INFLUENCE OF STORAGE CONDITIONS ON THE SHELF-LIFE OF MICROBIAL INOCULANTS AND THEIR BIOCONTROL ACTIVITY TOWARDS DAMPING-OFF PATHOGENS

D.C. Covert, L.M. Funk, N.P. Seresinhe, M.S. Reddy and G.L. Brown
Cominco Fertilizers Ltd., Ag Biologicals, 402-15 Innovation Blvd.,
Saskatoon, SK, S7N 2X8

Abstract

Select microbial inoculants (Ral-3, 64-3, 63-28, and G8-32) were produced in proprietary liquid formulation, placed into commercial packages and stored at different temperature regimes. Shelf-life of bacteria and level of antagonism were evaluated against the damping-off pathogens Pythium ***paracondrium, Rhizoctonia solani, Fusarium solani,*** and ***Cylindrocarpon destructans*** when retreived from the commercial packages after storage. Although differing in their antibiosis mechanisms, strains Ral-3 and 63-28 were significantly consistent in their antagonistic activity towards the fungi tested, irrespective of their storage conditions. Strains 64-3 and G8-32 did differ in their activity against the fungi. The storage temperature of 40°C affected the shelf-life of 64-3 by reducing it to Log 4.3 cfu/ml and the antagonistic activity of G8-32 was low against ***R. solani*** at all temperatures. However, the storage temperature of -20°C did not affect the activity or shelf-life of the strains.

Introduction

Bacterial strains Ral-3 and 64-3 suppress fungal pathogens in conifer seedling nurseries and improve the growth of seedlings on reforestation sites. Strains 63-28 and G8-32 suppress damping-off pathogens of vegetables and omamentals grown in greenhouse environments. For the commercial success of these products, studies to foresee what occurs to the product before it reaches the customer must be known. After the inoculant is packaged, it will undergo many types of stresses prior to it being used. Such stresses are induced by changes in storage temperature and the length of storage.

Shelf-life of inoculants is very important under different storage conditions. This all depends on formulation and packaging. Government regulations suggest that the commercial product must have a minimum shelf-life of 1 years at room temperature, and also be easy to handle, insensitive to abuse and be stable over the range of -5°C to +30°C (1). Biological control of damping-off pathogens by the use of in vitro means test the efficacy of the microbial inoculants after being in these environments. In recent years, there have been many reports on the role of Pseudomonads that promote plant growth and suppress diseases caused by soil-borne plant pathogens. The production of sideophores, hydrogen cyanide, and antibiotics have been some of the mechanisms of action postulated (2). Some of the antibiotic compounds have been chemically characterized and include pyrrol-type antibiotics, N-containing heterocycles such as phenazines, pyo-compounds and indole derivatives (3).

In view of these, the following experiments were set up to investigate, i) shelf-life of select microbial inoculants in commercial packages stored in different temperature regimes and ii) efficacy of the strains towards damping-off fungal pathogens when retrieved from storage at various temperature regimes.

Materials and Methods

Source of microbial inoculants and fngal pathogens

Four bacterial strains: Ral-3 ***(Pseudomonas cepacia), 64-3 (Pseudomonas fluorescens), 63-28 (Pseudomonas auerofaciens),*** and ***G8-32 (Pseudomonasputida),*** were selected for this study because of their activity against several fungal pathogens in vitro and in vivo. Bacterial cultures were maintained at -80°C in a nutrient broth amended with 5% glycerol prior to use. For use in experiments, the inoculants were streaked onto Pseudomonas agar F (PAF) and checked for purity after incubation for 24 h at 30°C. Purified single colonies of 64-3 and Ral-3 were grown for 48 h in a commercial fermentor under submerged culture conditions with 3 L volumes harvested into polymer packaging. The 3 L packages were placed at 5 °C, 18 °C, and 40 °C up to a 3 week period and at -20 °C for up to a week. For strains 63-28 and G8-32, purified single colonies were grown in a lab scale fermentor for 48 h with 100 ml volumes harvested into polymer packaging. These packages were then placed at 5 °C, 22 °C, 30 °C and -20 °C for up to approximately 7 weeks. The fungal pathogens, ***P. paracondrium, R. solani, F. solani,*** and C. ***destructans*** were selected for this study, and these were maintained on potato dextrose agar (PDA) at room temperature prior to use.

Shelf-life of inoculants in commercial packages under various temperature regimes

Samples were obtained by aseptically withdrawing 1 ml of inoculant from the replicated packages and placing it into a 9 ml buffer of posphate salts. After lo-fold serial dilutions, 0.05 ml was spread-plated in duplicate onto PAP plates, and then incubated for 48 h at 30°C. There were 4 replicates for each bacterial strain used to assess the shelf-life in the packages for each of the temperature regimes.

Antagonistic activity

Antagonism of the strains retrieved from the packages after storage at various temperatures was tested by a dual plate technique. For this, plugs of mycelium were cut from the edge of an actively growing fungi on PDA and one plug was placed in the centre of a PDA plate. Two parallel 5 cm long streaks of bacteria from the samples were then made 6 cm apart on opposite sides of the fungal mycelium plug. Duplicate plates from the 4 above reps were then incubated in the dark for 2-7 days at room temperature after which time, the mycelia radius was measured and compared to that of colonies emerging from plugs not challenged by bacterial strains. Bacterial storage conditions and specific pathogen challenges are listed in Table. 1 for the four strains.

TABLE 1. Storage Conditions

Bacterial Strains	Incubation Period	Storage Temperature (“C)	Fungal Isolates
63-28	3 weeks	5	P. paracondrium
G8-32	2 weeks	22	R. solani
	1 week	30	
	24 hours	-20	
	4 days	22	
Ral-3	2 days	-20	R. solani
64-3	1 week	-20	F. solani
	1 week	18	C. destructans
	3 days	40	
	4 days	18	
	1 week	5	

Populations of the bacteria were recorded as logrithmic colony-forming units per milliliter (Log ***cfu/ml). Antagonistic*** activity with each bacterial strain against each pathogen was represented as a percent inhibition compared to control. Statistical analysis was done on StatView using the unpaired T-test at p+0.05, with significance indicated with an asterisk (*).

Results

The initial population of the bacterial strains stored at the different temperatures in the commercial packages were Log 9.4 cfu/ml for Ral-3, Log 8.3 for 64-3, Log 9.1 for 63-28 and Log 9.0 for G8-32. As shown in Figs. l-3, the shelf-life stability of all the strains maintained an acceptable level of above Log 8.0 cfu/ml irrespective of their storage at various temperature regimes. However, the population of strain 64-3, dropped significantly when stored at 40°C, but was back up when stored at 18 °C for 4 days.

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Figure 1 Shelf-life of strains Ral-3 and 64-3 after storage at different temperatures.

Figure 2 Shelf-life of strains Ral-3 and 64-3 after storage at freezing temperatures.

Figure 3 Shelf-life of strains 63-28 and G8-32 after storage at different temperatures.

Antagonistic activity of Ral-3 against ***R. solani, F. solani,*** and C. ***destructans*** after retrieval from the storage regimes was shown in Figs. 4 and 5. Strain Ral-3 significantly suppressed the radial growth of the fungi tested irrespective of its storage temperature.

Figure 4 Antagonistic activity of Ral-3 after retrieval from storage at various temperatures.

Figure 5 Antagonistic activity of Ral-3 after retrieval from storage below freezing.

The antagonistic activity of 64-3 was also significant against the three fungal pathogens (Figs. 6 and 7). However, Fig. 6 shows that at 40 °C storage it did not inhibit ***Cylindrocarpon.*** When stored at -20 °C for 1 week, the antagonistic activity of 64-3 was not not changed (Fig. 7).

Figure 6 Antagonistic activity of 64-3 after retrieval from storage at various temperatures.

Figure 7 Antagonistic activity of 64-3 after retrieval from storage below freezing.

Strains **63-28 also** significantly suppressed the growth of ***P. paracondrium*** and ***R. solani,*** irrespective of its storage regime (Fig. 8), where as, G8-32 had variable activity (Fig. 9).

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Figure 8 **Antagonistic activity of 63-28 after retrieval from different temperatures.**

Figure 9 **Antagonistic activity of G8-32 after retrieval from different temperatures.**

Conclusion

The shelf-life stability of the four strains was not altered. They were neither increased or decreased, irrespective of storage at the various temperatures in the commercial packages. However, strain 64-3's population dropped significantly when stored at 40 °C for 3 days, but its population was back up after storage at 18 "C.

Storage regimes at below freezing temperatures did not influence the populations of the bacteria evaluated.

The efficacy of bacterial strains, Ral-3 and 64-3, against *Rhizuctoniu, Fusarium* and *Cylindrocarpon was* not altered irrespective of the bacterial storage at the various temperature regimes.

The antagonistic activity of strain 63-28 against *Pythium* and *Rhizoctoniu* was not changed, after retrieval from the various temperature regimes.

Strain G8-32, significantly suppressed the growth of *Pythium* irrespective of its storage. But, no activity against *Rhizoctoniu* at all temperature regimes.

References

Lethbridge, G 1989 An industrial view of microbial inoculants for crop plants. Soc. of Gen. Micro. 1 l-28.

Vincent, Marcia N. et al., 1991 Genetic Analysis of the Antifungal Activity of a Soilborne *Pseudomonas uureofuciens Strain.* Appl. Environ. Microbiol. 57:2928-2934.

Shanahan, Phil et. al., 1992 Isolation of 2,4-Diacetylphloroglucinol from a Fluorescent Pseudomonad and Investigation of Physiological Parameters Influencing Its Production. Appl. Environ. Microbiol. 58: 353-358.

Fig. 1 Shelf-life of strains Ral-3 and 64-3 after storage at different temperatures.

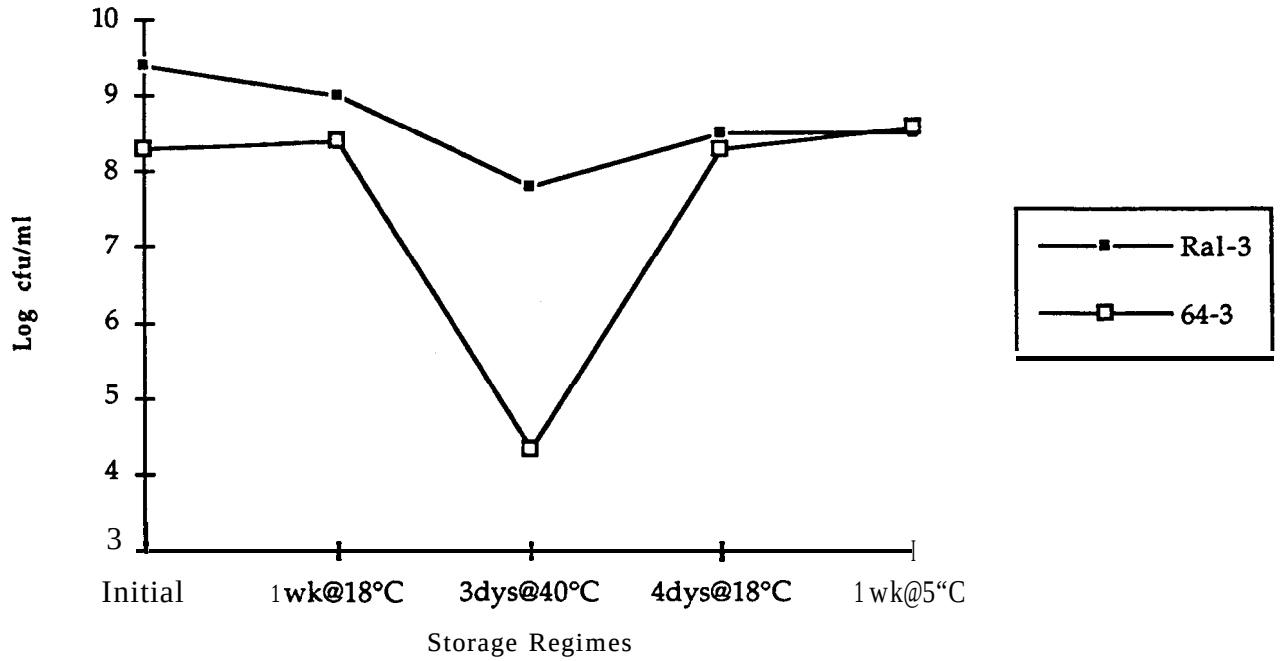

Fig. 2 Shelf-life of strains Ral-3 and 643 after storage at freezing temperatures.

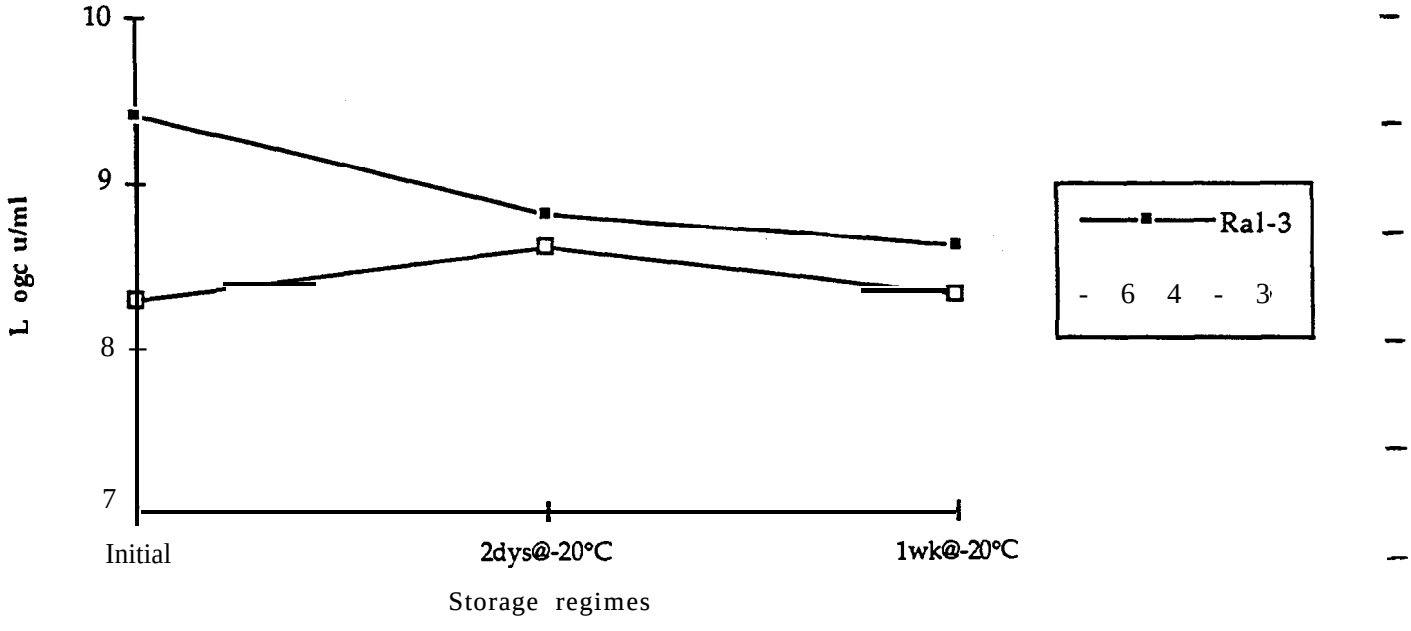

Fig. 3 Shelf-life of strains 63-28 and G8-32 after storage at different temperatures.

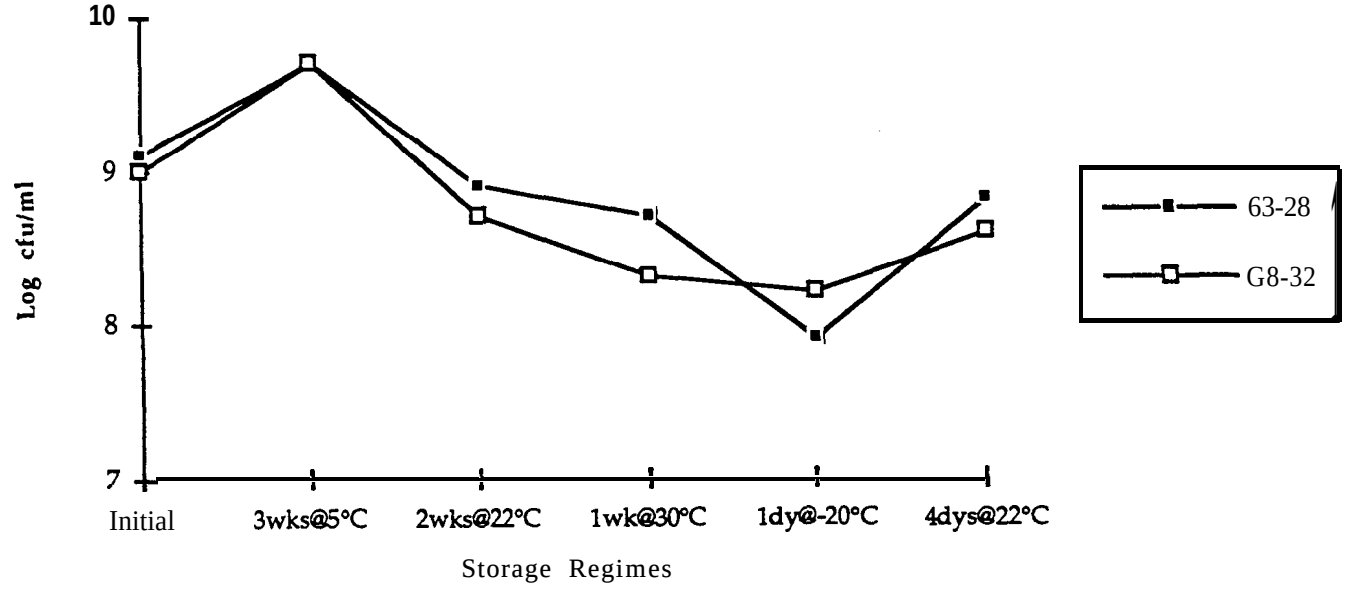

Fig. 4 Antagonistic activity of Ral-3 after retrieval from storage at various temperatures.

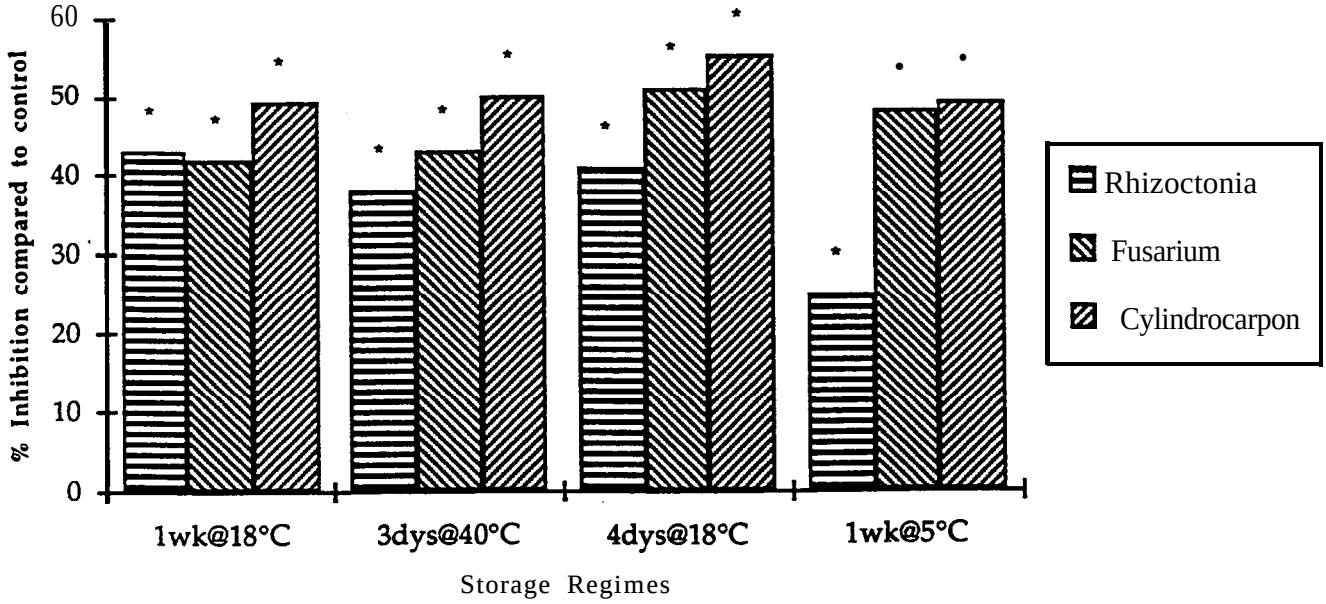

Fig. 5 Antagonistic activity of Ral-3 after retrieval from storage below freezing.

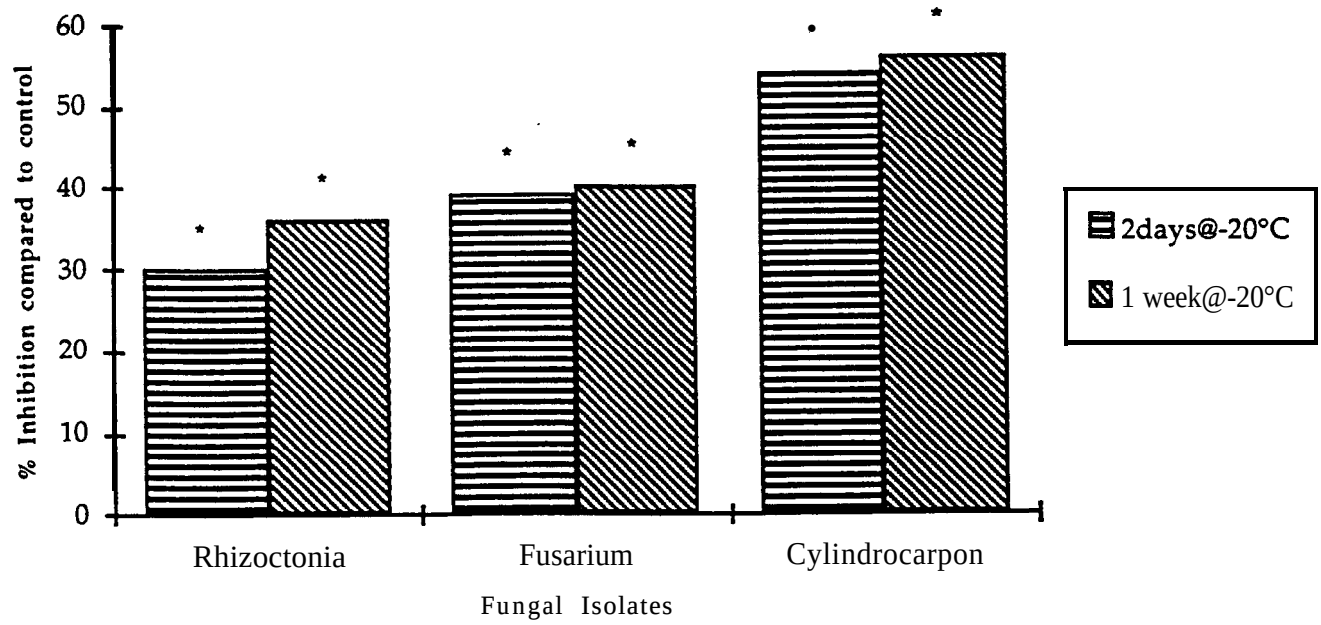

Fig. 6 Antagonistic activity of 64-3 after retrieval from storage at various temperatures.

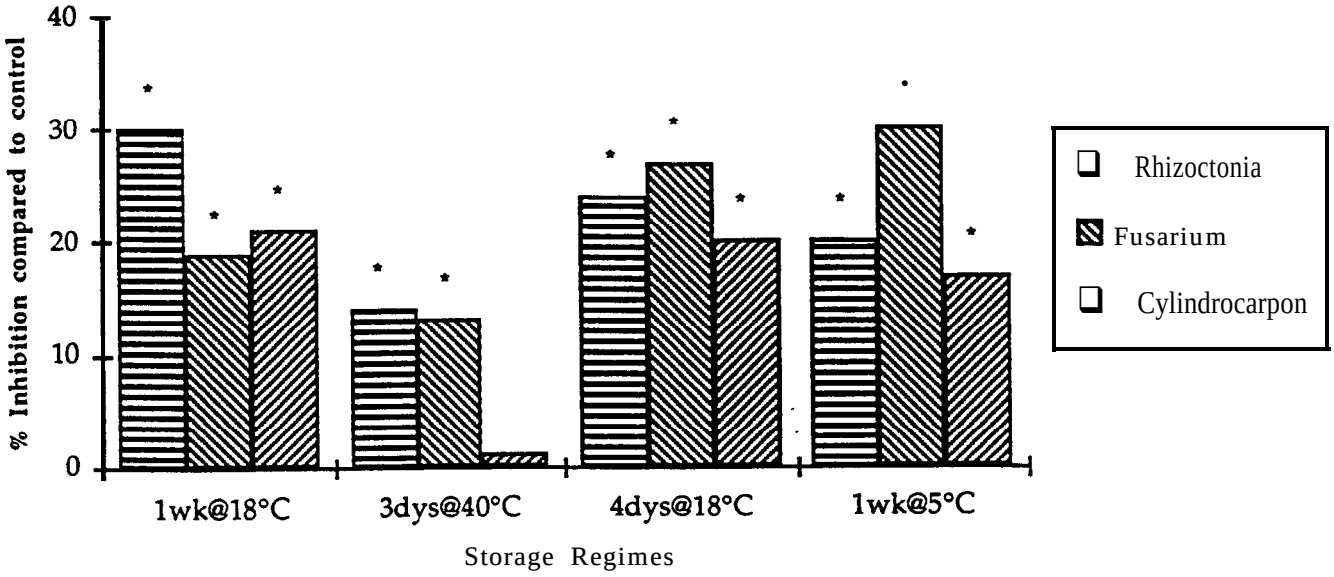

Fig. 7 Antagonistic activity of 64-3 after retrieval from storage below freezing.

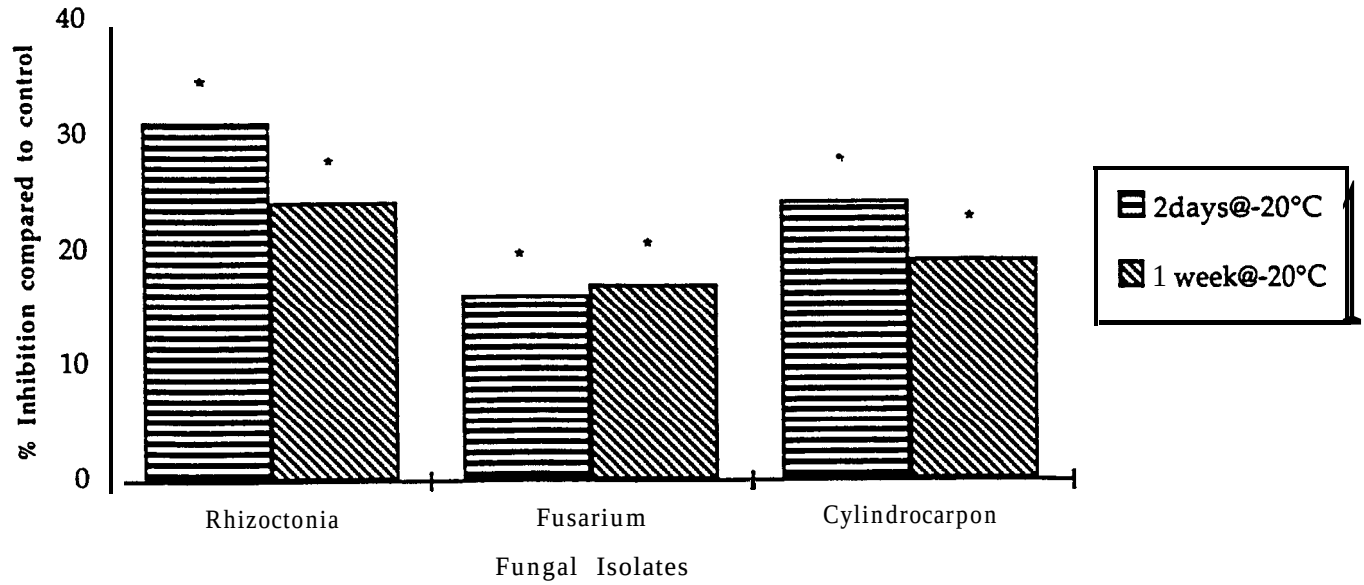

Fig. 8 Antagonistic activity of **63-28** after retrieval from different temperatures.

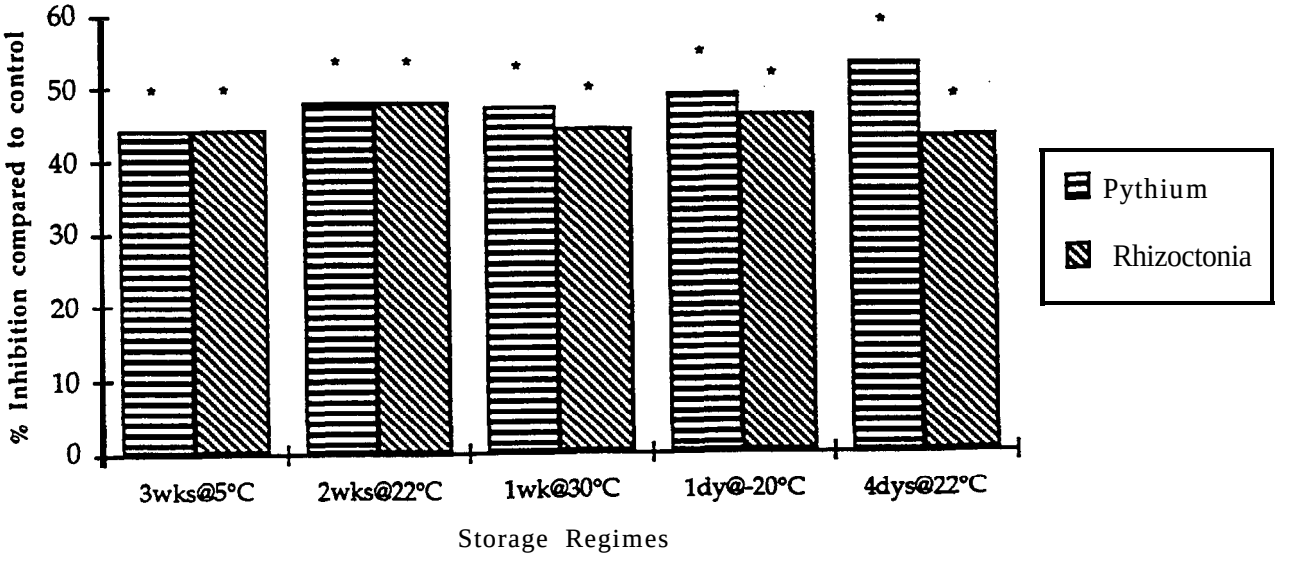

Fig. 9 Antagonistic activity of **G8-32** after retrieval from different temperatures.