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Evaluation of different treatments on break seed dormancy of Dodder (*Cuscuta campestris* Yunck)

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ABSTRACT

A pot experiment was conducted under field conditions in Al-Qizuina district-province of Najaf during the 2017 growing season in order to pre-planting control of field dodder (*Cuscuta campestris*) by breaking its seed dormancy and reducing seed banks in the soil. The experiment tested 56 treatments (chemical, biological and physical) in a complete randomized design with four replicates. Results showed significant differences between treatments. Gibberelic acid at 800 ppm resulted in the highest germination rates of the dodder seeds (92%), while KNO_3 had the highest speed of seed germination (7.6 days). Some other concentrations of Gibberellic acid, Salicylic acid, Ethrel and KNO_3, and seed extracts of Maize, Barley and Alfalfa as well as the physical treatment of boiling water had significant effects on the dodder seeds in terms of germination rates which ranged from 19 to 51% and germination speed that ranged from 8.4 to 15.4 days compared to the control treatment which resulted in 0.75% and 20.82 days, respectively. The study confirmed that using high efficient treatments in breaking dormancy and increasing seed germination of dodder as pre-planting measurements can be practically applied in the integrate management of this pest.

Introduction

Cuscuta campestris is a parasitic weed species of the family Convolvulaceae with many common names (dodder, strangle weed, field dodder, and golden dodder) (Dawson et al. 1994). In Iraq *C. campestris* is known by various common names, such as dodder, cancer and troublesome weed. Hutchinson and Ashton (1980) reported that most seedlings of *C. campestris* emerge from the top (1-3 cm) of the soil, while those located at 4-5 cm depth emerge in 6 days (Stojanovic and Mijatovic,

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1973). After germination, the dodder seedling circumnutates in anticlockwise direction in search of a host stem, if no suitable host is found within 3 to 8 days, the seedling will die (Sitkin, 1976). Dodder is considered to be plant parasitic, pest and pathogen at the same time; it causes physiological, chemical and biological damage on the host plants, which leads to the decrease of crop production or sometimes to the death of host plants (Smith et al. 2013). Dodder is widespread worldwide due to expansion of vegetative reproduction (Shen et al. 2013.), in addition to the production of large quantities of seeds, up to 116,973 per plant, with low weight (Holm et al. 1997). Agronomically, Mamluk and Weltzien (1978) reported that 26% of fields cultivated with eggplant (*Solanum melongena*), onion (*Allium cepa*), cucumber (*Cucumis sativus*), pepper (*Capsicum annuum*), potatoe (*Solanum tuberosum*), chili (*Capsicum annuum*), as well as alfalfa (*Medicago sativa*) and tomatoe (*Solanum lycopersicum*) were infected by dodder in the Near and Middle East. Therefore, in a study of weeds in Iraq classified dodder within the primary noxious weeds (Al-Jbouri et al. 1985). Difficulty of dodder control is due to the dormancy imposed by the hard seeds and its long duration, for up to 50 years or more (Costea and Tardif, 2004), unlike the parasitic weeds of the genera *Orobanche* or *Striga* which do not require host root exudates to stimulate germination (Benvenuti, 2003). Important feature ensuring the success of dodder as a crop parasite is seed dormancy (Hutchinson and Ashton, 1980).The dormancy influences germination dynamics, allowing late emerging plants to escape control practices, including cultivation and herbicide application. Dormancy is due to the presence of a hard seed coat, impermeable to water and oxygen (Gehan-Jayasuriya et al. 2008). Moreover, the phytohormone abscisic acid (ABA) has been shown to be involved in the establishment of primary-dormancy within seed development (Bewley, 1997), and the expression of several genes are affected by the ABA concentration in the embryonic tissues (Nicolás et al. 1997). The current study aims to control *C. campestris* at pre-planting by breaking dormancy of seeds to reduce seed bank in the soil and reducing the damage of dodder in crop cultivation fields in Iraq.

Materials and Methods

Diagnosis of C. campestris

C. campestris samples were collected from infected eggplant in Babylon, at the complete growth-stage (flowers and seeds). *C. campestris* was diagnosied the phenotypically by anatomical microscope according to the classification key suggested by Spaulding (2013), and molecular diagnosis of *C. campestris* was recorded as a new strain at the National Center for Biotechnology Information (NCBI) under the new accession number (MG669315).

Experimental procedure and treatments

The loamy soil was sterilized by heat (soil cooking) and used to fill 0.25 L pots. At depth of 1cm each pot was planted with 100 seeds of dodder (*C. campestris* MG669315). Dodder seeds were originally collected from infected eggplants. Pots were arranged in a Completely Randomized Design (CRD) with 4 replicates representing 56 treatments. The 224 experimental units were maintained on 20 cm benches in the field conditions and were irrigated as needed where irrigation started at day 3 post treatment. During three weeks experiment, data were recorded according to each experimental treatment. The experimental pots were treated with 50 ml according to each treatment.

Treatments were:

A. Control: loamy soil in the pot only treated with 50 ml of water.

B. Chemical treatments:

1- Ethrel (10, 30, 50 and 70 ppm).

2- Gibberellic acid (GA_3) (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900 and 1000 ppm).

3- Sulfuric acid concentration (5, 10, 15, 20 and 25%).

4- Thiouria (600, 800 and 1000 ppm).

5- KNO_3 (600, 800 and 1000 ppm).

6- Salicylic acid (SA) (100, 200, 300 and 400 ppm).

7- Herbicides Pre-Sowing

7-1- Paraquat SL 20% (3 L ha^{-1}).

7-2- Trifluralin EC 48% (2.5 L ha^{-1}).

7-3- Pendimethalin EC 33% (4 L ha^{-1}).

C. Natural treatments:

1. The seeds were dried at 70°C for 2 days in an oven. They were then macerated to powder with a grinder. About 500 grams of the seed powder were placed in 500 ml distilled water to reach a concentration of 100% (W/V). The solution was left for 24 hours so that alkaloids, flavonoids and other constituents if present will get dissolved. The water extract was filtered using Whatman No.1 filter paper and the residue was removed.

1.1. Water extract of crop seeds at concentration of 100 % (White corn, Alfalfa, Maize, Caster bean, Liquorice, Barley, Forage corn and Sunflower).

1.2. Water extract of weed seeds at concentration of 100 % (*Cynodon dactylon* L., *Imperata cylindrica* L., *Sorghum halepense* L., *Datura stramonium* L., *Melilotus indica* and *Phragmites communis* L.).

2. Organic fertilizer in the pot only

D. Physical treatments:

1. Boiling water (temperature of 100 °C).

Data collection

Germination rate was calculated using this equation:

$$Germination\ Rate\ (\%) = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} \times 100$$

Speed of germination in 21 days, according to the equation by Matlob et al. (1989).

$$Speed\ of\ germination = \frac{1a1b + 2a2b + 3a3b + \cdots}{1a + 2a + 3a + \cdots}$$

Where a= the number of seeds that germinate per day and b= The number of days from the start of the experiment.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) test. The means were compared according to Least Significant Difference (LSD) at 0.05 probability level. The Statistical Analysis Software (SAS/STAT Edition 6.12, 2012) was used to conduct all the statistical analysis.

Results and discussion

Table (1) shows that gibberellic acid at 800 ppm was superior to all treatments in dodder seed germination rates. From the highest to the lowest effect, treatments of gibberellic acid 650 ppm and gibberellic acid 500 ppm, salicylic acid 100 ppm, ethrel 30 ppm and KNO_3 800 ppm increased germination rates compared to the lowest germination rate induced by thiouria 600 ppm or no germination at all in the Sulfuric acid treatments. Among the plant extracts treatments, the maize seed extract treatment was the only treatment with a positive effect on seed germination. While, there was no germination in all weed seed extract treatments. Physically, treatment of boiling water had positive effect by increasing the germination rate of dodder seeds compared to control (loamy soil) treatment. However, the data in table (1) showed

the ineffectiveness of the chemical herbicides used where the germination rate was 0.00%, whereas 99.25% of the seeds did not germinate in control (loamy soil) treatment.

Table 1. The effect of treatments on seed germination rates of *Cuscuta campestris* MG669315.

Treatments	Germination%	Treatments	Germination%
Ethrel 10 ppm	0.00	Thiouria 1000 ppm	4.00
Ethrel 30 ppm	41.00 *	KNO_3 600 ppm	25.00 *
Ethrel 50 ppm	4.00	KNO_3 800 ppm	32.00 *
Ethrel 70 ppm	0.00	KNO_3 1000 ppm	11.00
Gibberellic acid 50 ppm	11.0	Salicylic acid 100 ppm	42.00 *
Gibberellic acid 100 ppm	3.00	Salicylic acid 200 ppm	6.00
Gibberellic acid 150 ppm	5.00	Salicylic acid 300 ppm	22.00 *
Gibberellic acid 200 ppm	3.00	Salicylic acid 400 ppm	8.00
Gibberellic acid 250 ppm	4.00	White corn	0.00
Gibberellic acid 300 ppm	7.00	Maize	23.00 *
Gibberellic acid 350 ppm	5.00	Alfalfa	3.00
Gibberellic acid 400 ppm	4.00	Castor bean	0.00
Gibberellic acid 450 ppm	10.00	Sunflower	8.00
Gibberellic acid 500 ppm	46.00 *	Liquorice	0.00
Gibberellic acid 550 ppm	21.00 *	Barley	21.00 *
Gibberellic acid 600 ppm	22.00 *	Forage corn	7.00
Gibberellic acid 650 ppm	51.00 *	*Mililotus indica*	0.00
Gibberellic acid 700 ppm	17.00	*Datura stramonum*	0.00
Gibberellic acid 800 ppm	92.0 *	*Cynodon dactylon*	0.00
Gibberellic acid 900 ppm	15.00	*Imperata cylindrica*	0.00
Gibberellic acid 1000 ppm	30.00 *	*Sorghum halepense*	0.00
Sulfuric acid 5%	0.00	*Phragmites communis*	0.00
Sulfuric acid 10%	0.00	Boiling water	19.00 *
Sulfuric acid 15%	0.00	Organic fertilizer	1.00
Sulfuric acid 20%	0.00	H-Paraquat	0.00
Sulfuric acid 25%	0.00	H-Trifluralin	0.00
Thiouria 600 ppm	4.00	H-Pendimethalin	0.00
Thiouria 800 ppm	5.00	Control (loamy soil)	0.75
LSD (0.05)= 16.750	F Value= 2112.82	Pr > F = <0.0001	
Mean Square= 1166.45057		R Squares= 64154.78125	

(*) Significantly different from the untreated control (loamy soil + water).

Table (2) shows that dodder seeds germination speed were the highest (low number of days) in treatment of KNO_3 800 ppm followed by treatments of KNO_3 600 ppm, salicylic acid 100 ppm and ethrel 30 ppm, salicylic acid 300 ppm and gibberellic acid 350 ppm, respectively. While,

treatment of alfalfa and barley extracts had the highest positive effect on seeds germination speed compared to other extracts treatments. In terms of physical effect, the boiling water treatment had higher effect on seeds germination speed compared to control (loamy soil) but the differences were not significant.

Table 2. *Cuscuta campestris* MG669315 speed of seed germination during 21 days.

Treatments	Days to germinate No.	Treatments	Days to germinate No.
Ethrel10 ppm	21.00	Thiouria 1000 ppm	12.82 *
Ethrel 30 ppm	10.75 *	KNO_3 600 ppm	8.40 *
Ethrel 50 ppm	15.00 *	KNO_3 800 ppm	7.60 *
Ethrel 70 ppm	21.00	KNO_3 1000 ppm	12.30 *
Gibberellic acid 50 ppm	12.30 *	Salicylic acid 100 ppm	10.20 *
Gibberellic acid 100 ppm	19.52	Salicylic acid 200 ppm	12.60 *
Gibberellic acid 150 ppm	19.80	Salicylic acid 300 ppm	10.90 *
Gibberellic acid 200 ppm	19.42	Salicylic acid 400 ppm	13.90 *
Gibberellic acid 250 ppm	18.20	White corn	21.00
Gibberellic acid 300 ppm	15.40 *	Maize	15.40 *
Gibberellic acid 350 ppm	11.20 *	Alfalfa	15.30 *
Gibberellic acid 400 ppm	19.10	Castor bean	21.00
Gibberellic acid 450 ppm	16.30	Sunflower	17.60
Gibberellic acid 500 ppm	14.80 *	Liquorice	21.00
Gibberellic acid 550 ppm	14.60 *	Barley	14.12 *
Gibberellic acid 600 ppm	17.05	Forage corn	18.20
Gibberellic acid 650 ppm	18.70	*Mililotus indica*	21.00
Gibberellic acid 700 ppm	17.10	*Datura stramonum*	21.00
Gibberellic acid 800 ppm	14.60 *	*Cynodon dactylon*	21.00
Gibberellic acid 900 ppm	19.20	*Imperata cylindrica*	21.00
Gibberellic acid 1000 ppm	19.05	*Sorghum halepense*	21.00
Sulfuric acid 5%	21.00	*Phragmites communis*	21.00
Sulfuric acid 10%	21.00	Boiling water	17.90
Sulfuric acid 15%	21.00	Organic fertilizer	20.82
Sulfuric acid 20%	21.00	H-Paraquat	21.00
Sulfuric acid 25%	21.00	H-Trifluralin	21.00
Thiouria 600 ppm	12.72 *	H-Pendimethalin	21.00
Thiouria 800 ppm	12.50 *	Control (loamy soil)	20.82
LSD (0.05)= 4.628	F Value= 720.90	Pr > F = <0.0001	
Mean Square= 62.206431		R Squares= 3421.353732	

(*) Significantly different from the untreated control (loamy soil + water).

The *Cuscuta* spp. seeds, especially of *C. campestris*, are characterized by having a solid seed coat because of their containment of two outer palisade cell layers which are non-permeable to water, oxygen and gas diffusion (Benvenuti, 2003; Gehan-Jayasuriya et al. 2008). The differences in the internal chemical content, dark color and low weight are reasons for seeds dormancy for many years, which have been confirmed by previous studies (Zhang et al. 2008). The dormancy of dodder seeds and their persistency in the soil for decades are the most reasons behind ineffectiveness of pre-planting herbicides used (Mishra, 2009). In this experiment, gibberellic acid had a clear increasing effect on germination rate of dodder seeds because gibberellic acid encouraged seed germination growth by increasing amylase enzyme synthesis and expansion of cell wall followed by hydrolysis of cell stored starch allowing more water to enter the cell and consequently leading to cells elongation (Sun and Gubler, 2004; Finch-Savage and Leubner-Metzger, 2006). Mustafa et al. (2015) noted that 250 ppm of GA_3 has the ability to break dormancy of dodder seeds under laboratory conditions. However, in this experiment the 800 ppm GA_3 was the best treatment in dodder seed germination rates. Accordingly, it is the most suitable concentration to increase the dodder seed germination percentage in the field (Table 1).

Salicylic acid increases metabolic activity in seeds and maintains the levels of auxin and cytokinin in the plant tissue, thereby promoting cell division. Salicylic acid at concentration of 100 ppm was most appropriate in increasing the percentage and speed of germination of dodder seeds, these results are in agreement with those of Sakhabutdinova et al. (2003.) and Basra et al. (2006). Adams and Tebeest, (2016) noted that ethrel is effective in increasing seeds germination. Results of this study showed that ethrel at 30 ppm have a clear effect on the rate and speed of germination of dodder seeds. Ethrel influences the seeds by regulating their metabolic processes and gene expression as well as stimulating proteins synthesis through water absorption, which leads to break seeds dormancy and increase germination rates (Kieber, 1997; Asano et al. 1999). It was confirmed by Khan and Shah (2011) that thiouria was effective in breaking the dormancy of weed seeds. While the thiouria concentrations used in the current experiment had similar slight effects on the rate and speed of germination of dodder seeds. This effect is because thiouria was involved in reducing the preventive effect of the seed coat and inhibiting effect of deep embryo dormant, agreeing with Hartmann et al. (1997) and Çetinbas and Koyuncu, (2006). Narwal, (2010) and Morris et al. (2009) noted that allelopathic compounds released from the plant parts had an effect on the seed germination. While, Seyyedi et al. (2013) reported that maize shoot extract had an inhibitory effect on the germination of dodder seeds, but in this current study compared to other extracts, the maize seed extract had a significant stimulatory effect on the germination rate of dodder seeds. The treatments of alfalfa and barely extracts had a clear positive effect on

germination speed of dodder seeds compared to the other plants extract treatments, which is consistent with Aliyas et al. (2014). Results also showed that KNO_3 800 ppm treatment resulted in earlier seeds germination over the other treatments. This is because KNO_3 helps in stimulating metabolic activity in seeds, and thus breaks down seed dormancy and stimulates germination (Golmohammadzadeh et al. 2015) (Table 2).

Results of this experiment may confirm that some of plants extracts treatments (weeds, white corn, castor bean and liquorice) had an inhibitory effect on dodder seed germination because the control treatment resulted in 0.75% of seeds germination. This was due to the thickness of the seed coat and lowering the level of water absorption leading to continuous dormancy (Lyshede, 1992). Several studies (Menlebrouck et al. 2008.; Haidar et al. 2010; Mustapha et al. 2015) noted that sulfuric acid at different concentrations (10% - 90%) was effective in chemical scarification to break seed dormancy and to stimulate water uptake and thus dodder seed germination. But, in the current study there was no definitive role for sulfuric acid to stimulate seed germination in the field. The physical scarification factor in the boiling water treatment had a significant effect on the proportion and speed of dodder seed germination because hot water scrapes the hard coat of seeds and thus enhances water and oxygen permeability (Aydin and Uzun, 2001; Gehan-Jayasuriya et al. 2008).

Conclusion

Pre-planting germination of dodder seeds is possible by using some treatments and the most effective is GA_3 at 800 ppm followed by KNO_3 at 800 ppm and some other treatments. While using of sulfuric acid, some plant extracts and some herbicides inhibited seed germination. Accordingly, some of these treatments may be implemented in some vegetable fields as integrated weed management (IWM) program.

Conflict of Interest

Authors declare no conflict of interest.

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