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Versha Upadhyay
Department of Botany,
Maya Group of Colleges,
Selaqui, Dehradun,
Uttarakhand, India

Corresponding Author:
Versha Upadhyay
Department of Botany,
Maya Group of Colleges,
Selaqui, Dehradun,
Uttarakhand, India

Ageratum conyzoides on seed germination of mung (*Vigna rediata*)

Versha Upadhyay

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Abstract
Allelopathy is the inhibition or stimulation by chemicals which is secreted by plants and microorganisms on the growth of seed germination of other plants. Use of chemical herbicide in exes amount to control the growth of weed. These herbicide increase weed resistant and create harsh environmental pollution. *Ageratum conyziodes* is one of the weed which effect the seed germination. In this experiment observe the seed germination percentage on various concentration And it was examine on different parameter like GP (Germination Percentage), GI (Germination Index), GRI (Germination Rate Index), MGT (Mean of Germination Time), GE (Germination energy), and Sum #germination. Seed Germination were reduced by 90 %, 60 % and 40 % and there is no germination on 100% concentration compared to the control, respectively.

Keywords: Allelopathy, inhibition, microorganism's herbicide, *Ageratum conyziodes* germination

1. Introduction
Allelopathy is the phenomenon of inhibition or stimulation by chemicals which is secreted by one plant/microorganism on the growth on seed germination of other plants (Farooq *et al.*, 2011) [6]. Use of chemical herbicide in exes amount to control the growth of weed. These herbicide increase weed resistant and create harsh environmental pollution. Other weed management activities are eco-friendly but cost effective and time demanding issue throughout the world. Phytotoxic plants might help in resolving the problems created by synthetic herbicide as they possess growth retarding substances. Recently increasing interest into the researchers for the work on medicinal plants because it is easily screened from medicinal plants. The increasing interest on medicinal plants could be due to either (i) the easier screening process of phytotoxic plants from medicinal plants or (ii) the possibility to have more bioactive compounds in medicinal plants than other plants. These phytotoxic plants could be used in several ways to control weeds, for example, (i) sowing/transplanting them as relay or cover crops with main crops, (ii) direct application of their crude extracts as bio herbicides, or (iii) isolation and characterization of their active substances and using them as a tool for new natural and biodegradable herbicides development Mominul & Noguchi (2014) [11].

Nutritional value: Mung beans contain highly nutritive value. It contain 65% carbohydrate in 650g/kg dry weight and it is rich in protein, vitamins, and minerals. Around 20% -50% protein of total dry weight, with globulin 62% and albumin 27% are strongest proteins in mung beans. Mung beans are considered to be strongest protein and substantive source of dietary proteins (Yi-Shen, *et al*. 2018) [18]. The proteolytic cleavage of these protein are even higher during sprouting. Bean's carbs are easy to digest, which causes less flatulence in human compared to other forms of legumes. Both seed and sprout of mung bean produce lower calories compares to other cereals, which is more useful for obese and diabetics persons Piotr *et al*. (2008) [16].

2. Material and Methods
2.1. Plant Materials
Ageratum conyzoides: *Ageratum* is weed plant and presently a major problem for environmentalists, ecologists, farmers and animal scientists. A number of studies have been allied on its control as a weed.

(Anju *et al*. 2019) [2]. Its biological properties of the extracts and the constituents might provide incentive for proper evaluation of the use of the plant in medicine and in agriculture. Test conducted in mice and rats for anti-inflammatory, analgesic, antipyretic, antibacterial, anti-fungal, anti-ulcer, radio-protective activities have shown significant results without adverse side effects Riddiford. (2020) [17]. Similarly, clinical trials with arthritis patients conducted with the aqueous extract of the whole plant did not show any side effect (Paulrayer 2017) [15]. The major constituents of the essential oil have been shown to produce precocious metamorphosis in insect larvae as well as sterility, moribund and dwarfness in adult insects. Jens Rolff, *et al*. (2019) [9]. The ß flavonoids possess wide range of biological activities like its effects on CVS, diuresis, antiviral, spasmolytic, ant-inflammatory properties of the ß flavonoids isolated from the plant need to be studied. Precocenes and coumarins have been seen as fourth-generation insecticides, also need to be studied (Kumar & Pandey 2013) [10]. Traditional communities in India use this species as an ant dysenteric and ant lithic and in Asia, South America and Africa aqueous extract of this plant is used as an antibacterial agent (Almagboul, 1985) [3].
Ageratum conyzoides L., is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from this species. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities. The whole plant is only used for medicinal purposes and has a long history in the folk medicine of different countries. Various extracts of the plant, including water and methanol have been shown to inhibit the growth of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and H. pylori* (Ndip *et al*., 2007) [14].
Durodola (1977) [4] demonstrated the effectiveness of crude extract of this plant in inhibiting the growth of *Staphylococcus aureus*a major wound pathogen in in-vitro cultures of the organism.
Plants of *Ageratum conyzoides* was collected from Dehradun valley. The plant was identified by Botanical Survey of India, Dehradun. The roots of *A. conyzoides* were chopped off leaving the aerial part which was shredded into tiny bits.

Material
Plant material, Methanol, what mann filter paper, grinder, beakers, funnel, laminar air flow, autoclave, Distilled water.

Fig 1: *Ageratum conyzoides*

2.2 Extraction Procedure
Method
The plant material was washed gently with Tin twenty to remove any dirt and to free it from any microbe and was air-dried under shade for a week. The sample was powdered with an electric grinder into a coarse form and stored in airtight containers.

Plant Material	Fresh weight	Dried weight
Ageratum conyzoides L.	500 grams	150 grams

After grinding, 30 gm of plant material was extracted in 130 ml of methanol for 24 hours. The extracts were filtered through Whatmman filter paper and were evaporated to dryness using a hot plate at a much reduced temperature (40 ℃). The residues obtained were dissolved in methanol. The weights of the extract was determined and stored below ambient temperature.

(A) (B)

(C) (D)

Fig 2: (a) shredding of plant parts, (b) & (c) preparation of crude methanol extract, (d) Methanolic extract of plant (green in colour)

2.3. Germination Bioassay
Plant extract was diluted into small amount of distil water to prepare four assay concentration 25 %, 50 %, 75 %, and 100 % and then was spray on filter paper No 1 in petri dishes. Ten seeds of mung was use for germination
Ten seeds of mung placed on the filter paper in Petri dishes. Control Petri dishes were also maintained in each experiment using 10, that is, without plant extracts. The Petri dishes were then incubated in dark at 25 °C. Germination was measured at every 0.5-day interval up to 7 days (the time when no further seeds germinated) and was

considered when the radical emerge by rupturing the seed coat as per (Islam and Kato-Noguchi 2012) [8].

Eight germination indices, that is, germination percentage (GP), germination index (GI), germination energy (GE), mean germination time (MGT), were calculated GP index indicated the total germination percent of a seed lot after certain period of time when germination became constant. As it is measured by total germination relative to total number of seeds set for germination, GP cannot explain the delayed germination. In contrast, GI is a measure of both percentage and speed of germination and assigns maximum arithmetic weight to seeds that germinate during first count and less weight to those that germinate later. The higher the GI, GE, values compared to control, the lower the inhibition, and vice versa. But the meaning is reversed for MGT.

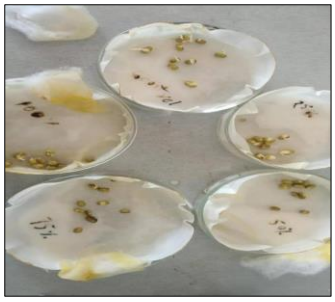
Germination percentage (GP) = No of seed germinated/Total no of seed tested*100he Germination Index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) [1] by following formula:

$$\text{Germination Index} = \sum (GT / Tt) \text{ or } \left[\frac{\text{No. of germinated seed}}{\text{Days of first count}}\right] + \ldots\ldots\ldots + \left[\frac{\text{No. of germinated seed}}{\text{Days of final or last count}}\right]$$

Germination energy (GE) = No of seed germinated on day/ Total no of seed tested*100

Germination Rate Index (GRI) = (G1/1) + (G2/2) + (G3/3) + (Gi/i)+

Mean germination time (MGT) = MGT = ∑(n × d) / Nn = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment (Ellis and Roberts, 1981) [5].



2.4. Growth Bioassay

The Petri dishes and the extracts were prepared as described above. Ten peregrinated seeds of mung, (germinated in the darkness at 25°C for 1–3 days after overnight soaking) were placed on the filter paper in Petri dishes. The shoot and root lengths of each seedling were measured after incubation in dark condition for 2 days at 25°C. Control Petri dishes were also maintained as germination bioassay.

2.5. Statistical Analysis

The bioassay experiments were conducted as completely randomized design (CRD) with three replications. The experiments were repeated twice to avoid any experimental error. The data calculated on different parameter for its verification.

Table: Calculation on Seed Germination

25% concentration														
Seed Mung	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Total No of Seed**	**SUM # Germ.**	**GRI**	**MGT**	**GP**	**GI**	**GE**
Trial on 10 Seed	10	10	10	10	10	10	10	10						
R1	0	0	3	4	6	7	9	10	29	15.99	17.77	90	5.65	290
R2	0	0	4	5	7	7	8	10	31	16.57	20.62	80	4.89	310
R3	0	0	3	3	4	8	9	10	27	16.74	16.88	90	5.16	270
50% concentration														
Seed Mung	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7							
Trial on 10 Seed	10	10	10	10	10	10	10	10						
R1	0	0	3	3	4	5	6	10	21	11.95	18	60	4.24	210
R2	0	0	2	5	5	5	5	10	22	9.64	23.2	50	4.46	220
R3	0	0	4	4	4	6	6	10	24	12.704	21	60	4.99	240
75% concentration														
Seed Mung	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7							
Trial on 10 Seed	10	10	10	10	10	10	10	10						
R1	0	0	2	4	4	4	4	10	18	8.84	23.5	40	3.7	180
R2	0	0	3	3	3	4	4	10	17	8.73	22	40	3.58	170
R3	0	0	3	3	4	4	4	10	18	8.93	23.25	40	3.78	180
100% concentration														
Seed Mung	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7							
Trial on 10 Seed	10	10	10	10	10	10	10	10						
R1	0	0	0	0	0	0	0	10	0	0	0	0	0	0
R2	0	0	0	0	0	0	0	10	0	0	0	0	0	0
R3	0	0	0	0	0	0	0	10	0	0	0	0	0	0
Controlled														
Seed Mung	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7							
Trial on 10 Seed	10	10	10	10	10	10	10	10						
R1	1	2	4	5	7	8	10	10	37	21.6	19	100	8.74	370
R2	2	3	5	6	7	7	10	10	40	23.51	19.4	100	10.66	400
R3	1	3	4	6	6	8	9	10	37	20.72	20.37	100	9.15	370

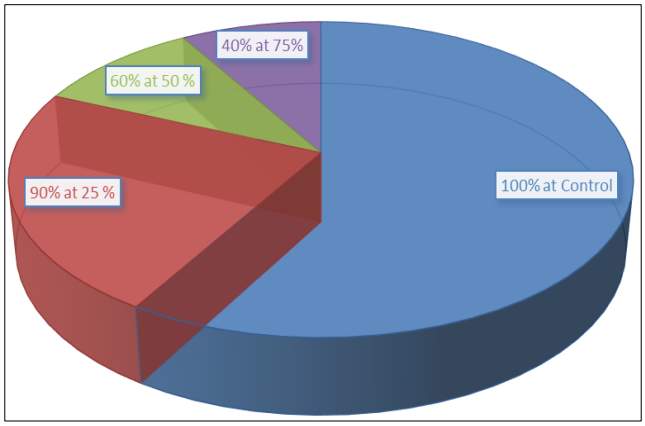


Fig 3: Allalopathic effect of A. CONY zoides

Conclusion

The chemical components analysis of different extracts of *Ageratum conyziodes* in our study, I found that *Ageratum conyzoides* whole plant significantly inhibited the germination and reduced the varieties and biomass of weeds in the field, when it was applied as a fertilizer originally. Therefore, I think that certain allelochemicals present in might inhibit the growth of weeds. To investigate the possible allelochemicals in *Ageratum conyzoides*, solvents (methanol) was used to extract the metabolites in *Ageratum conyzoides* leaves. The crud extract was analyzed as shown in Table 1 that extract reduced germination of weed species tested. Seed Germination were reduced by 90%, 60% and 40% and there is no germination on 100% concentration compared to the control, respectively.

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