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ALLELOPATHIC EFFECTS OF VOLATILE COMPOUDS FROM *CULLEN CORYLIFOLIA* MEDIK. ON SEED GERMINATION AND SEEDLING GROWTH OF *CUCUMIS SATIVUS* L.

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ABSTRACT:
The allelopathic effects of 4-relatively abundant volatile compounds (Phenolic meroterpene, bakuchiol, limonene and terpineol) present in the *Cullen corylifolia* Medik. (*Psoralea corylifolia* Linn. plant placed in the air tight jars containing soil and water were studied on the seed germination, seedling growth of *Cucumis sativus* L. The inhibitory effects of volatile compounds released in water have more inhibitory effects on seed germination and seedling growth of studied plant. These volatile compounds at high concentrations inhibited the seed germination, shoot length, seedling height but low concentrations were less inhibitory or stimulatory. All these volatile compounds at low concentrations were slightly stimulatory to seedling *of Cucumis* plant. Water kept with plant material of *Cullen* in airtight jar affects seed germination and seedling growth significantly than soil which indicates that water absorbs some volatile substance(s) more from the weed plant which may be responsible for allelopathic potential of this weed plant. The plant is chemically analyzed for the presence and confirmation of stearic acid by HPTLC method. However, the presence of this chemical compound in the plant parts of *Cullen corylifolia* Medik. was well correlated with the degree of their phytotoxicity.

Keywords: Allelopathic effects, *Cullen corylifolia*, volatile compounds.

INTRODUCTION:

Molisch in 1937 first coined the term allelopathy, which refer to biochemical interactions between all types of plants including microorganisms. The term allelopathy was derived from Greek word, which means mutual harm. This term covers both the detrimental and beneficial reciprocal biochemical interactions. Rice in 1984 also defined allelopathy as any direct or indirect harmful or beneficial effect of plants, including microbes, on another plant through release of chemicals that escape into the environment. Allelochemicals were suspected in 19th century in agriculture because of many observations of 'soil sicknesses of farmlands. If a piece of farm land is continually cropped to one plant, the yields often decrease and cannot be improved by additional fertilizers. Fruit trees, for example, often do poorly in farm land where the same species has grown before. Furthermore, it is common for one plant to harm another plant grown in its vicinity a phenomenon called allelopathy. Allelochemicals refer mostly to be the secondary metabolites produced by plants and are by-products of primary metabolic growth and development of the same plant or neighboring plants. Some of them are accumulated at various stages of growth, while some depends upon time of day or season.

Allelochemicals selectively inhibit the growth of soil microorganisms or other plants or both. They play an important role in chemical warfare between plants (allelopathic interactions) and include natural herbicides, phytoallexins (microbial inhibitors) and inhibitors of seed germination. Although many allelochemicals are strictly defensive substances, others are offensive compounds that act directly in weed aggressiveness, competition and the regulation of plant diversity.

It is expected that in the near future many allelochemicals may be used commercially as herbicides, insecticides, and nematocides or as bio regulators.

MATERIAL & METHODS:

Morphological Description of Studied Plant:

Cullen corylifolia, Linn. :

Cullen corylifolia belongs to family Fabaceae. It is found in waste places and in agricultural field crops. In vernacular it is known as 'Bavachi'. It is an erect annual herb grows upto 60- 120 cm in height with grooved and gland dotted stems and branches and found in monsoon. Stem and branches are covered with white hairs and red glands. Leaves are imparipinnately compound 2.3-7.0 x 2.0-5.5cm, broadly elliptic nigro- punctate beneath. The leaf

has inciso-dentate margins with rounded base and mucronate apex. Flowers are 10-30 in dense axillary racemes. Petals are bluish purple in colour. Pods are ovoid to oblong, closely pitted, apiculate and one seeded. Fruits are very small, 3-4mm long and 2.3mm broad, which are sometimes wrongly referred to as seeds. They are dark chocolate to black with oily pericarp attached to seeds. The seeds are non endospermic, oily and free from starch. Flowers and fruits are found during August-December (Singh et al., 2001). *Cullen corylifolia* is an important medicinal plant which is used in several traditional medicines to cure various diseases. The plant extracts have been reported to possess antibacterial, antitumor, antioxidant, anti-inflammatory, antifungal and immunomodulatory activity. A wide range of chemical compounds including psoralen, isopsoralen, bakuchiol, psoralidin, bakuchalcone, bavachinin, flavones, volatile oils, lipids etc.

Medicinal and other uses:

Plants have been the basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind. Plants have been one of the important sources of medicines since the beginning of human civilization. The recent resurgence of plant remedies resulted from several factors, such as effectiveness of plant medicines and lesser side effects compared with modern medicines. *Cullen corylifolia*, commonly known as babchi, is a popular herb, which has since long been used in traditional Ayurvedic and Chinese medicine for its magical effects to cure various skin diseases. This plant is also pharmacologically studied for its chemoprotective, antioxidant, antimicrobial, and antiinflammatory properties. Common medicinal uses are:

1. Fruits are used in Central and Northern India for the preparation of certain types of medicated oils and incense preparation.
2. Most of the ancient Indian Physicians and Ayurvedic practioners utilize the fruits externally as well as internally for several diseases like psoriasis, leucoderma and leprosy.
3. Fruits are also used like laxative, aphrodisiac, anthelmentic, anti-inflammatory and diuretic in ferbrile conditions (Chopra, R N 1933).
4. The roots are used in the carries of teeth and leaves are useful in diarrhoea. (Krishna and Badhwar, 1949).
5. In recent days this drug is largely used in Allopathy, Ayurveda and Homeopathy for the cure of leucoderma and other skin diseases. Several formulations from tablets, capsules, ointments and tinctures are available in the market. The activity is mainly due to mixture of two furo-coumarins, psoralen and isopsoralen.
6. The oil has an irritant and specific effect on the skin and mucous membrane. It has a powerful effect against the skin Streptococci.
7. Seeds are useful in bilious affections, and are also used to make perfumed oil, and its powder is especially recommended by Vaidyas in leprosy and leucoderma internally, and is also applied in the form of paste or ointment externally.
8. The drug has been considered to be so efficacious in leprosy that it was given the name of 'Kushtanashini' (leprosy destroyer).
9. It is a good hair tonic.

The problem of weed is as old as cultivation of crop plants itself. The weeds are cosmopolitan in distribution. They occupy diverse habit as well as habitat. They create various problems in agriculture. Under field condition weed infestation is one of the major causes of yield reduction in crops. Historically, most investigators have attributed these losses to various forms of competition between the weeds and crops and allelopathic interaction between them were not considered. However, findings after 1950's have shown that allelopathic interactions between the crops and weeds were also partly responsible for such losses in crop yields. Most of the weed species have inhibitory effects on crops, yet some weed species also exhibited stimulatory effects on the seed germination, growth and yield of crops.

Hence, in the present investigation it has been decided to carry out Allelopathic study of this medicinal plant weed of Agricultural fields in Pune district, which constitutes the dominant weed flora of the coverage area.

Volatilization Bioassay:

In few cases, allelochemicals may volatilize and absorbed directly from the atmosphere by neighboring plants and absorbed from condensate in dew, be absorbed on the soil particles and subsequently taken up by contact with plants or from the soil solution (Muller, 1966). Apparently several terpenoids transfer in these ways; higher plants produce a variety of essential oils of plants. These compounds continuously released in to the atmosphere in hot weather, therefore, this phenomenon is observed in the arid regions of the world. Thus a number of species from several climatic regions may express allelopathy through volatilization (Rice, 1974 and Horsley, 1977). Muller (1966) reported that terpenes volatilized by several species inhibited the growth of nearly plants.

To test volatile substances from the medicinal plant weeds, if any involved in the inhibitory effect on neighboring crop plants, fresh plants of *Cullen corylifolia* were collected from different localities of agricultural fields. All fresh plants parts were made clean with brush to remove soil and dust particles. Fresh plant parts (150g) were kept in surface sterilized air tight jar (Desicator) for 48 hrs. Soil (150g) and water (150ml) were kept inside the glass jar separately in such a way that they are not in direct contact with plant materials, and the air inside only acts as a carrier for volatile substance. The soil and water, thus obtained were used for further bioassay using test crop. For laboratory bioassay 10 surface sterilized seeds of *Cucumis sativus* were placed in sterilized petridish (11cm) containing a Whatman No. 1 filter paper. 10ml of water obtained from glass jar was utilized to moisten the filter paper, and 10gm soil obtained from glass jar was used a layer and made wet with distilled water. A petridish containing Whatman No 1 filter paper moistened with 10ml ml of distilled water and control soil (free from experimental plant) moistened with 10ml of distilled water served as control. Each set of petridish containing 10 seeds of *Cucumis* were kept in triplicate at room temperature (28 ± 2^{o}C). All the petridishes were wrapped by brown paper to avoid direct sunlight.

The observation in the form of seeds germination simultaneously hypocotyl and radicle lengths were recorded at 7 days after sowing.

Histochemical study:

For the histochemical studies free hand cut sections about 20-25µ of the root, stem and leaves were of different phases of life cycle of selected weeds taken and tested with respective reagents for the detection and localization of chemical constituents such as Starch, Proteins, Tannins, Saponins, Fat, Sugars, Alkaloids, and Glycosides (Johansen D A, 1940 and Krishnmurty K V, 1988).

Phytochemical study:

Preliminary phytochemical tests were carried out for the confirmation of Starch, Proteins, Tannins, Saponins, Reducing Sugars and Anthroquinones on water extractives, while Alkaloids, Glycosides and Flavonoids in alcoholic extractives. The chemical constituents were also confirmed with the help of HPTLC.

High Performance Thin Layer chromatography (HPTIC)

HPTLC technique was followed for the qualitative analysis and the confirmation of chemicals present in the studied plants (Passera, et al., 1964).HPTLC is a versatile separation technique included various steps as given below:

i) Selection of HPTLC plates and sorbent
ii) Sample preparation.
iii) Application of sample
iv) Development (separation)
v) Detection including post-chromatographic derivable on
vi) Quantitation
vii) Documentation

RESULT & DISCUSSION:

Results of Volatilization Bioassay:

Water kept with plant material of *Cullen* in airtight jar affects seed germination and seedling growth significantly than soil. This indicates that water absorbs some volatile substance (s) more from the weed plant which may be responsible for allelopathic potential of this weed plant.

Results of Histochemical studies:

Starch, tannins, saponins, fats, glycosides and alkaloids are detected in stem and leaf where as proteins and fats were not found in stem, root and leaf respectively. Tannins are totally absent in the leaf. The starch, tannins, saponins and glycosides are only localized in stem. Fats are detected only in stem, where as proteins were only found in leaf.

Results of phytochemical studies:

Starch, tannins, saponins, fats, glycosides and alkaloids are detected in root, stem and leaf where as proteins and fats were not detected in stem, root and leaf respectively. Tannins are totally absent in the leaf. The starch, tannins, saponins and glycosides are found in stem. Fats are detected only in stem. While proteins are only found in the leaf.

The stem and root contains phytochemicals such as tannins, saponins and glycosides. The inhibitory effect might be due to presence of these allelochemicals. The essential oil contains Phenolic meroterpene, bakuchiol, limonene, terpineol.

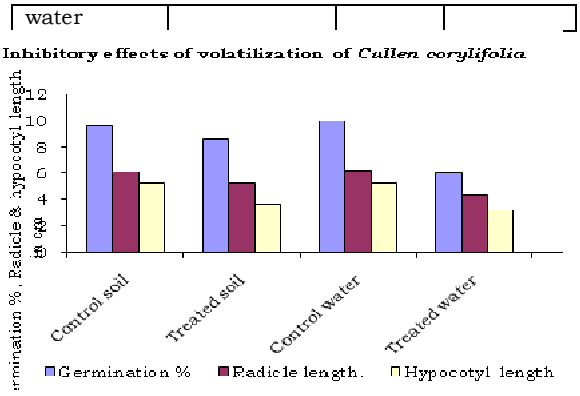
Results of High Performance Thin Layer Chromatography (HPTLC):

With the help of HPTLC technique, one phytoconstituent is identified and confirmed from root, stem and leaves of studied plant. Stem and leaves of *Cullen corylifolia* contains stearic acid. However, the above said phytochemical was phytotoxic. The presences of these chemical compounds in the plant parts of studied plant were well correlated with the

degree of their phytotoxicity. These studies and results indicated that, the allelopathic activity exists in the studied plant part(s) are because of these phytotoxic chemicals.

Table 1: Inhibitory effects of Volatilization of *Cullen corylifolia*.

Type of bioassay	Germination (%)	Average Seedling length of *Cucumis* (cm)	
		Radicle	Hypocotyl
Control Soil	96.02	6.100	5.300
Treated Soil	85.65	5.325	3.637
Control Water	99.64	6.210	5.280
Treated	60.48	4.400	3.183



Graph 1: Inhibitory effects of Volatilization of *Cullen corylifolia,* Linn.

Table 2: Histochemical Tests of *Cullen corylifolia*.

Test	Reagent	*Cullen corylifolia.*								
		Root	Colour	Location	Stem	Colour	Location	Leaf	Colour	Location
1. Starch	Iodine (I_2KI)	+ve	Blue	Hypo,xylm.	+ve	Blue	Xylm,m.r.	+ve	Blue	Palisade Tissue.
2. Proteins	Potassium ferocynide and $FeCl_3$	-ve			-ve			+ve	Intensive Yellow	Epi,meso. Tissue.
3. Tannins	10%Aq. $FeCl_3$	+ve	Blue Green	Cortex.	+ve	Blue Green	Xylm.tracheids.	+ve	Blue Green	Epi, Spongytissue.
4.Saponins	$Ba(OH)_2$ $CaCl_2$ and $K_2Cr_2O_{7,OR}$ Conc. H_2SO_4	+ve	Yellow-red	Epi,cortex, Endo.	+ve	Yellow-red	Cortex, xylm.vesl.	+ve	Yellow - red	Epi,spongy Tissue.
5. Fats	Sudan III/ IV	-ve			+ve	Red	Epi,cortex.	-ve		
6. Sugars	Copper Tartarate and Cuprous oxide	-ve			-ve			-ve		
7.Glycosides	2.5%Aq. $FeSO_4$and 20%Aq. $FeCl_3$	+ve	Deep blue	Cortex,V.B.	+ve	Deep blue	Cortex,m.r.	-ve		
8. Alkaloids	Mayer's reagent	+ve	Brown	Epi,cortex.	+ve	Brown	Epi,cortex.	+ve	Brown	Epi,spongy.
	Wagner's reagent	+ve	Brown	Epi,cortex.	+ve	Brown	Epi,cortex.	+ve	Brown	Epi,meso.
	Dragendorff's reagent	+ve	White	Epi,cortex.	+ve	White	Epi,m.r.	+ve	White	Epi,meso.

Table 3: Results of Phytochemical Tests of *Cullen corylifolia*.

Test	*Cullen corylifolia.*		
	Root	Stem	Leaves
A)WATER EXTRACTS			
Starch	-ve	-ve	-ve
Proteins	-ve	-ve	-ve
Tannins	-ve	+ve	+ve
Saponins	+ve	+ve	+ve
Anthroquinones	-ve	-ve	-ve
Reducing sugars	-ve	-ve	-ve
B)ALCOHOL EXTRACTS			
Flavanoids	-ve	-ve	-ve
Alkaloids	+ve	+ve	+ve
Dragendorff's Reagent	+ve	+ve	+ve
Mayer's reagent	-ve	-ve	-ve
Wagner's reagent	-ve	-ve	-ve

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b) Individual *Cullen corylifolia* plant.

a) Habit of *Cullen corylifolia* Medik.

c) *Cullen corylifolia* showing dominance in the field.

Detection of Stearic acid in *Cullen corylifolia*

Before derivatisation :

Image (254 nm)

Image (366 nm)

After derivatisation :

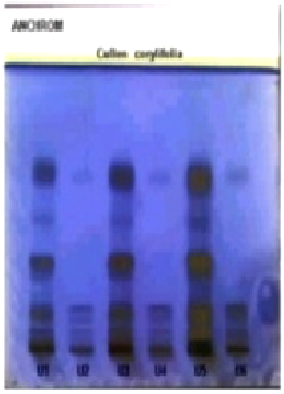

Image (visible)

U1 : *Cullen corylifolia* (Leaves) : 5 µl
U2 : *Cullen corylifolia* (Stem) : 5 µl
U3 : *Cullen corylifolia* (Leaves) : 10 µl
U4 : *Cullen corylifolia* (Stem) : 10 µl
U5 : *Cullen corylifolia* (Leaves) : 20 µl
U6 : *Cullen corylifolia* (Stem) : 20 µl