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Biological Control of *Striga hermonthica* Del. Bendth: Screening for Bacteria Scavenging Strigol

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Abstract Addition of bacterial suspensions to the root exudates of the host plant (*Sorghum bicolor*) significantly produced reduction in the ability of the Sorghum root exudates to induce the germination of *Striga* seeds. Bioassaying the treated root exudates using *Striga* seeds gave up to 100% reduction in germination. Most active were bacteria labelled B obtained from Safra Gadarif *Sorghum* strain. The germination of *Striga* seeds was inhibited by up to 100%. The active bacteria B could be applied as dry seed treatment before planting. Growth of the bacteria with production of the active enzyme could result in germination stimulant being destroyed as fast as it is produced. Bacteria B showed pronounced activity against the haustoria induction stimulants which was reflected on the number of haustoria formed. Bacteria B were found to belong to genus *Pseudomonas*.

Keywords Biological Control, Growth Stimulants, Microorganisms, Noxious Weeds, *Striga*, Strigol

1. Introduction

The genus *Striga* comprises parasites on many cereals and legumes crops and causes considerable yield losses of various crops in tropical and subtropical countries[1]. These hemiparasites infest two-thirds of arable land Africa and constitute the biggest single biological cause of crop damage in the continent[2]. In countries such as Ethiopia and Sudan, losses of 65 - 100% are common in heavily infested field.

To germinate, *Striga* seeds must go through an after-ripening process, and then pre-treatment (conditioning) in a warm moist environment for several days, and finally exposure to exogenous germination stimulants. These include germination stimulants, haustoria induction stimulants and development stimulants[3][4]. The nature of these chemicals was first demonstrated by Cook *et al.*[5] who reported the isolation of (+)-strigol (1) from the roots of cotton (*Gossypium hirsutum* L.), a non-host plant which nevertheless has a strong stimulatory effect on Striga seed germination. Cotton seedlings showed average strigol production levels of 15 pg/plant/day, with peak daily production of 30 pg/plant around days 5–7[6]. In another study four sesquiterpene lactones that share structural features with the lactone rings of strigol were shown to stimulate *Striga* seed germination at concentrations comparable to those of strigol[7]. It was later shown[8] that strigol was the main germination stimulant secreted by a number of the most commercially important Striga hosts, including maize (*Zea mays* L.) and millet (*Pennisetum* spp.). Strigol has also been identified in the root exudates of other cereals e.g. *Sorghum bicolor* L. and several dicotyledonous plants including *Menispermum dauricum* DC[9].

Classically the strigolactones have been described as sesquiterpene lactones[10]. The role played by molecules of the strigolactone family in stimulating the germination of seeds of parasitic weeds of the genera Striga, orobanche and Alectra has never been clearly elucidated[2]). However, little was known about their biogenesis until very recently, when a study by Matusova *et al*., (2005) established that (+)-strigol is in fact a product of the carotenoid biosynthetic pathway.

The objective of this work was to isolate naturally occurring microorganisms that could scavenge strigol as it exudes from the host plant root before it reaches the *Striga* seed. Our basic idea originated from a generally accepted microbiological rule that there is no naturally occurring organic substance that cannot be destroyed by some microorganism.

2. Materials and Methods

2.1. Bioassaying

Preconditioned (at 25℃ for 10 days) sterile *Striga* seeds (Shambat 91) were used to monitor the effect of different exudates collected from *Sorghum bicolor* (variety Abu

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Sabeen) as plant material source of *Striga* germination stimulants, and to study the effect of diverse types of bacteria on those exudates. Bacteria tested for their activity against *Striga* germination stimulants, were obtained from soaked *Sorghum* seeds (3 - 10 days). Bioassaying was carried out by applying the preconditioned *Striga* seeds in filter paper discs and damped with the tested exudates (control or treated with bacteria) for 24 hr at 33℃.

Germination of *Striga* seeds was counted as a percentage under microscope. Germination % was calculated as the percentage of *Striga* seeds induced to germinate in relation to the total number of *Striga* seeds per disc.

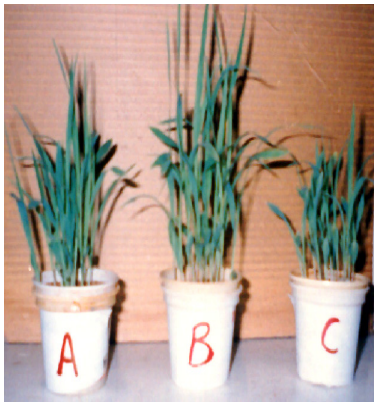
2.2. Cup Experiment

Fifteen of sterilized *Sorghum* seeds were planted in each ice-cream cup, which was initially packed with sterilized sand. These were finally thinned to 3 - 4 seedlings per cup. Buchnnar funnel was used to extract exudates, under water pump pressure, from host roots after 7 days of growth[11]. In control experiment ice-cream cups were irrigated with sterile distilled water for 7 days before extraction. Treated ice cream cups were irrigated with suspension of different bacteria in sterile distilled water for 7 days before extraction. Exudates extracted from both controlled and treated cups were bioassayed to test the effect of each bacteria studied on the host root exudates.

2.3. Pot Experiment

Nine pots of 9cm in diameter were prepared and packed with mixed soil made up of sand + clay. These were then sterilized by heating in an oven at 160℃ for 3 hr. The sterile soil of the prepared pots were seeded with 0.03 gm of *Striga* seeds. Abu Sabeen was then planted at different ratios not exceeding 20 seeds per pot, these were later thinned after 7 days to only 2 seedlings per pot[11].

Pots were then divided into 3 groups according to their irrigation methods: Group I, was irrigated with sterilized tap water for 45 days, Group II, was irrigated with sterilized tap water + bacteria suspention (B) from Safra Gadarif soaked seeds, for 45 days, and Group III, was irrigated with sterilized tap water + bacteria suspention (E) from Abu Sabeen soaked seeds, for 45 days.



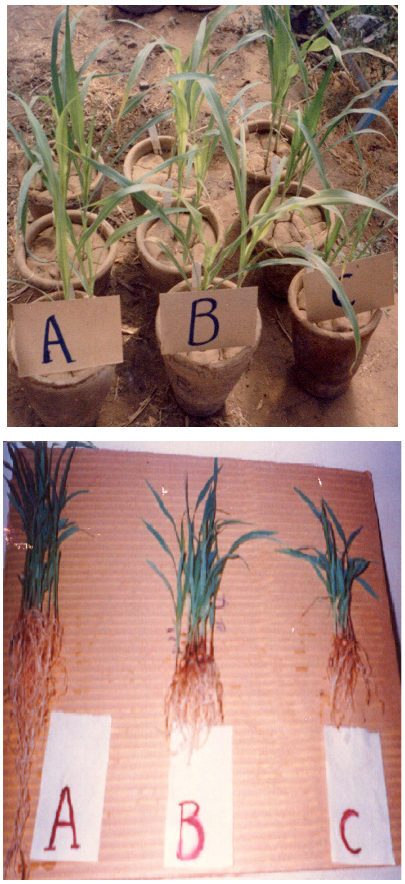


Figure 1. A = Pots irrigated with sterilized tap water only (Control) B = Pots irrigated with sterilized tap water + pure bacteria Type B from Safra Gadarif C = Pots irrigated with sterilized tap water + bacteria innocula from Safra Gadarif soaked seeds

2.4. Thin Layer Chromatography (TLC)

Exudates obtained from different treatments and control experiments were pooled in parts and concentrated using rotor evaporator under reduced temperature and pressure. 20 ml of each exudate was subjected to physical fractionation using separating funnel against equal volume of ethyl acetate [8]. The ethyl acetate fractions were pooled and concentrated to be examined using TLC.

Standard chromatograms of the extracted stimulants were prepared by applying 20-µl solution (5 mg/ml) to a silica gel plate and developing it in different solvent systems depending on the type of extract. These include toluene: ethyl acetate: formic acid (5:4:1), DCM: MeOH (9:1) and different ratios of H_2O: MeOH for RP silica gel. Chromatograms were detected under UV light (254 and 366) and sprayed with diagnostic reagents, which include: vanillin-H_2SO_4 reagent and Natural Product Reagent.

3. Results and Discussion

3.1. Effect of Bacteria Inocula on *Striga* Germination

The addition of bacteria suspensions to the root exudates of the host plant (*Sorghum*, Abu Sabeen) significantly

produced reduction in the ability of the exudates to induce the germination of *Striga* seeds. Results of bacteria suspensions studied on the germination (G%) of *Striga* are summarized in Table (1). Bioassaying the treated root exudates using *Striga* seeds gave up to 0% germination.

These results confirm the idea of existence of microorganisms that are able to abolish the activity of strigol and its analogues. Exudates from Sorghum roots were previously reported to contain analogues of strigol e.g. sorgolactone and they are known collectively as strigolactones[12][13]). The lactone rings, the linkage between them and the substituents on them, seem to be of crucial importance for the germination stimulant activity [7][13]). The terminal lactone ring of the strigol and its analogues are reactive and instable. They could readily be inactivated by non-specific esterase enzymes, apparently by hydrolysing them resulting in their loss of stimulant activity [13]. It is possible that the studied bacteria produce esterases or more specific lactonases that inactivated strigol lactones.

Table 1. Effects of mixed bacteria inocula from soaked seeds from Safra Gadarif or Abu Sabeen on germination percent (G%) of *Striga* seeds

Experiment No.	Control G%	Treated G%
1	75	1
2	82	0
3	64	0.5
4	35	5
5	80	4
6	70	1

Experiments 1, 2, 3 treatment was innocula from Safra Gadarif
Experiments 4, 5, 6 treatment was innocula from Abu Sabeen

In another observation it was found that when the bacteria suspension was applied one week after Sorghum germination, they had no effect on the G% of *Striga* seeds. It was also found that best results were obtained from innocula obtained by soaking Safra Gadarif seeds for ten days.

3.2. Effect of Pure Bacteria Isolates on *Striga* Germination

Different bacteria species were isolated from the innocula obtained from soaked seeds of Safra Gadarif and Abu Sabeen. Results of the effects of suspensions of these isolates on the germination of *Striga* seeds are summarized in Table (2).

Most active were bacteria labelled B and C obtained from Safra Gadarif strain. The germination of *Striga* seeds was inhibited up to 100%. Inoculum C was not studied further because it was not obtained in 100 % pure form.

In another experiment the active bacteria B was used as dry seed treatment and the results of this experiment were summarized in Table (3). Bacteria B showed strong activity against *Sorghum* root exudates when bioassayed using *Striga* seeds. This show that active bacteria could be applied as dry seed treatment before planting in the same way as *Rhizobium* is applied to legume seeds. Growth of the bacteria with production of the degradative enzyme could result in germination stimulant being destroyed as fast as it is produced. Germination of *Striga* seeds might be selectively prevented or slowed down.

Table 2. Effect of pure bacteria isolates on germination percent (G%) of *Striga* seeds

Experiment	A	B	C	D	E
I	70.40	0	0	11.20	0
II	38.91	7.22	12.11	27.51	27.27
III	54.92	2.22	20.11	30.51	32.51

- A = control irrigated with distilled water
- B = irrigated with distilled water + pure bacteria from Safra Gadarif type B
- C = irrigated with distilled water + pure bacteria from Safra Gadarif type C
- D = irrigated with distilled water + pure bacteria from Safra Gadarif type D
- E = irrigated with distilled water + pure bacteria from Abu Sabeen type E

Table 3. Effect of bacteria inocula applied as dry seed treatment on germination percent (G%) of *Striga* seeds

Experiment No.	Control G%	Treated G%
I	36.47	17.22
II	52.59	11.00
III	63.06	12.29

3.3. Effect of Bacteria on Haustoria Formation

Bacteria innocula and pure isolate (bacteria B) were tested for their activity against haustoria induction stimulant, and the results are summarized in Table (4). Haustoria induction stimulant is the second important *Sorghum* root exudate which affects *Striga* germination cycle[12]. Bacteria B showed pronounced activity against the haustoria induction stimulants which was reflected on the number of haustoria formed.

Table 4. Effect of pure bacteria isolate from Safra Gadarif on haustoria formation (number of attachments) of *Striga*

Experiment	I	II	III
A	160.66	139.00	263.33
B	58.33	97.66	164.67
C	143.66	161.33	233.33

3.4. Characterisation of Bacteria B

Isolates of bacteria B, which were proven to be active against different S*triga* germination stimulants from Sorghum root exudates, were subjected to different microbiological tests with an attempt of identifying it. Bacteria studied were found to be blue-greenish in colour which was clearly reflected in the growing media. Other characteristics of the studied bacteria included: rod shaped; Gram negative; non sporing; motile; Catalase positive and Oxidase positive. The studied bacteria were suspected to be a member of genus *Pseudomonas*.

3.5. Chromatography of the Root Exudates of *Sorghum*

The TLC of the ethyl acetate fraction from control exudates showed a range of phenolic compounds when studied using polyamide and C_{18} silica plates under short wave UV light. Pronounced were the major phenolic compounds when sprayed with $FeCl_3$. The TLC pattern of the ethyl acetate fraction of the treated root exudates was different from those of the control as far as the major

phenolic compounds were concerned. This shows that the major phenolic compounds of the treated root exudates fraction were subjected to chemical changes involving different sites within their chemical structures. This again confirms the effect of the bacteria on the stimulant produced by *Sorghum* roots.

4. Conclusions

Addition of bacteria suspensions to the root exudates of the host plant (*Sorghum*, Abu Sabeen) significantly reduced the ability of the exudates to induce germination of *Striga* seeds. Bioassaying the treated root exudates using *Striga* seeds gave up to 0% germination.

Most active were bacteria labelled B and C obtained from Safra Gadarif *Sorghum* strain. The germination of *Striga* seeds was inhibited up to 0%. Innocula of bacteria C was not studied further because it was not obtained in 100 % pure form. The active bacteria B could be applied as dry seed treatment before planting as *Rhizobium* is applied to legume seeds (Table 1). Growth of the bacteria with production of the degradative enzyme could result in germination stimulant being destroyed as fast as it is produced. Bacteria B showed pronounced activity against the haustoria induction stimulants which was reflected on the number of haustoria formed.

Bacteria B were found to belong to the genus *Pseudomonas*, and attempts are made to classify them down to the species level. Further bacterial isolates together with different plants extracts are under similar tests with the aim of biologically control of *Striga* weeds.

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