



Article

Evaluation of Some Bioagents and Calcium Carbonate on Onion Black Mold Management

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Abstract: This study evaluated the field application of biological control agents (*Trichoderma harzianum* and *Chaetomium globosum*) (30×10^6 CFU/mL, applied as 1 L/100 L) and inorganic salts (calcium carbonate, at 50, 70, and 90 mM) to manage black mold infection caused by *Aspergillus niger* and preserve bulb quality during ambient storage. Field trials were conducted over two consecutive winter growing seasons (2022/2023 and 2023/2024) at the El-Sadat experimental farm, Menoufia Governorate, Egypt, on onion cultivar Giza 6. Treatments were applied as a 10-minute seedling dip prior to transplanting, followed by bi-weekly foliar applications (seven applications in total) starting mid-January. Results demonstrated that biological agents significantly reduced disease incidence and severity. *T. harzianum* achieved the highest efficacy in reducing *A. niger* infection rate (44.7% efficacy), while *C. globosum* suppressed overall disease severity most effectively (44% efficacy). Calcium carbonate treatments, particularly at 90 mM, markedly suppressed bulb decay (83.1% efficacy) and reduced total weight loss (63.8% efficacy compared to control). Furthermore, treatments enhanced biochemical defenses, significantly elevating peroxidase and polyphenol oxidase enzyme activities, as well as preserving total soluble solids (TSS) and bulb firmness throughout storage. These findings highlight the potential of combining biocontrol agents and calcium applications as an effective integrated management strategy against black mold and post-harvest onion decay.

Key words: *Allium cepa*, *Aspergillus niger*, biological control, black mold, calcium carbonate, *Chaetomium globosum* and *Trichoderma harzianum*.

1. Introduction

Onion (*Allium cepa* L.) is one of the most important commercial vegetable crops globally, ranking second among vegetable crops by total production volume. According to recent global agricultural

database statistics (FAO, 2025), global production reaches approximately 108.3 million tonnes harvested across a total area of over 5.2 million hectares, with leading producers including India, China, the United States, and Egypt. Egypt is one of the world's leading producers and exporters of dry and fresh onions, with a cultivated area 97257 ha, 3.4 million metric tons, which makes Egypt one of the top 10 onion producers worldwide and ranks it No. 1 in Africa (MALR, 2025 and GOEIC, 2025).

Prolonged storage under ambient conditions is common practice to extend market supply, but it exposes bulbs to weight loss, softening, sprouting and rot, which poses a constant threat to bulb quality, shelf life, and economic yield (Sharma and Lee, 2016). Several fungal diseases attack onion. *Aspergillus niger* van Tieghem, the causal agent of black mold disease is particularly destructive, thriving in warm ambient storage conditions and causing extensive tissue soft rot, surface black sporangial dusting, and loss of commercial value in either local consumption or exportation (Hayden *et al.*, 1994).

Synthetic fungicides have traditionally served as the primary tool for disease management; however, concerns regarding chemical residues, environmental safety, pathogen resistance, and consumer health necessitate the development of safe, sustainable alternatives (Wisniewski *et al.*, 2016). Biological control agents, such as *Trichoderma harzianum* and *Chaetomium globosum*, offer promising non-chemical alternatives by deploying mechanisms such as mycoparasitism, competitive exclusion, antibiosis, and systemic resistance induction in host tissues (Sharma *et al.*, 2025, Druzhinina *et al.*, 2011 and Harman *et al.*, 2004).

In parallel, inorganic calcium salts like calcium carbonate (CaCO₃) contribute to structural integrity and disease suppression (Bakshi *et al.*, 2005). Calcium plays an essential role in stabilizing plant cell walls and middle lamellae by cross-linking pectins, thereby rendering plant tissue less susceptible to enzymatic degradation by necrotrophic pathogens (White and Broadley, 2003 and Hammerschmidt *et al.*, 1982).

The present study was therefore designed to compare under field conditions the influence of *T. harzianum*, *C. globosum*, and varying concentrations of calcium carbonate on infection by *A. niger*, disease severity, weight loss, bulb firmness, total soluble solids, and defense-related enzyme activities in stored onion bulbs for seven-months under ambient storage.

2. Materials and Methods

Calcium Carbonate

Calcium carbonate (CaCO₃), an inorganic calcium salt of carbonic acid, was obtained from El Nasr Pharmaceutical Chemicals Co., Abu Zaabal, Egypt (ADWIC). Because CaCO₃ is insoluble in water, it was dissolved in 5% citric acid prior to application. Three test concentrations were evaluated: 50, 70 and 90 mM (Kang, 2024).

Antagonistic Microorganisms

Isolates of *Trichoderma harzianum* and *Chaetomium globosum* were kindly provided by the Biological Control Production Unit, Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Centre (ARC), Giza, Egypt. Each fungus was cultured for 14 days at 25±2 °C in liquid Potato Dextrose Agar medium. Fungal cultures were homogenized in an electric blender for 2 min, adjusted to a concentration of 30×10⁶ CFU/mL, and diluted at a 1:100 ratio prior to field application.

Field Application and Experimental Design

Field experiments were conducted at the El-Sadat experimental farm, Menoufia Governorate, Egypt, during the 2022/2023 and 2023/2024 winter growing seasons. Experiments followed a randomized complete block design with three replicate plots per treatment and control. Each experimental plot measured 10.5 m² (3.0×3.5 m), contained 5 rows (50 cm apart). Each plot was planted with 300 transplants (50 days old, cv. Giza 6). Seedlings were transplanted on December 1 Following a 10-minute root dip in the respective biocontrol suspensions or CaCO₃ solutions. Foliar sprays of the corresponding treatments were subsequently applied every two weeks for a total of seven applications,

starting on 15 January from each season. Only water was used for control in either dipping or spraying. Standard agricultural practices were uniformly maintained across all plots until harvest in May.

a) Harvest determinations

After one week of the final spray in each season, peroxidase (PO) and polyphenol oxidase (PPO) activities, as defense-related enzymes were measured in onions spectrophotometrically (Model 9100 B, LabTech), and expressed as units/mg protein.

Enzyme assays were conducted following the methodologies described by **Hammerschmidt *et al.* (1982)** for PO, and **Benjamin and Montgomery (1973)** for PPO.

b) Post-Harvest Storage and Quality Determinations

Following the standard harvesting process, onion bulbs with mechanical injury, physiological defects, insect damage, or rot symptoms were discarded to ensure uniformity. Bulbs were grouped into 5 kg samples, transferred to woven polypropylene bags, and stored under ambient room conditions for seven months prior to evaluating post-harvest parameters (**Patil *et al.*, 2024**).

1- Disease Assessment

Infection percentage, overall disease severity, and severity on infected bulbs were calculated in accordance with **Ko *et al.* (2002)**.

2- Weight Loss (%)

Onion bulbs per replicate were weighed immediately after harvest (initial weight) and at the end of the seven-month storage period (final weight) using a digital balance. Percentage weight loss was calculated relative to the initial weight (**Abdelgawad *et al.*, 2025**).

3- Decay (%)

Stored onion bulbs were visually inspected to quantify the percentage of decayed bulbs after storage (**Abdelgawad *et al.*, 2025**).

4- Firmness

Onion bulb firmness was measured using a McCormick penetrometer (Model FT-011, 0-11 lbs capacity) equipped with a 5/16-inch (7.94 mm) plunger tip. Two opposing equatorial measurements were taken per peeled bulb, and force readings were converted to pressure and expressed as grams per square inch (g/inch²) according to **Bolat *et al.* (2022)**.

5- Total Soluble Solids (TSS %)

Total soluble solids (TSS) were determined from juice extracted from fresh onion bulb tissues using a hand-held refractometer (Model PZORR) equipped with a Brix scale. Readings were recorded directly as a percentage (%TSS/Brix) at room temperature.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SAS software version 9.0. Treatment means were compared using the Least Significant Difference (LSD) test at the 5% significance level (**SAS, 2014**).

Results

Disease Infection Rate

Biological control treatments markedly reduced post-harvest infection rates by *A. niger* compared to the control as shown in table 1. Over the two seasons, *T. harzianum* consistently maintained the lowest mean infection rate (11.2%), yielding the highest overall efficacy (44.7%). *C. globosum* also reduced infection (14.9% mean infection; 26.7% efficacy). In contrast, CaCO₃ applications did not suppress overall infection rates relative to the control (20.0%).

Table (1). The influence of *T. harzianum*, *C. globosum*, and calcium carbonate application* under field conditions at El-Sadat experimental farm, Monufia Governorate, Egypt, during the 2022/2023 and 2023/2024 winter growing seasons on onion cv. Giza 6 infection by *A. niger* after storage for seven months under ambient conditions

Treatments	Conc.	Infection (%)			
		2022-23	2023-24	Mean	Efficacy (%)
<i>T. harzianum</i>	30×10 ⁶ CFU/ml (1L/100L)	10.9	11.5	11.2	44.7
<i>C. globosum</i>		15.0	14.7	14.9	26.7
CaCO₃	50 mM	22.1	25.9	24.0	-18.5
	70 mM	21.8	23.0	22.4	-10.6
	90 mM	19.5	20.7	20.1	0.7
Control	-	18.5	22.0	20	-
LSD at 5%	-	2.10	2.11	-	-

* Dipping for 10 min, followed by seven foliar sprays at two-week intervals, starting on January 15.

Disease Severity

Both biocontrol agents significantly lowered overall disease severity compared to the untreated control (1.25%). *C. globosum* exhibited the greatest reduction in disease severity (0.7% mean severity; 44% efficacy), closely followed by *T. harzianum* (0.8% mean severity; 36% efficacy). CaCO₃ treatments provided slight reductions in disease severity (4% to 8% efficacy). When evaluating severity specifically on infected bulbs, *C. globosum* reduced severity to 4.7% (18.97% efficacy), whereas CaCO₃ at 50 mM achieved 4.95% severity (14.66% efficacy).

Table (2). The influence of *T. harzianum*, *C. globosum* and calcium carbonate application* under field conditions at El-Sadat Experimental Farm, Monufia Governorate, Egypt, during the 2022/2023 and 2023/2024 winter growing seasons on disease severity on onion cv. Giza 6 infected by *A. niger* after storage for seven months under ambient conditions

Treatments	Conc.	Overall Severity (%)				Severity on infected bulbs (%)			
		2022-23	2023-24	Mean	Efficacy (%)	2022-23	2023-24	Mean	Efficacy (%)
<i>T. harzianum</i>	30×10 ⁶ CFU/ml (1L/100L)	0.8	0.8	0.80	36.00	4.2	7.4	5.80	0.00
<i>C. globosum</i>		0.7	0.7	0.70	44.00	5.0	4.4	4.70	18.97
CaCO₃	50 mM	1.0	1.4	1.20	4.00	4.3	5.6	4.95	14.66
	70 mM	0.9	1.4	1.15	8.00	4.7	7.6	6.15	-6.03
	90 mM	1.0	1.4	1.20	4.00	5.1	6.5	5.80	0.00
Control	-	1.2	1.3	1.25	-	5.6	6.0	5.80	-
LSD at 5%	-	0.05	0.06	-	-	0.22	0.42	-	-

* As in Table 1.

Weight Loss

All tested treatments dramatically reduced bulb weight loss after seven months of ambient storage compared to the control group, which lost 33.2% of initial mass. High-concentration calcium carbonate (90 mM) yielded the lowest overall weight loss (12.0% mean; 63.8% efficacy). *T. harzianum*, *C. globosum*, and CaCO₃ at 70 mM all demonstrated equivalent protection (12.5% mean weight loss; 62.3% efficacy).

Table (3). The influence of *Trichoderma harzianum*, *Chaetomium globosum* and calcium carbonate application* under field conditions at El-Sadat Experimental Farm, Monufia Governorate, Egypt, during the 2022/2023 and 2023/2024 winter growing seasons on weight loss of onion cv. Giza 6 bulbs after storage for seven months under ambient conditions

Treatments	Conc.	Weight loss (%)			
		2022-23	2023-24	Mean	Efficacy (%)
<i>T. harzianum</i>	30×10 ⁶ CFU/ml (1L/100L)	12.0	13.0	12.5	62.3
<i>C. globosum</i>		12.0	13.0	12.5	62.3
CaCO ₃	50 mM	13.0	13.0	13.0	60.8
	70 mM	12.0	13.0	12.5	62.3
	90 mM	12.0	12.0	12.0	63.8
Control	-	31.3	35.0	33.2	0.0
LSD at 5%	-	0.66	0.68	-	-

* As in Table 1.

Post-Harvest Bulb Decay

Visual examination following ambient storage revealed substantial suppression of bulb decay across all treatments relative to the control (29.8% decay). CaCO₃ at 90 mM was the most effective treatment, limiting decay to 11.1% (83.1% efficacy). Also, CaCO₃ at 70 mM and *C. globosum* also suppressed decay effectively, achieving 71.9% and 69.1% efficacy, respectively.

Bulb Firmness (Hardness)

Biocontrol agents and calcium applications effectively preserved bulb firmness after seven months of storage relative to untreated controls (7.1 g/inch²). *C. globosum* maintained the highest tissue firmness (8.3 g/inch², 16.9% efficacy), followed by *T. harzianum* (8.1 g/inch², 14.1% efficacy) and 50 mM CaCO₃ (8.0 g/inch², 12.7% efficacy).

Table (4). The influence of *Trichoderma harzianum*, *Chaetomium globosum* and calcium carbonate application* under field conditions at El-Sadat Experimental Farm, Monufia Governorate, Egypt, during the 2022/2023 and 2023/2024 winter growing seasons on decay of onion cv. Giza 6 bulbs after storage for seven months under ambient conditions

Treatments	Conc.	Decay (%)			
		2022-23	2023-24	Mean	Efficacy (%)
<i>T. harzianum</i>	30×10 ⁶ CFU/ml (1L/100L)	29.6	30.0	29.8	54.7
<i>C. globosum</i>		18.52	22.22	20.4	69.1
CaCO ₃	50 mM	14.81	33.33	24.1	63.4
	70 mM	22.22	14.81	18.5	71.9
	90 mM	11.5	10.7	11.1	83.1
Control	-	65.0	66.7	29.8	-
LSD	-	2.41	2.48	-	-

* As in Table 1.

Table (5). The influence of *Trichoderma harzianum*, *Chaetomium globosum* and calcium carbonate application* under field conditions at El-Sadat Experimental Farm, Monufia Governorate, Egypt, during the 2022/2023 and 2023/2024 winter growing seasons on firmness of onion cv. Giza 6 bulbs after storage for seven months under ambient conditions

Treatments	Conc.	firmness (g/inch ²)			
		2022-23	2023-24	Mean	Efficacy (%)
<i>T. harzianum</i>	30×10 ⁶ CFU/ml (1L/100L)	7.4	8.8	8.1	14.1
<i>C. globosum</i>		8.2	8.4	8.3	16.9
CaCO ₃	50 mM	7.3	8.7	8.0	12.7
	70 mM	7.0	8.9	7.9	12.0
	90 mM	7.9	7.4	7.7	7.7
Control	-	7.1	7.1	7.1	0.0
LSD at 5%	-	0.11	0.12	-	-

* As in Table 1.

Total Soluble Solids (TSS) and Defense Enzymes

As presented in Table 6, the application of *Trichoderma harzianum*, *Chaetomium globosum*, and calcium carbonate (CaCO₃) differentially affected total soluble solids (TSS) and the activities of the defense-related enzymes peroxidase (POD) and polyphenol oxidase (PPO) across the two successive seasons. Application of biological agents elevated host defense enzyme activities and preserved TSS during storage. *C. globosum* induced the highest peroxidase (POD) activity (7.0 and 6.0 units/mg protein in) and polyphenol oxidase (PPO) activity (3.1 and 3.2 units/mg protein) in the two successive seasons respectively. High TSS values were maintained in *T. harzianum* (13.5 and 13.1%) and *C. globosum* (12.5 and 12.0%) compared to the control group (11.3 and 11.2%).

Overall, the results presented in Table 6 demonstrate complementary treatment effects: *T. harzianum* was the most effective treatment for maintaining TSS, whereas *C. globosum* produced the highest POD activity and comparatively high PPO activity in both seasons. These findings support the potential use of biological agents as components of integrated postharvest disease management strategies. Nevertheless, the overall effectiveness of these treatments should be assessed in conjunction with disease incidence, disease severity, weight loss, firmness, and other fruit-quality parameters.

Table (6). The influence of *Trichoderma harzianum*, *Chaetomium globosum*, and calcium carbonate application* under field conditions at El-Sadat Experimental Farm, Monufia Governorate, Egypt, during the 2022/2023 and 2023/2024 winter growing seasons on total soluble solids and defense-related enzyme activities in onion cv. Giza 6**

Treatments	Conc.	TSS (%)		Enzymatic activity (unit/mg protein)			
		2022-23	2023-24	Peroxidase (PO)		Polyphenoloxidase (PPO)	
				2022-23	2023-24	2022-23	2023-24
<i>T. harzianum</i>	30×10 ⁶ CFU/ml (1L/100L)	13.5	13.1	6.0	5.3	2.9	2.7
<i>C. globosum</i>		12.5	12.0	7.0	6.0	3.1	3.2
CaCO ₃	50 mM	11.5	11.3	6.3	5.3	2.6	2.3
	70 mM	12.3	11.9	6.2	5.9	2.7	2.5
	90 mM	12.3	12.5	6.3	6.0	2.9	2.7
Control	--	11.3	11.2	5.4	4.7	2.7	2.67
LSD at 5%	--	0.73	0.95	0.78	0.53	0.55	0.45

* As in Table 1.

** PO and PPO were determined one week from last spray, while TSS at the end of experiment.

4. Discussion

This study underscores complementary strategies for managing post-harvest onion decay caused by *A. niger*. Biological agents demonstrated superior performance in suppressing pathogen establishment and disease expansion, while calcium applications excelled at maintaining physiological tissue integrity and reducing post-harvest storage decay, and both converge on reduced weight loss and enzymes (Amin *et al.*, 2016, Bakshi, *et al.*, 2005, Harman *et al.*, 2004 and White and Broadley, 2003).

The high disease suppression exhibited by *T. harzianum* (44.7% efficacy against infection) and *C. globosum* (44% efficacy against overall severity) aligns with their known modes of action (Sharm *et al.*, 2025, Druzhinina *et al.*, 2011 and Harman *et al.*, 2004). *Trichoderma* spp. act via rapid hyphal growth, competition for spatial and nutritional niches, and production of lytic enzymes (e.g., chitinases, beta-1,3-glucanases) that digest fungal cell walls (Druzhinina *et al.*, 2011 and Harman *et al.*, 2004). *C. globosum* produces bioactive secondary metabolites, such as chaetocin and globosin that restrict lesion expansion and inhibit spore germination and hyphal development of necrotrophic pathogens (Sharm *et al.*, 2025).

Furthermore, both bio-agents triggered systemic defense responses in onion plants, as evidenced by significantly heightened POD and PPO activities. Peroxidase is instrumental in cell wall lignification and cross-linking, establishing physical barriers against hyphal penetration, whereas polyphenol oxidase catalyzes the oxidation of phenolics into antimicrobial quinones (Hammerschmidt, *et al.*, 1982 and Benjamin and Montgomery, 1973).

On the other hand, calcium carbonate played a vital physical and structural role (Bakshi *et al.*, 2005). Calcium ions bind to the galacturonic acid residues of cell wall pectins to form calcium pectate complexes, reinforcing structural rigidity and conferring resistance against pectolytic enzymes secreted by *A. niger* (Romanazzi *et al.*, 2016 and White and Broadley, 2003). Consequently, calcium treatments, especially at 90 mM, reduced post-harvest weight loss to 12% (compared to 33.2% in controls) and restricted bulb decay to 11.1% (83.1% efficacy) (Bakshi, *et al.*, 2005 and White and Broadley, 2003).

Higher TSS is commercially desirable for pungency and processing, and here it co-occurred with lower loss, dispelling any trade-off between quality and storability in this dataset. (Sharm *et al.*, 2025).

Preserving TSS and firmness during extended storage is essential for maintaining retail quality and economic value (Chope *et al.*, 2013). Treatments that reduced respiratory losses and fungal degradation retained higher carbohydrate concentrations, resulting in elevated TSS values in bio-agent treatments (up to 13.5%) relative to untreated controls. Integrating pre-harvest applications of biological agents (*T. harzianum* or *C. globosum*) alongside calcium salt sprays offers a balanced approach: bio-agents suppress primary pathogen colonization and prime host metabolic defenses, while calcium fortifies cellular infrastructure to minimize physiological weight loss and post-harvest decay during prolonged ambient storage, so future work should test the combination between calcium with bioagents to capture both spectra. (White and Broadley, 2003 and Conway *et al.*, 1994).

5. Conclusion

Over two consecutive winter growing seasons under field conditions, preharvest application of *Trichoderma harzianum* and *Chaetomium globosum* significantly reduced onion black mold infection and severity, meanwhile calcium carbonate-especially 90 mM reduced postharvest decay. All treatments halved weight loss, increased firmness and TSS, and elevated defense-related POD and PPO. Integrating bioagents with calcium nutrition offers a sustainable alternative to synthetic fungicides for long ambient storage of onion cv. Giza 6 in Egypt.

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