

## NOTE

# ALLELOPATHIC ACTIVITY AND ALLELOPATHIC SUBSTANCE IN JACKFRUIT LEAVES

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**KATO-NOGUCHI H & TAKAMI Y. 2015. Allelopathic activity and allelopathic substance in jackfruit leaves.** Jackfruit (*Artocarpus heterophyllus*) has traditionally been used in agroforestry system. However, there is very limited information on the allelopathic property of the species. Therefore, we investigated possible allelopathic activity and allelopathic active substances in jackfruit leaves. An aqueous methanol extract of jackfruit leaves inhibited the growth of roots and hypocotyls/coleoptiles of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), timothy (*Phleum pratense*), ryegrass (*Lolium multiflorum*) and *Echinochloa crus-galli* seedlings. The extract was purified and a main allelopathic active substance was isolated. These results suggest that jackfruit may have allelopathic property and contain an allelopathic active substance. Jackfruit leaves may be useful as soil additive materials to control weeds for sustainable agriculture.

Keywords: Agroforestry, allelopathy, *Artocarpus heterophyllus*, bioactive compound, weed control

Jackfruit (*Artocarpus heterophyllus*), originating from South Asia, belongs to the Moraceae family and is widely cultivated in tropical lowland areas as fruit crop and timber tree (Thomas 1980, Pradeepkumar et al. 2008, Khan et al. 2010). The species has traditionally been used in agroforestry systems for agriculture production, whereby crops are raised under the tree canopies and within shady environment (Hasan et al. 2008). Biodiversity in agroforestry systems is typically higher than that in conventional agricultural systems and the system creates a more complex habitat for a variety of birds, insects and other animals (Tschardt et al. 2012). In addition, the jackfruit–pineapple agroforestry system increased crop productions and reduced the damage of crops caused by insects and herbivorous animals (Hasan et al. 2008). Therefore, the agroforestry system is one of the options to develop sustainable agriculture (Tschardt et al. 2012).

During the development of sustainable agriculture systems, some plants were found to provide excellent weed control ability in intercropping and/or as soil additives due to their allelopathic property (Weston 1996, Semidey 1999, Caamal-Maldonado et al. 2001). Plants

produce hundreds of secondary metabolites, some of which have allelopathic activity. These substances are released into neighbouring environments through volatilisation, root exudation, leaching and decomposition of plants, and inhibited the growth of neighbouring plants (Duke et al. 2000, Field et al. 2006, Belz 2007). Therefore, allelopathy of plants is useful for weed management options in several agriculture settings to reduce dependency on commercial herbicide (Putnam 1988, Weston 1996, Narwal 1999). The interaction between jackfruit and crops in an agroforestry system was evaluated in terms of allelopathy (Kumar et al. 2008). Thus, the information on allelopathy is important for the development of sustainable agriculture in jackfruit agroforestry system. However, there is very limited information on the allelopathy of jackfruit. Only allelopathic activity of leaf leachates of jackfruit against vegetables was reported (John et al. 2007). The objective of this study was to investigate the allelopathic property of extracts of jackfruit leaves on three plants and three weed species, and isolation of a main allelopathic active substance in jackfruit leaves.

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Leaves of jackfruit (*A. heterophyllum* cv. BARI Jackfruit-1) were collected from Bangladesh Agricultural Research Institute (Gajipur, Bangladesh) in 2010 and dried in the sun. The seeds of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*) were chosen as test plants for bioassay due to their known seedling growth behaviour. Weed species, timothy (*Phleum pratense*), ryegrass (*Lolium multiflorum*) and *Echinochloa crus-galli* were also chosen for bioassay. Jackfruit leaves (100 g dry weight) were extracted with 800 mL of 80% (v/v) aqueous methanol for 2 days. After filtration using filter paper, the residue was extracted again with 800 mL of methanol for 2 days and filtered. The two filtrates were combined.

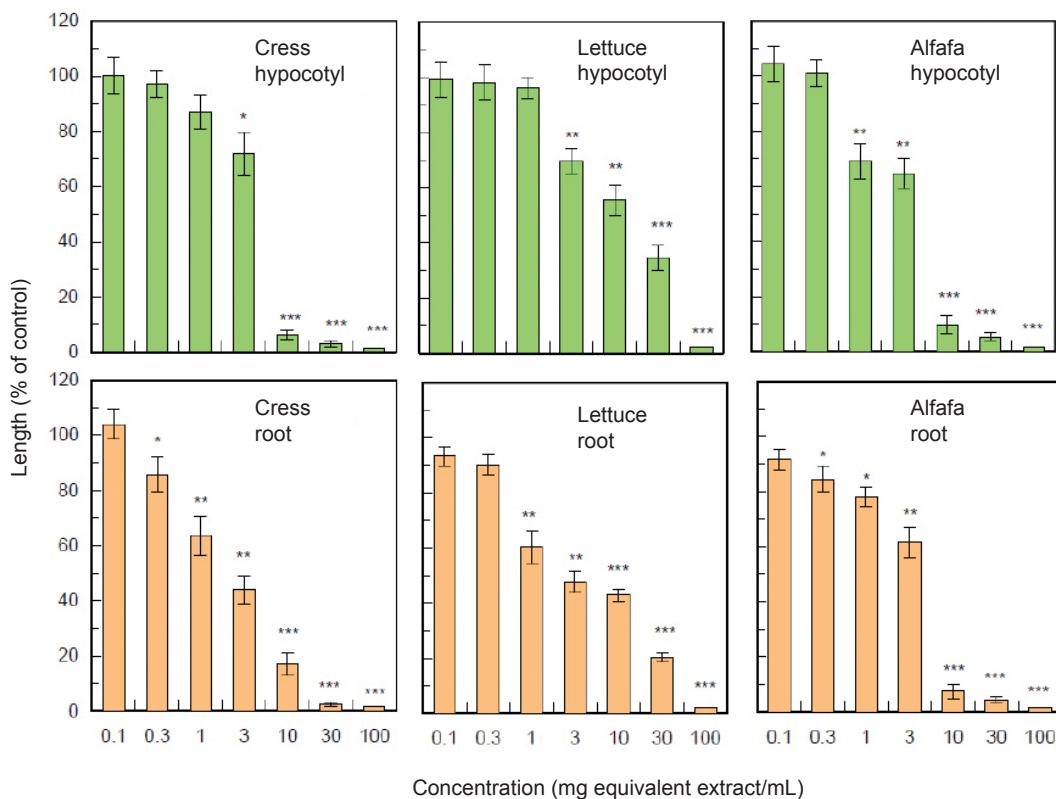
An aliquot of the extract (final assay concentration of tested samples corresponded to the extract obtained from 0.1, 0.3, 1, 3, 10, 30 and 100 mg dry weight of jackfruit leaves per mL) was evaporated to dryness, dissolved in 0.2 mL of methanol and added to a sheet of filter paper in a 3-cm Petri dish. Methanol was evaporated in a fume hood. The filter paper in the Petri dishes was moistened with 0.8 mL of 0.05% (v/v) aqueous solution of polyoxyethylene sorbitan monolaurate (Tween 20). Ten seeds of cress, lettuce or alfalfa, or 10 seedlings of timothy, ryegrass or *E. crus-galli* after germination in the dark at 25 °C for 36–48 hours were placed in the Petri dishes. The length of roots and hypocotyls/coleoptiles of these seedlings were measured after 48 hours of incubation in the dark at 25 °C, and compared with control seedlings. Controls were treated exactly as described above with the exception that 0.2 mL methanol was used instead of jackfruit extract. The bioassay was repeated five times using randomised design with 10 plants for each treatment. Significant differences were examined by Welch's t-test.

Jackfruit leaves were extracted and filtrated as described above. The two filtrates were combined and concentrated at 40 °C in vacuo to produce aqueous residue. The aqueous residue was adjusted to pH 7 with 1 M phosphate buffer, partitioned five times against an equal volume of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and separated on a column of silica gel (100 g, silica gel 60, 70–230 mesh),

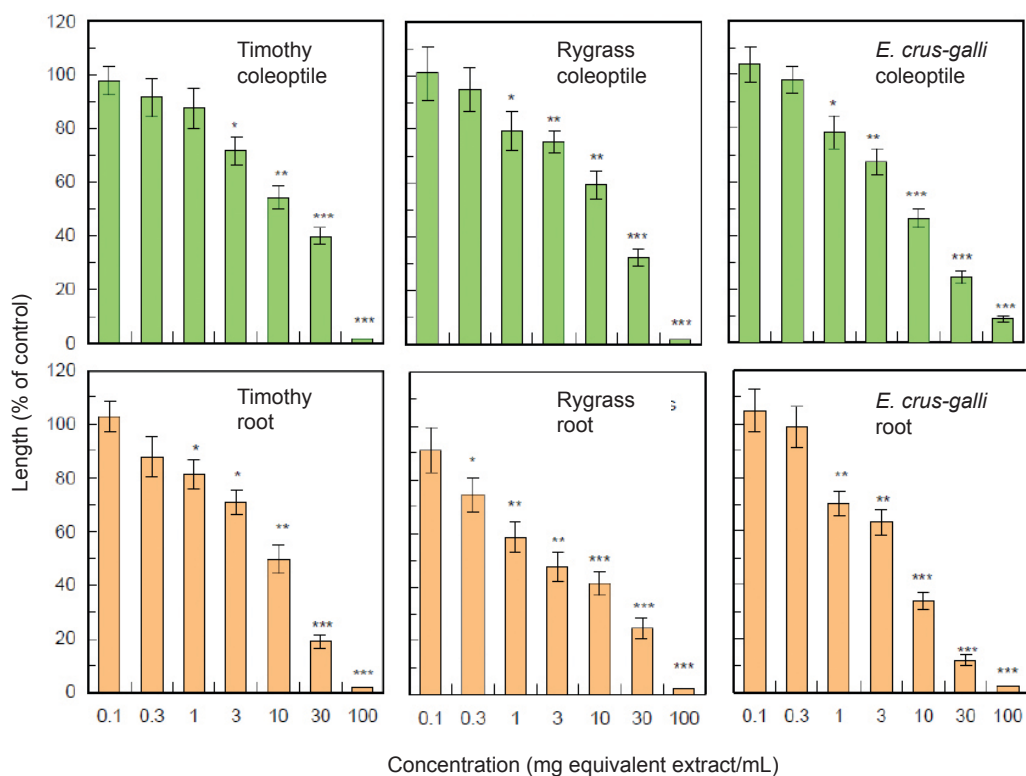
eluted stepwise with *n*-hexane containing increasing amount of ethyl acetate (10% per step, v/v; 100 mL per step) and methanol. The biological activity of the fractions was determined using a cress bioassay as described above. The activity was found in fractions obtained by elution with 80% ethyl acetate in *n*-hexane and evaporated to dryness. The residue was dissolved in 20% (v/v) aqueous methanol (2 mL) and loaded onto reverse-phase C<sub>18</sub> cartridges. The cartridge was eluted with 20, 40, 60 and 80% (v/v) aqueous methanol and methanol (15 mL per step). The active fraction was eluted by 40% aqueous methanol and evaporated to dryness. The residue was finally purified by reverse-phase high performance liquid chromatography eluted at flow rate of 1.5 mL min<sup>-1</sup> with 55% aqueous methanol and detected at 220 nm. Inhibitory activity of all peak fractions was determined by cress bioassay.

The aqueous methanol extract of jackfruit leaves had inhibitory effect on test plant and weed species (Figures 1 and 2). Inhibition was concentration dependent. The extract obtained from 30 mg jackfruit leaves inhibited hypocotyl growth of cress, lettuce and alfalfa by 2.9, 34.5 and 5.4% of control hypocotyl growth respectively, and inhibited root growth of cress, lettuce and alfalfa by 2.5, 20.2 and 4.5% of control root growth respectively (Figure 1). The extract obtained from 30 mg jackfruit leaves inhibited coleoptile growth of timothy, ryegrass and *E. crus-galli* by 39.3, 32.2 and 24.5% of control coleoptile growth, respectively, and inhibited the root growth of timothy, ryegrass and *E. crus-galli* by 19.2, 24.7 and 12.1% of control root growth respectively (Figure 2).

The concentrations required for 50% growth inhibition ( $I_{50}$ ) of jackfruit extracts on roots and hypocotyls/coleoptiles of six plants were determined by logistic regression analysis based on the concentration response bioassay (Table 1). These  $I_{50}$  values indicated that the extracts obtained from 2.2–21.5 mg jackfruit leaves inhibited the growth of hypocotyls/coleoptiles of six plants by 50%. Extracts obtained from 2.9–9.9 mg jackfruit leaves inhibited the growth of roots of these six plants by 50%. These results suggest that jackfruit leaves may possess allelopathic property and contain



**Figure 1** Effects of aqueous methanol extracts of jackfruit leaves on hypocotyl and root growth of plant species; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$



**Figure 2** Effects of aqueous methanol extracts of jackfruit leaves on coleoptile and root growth of weed species; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

**Table 1**  $I_{50}$  values (mg equivalent extract/mL) of jackfruit extracts on hypocotyl/coleoptile and root growth of plant and weed species

| Plant/weed           | Hypocotyl/coleoptile | Root |
|----------------------|----------------------|------|
| Cress                | 2.2                  | 4.5  |
| Lettuce              | 11.3                 | 3.5  |
| Alfalfa              | 3.1                  | 2.9  |
| Timothy              | 17.4                 | 7.1  |
| Rygrass              | 13.7                 | 9.9  |
| <i>E. crus-galli</i> | 21.5                 | 6.4  |

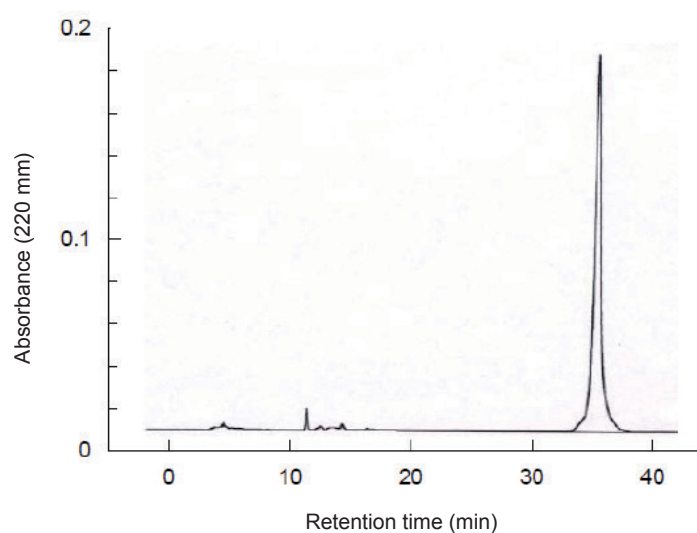
allelopathic active substances. By comparing  $I_{50}$  values, effectiveness of extracts on roots of each plant was greater than that on respective hypocotyls/coleoptiles.

Allelopathic activity was found at a peak fraction between 35 and 36 min (Figure 3). It is the first report of the presence of allelopathic active substance in jackfruit leaves. However, it is necessary to determine the chemical structure of the allelopathic substance. Phytotoxic substances in plants can be released into soil, either as exudates from living plant tissues or by decomposition of plant residues and act as allelopathic substances which inhibit seed germination, seedling establishment and plant growth (Bais et al. 2006, Bonanomi et al. 2006, Belz 2007). Allelopathic active substance in jackfruit leaves may also be released into the soil by exudates and/or by decomposition of plant residues.

As the use of chemicals increases, agricultural weed control alternatives to the present synthetic herbicide-dominated programme are now being given wide consideration. Controlling weeds through allelopathy is one strategy to reduce dependency on synthetic herbicides (Putnam 1988, Narwal 1999, Duke et al. 2000, Macías et al. 2007). It has been shown that certain plant residues and extracts may function as weed suppressive agents (Weston 1996, Semidey 1999, Caamal-Maldonado et al. 2001). It is possible that jackfruit is useful as a weed suppressive residue or soil additive material in a variety of agricultural settings in sustainable agriculture.

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**Figure 3** High performance liquid chromatogram of active substance purified from jackfruit leaves

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