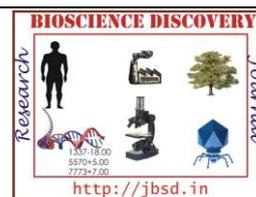


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Research Article



Allelopathic effect of *Eupatorium odoratum* L. on amylase activity during seed germination of *Cicer arietinum* L. and *Cajanus cajan* (L) Millsp.

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Abstract

Weeds are wild plants that grow and interfere with the cultivated plants through allelochemicals. The weed, *Eupatorium odoratum* is dominant in legumes fields and cause damage to crops. Therefore, the purpose of this study was to assess the allelopathic effect of leaf extract of *Eupatorium odoratum* on amylase activity. Different concentrations of plant extract were prepared with water as 1%, 10%, 20%, and 30%. An increasing in concentration decreased the amylase activity in *Cicer arietinum* at all seed soaking periods. In a case of *Cajanus cajan* increase in concentration as well as seed soaking period decreases the amylase activity. This indicates that some allelochemicals are present in the aqueous extract of *Eupatorium odoratum* and it works as plant growth inhibitors.

INTRODUCTION

The allelopathy described as biochemical interactions that inhibit the growth of nearby plants by another plant due to the release of chemical compounds (Molisch, 1937). The weeds are undesirable plants competing for moisture, light, water, nutrients and space with crop plants (Anonymous, 1994). It affects a crop growth dynamics by releasing chemical compounds called allelochemicals (Kadioglue *et al.*, 2005). The root, rhizome, stolon, stem, leaves, branches, flower, fruit and seeds of weeds have allelopathic potentiality. These parts possess allelochemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, amino acids and have an inhibitory or stimulatory effect on the seed germination of crop plants (Mali and Kanade, 2004 and Ghodakeet *et al.*, 2012). The leaf extract have much allelochemicals property studied by (kumbhar and Patel, 2012).

Carbohydrates are the most important compound in seed hydrolyzed by the hydrolytic enzymes into glucose. Amylase is an important hydrolytic enzyme synthesized during seed germination in plants. It is abundant in the

germinating seeds and catalyses a random hydrolysis of α -1, 4 glucosidic linkage in the starch component (Kengaret *et al.*, 2014). According to Weber *et al.*, (1998) a seed development is closely associated with seed metabolism and transport processes. It is involved in the mobilization of starch reserves which are transported as sugars and utilized by the growing embryo (Ernst and Floyd, 1971). The enzyme most commonly endorsed with the initial attack on starch granules is α -amylase (Trethewey and Smith, 2000).

Cajanus cajan commonly known as Pigeon pea, and *Cicer arietinum* commonly known as Chickpea, it belongs to family papilionaceae Pigeon pea and chickpea are major pulses grown in India. The weed, *Eupatorium odoratum* L. is an exotic flowering shrub grows in field of legumes crops. According to (Patil, 2011) was studied the effect of leaf leachates of invasive weed *Eupatorium odoratum* on some crop plants, the results indicate that all the studied crops are sensitive to leachate of *Eupatorium odoratum* at germination stage.

Eupatorium odoratum leaf extracts caused inhibitory effect on seedling development of some legumes crops (Rafiqul *et al.*, 2003). Therefore, the main aim of this study is to evaluate the effect of aqueous extract of leaves of *Eupatorium odoratum* L. on enzyme activity of amylase during seed germination of *Cicer arietinum* and *Cajanus cajan*.

MATERIALS AND METHODS:

Preparation of aqueous leaf extracts:

Fresh leaves of *Eupatorium odoratum* were collected from agricultural fields around Karad tehsil and brought to the laboratory. All leaves were washed with tap water to remove soil particles and shade dried for 10 days. Dried leaves were powdered with the help of grinder and stored in polyethene bags. *Eupatorium odoratum* shade dried leaf powder (1, 10, 20 and 30 g) was soaked separately in 100 ml distilled water for 24 h. After soaking the extract was filtered through a double-layered muslin cloth. The filtrate was a stock solution and then prepared 1, 10, 20, and 30% concentration with distilled water. Healthy uniform seeds of *Cicer arietinum* variety 'Vijay' and seeds of *Cajanus cajan* variety 'ICPL87' were surface sterilized with 1% sodium hypo-chloride for 10 minutes, then rinsed with distilled water for several times to remove excess of a chemical. Then surface sterilized seeds were soaked for treatments in different concentrations of plant extracts such as 6, 12 and 24 h. and distilled water was used as a control. These treated seeds were placed in petri plate containing wet blotting paper and covered with a lid. At each concentration and incubation period, triplicate sets were arranged and placed in the lab in normal temperature for germination, for 120 h.

Preparation of crude enzyme extract:

After 120 hours of germination, 0.5 g germinated seeds of *Cajanus cajan* and *Cicer arietinum*, were grinded separately in a mortar with a pestle in cold 0.1M phosphate buffer of respective PH (for amylase- PH 6.7) using a homogenizer. The temperature was maintained at 4^o C by ice in the outer chamber of the homogenizer. The suspension was then filtered through few layers of cheese cloth in the cold room. The filtrate was collected and clarified by centrifugation in a refrigerated centrifuge at 10,000 rpm for 15 min at 4^o C. The amylase activity was assayed as per method suggested by Jayaraman (1981). The readings were plotted in observation table.

The amylase activity was measured by estimating the release of maltose obtained from the standard curve. One unit of amylase activity was defined as the amount required for liberating 1 mg of maltose in 30 min at 37^o C.

Statistical analysis

The analysis was carried out in three replicates for all determinations. The mean and standard of deviation were calculated. The data was analyzed by one-way analysis variance ANOVA. The treatment means was performed by Duncan's New Multiple Range Test. Significance between control and treatment was compared at 0.05 probability levels.

RESULTS AND DISCUSSION

Amylase activity (Maltose unit/g) germinated of *Cicer arietinum* and *Cajanus cajan* seeds. 1 maltose unit is equivalent to the enzymatic activity, which liberates 1 mg of maltose in 30 min. Means values within a column followed by the same superscript letter are not significantly different at 0.05 probability letter.

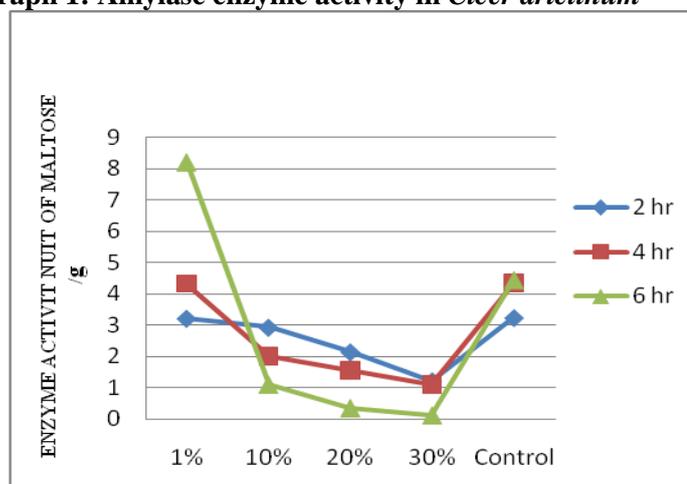
Table 1: Effect of aqueous leaf extract of *Eupatorium odoratum* on amylase activity in *Cicer arietinum* seeds during germination with various soaking period.

Concentrations	Seed soaking Period		
	2h	4h	6h
1%	3.21 ± 0.09 ^a	4.32 ± 0.07 ^a	8.21 ± 0.04 ^c
10%	2.92 ± 0.09 ^b	2. ± 0.06 ^b	1.11 ± 0.02 ^a
20%	2.13 ± 0.09 ^b	1.56 ± 0.04 ^a	0.36 ± 0.06 ^a
30%	1.20 ± 0.09 ^c	1.10 ± 0.03 ^a	0.125 ± 0.01 ^a
Control (D.W)	3.23 ± 0.09 ^a	4.35 ± 0.05 ^b	4.458 ± 0.07 ^b

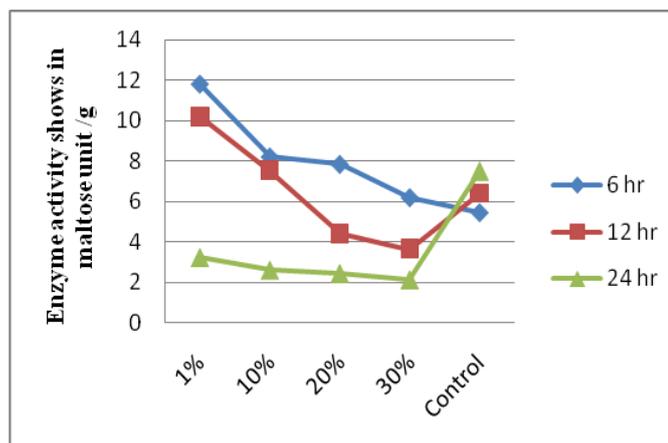
Table 2: Effect of aqueous leaf extract of *Eupatorium odoratum* on amylase activity in *Cajanus cajan* seeds during germination with various soaking period.

Concentrations	Seed soaking period		
	6h	12h	24h
1%	11.80 ± 0.03 ^c	10.21 ± 0.04 ^c	3.21 ± 0.04 ^a
10%	8.21 ± 0.04 ^b	7.52 ± 0.08 ^b	2.58 ± 0.06 ^a
20%	7.84 ± 0.06 ^b	4.39 ± 0.06 ^a	2.41 ± 0.09 ^a
30%	6.20 ± 0.04 ^a	3.62 ± 0.04 ^a	2.10 ± 0.07 ^a
Control (D.W)	5.46 ± 0.09 ^a	6.38 ± 0.06 ^a	7.49 ± 0.08 ^b

Graph 1: Amylase enzyme activity in *Cicer arietinum*



Graph 2: Amylase enzyme activity in *Cajanus cajan*



An effect of aqueous leaf extracts of *Eupatorium odoratum* on amylase activity in *Cicer arietinum* and *Cajanus cajan* was recorded in table 1 and 2 respectively. The activity of amylase in *Cicer arietinum* was decreased after increases concentration at 2, 4 and 6 h seed soaking periods. In 1% extract treatment maltose unit /g was increases as per increasing seed soaking period. The

maximum amylase activity 8.21 maltose unit /g in the 1% extract of *Eupatorium odoratum* was recorded in 6h after seed soaking period in *Cicer arietinum*. However, other treatments reported for retardation in amylase activity which indicates that *eupatorium odoratum* drastically affected on carbohydrate metabolism in higher concentration.

In case of *Cajanus cajan* (table 2) Amylase activity (maltose unit/g) was decreased as per increasing concentration as well as soaking period. In 6 h seed soaking period amylase is more than control. In 12 h seed soaking period amylase activity is less than control in 20 and 30 concentration. 24 h seed soaking period amylase activity is less than control in all concentration.

The results of the present investigation were supported by various researchers. Pawar and Chavan (2007) reported that the effect of leaf leachates of *Eucalyptus globulus*, *moringa olerifera*, *Parthenium hysterophorus* and *Glycine max* decreased the activity of alpha-amylase and invertase in germinating seeds of *Sorghum bicolor* (L.) Moench. The applications of allelopathic plants extracts at low concentration improved the performance of maize which might be due to the presence of various secondary metabolites (Casimiro *et al.*, 2001). Leaf leachates of *Gmelina arborea* inhibition the activity of some hydrolytic enzymes amylase, catalase and acid phosphatase in legumes seeds (Ramakrishnan *et al.*, 2014). Effect of leaf leachate of invasive weed *Eupatorium odoratum* is studied on some crop plants, the results indicate that all the studied crops are sensitive to leachate of *Eupatorium odoratum* at germination stage (Patil, 2011).

The present study revealed that the amylase activity in germinating seeds of *Cicer arietinum* was stimulated in 1% extract of *Eupatorium odoratum* at 6 h seed soaking period. However, amylase activity was stimulated in *Cajanus cajan* in all the concentrations of *Eupatorium odoratum* at 6 h seed soaking period as compared to control. This increase or decrease in activity of amylase is due to allelochemicals present in *Eupatorium odoratum*. It needs the further screening of allelochemicals and their characterization for detail study.

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