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Antimicrobial Activity of *Coriandrum sativum* L. and Its Effect on Microbiological Properties of Yoghurt

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ABSTRACT

In this study, the antimicrobial effect of *Coriandrum sativum* L. leaves against 30 different foodborne microorganisms, including 20 bacteria, 7 fungi, and 3 yeast species was tested, and some microbiological properties of yoghurt produced with lyophilized *C. sativum* L. were investigated. Yoghurts were prepared at three different concentrations of *C. sativum* L. powder (0.1, 0.2, and 0.3% w/w) and stored at 4°C for 21 days. Ethanol extract of the fresh plant showed antimicrobial activity against only 9 bacteria and 1 yeast with different inhibition zone diameter changing from 8 to 12 mm, but had no effect on moulds used in this study. The addition of *C. sativum* L. had no statistical significance on the growth of yoghurt bacteria compared with the control sample and they remained over 8 log CFU g^{-1} in all yoghurts throught the storage period. On the other hand, an increase was observed in the number of *Lactobacillus bulgaricus* and a decrease in the number of *Streptococcus thermophilus* depending on the concentration of *C. sativum* L., but this was not statistically significant (P> 0.05). However, significant differences in yeast and mould counts were observed (P< 0.05). Yeasts and moulds were not detected in any of the samples containing *C. sativum* L. powder, whereas the counts of yeast and mould increased in control throught the storage period. These results show that the powder of *C. sativum* L. leaves can be used an agent for preventing the growth of yeast and mould, which are shelf-life and quality spoilage microorganisms of yoghurt.

Keywords: Foodborne microorganisms, **S**helf-life, Spoilage microorganisms, Yoghurt bacteria.

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INTRODUCTION

Functional foods are produced to be consumed as a part of the diet and they contain biologically active components that reduce the risk of degenerative diseases. Yogurt is one of these foods due to the high digestibility and bioavailability of its protein, calcium, potassium and vitamin B (El-Fattah *et al*., 2018). Yoghurt, the most known and consumed fermented dairy product worldwide, contributes to health due to nutritional profile and probiotic properties (Tamime and Robinson, 1999; Michael *et al*., 2010; Rutella *et al*., 2016). The popularity of yoghurt is not only related to its health benefits but also as a suitable food for preparation with different taste and needs (Perna *et al*., 2014). For example, with respect to preparation of natural and healty foods, yoghurt is commanly used as a an important carrier for food components such as fibers, fatty acids, and antioxidants, which are useful for improving physiological properties of the body (Pop *et al*., 2015). For this reason, there has been an increasing

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interest in the use of natural food additives that have antioxidant, antimicrobial, antifungal or anti-yeast properties and incorporation of health-promoting substances into the diet (Varga, 2006). For instance, spices and herbs are commonly used for seasoning and increasing shelf life of food and restoring of health (Bhat *et al*., 2014). Also, they are a good source for inhibiting bacteria, yeasts, and moulds (Smid and Gorris, 1999).

Coriander has been used for nutrition, medicine, beverages, flavorings, repellents, coloring, cosmetics, fragrances, smoking, charms and industrial uses as well (Nadeem *et al*., 2013). Leaves of *C. sativum* L. (cilantro) are one of the most popularly used plants for culinary and medicinal purposes (Sarimeseli, 2011; Yildiz, 2015). The leaves and fruits are highly fragrant and contain nutrients like fat, proteins, vitamins minerals etc. Its health benefits activities range from antibacterial to anticancer activities. Due to its multifunctional uses and protective and preventive action against various chronic diseases, this herb is righty called as “herb of happiness” (Bhat *et al*., 2014; Yildiz 2015). All parts of this herb are in use as flavoring agent and/or as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations (Sahib *et al*., 2013). Previous studies have reported that *C. sativum* has antimicrobial, antioxidant, anti-inflammatory, hypoglycemic, and anti-carcinogenic activities (Laribi *et al*., 2015; Yildiz, 2016).

The use of foods that promote a state of well-being, better health, and reduction in the risk of diseases has become popular as consumers become more health conscious. In the last few years, this tendency has driven the manufacturers to develop and produce a wide variety of these products with different characteristics (Romano *et al*., 2014). On the other hand, the food industry has a difficulty to produce these products without affecting their consumer acceptance. The development of health benefits of yogurt can be increased, depending on the addition of various materials such as herb, fruit, vegetable, seed, and spice. Although there have been many studies on yoghurt fortified with fruits, little work has been done on herby yoghurt. Based on our knowledge, there is no study reported for *Coriandrum sativum* L. in the production of yoghurt.

Therefore, this study aimed to investigate the antimicrobial properties of *C. sativum* L. on 30 selected food-borne microorganism and assessing of microbiological properties of yoghurt fortified with 0.1, 0.2, and 0.3% of *C. sativum* L during the period of 21 days storage.

MATERIALS AND METHODS

Plant and Milk Material

Fresh *C. sativum* L. leaves were purchased in autumn from local producers in Erzurum Province, which is located at the east of Turkey, and brought into the laboratory as soon as possible. After cleaning and sorting, they were washed, cut, and dried via lyophilisator. Later, they were grinded and kept at 4±1°C in refrigerator until used. It was used at three concentrations (0.1, 0.2, and 0.3%). For the yoghurt production, ultra-high temperature-treated milk (Dost; AkGıda Co., Sakarya, Turkey) was purchased from a local market in Gumushane, and skim milk powder was obtained from Pinar Dairy Co. (Izmir, Turkey).

Microorganisms

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* (DVS YC350) were used for yoghurt production and purchased from Chr. Hansen, Istanbul, Turkey. Thirty test strains that are food-associated used for antimicrobial tests were provided by the Food Microbiology Laboratory, the Department of Food Engineering, Faculty of Agriculture at

Ataturk University, Erzurum, Turkey. The identification of the microorganisms used in this study was confirmed by the Microbial Identification System (SherlockMicrobial Identification System version 4.0, MIDI Inc., Newark, DE, USA), API (BioMerieux, Craponne, France), BIOLOG (MicroStation™ IDSystem,Biolog Inc., Hayward, CA, USA), and classical identification test (Bergey's Manual of Determinative Bacteriology). The list of microorganisms used is given in Table 1. The bacteria strains were incubated for 24 hours at 37°C on Nutrient Agar (NA), whereas fungus and yeast strains were on Potato Dextrose Agar (PDA) medium (Merck) at 25°C.

Extraction of Plant Material

Twenty-five g of the powdered plant leaves were soaked in 75 mL of 90% for 24 hours. This mixture was passed through Whatman filter paper no. 1 (Whatman, England). The extract was obtained by concentration of mixture in vacuo by using rotary evaporator at 30°C. A quantity of 25 g of the grinded leaves that was kept in refrigerator was weighted, and 75 mL of 90% alcohol was added and extracted at 150 rpm and 20°C for 24 hours. After filtered, extracts were concentrated at 40°C, 175 mbar and 200 rpm in a rotary evaporator (Buchi R 210) for removing of alcohol.

Table 1. Antimicrobial activities of *Coriandrum sativum* L.

Microorganisms	Inhibition zone (mm)
Bacteria	
Acinetobacter lwoffi BC 2819	-
Alcaligenes piechaudii BC 0236	10
Bacillus cereus BC 6830	-
Bacillus subtilis ATCC 6633	-
Enterococcus faecalis ATCC 29122	10
Escherichia coli 1402	10
Escherichia coli O157:H7	-
Flavobacterium indologenes BC 1520	8
Klebsiella pneumoniae BC 34	10
Klebsiella pneumoniae ozaenae BC 30	-
Klebsiella pneumoniae ozaenae BC 32	-
Listeria monocytogenes BC 8353	-
Proteus mirabilis BC 2644	11
Pseudomonas aeruginosa BC 4372	-
Pseudomonas fluorescens BC 7324	-
Pseudomonas pseudoalcaligenes BC 3445	9
Salmonella Typhimurium RSSK 95091	-
Staphylococcus aureus ATCC 29213	-
Streptococcus pyogenes ATCC 176	12
Yersinia enterocolitica	10
Yeasts	
Candida albicans ATCC 1223	9
Candida albicans ATCC 90029	-
Candida crusei ATCC 14243	-
Molds	
Aspergillus niger BC 102	-
Cladosporium herbarum BC 106	
Paecilomyces variotii 108	-
Penicillium brevicompactum BC 109	-
Penicillium roquefortii BC 111	-
Penicillium roquefortii BC 113	-
Trichothecium roseum BC 116	-

Later, concentrated extracts were transfered to the Eppendorf tube containing 300 µg extract for every disc, and 10% of DMSO was added.

Antimicrobial Activity Tests

The antimicrobial activity test of the extracts was carried out with the disc diffusion method. For this purpose, 100 µL of suspension containing 10^8 CFU mL^{-1} of bacteria was diffused on Nutrient Agar (NA) medium. Sterile filter paper discs (6 mm) were soaked with 300 µg sterile test material and placed onto nutrient agar. Negative control was prepared with the same solvent used for dissolving the plant extracts. After inoculation, plates were incubated at 27°C for 24 hours. The antibacterial activity was expressed as a diameter of clear zone, which means growth inhibition of microorganism. Five discs were used for each plate and plates were triplicate. (Djipa *et al*., 2000).

Production of Yoghurts

UHT cows' milk contents of protein, fat, total solid, pH and titratable acidity were 2.87%, 3%, 11.32%, 6.55, and 0.20%, respectively. Skim milk powder was added at a rate of 20 g L^{-1} for enhancing of dry matter. Then, milk was heated at 85°C for 20 minutes and cooled down to 44 ±1°C immediately. Starter culture containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* was added and mixed. It was separated into four parts and incubated at 45°C until pH reached 4.6. After fermentation, they were immediately cooled 4±1°C for 24 hours. The third part of yoghurt samples was supplemented with 0.1, 0.2, and 0.3% *Coriandrum sativum* L. Powder, respectively, and stirred well to get a homogenous structure. The fourth one was accepted as a control without adding *C. sativum*. All yoghurt samples were divided into approximately sterilized 250 mL of glass and kept at 4±1°C for 21 days and analyzed at 1, 7, 14, and 21 days of storage for microbiological properties. The yoghurts were manufactured in duplicate.

Enumeration of Bacteria Used for Yoghurt Production

L. delbrueckii ssp. *bulgaricus* was enumerated on M-17 agar (Oxoid) and incubated anaerobically at 45°C for 72 hours. For the enumeration of *S. thermophilus*, M-17 agar (Oxoid) and aerobic incubation at 37°C for 24 hours were used (Dave and Shah, 1996).

Enumeration of Yeast and Mould

For enumeration of yeast and mould, Potato Dextrose Agar (PDA) was used and plates were incubated at 25±1ºC for 5 days (Harrigan, 1998).

Statistical Analysis

The experiment was based on randomized design with two replications. Data were subjected to the Analysis Of Variance (ANOVA) and means were evaluated by Duncan multiple range test at P< 0.01 significant level.

RESULTS AND DISCUSSION

Antimicrobial Activity of *Coriandrum Sativum* L.

The diameters of the inhibition zones obtained with the leaf extracts of *C. sativum* L. against twenty bacteria, three yeasts, and seven molds are presented in Table 1.

As seen in Table 1, screening of the antimicrobial activity of *C. sativum* L. leaf extract revealed activity against 9 bacteria and 1 yeast. On the other hand, *C. sativum* L. leaf did not inhibit the growth of any of fungi used in the study. While *Streptococcus* *pyogenes* (12 mm) showed the widest spectrum, *Flavobacterium indologenes* (8 mm) was the narrowest. In the literature, there are different results related to antimicrobial activity of *Coriandrum sativum* L. For example, Chaudhry and Tariq (2006) reported that coriander had no antimicrobial activity against *Escherichia coli* and *Alcaligenes* spp., but in our study it showed 10 mm zone inhibition against both microorganism. On the other hand, in the same study, the results which coriander did not show antimicrobial effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus* paralleled our study. Another study indicated that the coriander oil kills both Gram-positive and Gram-negative bacteria by disrupting membrane function (Silva *et al*., 2011a). Kim *et al*. (2001) have shown that aqueous and ethanol extracts of fresh *Coriander sativum* leaf are effective for inhibiting growth of *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Salmonella* Typhimurium, *Lactobacillus. plantarum*, *Leuconostoc mesenteroides,* and *Pseudomonas fluorescens* (Wong and Kitts, 2006). It could be concluded that these different results can be because of plant origin, different extraction methods, fresh plant or seeds, and oils compositions. Ateş and Ertuğrul (2003) tested the antimicrobial activity of *Coriandrum sativum L.* seeds against *Bacillus brevis*, *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Bacillus subtilis* var. *niger, Enterococcus faecalis, Klebsiella pneumoniae, Listeria monocytogenes, Micrococcus luteus, Mycobacterium smegmatis, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Yersinia enterocolitica* and showed no inhibition zone. Yildiz (2016) reported that the essential oil of *C. sativum* showed significant antimicrobial activities against many microbial strains, but the ethanol extract of the plant showed weaker antimicrobial activities against microbial strains.

On the other hand, lyophilized fresh *Coriandrum sativum* L. powder exhibited 9 mm zone against *Candida albicans* ATCC 1223 from the three *Candida* species tested in this study. A study revealed that essential oil from *Coriandrum sativum* L. exhibited antimicrobial activity against five *Candida* species except *Candida tropicalis* CBS 94 and it was said that the EO and its fractions could be used as potential antimicrobial agents to treat or prevent *Candida* yeast infections (Begnami *et al*., 2010). The anti-yeast properties of coriander essential oil have been reported on pathogenic species such as *Candida albicans* in previous studies (Silva *et al*. 2011b; Yildiz, 2016). *Candida* species can adopt a pathogenic attitude and cause disease (candidemia), ranging from superficial mucosal to hazardous systemic infections (Banjara *et al*., 2016).

A study by Minija and Thoppil (2001) revealed that *Coriandrum sativum* L. essential oil had no effect against *Aspergillus niger*. This correlated with our study, although we used the fresh plant leaves. In another study, the antimicrobial activities of 12 plant species containing *C. sativum* were tested *in vitro* against 2 fungi and 8 bacterial species and it was found that coriander showed the least antibacterial effect among the tested plants. Authors emphasized the difference antimicrobial effects of different plant could be due to the phytochemical differences between species and collection site, the cell wall structure, species, and subspecies (Keskin and Toroglu, 2011).

Microbial Properties of Yoghurt Samples

As seen in Table 2, while yeast and mould were not detected in samples containing *Coriandrum sativum* L. at any storage day, it was detected in the control sample throughout the storage, except the first day.

The most common yeasts isolated from plain and fruity yoghurts were Saccharomyces cerevisiae, Debaryomyces hansenii, Saccharomyces exiguus, Kluyveromyces marxianus, Yarrowia lipolytica, and Rhodotorula glutinis, in a

Table 2. Effect of treatments and storage periods on some microbiological properties of yoghurt samples (log CFU g^{-1}).

Variants	Storage days	Yoghurt samples [a]			
		A0	A1	A2	A3
S. thermophilus	1	8.84±0.12 [aA]	8.66±0.44 [aA]	8.53±0.40 [aA]	8.48±0.10 [bcA]
	7	8.79±0.10 [aB]	8.43±0.35 [aAB]	8.28±0.27 [aA]	8.13±0.10 [aA]
	14	8.71±0.11 [aC]	8.56±0.07 [aB]	8.33±0.05 [aA]	8.77±0.04 [cC]
	21	8.58±0.40 [aA]	8.52±0.26 [aA]	8.32±0.30 [aA]	8.31±0.34 [abA]
L. bulgaricus	1	8.66±0.08 [aA]	8.36±0.33 [abA]	8.38±0.08 [abA]	8.46±0.18 [abA]
	7	8.51±0.09 [aA]	8.58±0.07 [bA]	8.64±0.21 [bA]	8.67±0.11 [cA]
	14	8.34±0.22 [aA]	8.42±0.18 [abA]	8.45±0.38 [abA]	8.49±0.07 [bcA]
	21	8.32±0.38 [aA]	8.18±0.19 [aA]	8.24±0.13 [aA]	8.28±0.07 [aA]
Yeast-mould	1	-	-	-	-
	7	2.16±0.16a	-	-	-
	14	3.6±0.57b	-	-	-
	21	4.94±0.40c	-	-	-

[a] C: Control (no additives); A1, A2, A3 yoghurts with respectively 0.1, 0.2, and 0.3% of Coriandrum sativum L. [A-C] Means within a row with no common superscript differ (P< 0.05); [a–c] Means within each column of each category followed by the different letters are significantly differ (P< 0.05).

study conducted by Viljoen et al. (2003). The same researchers noted that the lower pH levels of the yoghurts supported the growth of yeasts and could inhibit the growth of undesired bacterial species. As yeasts can cause spoilage and affect desirable biochemical properties, they are important in dairy products.

Vahedi *et al*. (2008) reported that there was no growth of mold and yeast in yoghurts with added 10% apple during 28 days of storage, but yeast growth was seen in samples containing 13% strawberry up to the 7th day of storage and disappearing completely after 14 days. At the same time, the pH value of strawberry yoghurt increased after 14 days.

In a study by Tarakci and Kucukoner (2003), the number of yeast and mold in yoghurt samples prepared with red pepper, rosehip, cherry marmalade, grape molasses, palm pulp, yoghurt without fruit (control group) was between 2.10-2.89 CFU g^{-1}.

In a study, yoghurt samples prepared with red pepper, rosehip, cherry marmalade, grape molasses, palm pulp and yoghurt without fruit (control group) were stored at 5°C for 10 days and, the number of yeast and mold in yoghurt samples was 2.10-2.89 CFU g^{-1} (Tarakci and Kucukoner, 2003). In our study, an increase was observed in the number of yeast and mold (2.16-4.94 log CFU g^{-1}) in the control group during storage, but it was not determined in the yoghurt samples with *Coriandrum sativum* L. (Table 2). Taking into consideration the literature data, it can be said that the fruit and plant added to yoghurts were effective on the growth of yeast and mold. Therefore, *Coriandrum sativum* L. addition to yoghurt is thought to prevent the growth of yeast and mold.

Table 2 shows the number of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* in yoghurt samples during storage period of 21 days. There was no significant difference between the control and samples containing *Coriandrum sativum* L. as of these two bacteria.

The number of *L. bulgaricus* (8.66 log CFU g^{-1}) and *S. thermophilus* (8.84 log CFU g^{-1}) were the highest in the control sample on the first day of storage but they decreased throughout the storage. Addition of *Coriandrum sativum* L. to the yoghurts

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decreased the number of these lactic acid bacteria when compared to the control sample, but they were not different statistically. At the same time, in yoghurt samples containing *C. sativum* L. powder, as the ratio of *C. sativum* L. increased, the number *S. thermophilus* decreased but the number of *L. bulgaricus* increased, except the first day of storage. However, these changes did not have statistical significance. The number of *L. bulgaricus* ve *S. thermophilus* was suitable to the Turkish standards at the end of 21 days of storage (Table 2). It has been reported that the addition of carrot juice has no effect on the number of *L. bulgaricus* and *S. thermophilus* in yoghurt samples (Salwa *et al*., 2004). Varga (2006) reported that the addition of 1.0 to 5.0% of acacia honey did not significantly affect the survival of the characteristic microorganisms (*L. bulgaricus* ve S. *thermophilus*) in the yoghurt at 4°C during 6 weeks of storage. It was stated that the addition of green, white, and black tea extracts did not lead to a significant change in the mean live cell count of *L. bulgaricus* ve S. *thermophilus* in yoghurt samples after 21 days of storage (Muniandy *et al*., 2015). These studies are similar to our study that revealed that the addition of *Coriandrum sativum* L. was not effective on the characteristic microorganisms of yoghurt. In a study, the effect of a plant extract prepared from olive, garlic, onion, and citrus on the viability of yoghurt starters was investigated and a significant decrease in the number of *L. bulgaricus* was observed with storage at 5°C for 50 days, while the number of *S. thermophilus* was > 6 log CFU mL^{-1} for all yoghurt samples (Michael *et al*., 2010). In our study, the number of *L. bulgaricus* and *S. thermophilus* was > 10^7 CFU g^{-1}during storage. Jimborean *et al*. (2016) developed a new type of yoghurt by the addition of essential oils from *Citrus sinensis* and found that the quality parameters of the new product and the viability of the lactic acid bacteria did not change throughout the storage period. Another study results indicated that rich additives such as polyphenolic compounds, fibers, and vitamins could be potentially used as a source for the yoghurt enrichment (Pop *et al.,* 2015).

CONCLUSIONS

In recent years, consumers demand new food products containing natural components and providing health benefits. Fermented dairy products like yoghurt are frequently consumed because of their high nutritional value and sensorial properties, and yoghurt is one of primary, convenient, and preferable foods for the development of a new food and enrichment with the natural sources. Any part of plants such as fresh leaves, seed, or its extracts are of great interest because of being a natural substance. Generally, in food industry, plant extracts are used for their antimicrobial, antioxidant properties, and some biological activities, instead of synthetic antioxidants and antibiotics. Therefore, they can improve the shelf life of the product. Due to the growth of yeast and mold, the shelf life of yogurt is limited. In this study, the use of lyophilized fresh *C. sativum* L. leaves inhibited the growth of yeast and mould in yoghurts throughout the storage and had no effect on the yoghurt starter culture. This study showed that *C. sativum* L. can be used as a good source for inhibiting yeast and moulds growth and flavoring of yoghurt.

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فعالیت های ضد میکروبی *Coriandrum sativum* L. و اثرهای آن روی خواص میکروبیولوژیکی ماست

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چکیده

در این پژوهش، اثرهای ضد میکروبی برگ *Coriandrum sativum* L. روی ۳۰ ریزجاندار (میکروارگانیزم) ناشی از غذا (food borne) شامل ۲۰ باکتری، ۷ قارچ، و سه گونه مخمر آزموده شد و برخی خواص میکروبیولوژیکی ماست تولید شده با خشک کردن انجمادی(lyophilized) *C. sativum* L. بررسی گردید. ماست ها با سه غلظت از پودر *C. sativum* L. (۰/۱ ٪، ٪۰/۲ ،٪

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۰/۳٪) تهیه شد و در ۴ درجه سانتی گراد به مدت ۲۱ روز نگهداری شد. عصاره اتانول از گیاه تازه دارای فعالیت های ضد میکروبی علیه فقط ۹ باکتری و یک مخمر بود که قطر ناحیه بازدارندگی (inhibition zone) بین ۸ تا ۱۲ میلی متر تغییر می کرد ولی هیچ اثری روی کپک های استفاده شده در این پژوهش نداشت. افزودن *C. sativum* L. در مقایسه با تیمار شاهد هیچگونه تاثیر معنادار آماری روی رشد باکتری های ماست نداشت و آنها در حدی بیش از 8 log CFU/g در تمام ماست ها در طی دوره انباری ثابت ماندند. از سوی دیگر، افزایشی در شمارش *Lactobacillus bulgaricus* و کاهشی در *Streptococcus thermophilus* مشاهده شد که به غلظت *C. sativum* L. وابستگی داشت ولی این امر از نظر آماری معنادار نبود($p>0.05$). با این وجود، تفاوت های معناداری بین شمارش مخمرها و کپک ها مشاهده شد ($p<0.05$). در هیچ یک از نمونه هایی که دارای پودر *C. sativum* L. بودند مخمر و کپک شناسایی نشد، در حالیکه شمارش مخمر ها و کپک ها در تیمار شاهد در طی دوره انبارداری افزایش یافت. این نتایج نشان می دهد که پودر برگ *C. sativum* L. را می توان به عنوان عاملی برای جلوگیری از رشد مخمر و کپک که ریزجانداران مخرب کیفیت ماست در دوره انبارداری هستند به کار بست.

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