery of allelopathic compounds from natural sources has played a major role in the development of organic chemistry. The ecological role of these allelochemicals as herbicides for ecological/organic sustainable agriculture has recently drawn great consideration, due to increasing public anxiety against the use of synthetic (Narwal1 & Sampietro 2009). The results of the current research showed that the inhibitory activity was proportional to the concentrations of the extracts of rice and higher concentration had a stronger inhibitory effect on the germination and seedling growth of *L. sativum* and *E. crus-galli*. Seed germination is extensively used bioassay in allelopathy and studies refer to the use of this bioassay and its general suitability for the determination of allelopathic activity among species (Leather & Einhellig 1986; Inderjit 1995; Romeo & Weidenhamer 1988). Ethyl acetate phase of rice plant extracts completely inhibited germination of *L. sativum*, and *E. crus-galli* germination was 16% with respect to control. Four biologically active compounds, syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), (-)-loliolide, 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one and 3-hydroxy-*β*–ionone, were isolated and identified initially from rice plant as allelochemical where active fractions completely inhibited the germination of *L. sativum.* Biological activity results showed the identified compounds were too active at a very low concentration (10 μM) on the seedling growth of *E. crus-galli*, which should be exposed to them in nature. Similarly, growth inhibitory compounds identified from rice inhibited the growth of *Cyperus difformis*, *C. iria, E. crusgalli, Eclipta prostrata* and *Leptochloa chinensis* weeds associated with rice (Kong et al. 2004a; Macias et al. 2006; Kong 2007b; Yang et al. 2017). These results suggest that allelopathy offers a real promise for practical weed management. The mixture of the four compounds enhanced inhibition greatly, which implies that the four compounds acted synergistically to strongly reduce the growth of test species. Considering the estimated endogenous level and the inhibitory activities, all four compounds might provide a facilitation to rice plants through the inhibition of the growth of adjacent and succeeding weed species. Comparable consequences were also stated by many researchers (Einhellig 1995; Gealy et al. 2000; Chung et al. 2001; Kato-Noguchi et al. 2011).

Allelochemicals primarily consist of secondary metabolites which enter the environment either volatilization, leaf leaching, residue decomposition, and/or root exudation (Birkett et al. 2001). Therefore, laboratory bioassay alone does not sufficiently prove that allelopathy is effective in the field due to the complexity of field interactions and retorts (Inderjit & Weston 2000). However, allelopathy has been demonstrated, in plant communities, to be a factor of ecological significance by influencing plant succession, dominance, climax formation, species diversity, the structure of plant communities and productivity (Whittaker & Feeney 1971; Rice 1984; Chou 1989). Therefore, under no weed control method, six varieties namely, "Boterswar', 'Goria', "Biron' and 'Kartiksail' as most allelopathic, 'Hashikolmi' as weakly allelopathic, and 'Holoi' as non-allelopathic were raised for field study. There were seven weeds *viz*., *E. crus-galli.* var. *oryzicola*, *Brachiara syzigachne*, *Scirpus fluviatilis* A. Gray, *C. difformis*, *E. prostrata* L., *Lindernia dubia* Philcox and *Dopatrium junceum* Hamilt infested rice field in different growing periods of rice. The infestation levels of these seven weeds species were calculated using Simpson's Diversity Index (*SDI*) which, ranged from 0.2 to 0.56. Remarkably, a significant correlation co-efficient (0.87, P <0.001) was obtained from these field data by comparing with the root inhibition (%) from the in vitro bioassay. Varieties 'Boterswar' and 'Biron' were found as the most allelopathic which significantly reduced the vegetative growth and delayed the reproductive organs initiation of weeds that will must reduce seed production, and any reduction in weed vigour is an advantage (Cousens & Mortimer 1995).

The implication of allelopathy to the ecological concept is boundless. Plant interactions such as resource competition and allelopathy play simultaneously in the species performance. Therefore, a rigorous proof is desired that the effect of this variety in the field on weeds is not because of its competition effects but only due to its allelopathic influence. Competitive index analysis from glasshouse hydroponic experiments showed that 'Boterswar' was a stronger competitive potential against *E. oryzicola* than 'Hashikolmi' which ultimately gave facilitation for 'Boterswar' but competition for 'Hashikolmi' in plant-plant interaction. In that event, allelopathy provides plants with an advantage for competing in plat-plant interaction (Singh et al. 1999; Bruin & Dicke 2001; Fitter 2003; He et al. 2012; Gioria & Osborne 2014). The allelopathic effect was the absolutely leading factor in 'Boterswar'- *E. oryzicola* interactions and had more powerful interaction with *E. oryzicola* than 'Hashikolmi' which suggests that 'Boterswar' had strong allelopathic potential. The exudates solution from 'Boterswar'/*E. oryzicola* mixed cultures had great inhibition effects on *E. oryzicola* growth which confirmed allelopathy by excluding possible complexity in agroecology where the allelopathic effect of

'Boterswar' was much bigger than its resource competition on *E. oryzicola* growth. Inhibitions also found on the nutrient content of *E. oryzicola* in the same order of magnitude due to putative allelochemicals as exudates in 'Boterswar'/*E. oryzicola* mixed culture. This result is accomplished with Muller (1966) that tiny extents of allelochemicals may be responsible for vast decreases in plant growth and in water or mineral absorptions.

A huge opportunity is available to use allelopathic rice residues as mulching and/or incorporation into the soil or as a source of bioherbicides. The phytotoxicity ascribed to the application of allelopathic rice 'Goria' straw into the soil was investigated. It was found that the phytotoxic magnitudes were the utmost @ 1.5 to 2 t ha^{-1} incorporation in which significantly induced inhibitory effects on the growth and dry matter of *E. oryzicola*. Therefore, if the allelopathic rice straw incorporated to the soil, a greater decrease of weeds might be achieved (Inderjit 2001; Xuan & Tsuzuki 2001). Beneficially, an expectant improvement was found on the growth and yield parameters of rice due to the straw incorporation into the soil which renounces autotoxicity effect against rice plant. Moreover, it returned plant nutrients in the soil. These results are consistent with Xuan et al. (2005), and Dobermann and Fairhurst (2002) who reported benefits of rice straw incorporation into the soil. The aqueous methanol extract test of 'Goria' straw showed substantial inhibitions on the root and shoot growth of *L. sativum* and *E. oryzicola*. Successively ethyl acetate phase of aqueous methanol extract of 'Goria' straw (2.4 kg) yielded two biologically active compounds, (-)-loliolide (3.8 mg) and 3*β*-hydroxy-5*α*,6*α*-epoxy-7-megastigmen-9-one (1.5 mg) through several chromatographic steps and spectroscopic analysis. The concentrations causing approximately 50% growth inhibition in the assay (defined as I_{50}) for *E. oryzicola* roots and shoots were 64.62 and 162.92, 43.28

and 137.64, and 2.31 and 17.86 μM for (-)-loliolide, 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one and their mixture, respectively. Proportionately, a magnitude concentration of phytotoxins will be achieved in soil water if this allelopathic rice straw is incorporated, which will give a complete or significant inhibition on the growth of rice weeds (Xuan et al. 2005). Moreover, allelopathic rice straw gives another opportunity as a source of phytotoxins by which bioherbicides could be developed as these isolated allelochemicals were very inhibitive to rice weed.

Modern agricultural weed management practices rely intensively on the use of herbicides. Despite the effective weed control attained with synthetic, many weed species eventually grew resistance to some of these compounds. Natural products have truthfully been a valuable basis of many pesticides, used either directly as crude preparations, as pure compounds, or as structural leads for the discovery and development of natural product-based pesticides (Dayan 2002). However, the probable benefits of natural product-based herbicides remain undervalued (Dayan & Duke 2006). The comparison study of this research on allelochemicals (*trans*-cinnamic acid and syringaldehyde) and a herbicide ('Nominee' a.i. 100 g/L bispyribac-sodium) showed that syringaldehyde inhibited germination of the *E. crus-galli* by delaying and affecting the seed germination indices. The bioassay species grown in a concentration of more than 100 μM syringaldehyde demonstrated stronger inhibition effects on root and shoot growth in both with- and without-nutrients media. It was also observed that test species were considerably smaller compared to the control plants in growth chamber bioassay and demonstrated leaf blade wilting, chlorosis and necrosis as well as inhibited quantity of roots, root and root hair growth. Root anatomy depicted a significant contraction or reduction of root pith cells, and fewer and larger vacuoles compared to the control at the highest concentration (1000 μM) and these symptoms are a good candidate for allelochemical interactions (Lotina-Hennsen et al. 2006). There are many other studies of allelochemicals that are found allelopathic compounds had significant inhibitory effects on growth, physiology and biochemical content of test species and have been found later to be much more active on other metabolic processes (Meazza et al. 2002). Therefore, these findings confirm the phytotoxicity of syringaldehyde as well as other identified allelochemicals from Bangladeshi indigenous rice plant and straw, and those can be developed as bioherbicides for future rice weed management strategies.

Bangladesh own a great richness in terms of rice species. This monograph results showed an enormous feasibility for using Bangladesh indigenous rice as weed suppressive or as a source of allelochemicals. The most interesting finding of the present work is identifying Bangladesh indigenous rice variety 'Boterswar' as allelopathic by which effective biologically active allelochemicals were isolated and identified initially from rice. The in vitro bioassay results were also successfully verified by comparing with field performance in terms of weed control, and successfully distinguished allelopathic effects from competition in crop-weed interference. This elite allelopathic rice genotype could be used by breeding efforts to improve weed suppression traits in commercial varieties. Similarly, the results showed the opportunities for achieving a non-herbicidal weed control as well as improving soil health by using allelopathic rice straw. Another interesting finding was justifying allelochemical as bioherbicide by which significance in nature of allelochemicals was found and attributed the constant need for new chemistries and new target sites. Therefore, this Ph.D. projects will be very beneficial for the resource-poor farmers of Bangladesh as well as for the researchers who work for the development of the environmentally friendly weed management options. Furthermore, it will offer a better understanding of the communication networks that are allelochemically interceded between different species.

Apparently, this is a currently underexplored line of research. Therefore, studies on the fate of allelochemicals in the environment with relation to soil microorganism activities are required, to identify the retention, degradation and transport mechanisms of the compounds in the environment, which are determinants of their persistence in the soil and their efficacy on target weeds. The desired expansion of allelopathic knowledge might be useful to improve rice production systems. Consequently, improved agricultural productivity would boost food production and help to discourse the world's food demand.

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ACKNOWLEDGEMENT

Alhamdulillah, all praises are due to the almighty **Allah Rabbul Al-Ameen** for **His** gracious kindness and infinite mercy in all the endeavors the author to let him perform higher education and to successfully complete the research work as well as to submit this dissertation for the degree of Doctor of Philosophy. I am greatly acknowledged to the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, for providing a scholarship which had given me the opportunity to carry out doctoral course in Japan. I am also greatly acknowledged to the Bangladeshi government for giving permission on study leave.

I would like to express deepest sense of gratitude, gratefulness, sincere appreciation and profound regards to my major advisor Professor Dr. Md. Amzad HOSSAIN, vice advisors Professor Dr. Hikaru AKAMINE and Professor Dr. Jun-Ichi SAKAGAMI, the United Graduate School of Agricultural Sciences Kagoshima University, Japan for their scholastic guidance and constructive criticism throughout the research work and during producing this dissertation.

Likewise, grateful appreciation is conveyed to Professor Dr. Takahiro ISHII for his affectionate encouragement and kind cooperation in characterization and determination of the allelopathic compounds, which were reported in the dissertation. With profound regard, I also would like to Thank Professor Dr. Takara KENSAKU and Professor Dr. Toshihiro KONNO for their valuable suggestions and hands-on training on laboratory procedures and microscope operations. I would like to extend my Thanks to Professor Dr. Ichiro NAKAMURA for his kind cooperation for giving me an opportunity to grow rice in the field. I would like to thanks all the Professors, Officers and Technicians of Sub Tropical Field Science Center, Faculty of Agriculture, and Center for Research Advancement and Collaboration, University of the Ryukyus, Japan.

I gratefully remember the help and cooperation of my tutor Makiko Furuhashi, and my friends Rikiya Uchida, Rika Miyagi, Chisei Tanaka, Christal Burnette, Doi, and so on. Special thanks to all other friends and lab mates for their assistance and cooperation throughout the study period. Special thanks to Bangladeshi Community in Okinawa, Japan for their support and encouragement to complete this study. I extent special thanks to "Okinawa Muslim community" for offering me a favorable environment to practice my religion properly.

I would like to express my gratitude to Professor Dr. Md. Hazrat Ali for his endless encouragement and prayer during my staying in Japan. I would like to thank all of my Teachers, colleagues, officials, technicians and student of Sher-e-Bangla Agricultural University, Bangladesh. I would like to give thanks to Dr. Mirza Hasanuzzaman, Dr. Md. Sirajul Islam Khan, Abdul Muktadir Himu, Md. Hasanuzzaman, Dr. Zohurul Islam and Md. Kamrul Islam Himel for supporting in my study.

Last but not the least, I gratefully acknowledge with regards and deepest sense of gratitude to my Father, Mother, wife, sisters, brothers-in-law and all of my family members, relatives and friends for their sacrifice, continuous mental support and keeps me always cheerful.

Sheikh Muhammad Masum