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EFFECTS OF STIMULANT PH, LIGHT AND SOIL MOISTURE ON *Orobanche solmsii* SEED GERMINATION

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ABSTRACT

In order to investigate effect of soil moisture, *Orobanche* seeds were pre-conditioned in three different soil moisture conditions. The germination percentage was found to be highest (48.31%) in normal soil moisture condition and lowest (4.6%) in flooded soil moisture condition. This indicates that *Orobanche* seeds are unable to survive for a long period in water logged conditions. In response to stimulant pH, *Orobanche solmsii* seeds showed significantly a high germination percentage (65.27%) at pH value 6.5 and it declined progressively with the increase of acidic and alkaline conditions. However, seeds appeared to be more sensitive to alkaline rather than acidic condition. The study of effect of light showed that exposure of seeds to continuous light during pre and post-conditioning period inhibited seed germination. The inhibition was more effective when seeds were exposed to light during post conditioning phase rather than during pre- conditioning phase.

Key words: Stimulant, pre-conditioning, post-conditioning.

INTRODUCTION

Orobanche solmsii (Broomrape) is a holoparasite causing severe damage to solanaceous crops like tobacco, tomato and brinjal. Tobacco plant is seriously attacked by this weed in Terai regions of Nepal. Broomrapes have an enormous seed production potential and seeds remain viable for longer period in the soil (Puzzilli 1983). Due to this, the available method of control against broomrape have not proven as effective, economical and applicable as predicted (Perez-de-Luque *et al.* 2008, Goldwasser *et al.* 2004).*Orobanche* attach to the roots of host plants from which they drain water, minerals and photo assimilate resources. *Orobanche* species can attack a wide range of host plants, and those that infect crops can cause considerable agricultural damage. As obligate parasites, broomrape seedlings can only survive for a few days after germination before connecting to a host root. When stimulated after a conditioning period of several days under appropriate temperature and humidity, seeds germinate and produce a radicle that can adhere to a host root. The stimulants interact with different factors and obviously, the soil pH, which might affect activeness of the chemicals (Saghir 1986, Whitney 1986). Therefore, in the light of above presumption, present study was under taken to explore the pH, soil moisture and light condition that *Orobanche* prefers most in agronomic condition of Nepal.

MATERIALS AND METHODS

In all the experiment, *Orobanche solmsii* seeds were sterilized in 5% sodium hypochlorite solution for 5 minutes and washed several times with distilled water (Ben-Hod *et al.* 1993).

Treatment for pH experiment: In this experiment, the synthetic growth regulator (GR_{24}) of different pH was prepared using either 0.001 M of KH2PO4 or NaOH and distilled water. The experiment was based on the fact that the buffer solution of 0.001 M Potassium dihydrogen phosphates and 0.001 M Sodium hydroxide do not affect the process of *Orobanche* seed germination (Pieterse 1981). GR_{24} solution (10 ppm) of six different pH value (4.5, 5.5, 6.5, 7.5, 8.5 and 9.5) were prepared. Sterilized seeds were pre-conditioned in distilled water having pH value of 6.7 at 20-22ºC for 10 days in dark. After this, the seeds were washed with distilled water and put in a bottle containing 10 ml distilled water. Then the seeds were post-conditioned in GR_{24} solution of different pH values at 24-26ºC for 10 days. Each treatment was replicated three times. Germinated seeds were counted under binocular microscope after 10 days of post-treatment.

Treatment for light experiment: In this experiment the sterilized seeds of *O. solmsii* were pre-conditioned in light and dark condition in the petridishes containing double layer of filter paper moistened with distilled water (5 ml). There were two sets of petridishes and each set consisted of six petridishes. One of the set of petridish was incubated in the continuous dark and another in continuous light at room temperature (23-25ºC). After 10 days, the petridishes were post-conditioned in 10 ppm GR_{24} solution. Out of six petridishes subjected to continuous dark during pre-conditioning, three were post-conditioned in dark and three in continuous light.

Treatment for soil moisture experiment: In this experiment, *Orobanche solmsii* seed germination was observed after exposing the seeds in different soil moisture condition. Soil was collected from the highly infested field of tobacco research center in Belachhapi of Dhanusa district. Three plastic pots were filled with infested soil after making a hole at the bottom for drainage. *Orobanche seeds* (10 mg) were packed in a small piece of nylon cloth and tied with nylon thread. The seed packets were buried in the soil at the depth of 5 cm leaving other end of threads out of the soil. There were three packets of seeds in each plastic pot. The three pots were subjected to different moisture conditions: dry, normal and flooding by adding 50 ml, 150 ml and 350 ml of water, respectively. The plastic pots were covered with polythene sheets and placed in the dark at room temperature (24-26°C).

Preparation of root extract: Root of tomato plant grown in pots was taken out carefully and washed thoroughly with tap water and then with distilled water. It was air dried for some time, cut into small pieces and then ground with mortar and pestle by adding ethyl acetate (at 1:30 ratio). The supernatant was decanted and then passed through a column filled with anhydrous sodium sulphate to obtain root extract in ethyl acetate.

Post-treatment: After 10 days of pre-conditioning period, packets of seed from each plastic pot were retrieved, sterilized and post conditioned in tomato root extract (extracted in ethyl acetate) and GR_{24} separately. Each GFFP (Glass Fiber Filter Paper) in the petridishes were treated with either 5 µl tomato root extract or with GR_{24} (10 ppm). The disks treated with root extract were left as such for some time in order to allow ethyl acetate to evaporate. Altogether, there were ten petridishes (replications) for each soil moisture treatment (treatments): five for root extract and five for GR_{24}. Each treatment consists of a set of petridish without stimulants (as a control). All the petridishes were packed inside the polythene bag and incubated in the dark at 25-26ºC for 10 days. Seeds were considered germinated only when the radicle emerged from the seed. Germinated seeds were counted under binocular microscope.

DATA ANALYSIS

The seed germination (%) data obtained from these experiments were processes through ANOVA and Duncan's Multiple Test (DMRT) to understand amount of difference between the values of percentage of *Orobanche* seed germination resulted from different treatment.

RESULTS AND DISCUSSIONS

In response to different pH conditions, *Orobanche* seeds gave significantly a high percentage (65.27%) of germination at pH value, 6.5 (Fig. 1). It declined progressively with the increase of acidic and alkaline conditions. However, the rate of decline was sharper with the increase of alkaline condition than with acidic condition. Little emphasis has been given in the study of soil pH in the parasitization process of *Orobanche* species. In the field condition, the parasite germinates only when the host releases stimulant in the soil. The results of the present study showed that slightly acidic condition of the stimulant was better for *Orobanche* seed germination than the neutral or alkaline conditions. The finding is in agreement with the report of (Brown *et al.* 1951) that stimulants for *Orobanche* seed germination are highly sensitive to alkaline pH and less sensitive to acidic condition above pH 4. The report of Bischoff and Koch (1973) also supports that best germination occurs in slightly acidic soils. Similar results were also obtained by Brown *et al.* (1965) and Johnson *et al.* (1976) in *O. minor* in which seed germination was inhibited by the alkaline solution but retarded in the acidic medium. The study suggests that application of agricultural lime in the acidic field might help to reduce *Orobanche* problem to some extent.

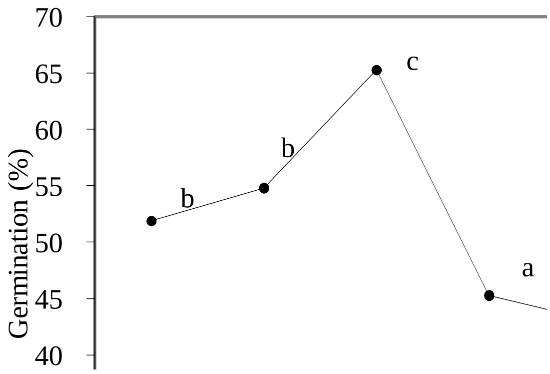

Fig. 1. Effects of different pH on *O. solmsii* seed germination.

(Treatment values with same letters do not differ significantly at P= 0.05 according to Duncan's Multiple Range Test)

The influence of light on *Orobanche* seed germination has received less importance than other ecological parameters (Sauerborn *et al.* 1991).There is a wide variation in the germination of seeds to light. Some seeds germinate well in light and some in dark, still there are seeds, which are indifferent to light (Khan 1977). In the present study, *Orobanche solmsii* seeds showed significantly high percentage of germination (68.87%) in the light-dark treatment than in the light–light treatment (31.98%) under the stimulation of GR_{24}. The finding is in agreement with the reports of Hiron (1973) and Jain and Foy (1987) who concluded that light might act as an inhibiting factor at least in older seeds of *O. ramosa* and *O. crenata*. However, Izard and Hitier (1958) and Privat (1960) contradicted with the present results as they reported that *O. ramosa* seed germination was indifferent of light and dark conditions. Spontaneous germination (control) of the seeds in response to the light and the dark conditions also gave more or less similar results except in light-light treatment (Table 1).

An additional aspect of the present study is that presence or absence of light is important during post-conditiong phase rather than in pre-conditiong phase of *O. solmsii* seed germination. This is in contrast with the findings of Egley (1972) who reported that light provided during pre-conditioning phase has inhibitory influence on *Striga* seed germination. Furthermore, dark period provided during post-conditiong phase seemed to have favoured *Orobanche* seed germination. Saunders (1993), Worsham *et al.* (1964) and Egley (1972) have also reported similar results in *Striga asiatica*.

Table 1. Effects of light and dark conditions on *O. solmsii* seed germination

Treatments		Germination (%)	
Pre-conditioning	**Post-conditioning**	**Control**	**GR_{24}**
Light	Light	1.08 a	31.98 a
Dark	Light	5.14 b	56.64 b
Dark	Dark	6.86 b	59.7 bc
Light	Dark	6.33 b	68.87 c

(Mean values followed by same letters in vertical columns do not differ significantly at P= 0.05 according to Duncan's Multiple Range Test)

When the seeds were treated with different soil moisture condition, the germination percentage was significantly high in the seeds pre-conditioned in normal soil moisture condition and post conditioned in GR_{24} as well as in tomato root extracts (Fig. 2). Maximum seed germination (48.31%) was observed in seeds pre-conditioned in normal soil moisture condition. The necessity of optimum soil moisture is indicated by the maximum germination of seeds in normal soil moisture condition. This is supported by the findings of Pearson (1913) who reported that *Striga asiatica* seeds germinated much less in soil capable of retaining water than in the soil that quickly dried out. Spontaneous germination (without GR_{24} and root extract) was found highest (21%) in normal soil moisture condition. Probably this is due to the fact that *O. solmsii* seeds synthesized gibberellins like substance during pre-conditioning period. *Orobanche solmsii* seeds were found to be sensitive to soil moisture requirement during pre-conditioning period. A similar result were observed by Babiker *et al.* (1982, 1987) in the germination of *Striga hermontheca* seeds in response to the strigol analogs GR_7 and GR_{24} and was influenced by soil moisture prior to, during, or after stimulation. The necessity of optimum soil moisture is indicated by the maximum germination of seeds in normal soil moisture condition. It shows that once the field is irrigated *Orobanche* seeds become capable of germination until the soil can hold water.

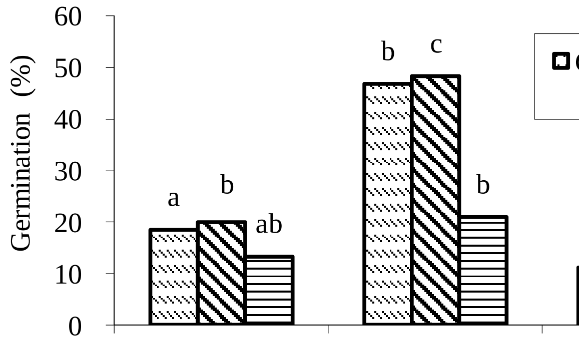

Fig. 2. Response of *O. solmsii* seed germination to different soil moisture conditions.

(Treatment values having same letters in vertical columns do not Differ significantly at P= 0.05 according to Duncan's Multiple Range Test)

Significantly low germination in flooding soil condition in *in vitro* might be due to direct effect on the dilution of endogenous chemicals responsible for the receptiveness of stimulant produced during pre-conditioning period. In the field condition the dilution of root exudates and reduction of oxygen supply could occur due to flooding soil condition. This might be the reason for less *Orobanche* problem in the moist soil (Cicerone and Piglionica 1979). So the rice crops that require flooding are rotated with host plants to reduce *Orobanche* infestation (Cubero 1983). It has been reported that exposure of seeds to excessive soil moisture content (70% w/w) during conditioning resulted in a low response to GR_{24}. Furthermore, transfer of seeds from soil at 70% moisture to lower moisture level (47%) for 2 days or more improved the response to GR24 (37% germination), while air-drying restored germination (Babiker *et al.* 1982).

On the basis of this *Orobanche* seed germination study, it can be concluded that light during pre-conditioning has little effect than post-conditioning period as in other seeds. In response to different pH, seeds appeared to be more sensitive to alkaline condition rather than acidic condition. This suggests that the application of agricultural lime in the acidic field can reduce the *Orobanche* problem to some extent. The result of the soil moisture study suggests that *Orobanche* seeds are unable to survive for a long period in waterlogged condition and this might be the reason for low incidence of *Orobanche* problem in rice field.

REFERENCES

Babiker, A.G.T. and A.M. Hamdoun. 1982. Factors affecting the activity of GR7 in stimulating germination of *Striga hermonthica* (Del.) Benth. *Weed Research* **22(2):**111-115.

Babiker, A.G.T., A.M. Hamdoun, A. Rudwan, N.G. Mansi and H.H. Faki. 1987. Influence of soil moisture on activity and persistence of the strigol analogue GR 24. *Weed research* **27(3):**173-178.

Ben-Hod, G., D. Losner, D.M. Joel and A.M. Mayer. 1993. Pectin methylesterase in calli and germinating seeds of *Orobanche aegyptiaca*. *Phytochemistry* **32(6):**1399-1402.

Brown, R., A.D. Greenwood, A.W. Johnson, A.G. Long, and G.J. Tyler. 1951. The stimulant involved in the germination of *Orobanche minor* Sm. 2. Chromatographic purification of crude concentrates. *Biochemical Journal* **48(5):**564.

Brown, R. 1965. The germination of angiospermous parasite seeds. In: *Differenzierung und Entwicklung/ Differentiation and Development*. Springer Berlin Heidelberg. pp. 2572-2579.

Bisscof, F. and W. Koch. 1973. Einige Beitrage zur Biologie von *Orobanche aegyptiaca* L. In: *Symp. on Parasitic Weeds, European Weed Research Council, Malta*, pp. 48-54.

Ciccarone, A. and V. Piglionica. 1979. Attempts to control *Orobanche* in southern Italy. Some current research on *Vicia faba* in Western Europe, edited by DA Bond, GT Scarascia-Mugnozza and MH Poulsen; sponsored by the Commission of the European Communities, Directorate-General for Agriculture, Coordination of Agricultural Research.

Cubero, J.I. 1983. Parasitic diseases in *Vicia faba* L. with special reference to broomrape (*Orobanche crenata* Forsk.). *The Faba Bean* pp. 493-519.

Egley, G.H. (1972). Influence of the seed envelope and growth regulators upon seed dormancy in witchweed (*Striga lutea* Lour.). *Annals of Botany* **36(4):**755-770.

Goldwasser Y. and Y. Kleifeld. 2004. Recent approaches to *Orobanche* management: A review. In: *Weed Biology and Management*. (ed.) Inderjit, S. Amsterdam, The Netherlands, Kluwer, pp. 439–466.

Hiron, R.W.P. 1973. An investigation into the processes involved in germination of *Orobanche crenata* using a new bio-assay technique. In: *Proc. Eur. Weed Res. Council Symp. Parasitic Weeds*. pp. 76-88.

Izard, C. and H. Hitier, H. 1958. Action Du I. 3-dichloropropane I. 2-dichloropropene, De La Rindite Et De La Gibberelline, Sur La Germination Des Graines De Lorobanche, Parasite Du Tabac. Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences, **246(18):**2659-2661.

Jain, R. and C.L. Foy. 1987. Influence of various nutrients and growth regulators on germination and parasitism of *Orobanche aegyptiaca*. In: *Proceedings of the 4th International Symposium on Parasitic Flowering Plants*. Marburg, Germany, Philipps-Universitat, pp. 427-436.

Johnson, A.W., G. Rosebery and C. Parker. 1976. A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. *Weed Research* **16(4):**223-227.

Khan, A.A. 1977. Seed dormancy: Changing concepts and theories. *Physiology and Biochemistry of Seed Dormancy and Germination*.

Pearson, H.H.W. 1913. The problem of Witchweed. *Agric. J. Union S. Africa* **6:**803.

Pérez-de-Luque, A., M.T. Moreno and D. Rubiales. 2008. Host plant resistance against broomrapes (*Orobanche* spp.): Defence reactions and mechanisms of resistance. *Annals of Applied Biology* **152(2):**131-141.

Pieterse, A.H. 1981. Germination of *Orobanche crenata* Forsk. seeds in vitro. *Weed Research* **21(6):**279-287.

Pieterse, A.H. 1981. Germination of Orobanche crenata Forsk. seeds in vitro. *Weed Research* **21(6):**279-287.

Privat, G. (1960). Recherches sur les phanerogames parasites. (Etude d'Orobanche hederae Duby). *Annls. Sci. Nat. Bot.* **1:**721-871.

Puzzilli, M. 1983. Tobacco broomrapes and their control and some useful references to other parasite and host species. *Rivista di Agricoltura Subtropicale e Tropicale* **77(2):**209-248.

Saghir, A.R. 1986. Dormancy and germination of *Orobanche* seeds in relation to control methods. In: *Proceedings of a Workshop on Biology and Control of Orobanche, Wageningen (Netherlands), 13-17 Jan 1986*. Landbouwuniversiteit.

Sauerborn, J., G. Wyrwal and K.H. Linke. 1991. Soil sampling for broomrape seeds in naturally infested fields. *Progress in Orobanche Research, Eberhard-Karls Universität: Tübingen, Germany*, pp. 35-43.

Saunders, A.R. 1993. Studies on phanerogarmic parasitism with particular reference to *Striga lutea.* South African Department of Agriculture Bulletin, 128 p.

Worsham, A.D., D.E. Moreland and G.C. Klingman. 1964. Characterization of the *Striga asiatica* (Witchweed) Germination Stimulant from *Zea mays* L. *Journal of Experimental Botany* **15(3):**556-567.

Whitney, P.J. 1986. Factors affecting *Orobanche* seed germination. In: *Proceedings of a Workshop on Biology and Control of Orobanche*, Wageningen (Netherlands), 13-17 Jan 1986. Landbouwuniversiteit.