Asian Journal of Agricultural Sciences 2(3): 94-98, 2010
ISSN: 2041-3890

Submitted Date: April 09, 2010 Accepted Date: April 22, 2010 Published Date: July 05, 2010

Effect of *Klebsiella* Spp. and Different Ethylene Inhibitors on *Striga hermonthica* Benth. (Del.) Seeds Germination

[1]M.M. Hassan, [2]M.E. Abdelgani, [3]A.E. Babiker and [4]M.G. Osman
[1]Environment and Natural Resources Research Institute, Khartoum, Sudan
[2]National Centre for Research, Khartoum, Sudan
[3]Faculty of Agriculture, Sudan University of Science and Technology, Khartoum, Sudan
[4]Commission for Biotechnology and Genetic Engineering, Khartoum, Sudan

Abstract: The aim 0f this study was undertaken to evaluate the effect of the bacteria strain (*Klebsiella* spp.), silver nitrate, cobalt chloride and ACC in germination of *Striga* seeds. *Striga* seeds required after- ripening, pretreatment (conditioning) in moist warm environment for 2-14 days and subsequently exposure to an exogenous stimulant. In the present study, an inhibitor of ethylene biosynthesis, $AgNO_3$ and $CoCl_2$, was found to inhibit germination. Germination was found to be inhibited by higher concentrations of $AgNO_3$ and $CoCl_2$. Combination of ACC with $AgNO_3$ and $CoCl_2$ increased *Striga* seeds suppression as compared with control. Also this study investigates the effect of microorganism in *Striga* germination. *Klebsiella* spp. strains known to produce large amounts of ethylene were tested for efficacy in germinating seeds of *Striga hermonthica* (Del.) Benth. Incubation of conditioned *S. hermonthica* seeds over a medium inoculated with a *Klebsiella* sp. resulted in considerable germination (34-49%) this indicates an involvement of a volatile substance. Unconditioned *S. hermonthica* seeds were not stimulated.

Key words: Biological control, ethylene biosynthesis, *Striga hermonthica*

INTRODUCTION

Witchweeds (*Striga* spp.) are root hemi-parasites which cause significant loses to food crops in Asia and Africa. In Africa, up to 45 million ha of arable land is threatened by these weeds (Sauerborn, 1991). The use of bacteria for the biological control of *S. hermonthica* is recent (Babalola *et al.* 2002). Studies indicate that *Striga asiatica* seed may remain viable in the soil for 14 years or more (Bebawi *et al.*, 1984) indicating that the venture of inducing *Striga* suicidal germination is worth undertaking. This approach is environmentally friendly. Knowledge of the phytotoxicity of microorganisms is very useful in developing a sound biological management program for *S. hermonthica*. Numerous studies have been conducted on the use of trap crops in the rotation to deplete *Striga* seed reservoir in the soil (Khan *et al*., 2008). Use of a soil-borne pathogen to control the parasite will reduce both the *Striga* seed bank and emerged *Striga* plants and should contribute to the reduction of the yield losses due to *Striga*. Berner *et al*., (1999) studied the efficacy of *Pseudomonas syringae* pv. *glycinea* strains, ethylene gas and root pieces of cowpeas in stimulating germination of several *Striga* spp. He reported that the bacterium strain were consistently better stimulators of seeds germination than exogenously applied ethylene gas or root pieces of cowpeas. Researchers have come to understand that no single methodology will be completely effective in eliminating *Striga* infestations in farmer's fields. Meanwhile research efforts by scientists employ multidisciplinary approach in the combat of *Striga*.

Ethylene in higher plants as well as in many seeds is synthesized from s- adenosyl -L- methionine via the intermediatede 1- aminocyclopropane -1- carboxylic acid (ACC). The key enzymes in ethylene biosynthesis are ACC synthase and ACC oxidase (ACCO). In conditioned *Striga* seeds germination stimulants induce ACC synthase and increased capacity of the seeds to oxidize ACC to ethylene (Babiker *et al*., 1993; Babiker *et al*., 2000). The present work was undertaken to investigate the effect of bacteria strain, silver nitrate, cobalt chloride and ACC in germination of *Striga* seeds.

MATERIALS AND METHODS

This experiment was conducted at the laboratory of Environment and Natural Resources Research Institute (ENRRI), the National Centre for Research, Khartoum; in the period August to October 2008. A study was conducted to investigate the response of *Striga* seeds to volatile(s) from a *Klebsiella planticola*. In this experiment, treatments were arranged in a factorial experiment with randomized complete design with five replicates.

Corresponding Author: M.M. Hassan, Environment and Natural Resources Research Institute, Khartoum, Sudan

Striga hermonthica seeds were collected from parasitic plants growing under sorghum fields at the Gezira Research Station Farm of the Agricultural Research Corporation in Wad Medani. Seeds were surface disinfected as described by (Hassan *et al*., 2008). The seeds were stored in sterile glass vials and kept at room temperature until used.

Tests solutions: 1-aminocyclopropane-1-carboxylic acid (ACC) was obtained from Sigma Ltd. Methionine was obtained from Sigma Ltd.

Klebsiella planticola was obtained from the Environment and Natural Resources Research Institute (ENRRI), the National Centre for Research, Khartoum.

A general-purpose medium [Nutrient Agar (2.8%)] was used for the growth of bacterial strains and isolates. Media were sterilized by autoclaving at 121ºC for 15 min, whereas glass- ware was sterilized using an oven set at 160ºC for 2 h. and subsequently by autoclaving as above.

$AgNO_3$, 1 and 2 mg/L and $CoCl_2$ were applied to *Striga* seed conditioned at two concentrations 1 and 2 mg/L, at the first experiment, however in the second one were used 2 and 4 mg/L.

***Striga hermonthica* seeds conditioning:** *Striga* seeds were conditioned as described by Babiker *et al*. (1993). Briefly glass fiber filter papers (GF/C) discs (8 mm diameter) were cut, wetted thoroughly with water and placed in an oven at 100ºC for 1 h to be sterilized and ready for further use. The sterilized discs, placed in 9 cm petri dishes lined with glass fiber filter papers (GF/C), were moistened with 5 mL disitilled water. About 25-50 surface disinfected *S. hermothica* seeds were sprinkled on each of the glass fiber discs in each petri dish. The dishes, sealed with para film, placed in black polythene bags were incubated at 30ºC in the dark for 8 days.

The Two experiments were undertaken to investigate the influence of volatiles from the bacterium *per se*, and the bacterium fed with ACC or methionine on germination of *S. hermonthica* seeds.

Effect of the *Klebsiella* strain in *Striga* germination: In the first experiment conditioned *Striga* seeds, on moistened glass fibre discs, were transferred to the walls of tightly capped 10 mL glass vials (5 discs/vial). Three ml of bacterial suspension (*Klebsiella* sp.) were placed in each vial. The vials, closed with air-tight caps, were wrapped with aluminum foil and incubated in the dark at 30ºC for 7 days. Subsequently the discs were transferred to Petri dishes and examined for germination.

Effect of the *Klebsiella* strain and different substrate in *Striga* germination: In the second experiments *Striga* seeds conditioned were transferred and placed on walls of tightly capped glass vials as described in the first experiment. Three mL aliquots of the bacterium suspension containing ACC or methionine each at 0, 20, 40, 80 and 100 µM were placed in each vial. The vials, closed and wrapped in aluminum foil, were incubated in the dark at 30ºC for 3 days. The seeds were then examined for germination.

Effects of $AgNO_3$ and $CoCl_2$ on ACC - induced germination of *S. hermonthica*: $AgNO_3$ and $CoCl_2$ and combination with ACC were studied for their ability to inhibit ACC-induced germination of *S. hermonthica* seeds. *Striga* seeds were conditioned as in above mention. The discs containing *Striga* seeds were treated with aliquots (20 µl) of ACC, $AgNO_3$, $CoCl_2$ and their combination. Both of these chemical ($AgNO_3$, $CoCl_2$) were applied at to concentrations level 1mg/30 mL water and 0.5 mg/15 mL. With respect to ACC it was applied at to level 5 and 10 µM displayed negligible germination µM. The seeds were reincubated and examined for germination as described above.

Effects of $AgNO_3$ and $CoCl_2$ in combinations on *S. hermonthica* seeds germination: To determine the effects of ethylene inhibitors, $AgNO_3$ and $CoCl_2$ increased their concentrations 2 mg/15 mL and 4 mg/30 mL water for the both. ACC, $AgNO_3$ and $CoCl_2$ Were combination and treated to conditioned *Striga* seeds as described above.

In all experiments, treatments were arranged in a randomized complete design with 4 replicates. Data on percentage germination was calculated for each disc, transformed to arcsine (Gomez and Gomez, 1984) and subjected to analysis of variance (ANOVA). Means were compared with the Least Significance Difference (LSD) at 5% level. The data were back transformed and tabulated.

RESULTS

Effects of volatile from *Klebsiella* sp. and conditioning on germination of *S. hermonthica* seeds: *Striga* seeds conditioned in water and incubated over nutrient broth displayed negligible germination. Seeds similarly conditioned in water and placed over a medium inoculated with *Klebsiella* strain displayed 49% germination (Table 1). Un-conditioned seeds similarly incubated over a medium inoculated with the bacterium displayed low (13%) germination.

Effects of *Klebsiella* sp. volatiles, ACC and methionine on S. *hermonthica* germination: *Striga* seeds conditioned in water and incubated over an aqueous medium and aqueous solutions of ACC or methionine (20-100 µM each) displayed negligible (0-15%) germination (Table 2). Seeds conditioned in water and

Table 1: Effects of volatile from *Klebsiella* sp. and conditioning on germination of *S. hermonthica* seeds

	Germination (%)	
Treatments	Conditioned seeds	Unconditioned seeds
Klebsiella spp.	49 (48.98)	13 (12.4)
Water	0.0 (0.71)	0.0 (0.71)
LSD	±7.37	±1.16

( ) indicates arcsine transformed data

Table 2: Effects of *Klebsiella* sp. volatiles, ACC and methionine on germination of *S. hermonthica*

Treatments	Germination (%)
Klebsiella sp.	34 (33.85)
Klebsiella sp.+ ACC (100 µM)	16(16.67)
Klebsiella sp.+ ACC (80 µM)	18 (18.06)
Klebsiella sp.+ ACC (40 µM)	19 (19.12)
Klebsiella sp.+ ACC (20 µM)	24 (25.54)
Klebsiella sp.+ Methionine (100 µM)	17 (16.76)
Klebsiella sp.+ Methionine (80 µM)	19 (19.52)
Klebsiella sp.+ Methionine (40 µM)	20 (19.73)
Klebsiella sp.+ Methionine (20 µM)	25 (22.53)
Aqueous+Methionine (100 µM)	14 (14.3)
Aqueous+Methionine (80 µM)	14(13.49)
Aqueous+Methionine (40 µM)	13 (12.83)
Aqueous+Methionine (20 µM)	13 (12.54)
Aqueous+ACC (100 µM)	15 (14.44)
Aqueous+ACC (80 µM)	14 (14.11)
Aqueous+ACC (40 µM)	13 (13.18)
Aqueous+ACC (20 µM)	13 (12.83)
Aqueous control	0.00 (0.71)
LSD	(5%) (±4.20)

( ) indicates arcsine transformed data

placed over a medium inoculated with *Klebsiella* sp. and fortified with ACC or methionine displayed 16-25% germination. *Striga* seeds similarly conditioned and incubated over a medium inoculated with *Klebsiella* sp. displayed 34% germination (Table 2).

Effects of $AgNO_3$ and $CoCl_2$ on ACC-induced germination of *S. hermonthica*: In all experiments, seeds treated with distilled water displayed negligible germination of *Striga* seeds. *Striga* seeds exhibited high germination (89-92%) in response to a terminal ACC treatment, irrespective of the concentration (Table 3). $AgNO_3$ and $CoCl_2$ (ethylene inhibitors) applied to *Striga* seed conditioned in water displayed no germination, irrespective to concentrations level. All ethylene inhibitors significantly reduced germination, in response to the highest ACC concentration (10 µM). $AgNO_3$ in mixture with ACC resulted in little germination, irrespective to concentrations level. Combination of $AgNO_3$ (1 mg/30 mL water) with ACC at 5 µM reduced *Striga* seed germination 69%, significantly as compared with ACC alone (irrespective to concentration) (Table 3). A combination of 1mg/30 mL $CoCl_2$ and 5 µM was the most reduction of *Striga* seed (30%) in comparison to the corresponding control. Generally increasing ethylene inhibitor ($AgNO_3$ and $CoCl_2$) concentration increased *Striga* seeds suppression.

Effects of $AgNO_3$ and $CoCl_2$ in combinations on *S. hermonthica* seeds germination: In a different experiment, ACC at 10 and 5 µM, effectively induced germination of conditioned seeds in a dose- dependent manner. Seeds conditioned for 10 days and then treated with 10 µM, silver nitrate and cobalt chloride (at higher concentration of silver nitrate) completely inhibit *Striga* seed germination (Table 4). In among all chemicals used $AgNO_3$ and $CoCl_2$ at higher level, irrespective to ACC concentrations were the least inhibitory to *Striga* seeds germination. $AgNO_3$ at lower concentration and $CoCl_2$, irrespective to concentrations, and ACC at lower level produced 30% seeds germination.

DISCUSSION

Root parasitic weeds generally damage their hosts' plant even before they emerge above ground. Germination of conditioned *Striga* seeds is known to be triggered by several synthetic and natural compounds, which are structurally unrelated (Worsham, 1987). Ethylene is claimed to be the actual inducer of germination in *Striga* seeds and that all *Striga* germination stimulants act through promotion of ethylene biosynthesis in the parasite seeds. All germination stimulants known to date inhibit germination when applied to unconditioned seeds (Hsiao *et al*., 1981; Babiker *et al*., 1993; Babiker, 2007). In essence *Striga* germination is modulated (inhibited or stimulated) by germination stimulants pending the conditioning status of the seeds. Ethylene was reported to induce germination of conditioned *Striga* seeds and to delay and/or reduce germination of unconditioned seeds (Hsiao *et al*., 1981; Logan and Stewart, 1991; Babiker *et al*., 1993 Babiker *et al*., 2000; Hassan *et al*., 2009b). The ubiquitous nature of ethylene producing bacteria in soil may explain the relative preponderance of bacteria capable of suppressing *Striga* germination noted in the present study. Of the 757 soil inhabiting bacteria screened for ability to produce ethylene 229 isolates were reported to be capable of producing the phytohormones (Nagahama *et al*., 1992).

The present study revealed that conditioned S. *hermonthica* seeds placed in gas tight vials over a culture of a *Klebsiella* spp. displayed germination comparable to that induced by GR24 (Table 1). This finding is in line with previous reports on induction of *S. asiatica* and *S. gesnerioides* (Babalola *et al*., 2003; Babalola, 2002) by some soil borne bacteria. The results indicate involvement of a volatile substance, properly ethylene, in the germination induced by the bacterium. It's noteworthy that several *Klebsiella* species and strains were reported to produce ethylene (Frankenberger and Arshad, 1995). Inclusion of methionine and ACC in the medium with the bacterium reduced *Striga* germination

Table 3: Effects of silver nitrate and cobalt chloride on *S. hermonthica* seeds germination in response to ACC

Treatments	Germination (%)					
ACC µM	$CoCl_2$ [a]	$CoCl_2$ [b]	$AgNO_3$ [a]	$AgNO_3$ [b]	ACC	means
	54.43	44.41	41.95	41.67	74.02	51.30
10	(66.11)	(48.99)	(44.83)	(44.22)	(92.19)	(59.27)
	51.70	40.58	32.11	30.03	70.85	45.05
5	(61.56)	(42.39)	(28.33)	(25.12)	(89.23)	(49.33)
	53.07	42.50	37.04	35.85	72.43	
mean	(63.84)		(45.69)	(36.58)	(34.67)	(90.71)

LSD interaction± 8.01, LSD ethylene inhibitor± 5.66, LSD con± 3.03, [a]: 2 mg/L, [b]: 1 mg/L
Data out of ( ) indicates arcsine transformed data

Table 4: Effects of the combinations on *S. hermonthica* seeds germination

Treatment (µM)	Mean
$AgNO_3$[a]* $CoCl_2$[a] * ACC 10 µM	0.71 (0)
$AgNO_3$ [a] * $CoCl_2$[b]* ACC 10 µM	0.71(0)
$AgNO_3$ [a] * $CoCl_2$ [a] * ACC 5 µM	0.71 (0)
$AgNO_3$ [a] * $CoCl_2$ [b] * ACC 5 µM	0.71(0)
$AgNO_3$ [b] * $CoCl_2$ [a] * ACC 10 µM	17.74 (10)
$AgNO_3$ [b] * $CoCl_2$ [b] * ACC 10 µM	13.55 (8.75)
$AgNO_3$ [b] * $CoCl_2$ [a] * ACC 5 µM	32.31 (30)
$AgNO_3$ [b] * $CoCl_2$ [b] * ACC 5 µM	14.66 (7)
$AgNO_3$ [a] * $CoCl_2$ a	0.71 (0)
ACC 10 µM	74.78 (93.5)
ACC 5 µM	77.64 (90.5)

LSD: 5.28, [a:] 4 mg/L, [b]: 2 mg/L

(Table 2). This may be due to effects of these compounds on growth and/or activity of the bacterium. Ethylene biosynthetic pathway in microorganisms differs from that in higher plants (Frankenberger and Arshad, 1995). A wide range of structurally unrelated compounds have been reported as possible precursors for ethylene generation by different microbial isolates (Arshad and Frankenberger, 1989). Involvement of structurally unrelated compounds in microbial ethylene biosynthesis indicates that more than one pathway may be implicated in production of the phytohormone by micro-organisms. Production of ethylene by *Escherichia coli* and several other bacteria from methionine (MET) proceed through deamination of MET to 2-keto-4-methylthiobutyric acid (KMBA) which is enzymatically degraded to ethylene (Arshad and Frankenberger, 1989). However, production of ethylene from MET often requires sucrose to activate the pathway (Frankenberger and Arshad, 1995).

Both inhibition and promotion of *Striga* germination can be achieved by manipulation of ethylene biosynthesis, ethylene action, or by promotion of ethylene metabolism or that of its immediate precursor ACC. Aminoethoxyvinyl Glycine (AVG), an ACC synthase inhibitor, curtails ethylene biosynthesis and germination in *Striga*. The present study revealed that of the ethylene inhibitors used ($AgNO_3$ and $CoCl_2$) screened inhibited germination in response to ACC (inhibited ACC elicited germination) (Table 3). These observations may indicate that may reduce ethylene biosynthesis by influencing both ACC synthesis and oxidation (Hassan *et al*., 2009a). Our results suggested that the low germination might be due, at least in part, to curtailment of ACC synthesis. The inhibitory effects of silver and cobalt could be attributed to inhibitors of ethylene biosynthesis, inhibitors of ethylene actions.

CONCLUSION

- Special attention should be given to ethylene producing bacteria. *Klebsiella*, which is known to be an ethylene producer, elicited considerable germination of the parasite. Microbes - derived ethylene could be used to induce suicidal germination of the parasite and thus deplete the seed reserves.
- Future research should focuses on; i) re-screening of the effective bacteria and bacterial isolates and rank them according to their ability to suppress or promote specific stages in *Striga* life cycle ii) Screen various inexpensive compounds and local materials as physiological precursors for the respective phytohormones; iii) Identify the most prolific phytohormones producer

REFERENCES

Arshad, M. and W.T. Frankenberger, 1989. Biosynthesis of ethylene by *Acremonium falciforme*. Soil Biol. Biochem., 21: 633-638.

Babalola, O.O., E.O. Osir, A.I. Sanni, G.D. Odhiambo and W.D. Bulimo, 2003. Amplification of 1-amino-cyclopropane-1-carboxylic (ACC) deaminase from plant growth promoting rhizobacteria in *Striga*-infested soil. Afr. J. Biotechnol., 2: 157-160.

Babalola, O.O., 2002. Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination in *Vigna unguiculata*. Ph.D. Thesis, University of Ibadan, Ibadan.

Babiker, A.G.T., Y. Ma, Y. Sugimoto and S. Inanaga, 2000. Conditioning period, CO_2 and GR24 influence ethylene biosynthesis and germination of *Striga hermonthica*. Physiol. Plantarum, 109: 75-80.

Babiker, A.G.T., L.G. Butler, G. Ejeta and W.R. Woodson, 1993. Ethylene biosynthesis and strigol-induced germination of *Striga asiatica*. Physiol. Plantarum, 88: 359-365.

Babiker, A.G.T., 2007. *Striga*: The spreading scourge in Africa. Japanese Soc. Chem. Regul. Plants, 42: 74-87.

Bebawi, F.E., R.E. Eplee, C.E. Harris and R.S. Norris, 1984. Longevity of witchweed (*Striga asiatica*) seeds. Weed Sci., 32: 494-497.

Berner, D.K., N.W. Schaad and B. Volksch, 1999. Use of ethylene producing bacteria for stimulation of *Striga* spp. seeds germination. Biol. Control, 15: 274-282.

Frankenberger, W.T. and J.R.M. Arshad, 1995. Phytohormones in Soil Microbial Production and Function. Marcel Deker, New York, pp: 503.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., A Wiley-Interscience Publication. John Wiley and Sons, Inc., Singapore, pp: 734.

Hassan, M.M., M.E. Abdel Gain and A.G.T. Babiker, 2009a. Potential of Bacterial Strains and Nitrogen in Reduction of *Striga hermonthica* (Del.) Benth. Infesting Sorghum. Adv. Environ. Biol., 3(1): 1-9.

Hassan, M.M., 2008. Evaluation of some soil rhizosphere bacteria for biological control of *Striga hermonthica* (Del.) Benth. infested sorghum. Ph.D. Thesis, Sudan Academy of Sciences (SAS), Khartoum, Sudan, pp: 135.

Hassan, M.M., M.E. Abdel Gain and A.G.T. Babiker, 2009b. Management of *Striga hermonthica* in sorghum using soil rhizosphere bacteria and host plant resistance. Int. J. Agr. Biol., 11: 367-373.

Hsiao, A., A.D. Worsham and D.E. Moreland, 1981. Regulation of witchweed (*Striga asiatica*) conditioning and germination by dl-strigol. Weed Sci., 29: 101-104.

Khan, Z.R., J.A. Pickett, A. Hassanali, A.M. Hooper and C.A.O. Midega, 2008. *Desmodium* species and associated biochemical traits for controlling *Striga* species: present and future prospects. Weed Res., 48: 302-306.

Logan, D.C. and G.R. Stewart, 1991. Role of ethylene in the germination of the hemiparasite *Striga hermonthica*. Plant Physiol., 97: 1435-1438.

Nagahama, K., T. Ogawa, Fujii and H. Fukuda, 1992. Classification of ethylene producing bacteria in terms of biosynthetic pathways to ethylene. J. Ferment. Bioeng., 73: 1-5.

Sauerborn, J., 1991. The Economic Importance of the Phytoparasitic *Orobanche* and *Striga*. In: Ransome, J.K., L.J. Musselman, A.D. Worshman and C. Parker (Eds.), Proceedings of 5th International Symposium on Parasitic Weeds, Nairobi, Kenya, pp: 127-133.

Worsham, A.D., 1987. Germination of Witchweed Seeds. In: Musselman, L.J. (Ed.), Parasitic Weeds in Agriculture. Vol. 1, CRC Press, Boca Raton, FL, pp: 45-61. ISBN: 0-8493-6272-5.