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RESEARCH ARTICLE

INHIBITION OF SEED GERMINATION OF WEEDS BY NATURAL INHIBITOR- CATECHIN

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ABSTRACT

Weeds are unwanted and undesirable plants, which interfere with the utilization of land and water resources and thus adversely affect human welfare. Most of these plants propagate vegetatively by stolon and sexually by seed germination process. The Pectin methylesterase (PME) plays important role in the seed germination process. The PME, acts on the homogalactouran pectin of the cell wall affecting its porosity and elasticity to induce the water uptake initiating the seed germination. In the previous works, we have characterized PME, form *Arabidopsis thaliana* and identified natural potent inhibitor as catechin by using *in-silico* approaches. In the, continue of previous work here we have, studied the effect of catechin isolated from the Green tea on the seed germination of selected weed plants.

INTRODUCTION

Weeds are unwanted and undesirable plants, which interfere with the utilization of land and water resources and thus adversely affect human welfare. Weeds compete with the beneficial and desired vegetation in crop lands, forests, aquatic systems etc., and poses great problem in non-cropped areas like industrial sites, road or rail lines, air fields, landscape plantings, water tanks, water ways etc. Most of weed plants propagate vegetatively by stolon and sexually by seed germination process. Seed germination is a mechanism, in which morphological and physiological alterations result in activation of the embryo in plant growth and development. Before germination, seed absorbs water, resulting in the expansion and elongation of seed embryo (M. Miransari *et al.,* 2014). The plant cell wall consists mainly of a hydrated gel matrix of hemicellulosic and pectic polysaccharides, as well as cellulose, along with proteins and aromatic substances. Cell wall pectins are found either as homogalacturonans or as substituted molecules, the rhamnogalacturonans I and II as well as xylogalacturonan. Composed of a linear chain of 1, 4-linked a-D- galacturonic acid (Gal UA) residues, the homogalacturonans can be methylesterified at the C-6 carboxylic acid groups of the Gal UA residues (Fry SC, 2005, Knox JP, 2008 and S. Wolf *et al.,* 2003). Pectin methyl esterase (PME) (EC 3.1.1.11) is a ubiquitous cell wall-associated enzyme catalyzes reactions according to the double-displacement mechanisms, de- esterification through transferring the C6 carboxyl groups in the pectin -PME complexes to water molecules altering the degree and pattern of methyl esterification and trans acylation through transferring the C6 carboxyl groups to the hydroxyl groups of another pectin molecules and resulting in the formation of high molecular weight pectins with new non-methoxy ester linkages which facilitate plant cell wall modification and subsequent breakdown allowing water uptake (Jiang *et al.,* 2001). In this reaction methanol is produced as a byproduct in addition with pectic substances. This enzyme is widely used in juice and fruit beverage industries to improve the quality of the process (Kohli *et al.,* 2015). Pectinase preparations (such as Olivex) are also used in olive oil industry to increase the oil extraction output and to improve certain olive oil quality indicators (Kashyap *et al.,* 2001 and Vierhuis *et al,* 2003). Another application of combinational use of PME, other pectinases and cellulases is the peeling of fruits. The degree of pectin methylesterification of the cell walls of seed tissues influences the rate of germination of the seeds. Several PMEIs from *Arabidopsis thaliana*and other plant species have been characterized (Kohli *et al.,* 2015 and Wolf *et al.,* 2003). However, the use of proteinaceous inhibitors is complex and hence not trivial. Small molecule inhibitors would be more tractable as applied enzyme inhibitors (A. Raiola *et al.,* 2004). Recently Lewis *et al.,* identified the green tea catechin epigallocatechin gallate as a natural inhibitor of pectin methyl esterases by gel assay in tomato (*Solanumlycopersicum*) and citrus (Lewis *et al.,* 2008). Green tea is a rich source of catechins, which account for up to 30% of the leaf dry weight (H. N. Graham 1992). In the previous work we have characterized the PME of *Arabidospis thaliana* and identified the novel inhibitors using bioinformatics tools. The PME of *A. thaliana* contains 595 amino acids and the physicochemical

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properties depict that the PME is alkaline, stable, cationic protein. The secondary structure reveals that PME consist of a helix, a sheet and random coil structure within its short stretch of residues. The3D structure predicted by SWISS-MODEL was validated using PROCHECK, the percentage of most favorable region was 86.1% (Joshi *et al.,* 2016). Further we have constructed the ligand library against the PME and identified the two potent inhibitors with the docking score, Epicatechin gallate (Docking score= 285.53) and 3-Galloylcatechin (Docking score= 281.83) (P. S. Chegu *et al., ,* 2017). Continuing the previous works, have isolated the catechin from the Green tea by using the chloroform and ethyl acetate extraction and confirmed by absorption peak at 272 nm in UV-VIS spectroscopy. The isolated 50 mg catechin is dissolved in 10% methanol and used for the seed germination studied against, *Senna occidantalis, Datura stramonium, Calotropis procera, Parthenium hysterophorus* and *Cassia tora.*

MATERIALS AND METHODS

Collection of seeds of weeds and poisonus plants from reagion of solapur: The dried seeds of selected plants (*Senna occidantalis, Datura stramonium, Calotropis procera, Parthenium hysterophoru, Cassia tora*) of known identity were collected from Solapur, Maharashtra, India. Then the seeds were washed with Distilled water for 2-3 times and used for further process.

Isolation and Confirmation of catechin from green tea: Green tea powder was obtained from local market (Big Bazar) in Solapur in powder of dried leaves stored in sachets. The 9 gmof Green tea powder was weighed and boiled in 180 ml of distilled water at 80˚C in water bath for 80 min. Extraction was filtered with filter paper of pore size 5µm. Then the filtrate was partitioned with equal amount of chloroform in separating funnel for 1 hr. The above step was repeated for 2-3 times to remove the remaining traces of proteins, carbohydrates and lipids and the second partition was done by ethyl acetate to get the pure form of catechin and confirmed by UV- VIS spectroscopy.

Inhibition of the seed germination of selected plants by catechin: The 10 seeds of each selected plants were soaked in 5ml distilled water and catechin solution (0.5 mg in 10% methanol) (Inderjit *et al.,* 2008) in each two different test-tubes respectively for 24 hours and labelled as control and test. The overnight soaked seeds were distributed on filter papers moistened with distilled water andcatechin into two different petri plates and observed for germination for 24 hours. After the sproutening the seeds were sowed in the soil in germination tray and observed for growth provided with sufficient water daily.

RESULTS AND DISCUSSION

Inderjit *et al.,* in 2008 studied the phytotoxic effect of catechin on the root growth of Bambusa and Koeleria seedlings and he found the significant inhibition of root growth root growth of Bambusa and Koeleria seedlings at 50 µg /ml (Inderjit *et al.,* 2008).

Collection of Seeds: The dried seeds of *Senna occidantalis, Datura stramonium, Calotropis procera, Parthenium hysterophorus, and Cassiatora* were collected from the region of Solapur, Maharashtra, India and used for the inhibition of seed germination study. The collected seeds of selected plants are shown in Fig. 1.

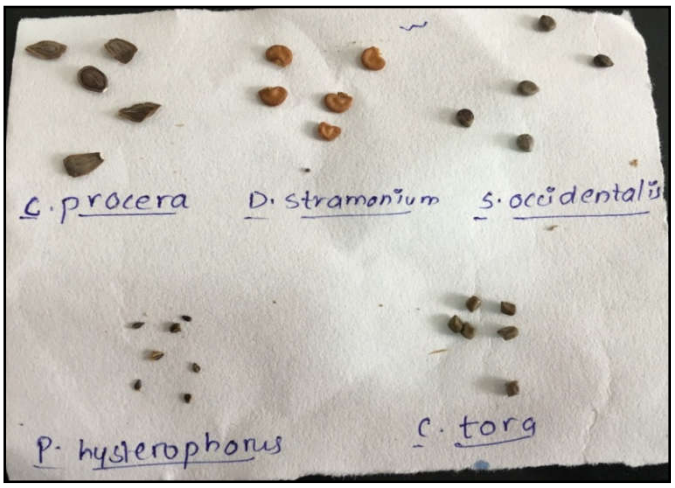
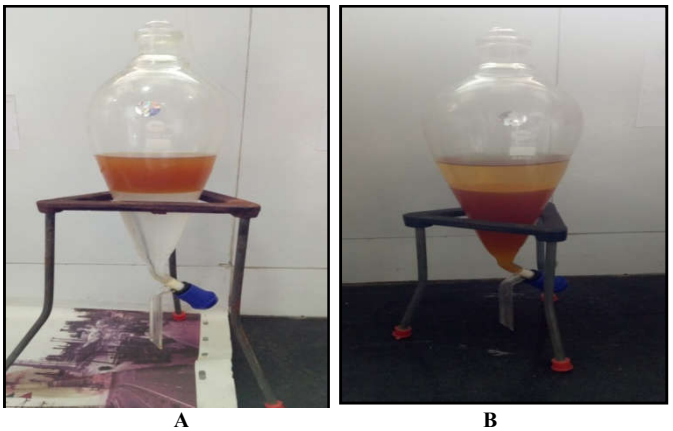


Fig. 1. Seeds of selected weed plants.

Isolation and identification of catechin from green tea: After boiling the Green tea powder in the D/W, the brown coloured extract was subjected for the filtration to remove the unwanted debris. In the chloroform extraction step the two layers were observed in the separating funnel, upper aqueous layer lower chloroform layer in which the unwanted lipids, carbohydrates and proteins were get removed. Further in the ethyl acetate extraction, the catechin gets displaced in to the upper ethyl acetate layer from the lower aqueous layer leaving the pigment and small molecules.



A B

Fig. 2: Isolation of Catechin. A: Chloroform Extraction, B: Ethyl acetate extraction

Fig. 3. Isolated catechin powder

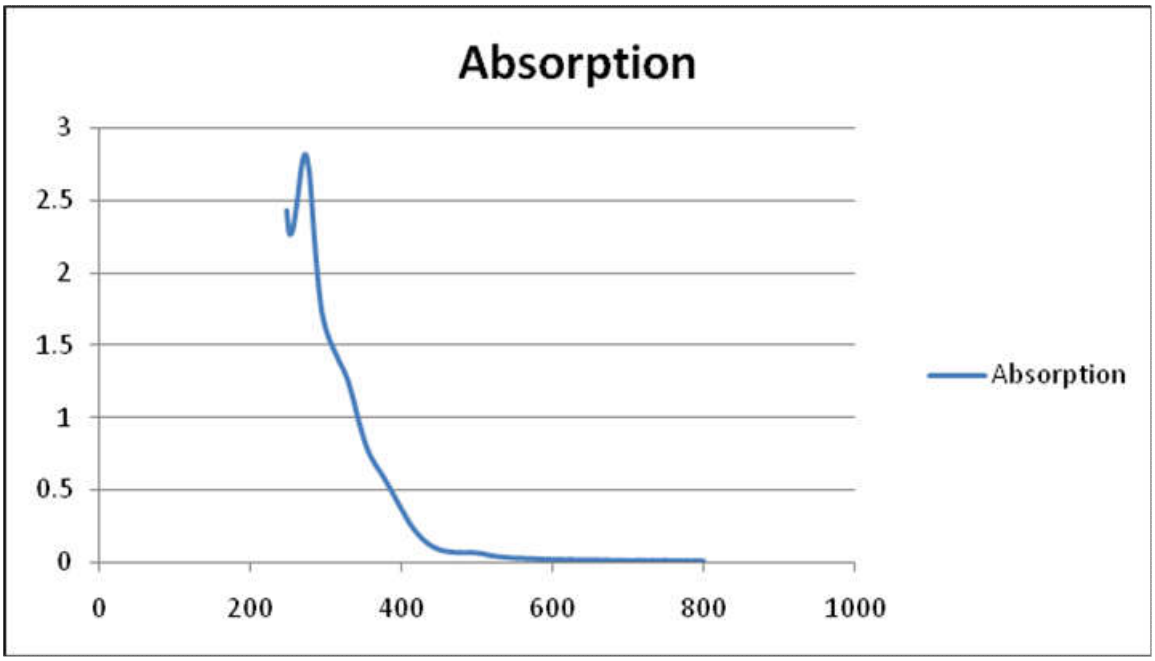


Fig.4. UV-VIS absorption spectra of catechin

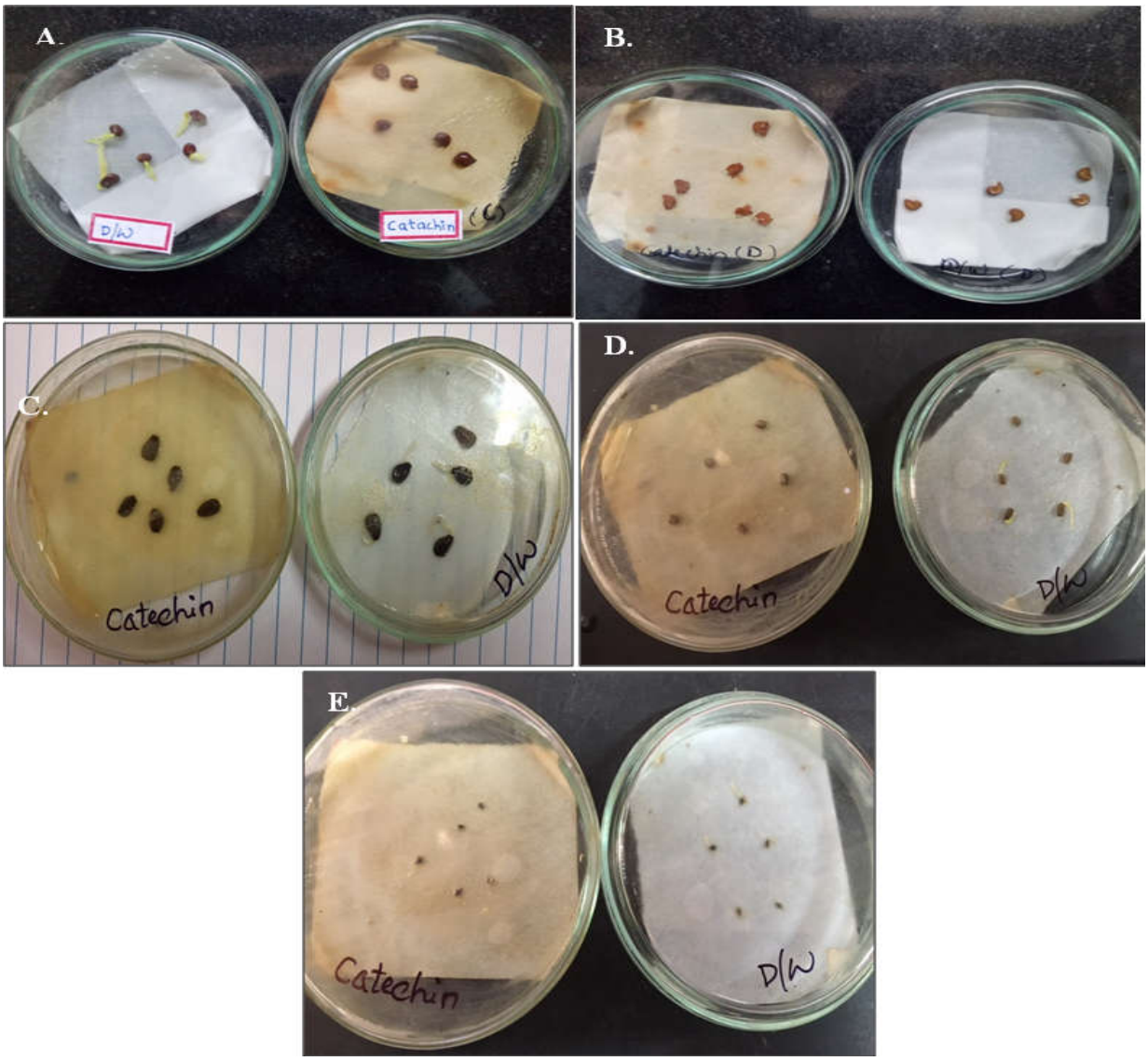


Fig. 5. Inhibition of seed germination by catechin

A: ***Sennaoccidantalis*****B:** ***Daturastramonium*****, C:** ***Calotropis. procera,*** **D:***Cassia tora,* **E:** ***Partheniumhysterophorus.***

The chloroform and ethyl acetate extraction are shown in the fig. 2. Further the extraction is evaporated at 80° C to get the brown coloured catechin powder as shown in Fig. 3. The UV-VIS absorption spectra of catechin dissolved in 1% methanol is shown in the Fig.4. The maximum absorption at 272nm indicates the presence of catechin in the solution.

Inhibition of the seed germination of selected plants by catechin: After 24 hrs of incubation there was no seed swelling observed in the seeds soaked in the TT (Test-tubes) containing catechin (Test) when compared with the seeds soaked in the TT containing D/W (Control). As no swelling was observed in the catechin treated seeds, no germination was observed when put onto the wet filter paper into Petri plate compared with the control Petri plate. The seed germination of catechinand D/W seeds in the Petri plates are shown in the Fig. 5.

Conclusion

The purified catechin from the Green tea can inhibit the seed germination of the *Senna occidantalis, Datura stramonium, Calotropis procera, Parthenium hysterophorus* which is poisonous to the cattle and children. The no germination was observed when the seeds were soaked in catechinand distributed on the filter paper in the Petri-plate compared with the seeds soaked in the distilled water. This study paves the way for further attention and research to study the inhibition effect of catechin on the seed germination of the unwanted (weeds) and poisonous terrestrial and aquatic plants which interfere with the utilization of land and water resources and thus adversely affect human welfare causing health and environmental hazards.

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