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Potency of Some Synthetic Stimulants and Root Exudates on the Germination of *Phelipanche* spp.

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ABSTRACT

Broomrapes (*Orobanche/Phelipanche* spp.) are considered as the most important problem of some cultivated plants, especially belonging to Solanaceae and Fabaceae families. *Phelipanche ramosa* L. and *Phelipanche aegyptiaca* (L.) Pers. cause serious problems especially in tomato grown areas in Turkey and the other Mediterranean countries. This study was conducted to determine the effect of some synthetic stimulant substances and root exudates on the germination rate of the broomrape species under controlled laboratory conditions. GR24 (0.1-1 ppm), GR7 (0.1-1 ppm) and GA3 (10 ppm) were used as synthetic germination stimulant substances; otherwise flax, cotton, soybean, bean, pea, cowpea, tomato, lentil, cucumber and tobacco seedlings were used for root exudates. According to average results of the trials; synthetic stimulants and root secretions increased the germination rate of *P. ramosa* at the range of 50-77% and 0-16% respectively, otherwise average data for *P. aegyptiaca* are 62-95% and 0-63%. As a result of the studies it was determined that synthetic stimulants were significantly increased the germination rate of *Phelipanche* species.

Keywords: Broomrape; *Orobanche/Phelipanche*; Parasite plant; Germination stimulants; Trap crops

Phelipanche spp. Çimlenmesi Üzerine Bazı Sentetik Stimulantların ve Kök Salgılarının Potansiyel Etkisi

ESER BİLGİSİ

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ÖZET

Canavar otları, özellikle Solanaceae ve Fabaceae familyalarına ait bazı kültür bitkilerinin en önemli sorunu olarak değerlendirilmektedir. *Phelipanche ramosa* L. ve *Phelipanche aegyptiaca* (L.) Pers. Türkiye ve diğer Akdeniz ülkelerinde daha çok domates üretim alanlarında ciddi sorunlara neden olmaktadır. Bu çalışma bazı bitki kök salgılarının ve sentetik

stimulant maddelerin, bu canavar otu türlerinin çimlenme oranına etkisini belirlemek amacıyla kontrollü laboratuvar koşullarında yürütülmüştür. Çalışmada sentetik çimlenme stimulantları olarak GR24 (0.1-1 ppm), GR7 (0.1-1 ppm) ve GA3 (10 ppm), ile keten, pamuk, soya, fasulye, bezelye, börülce, domates, mercimek, hıyar ve tütün fidelerine ait kök salgıları kullanılmıştır. Denemelerden elde edilen ortalama sonuçlara göre; sentetik stimulantlar ve kök salgıları *P. ramosa* çimlenme oranını sırasıyla % 50-77 ve 0-16 arasında artırırken, *P. aegyptiaca* için elde edilen ortalama değerler % 62-95 ve % 0-63 arasında olmuştur. Sonuç olarak, sentetik stimulant maddelerin canavar otlarının çimlenme oranını önemli derecede artırdığı belirlenmiştir.

Anahtar Kelimeler: Canavar otu; *Orobanche/Phelipanche*; Parazit bitki; Çimlenme stimulantları; Tuzak bitkiler

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1. Introduction

Broomrapes (*Orobanche/Phelipanche* spp.-Orobanchaceae) are obligate root parasite weeds with a widespread distribution and cause significant yield losses in the Mediterranean region including Turkey. It is problematic in most cultivated crops; mainly tomato, lentil, sunflower, tobacco. The weed produces thousands of minute seeds that are highly persistent in the soil and can easily spread to new areas. Moreover, due to the intimate connection between these holoparasitic weeds and their hosts no economically viable and effective control system against the parasites could be developed. The insufficiency of countermeasures against broomrapes leads to a continuous increase in the importance of these weeds in agricultural areas.

Seed germination of broomrape depends on compounds that are produced by the roots of their hosts. Clearly, the chemical signaling involved in this first step of the life cycle is crucial in the life of these parasitic plants (Bouwmeester et al 2003). Therefore, many research groups have studied the chemistry of this interaction. Up to now, several germination stimulants were identified from host and non-host plants. Most of these compounds belong to one chemical class, called the strigolactones (Matusova & Bouwmeester 2005).

Natural strigolactones isolated and identified to date are Strigol, Sorgolactone, Alectrol and Orobanchol (Matusova et al 2005; Yoneyama 2009). They are examples of very potent germination stimulants for parasitic weeds of the genera *Striga*, *Alectra* and *Orobanche/Phelipanche* (Zwanenburg

et al 1997; Matusova et al 2005; Yoneyama 2009). Strigol, the first strigolactone, was a *Striga* germination stimulant isolated from the root exudates of a false host, cotton (*Gossypium hirsutum* L.) (Cook et al 1972) and then identified in the root exudates of *Striga* hosts, sorghum [*Sorghum bicolor* (L.) Monch], maize (*Zea mays* L.) and proso millet (*Pennisetum glaucum* R.Br.) (Siame et al 1993). Sorgolactone and Alectrol were isolated from the root exudates of sorghum (Hauck et al 1992) and cowpeas (*Vigna unguiculata* Auct.) (Muller et al 1992), respectively. Some germination stimulant(s) have also been identified for the *Orobanche* spp. and these also belong to the strigolactones. Alectrol, Orobanchol and a third unidentified compound were isolated from the root exudate of red clover (*Trifolium pretense* L.) (Yokota et al 1998; Matusova et al 2005; Yoneyama 2009).

Once broomrape seeds have germinated, they must establish contact with the host roots rapidly in order to derive nutrients and water for further growth and development. This germination phase is a critical period in the life cycle of the parasite (Musselman & Press 1995; Dhanapal et al 1996). If broomrape seeds are stimulated to germinate in the absence of host plants, the parasite seedlings die because of lack of nutrition. By stimulating the germination through chemicals in the absence of hosts or through natural stimulants by exposing seeds to trap crops, the seed bank can be reduced. This suicidal germination is a basis for these chemical and biological control.

Suicide germination by natural or synthetic germination stimulants has emerged as an attractive

method for controlling broomrape. Due to their low concentrations, strigolactones cannot be isolated and used for field application in order to induce suicide germination in the absence of a host. Strigolactones, however, are exuded also by non-host plants (trap crops). This strategy consists in the use of non-host species that produce germination stimulants, inducing massive suicidal germination of the parasite. Cultivation of trap crops is a means for reducing the broomrape seed bank in the soil. Due to the specificity of root exudates, more research is required to identify specific trap crops for each broomrape species (Wegman 2006). The non-host plants promote the germination of broomrape without being parasitized and because of this characteristic, these false non-host plants are known as trap crops. Flax, bean, soybean, pea and cowpea are some of the trap crops of *Phelipanche ramosa* and *P. aegyptiaca* (Sauerborn 1991).

Another approach for the induction of suicidal germination is the use of chemicals. They could be used to induce suicidal germination by treating the soil before the crop is sown (Lopez et al 2008). Because the isolation and purification of strigolactone in large amounts from root exudates appears impractical; an artificial synthesis of strigolactone is necessary to obtain enough compounds for commercial use (Dhanapal & Struik 1996). The application of germination stimulants to induce suicidal seed germination of parasitic weeds appears attractive for biosafety reasons, rapid soil decomposition, and high biological activity at very low application rates (Elzein & Kroschel 2004). Compounds structurally related to strigolactone are potent synthetic germination stimulants for many *Orobanchaceae* species (Zwanenburg & Wigchert 1998; Wigchert et al 1999). Synthetic strigolactone analogues of the strigolactones have been synthesized by several working groups: Johnson, Welzel, Zwanenburg (Wegman 2006). Prominent examples of synthetic analogues include GR24, GR7, DMSL and Nijmegen 1. These analogues exhibit appreciable or high activity in the stimulation of the germination of seeds of root parasitic weed species (Zwanenburg et al 1997).

The induction of the germination of parasitic plants using germination stimulants has been studied by many groups as a possible target for control measures. For example, Zwanenburg and coworkers worked on the development of synthetic germination stimulants to induce suicidal germination under field conditions. Ejeta and coworkers selected sorghum lines with reduced induction of germination. Also, the wide use of trap crops, used in monoculture or in intercropping, and catch crops is a control measure partly based on the suicidal induction of germination (Matusova et al 2005).

This study was carried out to determine the effects of different synthetic stimulants and root exudates on the germination of *Phelipanche* seeds under controlled conditions. GR24, GR7 and GA3 (Gibberellic acid) were used as synthetic germination stimulants and, flax, cotton, soybean, bean, pea and cowpea were used for exudates. The results of the study provide the basis for studies of broomrape management.

2. Material and Methods

Phelipanche ramosa (Hemp broomrape) seeds were collected from tomato fields in 2006 and *Phelipanche aegyptiaca* (Egyptian broomrape) seeds from lentil fields in 2004. Three synthetic stimulant substances and ten plant exudates were investigated on both broomrape species (*Phelipanche ramosa* and *P. aegyptiaca*). The petri dish trials were conducted with 16 characters for each broomrape species and repeated twice. The first experiment was done in Weed Science Laboratory of Biological Control Research Center while the second one was done in the Department of Plant Protection, Faculty of Agriculture, Çukurova University-Adana in 2007. During the trials, GA3 was applied in a 10 ppm dose while GR7 and GR24 were applied in 0.1 and 1 ppm doses. For the purpose of comparing the effect of synthetic substances and plant exudates, pre-incubated broomrape species were taken as control applications. The synthetic strigolactone analogues GR24 and GR7 were kindly provided by Professor B. Zwanenburg, University of Nijmegen, the Netherlands. The synthetic stimulants and plant exudates (root secretions) applied to pre-incubated

Phelipanche seeds and germinated seeds were counted at the end of the trial period (Kroschel 2001).

2.1. Preconditioning of parasite seeds prior to exposure to the stimulant

Sterile petri dishes (9 cm diameter) were lined with two layers of Whatman glass microfiber papers. Small Whatman paper disc with 1.5 cm diameter was placed in the center of the petri dish. Minimum 100 seeds of the parasite species were sprinkled on each small disc. Each petri dish was moistened with 5.5 mL of deionized water containing 100 ppm of GA3 to enhance preconditioning in the *Phelipanche* seeds. After the petri dishes were closed, they were sealed in small, lightproof black plastic bags in order to prevent the loss of water. These prepared seeds were kept in a dark incubator at 23 °C for 10 days to respond to the stimulants.

2.2. Preparation of the germination stimulants

2.2.1. Preparation of root exudates

The host and non-host (trap) plants were grown in small (23x32 cm) plastic tubs filled with pure sterile sand. 100 seeds of each plant were sown in the tubs with the appropriate number depending on the size of the seeds. After 10-12 days the seedlings were removed and the roots were carefully washed to remove sand. The roots of 20 seedlings were immersed in 100 mL of deionized water in a 100 mL flask or erlenmeyer flask, depending on the size of the seedlings. Small delicate seedlings were supported with non-absorbent cotton, so that the roots were immersed in water. The seedlings were allowed to grow under the same growth conditions for two days. The water losses due to transpiration were compensated. After two days the seedlings were discarded, and the solutions were used in germination experiments.

2.2.2. Preparation of synthetic germination stimulants

Among the synthetic stimulants, GA3 was prepared as a dose of 10 ppm by diluting it with distilled water. To prepare GR7 and GR24 at doses of 0.1 and 1 ppm, primarily the stock solutions were prepared

by dissolving an accurately weighed amounts of 1 mg of GR using a five-decimal balance amount in 1 mL acetone measured with a measuring pipette and distilled water was added to this solution until a total volume of 100 mL was reached. In this way the stock solutions, containing 10 ppm of GR (10 mg L⁻¹), were obtained.

From this stock, 1 ppm and 0.1 ppm dilutions were prepared by adding the appropriate volumes of distilled water (for 1 ppm GR, by adding distilled water to 10 mL stock solutions until a total volume of 100 mL was reached and for 0.1 ppm GR, by adding distilled water to 1 mL stock solutions until a total volume of 100 mL was reached).

2.3. Stimulation of germination and evaluation

Germination stimulants were added at a dose of 5.5 mL to each petri dish containing the pre-conditioned broomrape seeds. Thereafter, the closed petri dishes were put in black plastic bags again and kept in a dark incubator at 25 °C for seven days to respond to the stimulants.

The broomrape seeds were checked using binoculars and scored as germinated if the root tip (radicle) had protruded through the seed coat; in other words, germination tubercles had been created. The germination rate was determined by counting 100 seeds in each petri dish. The analysis of variance and Duncan's multiple comparison test at a 0.05 significance level using an MSTAT-C program was applied to the results of the study.

3. Results and Discussion

The results showing the effects of some synthetic stimulants and plant root exudates on the germination rate of *P. ramosa* and *P. aegyptiaca* were given in Table 1 and Table 2. The germination of *Phelipanche aegyptiaca* (L.) Pers. was at a higher rate than that of *P. ramosa*, which is considered to be related to the seed age: the *P. ramosa* seeds were collected in 2006 while the *P. aegyptiaca* seeds were collected in 2004. Also, broomrape seeds need a period to germinate after maturation and this period may take a few years (Edwards 1972; Saghir & Dastgheib 1978).

Table 1- Effect of some applications on the germination rate of *P. ramosa* seeds

Çizelge 1- Bazı uygulamaların *P. ramosa* tohumlarının çimlenme oranına etkisi

Applications	Germination rate (%)		
	1 st trial	2 st trial	Average
GA ₃ (10 ppm)	56.5 b	44.0 d	50.3 c
GR ₇ (0.1 ppm)	75.5 a	63.5 c	69.5 b
GR ₇ (1 ppm)	75.5 a	77.5 ab	76.5 a
GR ₂₄ (0.1 ppm)	63.5 b	71.5 bc	67.5 b
GR ₂₄ (1 ppm)	72.5 a	82.0 a	77.3 a
Soybean (<i>Glycine max</i> (L.) Merr.)	0.0 e	0.0 f	0.0 g
Flax (<i>Linum usitatissimum</i> Linnaeus)	8.5 de	13.5 e	11.0 de
Cotton (<i>Gossypium hirsutum</i> L.)	0.3 e	0.3 f	0.3 fg
Bean (<i>Phaseolus vulgaris</i> L.)	0.0 e	0.5 f	0.3 fg
Pea (<i>Pisum sativum</i> Linnaeus)	0.0 e	0.0 f	0.0 g
Cowpea (<i>Vigna unguiculata</i> (L.) Walp.)	5.5 e	0.0 f	1.4 fg
Tomato (<i>Lycopersicon esculentum</i> Mill.)	32.5 c	0.0 f	16.3 d
Cucumber (<i>Cucumis sativus</i> L.)	0.3 e	5.5 ef	2.9 fg
Lentil (<i>Lens culinaris</i> Medik.)	0.0 e	0.0 f	0.0 g
Tobacco (<i>Nicotiana tabacum</i> L.)	15.5 d	0.0 f	7.8 ef
Control	0.0 e	0.3 f	0.1 g

There is no significant difference between the applications as they have the same letter in according to Duncan's multiple comparison test at the 0.05 significance level.

Table 2- Effect of some applications on the germination rate of *P. aegyptiaca* seeds

Çizelge 2- Bazı uygulamaların *P. aegyptiaca* tohumlarının çimlenme oranına etkisi

Applications	Germination rate (%)		
	1 st trial	2 st trial	Average
GA ₃ (10 ppm)	77.5 c	47.5 d	62.5 d
GR ₇ (0.1 ppm)	89.5 b	79.0 b	84.3 c
GR ₇ (1 ppm)	94.0 ab	81.5 ab	87.8 bc
GR ₂₄ (0.1 ppm)	97.5 a	85.0 ab	91.3 ab
GR ₂₄ (1 ppm)	97.5 a	92.5 a	95.0 a
Soybean (<i>Glycine max</i> (L.) Merr.)	0.0 g	0.0 f	0.0 f
Flax (<i>Linum usitatissimum</i> Linnaeus)	70.5 d	54.0 cd	62.3 d
Cotton (<i>Gossypium hirsutum</i> L.)	4.0 g	0.0 f	2.0 f
Bean (<i>Phaseolus vulgaris</i> L.)	0.0 g	0.0 f	0.0 f
Pea (<i>Pisum sativum</i> Linnaeus)	1.0 g	1.5 f	1.3 f
Cowpea (<i>Vigna unguiculata</i> (L.) Walp.)	3.3 g	29.0 e	16.1 e
Tomato (<i>Lycopersicon esculentum</i> Mill.)	12.0 f	0.8 f	6.4 f
Cucumber (<i>Cucumis sativus</i> L.)	1.0 g	0.5 f	0.8 f
Lentil (<i>Lens culinaris</i> Medik.)	63.5 e	63.5 c	63.5 d
Tobacco (<i>Nicotiana tabacum</i> L.)	5.0 g	0.0 f	2.5 f
Control	0.8 g	0.5 f	0.6 f

There is no significant difference between the applications as they have the same letter in according to Duncan's multiple comparison test at the 0.05 significance level.

According to average results of the trials; synthetic stimulants (GR24, GR7 and GA3) and root secretions increased the germination rate of *P. ramosa* at the range of 50-77% and 0-16% , respectively, whereas average data for *P. aegyptiaca* are 62-95% and 0-63%. Similarly, Matusova et al (2004) recorded that GR24 at the same concentrations (0.1 and 1 mg L⁻¹ = 1 and 10 ppm) usually induced a high germination rate for the other broomrape species (*Orobancha cumana*) regardless of the preconditioning period, 5-40 days. GR7 and GR24 are most active at 0.1 to 1.0 mg kg⁻¹ on *Orobancha* seed germination (Saghir 1979; Spelce & Musselman 1981; Saghir 1986; Parker & Riches 1993). Jacobsohn et al (1988) observed over 90% germination of broomrape conditioned in water and then stimulated to germinate by GR24. As the results of the study, GR24 and GR7 stimulated the germination of broomrape seeds to the same degree; this was higher for the 1 ppm dose than for the 0.1 ppm dose. The germination rates of broomrape were not statistically different for 1 ppm doses of GR24 and GR7. GR24 was not statistically different from GR7 for the 1 ppm and 0.1 ppm doses for *P. aegyptiaca*.

According to Aly (2007), Elzein & Kroschel (2003) reported that the application of germination stimulants to induce suicidal seed germination of parasitic weeds appears attractive for biosafety reasons; there is rapid soil decomposition and high biological activity at very low application rates. Compounds structurally related to strigolactone are potent synthetic germination stimulants for many *Striga* and *Orobancha/Phelipanche* species (Zwanenburg & Wigchert 1998; Wigchert et al 1999) but their usage in the field has not been practical up to now due to the high cost involved in their synthesis and also their instability in soils with high pH (Dhanapal & Struik 1996; Kroschel 2001; Lopez-Raez et al 2008). Therefore, detailed studies are needed to solve the issues about the cost

and the formulation of the substances, which are affecting the efficiency and stability of them in the field conditions.

Gibberellic acid at 10 ppm stimulated the germination of both broomrape species. This is consistent with the literature (Al-Menoufi 1986; Hiron 1973). Al-Menoufi (1986) also reported that Gibberellic acid induced germination of *Phelipanche ramosa* seeds. However, GA3 was less effective and statistically different than GR24 and GR7 at 1 and 10 ppm in stimulating the germination of broomrape seeds. Dhanapal & Struik (1996) indicated that GR24 was most effective in stimulating the germination of *Orobancha cernua* seeds and followed by gibberellic acid among several chemicals, similar to that used in our study.

For the data on host plants of *Phelipanche* species (tomato, cucumber, lentil, tobacco); the highest values were obtained in tomato (16.3%) for *P. ramosa* and in lentil (63.5%) for *P. aegyptiaca*, statistically different from the other host plants. Compared to the host and trap crops with each other, the results of lentils, cucumbers, beans, peas, cotton and soybeans were not different from those of the control for *P. ramosa* while the results of tobacco, cucumbers, peas, beans, cotton and soybeans were not different from those of the control for *P. aegyptiaca*.

Among the trap crops (soybean, flax, cotton, bean, pea, cowpea) flax and cowpea provided the maximum germination rates for the broomrape species: 11.0 - 2.7% for *P. ramosa* and 62.3 - 16.1% for *P. aegyptiaca*, respectively. Flax and cowpea are found to be promising trap crops whereas soybean, cotton, bean and pea trap crops were found to be less effective in inducing the germination of *P. ramosa* and *P. aegyptiaca* seeds. Although, Sauerborn (1991) stated that flax, bean, soybean, peas and cowpea are the trap crops of *P. ramosa* and *P. aegyptiaca* and the results on the efficacy of the substance may extremely variable and contradictory (Pieterse 1981; Riches & Parker 1995).

The effective result of flax as a trap crop was consistent with the literature. According to

Wegmann (1994), Kuijt (1969) reported that flax (*Linum usitatissimum* L.) may use for broomrape control as a trap crop. Kleifeld et al (1994) conducted pot and field experiments over two years to determine whether some trap plants reduce the rate of *Phelipanche aegyptiaca* Pers. infestation. The results showed that only flax cultivated for two years or flax cultivated with Jerusalem beans (*Phaseolus aureus* Roxbg.) significantly reduced the infestation rate, but the using of trap crops in areas highly infested with broomrape provided limited success. Similarly, it has been concluded that detailed field trials on the cultivation of host plants of broomrape with hopeful and economic trap crops using a rotation system in Turkey will be useful, hereafter, for controlling the weeds.

Abebe et al (2005) investigated the effects of 10 potential trap crops (fenugreek, linseed/flax, alfalfa, cotton, onion, garlic, pepper, snap bean, maize, sesame and tomato to act as a check) to broomrape (*P. ramosa* and *O. cernua*) seed bank. After the field trials, maize (*Zea mays* L.) and snap bean (*Phaseolus vulgare* L.) showed better performance in terms of stimulating the germination of the broomrape seed bank and raised the germination by 74 and 71%, respectively. Snap bean was found to be very effective contrary to our study, otherwise cotton was not effective similar to our study. Similarly, numerous pesticides, chemically pure substances, nutrients and growth regulators have been tested for their efficacy on broomrape seed germination; only few of them could induce germination of broomrape seeds and the results are extremely variable and contradictory (Pieterse 1981; Riches & Parker 1995).

4. Conclusions

Three synthetic stimulant substances (GR24, GR7 and GA3) significantly increase the germination rate of both *Phelipanche* species when compared to plant root secretions under laboratory conditions; especially GR compounds are able to stimulate the germination of broomrape by over 95%; the effects of host and trap crops on the germination rate of broomrape may be very low or variable; in

addition flax and cowpea, as trap crops, provide the maximum germination rates. An insight into the mechanism of action of strigol and its analogues may help to develop chemicals that have similar activity and can be synthesized economically. The isolation and identification of the factor in host root exudates may also open new avenues into the chemistry of germination stimulants.

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