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In vitro Studies of Imazethapyr and GR24 Effects on Early Developmental Stages of *Striga hermonthica* (Del.) Benth.

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Authors' contributions

This work was carried out in collaboration with all authors. Authors RMAA and AHE carried out the experimental laboratory work. Author MMH designed the study. Author RMAA performed the statistical analysis. Authors RMAA and MMH performed and wrote the protocol and the first draft of the manuscript. Author MMH managed the analyses of the study, read and approved the final manuscript.

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ABSTRACT

Aims: A series of laboratory experiments were conducted to investigate the effects of Imazethapyr on *Striga* germination and haustorium initiation in response to different chemical stimulants.
Study Design: A completely randomized design with five replicates.
Place and Duration of Study: A series of laboratory experiments was undertaken at the College of Agricultural Studies, Sudan University of Science and Technology (SUST) at Shambat, during the season 2012/2013.
Methodology: *Striga* seeds were sprinkled on (GFFPD), in Petri-dishes. Aliquots (5ml) of the

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herbicide at 10, 20, 40, 60, and 80 µM were added to each petri-dish. The Petri-dishes, were sealed with Parafilm, wrapped in aluminum foil and incubated in the dark at 30°C±2 for 14 days. A control with water was included. The seeds were subsequently treated with 20µl (aliquots) of GR24 at 0.01 and 0.1 ppm per disc. A piece of filter paper, moistened with sterilized distilled water, was placed in the centre of each petri-dish to maintain moist condition. The Petri-dishes were sealed with parafilm, wrapped in aluminum foil and incubated in the dark at 30°C±2 for 24 h.

Results: Seeds conditioned in Imazethapyr at 10-80 µM displayed inconsistent germination in response to GR24 and the germination was poorly correlated with herbicide concentration (r= -0.2). Seeds conditioned in imazethapyr at 60-80 µM for 10 or 15 days prior to treatment withGR24 at 0.01 ppm.reduced*Striga* germination comparable to the respective control. *Striga* germilings resulting from seeds conditioned in imazethapyr at 40 µM exhibited significant reductions (36%) in haustorium initiation in response to DMBQ as compared to the control.

Conclusion: Imazethapyr, irrespective of concentration reduced *Striga* germination, radicle length and haustorium initiation significantly.

Keywords: DMBQ; GR24; germination; haustorium; imazethapyr; reduction; Striga.

1. INTRODUCTION

Striga spp. are hemiparasites of many economically important cereals crops. As weeds, they cause reductions in crop yield, adversely affect crop quality, and result in loss of cultivated land due to reduced crop alternatives. *Striga* seed germination occurs only in response to a chemical signal from the host root. Before germination, *Striga* seeds must undergo conditioning under suitable temperature and moisture conditions. The conditioning phase may range from two to several days, depending on the species. Following the conditioning phase, the seed produces a 'germ tube' or radicle in response to a chemical stimulant from the host root. The stability of the chemical stimulant is very short-lived in the soil. Several factors influence germination of *Striga* in the soil including temperature, moisture, pH, nutrients, soil type, and stimulants produced by host plants. Parker and Riches [1] and Foy et al. [2] viewed that after germination the radicle elongates by cell division and extension and attaches to host roots mainly in the region of root elongation and absorption. The tip of the radicle enlarges as soon as it attaches to the host root and forms a 'haustorium'. Subsequently, the haustorial tissue penetrates the host root by enzymatic degradation, rather than mechanical destruction (Dörr and Kollman) [3], and establishes connections with the host vascular system. It is by these connections that the parasite derives its nutrients and water from the host.

Weed management is essential for any current system of agricultural production, especially for large monoculture areas, which exert high pressure on the environment. Chemical strategies have been used to control *Striga* either directly or indirectly. Direct involvement is by reduction or destruction of *Striga* seed reserves in the soil, prevention of or negative influence on the germination of *Striga* seeds and attachment to the host root. Measures such as soil fumigation, germination stimulants, and certain pre-plant or pre-emergence herbicides act directly on *Striga*. Indirect control is aimed at suppressing growth of the parasite after attachment and penetration of the host root. Imazethapyr and imazapic are frequently applied to reduce the emergence of weeds during the fallow period and/or associated with the herbicide 2, 4-D on burn down, about 15-20 days before the sowing, for dicotyledonous management of complex control by glyphosate. García-Torres and López-Granados [4], Jacobsohn and Eldar [5], and García-Torres et al. (1996) [6], showed that chlorsulfuron and imazethapyr were effective against broomrape. No single method has so far been found to successfully control *Striga*. For this reason, an integrated management approach is required. Crop rotation, crop cultivar and clean crop seeds should be the backbone of each package. Other components namely fertilizers, water harvest, catch cropping, herbicides and resistant or tolerant crop cultivars could be adjusted according to needs and expected returns [7,8,9,10,11].

The objective of this research was to determine the effect of Imazethapyr on pre-conditioned witchweed seeds, on germination, radicle length and haustorium initiation of *Striga* in response to chemical stimulants.

2. MATERIALS AND METHODS

2.1 Laboratory Experiments

A series of experiments was undertaken to investigate the effects of Imazethapyr (Pursuit) on early developmental stages of the parasite including: i) germination, ii) radicle extension, iii) haustorium initiation. i) GR24 induced germination of *S. hermonthica*, ii) haustoria initiation in response to 2, 6-dimethoxybenzoquinone (DMBQ).

2.2 Plant Material

An experiment was conducted at the *Striga* Research Laboratory, College of Agricultural Studies, Sudan University of Science and Technology at Shambat. *Striga* seeds used for this investigation were collected from under sorghum from the Gezira, Sudan in 2011. In all experiments, treatments were arranged in factorial experiment with randomized complete design with 5 replicates.

2.3 Preparation of GR24 Stock Solution

The synthetic *Striga* germination stimulant GR24 (a strigol analogue), provided by Prof B. Zwanenberg, the University of Nimijhen, the Netherlands was used. Stock solution of the stimulant was prepared by dissolving 1mg in 1ml of acetone and subsequently completing to size (100 ml) with sterile distilled water to obtain the desired concentration (10 ppm).

2.4 Preparation of DMBQ Stock Solution

DMBQ was a gift from Dr. Sugimoto, Y. from Koby University, Japan. A stock solution (100 µM) was prepared by dissolving 1.68 mg in 1 ml of acetone and completing to volume (100 ml) with sterile distilled water.

2.5 Imazethapyr Stock Solution

A stock solution (100 µM) of imazethapyr was prepared by taking 29 µl of the 10% formulation (Pursuit) (2.89 mg a.i.) and completed to 10 ml with sterile distilled water.

2.6 Sterilization and Conditioning of *Striga* seeds

Striga seeds were cleaned by washing in a measuring cylinder (1000 ml^3), filled with tap water to which Tween 20 (1-2 ml) was added. The measuring cylinder was occasionally swirled, seeds were allowed to settle and water containing debris and light seeds were decanted. *Striga* seeds were transferred to a fine sieve (70 µm), washed with tap water, several times, to remove the detergent. Then seeds, plotted dry on Whatman No. 1 filter papers, were air dried and stored till used. Seeds were surface sterilized by immersion, for 3 min, in sodium hypochlorite solution (1%), obtained by appropriate dilution of a commercial sodium hypochlorite solution (Bleash containing 5% NaOCl). Sodium hypochlorite was drained off and seeds were washed, under suction, with sterilized distilled water, several times, until the yellow colour disappeared. *Striga* seeds, plotted on Whatman No. 1 filter papers, were air-dried and stored till used.

2.7 Effects of Imazethapyr on *Striga* Germination and Radicle Length

Striga seeds were sprinkled on 8 mm glass fiber filter papers (GFFPD), discs (25-30 seeds/disc) in Petri-dishes. Aliquots (5ml) each of aqueous solution and/or suspension as appropriate of the herbicide at 10, 20, 40, 60, and 80 µM was added to each petri-dish. Petri-dishes, were sealed with Parafilm, wrapped in aluminum foil and incubated in the dark at 30°C±2 for 14 days. A control in which the seeds were conditioned in water was included. The seeds were subsequently treated with 20 µl (aliquots) of GR24 at 0.01 and 0.1 ppm per disc. A piece of filter paper, moistened with sterilized distilled water, was placed in the centre of each petri-dish to maintain moist condition. Petri-dishes were sealed with parafilm, wrapped in aluminum foil and incubated in the dark at 30°C±2 for 24 h. Seeds treated with water alone were included as control. The seeds were then examined for germination and radicle length. The negative control consisted of sterile distilled water without GR24 and the herbicide.

2.8 Measurements

After 10 days of treatment, the percentage of seed germination and radicle length were established for each GFFP disc by using a stereoscopic microscope at 30 × magnification. Germination percentage was determined by scoring the number of seeds with an emerged radicle through the seed coat. Radicle length was measured in 10 randomly selected germinated seeds from each disc.

2.9 Effects of Imazethapyr on *Striga* Haustorium

laboratory experiment was conducted at JICA laboratory to study the effects of Imazethapyr on haustorium initiation. Surface sterilized *Striga* seeds, placed on 8 mm glass fiber discs conditioned in presence and absence of the herbicide as described above, were dapped on filter papers (Whatman No. 1) and transferred to sterile Petri dishes. The discs containing *Striga* seeds were treated, each, with 20 µl GR24 solution (0.1 ppm) to induce germination. Petri dishes sealed with parafilm and placed in black ploythene bags, were incubated in the dark at 30ºC for 48 h. *Striga* germilings dapped on a filter paper, were placed, and inverted top-down on similar discs without *Striga* seeds. The Pairs of discs were treated either with 40 µl solution of DMBQ (10 µM). *Striga* germilings resulting from seeds conditioned in distilled water similarly treated with DMBQ was included as control for comparison. Petri dishes were again incubated in the dark at 30ºC for an additional 24 h then examined for haustorium initiation using a binocular stereomicroscope.

Data on percentage germination, radicle length and haustorial initiation were calculated for each discs, (Gomez and Gomez. 1984) and subjected to analysis of variance (ANOVA). Treatment means that were significantly different were compared using Duncan's Multiple Range Test (DMRT) at 5% level.

3. RESULTS

3.1 Effects of Imazethapyr on *Striga* Seeds Germination and Radicle Length

3.1.1 Effects on germination

Seeds conditioned in water for 5, 10 and 15 days and subsequently treated with GR24 at 0.1 ppm displayed 42.2, 63.6 and 80.8% germination, respectively. Seeds conditioned in imazethapyr at 10 µM for 5 days displayed a slightly higher germination (64.1%) than the respective aqueous control (Table 1). A further increase in the herbicide concentration to 20 µM increased germination significantly. Increasing imazethapyr concentration to 40 µM or more resulted in germination comparable to the aqueous control. *Striga* seeds treated with the herbicide solution at 10-80 µM for 10 or 15 days and subsequently challenged with GR24 at 0.1 ppm displayed comparable germination to the aqueous control (Table 1). Seeds conditioned in water for 5, 10 and 15 days and subsequently treated with GR24 at 0.01ppm displayed 41.5, 62.3 and 73.7% germination, respectively. Imazethapyr at 10-80 µM applied for 5 days on *Striga* seeds and subsequently treated with GR24 displayed comparable germination to the respective aqueous control (Table 1). While imazethapyr applied at 60-80 µM for 10 or 15 days prior to treatment with the GR24 at 0.01 ppm reduced *Striga* germination comparable to the respective control. Seeds conditioned in imazethapyr at 10 µM for 15 days and subsequently treated with GR 24 at 0.01 ppm displayed comparable germination to the respective aqueous control. A further increase in the herbicide to 20-60 µM reduced germination, but often not significantly. Seeds conditioned in imazethapyr at 80 µM, on the other hand, exhibited a significant reduction in germination. Germination and herbicide concentration displayed low negative correlation (r = -0.2).

3.2 Effects on Radicle Length

Striga germilings from seeds conditioned in imazethapyr at 10-80 µM for 5 days and subsequently treated with GR24 at 0.1 ppm exhibited comparable RL to the respective aqueous control (Table 2). Germilings from seeds conditioned in imazethapyr at 10-80 µM for 10 or 15 days and subsequently treated with GR24 displayed inconsistent reductions in RL. *Striga* germilings from seeds conditioned in imazethapyr at 10-80 µM for 5 days prior to treatment with GR24 at 0.01 ppm displayed insignificant reductions in RL when compared with aqueous control.

Germilings from seeds conditioned in the herbicide at 10 µM for 10 or 15 days prior to treatment with the stimulant displayed significant reductions (38.5-49.1%) in RL. Further increase in the herbicide concentration to 20-80 µM, had no further significant effects on RL (Table 2). Radical length and imazethapyr concentrations were poorly but negatively correlated (r = -0.3). Conditioning period and RL were poorly but positively correlated (r = 0.1).

3.3 Effects of Imazethapyr on *S. hermonthica* Haustorium Initiation

DMBQ applied to *Striga* germilings resulting from seeds previously conditioned in water and treated with GR24 induced 66% haustoria (Table 3). *Striga* germilings from seeds conditioned in

Table 1. Effects of imazethapyr on *S. hermonthica* germination in response to GR24

Imaz. (µM)	Germination (%)					
	GR24 0.1ppm			GR24 0.01ppm		
	Conditioning period (Days)					
	5	10	15	5	10	15
DW	42.2±8.6^{b}	63.6±4.8^{a}	80.8±4.8^{a}	41.5±8.0^{a}	62.3±6.9^{ab}	73.8±5.2^{a}
10	64.1±4.5^{ab}	72.8±3.9^{a}	74.4±3.3^{a}	60.4±8.3^{a}	70.4±5.7^{ab}	73.±57.0^{a}
20	73.3±6.8^{a}	72.2±3.8^{a}	69.4±3.9^{a}	41.35±6.3^{a}	73.4±8.3^{a}	49.1±7.1^{bc}
40	58.1±6.0^{ab}	66.6±4.7^{a}	77.1±3.3^{a}	44.9±8.3^{a}	62.7±6.1^{ab}	68.±7.0^{ab}
60	43.9±14.2^{b}	55.3±7.3^{a}	75.3±4.8^{a}	38.5±5.1^{a}	53.3±7.0^{ab}	62.1±8.8^{ab}
80	62.33±4.5^{ab}	67.34±7.3^{a}	78.4±6.9^{a}	43.1±2.4^{a}	49.2±5.8^{b}	39.6±6.4^{c}
3- way ANOVA						
Imaz.	3.7**					
GR24, G	23.4***		Imaz.^{vs}D		2.38**	
Days, D	22.8***		G ^{vs} D		2.08^{NS}	
Imaz.^{vs}G	2.8*		Imaz.^{vs}G^{vs}D		0.71^{NS}	

*± Standard errors of means. Means within a column having the same superscript letter(s) are not significantly different according to DMRT. *= P ≤ 0.05; ** = P ≤ 0.01; ***= P ≤ 0.001*

Table 2. Effects of imazethapyr on *S. hermonthica* radicle length in response to GR24

Imaz. (µM)	Radicle length (mm10^{-2})					
	GR24 0.1ppm			GR24 0.01ppm		
	Conditioning period (Days)					
	5	10	15	5	10	15
DW	14.0±1.8^{a}	14.6±2.1^{ab}	20.8±1.8^{a}	14.4±0.9^{a}	19.2±1.2^{a}	21.2±1.7^{a}
10	12.2±1.5^{a}	8.4±0.9^{bc}	9.6±1.5^{b}	12.6±1.5^{a}	11.8±0.7^{b}	10.8±2.1^{b}
20	11.8±1.4^{a}	15.0±1.5^{a}	11.8±1.7^{ab}	11.8±1.3^{a}	11.4±0.5^{b}	10.6±1.5^{b}
40	11.2±1.1^{a}	8.8±1.5^{bc}	9.8±1.4^{ab}	14.6±0.7^{a}	11.2±1.5^{b}	8.8±1.3^{b}
60	11.2±0.4^{a}	9.8±1.5^{c}	15.8±4.0^{ab}	8.8±1.2^{a}	9.4±1.3^{b}	11.0±1.6^{b}
80	11.2±1.2^{a}	13.4±1.6^{abc}	12.0±1.5^{ab}	12.2±1.6^{a}	12.2±1.2^{b}	11.0±1.2^{b}
3- way ANOVA						
Imaz.	16.1 ***					
GR24, G	0.03^{NS}	Imaz.^{vs}D	3.72***			
Days, D	0.68^{NS}	G ^{vs} D	1.31^{NS}			
Imaz.^{vs}G	2.23^{NS}	Imaz.^{vs}G^{vs}D	0.72^{NS}			

*± standard errors of means. Means within a column having the same superscript letter(s) are not significantly different according to DMRT. ***= P ≤ 0.001*

Table 3. Effects of imazethapyr on haustorium initiation in response to DMBQ

Imazethapyr (µM)	Haustorium initiation
DW	66.0^{a}
10	62.9^{b}
40	43.5^{b}
SE ±	4.4
LSD	14.4

Means with in a column having the same superscript letter(s) are not significantly different according to DMRT

imazethapyr at 10 and 40 µM, induced to germinate with GR24 at 0.1 ppm and subsequently, challenged with DMBQ at 20 µM, displayed significant reductions (5- 36%) in haustorium initiation.

4. DISCUSSION

Parasitic plants are becoming a severe constraint on major agricultural crops in Mediterranean and tropical countries and the efficacy of available means of control is minimal. In general, the most limiting factor in the use of the promising herbicides is their degree of selectivity among the crops at the required rate for parasite control, and the critical time of application, especially of the foliar applied systemic herbicides. Imazaquin is highly effective and selective; it is one of the most commonly used herbicides. It is classified as an imidazolinone herbicide that controls weed

growth through the inhibition of specific amino acids that prove to be vital for plant growth [12]. Imazaquin inhibits the acetohydroxy acid synthase (AHAS) enzyme accountable for synthesis of the amino acids valine, leucine, and isoleucine. The results of this investigation indicated that seeds conditioned in Imazethapyr at 80 µM displayed inconsistent germination in response to GR24 and the germination was poorly correlated with herbicide concentration (r= -0.2). The findings are consistent with previous reports that conditioning of *S. asiatica* and *S. hermonthica* in the presence of germination stimulants reduced germination in response to a terminal stimulant treatment [13], [14] and [15]. The herbicide imazethapyr (10-30 g a.i. /ha) delayed *Orobanche* emergence and reduced infestation. It would appear that the action of imazethapyr on witchweed germination is complex and is influenced by a multitude of factors including a dilution effect, penetration and accumulation of imazethapyr in the seeds, induction and/or activation of enzymes, stimulation of germination as well as toxic effects. Herbicide application is too technical and expensive in subsistent farming. In the present investigation results displayed that, application of Imazethapyr at 20 or 80 mM affected negligible or no abnormal germination, in response to the higher concentration of GR24.Increasing the concentration of Imazethapyr reduced germination significantly in response to GR24 at 0.01 ppm. A further increase in the herbicide to 20-60 µM reduced germination, irrespective to duration time. Seeds conditioned in imazethapyr at 80 µM, on the other hand, exhibited a significant reduction in germination. This observation may be explained by slow penetration of imazethapyr into the seed. The magnesium salt of imazapyr was optimal for seed dressings (drenching, priming, and coating), for preventing field damage from parasitic *Striga hermonthica* (witchweed) [16]. García-Torres and López-Granados (1998) [4], reported that lentil seed treatments with imazapyr by coating seeds at rates equivalent to 5–10 g ha^{-1} or by soaking for 5 min in 0·25% solutions did not affect germination or crop growth, and controlled 85–95% of broomrape. Germilings from seeds conditioned in the herbicide at 10-80 µM for 10 or 15 days prior to treatment with the GR24 at 0.01 ppm displayed significant reductions RL relative to the control. This drastic growth inhibition was increased with an increase in the dose of imazethapyr. It is known that parasitic plants use quinines to initiate the development of haustorium. It deserves mentioning that imazethapyr, an imidazolinone, is ALS inhibitors. The present study revealed that, DMBQ applied to *Striga* germilings resulting from seeds previously conditioned in imazethapyr at 10 and 40 µM and treated with GR24 reduced haustoria significantly, as compared to the corresponding control. Simple seed coating with imazapyr and pyrithiobac controls *Striga* at early stages of growth, reduces crop losses, depletes *Striga* seed bank in soil, presents no carryover problem, is cost effective and is compatible with the existing cropping systems, particularly intercropping with legumes [16]. The herbicide imazethapyr irrespective of rates, effected considerable to significant reductions on early developmental stages of *Striga hermonthica*.

5. CONCLUSION

Striga is a difficult weed to control. *Striga* management requires integrated practices comprising different components.

Imazethapyr, irrespective to concentration reduced *Striga* germination and radicle length signicantly.

Imazethapyr effected significant reduction in haustorium initiation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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