BIOLOGICAL CONTROL OF NECK ROT AND BLACK MOULD OF ONION

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(Manuscript received 23 February 1998)

Abstract

Control of onion neck rot caused by *Botrytis allii* and a decrease in black mould caused by *Aspergillus niger* were obtained on onion (cv Giza 20) with promot. Promot strongly inhibited radial growth of pathogens on potato dextrose agar when it was conditioned at 20°C for 12 hr or at 30°C for 4 hr. Moreover, area of inhibition increased when promot was conditioned at 20°C or 30°C for 12 hr.

On dry onion, however, promot was ineffective on dry onion fleshy fresh scales after dipping in promot (conditioned at 22 ± 2°C for 4, 12 or 24 hr). It promoted the growth of the inoculated discs, of *B.allii or A.niger*, on the concave surface of scales giving high infection rate with expanded rotted area.

The level of control of neck rot through post harvest simulation was determined at room temperature for the bulbs resulting from dip treatment of onion seedlings in promot suspension before planting followed by three spray applications at 15 day intervals four months after planting. Infections were significantly reduced to 27% and 23%. The dip treatment in Benlate solution (half dose 0.5 g./L) followed by the same spray application of promot was not satisfactory.

Similar results were obtained in the control of black mould rot, where the percentage of infection of the same dip and spray treatments with promot was approximately 50% of the control treatment, Meanwhile, spray treatments with both of Ridomil plus (half dose 75 g./L) and promot was comparable. On the other hand, the treatment of Ridomil using recommended dose (150 g./L) was much less effective.

According to the obtained results Trichoderma product (promot) as dip or spray could protect onion plants against *B.allii* and *A.niger* and could result in enhanced plant growth and better bulb development, thus help in escaping infection.

INTRODUCTION

Success in biocontrol has remarkably increased in the past few years for flower-infecting, post harvest diseases, pathogens of green house crops, turfgrass diseases as well as pathogens in crops residues. Even serious foliage diseases, which have long been considered not amenable to biocontrol in the field, are subjected to suppressive effects of microorganisms (Andrews, 1992 and Blackeman & Fokkema, 1982).

The use of *Trichoderma spp* as biocontrol agents is well established (Chet and Barker, 1981; Elad *et al.*; 1981, Harman *et al.*; 1980; Papavizas and Lewis, 1981 and Sivan *et al.*, 1984). In previous studies, *Trichoderma harizianum* was considered to be effective against *Sclerotium cepivorum*, the causal organism of white rot of onion when added to the soil (Abd El-Moity, 1981).

It was also demonstrated that *T.harzianum* was effective in controlling *S.rolfsii* and *Rhizoctonia solani* and there was a positive correlation between the amount of *T.harzianum* preparation and disease reduction and that an integrated control of *S.rolfsii* was tried by using a combination of *T.harzianum* preparation and the fungicide Pentachloronitrobenzene (PCNB) at low dosage. The obtained results indicated a synergistic interaction between both treatments (Chet et al., 1979).

It is abundantly clear that the control of post harvest problems requires a combination of pre-and post harvest applications involving an array of chemical and non chemical treatments (Spotts, 1984; Leach, 1984; Blank *et al.*, 1987 and Gesson *et al.*, 1988). Moreover, preharvest control measures that are applied before harvest will influence post harvest disease losses (conway, 1984), either by reducing pathogen populations on crops (Ogawa & Manji, 1994) or where infection occurs in the field (Booth-Burden, 1983 and Noon, 1984).

This work was undertaken to evaluate a commercial product of *Trichoderma,* marketed under the trade name "Promot". Efficiency of the product, either singly or in combination with other fungicides, against neck rot and black rot of onion was tried. The possible mode of action in biocontrol this product is also discussed.

MATERIALS AND METHODS

2.1. Source and preparation of the product (Promot):

Promot is a microbiological growth promotive dry formulation produced by fermentative processes from beneficial *Trichoderma* spp. It contains not less than $3x10^7$ spores/gram of *Trichoderma Koninjii* and $2x10^7$ spore/gram of *Trichoderma harzianum.* It is manufactured by JH Biotech. Inc., Ventura, California USA and was provided by Danton Egypt company.

An active suspension of promot was prepared in distilled water in 250 Erlenmeyer flasks from the dry formula to give the desired concentration (10g/L distilled water) and then shacken on a rotary shaker (Lab. Line Oerbit Environ-Shaker) at 150 rpm overnight at 25°C. It was prepared fesh for each use.

2.2. Inocula preparation:

Cultures of *Botrytis allii* and *Aspergillus niger* were isolated from infected onion bulbs and maintained on potato dextrose agar (PDA) at 20°C for *B.allii* and at 27°C for *A.niger* with periodical transfer through onion bulbs. Conidial suspensions of $4x10^5$ and $7x10^8$ spores/ml were prepared for each of *B.allii* and *A.niger* respectively from 7 day-old cultures grown on PDA as described by Janisiewies and Marchi (1992).

2.3. Plant materials:

Fresh onion seedlings and dry onion bulbs (cv. Giza 20) were provided by onion breeding Dept., Field Crops Res. Inst., ARC. Healthy seedlings were planted immediately in a 25 cm diameter-pots in clay soil, five seedlings per pot. Bulbs used for post harvest studies (on onion scales) were chosen on the basis of equal sizes and absence of physical injuries or infection and then stored at 20 ± 2°C till use.

2.4. Effect of promot on the pathogen:

a. *In vitro* studies

The effect of incubation period and temperature of promot suspension on the resulting radial growth of *B.allii* and *A.niger* was determined by two methods:

i. Promot suspension (10 g/L.) was incubated at 20°C or 30°C each for 4, 12 and 24 hr. One ml of the prepared suspension was mulched on PDA plates (10 ml. Media/ plate) followed by placing 4 mm disc of each of *B.allii* and *A.niger* in the center of the plate, 15 minutes after mulching.

ii. Sterilized filter paper discs impregnated with promot suspension for 5 minutes were placed opposite to each other at a fixed distance on PDA plate. A disc (0.4 mm) of the pathogens in concern was placed in the middle of the distance between the promot discs. Control treatments without promot were included. The plates contained either *B.allii* or *A.niger* were incubated at 20°C ± 2 and 27°C±2 respectively. Four replicates served for each treatment. Radial growth of fungi on promot mulch as well as inhibition zones were determined in (mm.).

b. On onion scales

Medium size onion bulbs were longitudinally cut into four equal parts of the out er fleshy scales (originated from different positions of the same bulb).

Slices were immersed in the prepared promot suspension (10 g/L.), incubated

at room temperature (25°C ± 2) for 4,12 and 24 hr. Treated slices were then inoculated with a 4 mm. disk of each of *B.allii* or *A.niger* on the concave surface of the slice and placed in a sterilized glass jars, then incubated at 20°C ± 2 for *B.allii* and 27°C ± 2 for *A.niger*. Three jars with four onion slices each were used for each treatment. The development of necrotic zones was checked after seven days and then measured and expressed in mm.

2.5. Effect of pre harvest treatments on the development of Neck rot and Black mould rot after harvest:

Giza 20 onion transplants, 60 days old were treated against neck rot and black mould rot before planting and throughout the growing period by certain combinations of the promot preparations and low rates of the recommended fungicides.

The dipping treatments against *B.allii* were made as follows:

1. Dipping onion seedlings in promot suspension (conditioned at 25°C ±2 for 4 or 12 or 24 hr.) for 15 minutes and spraying with promot suspension (conditioned at 25°C ± 2 for 12 hr.), four months after planting three times at 15 day intervals.
2. Dipping onion seedlings in Benlate solution (50%), at the recommended dose is 1g./L.) for 15 minutes before planting.
3. Dipping onion seedlings in Benlate solution (50%), at half the recommended dose (conditioned at 25°C ± 2 for 12 hr.) four months after planting three times at 15 day intervals.

Onion plants were artificially insoulated by drenching with *B.allii* ($4x10^5$ conidia/ml), two weeks at the end of each treatment.

The dipping treatments against *A.niger* were made as follows:

1. Dipping in promot suspension (conditioned at 25°C ± 2 for 4 or 12 or 24 hr.) each for 15 min. and three sprays at 15 day intervals, four months after planting.
2. Spraying onion plants tarice with Ridomil plus at the recommended dose (150g./L.), four moths after planting.
3. Sprayings of Ridomil plus, at half the recommended dose (75g./L.) alternatively with promot suspension (conditioned at 25°C ± 2 for 12 hr.) four months after-planting.

Onion plants were artificially inoculated by spraying with a suspension of *A.niger* ($1x10^8$ spore/ml.) two weeks before the end of each treatment.

Grown bulbs were harvested after 6 months and stored in storage room at 20°C±2 for the bulbs treated against *B.allii* and at room temperature for those against *A.niger*. Bulbs were then screened for decay percentage after 2, 4 and 6 month-storage periods and each experiment was repeated twice.

2.6. Data analysis:

Data were statistically analyzed, using Duncans multiple range test following SAS/GLM procedures to test for separation of means at P=0.05. Sample size was n=4 for testing replication effects and n = 7 for testing treatment effects.

RESULTS

3.1. Effect of promot on the growth of *Botrytis allii* or *Aspergillus niger* in vitro:

The interaction between different treatments of conditioned promot with the two fungi in concern i.e. *B.allii* and *A.niger* is shown in tables (1a, 1b).

Table 1. Effect of promot conditioning at 20°C or 30°C for 4, 12, and 24 hr. on radial growth (1a) and inhibition zone (1b) of *B.allii* or *A.niger*.

Pathogen	Radial growth (cm)						
	Promot at 20oC			Promot at 30oC			PDA
	4hr.	12hr.	24hr.	4hr.	12hr.	24hr.	
B.allii	2.33D	0.00E	2.45D	0.45E	2.18D	2.73D	9.00A
A.niger	7.07B	5.15C	9.00A	7.03B	7.68AB	7..43AB	9.00A

Within rows values followed by different letters are significantly different (P=0.05) according to Duncans' multiple range.

Table (1b)

Pathogen	Radial growth (cm)						
	Promot at 20oC			Promot at 30oC			PDA
	4hr.	12hr.	24hr.	4hr.	12hr.	24hr.	
B.allii	0.73DEFG	1.20CDE	0.83CDEFG	0.28GH	1.10CDE	0.33FGH	0.00 H
A.niger	0.98CDEF	1.43BC	1.88AB	0.58EFGH	2.38 A	1.28BCD	0.00 H

Within rows values followed by different letters are significantly different (P=0.05) according to Duncans' multiple range.

In vitro studies showed that conditioning promot at certain temperature i.e. 20°C or 30°C for 4, 12, 24 hr., affected the fungal growth as well as its inhibitory effect to both *B.allii* and *A.niger*. Radial growth of *B.allii* was significantly ($P<0.05$) reduced in the presence of promot with complete inhibition ($p = 0.00$) when promot was incubated for 12 hr. at 20°C, while it was remarkably reduced for *A.niger* in the presence of promot when it was conditioned for 12 hr. at 20°C compared with growth in the control (plain PDA).

B.allii appeared to be more sensitive to promot than *A.niger*; the restriction of the radial growth of *B.allii* with certain conditions of promot was greater than that of *A.niger* (Table 1a). On the other hand, zones of growth inhibition of both of *B.allii* or *A.niger* were largely detected on plates treated with promot and incubated for 12 hr. at at 20°C or 30°C ($p = 0.00$). The effect was clearer on *A.niger* than that on *B.allii* (Table 1b). Relatively lower effect was obtained on both *A.niger* or *B.allii* when promot was conditioned for 4 hr. at 20°C or 30°C.

3.2. Effect of conditioning of promot on the growth of *B.allii* or *A.niger* on onion scales.

Decay percentage and developed rotted tissues were determined on fleshy onion scales which were treated with promot (10 g./L.) conditioned for 4, 12, 24 hr. at 22°C ± 2°C.

In general, non-significant reduction ($P>0.05$) was obtained regarding the incidence of both decay and developed rotted area by each of *B.allii* and *A.niger* ($P = 0.328$).

Conditioning of promot for 4 or 12 hr. at 22°C ± 2°C strongly enhanced the growth of both *B.allii* and *A.niger* over the treated scales, the extent of enhancement doubled that obtained on scales treated with water and loaded with inocula of each of the tested fungi (control).

Similar effects concerning decay and rotted areas were observed when the scales were treated with promot conditioned for 24 hr. at 22°C ± 2°C Table (3.b).

Statistical analysis showed no significance ($P>0.05$) between *B.allii* and *A.niger* ($P= 0.082$) in case of decay percentage, while a significant effect was detected for the development of rotted area ($P<0.05$) as the treatments of promot were significantly different among each others ($P=0.017$).

Table 2. Effect of promot conditioning at 20°C or 30°C for 4, 12, and 24 hr. at room temperature (25°C±2°C) on decay percentage and developed rotted area by *B.allii* and *A.niger* on onion scales.

Treatment	% Decay			Rotted Area		
	B.allii	*A.niger*	Mean	*B.allii*	*A.niger*	Mean
Check (water)	33.33	66.67	50.00 b	0.100	0.580	0.340 b
Promot (4hr.)	100.00	100.00	100.00 a	0.300	0.567	0.433 b
Promot (12hr.)	100.00	100.00	100.00 a	0.400	0.817	0.608 a
Promot (24hr.)	50.00	100.00	75.00 ab	0.093	0.613	0.353 b

Scales were dipped in a suspension of various treatments of promot or water (control) and then loaded with discs of each fungus.

L.S.D. for	Decay	Rotted Area
Fungi	N.S.	0.087
Treatment	33.63	0.123
Fungi x Treatment	N.S.	N.S.

3.3. Effect of Preharvest application of Promot and/or Low rates of recommended fungicides on the development of neck rot and black mold rot after harvest.

Pre-harvest treatment with Promot conditioned at 22°C ± 2 for 4,12 and 24 hr. individually or combined with low rates of Benlate or Ridomil were conducted for controlling post harvest rots caused by *B.allii* and A.niger respectively after 2 and 4 months of harvesting.

Significant reduction of infection caused by *B.allii* was achieved on bulbs stored for four months and originally obtained from seedlings treated by dipping in Promot (conditioned at 22°C ± 2 for 4 hr. and 24 hr.) and afterward sprayed with Promot (conditioned at 22°C ± 2 for 12 hr.) three times, four months after planting. The level of control reached 27%, 23% respectively of that obtained for those artificially inoculated with *B.allii* and received no treatment (P = 0.00).

The combination of Promot with Benlate (at half the recommended dose 0.5 g./l, as dipping treatment) followed by spray treatment with promot (conditioned at

22°C ± 2 for 12 hr.) achieved a reasonable level of protection against *B.allii* (35.25%) (Table 3a).

Table 3a. Effect of dipping application of various combinations of promot and Benlate during growing period on infection percentage by *B.allii* after two and four month-storage of onion bulbs.

Treatment (Dipping)	% Infection	
	After 2 mo.	After 4 mo.
Promot (4hr. incubation)	6.25 EF	27 EFG
Promot (12hr. incubation)	0.00 F	58 BCDEF
Promot (24hr. incubation)	0.00 F	23.75 FG
Benlate (1 g./L.)	0.00 F	14.5 G
Benlate (0.5 g./L.)	0.00 F	35.25 DEFG
Check (4x105/conidia/ml.)	0.00 F	100 A

** The plants of all treatments were sprayed with promot suspension incubated for 12 hr. during growing period, three times at 15 days intervals, except the plants treated with Benlate (1g./L.) as well as check plants.

Similar results were obtained in *A.niger* treatments (Table 3b) where significant reduction in infection occurred after four months of storage of the bulbs resulting from seedlings treated either by dipping in promot (conditioned at 22°C ± 2 for 4 hr., 24 hr.) and spray by promot (conditioned at 22°C ± 2 for 12 hr. respectively) or by a combination treatment (plants sprayed with Ridomil at half the recommended dose 75g./L., after four months of planting followed by 3 sprays with promot conditioned at 22°C ± 2 for 12 hr. three times) at 15 day intervals. The infection level was approximately reduced to 50% of that of the control.

Table 3b. Effect of Spray & dipping application of various combinations of promot and Ridomyl during growing period on infection percentage of *A.niger* after two and four month-storage of onion bulbs.

Treatment (Dipping)	% Infection	
	After 2 mo.	After 4 mo.
Promot (4hr. incubation)	31.25 BCDE	39.5 CDEFG
Promot (12hr. incubation)	47.5 BC	65 ABCDE
Promot (24hr. incubation)	20.75 CDEF	41 CDEFG
Ridomil (150 g./100L.)	79.5 A	77 ABC
Ridomil (75 g./L.)	32.75 BCDE	40 CDEFG
Check (1x10-8/spore/ml.)	56 AB	83.25 AB

* Note: The plants of all treatments were sprayed with promot spore suspension (incubated for 12 hr.) three times at 15 day-intervals except the plants treated with Ridomyl (150 g./100 L.) as well as check plants.

DISCUSSION

Two mechanisms have been proposed most frequently to explain the nature of the protective action due to the application of certain *Trichoderma spp.* agaist plant pathogens. The first, is that enhanced plant growth resulting from amendment of soil with *Trichoderma* spp. be attributed to a direct effect of these *Trichoderma* spp. on the plant. The second is that contributed by a secondary effect due to control of minor plant pathogens (Kloepper and Schroth, 1981).

The experiments described here were performed with the *Trichoderma* product "promot" *in vitro*, on onion scales and in pots to test its potential against the development of the causal pathogens of neck rot and black mold rot on onion bulbs after harvest.

Pilot tests involving the application of the product Promot against the tested organisms on plates revealed that conditioned Promot significantly decreased the development of both tested fungi *in vitro.*, while it was not satisfactory when applied on onion scales as no reduction percentage of decay and lesion diameter was observed when both fungi were inoculated on treated scales.

Based on the fact that preharvest control measures will affect the postharvest disease losses (Conway, 1984), largely through affecting pathogen population on crops (Ogawa & Manji, 1984) where infection occurs in the field (Booth & Burden, 1983) and (Noon, 1984), in addition to the possible production of growth regulating metabolites by biocontrol agents which subsequently affect plant growth (Windham *et al.*, 1985), some trials were made.

Treatment of onion seedlings grown in autoclaved soil-containing Promot showed promising results in controlling the development of *B.allii* and *A.niger* with a greater effect on *B.allii* infection after harvest.

In field studies in the Netherlands, species of *Gliocladium, Trichoderma, Penicillium* and other antagonists applied as spore suspension to wounds on succulent leaf bases of onions (the wounds were produced by a simulation of the harvest practice of foliar topping) suppressed the incidence of neck rot caused by *Botrytis aclada* during subsequent storage (Khol *et al.*, 1991). The treatments were moderately suppressive even though a high concentration of *B.aclada* was applied immediately after application of the antagonists.

Moreover, preharvest experiments enhanced bulb growth in treatments involving promot could be attributed to the control of minor plant pathogen as explained by Windham *et al.*, (1984).

To overcome the variability in the performance of Promot alone, low concentrations (0.5 g./L, 75g./L,) i.e. half dose of the commercially recommended concentrations of Benlate and Ridomil were applied against *B.allii* and *A.niger* respectively in addition to Promot applications. The results suggest that in order to achieve high efficacy comparable to the standard commercial rate of fungicides, Promot should be used with a low concentration of both tested fungicides.

Although the combinations of Promot plus low rates of either Benlate or Ridomil against neck and black mould rot diseases did not always results in a significantly lower inidence of decay compared with the commercial rate of both fungicides, Promot plus fungicides is preferable because it allows a much lower level of chemical fungicides and resulted in a more consistent effect.

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المقاومه الحيويه لعفن الرقبه والعفن الاسود على البصل باستخدام منتج التريكودرما "البروموت"

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أمكن مقاومه عفن الرقبه المتسبب عن الفطر *Botrytis allii* و خفض الاصابه بالعفن الاسود المتسبب عن الفطر *Aspergillus niger* بصنف البصل جيزه ٢٠ باستخدام البروموت بتركيز ١٠ جم/لتر حيث ساعد البروموت (المحضن على ٢٠ م لمده ١٢ ساعه أو على ٣٠ م لمده ٤ ساعات) على التثبيط الشديد للنمو الميسليومى للفطرين عند اختباره على بيئه اجار دكستورز البطاطس وكذلك ادى لزياده منطقه التثبيط الناتجة عن كلا الفطرين (عند تحضينه على ٢٠ م أو ٣٠ م لمده ١٢ ساعه) ولكن لم يكن للبروموت (المحضن على ٢٥ ± ٢ م لمده ٤ أو ١٢ أو ٢٤ ساعه) فعالية عند غمر الاوراق اللحميه الطازجه للابصال بمعلق منه حيث ادى المبروموت لتنشيط لقاح الفطرين على اسطح الاوراق اللحميه وبالتالى زياده معدل ومساحة الإصابة.

وبدراسه تقييم المقاومه لعفن الرقبه للابصال الناتجه عن الشتلات التى تم غمرها قبل الزراعه فى معلق البروموت (المحضن على ٢٥ ± ٢ م لمده ٢٤ ساعه) بعد اربعة شهور من الزراعه ثلاث رشات كل ١٥ يوم ادى ذلك لانخفاض معنوى فى نسبة الاصابة حيث وصلت الى ٢٣٪ و ٢٧٪ من النسبة الناتجه للمقارنه فى حين لم تكن المعامله بالغمر فى محلول البنليت (٠,٥ جم / لتر نصف الجرعه الموصى بها) يتبعها معاملات الرش السابقة بالبروموت على نفس المستوى. وبنفس التأثير، ادت نفس معاملات الغمر والرش السابقة بالبروموت – أو بالغمر فى الريدوميل (٧٥ جم /لتر نصف الجرعه الموصى بها) يتبعها الرش بالبروموت الى خفض الاصابة بالعفن الاسود حيث سجلت نسبه الاصابه ٥٠ ٪ تقريبا من النسبة المتحصل عليها للمقارنه رغم ان المعاملة بالريدوميل فقط (١٥٠ جم/لتر جرعه موصى بها) كانت اقل فعالية. والخلاصة هى ان منتج التريكودرما: "البروموت" يمكن ان يوفر الحماية لنبات البصل ضد فطرى *Botrytis allii* و*Aspergillus niger* بواسطه معاملات الغمر والرش التى تستميل نمو النبات والابصال وبالتالى تساعد على الهروب من الاصابه.