JASEM ISSN 1119-8362

Full-text Available Online at www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *June*, 2007
Vol. 11 (2) 165 - 168

Control of Bean Rust using Antibiotics Produced by *Bacillus* and *Streptomyces* species - Translocation and Persistence in Snap Beans

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ABSTRACT: Antibiotic culture filtrates produced by *Bacillus* (CA5) and *Streptomyces* spp. were tested for translocation and persistence when applied on snap beans inoculated with rust (*Uromyces appendiculatus*) in greenhouse pot experiments. The antibiotics were applied on the first trifoliate leaves and translocation was assessed as the number of rust pustules on non-treated leaflets or trifoliates while persistence was assessed as the number of rust pustules on rust infected plants at different times after antibiotic treatment. The treatments were replicated three times, each replicate consisting of a pot containing three plants. Antibiotics from both *Bacillus* and *Streptomyces* were found to have up to 100% trans-lamina and leaflet-to-leaflet translocation but no significant trifoliate-to-trifoliate translocation. The antibiotic culture filtrates also retained significant rust control for up to 10 days after application on the bean plant. However, no significant rust control was found on the plants after 16 days of treatment. The study indicated that the antibiotics produced by antagonistic *Bacillus* and *Streptomyces* species possess systemic activity that can persist within the plant for over one week. These metabolites are potential bean rust control products that could be incorporated in integrated disease management programs. @JASEM

Snap bean is an important crop in Kenya, contributing about 62% of the vegetable exports (HCDA, 1999). Bean rust is a common and potentially serious disease of dry and snap beans with a worldwide distribution but is most prevalent in tropical and sub-tropical areas (Robert, 1991). It causes 25 - 100% losses depending on stage of infection and the prevailing weather conditions (Schwartz et al., 2004; Robert, 1991). Severe rust infection results in defoliation, stunted growth and subsequent reduced yields while infected pods may be rejected in the market due to the development of disfiguring lesions (Jacques, 2002; Partridge, 1997).

Although chlorothalonil fungicides have been effective in the control of bean rust, various problems have arose including residues on produce, environmental pollution, development of new physiological races and the prohibitive cost (Gerhardson, 2002; Ken et al., 1987). Additionally, they have negative effects on human health and kill possible antagonists (ICIPE, 2006). The main European markets are increasingly becoming intolerant to residues in the horticultural produce (Cesnik, 2006; Shopper, 2006; Mulandi, 1998).

This study was carried out to investigate translocation and persistence of antibiotics from *Bacillus* and *Streptomyces* species in controlling bean rust (*Uromyces appendiculatus)* in snap bean.

MATERIALS AND METHODS

Antibiotics were produced in Tschen's medium containing glucose 15g, glycerol 15mls, soybean meal 15g, NH_4SO_4 5g, yeast extract 1g, Nacl 5g, $Caco_3$ 5g, per litre (Tschen & Kou, 1984) for *Bacillus* sp. and glucose-soyabean medium (30g soybean meal, 20g commercial glucose per litre (Loeffler *et al*., 1986) for *Streptomyces* sp. The cultures were incubated at room temperature on a circulatory shaker at 125 rpm for 7 days. Culture filtrates were harvested by centrifugation and concentrated to 30% by evaporation under vacuum at 60^{o}C. The filtrates were assayed for antibiotic activity by the paper disc method (Abdel & Sinclair, 1984) using *Fusarium oxysporum* and *Pythium* sp. as the test pathogens for filtrates from *Bacillus* sp. and *Streptomyces* sp., respectively. Antibiotic activity was determined by measuring the diameter of clear inhibition zones formed around the paper discs after 48 hours of incubation.

Translocation and persistence experiments were carried out in the green house using snap bean, variety Samantha. Antibiotic culture filtrates were applied at three weeks after germination. The plants were inoculated with bean rust urediospore suspension ($2x10^6$ spores/ml) containing a few drops of tween 80. For translocation experiments, inoculation was at two days after application of antibiotics and repeated once at 4 days while for the persistence experiments inoculation was done on same day as the antibiotic and repeated at 2, 4, 8, 12 and 16 days. Control plants were sprayed with distilled water. Three aspects of translocation were investigated (i) trans-lamina where the filtrate was applied on the upper surface of the first trifoliate leaf and rust infection observed on the lower surface (ii) leaflet-to-leaflet where filtrate was applied on the first leaflet of the first trifoliate and infection observed on the other leaflets of the same trifoliate (iii) trifoliate-to-trifoliate where filtrate was applied on the first trifoliate and infection observed on the other trifoliate leaves. The experiment was laid out in a completely randomized design (CRD) with 3 replications each containing 3 plants. Disease

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assessment started with the first appearance of rust symptoms and was determined as the number of rust pustules per leaf. Data was analysed using Genstat[R] 3.0 statistical software.

RESULTS AND DISCUSSION

Both culture filtrates from *Bacillus* (CA5) and *Streptomyces* (CS35) inhibited the growth of *Fusarium oxysporum* and *Pythium* spp in culture. Culture filtrates from *Bacillus* were more effective in suppressing growth of *Fusarium oxysporum* while culture filtrates from *Streptomyces* were more effective against *Pythium* spp. (Table 1). The inhibition zones produced by the two culture filtrates were very clear. Combining the culture filtrates from *Bacillus* sp. and *Streptomyces* sp. did not result in increased antibiotic activity. The inhibition zones produced by the combined filtrates against both *Fusarium oxysporum* and *Pythium* spp. were faint to moderately faint.

Table 1: Inhibition zones (mm) produced by culture filtrates from *Bacillus* and *Streptomyces* spp. and their combination against *Fusarium oxysporum* and *Pythium* spp.

	Fusarium oxysporum	*Pythium* spp.
Distilled water	0.000c	0.000c
Bacillus (CA5)	14.111a	11.72b
Streptomyces (CS35)	12.000b	15.53a
Combined CA5 + CS35	12.083b	12.14b
Mean	9.549	9.85
LSD (0.05)	0.4031	0.728

Values followed by the same letter within columns are not significantly different (p=0.05)

The antibiotics produced by *Bacillus* CA5 and *Streptomyces* CS35 were translocated from upper to lower leaf surface and within leaflets of the same trifoliate leaf. Rust infection was completely inhibited on the treated leaves (Table 2). No translocation was observed from one trifoliate leaf to other leaves within the plant. However, the treated leaves exhibited some phytotoxicity, which was expressed as thickening, scorching and early senescence of the leaves. Both culture filtrates from *Streptomyces* and *Bacillus* showed significant persistence by inhibiting rust infection for up to 8 days after application. Culture filtrate from *Bacillus* reduced rust infection by 94.9%, 81.7% and 99.81% on plants inoculated at 0, 4, and 8 days after application while culture filtrates from *Streptomyces* reduced infection by 91.7%, 81.7% and 66.7% respectively (Table 3). No antibiotic activity was observed when inoculation was done 16 days after application of both culture filtrates. The culture filtrates from *Bacillus* and *Streptomyces* gave an overall reduction in rust severity of 47.2% and 27.8%, respectively.

Table 2: Average number of rust pustules on snap bean leaves treated with culture filtrates from *Bacillus* (CA5) and *Streptomyces* (CS35) in greenhouse after inoculation with *Uromyces appendiculatus*.

	Bacillus CA5	*Streptomyces* CS35	Distilled water
Trans-lamina translocation	0b	0b	20.21a
Leaflet-to-leaflet translocation	0b	0b	10.03a
Trifoliate-to-trifoliate translocation	34.7a	41.4a	32.4a
Mean	11.56	13.8	20.88

Values followed by the same letter(s) within rows are not significantly different (p=0.05).

The results of this study showed that culture filtrates of *Bacillus* CA5 and *Streptomyces* CS35 possess antibiotic activity against different fungal pathogens. The filtrates inhibited the growth of *Fusarium oxysporum* and *Pythium in vitro* and bean rust on inoculated plants. The activity of the antibiotic culture filtrates from both *Bacillus* and *Streptomyces* against the test fungi differed both *in vitro* and *in vivo*. The effectiveness of antibiotics produced by a given antagonist depends on the test pathogen and the type of antibiotic produced (Lee et al., 2005; Yeo and Hol, 1997; Campbell, 1989; Loefler et al., 1986). The antibiotics produced by *Bacillus* CA5 and *Streptomyces* CS35 possess limited systemic action as indicated by their translocation within the treated bean leaves. Lack of significant upward translocation could be attributed to dilution effect of weakly active compounds (Dernoeden, 2002). Absorption and translocation of antibiotics can be enhanced by dissolving the active compounds in free oil (Moss, 1989). The activity of antibiotics produced by *Bacillus* CA5 was more persistent than that of *Streptomyces* CS35. Persistence of antibiotic activity of commercial formulations of antagonistic bacteria

has been reported elsewhere (Kiewniek and Jacobsen, 1998). The systemic action and persistence of the antibiotics is of great significance in plant disease control. The active metabolites get absorbed into the plant cells where they are retained and protect the plant from new infections for about 10 days. However, prolonged persistence of chemicals on food crops is not recommended due to current international regulations on maximum residue levels and pre-harvest interval of the treated produce (Grandison, 2006; Wandiembe and Adipala, 2001; Mulandi, 1998; Watson and Koon, 1997). The results indicated that *Bacillus* (CA5) and *Streptomyces* (CS35) produced extracellular metabolites that could be useful in the integrated management of rust (*Uromyces appendiculatus*) in snap beans.

Table 3: Average number of rust pustules on snap bean leaves inoculated at different times with rust spores after treatment with antibiotic culture filtrates from *Bacillus* (CA5) and *Streptomyces* (CS35) species

Time of inoculation (days)	Treatment	Time after inoculation (days)										
		10	12	14	16	18	20	22	24	26	28	Mean
0	water	7a	9a	11a	13a	18.7a	33a	48a	65.7a	76.7a	93.3a	37.5a
	CS35	0a	0b	0b	0.5b	1.2b	1.5b	2.3b	2.3b	8.2b	15.2b	3.1b
	CA5	0a	0.2b	0.2b	0.2b	1.2b	1.2b	1.2b	3.7b	4.8b	6.3c	1.9b
4	water	1.7a	3.5a	5.7a	16.3a	28a	42a	55.5a	65.8a	79.5a	93.5a	39.1a
	CS35	0a	0.3a	1.3a	2.3b	3.5b	6.3b	8.7b	10.7b	17b	23.8b	7.4b
	CA5	0a	0a	0a	2b	3.2b	5.5b	9.3b	13.8b	16.5b	23.8b	7.4b
8	water	0.2a	7.3a	33.5a	58.3a	73.3a	90.3a	105.3a	132a	149.8a	177.7a	82.8a
	CS35	0.2a	1.3a	10.3b	19.5b	23.8b	29.7b	35.3b	42.7b	51b	62.3b	27.6b
	CA5	2a	3a	6.7b	9.7b	11.7b	14b	16.8c	22.3c	30.2c	38c	15.4b
12	water	21.8a	27.3a	34.5ab	42b	49.2b	86a	125.5a	151.2a	191.2a	231.2a	96.0a
	CS35	24a	34.3a	49.2a	62.7a	83a	103.7a	125.3a	149.7a	174.7a	206b	101.2a
	CA5	6.2a	19.2a	23.8b	28.5b	31.3b	51.8b	67.3b	88b	113.5b	157.8c	58.8b
	CS35	33.2a	46.3a	66.2a	77a	94.5a	117.3a	147.5a	176.5a	211.8a	238.5a	120.9a
	CA5	18.8a	36.7a	57.7a	62ab	81.3a	102.2ab	130ab	159.2a	189.3b	230a	106.7a

Values followed by same letter(s) within columns (same day of inoculation) are not significantly different (p=0.05)

Acknowledgement: This study was funded by the University of Nairobi and the Agricultural Research Fund (ARF) of the Kenya Agricultural Research Institute (KARI).

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