

RESEARCH ARTICLE

ALLELOPATHIC EFFECT OF LEAF EXTRACTS OF ANAMIRTA COCCULUS, ANDROGRAPHIS PANICULATA AND HELICTERES ISORA ON GERMINATION OF VIGNA RADIATA SEEDS.

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Abstract

A study was conducted to ascertain the allelopathic effect of leaf extracts of three medicinal plants – *Anamirta cocculus, Andrographis paniculata* and *Helicteres isora* on seed germination of *Vigna radiata*. Aqueous leaf extracts of these plants were prepared and sterilized, healthy green gram seeds were allowed to germinate in these extracts in lab conditions along with a control. Enumerations were done after 24 and 48 hours. The data were recorded for statistical analysis. From the study it was observed that *Andrographis paniculata* leaf extract showed maximum negative allelopathic effect and Helicteres isora showed minimum effect. *Anamirta cocculus* leaf extract was moderate in providing inhibitory effect. The study was a useful prelude for similar research in this field and also to the farmers cultivating green gram.

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Introduction:-

Plants of natural ecosystems show subtle interactions with each other for their sustenance. This can be even noticed in artificial monoculture systems like agriculture where the interaction is developed between crops and weeds. Upon invasion, weeds compete with crop plants causing adverse effects to them and other natural vegetations (Rice, 1984). Weeds arrest the crop productivity both quantitatively and qualitatively. One of the reasons for invasive nature of weeds may be their allelopathic property. Allelopathy is a biochemical phenomenon by which an organism produces one or more bio chemicals that have a positive or negative influence on the growth, survival and reproduction of other organisms. The term allelopathy, derived from two Greek words- allele and pathy (meaning mutual harm or suffering), was first used in 1937 by the Austrian professor Hans Molisch. Allelopathy plays an important role in community characteristics of tropical flora. While implementing rural and urban forestry programmes, a thorough knowledge about the mechanism of allelopathy is important because the allelopathic factors are species specific in many a times. Germination, growth, metabolism, development, distribution, behaviour and reproduction etc. are various phases of plant life that are influenced by allelopathic chemicals (Narwal et al., 1997). Terpenoids and Phenolics are the main secondary metabolites which act as suppressive allelochemicals with specific actions (Khanh et al., 2007). The diversity, richness, dominance, important value index, climax community characteristics etc. of species in a community are directly or indirectly determined by allelopathic factors.

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Allelochemicals are synthesised in the plant body during mature stages of the plant and their water soluble nature make them influential on the growth and reproduction of associated plants (Inderjit, 2009). The invasion by alien

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species can be naturally controlled if native species are allelopathic in nature. Moreover, herbivore defence mechanisms by native flora boost allelopathic behaviour and found to be effective against weed invasion. Of various parts of the plant, leaves are the main source of allelopathic constituents though other parts also have their presence in low concentrations. Different concentrations of leaf extract of *Albizia lebbeck* are capable of inhibiting seed germination in crop plants and complete inhibition is observed in certain concentrations (Copping, 1996). The most studied compound with allelopathic effect could be benzoxazinoids present in many plants which has herbicidal, fungicidal, antimicrobial and insecticidal properties. Sorgoleone is another primary allelochemical present in the root exudates of Sorghum which prevents the growth of crops during crop rotation. The residue of *Chenopodium murale* has allelopathic effect on growth of Chick pea as it caused reduced root and shoot length (Batish et al.2007). Extracts from various plant parts of *Moringa oleifera* showed its inhibitory effect on seed germination of *Vigna radiata* in laboratory condition irrespective of its concentration levels (Md. Moktar Hossain et al, 2012).

Vigna radiata (L) Wilczek, of family Fabaceae is one of the important pulse crops of developing countries because of its short duration, protein richness, low fertilizer needs and nitrogen fixing character. Anamirta cocculus (Linn.) is a wild woody climber belonging to the family Menispermaceae distributed throughout India as well as south-east-Asia. It is primarily a piscicidal plant which is traditionally used by indigenous population of this area. Picrotoxin (cocculin) is the major reported toxic component of the seed and altogether five sesquiterpene lactones viz., picrotoxinin, methyl picrotoxate, picrotin, dihydroxypicrotoxinin and picrotoxic acid were isolated from the seeds of this plant. Presence of these chemicals can induce allelopathic effect in this plant. Andrographis paniculata (Burm.f.) Nees; a medicinal plant belonging to the family Acanthaceae; is used in many countries to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection. The herb contains diterpenoids, flavonoids and polyphenols as the major bioactive components. The whole plant harbours over 20 diterpenoids and over 10 flavonoids which can impart allelopathic nature to its extracts. Helicteres isora Linn., the Indian screw tree is a member of family Sterculiaceae. It possesses an impressive range of nutritional and medicinal properties. Investigations have indicated that the plant has anti-oxidant, anti-cancer, anti-diabetic and antimicrobial properties. It is also used as an anti-gastrospasmodic, anthelmintic, antipyretic, antidiarrheal, antidysenteric and as a tonic after child birth. Cucurbitacin B and isocucurbitacin B were isolated from this plant which has cytotoxic activity. Six neolignans, the helicterins A-F were isolated from the aqueous extract of the fruits which also contains flavonoid glucosides (Suthar et al., 2002). Numerous studies have revealed the presence of phenols, flavonoids, alkaloids, glycosides, phytosterols, carotenoids, tannins, fixed oils and fats from different part of Helicteres isora, in varying concentrations. Hence the purpose of the present study is to ascertain the allelopathic effects of aqueous leaf extracts of Anamirta cocculus, Andrographis paniculata and Helicteres isora on the seed germination and radical length of Vigna radiata (green gram).

Materials And Methods:-

A total of three medicinal plants known for ability to produce allelochemicals were selected for the present study; namely *Anamirta cocculus* (Menispermaceae), *Andrographis paniculata* (Acanthaceae) and *Helicteres isora* (Sterculiaceae). The selection was based on the availability of these plants in the locality and they represented three morphological groups; namely climber, herb and shrub. Fully grown healthy leaves collected from these plants were washed thoroughly with distilled water. The leaves were shade dried for seven days and later chopped into small pieces. Ten gram of these leaves were separately weighed and ground with 40 mL distilled water for each by using mortar and pestle and the extracts were filtered through a clean, sterilized muslin cloth. This served as the stock solution and was stored in three separate glass bottles in refrigerator until further use.

Healthy uniform seeds of green gram (*Vigna radiata*) were collected with extreme care. They were handpicked through water floating analysis to ascertain their health and prospect of viability. Before germination test, the grains were surface sterilized with 1% sodium hypochloride for 1 minute, then rinsed with the distilled water for several times to remove excess of chemical. Twelve autoclaved petridishes were prepared with thin lining of sterilized cotton and 7.5 mL of test extract from the experimental plants were poured in it. The petridishes treated with distilled water were taken as control. The experiments were laid out in a Completely Randomized Design (CRD) with three replications for each plant extracts. In each petridish, 10 surface sterilized green gram seeds were placed. The setup is then kept undisturbed in room temperature $(24\pm2^{\circ}C)$ in the laboratory. The seed germination and the emerged radicle length were recorded after 24 hrs and 48 hrs respectively.

Results And Discussion:-

The germination patterns of Vigna seeds were found to be different in the case of three selected aqueous, medicinal plant leaf extracts. The emergence of radicle from the seed is considered as the germination and the mere breaking of the seed coat as non-germination. The rate of germination and the radicle length of *Vigna radiata* on different leaf extracts after first day and second day are tabulated as given in the following table.

S1.	Anamirta cocculus		Andrographis paniculata		Helicteres isora		Control	
No.	(Radicle ler	ngth in cm)	(Radicle len	gth in cm)	(Radicle length in cm)		(Radicle length in cm)	
Time	24 hrs.	48hrs.	24 hrs.	48hrs.	24 hrs.	48hrs.	24 hrs.	48hrs.
1	-	1.6	-	-	1.0	4.0	1.5	5.5
2	-	1.8	-	-	0.5	3.5	1.6	4.5
3	-	2.1	-	-	0.6	4.0	1.0	6.0
4	-	2.2	-	-	1.0	3.6	0.5	4.0
5	-	1.0	-	-	1.5	3.5	1.0	4.5
6	-	1.2	-	-	0.4	6.0	0.5	4.5
7	-	1.5	-	-	1.1	4.0	0.6	3.5
8	0.5	1.0	-	-	0.8	6.0	0.5	4.9
9	0.5	0.5	-	-	1.1	4.0	0.5	1.4
10	1.0	2.0	-	-	0.6	-	-	1.0
11	-	3.5	-	-	-	5.8	1.0	4.5
12	-	2.0	-	-	-	4.5	1.3	4.5
13	-	2.5	-	-	-	4.0	1.0	3.5
14	0.3	2.3	-	0.3	0.6	4.3	1.0	4.2
15	0.3	1.0	-	1.2	0.8	5.0	1.6	6.3
16	-	1.2	-	1.0	1.2	1.0	1.0	4.5
17	0.5	1.5	-	1.0	1.6	3.0	0.5	4.1
18	1.0	-	0.5	-	0.4	5.5	1.2	4.2
19	1.0	1.5	1.0	-	1.0	0.2	0.5	2.6
20	0.5	2.0	1.0	1.3	0.4	-	0.5	1.2
21	-	1.8	-	-	-	5.5	1.8	4.6
22	-	2.0	-	-	-	4.0	1.2	3.6
23	-	1.5	-	-	0.5	3.2	1.0	4.1
24	-	2.4	-	-	0.8	3.0	0.5	4.3
25	0.5	1.5	-	-	1.2	3.0	-	2.8
26	0.5	2.0	-	-	0.6	2.0	1.2	1.0
27	0.5	2.0	-	-	0.6	2.0	1.4	1.2
28	1.0	1.2	-	1.0	1.0	2.2	1.0	0.6
29	0.5	1.0	0.5	1.5	2.0	4.0	0.6	1.0
30	1.0	1.5	1.0	0.5	1.5	-	1.6	5.0

Table 1:-Radicle length (in c.m.) of Vigna radiata in each extracts after 24 and 48 hours.

The experimental results of allelopathic effect of *Anamirta cocculus, Andrographis paniculata* and *Helicteres isora* on the germination of *Vigna radiata* seeds is shown in the Table 1. It is obvious that the aqueous leaf extract of *Andrographis paniculata* inhibited the seed germination of *Vigna radiata* to the maximum extent. In the case of *Anamirta cocculus* leaf extract the inhibitory allelopathic effect was moderate and leaf extract of *Helicteres isora* was showing minimum inhibitory allelopathic effect. The maximum seed germination percentage was shown in the control where no extract was used which was 93% at 24 hours, 100% at 48 hours. The highest inhibitory allelopathic effect was caused by *Andrographis paniculata* (10gm/7.5ml) treatment which resulted in germination percentage of only 23% at 24 hours and 36% at 48 hours. This indicated sharp contrast with the control which showed 93 % seed germination at 24 hours and 100 % at 48 hours. In the case of *Anamirta leaf extract* the negative inhibitory allelopathic effect was moderate in this case. *Helicteres isora* leaf extract showed negligible inhibitory allelopathic effect which was evident from the germination percentages; 93% after 24 hours and 100% after 48 hours. It was clear that the leaf extract of *Helicteres isora* did not have inhibitory effect on Vigna seed

germination. So it can be a useful component in the organic fertilizers provided during green gram cultivation. Farmers are also advised not to include *Andrographis* plant parts while making organic fertilizers for Green gram. The negative allelopathic effect of *Andrographis* can be attributed to high amount of diterpenoids and flavanoids stored in its plant parts like leaves which are the secondary metabolites. Melkania et al. (1984) have observed similar findings on various agricultural crops.

The study revealed that the leaf extract of Andrographis paniculata decreased the radicle length of Vigna radiata compared to control. The lowest radicle length was registered in the Andrographis paniculata leaf extract, which was 0.13 ± 0.03 cm at 24 hours and 0.26 ± 0.11 cm at 48 hours time interval, which significantly differed from other extracts on Vigna radicle length. The highest radicle length was obtained in control group in both time intervals. The extract of *Helicteres isora* showed similar result as that of control which meant it had the enhancing effect on seed germination and radicle length when compared to others (0.76 ± 0.02 cm at 24 hours and 3.36 ± 0.47 at 48 hours). Anamirta cocculus showed moderate negative allelopathic effect with radicle length of 0.32 ± 0.02 cm at 24 hours and 1.64 ± 0.37 cm at 48hours. The study revealed that the Andrographis aqueous leaf extract had pronounced negative allelopathic effect on seed germination and radicle length of vigna radiata.

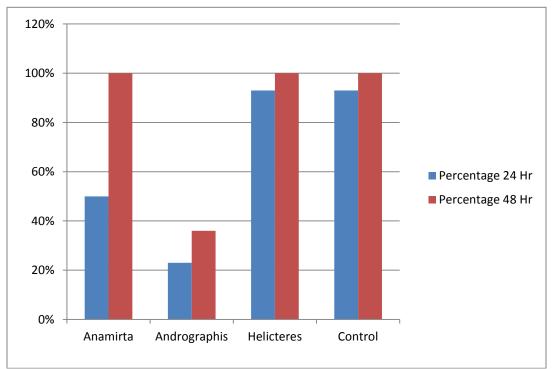


Fig. 1:-Effect of aqueous extract of Anamirta cocculus, Andrographis paniculata and Helicteres isora on the seed germination of Vigna radiata

Experimental parameters	Radicle length (in c.m)			
Time Duration	24 hours (Mean \pm S.E)	48 hours (Mean \pm S.E)		
Control in distilled water	0.92 ± 0.03	3.58 ± 0.87		
Anamirta cocculus leaf extract	0.32 ± 0.02	1.64 ± 0.37		
Andrographis paniculata leaf extract	0.13 ± 0.03	0.26 ± 0.11		
Helicteres isora leaf extract	0.76 ± 0.02	3.36 ± 0.47		

Table 2:-Effects of aqueous extracts of Anamirta cocculus, Andrographis paniculata and Helicteres isora on the radicle length of Vigna radiata.

The present study emphasized that the inhibition of growth parameters of seedlings was more pronounced than that of seed germination. This inhibitory action may be related to the presence of allelochemicals including tannins, alkaloids, long-chain fatty acids, terpenoids wax, flavonoides, phenolic acids etc. (Chou 1980, Rice 2012). Further,

the toxicity might be due to synergistic effect of consortium of chemicals rather than single one. These compounds exhibit wide range of inhibitory mechanisms and affect on DNA (alkaloids), photosynthetic and mitochondrial function (quinines), phytohormone activity, ion uptake and water balance (phenolics) (Einhellig, 2002). In addition, the allelochemicals are an important defence for certain plants against the interference of other plants of the same or different species, which can affect their growth and development (Larcher, 2000 and Uremis et al., 2009).

So the present study is a useful assay to know the initial stages of allelopathic effect of three well known medicinal plants of Mahe which have grown to the level of weeds in certain locations. The results of the experiment will give useful inputs to the farmers while making green manure for Vigna cultivation and also for making soil beds in agriculture. The present study can be a prelude to further experimental activities in this field. Allelopathic effect of extracts from stems, roots and other plant parts other than leaves can be carried out in future. The exact concentration of plant extracts at which the inhibitory effect is maximum in the case of these three medicinal plants and the elucidation of chemical nature of the 'active principle' are the further prospects in the study of allelopathic effect of these plants.

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