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Study of Allelopathic Interactions of weeds on of Wheat (*Triticum aestivum* L.) and Moong (*Vigna radiata*) using Equal-Compartment-Agar Method (ECAM)

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Abstract

Equal-Compartment-Agar Method (ECAM) is employed in the current work to understand the allelopathic nature of five common weeds of India on two major crops. The weeds under consideration were *Hyptis sauveolens* (L.), *Ricinus communis* (L.), *Alternanthera sessilis* (L.), *Ipomoea carnea* (Jacq.), *Malachra capitata* (L.) and *Cymbopogon citrutus* (Stapf) were used for the study and its effect on Wheat (*Triticum aestivum* L.) and Moong (*Vigna radiata*) are studied. The studies were carried out using agar method in six well Petri dish. The crop seeds were germination in sterilized agar using the powdered leaf extracts of the weeds at 10 and 50 mgs of dry leaf powder. The length of the radicles of both the crops were measured after three days and compared with a control. Overall Allelopathic Potential of the five weeds were derived from the rate of inhibition of radicle growth. Wheat was most affected by the weeds in the study while moong showed marginal effects. Weed litter at 50mgs affected both the crops severely.

Keywords: Compartment-Agar Method, Weeds, Crops, Overall Allelopathic Potential

INTRODUCTION

Allelopathy is a mechanism in which live or dead plant materials release chemical substances, which inhibit plant growth (1, 2). Allelopathy may also play an eminent

role in the intraspecific and interspecific competition and may determine the type of interspecific association. The plant may exhibit inhibitory or rarely stimulatory effects on germination and growth of other plants in the immediate vicinity.

Considerable amount of literature on allelopathy, its implications, methodology, and ecological significance is available (3, 4, and 5). A suppressive effect on weed, possibly mediated by the release of allelochemicals has been reported for a wide range of temperate and tropic crops. These include alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), clovers (*Trifolium* spp., *Melilotus* spp.) oats (*Avena sativa*) pearl millet (*Pennisetum glaucum*), rice (*Oryza sativa*) rye (*Secale cereale*), sorghums (*Sorghum spp*.), sunflower (*Helianthus annus*), sweet potato (*Ipomoea batatas*) and wheat (*Triticum aestivum*) (2). Laboratory bioassay is the first step used to investigate the possible involvement of allelopathy (6)

ECAM provides a rapid, simple, and inexpensive procedure for the initial screening of the allelopathic potential of a crop against a target weed species under laboratory conditions (7) in the current work the effect of leaf residues of weeds on two important crop seeds of India viz, Wheat (*Triticum aestivum* L.) and Moong (*Vigna radiata* L) has been studied using ECAM.

MATERIALS AND METHODS

Leaves of *Hyptis sauveolens (L.), Ricinus communis (L.), Alternanthera sessilis (L.), Ipomoea carnea (Jacq.), Malachra capitata (L.) and Cymbopogon citrutus (Stapf)* were used for the study. These are common weeds seen in the state of Maharashtra. The leaves of individual species were, cut into small pieces and dried at 60 degrees Centigrade, powdered, sieved and stored in Ziploc bags. Seeds of Wheat (*Triticum aestivum* L.) and Moong (*Vigna radiata*) were used to study the effect of weeds on seeds germination.

i) Overview of Sandwich Method:

The Sandwich Method involves the placing of pre-weighed samples of dried plant material into the wells of a six well plate. Each well has an area of 10 cm^2,which corresponds to 10 or 50mg powered litter in each well, which is equivalent to litter deposition rates of 10 gm^{-2} and 50 gm^{-2}. This is the low and high ends of natural litter deposition rates. Each well volume is close to 10 ml so the equivalent concentrations of 10 mg and 50 mg leaf material were therefore 1 mg ml^{-1} & 5 mg ml^{-1} that is 1 gl^{-1} and 5 gl^{-1} respectively.

ii) Sandwich Method Protocol. Powdered plant materials were carefully weighed then gently tipped into the wells of a six well multiwall plate .The top row of 3wells were filled with 10 mg of plant sample per well. The bottom three were filled with 50 mg plant sample per well. Three multi dishes were usually filled per sample, giving three replicates per sample, with three repeats. A control dish was set up for each experimental run, using agar only.

iii) Type Of Agar And Its Preparation

Following Fujii's protocol (8), low temperature agar was used. The agar 0.75% concentration w/v in water was boiled in a microwave to ensure that the agar melted properly and it was then decanted, cooled and five milliliters of the agar was then carefully pipetted into each of the sample and control wells using a pipette and allowed to set. This took approximately 30 minutes. A further 5 ml of agar was pipetted on top of the first layer. A sterilized needle was used to push the risen plant material below the surface so that a uniformly smooth upper surface was created when the agar set.

iii) Arrangement of seeds and incubation of multiwell plates

4 surface sterilized seeds of respective crop species were placed horizontally on the surface of the agar in each well using a pair of forceps.100 seeds of the crops were studied.

The seeds were arranged in a regularly spaced crisscross pattern. The lids of the plates were then closed and sealed with laboratory tape to prevent desiccation of the agar. The plates were wrapped in Aluminium foil to20°C for three days. The multiwell plates were opened for measurement. The number of germinating seedlings was recorded and then the seedlings with the longest and shortest radicles in each well were discarded in order to maintain the central tendency and normality of the data. The radicle and hypocotyl lengths of each of the remaining three seedlings were then measured. Percentage elongation relative to control was calculated and converted to percentage inhibition, where 0% represents no inhibition and 100% complete inhibition. In order to rank the data collected from separate experiments in terms of their allelopathic effects by plant organ and also by species, the concept of overall allelopathic potential (OAP) was applied for this study (4)

Calculations were made using the formula:

$$OAP = \text{mean } (I10 + I50) / 100$$

Where I10 = % inhibition of radicle growth compared to the control at 10 mg concentration and I50 = % inhibition of radicle compared to the control at 50 mg

concentration. Using the mean of the sum of the radical percentage inhibitions divided by 10, a score between 0.0 and 1.0 was obtained and the data were ranked according to this score. A maximum score of 1.0 would indicate that the test material had totally inhibited growth, while a score of 0.0 would indicate that no allelopathic inhibition had occurred.

RESULTS AND DISCUSSIONS:

Maximum inhition in the length of radicle of *Vigna radiata* was seen in seeds exposed to *Malachra capitata (L.) Ipomoea carnea (Jacq.),* and *Alternanthera sessilis (L.),* at 10 mg of dry weed litter. At 50mg more than 90% inhibition was seen for all the weeds. While in 50mg the trend in inhibiton of radicle grwoth was of radicle was *Alternanthera sessilis (L.), Malachra capitata (L.)* and *Ipomoea carnea (Jacq.).* Table 2.0 and Fig 1.0

Percentage inhibiton in radicle of *Triticum aestivum* after 3 days at 10 mgs of weed litter showed that *Alternanthera sessilis (L.) affected the most, followed by Cymbopogon citrutus (Stapf) and Hyptis sauveolens (L*.). at treatment of 50mgs of weeds litter all the weeds inhibited the growth of the radicle severely with more than 90% of inhibition in all weeds with the exception of *Ricinus communis(L.)* which showed 88% inhibition (Table 3 and Fig 2).

Overall Allelopathic Potential : In order to rate the allelopathic potentials of the weeds in the current study upon the crops under consideraton , concept of overall allelopathic potential (OAP) developed by Smith.O.P ,2013 is used. Where the formula compares the growth of the radicle within the weed residue concentrations. The formula depicts the inhibition of growth of seedlings at 10mg and 50mgs of weed matter in terms. The ratio thus obtained, if it is more towards 1 showed high allelopathic effect (Table 3).

It can be seen from Table 4, and Fig 3, the overall allelopathic potentials of the five weeds under study and two crops, that *Triticum aestivum* is more affected than *Vigna radiata.* The allelopathic effect of *Alternanthera sessilis (L.),* was more than *Cymbopogon citrutus (Stapf)* and *Hyptis sauveolens (L.) on wheat.* However all the weeds showed a high allelopathic potential on wheat with a value of more than 0.5 OAP.

TABLES AND FIGURES

Table 1: Effect of different concentrations of leaf litter on average length of radicle of *Vigna radiata* by ECAM in 3 days

Species	Control	Length in cms 10mg	Percentage inhibition	Length in cms 50mg	Percentage inhibition
Ipomoea	3.726	2.9	22	2.4	35
Cymbopogon	3.726	3.1	16	3	19
Ricinus	3.726	3.5	6	3.7	0.6
Hyptis	3.726	3.4	8	3.1	16
Malachra	3.726	2.6	30	2.6	30
Alternenthera	3.726	3	19	1.8	51

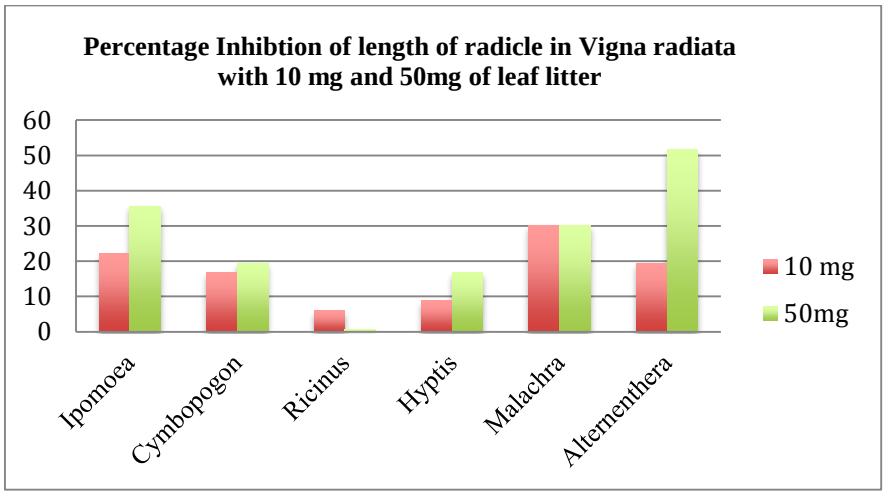

Fig 1: Percentage difference in length of radicle of *Vigna radiata* from control by ECAM in 3 days

Table 2: Average length of radicle and percentage inhibition in *Triticum aestivum* after 3 days by ECAM with 10mg and 50 mg of leaf litter

Plant species	Control	10mg	Percentage Inhibition	50mg	Percentage Inhibition
Ipomoea	5.425	3.9	26	0.2	95
Cymbopogon	5.425	2.5	53	0.2	96
Ricinus	5.425	3.9	27	0.6	88
Hyptis	5.425	3.5	35	0.2	95
Malachra	5.425	4.6	14	0.2	95
Alternenthera	5.425	2	63	0.1	97

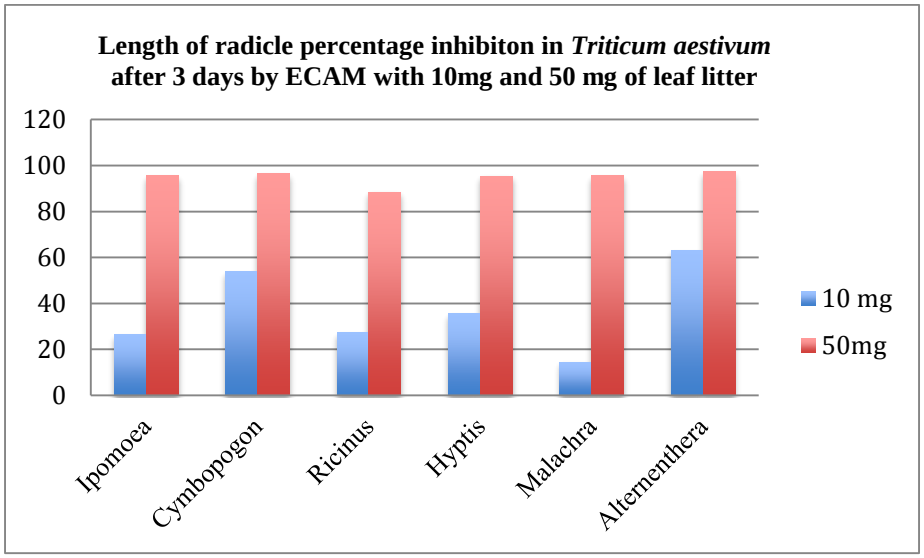

Fig 2: Length of radicle and percentage inhibiton in *Triticum aestivum* after 3 days by ECAM with 10mg and 50 mg of leaf litter

Table 3: Categories used to determine Allelopathic potential using OAP score

OAP SCORES	DESCRIPTION
0.00-0.25	NON-ALLELOPATHIC
0.26-0.50	MODERATELY ALLELOPATHIC
0.51-0.75	HIGHLY ALLELOPATHIC
0.76-1.0	EXTREMELY ALLELOPATHIC

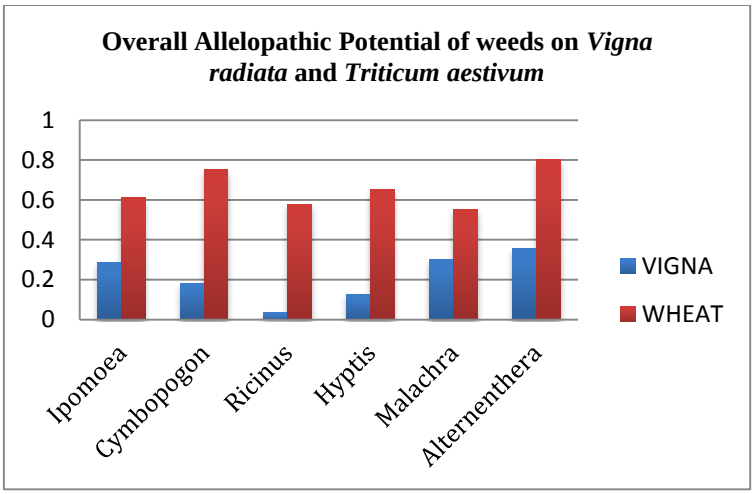

Fig 3: Overall Allelopathic Potential of weeds on *Vigna radiata* and *Triticum aestivum*

Table 4: Overall Allelopathic Potential of weeds on *Vigna radiata* and *Triticum aestivum*

Plant species	VIGNA	WHEAT
Ipomoea	0.288	0.610
Cymbopogon	0.181	0.751
Ricinus	0.033	0.578
Hyptis	0.127	0.654
Malachra	0.302	0.550
Alternenthera	0.355	0.801

The trend of OAP of weeds on *Vigna radiata* showed the following trend*, Malachra capitata (L.)* was greater than *Cymbopogon citrutus (Stapf).* Weeds had a more pronounced effect on *Triticum aestivum* than *Vigna radiate*. Reduction in seed vigor index was observed in a previous work by Petri plate method on both the crop species. (9, 10).

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