Pericarp of *Trapa natans* var. *bispinosa* (Roxb.) Makino as an organic herbicide

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**ABSTRACT**

A huge amount of crop yield has been lost due to weeds. Modern control strategies including biological techniques are needed to overcome this problem. Recent studies have suggested that allelochemicals can be used to control weeds and the pericarp of *Trapa natans* var. *bispinosa* Roxb. is a rich source of phytochemicals. The presence of weed species in cultivated fields might be the upshot of certain inhibitory substances produced by the crop plants. Therefore the allelopathic effect of ground pericarp of *T. natans* var. *bispinosa* was evaluated against the growth of *Cucumis sativus* L., *Lactuca sativa* L., *Raphanus sativus* L., *Spinacia oleracea* L. and *Triticum aestivum* L. by sandwich method. Three different concentrations (0.01, 0.05 and 0.1 g) of ground pericarp were used as treatments. All of them introvertedly inhibit the germination and growth of all the test plants in dose-dependent manner except *C. sativus* and *T. aestivum*. A higher concentration (i.e 0.1 g) showed more inhibitory effects on the germination and growth of the seeds. The results obtained indicate that the pericarp extract of *T. natans* var. *bispinosa* can be used as a bioherbicide but cannot be introduced to agricultural crops for weed control because of its suppressive growth effects. This application can limit the need for conventional weedicides which remain in soil for longer time as compared to natural compounds.

**Keywords:** Bioherbicide, *Lactuca sativa*, Phytochemicals, Sandwich method, *Trapa natans* var. *bispinosa*.

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INTRODUCTION

The increase of weed density in crop areas is a leading cause of decreasing crop yield. Alternative weed control strategies are required to replace current chemical, mechanical and cultural methods. Allelochemicals inhibiting the growth of weed species, released by growing crops are receiving greater attention for weed control (Leather, 1983). Evidences show the significant effects of allelochemicals on cell division, cell differentiation, ion and water uptake, water status, phytohormone metabolism, respiration, photosynthesis, enzyme function, signal transduction as well as gene expression (Macias et al., 2007; Inderjit and Duke, 2003).

These substances are released into the environment through volatilization, foliar leaching, root exudation, residue decomposition or death and decay of fallen plant parts via biotic and abiotic means or other processes in sufficient quantities (Islam and Kato-Noguchi, 2013; Bisio et al., 2011). The ecological, environmental and health problems possibly associated with synthetic pesticides boost up the interest of developing new classes of environmentally safe herbicides (Islam and Kato-Noguchi, 2013; Gonçalves et al., 2009; Dayan et al., 1999). The researchers have paid much attention to the plants for their inhibitory potential to set it as a tool for sustainable and eco-friendly weed control strategies (Khan et al., 2005). This could be achieved through either using their extracts as bio-herbicides, or isolated active substances.

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Trapa natans var. bispinosa (Roob.) Makino, is commonly known as “Water Chestnut” or “Water Caltrop”, Singhara belonging to the family Trapaceae (Shalabh et al., 2012). T. natans exists in two varieties; T. natans var. quadrissipina L. with four-horned fruit and T. natans var. bispinosa Roxb. with fruit having two stout curved horns (Malik et al., 2012; Crow and Hellquist, 2000; Herklots, 1972). T. natans var. bispinosa is an annual free-floating aquatic herb grown in ponds, marshy lands or shallow water fields in tropical and temperate zones of the world (Takano and Kadono, 2005). Fruit of Trapa ripens in winter season (Singh et al., 2011). Pericarp is thick, hard and jet black; with shape like a horn protruding from the buffalo head (Tulyathan et al., 2005). Its pericarp consists of 44.22% moisture, 37.23% fibre and 2.63% protein (Ciou et al., 2011).

Phytochemical screening of the pericarp extract revealed the presence of tannins, flavonoids, glycosides, alkaloids, saponins, steroids and phenolic compounds (Patel et al., 2010). These secondary metabolites could only be activated during the stress periods, caused by the attack of some microorganisms or by an environment poor in nutrients; representing a real chemical barrier against the attack of pathogens (Stoicescu et al., 2012). According to detailed research of scientists, this pericarp also exhibits anthelmintic, antioxidant, antibacterial and antimicrobial properties (Verma et al., 2013; Stoicescu et al., 2012; Parekh and Chanda, 2007). It is considered that this pericarp can serve beneficial purposes without any side effect (Stoicescu et al., 2012).

Phytochemicals directly inhibit or stimulate germination, growth and development of the neighbouring plant species near their vicinity and even the same plant itself (Kato-Noguchi et al., 2009; Bais et al., 2006; Duke et al., 2000). These substances indirectly affect plants through inhibition of microorganisms, including nitrogen-fixing, nitrifying bacteria or ecto-mycorrhiza (Walker et al., 1999). Therefore, the main objective of this work was to explore the allelopathic potential of the pericarp of T. natans var. bispinosa.

MATERIALS AND METHODS

Experimental site, Collection of pericarp and seeds

The experiment was conducted in Molecular Taxonomy Lab, Department of Botany, Lahore College for Women University, Jail Road, Lahore (LCWU) from November 2014 - July 2015. The fully matured fresh fruits of T. natans var. bispinosa were purchased from the local market of Lahore, Pakistan in November, 2014 and was taxonomically identified and authenticated as fruit of T. natans var. bispinosa by a taxonomist in the Department of Botany, Lahore College for Women University, Lahore, Pakistan. Any bruised or diseased fruits were discarded. The pericarp was removed from the kernel, washed under running potable water to remove the contaminants like surface dirt and other hazards. It was then air dried and stored in an air tight bottle. Cucumis sativus L. (Cucumber), Lactuca sativa L. (Lettuce), Raphanus sativus L. (Radish), Spinacia oleracea L. (Spinach) and Triticum aestivum L. (Wheat) belonging to different families such as Cucurbitaceae, Asteraceae, Brassicaceae, Chenopodiaceae and Poaceae, respectively were used as test plants in the bioassay for their ability to germinate within 3-4 days, small in size, easy to handle and purchase; and ability to be easily affected by the inhibitory and stimulatory allelochemicals. Their seeds were purchased from Punjab Seed Corporation and Federal Seed Corporation and were used as a test plant.

Bioassay

To determine the allelopathic activity of leachate from donor plant pericarp, the sandwich method was used (Fuji, 1994; Fuji et al., 2003; Fuji et al., 2004).

Experimental design and treatments

The experiment was designed following complete randomized design (CRD) with three replications where different concentrations of T. natans var. bispinosa were applied at the time of sowing seeds. The treatments were control (only agar medium), 0.01, 0.05 and 0.1g T. natans var. bispinosa pericarp for one set of experiment.

Preparation of growth medium and plant treatments

Agar powder (Navalai Tasque, Kyoto, Japan) with gelling temperature 30-31°C was used as growth medium. Agar solution (0.75% w/v) was prepared by dissolving the required amount of agar powder in distilled water and was autoclaved at 121°C for 15 min to sterilize. Dried pericarp of T. natans var. bispinosa were crushed into fine powder by using an electric grinder. Three different concentrations (0.01, 0.05 and 0.1 g) of dried pericarp were weighed and were used for the experimentation.

Sandwich preparation

Three different concentrations of pericarp were placed in Petri plates (40 mm x 12 mm area per each plate) (SIGMA-ALDRICH A part of Merck, Pakistan). By using a pipette (Gislon co. Ltd, Villiers-le-Bel, France), first 5 ml layer of agar was poured, as a result, dried plant material rise up and was allowed to gelatinize, on top of which a second 5 ml layer of agar was poured and allowed to solidify.
Table 1. Seedling germination and growth (length of radical and hypocotyl in cm) of all test plants against different treatments.

<table>
<thead>
<tr>
<th>Test plants</th>
<th>Control (only agar medium)</th>
<th>Different concentrations of pericarp of T. natans var. bispinosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.S</td>
<td>S.G</td>
</tr>
<tr>
<td>C. sativus</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>L. sativa</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>R. sativus</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>S. oleracea</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>T. aestivum</td>
<td>15</td>
<td>9</td>
</tr>
</tbody>
</table>

S.S, No. of seeds sown; S.G, No. of seeds germinated; R, radical; H, hypocotyl.
Values are expressed as means and means with different superscript letters within a column are significantly different.

**Seed sowing**

A total 60 seeds of every test plant were subjected to germination test (15 seeds per treatment). 5 seeds of every test plant were sown at equal distance from each other in each Petri plate lined with solidified doubled layered agar. Petri plates were covered by aluminum foil to protect them from light and were kept in the incubator (BIOTECH 300-L) (Shimadzu Rika Institute Co. Ltd, Kyoto, Japan) at 20-25°C for three days (72 h).

**Germination and growth measurement**

The germination percentage was calculated on 3<sup>rd</sup> day as prescribed by Dr. Fujii. The germination percentage of each treatment was compared with the control using the equation stated by Islam and Kato-Noguchi (2013):

\[
\text{Germination (\% of control) = } \frac{G_T}{G_0} \times 100
\]

Where \( G_T \), average number of seed germinated with treatment at the same time of measurements; and \( G_0 \), average number of seed germinated with control in each time of measurements.

Length of radical and hypocotyl were measured on 3<sup>rd</sup> day for every seedling. Average of all radical or hypocotyl length was taken and expressed in Centimeter. The growth promotion or inhibition was calculated using the following equation previously described by Islam and Kato-Noguchi (2012):

\[
\text{Inhibition or promotion = } \frac{(1 - \text{Length of shoot or root with treatment})}{\text{Length of shoot or root with control}} \times 100
\]

**Statistical analyses**

The data were expressed as means of the measurement obtained in Table 1 and were subjected to one-way analysis of variance (ANOVA) using COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company (Zar, 1984) to evaluate the significance of concentration on the growth of radical and hypocotyl. To determine the differences among the means, Duncan's Multiple-range test was used at 5% level of significance. Differences at \( p < 0.05 \) were considered statistically significant. The data was analyzed by Microsoft excel 2010, germination % and % inhibition of radical and hypocotyl of test plants under the influence of pericarp of T. natans var. bispinosa is presented by line and bar graphs respectively. Positive values show the suppression of growth against control and negative values present stimulation.

**RESULTS AND DISCUSSIONS**

The seedling germination and growth (i.e., length of radical and hypocotyl) of all the test plants belonging to different families under the influence of all four treatments are presented in Table 1. Bioassay results of the tested plant species revealed that pericarp of T. natans var. bispinosa have significant inhibitory effect towards the germination and growth of all test plants in dose-dependent manner except in C. sativus and T. aestivum (Table 1 and Figures 1 to 6). It indicated
Figure 1. Effect of 0.01, 0.50 and 0.1 g pericarp of *T. natans* var. *bispinosa* on seed germination of lettuce. Each value is the average of all the measurements; bar indicates mean ± SD.

Figure 2. Effect of different concentration of pericarp of *T. natans* var. *bispinosa* on root and shoot growth of lettuce. Each value is the average of all the measurements; bar indicates mean ± SD.
Figure 3. Effect of 0.01, 0.50 and 0.1 g pericarp of *T. natans* var. *bispinosa* on seed germination of radish. Each value is the average of all the measurements; bar indicates mean ± SD.

Figure 4. Effect of different concentration of pericarp of *T. natans* var. *bispinosa* on root and shoot growth of radish. Each value is the average of all the measurements; bar indicates mean ± SD.
Figure 5. Effect of 0.01, 0.50 and 0.1 g pericarp of *T. natans* var. *bispinosa* on seed germination of spinach. Each value is the average of all the measurements; bar indicates mean ± SD.

Figure 6. Effect of different concentration of pericarp of *T. natans* var. *bispinosa* on root and shoot growth of spinach. Each value is the average of all the measurements; bar indicates mean ± SD.
that the greater the concentration of plant material, the more would be the effect on germination of seeds and growth of seedlings as observed by Fujii et al. (2003), Gilani et al. (2010), Khan et al. (2008) Syed et al. (2014).

Recent studies have indicated the presence of diverse phytochemicals in the pericarp of *T. natans* var. *bispinosa* (Patel et al., 2010). So the inhibition growth pattern at all three concentrations of pericarp leachates could be attributed to the presence of active chemical compounds in pericarp which even at minimum dose are able to cause the damage to the test plants. The bioactive compounds (secondary metabolites) such as tannins, phenolic acids, lignins, alkaloids, flavonoids and terpenoids are present in all plant tissues including leaves, stems, roots, rhizomes, flowers, fruits and seeds, and even in pollen grains and are capable of suppressing the growth of other plants when released into the environment (Ahmad et al., 2011). Phenolic compounds exhibit morphological and physiological effects shown on susceptible plants. Morphological effects are decreased number of newly formed leaves and their enlargement and physiological effects are reduced net carbon assimilation and stomatal conductance (Patterson, 1989); reduced leaf water potential because of decreased osmotic potential and turgor pressure, lesser nutrient matter in roots and shoots (Einhellig et al., 1985) and by influencing different metabolic processes because of inactivation of enzymes taking part in these metabolic processes (Yu et al., 2001). Flavonoids affects the growth of test plant by inhibiting germination and cell development along with damage to adenosine triphosphate (ATP) and by disturbing the function of plant growth hormone: auxin (Berhow and Vaughan, 1999). Some of the phenolic compounds and flavonoids are experimentally proved to carry allelopathic behaviour (Tsuzuki and Yamamoto, 1987; Wu et al., 1998; Bias et al., 2003; Pervaiz et al., 2004; Golisz et al., 2007; Makoi and Ndakidemi, 2007).

Variation in concentration of phytochemicals with amount of plant parts was also observed by Khan et al. (2009). They also suggested phytotoxic effect of higher concentration. This indicated that plant inhibition is directly related to the concentration of allelochemicals. The growth inhibition of the test plant, in the presence of allelochemicals may be due to the reduction in cell division, elongation and expansion rate which are prerequisites (Einhellig, 1996; Ortega et al., 1988). The concentration-dependent inhibitory activity by allelopathic plant extracts was also reported by Nasir et al. (2005), Batlang and Shushu (2007) Teerarak et al. (2010) and Bich and Kato-Noguchi (2012).

In *C. sativus*, the germination % of seeds was enhanced 118% at 0.01 g, 127% at 0.05 g and 109% at 0.1 g (Table 1 and Figure 7) and the growth of seedlings was inhibited at 0.01 g, stimulated at 0.05 g and again inhibited at 0.1 g (Table 1 and Figure 8). It indicated that
a specific dose of allelochemicals can cause stimulation in growth but large quantity can cause phytotoxicity (Pelinganga and Mashela 2012). It is evident that there were some growth promoting substances which released some stimulatory allelochemicals to the environment and promoted the growth of recipient plant (Yamada et al., 2010).

From all test plant species, the germination of seeds was enhanced in cucumber (Table 1 and Figure 7) and wheat (Table 1 and Figure 9) and the growth of seedling was enhanced only in cucumber at specific dose (Table 1 and Figure 10) whereas, germination of seeds and growth of seedling was inhibited in rest of the test plants (Table 1 and Figures 1 to 6). However, the magnitude of inhibition for cucumber seeds was not as severe as was witnessed in rest of the seeds because seed size plays an important role in growth response of the test plant. Small seeds are more vulnerable to the effects caused by allelochemicals as compared to seeds larger in size. Seed size significantly effects the concentration of a particular allelochemical to produce a particular growth response (Perez et al., 1990). So small size seeds will be easily affected by the same concentration of allelochemicals as compared to the seeds large in size. This gives an insight to the fact why cucumber showed less inhibitory germination and growth activity as compared to other seeds when given same treatments.

Crop weeds are the main problem in agriculture, causing a worldwide an annual loss of about US$95 billion (FAO, 2009). The principal method to control weeds is the use of synthetic agrochemicals such as herbicides, fungicides, insecticides and nematocides (Dayan et al., 2009). Although these products have brought unquestionable gains to agriculture but their overuse causes nutrient deficiency and change the soil physiochemical properties, resulting in reduced crop quality and productivity (that is, less healthy plant and more expensive food) and environmental hazards which is a threat to human and livestock health (Chou, 2010). Furthermore, the continued use of synthetic herbicides has resulted in herbicide-resistant weeds (Vyvyan, 2002). Therefore, there is a growing demand to replace traditional agrochemicals with naturally occurring and environmentally friendly weed control agrochemicals for sustainable agricultural production (Dayan et al., 2009; Cantrell et al., 2012).

The possibility of weed control using allelochemicals is widely recognized (Rice, 1995; Souza et al., 2002; Piccolo et al., 2007; Dayan et al., 2009). Therefore, the concept of allelopathic properties of different plant species against common weeds of agricultural lands is now receiving much attention as an alternative weed control strategy (Leather, 1983). Allelochemicals are compounds from the secondary metabolism of plants,
and present several advantages over herbicides, such as less toxicity, a shorter half-life and higher water-solubility (Duke et al., 2000). Furthermore, because they have a lower specific action mechanism, they are less likely to select resistant biotypes (Reigosa et al., 2001; Durán-Serantes et al., 2002). Allelochemicals are present in different parts of plants in different concentrations; therefore, their mode of release is different from different positions. These allelochemicals may affect the growth and development of plants in a positive (stimulatory) or negative (inhibitory) way.

A main shortcoming of the commercial use of allelochemicals is their production at a large scale, because most donor plants are non-cultivated, poorly-
studied species. One possible solution is to determine the allelochemical compound and then artificially synthesize it; however, this is far beyond the reality of most farmers. Furthermore, the synthetic versions of natural compounds are not accepted in organic agriculture in most countries. Another solution, which has been successfully tested, is the use of crop residues as allelochemical sources (Rizvi et al., 1999). Crop residues are likely to be used at a commercial scale because they are available in large amounts. In Pakistan, yield of *T. natans* var. *bispinosus* crop per acre with a growth cycle of 6-8 months is approximately 93 Mounds (Malik et al., 2012). Therefore, residues from this crop might be an enormous source of allelochemicals because the water chestnut pericarp, frequently discarded after eating kernel inside, is rich source of phytochemicals.

**Conclusion**

Biochemical evaluation of *T. natans* var. *bispinosus* showed dose dependent suppressive effect on the germination and growth of radical and hypocotyl of all the test plants except *C. sativus* and *T. aestivum*. From the observed results it was concluded that germination and growth of most of the test plants was strongly inhibited with increasing concentration of plant material. Therefore it can be suggested that the pericarp extract of *T. natans* var. *bispinosus* can be used as a bioherbicide but it cannot be introduce with agricultural crops for weed control because of its suppressive effects on germination and growth of other plant species. The use of cover crops with increased production of allelochemicals could limit the need of conventional herbicides as natural or plant based chemicals are biologically degradable, hence do not toxify the soil by accumulating in it. *T. natans* var. *bispinosus* could also be used as natural herbicide in weed management by isolating allelochemicals from its pericarp.

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