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BIO-EFFICACY OF *EUPHORBIA HIRTA* L. ON THE GROWTH AND ANTI OXIDANT ACTIVITY OF *CICER ARIENTINUM* L.

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Abstract

Euphorbia hirta is a common weed found throughout the hotter part of India and are characterized by presence of milky latex. A study was carried out to check the allelopathic effect of *Euphorbia hirta* L. leaves on the growth of *Cicer arientinum* L (Chick pea) seeds. Leaves were collected to prepare extract, leachate and dried paste and *Cicer* seeds were treated for two hours. After 2 h., coated *Cicer* seeds were transferred to individual pots and kept for germination. The vegetative parameters were calculated after twenty-eight days. The study of these vegetative parameters indicated that there is positive plant to plant interaction. Treated *Cicer* plants showed significant increase in root-shoot length, number of leaflets, fresh and dry weight as compared to plant grown with distilled water. The effect of anti-oxidant enzymes showed significant rise in *Cicer* leaves treated with extract and leachate as compared to distilled water. It can be speculated that the anti-oxidant property, Flavonoids, terpenoids, phenols, essential oil and other compounds of fresh and dried leaves of *Euphorbia* showered positive effect on growth of *Cicer* plant. Further anti-fungal analysis of the *Euphorbia* fresh leaf extract on *Fusarium* species proved effective and the phytochemical extracted can be used to control fungal growth. GC-MS analysis showed presence of Penta decanal, Oleic acid and Neophytadiene, all inhibitory fungus antimicrobial compounds.

Key words: *Euphorbia hirta*, Amylase, Catalase, *Cicer arientinum*, Dehydrogenase, Flavonoids, Phenolics, Protease.

Introduction

Euphorbia hirta is used to cure several indicators of gastro intestinal disorders, respiratory diseases, urinary and various ocular ailments. The latex of the plant is often used against pathogen infection (Asha *et al*., 2014)

Euphorbia hirta is composed of Flavonoids, terpenoids, phenols, essential oil and other compounds (Kausar *et al*., 2016). The whole plant of *E.hirta* possesses anti-bacterial, anti-amoebic, anti-fungal, anti-viral, spasmolytic, anti-diarrheal, analgesic, anti-inflammatory, anti-malarial and anti-hypertensive properties (Nyeem *et al*., 2017)

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The oxidative defence systems include several anti-oxidant enzymes such as catalase, dehydrogenase, protease and amylase (Hatata and Darier, 2009). There are numerous reports that catalase, dehydrogenase, protease and amylase seem to play a vital role during germination and growth (Maiti *et al*., 2010).

Cicer (desi chana) is an important source of cheap protein with high energy and nutritive value (Cokkizgin, 2012). *Cicer* seeds contain Malic and Oxalic acids which lower blood cholesterol levels (Snafi, 2016).

The aim of the present study was to analyse the allelopathic potential of *Euphorbia* fresh and dried leaves as well as the effect of leachate on germination and growth of *Cicer* seeds. The Bioassays was carried out to study the effect on *Cicer* leaves and compared with control. The anti-fungal activity of *Euphorbia* leaves was also analysed against *Fusarium.*

Table 1: Quantitative analysis of Phytochemicals in *Euphorbia hirta* fresh leaf extracts (aqueous).

Total Phenolic content (mg/ml)	Total Flavonoid content (mg/ml)
3.0±0.01**	1.2±0.3**
Note: Each value is expressed as mean (n=3). Here ** stands for (p>0.05)	

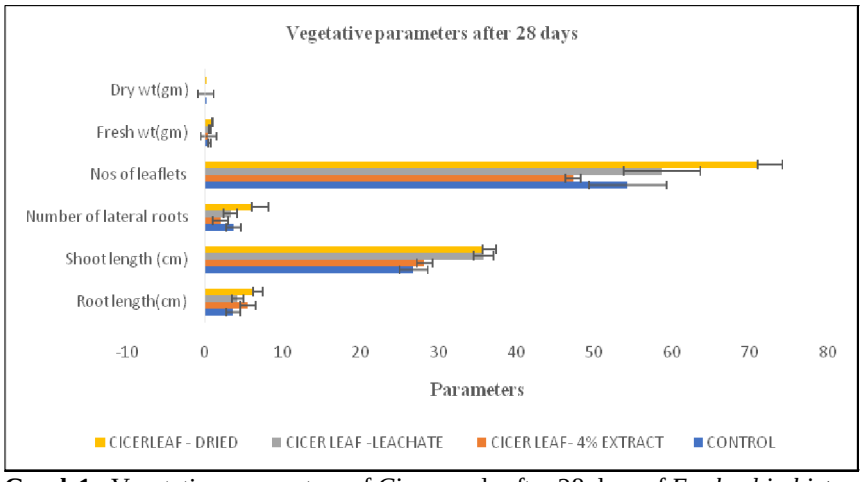
Materials and methods

Plant extracts

Disease free and fresh *Euphorbia hirta* L. plant was collected from Changa campus. The leaves were separated and washed under tap water. The fresh leaves were used to prepare 4% fresh leaf extract and leachate. 4% fresh leaf extract was prepared by crushing 4 g fresh leaves in distilled water. For Leachate preparation 250 g of *Euphorbia* fresh leaves were soaked for 24 h. in 500 ml distilled water. Similarly, 10 g of leaves were oven dried at 55-60ºC for 24 h. and used to make dried leaf paste with required quantity of water. The remaining 4% *Euphorbia* fresh leaf extract, fresh leaf leachate was stored in refrigerator at 5-10ºC and dried leaf paste was stored in oven. The study was carried out from June, 2017 to February, 2019.

Vegetative parameters

Cicer seeds (desi chana) were purchased from D Mart store, Ahmedabad. Ten non-sterilized *Cicer* seeds each were soaked in 4% *Amaranthus* fresh leaf extract, *Amaranthus* fresh leaf leachate and coated with Amaranthus dried leaf paste for 2 h. (Lalitha *et al*., 2012). After 2 h. the treated *Cicer* seeds was shifted to pots and kept for germination under natural conditions (25-29ºC). A total of three replications of treated *Cicer* seeds were kept undisturbed and watered at regular intervals. *Cicer* seeds with distilled water were kept as control. The readings for vegetative parameters of *Cicer* plant like germination percentage, root length, shoot length, number of lateral roots and leaflets, fresh and dry weight was taken after 28 days regularly.



Graph 1: Vegetative parameters of *Cicer* seeds after 28 days of *Euphorbia hirta*– 4% fresh leaf extracts, fresh leaf leachate and dried leaf paste treatment.

Table 2: Effect of *Euphorbia hirta* 4% fresh leaf extracts (aqueous) on *Fusarium* species.

Type	*Fusarium oxysporum* (sp. chlamydosporum, FOCh) (mm)	*Fusarium oxysporum* (sp. pallidoroseum, FOP) (mm)	*Fusarium oxysporum* (sp. vasinfectum, FOV) (mm)
Euphorbia fresh leaf extract (mg / ml)	2.4±0.2**	2.3±0.1**	2.1±0.3**
Note: Each value is expressed as mean (n=3). Here ** stands for (p>0.05)			

Bioassays

Dehydrogenase enzyme activity was determined by 2, 3, 5-Tetrazolium chloride reduction technique. One gram of leaf was mixed with 0.1 gram Calcium carbonate and 1 ml of 1% TTC solution. The mixture was shaken and incubated for 24 h. at 30ºC. The resultant slurry was extracted with methanol and later volume was made up to 50 ml by adding methanol. The OD was taken at 485 nm on Shimadzu UV Spectrophotometer (Ojha, 2013).

Similarly, Catalase enzyme activity was estimated by the rate decomposition of Hydrogen peroxide (H_2O_2). The reaction mixture containing 1.5 ml Phosphate buffer, 1.2 ml H_2O_2 and 300 µl enzyme extract with O D was taken at 240 nm on Shimadzu UV Spectrophotometer (Ojha, 2013).

Amylase enzyme activity was determined by method developed (Ojha, 2013). The supernatant (1 g of leaf was homogenized with 10 ml of 0.1M phosphate buffer (pH= 6.5)) was taken as the crude source of the enzyme. 1 ml of enzyme solution was mixed with equal volume of 0.1% starch solution in 0.1 M sodium acetate buffer, pH =5.0 incubated at 37ºC for 10 min. The reaction was stopped with 3 ml iodine-HCl solution and the intensity of blue colour was measured at 620 nm on Shimadzu UV Spectrophotometer.

Similarly, Protease enzyme activity was estimated by incubating the reaction mixture of 1ml enzyme extract, 0.1 ml 0.1M $MgSO_4.7H_2O$ and 1ml BSA for 1 h. at 37ºC followed by adding 1 ml 50% Trichloro acetic

Table 3: Important compounds identified in the GC-MS analysis of *Euphorbia hirta* fresh leaf extracts.

Peak	Retention time	Area %	Compound name	Mol. Wt.
1	19.94	69.28	Penta decanal	226
2	20.21	16.88	Oleic acid	282
3	20.44	13.84	Neophytadiene	278

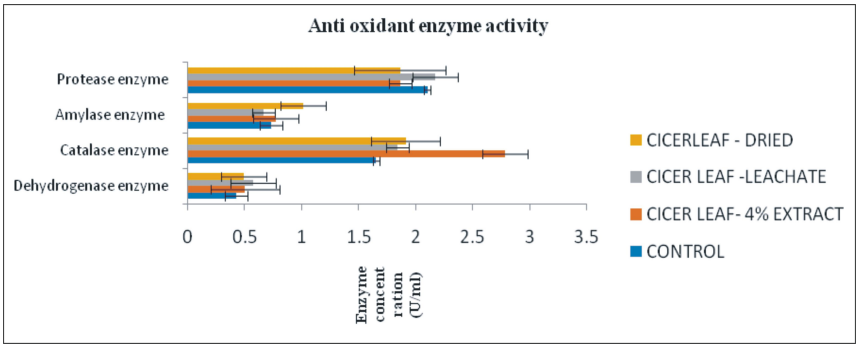
acid (TCA) and subsequent with 1 ml Folin reagent was carried out at 650 nm on Shimadzu UV Spectrophotometer (Ojha, 2013).

Phytochemical constituents

• Total phenolic content: Total phenolic content was estimated by Folin Ciocalteu method (Jha *et al*., 2014; Rebaya *et al*., 2014). 1 ml leaf extract was mixed with 5 ml distilled water, 1 ml sodium carbonate (20%) and 1 ml Folin Ciocalteu reagent. The mixture was allowed to stand in water bath at 40ºC for 30 min. The absorbance was measured at 765 nm using UV spectrophotometer. Standard graph was prepared by using different concentrations of phenol crystals.

• Total Flavonoid content: Flavonoid content was determined by Aluminium Chloride method (Jha *et al*., 2014; Rebaya *et al*., 2014) using Catechin as the reference compound. A volume of 125µl of extract is added to 75 µl of 5% $NaNO_2$ solution. The mixture is allowed to stand for 6 min, then 150 µl of Aluminium chloride was added and incubated for 5 min, followed by addition of 750µl of NaOH (1M). The final volume was adjusted to 2500 µl with distilled water. After 15 min the mixture turned to pink and the absorbance was measured at 510 nm.

• Gas Chromatography and Mass Spectroscopy Analysis: Analysis by GC-MS was performed using Thermo GC- Trace Ultra Ver: 5.0. Pyrolysis auto sampler interfaced to a Perkin Elmer Turbo mass Gold equipped with a fused silica capillary column. The fraction was pyrolyzed at 610ºC and then introduced to the GC column. Helium was employed as carrier gas (1ml / min).



Graph 2: Allelopathic effect of *Euphorbia hirta*– 4% fresh leaf extracts, fresh leaf leachate and dried leaf paste on anti-oxidant activity of *Cicer arientinum* leaves.

Table 4: Growth inhibition of *Fusarium oxysporum* sp. Chlamydosporum (FOCh).

FOCh growth (control) (g/100 ml)	FOCh growth + 4% extract (g/100 ml)
4.66±0.1*	1.96±0.01*
Note: Each value is expressed as mean (n=3). Here * stands for ($p<0.05$)	

Qualitative identification of the different constituents was performed by composition of the relative retention times and mass spectra with those of authentic reference compounds by retention indices (RI) and mass spectra. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) (Jha *et al*., 2014)

• Antifungal activity assay: Agar well diffusion method was used where a drop of culture suspension was placed in the centre of nutrient agar plate and spread all over the plate with sterile spreader. Three wells were made on the Potato dextrose agar medium containing plate with sterile cork borer and wells were filled with 100 µl of plant extract. Plates were observed for zone of inhibition by measuring the colony diameter (Jha *et al*., 2014). Sterile water was used as control. Further 100 ml Potato Dextrose Broth was prepared in conical flask and it was inoculated with *Fusarium oxysporum* sp. *chlamydosporum* and tested against 10 ml *Euphorbia* fresh leaf extract (aqueous).

• Statistical analysis: All the experiments were carried out in a randomized manner. The data for germination percentage, root length, shoot length, number of lateral roots; number of leaflets, fresh weight and dry weight were calculated using SPSS/PC software. One way analysis of variance (ANOVA) test was used to analyse all data and mean.

Results and Discussion

Leaf extracts

Cicer (Chick pea) plant has high nutritive value and with a view to increase this property - *Euphorbia* fresh leaves, fresh leaf leachate and dried leaves were used. Aqueous 4% extract of *Euphorbia* fresh leaf, *Euphorbia* leachate and *Euphorbia* dried leaf paste significantly increased the germination percentage, root and shoot length, number of leaflets, fresh as well as dry weight of treated *Cicer* plant as compared to distilled water in readings taken after 28 days (Graph 1). The low amount of weed powder increased the

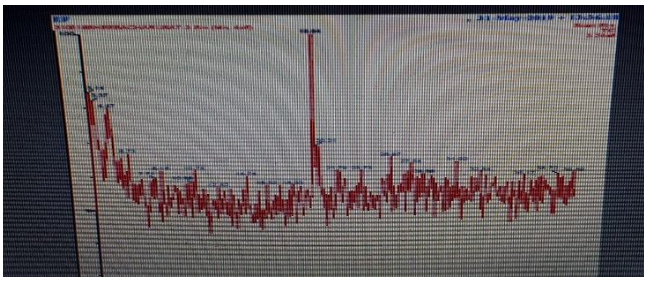


Fig. 1: GC-MS analysis of *Euphorbia hirta* fresh leaf extracts.

amount of organic matter in soil, resulting in the better growth than the control (Jabeen, 2013). The extracts of *Parthenium hysterophorus* root, *Datura stramonium* root and stem and *Argemone mexicana* leaf stimulated elongation of Wheat seedlings due to induction of growth promoting hormones (Gella *et al*., 2013). Allelochemicals can stimulate or inhibit plant growth depending on their concentration (Babar *et al*., 2009). The leaf extracts of *Celosia* and *Euphorbia* are highly rich in various essential macro and micro elements, bioactive compounds like terpenoids, steroids, flavonoids, pungent and bitter essential oils and phenols etc. All these might have caused the significant improvement in yield and yield parameters in mung bean, chick pea and sorghum acting in combination and individually (Plummer, 2017).

Anti-oxidant enzymes

Maximum increase in Dehydrogenase, Catalase, Amylase and Protease activity was recorded in *Cicer* leaves treated with Aqueous 4% extract of *Euphorbia* fresh leaf and Euphorbia fresh leaf leachate in contrast to control (Graph 2). Dehydrogenase enzymes catalyse the reversible reduction of pyruvate to lactate with $NADH_2$ as the co enzyme (Shora and Gawad, 2014). Anti-oxidant Catalase enzyme increases when plants are treated with phenolic compounds at low level (Balakireva and Zamyatnin, 2018). Amylase activity increases slowly during initial days of germination and convert starch to soluble sugars needed for growth (Laware, 2014). Proteases activate different signalling processes by carrying out controlled Proteolysis (Saswade, 2015).

Anti-fungal activity

The quantitative analysis of *Euphorbia* fresh leaf extract showed the presence of Phenolics and Flavonoids (Table 1). The aqueous *Euphorbia* fresh leaf extract was tested against *Fusarium oxysporum* (sp. *Chlamydosporum*, sp. Pallidoroseum and sp. *Vasinfectum*) and it was found to be effective. The zone of inhibition was highest in *Fusarium oxysporum* sp. Chlamydosporum (Fig. 2 & Table 2). Further analysis by GC-MS isolated 'Penta decanal', Oleic acid and Neophytadiene a phytochemical (Flavonoid) (Fig. 1 & Table 3). There is a possibility of using these Allelochemicals directly or as structural leads for the discovery and development of environment friendly herbicides (Nasrine, 2013). Fungal growth in potato dextrose broth was 4.66g/100ml and in flask with potato dextrose broth having extract was 1.96g/100ml (Table 4).

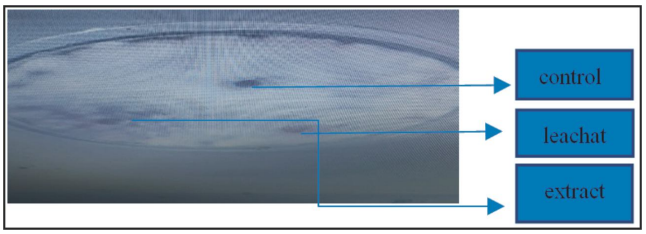


Fig. 2: Antifungal activity of *Euphorbia hirta* fresh leaf extracts (aqueous) on *Fusarium oxysporum* (sp. Chlamydosporum) (FOCh).

Conclusion

Allelopathy has been recognized in weed-crop interaction and its beneficial effects are being applied for obtaining better crops and yields in agricultural production (Ishak and Shahid, 2014). The germination parameter indicates that *Euphorbia* fresh, dried leaves and leachate can be used for germination in further experiments with different edible seeds. Carbohydrate, Proteins and lipids are the store house of food material. It shows that *Euphorbia* fresh, dried leaves and leachate have both the potential, one is germination and second is positive Biochemical parameters and also anti-fungal property. Allelopathy is a form of plant interference that can significantly influence ecosystem and agroecosystem dynamics (Trezzi *et al*., 2016).

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Conflict of interest

Conflict of interest declared none.

References

Asha, S., B. Deevika and S.A. Mohammed (2014). *Euphorbia hirta* Linn-A review on traditional uses. *Phytochemistry and Pharmacology.*, **3(4):** 1-27.

Babar, H.B., A. Tanveer, T. Muhammad, A. Ahsan, A.U.H. Ahmad, A.N. Muhammad and M.J. Muhammad (2009). Allelopthic potential of wild onion (*Asphodelus tenuifolius*) on the germination and seedling growth of chick pea (*Cicer arientinum*). *Weed Biology and Management.*, **9:** 146-151.

Balakireva, A.V. and A.A. Zamyatnin Jr (2018). Indispensable role of Proteases in Plant Innate community. *International Journal of molecular Sciences.*, **19(629):** 1-26.

Cokkizgin, A. (2012). Botanical characteristics of chick pea genotype (*Cicer arientinum* L.) under different plant densities in organic farming. *Scientific research and essay.*, **7(4):** 498-503.

Gella, D., A. Habtamu and N. Takele (2013). Allelopathic effect of aqueous extracts of major weed species plant parts on germination and growth of Wheat. *Journal of Agriculture and Crop research.*, **3:** 30-35.

Hatata, M.H. and S.M.E. Darier (2009). Allelopathic effect and oxidative stress induced by aqueous extract of *Achillea santolina*. L shoot on *Triticum aestivum*. L. *Egyptian Journal of Experimaent Biology (Botany).*, **5:** 131-141.

Ishak, M.S. and I. Shahid (2014). Allelopathic effects of the aqueous extract on the leaf and seed of *Leucaena leucocephala* on three selected Weed species. *AIP conference proceedings.*, **1614:** 659-664.

Jha, Y., R.B. Subramanian and S. Sahoo (2014). Antifungal potential of fenugreek, coriander, mint, spinach herbs extracts against *Aspergillus niger* and *Pseudomonas aeruginosa* phyto-pathogenic fungi. *Allelopathy Journal.*, **34(2):** 325-334.

Jabeen, N., M. Ahmed, S.S. Shaukat and Iram-Us- Slaam (2013). Allelopathic effects of Weeds on Wheat (*Triticum aestivum* L.) germination and growth. *Pakistan Journal of Botany.*, **45(3):** 807-811.

Kausar, J., D. Muthumani, A. Hedina, Sivasamy and V. Anand (2016). Review of the Phytochemical and Pharmacological activities of *Euphorbia hirta* Linn. *Pharmacognosy Journal.*, **8(4):** 310-313.

Lalitha, P., K. Shivani and R. Rama Rao (2012). *Parthenium hysterophorus*-An economical tool to increase the agriculture productivity. *International Journal of Life Science, Biotechnology and Pharmacy research.*, **1(1):** 113-127.

Laware, S.I. and S. Raskar (2014). Effect of Titanium Dioxide Nanoparticles on Hydrolytic and Anti-oxidant enzymes during seed germination in Onion. *International Journal of current Microbiology and Applied Sciences.*, **3(7):** 749-760.

Maiti, P.P., R.K. Bhakat and A. Bhattacharjee (2010). Evaluation of Allelopathic potential of an obnoxious weed using Mung bean as a Bioassay material. *International Journal of Science and Nature.*, **1(2):** 236-241.

Nyeem, M.A.B., M.S. Haque, M. Akramuzzaman, R. Siddika, S. Sultana and B.M.R. Islam (2017). *Euphorbia hirta* Linn. A wonderful miracle plant of Mediterranean region. *Journal of medicinal Plant studies.*, **5(3):** 170-175.

Nasrine, S., S.M.E.L. Darier and H.M.E.L. Taher (2013). Allelopathic effect of *Euphorbia guyoniana* aqueous extract and their potential uses as natural herbicides. *Sains Malaysia.*, **42(10):** 1501-1504.

Ojha, S. (2013). Chemical manipulation of seed viability, plant growth, metabolism, productivity and Phytochemical analysis of *Abrus precatorius* L. and *Dolichos biflorus* L. (Ph.D. thesis).

Plummer, D.T. (2017). An introduction to Practical Biochemistry. 3rd Edition, McGraw Hill Education (India) Private Limited, Chennai.

Rebaya, A., S.I. Belghith, B. Baghdikian, V.M. Leddet, F. Mabrouki, E. Olivier, J.K. Cherif and I.M.T. Ayad (2014). Total Phenolic, Total Flavonoid, Tannin content and Antioxidant capacity of *Halimium halimifolium* (Cisteraceae). *Journal of Applied Pharmaceutical Science.*,**5(1):** 52-57

Snafi, A.E.A. (2016). The medical importance of *Cicer arientinum*-A review. *International Organization of Scientific Research Journal of pharmacy.*, **6(3):** 29-40.

Shora H.M.E. and A.M.A.E. Gawad (2014). Evaluation of Allelopathic potential of *Rumex dentatus* root extract and Allelochemicals on *Cicer arientinum*. *Journal of Stress Physiology and Biochemistry.*, **10(1):** 167-180.

Saswade, R.R. (2015). Allelopathic potential of Celosia and Euphorbia leaf extracts on yield per plant (gm) in Mung bean, Chick pea and Sorghum. *International conference on Plant, Marine and Environmental Science (PMES-2015), Kuala Lumpur (Malaysia).*, **1-2:** 50-53.

Trezzi, M.M., R.A. Vidal, A.A.B. Junior, H.V.H. Bittencourt and P.D.S.S. Filho (2016). Allelopathy: driving mechanisms governing its activity in agriculture. *Journal of Plant Interactions.*, **11(1):** 53-60.