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Research Paper

ALLELOPATHIC EFFECTS OF AQUEOUS EXTRACTS OF FEW INDIAN HERBS ON GERMINATION AND GROWTH OF *Vigna radiata*

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Abstract

Allelopathy is defined as a phenomenon which involves the production of chemical compounds that escape into the environment and have a direct harmful or beneficial effect on a neighbouring plant or plants. In the present study, Allelopathic interactions of a few Indian herbs, namely *Piper nigrum* (Pepper), *Murraya koenigii* (Curry leaves), *Matricaria Chamomilla* (Chamomile dried flower), *Origanum vulgare* (Oregano) and a mixture of all of these, were studied on *Vigna radiata* (Moong) using the sandwich method. The germination rate of *Vigna radiata* was studied along with its plumule and radical length and the OAP values were determined by performing ANOVA and paired t-tests. There was significant growth inhibition observed in Moong seeds for all herbs especially *Murraya koenigii* which indicated this herb had the highest allelopathic potential. The current study attempted to exploit one of the several applications of Allelopathy and the responsible allelochemicals should be extracted and studied further.

Key words: Allelopathy, Allelochemicals, Indian herbs, Overall Allelopathic Potential (OAP), ANOVA.

INTRODUCTION

Allelopathy is defined as a phenomenon which involves the production of chemical compounds that escape into the environment and have a direct harmful or beneficial effect on a neighbouring plant or plants (Brown *et al.,* 1991). The chemicals released by allelopathic plants are called Allelochemicals and they are largely classified as secondary plant metabolites (Joshi *et al.,* 2016).

Allelopathy has effects on several aspects of plant ecosystem which includes occurrence of the plants, their growth, dominance, productivity, divergence and succession (Jain *et al.,* 2017) (Bhadoria P., 2011).

Various allelopathic effects have been observed in nature and its effects have been noticed affecting agricultural production, reduction of the input of chemical pesticides and consequent environmental pollution, and provision of effective methods for the sustainable development of agricultural production and ecological systems (Macias FA *et al.,* 2003).

The agro-ecological applications of Allelopathy provide alternatives to synthetic herbicides for weed management which increase attention towards Allelopathy (Barnes *et al.,* 1987). Different plants possess Allelochemicals, which could be utilized for suppressing weeds (Randhawa *et al.,* 2002). Research on the recognition and understanding of Allelopathy has been well documented over the past few decades (Rice, 1984; Rizvi *et al.,* 1992).

Indian Herbs have been used throughout the beginning of human history and played a pivotal role in the prevention and treatment of various diseases. Ancient, Indian medicinal systems viz. Ayurveda, Siddha, Unani, Amchi and local health traditions provide a strong base for the utilization of a large number of plants in terms of safety and effectiveness and leads for the management and treatment of different disease conditions. A large number of herbs & herbal formulations are used as rejuvenators (Singh and Dewasya, 2014).

According to the World Health Organization (WHO) 80% of the people in throughout the world currently uses herbal medicine for primary health care. Herbal medicine is a major component of Ayurvedic, homeopathic, naturopathic, traditional Chinese medicine, and Native American Indian medicine (Singh, Dewasya, 2014). Most commonly used Indian herbs include Pepper, Curry leaves, Oregano, Chamomile, Tulsi, Neem, turmeric, drumstick, cinnamon, saffron, cardamom, etc.

Peppers contain vitamin A, B6, folate and antioxidants (Butt MS., 2013). Curry leaves are rich in carbohydrates, fiber, calcium, phosphorous, irons and vitamins like vitamin C, vitamin A, vitamin B, vitamin E. They help in better heart function, fights infections (Firdaus SB., 2014). Oregano is useful in treating respiratory tract disorders, gastrointestinal (GI) disorders, menstrual cramps, and urinary tract disorders (Baranauskaite J *et al.,* 2017). Chamomile protects against diarrhoea, stomach ulcers, nausea and gas, likely due to its anti-inflammatory effects (Srivastava JK., 2010).

Plant Allelopathy is one of the modes of interaction between receptor and donor plants and may exert either positive effects (e.g., for agricultural management, such as weed control, crop protection, or crop re-establishment) or negative effects (e.g., autotoxicity, soil sickness, or biological invasion). To ensure sustainable agricultural development, it is essential to study cultivation systems that utilize the stimulatory or inhibitory influence of allelopathic plants to regulate plant growth and development and to avoid allelopathic autotoxicity. Allelochemicals can potentially be used as growth regulators, herbicides, insecticides, and antimicrobial crop protection products. Here, we reviewed

the plant Allelopathy management practices applied in agriculture and the underlying allelopathic mechanisms described in the literature (Cheng F., 2015).

The current study aimed at investigating allelopathic effects of *Piper nigrum* (Pepper), *Murraya koenigii* (Curry leaves), *Matricaria Chamomilla* (Chamomile dried flower), *Origanum vulgare* (Oregano) and a mixture of all the above on *Vigna radiata* (moong) using Sandwich method. This method consists of the use of a special six well multi-dishes in which dry leaf powder from the donor plant is placed above a layer of autoclaved agar cooled to 40˚C to 45˚C.

MATERIALS AND METHODS:

a) Selection of Plants

Mature and green leaves of Pepper, Oregano, Chamomile, Curry leaves and a mixture of leaves were studied for their effects of roots and shoots on *Vigna radiata* (Moong).

b) Plant Sampling and Preparation of extracts

Leaves were collected from a local area and washed several times with water. Further, leaves were dried in oven, grounded and sieved. Water extracts of the herb powder was prepared of 1%, 2%, 3%, 4% & 5% concentration and stored in bottles. In the 48-hour period, the bottles were shaken every 24 hours. The extracts filtered through muslin cloth and stored in dark bottles.

c) Sandwich method protocol

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

d) Type of agar and its preparation

Low temperature agar was used in accordance with Fujii's protocol. (Fujii Y., et al, 2004) 0.75% w/v of agar in water was boiled in a microwave to ensure that the agar melted properly and it was then decanted, cooled and 5 ml of the agar was then carefully pipetted into each of the sample and control wells. This took approximately 30 minutes for agar to set. Furthermore, 5 ml of agar was pipetted on top of the first layer. The risen plant material was pushed below the surface using a sterilized needle in order to obtain a uniformly smooth upper surface after the agar sets.

e) Arrangement of seeds and incubation

Using a pair of forceps, 4 surface sterilized seeds of the respective crop species were placed horizontally on the surface of the agar in each well. The seeds were arranged in a regularly spaced crisscross pattern. The lids of the plates were closed and sealed using a laboratory tape to prevent desiccation of the agar. The plates were wrapped in Aluminium foil and incubated at 20°C for three days. The multiwell plates were opened for measurement.

Calculations:

a) Percentage Inhibition

Observations were made for Germination, Radical and Plumule length. Percentage inhibition (%inhibition) was calculated using the formula below to observe the magnitude of inhibition by various extracts on radical and Plumule length.

$$Percentage\ Inhibition\ (\%\ inhibition) = \frac{A - B}{A} \times 100$$

Where, A= Effect on control, and B= Effect on test
Control and treatment means were compared using
Anova and Students t-test (α- 5%) for analyzing the significance of the difference between them.

b) OAP (Overall Allelopathic potential) values

The number of germinating seedlings was recorded. 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. 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. (Smith, O.P, 2013)

$$Overall\ Allelopathic\ Potential\ (OAP) = \frac{Inhibition\ of\ radical\ growth}{100}$$

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. (Jain *et al.,* 2017)

RESULTS AND DISCUSSION:

a) Length of Radicle

The radicle of test seedlings of all the herbs were significantly affected by *Vigna radiata* extracts as per ANOVA (Table 1). On performing paired t-test, a comparative study was done between the control and each concentration as indicted in table 2. A significant difference was observed between the control group and all the herbs at 3%, 4% and 5%. No significant difference was observed for Curry leaves and Oregano at 1%. Also, at 2% no significant difference was observed for Pepper and Curry leaves asindicated by Table 2. The root length for Oregano was the longest followed by Chamomile, mixture, Pepper and Curry leaves at 4%. (Table 4). At 3%, mixture had the longest root length followed by Oregano, Chamomile, Pepper and Curry leaves.

Table 1: ANOVA

Herb	Radicle
Pepper	6.020175*
Curry leaves	12.24413*
Chamomile	25.35337*
Oregano	7.006235*
Mixture	29.71347*

' * ' : Significant difference between control and herbs (5% level of significance)

Table 2: Paired T test

Herb	Radicle				
Concentration (%)	1	2	3	4	5
Pepper	7.326607*	1.257054	3.557908*	7.396044*	10.02284*
Curry leaves	1.630228	0.313656	2.374742*	6.869991*	11.22412*
Chamomile	9.284815*	7.031014*	5.696793*	14.93717*	28.13152*
Oregano	1.986882	4.642434*	8.77653*	16.84577*	8.728811*
Mixture	5.853178*	6.875024*	9.703808*	8.603102*	10.13422*

' * ' : Significant difference between control and herb (5% level of significance)

b) Length of Plumule

The plumules of test seedlings of all the herbs were significantly affected by *Vigna radiata* extracts as per ANOVA (Table 3). On performing paired t-test, a comparative study was done between the control and each concentration as indicted in table 4. A significant difference was observed between the control group and all the herbs at 2%, 3%, 4% and 5%. No significant difference was observed at 1% for all the herbs except Chamomile. The shoot length for Chamomile was the longest followed by Oregano, Curry leaves, mixture and Pepper at 4%. (Table 4).

Table 3: ANOVA

Herb	Plumule
Pepper	21.00073*
Curry leaves	17.44504*
Chamomile	14.28189*
Oregano	11.28194*
Mixture	31.28174*

' * ' : Significant difference between control and all herbs at different concentrations (5% level of significance)

Table 4: paired t Test

Herb	Plumule				
Concentration (%)	**1**	**2**	**3**	**4**	**5**
Pepper	-1.19259	4.512956*	5.652377*	12.93784*	11.19463*
Curry leaves	2.058158	12.31567*	4.902833*	15.54759*	11.12445*
Chamomile	2.261971*	2.395078*	3.026636*	69.44715*	37.7362*
Oregano	1.175369	3.986628*	9.067931*	26.5858*	28.31765*
Mixture	0.214358	5.267755*	7.946872*	14.2612*	18.22108*

' * ' : Significant difference between control and herb at respective concentration (5% level of significance)

c) Percentage Inhibition of Growth

The percentage inhibition of growth of all the herbs was calculated as indicated in Table 5. At 5% concentration, 100% inhibition was observed for Curry leaves and mixture of all leaves. A general trend was observed where an increase in concentration increased the inhibition of the test plant.

Table 5: % Inhibition of growth

Concentration	Category	Pepper	Curry leaves	Chamomile	Oregano	Mixture
1%	Root	36.82	22.12	55.39	21.77	38.75
	Shoot	-10.36	25.54	18.59	12.10	34.27
2%	Root	15.34	31.85	48.85	38.25	49.02
	Shoot	34.82	47.55	25.77	34.15	44.53
3%	Root	42.84	17.55	38.85	60.21	51.54
	Shoot	42.53	41.13	43.67	60.79	56.89
4%	Root	47.37	38.08	67.69	65.90	60.07
	Shoot	58.21	55.98	96.01	70.79	70.79
5%	Root	47.19	100	66.80	56.25	100
	Shoot	61.93	100	92.02	89.22	100

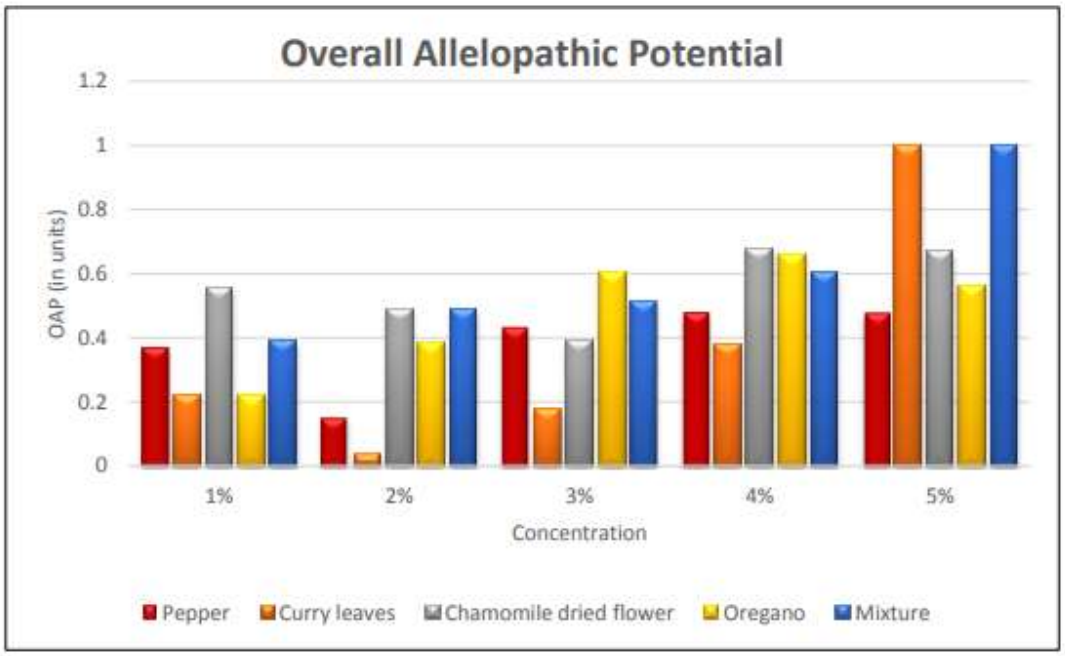
d) OAP Values.

The Overall Allelopathic Potential of *Vigna radiata* was calculated for *Piper nigrum*, *Murraya koenigii*, *Matricaria Chamomilla*, *Origanum vulgare* and mixture of all of these herbs at 1,2,3,4 and 5% concentration each.

Allelopathic effect was significantly observed on both plumule and radicle of *Murraya koenigii* and mixture of all herbs at 5% concentration, as indicated by the table 6.

Table 6: OAP values

Con centration	Pepper	Curry leaves	Chamomile	Oregano	Mixture
1%	0.3682	0.2212	0.5539	0.2177	0.3875
2%	0.1534	0.0385	0.4885	0.3825	0.4902
3%	0.4284	0.1755	0.3885	0.6021	0.5154
4%	0.4737	0.3808	0.6769	0.6590	0.6007
5%	0.4719	1	0.6680	0.5625	1



CONCLUSION:

In the current study, Sandwich method was used to study the allelopathic effect on Vignia radiata. The overall allelopathic effect indicated that radicle of all the herbs and plumule of all herbs except *Piper nigrum* were affected. Allelopathic effect was most significant in *Murraya koenigii* in both radicle and plumule at 5% concentration whereas, it was least observed in plumule of *Piper nigrum* and Mixture of all herbs at 1% concentration.

At 4%, the plumule length was longest for Chamomile > Oregano > Curry leaves > Mixture > Pepper where as the root length was found to be longest for Oregano > Chamomile > Mixture > Pepper >Curry leaves at 4% and at 3% the longest root length was for Mixture > Oregano > Chamomile > Pepper > Curry leaves.

100% inhibition was observed at 5% concentration for Curry leaves and Mixture of leaves. Mixture of leaves might have shown any effect due to the presence either any one of the leaves or a synergistic effect of any herbs. Hence, many other combinations of the same herbs can be used further to test which herb is responsible for the effect.

Determination and isolation of the responsible Allelochemicals should be carried out in order to take the study further towards development of herbicide. Also, several other herbs can be tested against *Vigna radiata* to have a broad spectrum of sensitive herbs. The current study attempted to exploit one of the several applications of Allelopathy.

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