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# Polarity nature of seed germination stimulants present in root extract of host plants of *Orobanche* spp.

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**ABSTRACT**

The seed germination of *Orobanche* is initiated by the stimulants present in the root exudates of host plants. Therefore, in order to find out the polarity nature of germination stimulants, root extract of tomato, tobacco and brinjal plants were assayed through different organic solvents of varying polarity by the flow chart method. Hexane and ethyl acetate fractions of root extract of tobacco, tomato and brinjal induced better germination than any other solvent tested. Tobacco root extract fractions isolated in ethyl acetate induced the highest germination compared to hexane. Tomato root extract fractions in different solvents also produced similar results. The performance of root extract of brinjal was more or less similar to that of tobacco and tomato root extracts except that the percentage of germination in both the solvents was greater than in other two host plants. The polarity of one form of stimulants matches with that of hexane (1.89) and polarity of another form with that of ethyl acetate (6.02).

*Orobanche* spp. (Broomrapes) are holoparasites that acquire all nutrition and water from their host through root connection. The seeds of *Orobanche* germinates in the soil only when they come in contact with chemical stimulants exuded by its specific host. It has been reported that the roots of host plants of *Orobanche* possess one or more types of chemicals that stimulate seed germination in the field condition.Three different types of compounds (dihydroquinones, sesquiterpene and strigolactone) have been identified as germination stimulants for parasitic plants (Bouwmeester *et al.* 2003). Different germination stimulants such as strigol, strigyl acetate, sogolactone, alectrol and orobanchol were isolated from root exudate of host and non-host plants. These compounds belong to strigolactones groups and are potent stimulants of both *Striga* and *Orobanche* (Bouwmeester *et al*. 2003). Other germination stimulants such as peapolyphenols A-C (Evidente *et al.* 2010), dehydrocostus lactone (Joel *et al.* 2011), or isothyocynates (Auger *et al*. 2012) have been identified. The sitmulant is required at optimum concentration, which if exceeded causes reduced germination (Brown *et al.*1951). The first germination stimulant from sunflower root exudate was identified as dehydrocostus lactone, a sesquiterpene lactone (Frank *et al.* 2013). Besides dehydrocostus lactone, costunolide, tomentosin, and 8-expixanthatin were purified and identified spectroscopically.

It is a general practice of using host root extract or exudation in laboratory study of *Orobanche* seed germination. Very few reports are available regarding the specific work on polarity nature of germination stimulant exuded by host root. Whitney (1979) has reported that the stimulant is soluble in die-ethyl ether but Chabrolin (1938) found that the stimulant is insoluble in organic solvents. Therefore, understanding polarity nature of stimulating chemicals could be a step towards the identification of stimulating chemicals present in the host plants. In this context, root extract of tomato, tobacco and brinjal, which are usual hosts of *O. solmsii*, were prepared by flow-chart method in different solvents of increasing polarity and were, tested in *invitro* germination of seeds of the parasitic species.

## Method of preparation of root extract

Root extracts of tomato and brinjal plants were prepared in different solvents in order of hexane®ethylacetate®butanol® water (**Figure 1**).

Similarly, tobacco root extracts were prepared by the same method but using two different orders of

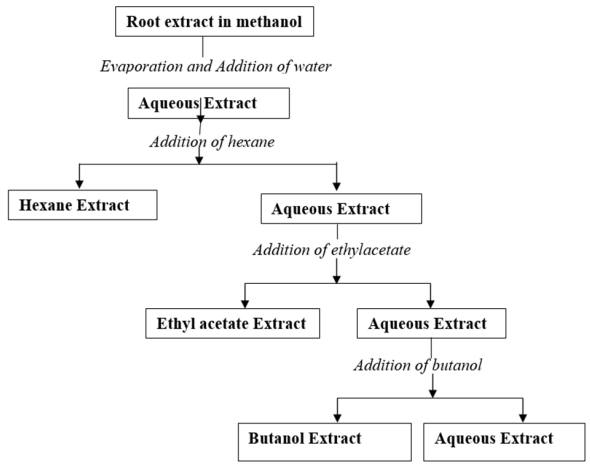



**Figure 1. Flow chart showing the method used in the separation of germination stimulants present in the host root extract by different solvents**

solvents.

(a) Hexane® benzene ® carbon-tetrachloride ®etylacetate

(b) Ethylacetate® benzene ®carbon-tetrachloride® hexane

Fresh roots of host plants (flowering stages) were taken out from the soil carefully so as to obtain maximum root parts and washed thoroughly with tap water and then with distilled water. Roots were cut into pieces and dried under laminar flow. Root extract was prepared by blending the dried roots with methanol at 1:30 ratio (1g root/30 ml methanol) at 20 $^0$C. The whole blended mass was shaken for few minutes in a vertical shaker and supernatant methanol fraction was removed. The extract was evaporated to dryness at 20 $^0$C with the help of aerator. The residue was taken out in 52.5 ml water to get aqueous extract, which was further extracted in different solvent in order of increasing polarities by solvent extraction method as shown in **Figure 1**.

**Treatment of *Orobanche* seeds with root extract in different solvents**

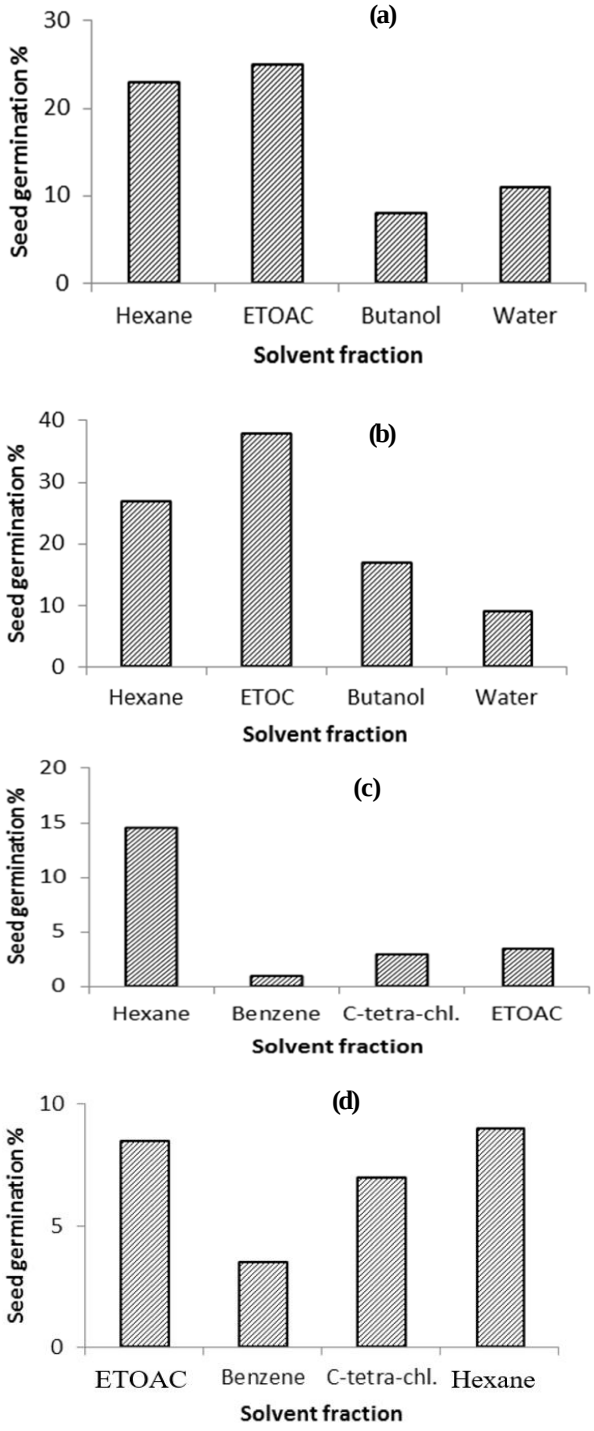
Before preconditioning, the *Orobanche* seeds were sterilized with 1% sodium hypochlorite solution for 5 minutes followed by washing with distilled water and then incubated at 22 $^0$C for 10 days. Preconditioning was done in small vial and distilled water in the vial was changed regularly at an interval of three days. After preconditioning period, the seeds were post-conditioned with different solvent extract prepared above. The concentration (amount) of the root extract used were 10, 20, 40, 80 µl and control (solvent). Solvent part of the extract was allowed to evaporate completely from GFFP disks before preconditioned seeds were put on the solvent treated disks. Each GFFP disks contained about 40 seeds and moistened with distilled water. All the treatments were replicated five times. The incubation was done at room temperature (25-27 $^0$C) for 10 days and germinated seeds were counted under binocular microscope.

In the present study, fractions of root extract of tomato in ethyl acetate at 80 µl (24.28%) and hexane at 10 µl (22%) stimulated the highest percentage of seed germination than the fractions in other solvents (**Figure 2a**). It can be inferred that there was a greater amount of ethyl acetate soluble stimulatory chemicals than the hexane soluble stimulatory chemicals in tomato root extract. The different solvent concentrations did not produce marked difference in the percentage of seed germination. In hexane fraction, there was a progressive decrease in percentage of germination with the increase of concentration. The result indicated that lower concentration of hexane fraction contains more stimulatory chemicals than the higher concentration.

In case of brinjal root extract, ethyl acetate induced better germination (38%) at 40 µl and hexane 27% at 80 µl (**Figure2b**). The result supports the former experiment where ethyl acetate gave the highest percentage of seed germination in tomato root extract. The performance of root extract of brinjal was found to be more or less similar to that of tobacco and tomato except that percentages of germination in hexane and ethyl acetate fractions were greater than in the other two host plants. This indicated that brinjal plants contain more stimulatory chemicals per unit root mass.

When tobacco root extract was essayed through different solvents in order of hexane, benzene, carbon tetra chloride and ethyl acetate, root extract in ethyl acetate stimulated higher seed germination than in hexane (**Figure 2c)**. Unlike previous order of solvents, fractions essayed in order of ethyl acetate, benzene, carbon tetra chloride and hexane, it was only ethyl acetate that produced better percentage (14%) of seed germination at 80 µl (**Figure 2d**). This indicated that a solvent of greater polarity, like ethyl acetate could dissolve stimulatory chemicals of lower polarity and, on the other hand, solvent of lower polarity, like hexane could not dissolve stimulatory chemicals of higher polarity. Furthermore, fraction in ethyl acetate induced germination better than the fraction in hexane. It indicated that the root stimulant(s) of tobacco plant is more soluble in ethyl acetate than in hexane and in, another words, ethyl acetate soluble form of stimulant is greater in amount than the hexane soluble form.

Low percentage of *Orobanche* seed germination in root extract fractions of other solvents like,




(The bars in the figure represent mean value of percentage germination in different concentration (µl) of each-solvent tested; ETOAC= ethyl acetate, C-tetra.chl= carbon tetrachloride)

**Figure 2. Germination of *O. solmsii* seeds in different solvent fractions of the root extract of (a) tomato (b) brinjal (c) tobacco prepared by using the order hexane-benzene-carbon-tetrachloride-etylacetate (d) tobacco prepared by using the order ethylacetate-benzene-carbon-tetrachloride-hexane**

benzene, carbon tetrachloride, di-ethylether, butanol and water was in contrasts with the findings of Sunderland (1960) and Whitney (1979) who found that the stimulants are soluble either in water or in ether.

In the present study, hexane and ethyl acetate fractions of root extracts of tobacco, tomato and brinjal induced germination better than any other solvents tested. This suggests that the stimulatory chemicals exist in more than one form in the host plants and possibly, in two main forms. On this basis, it can be said that the polarity of one form of stimulants matches with that of hexane (1.89) and polarity of another form with that of ethyl acetate (6.02).

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