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Allelopathic Activity of Coffee Against *Cicer arietinum* and *Triticum aestivum*

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Abstract: The present study aimed the evaluation of allelopathic effect of aqueous extract of coffee (*Coffea arabica*) against germination and radicle growth of *Cicer arietinum* and *Triticum aestivum* seeds. The extract at different concentrations was incubated in controlled conditions with the surface sterilized seeds of *C. arietinum* and *T. aestivum* and observed periodically for seed germination and radicle growth to assess the allelopathic behaviour. The extract mainly at higher concentrations demonstrated promising allelopathic potential by significantly affecting seed germination and radicle elongation of both *C. arietinum* and *T. aestivum* in a concentration dependent manner. *C. arietinum* was found to be more sensitive than *T. aestivum*. The present study demonstrated remarkable allelopathic potential of coffee against the test seeds. The effect was plausibly due to the alkaloid and polyphenols contents of coffee.

Key words: Allelopathic · Polyphenols · *Coffea arabica* · *Cicer arietinum* · *Triticum aestivum*

INTRODUCTION

Nowadays indiscriminate use of synthetic herbicides has resulted in herbicide-resistant weeds and environmental concerns about the safety of commonly used synthetic herbicides. Therefore, there is need for alternative weed management strategies which are less synthetic herbicide dependent or based on naturally occurring compounds [1]. Allelopathy holds promise for the environmentally friendly weed management. The phenomenon of allelopathy, where a plant species chemically interferes with the germination, growth or development of other plant species has been known for over 2000 years. Allelopathy can be defined as any direct or indirect harmful or beneficial effect of one plant on another through the production of chemicals that it releases into the environment [2]. In 1996, the International Allelopathy Society defined allelopathy as follows: "Any process involving secondary metabolites produced by plants, micro-organisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects" [3]. Chemicals released from plants and imposing allelopathic influences are termed allelochemicals or allelochemics. Most allelochemicals are classified as secondary plant metabolites which are biosynthetically derived from the primary metabolites of the plant [4]. When susceptible plants are exposed to allelochemicals, germination, growth and development may be affected. Allelochemicals are present in several parts of plants that are known to interfere with seed germination and growth of neighbouring or successional plants by releasing allelochemicals in their environment [1, 2]. The search and development of new herbicides through the identification of active compounds from allelopathic plants is an interesting research area [5]. These compounds can be regarded as 'natural herbicides'. Several higher plants are reported by the present authors to possess allelopathic potential and efforts are being made to apply them for weed control [6-8].

Coffee, also known as coffee bean consists of the dried ripe seeds of *Coffea arabica* Linn. (Rubiaceae). Coffee plant is an evergreen shrub or small tree grown wild in East Africa, and cultivated in several parts of the world including India. Coffee beverage is the widely consumed popular beverage worldwide. Traditionally, it has been used for several important medicinal purposes in different parts of the world [9]. Previous researchers have reported certain pharmacological properties on

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coffee [9-11]. Recently the present authors have reported *in vitro* anti-inflammatory effect of coffee bean [12]. The present study was conducted to assess the possible allelopathic effects of coffee extract on the germination and radicle growth of *Cicer arietinum* and *Triticum aestivum* seeds.

MATERIALS AND METHODS

Plant Material: Dried coffee beans (*Coffea arabica* Linn. Family: Rubiaceae) were procured in the month of July, 2011 from Sarkar & Sons., Kolkata, India. Just after procurement, the beans were ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

Preparation of Extract: The powdered plant material (50 g) was extracted with 400 ml distilled water by boiling for 45 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (AQCA, yield: 27.28%). The dry extract was kept in a vacuum desiccator until use in the study.

Test Samples: The test samples for allelopathic bioassay were prepared freshly from the dry extract. Different concentrations of AQCA (40, 20, 10, 5, 2.5, 1.25 mg/ml) were prepared by dissolving in double-distilled water immediately prior to use.

Collection and Preparation of Seeds: Healthy uniform seeds of gram (*Cicer arietinum* L., family: Fabaceae) and wheat (*Triticum aestivum*, family: Poaceae) were obtained from the Agriculture Seed Store (Govt. of West Bengal) Kalyani, West Bengal, India. The seeds were soaked in distilled water for one hour. Then the seeds were surface sterilized with 70% ethanol for 2 minutes, then rinsed with double-distilled water for several times for complete removal of the sterilant.

Exposure to Text Samples: This procedure was performed under aseptic conditions at laminar air-flow bench. The surface sterilized seeds were placed evenly in sterilized glass Petri dishes (9 mm). Each Petri dish contained 10 seeds. Then equal volume (5 ml) of varying concentrations of the test samples were introduced into each Petri dish. Similar volume of double distilled water was used as control. In case of wheat seeds the test liquids were decanted after 30 minutes. Then all the Petri dishes were incubated in dark at room temperature (24-26°C). Allelopathic behaviour was evaluated by recording the number of germinated seeds and radicle length using a millimetre ruler, after 48, 72 and 96 hrs in case of gram seeds, and after 24 and 48 hrs in case of wheat seeds. The indicating parameters viz., germination percentage and percentage inhibition of radicle growth were calculated by the following formulae:

Germination percentage = Number of germinated seeds/Total number of seeds ×100

% Inhibition of radicle growth= (X–Y)/X ×100.

where, X= Control mean radicle length and Y= Treated mean radicle length.

The extract concentration for 50% radicle length inhibition (IC_{50}) was determined form the dose response curve by plotting percentage inhibition of radicle growth with respect to control against treatment concentration [13].

Statistical Analysis: The data of radicle growth inhibition were expressed as the mean ± standard error of mean (SEM). Same data were analyzed for statistical significance by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test using Graph Pad InStat software.

RESULTS

The results of allelopathic effect of AQCA on *C. arietinum* are summarized in Tables 1 and 2. AQCA at all test concentrations inhibited germination of *C. arietinum* seeds in a concentration dependent way; however, about 80 to 90% seeds germinated at lower concentration (1.25 mg/ml) at the time interval 48 to 96 hours (Table 1). AQCA remarkably inhibited radicle growth at the all test concentrations in a time and concentration dependent manner. The effects were found to be prominent and significant during the whole observation period (Table 2).

The results of allelopathic effect of AQCA against *T. aestivum* are presented in Tables 3 and 4. AQCA at all test concentrations inhibited germination of *T. aestivum* seeds in a concentration dependent fashion; however, no germination was observed in case of highest concentration of AQCA (40 mg/ml) at each time interval (Table 3). AQCA significantly and concentration dependently inhibited radicle growth at all the test concentrations during 48 hrs of observation. No detectable radicle growth was observed at the highest

Table 1: Effect of AQCA on germination percentage of *C. arietinum*

Concentration (mg/ml)	After 48 hrs (%)	After 72 hrs (%)	After 96 hrs (%)
Control	100	100	100
1.25	80	90	90
2.5	60	70	80
5	50	60	80
10	20	40	50
20	0	20	20
40	0	0	0

Table 2:Effect of AQCA on radicle growth of *C. arietinum.*

	After 48 hrs		After 72 hrs		After 96 hrs	
Concentration (mg/ml)	Radicle length (mm)[§]	% Inhibition of radicle growth	Radicle length (mm)[§]	% Inhibition of radicle growth	Radicle length (mm)[§]	% Inhibition of radicle growth
Control	19.20 ± 2.50	-	39.50 ± 5.43	-	60.0 ± 5.45	-
1.25	3.20 ± 0.58*	83.30	4.30 ± 1.26*	89.11	8.13 ± 2.72*	86.45
2.5	2.87 ± 0.83*	85.02	4.28 ± 0.72*	89.16	7.30 ±1.64*	87.83
5	2.30 ± 0.33*	88.02	3.80 ± 0.87*	90.30	4.50 ± 0.80*	92.50
10	1.0 ± 0.23*	94.79	1.50 ± 0.29*	96.20	2.20 ± 0.30*	96.35
20	0	100	1.0 ± 0.17*	97.46	2.0 ± 0.19*	96.66
40	0	100	0	100	0	100

[§]Data are expressed as mean ± SEM. *$p < 0.001$ compared with control

Table 3: Effect of AQCA on germination percentage of *T. aestivum*

Concentration (mg/ml)	After 24 hrs (%)	After 48 hrs (%)
Control	60	90
1.25	50	70
2.5	50	60
5	30	50
10	20	50
20	20	50
40	0	0

Table 4:Effect of AQCA on radicle growth of *T. aestivum*

	After 24 hrs		After 48 hrs	
Concentration (mg/ml)	Radicle length (mm)[§]	% Inhibition of radicle growth	Radicle length (mm)[§]	% Inhibition of radicle growth
Control	2.17 ± 0.30	-	2.67 ± 0.47	-
1.25	2.0 ± 0.17[¶]	7.83	2.40 ± 0.40	11.11
2.5	2.0 ± 0.12[¶]	17.05	2.0 ± 1.0[¶]	25.09
5	1.80 ± 0.37[¶]	17.05	2.0 ± 0.57[¶]	25.09
10	1.0 ± 0.21*	53.91	1.80 ± 0.37[¶]	32.58
20	1.0 ± 0.19*	53.91	1.75 ± 0.47*	34.45
40	0	100	0	100

[§]Data are expressed as mean ± SEM. [¶]$p < 0.5$, *$p < 0.001$ compared with control

Table 5: IC_{50} values of AQCA on radicle growth of *C. arietinum* and *T. aestivum*

	IC_{50} (mg/ml)	
Treatment time	*C. arietinum*	*T. aestivum*
After 24 h	-	9.5
After 48 h	0.75	24.75
After 72 h	0.745	-
After 96 h	0.75	-

concentration of AQCA (40 mg/ml) even up to 48 hrs (Table 4). The IC_{50} values of AQCA for both *C. arietinum* and *T. aestivum* are summarized in Table 5.

DISCUSSION

Screening of plant extracts and their fractions for their effects on seed germination of various plant species are routinely used to evaluate their alleleopathic potential [5]. The present findings demonstrated negative allelopathic effects of aqueous extract of coffee (*Coffea arabica*) on the germination and radicle growth of *C. arietinum* and *T. aestivum* seeds. These seeds were selected for the present study because of their low cost, easy availability in healthy disease free state, short germination time, simple germination conditions and reproducible results.

The most frequently reported allelochemical-induced gross morphological effects on plants include inhibited or retarded seed germination, effects on coleoptile elongation and on radicle, shoot and root development [14]. Here, germination percentage and radicle growth were recorded to monitor the allelopathic behaviour. However, in the present study, radicle growth appeared to be the most sensitive parameter and IC_{50} values based on this parameter very clearly indicated the differential allelopathic effect of AQCA on both the test seeds (Table 5). From these values it becomes evident that *C. arietinum* was more sensitive to AQCA, being effective in lower concentrations; than *T. aestivum.*

Plants exhibit allelopathic activity due to release of allelochemicals of different chemical classes mainly polyphenolic compounds (tannins and flavonoids), cyanogenic glycosides and alkaloids [4, 15]. The inhibitory effect of the test extract on seed germination and seedling length may be due to the presence of putative allelochemicals. The main constituents of coffee bean are an alkaloid caffeine and polyphenolic compounds like tannins and a phenolic acid namely chlorogenic acid [16]. Polyphenols are well known natural products known to possess several notable biological properties [17]. Phenolic acids have been shown to be toxic to germination and plant growth processes [4, 18]. Coffee contains the alkaloid caffeine which has phytotoxic property [18, 19]. In the present study, allelopathic effect of coffee can be attributed to its alkaloid and polyphenols contents. The observed effect may be due to synergistic effect rather than single constituent.

From the present preliminary investigation, it can be concluded that coffee bean exhibited remarkable negative allelopathic potential by significantly affecting the germination and radicle growth of both *C. arietinum* and *T. aestivum. C. arietinum* was found to be more sensitive than *T. aestivum.* The observed allelopathic effect was plausibly due to its alkaloid and polyphenols contents. Further studies are necessary to determine the exact chemical constituents of coffee accounting for its allelopathic activity. Allelopathic effects of coffee bean under field conditions also need further research in pursuit of a new, effective and environment friendly natural herbicide.

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