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RESEARCH ARTICLE

Effects of *Tinospora tuberculata* leaf methanol extract on seedling growth of rice and associated weed species in hydroponic culture

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Abstract

The study was conducted to evaluate the responses of rice and rice weed seedlings (barnyardgrass and weedy rice) at the three-leaf stage to *Tinospora tuberculata* leaf methanol extract (3.12, 6.25 and 12.5 g L^{-1}) under hydroponic culture. It shows that the leaf methanol extract had various degrees effects depending on target plant species and each tested index (biomass, root length, shoot length, transpiration volume, chlorophyll *a*, chlorophyll *b* and carotenoid contents). The effective concentration of the leaf extract capable of reducing 50% of rice growth was higher than those of target weed species. Moreover, the root length was more tolerant to leaf methanol extract in comparison to the other plant parameters measured. A greater reduction was observed in chlorophyll *a* content compared to chlorophyll *b* and carotenoid. The results revealed that the reduction of transpiration volume closely coincided with the magnitude of growth inhibition of tested plants. Ultra-fast liquid chromatography analysis revealed 11 of 32 peaks in chemical profile, including benzoic acid, caffeic acid, chlorogenic acid, isoorientin, isovitexin, orientin, *p*-anisic acid, syringic acid, *trans*-cinnamic acid, *trans*-ferulic acid, and vitexin have the same retention time with those peaks of the extract. The amount of compounds was present in the range of between 4 817 and 115.5 mg kg^{-1} dry weight (DW). The concentration-response bioassay of all 11 individual compounds and their equimolar mixture against the seeds of barnyardgrass revealed their contribution in the allelopahic activity of *T. tuberculata* leaf extract. The examined compounds and their combination exhibited various degrees of growth inhibitory effects on the early growth of barnyardgrass. Therefore, the specific number, concentration, combination and inhibitory activity of bioactive compounds leads to allelopathy activity of *T. tuberculata* leaves which could be employable directly as a natural herbicide and its growth inhibitor compounds can be used as a template for producing new herbicides.

Keywords: allelochemicals, identification and quantification, natural herbicide, UFLC, weed control

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1. Introduction

Rice (*Oryzas ativa* L.) is the third most important crop in Malaysia and most widely consumed food for more than 557 million people in Southeast Asia (IRRI 2009). Global

rice yield needs to enhance 0.6 t ha^{-1} over the next decade to feed growing populations (Seck *et al.* 2012). Weed infestations are recognized as a serious biological constraint to rice production in both lowland and upland ecosystems (Saito 2010). Weed growth has been recognized to reduce grain yield of rice by 16–100%, depending on the method of rice establishment and the season (Rashid *et al.* 2012). Thus, weed management plays an important role in the global rice production.

The utilization of herbicides has increased over the recent years due to the high cost of labor for weed removal and the lack of appropriate weed control alternatives (Brethour and Weersink 2001). However, the constant use of chemical herbicides has led to the resistance development in weed species. It also has negative effects on both the environment and human (Beltran *et al.* 2012; Mahajan and Chauhan 2013). Hence, it is essential to replace agrochemicals with natural products to achieve sustainable agriculture. In this relation, scientists have tried to invert the trend of reliance on chemical weed managements through developing effective natural herbicides (Kato-Noguchi *et al.* 2013; Araniti *et al.* 2014b).

Allelopathy exploitation is one of the alternative methods. Allelopathy is a very sophisticated mechanism of plant defense. It has been defined as any direct or indirect effect of one plant on the survival, growth, and reproduction of another (Zeng 2014). Allelopathy can be employed as environmentally sustainable methods for weed management in sustainable agriculture systems (Tesio *et al.* 2011).

Many studies have been conducted using allelopathic plants for managing weed species. Irrigation with aqueous extracts of *Inula crithmoïdes* leaves caused a total inhibition of 27, 18 and 67% for the seedling length of lettuce, radish and thistle, respectively (Omezzine *et al.* 2011). Allelopathy activity of four medicinal plants (*Passiflora incarnate*, *Nerium oleander*, *Helianthus tuberosus*, and *Alocasia cucullata*) significantly suppressed the growth of rice weeds (Khanh *et al.* 2005). Similarly, *Ageratum conyzoides* and *Melia azedarach* as medicinal plants promoted the growth and yield of rice and greatly reduced the growth of weed species (Hong *et al.* 2004). Batish *et al.* (2007a) also observed significant adverse effects of *Tagetes minuta* on the emergence and growth of the rice field weeds. Application of *Anisomeles indica* as an allelopathic plant for controlling weed species in wheat field inhibited the emergence and growth of weeds as same as chemical herbicide (Batish *et al.* 2007b).

Tinospora tuberculata (*Tinospora rumphii* Boerl or *Tinospora crispa*), Malay name Batawali, belongs to the family Menispermaceae, and is a traditional medicinal plant (Forman 1981). It is a plant that grows in primary rainforests in all parts of Malaysia, Thailand and Indonesia. There are a few studies that dealt with allelopathic activity of *T. tuberculata* (Pukclai and Kato-Noguchi 2012; Aslani *et al.* 2013; Aslani *et al.* 2014). They observed the inhibitory activity of *T. tuberculata* against the germination and initial growth of crops and weed species.

The current study was performed to (i) determine the allelopathic potential of *T. tuberculata* leaf methanol extract on the growth and development of the rice (*Oryza sativa* L. *var*. MR219) and two common rice weeds, barnyardgrass (*Echinochloa crus-galli*) and weedy rice (*Oryza sativa*), at three-leaf stage under hydroponic conditions; and (ii) evaluate the role of phytotoxic compounds on the allelopathic activity of extract obtained from leaves of *T. tuberculata*.

2. Materials and methods

2.1. Test materials

T. tuberculata plants growing in the Herbal Garden of University Putra Malaysia, Selangor, Malaysia, was used. *T. tuberculata* leaves were cleaned several times with tap water and air-dried for 3 weeks, then ground to a fine powder in a laboratory blender and stored in a refrigerator at 4°C until use.

Commercial seeds of barnyardgrass (*Echinochloa crusgalli*) and weedy rice (*O. sativa*) were purchased from Herbiseed company (London, United Kingdom). Barnyardgrass seed coats were loosed by hand, one by one to improve the germination percentage. The seeds of rice (*O. sativa* L. *var*. MR 219) were manually collected in the rice field, Universiti Putra Malaysia. Rice and weedy rice seeds were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with distilled water. The rice seeds were cleaned, air-dried and all seeds stored in darkened, airtight containers at –18°C prior to their use. The germination of the seeds was randomly checked and was ~86–95%.

Syringic acid (98%), *trans*-ferulic Acid (99+%), *trans*-cinnamic acid (99+%), *p*-anisic acid (99+%), chlorogenic acid (from Eucommial leaf, 98%), vanillic acid (99%), coumarin (98%), gallic acid (99%), caffeic acid (99%) and benzoic acid (99.5%) were supplied from Chemtron Biotechnology Sdn. Bhd (Kuala Lumpur, Malaysia). Vitexin, isovitexin (98+%), orientin (97+%) and isoorientin (98+%) were obtained from Sigma-Aldrich (Kuala Lumpur, Malaysia). Methanol and acetic acid of HPLC grade were obtained from Friendemann (Kuala Lumpur, Malaysia). Water was twice distilled and all chemicals used were HPLC grade.

2.2. Application of leaf methanol extract under hydroponic culture (Experiment 1)

The experiment was conducted to determine the allelopathic