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POSTHARVEST TREATMENT OF CELERY APIUM GRAVEOLENS USING ESSENTIAL OILS AND COLD TREATMENT TO CONTROL SPRINGTAILS HYPOGASTRURA VERNALIS (COLLEMBOLA: HYPOGASTRURIDAE)

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Abstract

Australia produces celery (*Apium graveolens* var. *dulce*) for both home and international markets. Celery can be exported to other countries after postharvest treatment, which terminates springtail infestation. In the field, celery bunches act as a home for the springtail (*Hypogastrura vernalis*) (Collembola: Hypogastruridae), a native of Australia. Springtails are insects that dwell inside celery bunches but do not destroy the produce. However, springtails, which are regarded as a quarantine pest and have significantly affected exports of celery, are a concern. This experiment tested the effects of essential oils and cold treatments on springtail mortality in fresh celery. Eucalyptus and rosemary and their mixture at the concentration of 5% as well as four different cold treatment treatments 3, 5, 10, and 15ºC were used over four treatment periods 3, 5, 7, and 14 days. Springtail mortality in rosemary essential oil was 21.66, followed by 18.66 in the mixture of eucalyptus and rosemary compared with eucalyptus essential oil and was 15.33 mortality. The mortality of springtail was not affected by the 3, 5, 10, and 15ºC treatments for the treatment periods of 3, 5, 7 and 14 days. Phytotoxicity on celery bunches was absent with the use of essential oils. However, cold treatment damage was observed most noticeably on celery in the 3 and 5ºC treatments.

Keywords: Springtails, Fresh celery, Eucalyptus oil, Rosemary oil, Cold treatment.

معاملة الكرفس Apium graveolens بعد الجني لمكافحة حشرة ذات الذنب القافز Hypogastrura vernalis (Collembola: Hypogastruridae) باستخدام الزيوت العطرية ودرجات الحرارة المنخفضة

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الخلاصة

ينتج الكرفس (*Apium Gravolens*) في كثير من الدول ومنها استراليا للأسواق المحلية والعالمية. يصدر الكرفس من استراليا إلى دول أخرى بعد اجراء معاملات ما بعد الجني للتخلص من الآفات. تعد حشرات ذات الذنب القافز (*Hypogastrura vernalis*) المستوطنة في استراليا من الآفات التي تخضع الى الحجر الزراعي نتيجة اصابتها لكثير من المحاصيل الزراعية، ولا تعد افة رئيسية على الكرفس لكنها تعيش داخل باقة الكرفس ولا تسبب الضرر. أثرت تواجد حشرات ذات الذنب القافز بشكل كبير على صادرات الكرفس الى الأسواق العالمية، لذلك سببت خسائر مادية نتيجة رفض استيراد الكرفس من قبل العديد من الدول. أجريت هذه التجربة لبيان تأثير الزيوت العطرية ودرجات الحرارة المنخفضة على نسب موت حشرات ذات الذنب القافز التي تعيش داخل باقات الكرفس بعد الجني. استخدم زيت اليوكالبتوس وإكليل الجبل وخليطهما بتركيز 5٪ فضلا عن استخدام أربعة حرارة منخفضة وهي 3، 5، 10، و15°م وحسبت نسب الموت على أربع فترات لكل معاملة 3، 5، 7، و14 يوما. بلغ معدل نسب موت حشرة ذات الذنب القافز في معاملة زيت إكليل الجبل العطري 21.66%، يليه 18.66% في خليط اليوكالبتوس وإكليل الجبل بالمقارنة مع زيت اليوكالبتوس العطري وكان 15.33%. لم يتأثر معدل موت حشرات ذات الذنب القافز بمعاملات درجات الحرارة المنخفضة 3، 5، 10، و15°م لفترات المعاملة من 3، 5، 7، و14 يوما. كانت سمية الزيوت العطرية النباتية على أوراق وباقات الكرفس غائبة مع استخدام الزيوت الأساسية، فضلا عن لوحظ اضرار لباقات الكرفس في معاملات درجات الحرارة وخصوصا في معاملتي 3 و5°م.

كلمات مفتاحية: ذات الذنب القافز، كرفس، زيت اليوكالبتوس، زيت اكليل الجبل، معاملة التبريد.

Introduction

Celery Apium graveolens var. dulce is grown in most Australian states and is considered one of the country's highest-yielding and most valuable horticultural export crops (1). The celery growing area is around 300 hectares, and Australian celery production in 2008-2009 was more than 57 thousand tons, valued at 45 million Australian dollars (26). In 2017, celery exports increased to 3557 tonnes (18). This growing market is, however, threatened by a natural infestation of springtails, an insect native to Australia that is considered a quarantine pest (22), and which has significantly affected market access as springtails are considered a quarantine pest in many countries.

Many insects are classified as 'quarantine pests.' Every year, containers of fresh vegetables and fruits are rejected and destroyed because of quarantine pests, causing economic losses for exporters (23). Australia is in an excellent position to produce fresh vegetable products, such as the production of celery, for worldwide export (1 and 18), but this can only achieve with insect control and quarantine strategies. Phytosanitary treatment of fresh produce is compulsory for a wide variety of products as a means to prevent the unwanted transfer of insects during export. These treatments, however, add to the overall cost of export and sometimes reduce the quality of the product (2).

Collembola (Springtails) are small wingless insects that size between 1-3 millimeters in length with a bulbous, cylindrical shape. Collembola is one of the oldest groups among Arthropoda. The common name comes from their hinged spring, located on the underside of the abdomen. Springtails are found almost all over the place in Australia in a wide diversity of habitats, and the densities of springtails in Australia exceed 100,000 per square meter in some areas of nature depending on climate, season, and habitat (5). Springtails feed on decaying plant material and other organisms, such as fungi, algae, and occasionally dead animals. A few kinds of springtails were recorded as pests that can damage mushrooms and other crops. Some species prefer fresh plant material (8).

A few species of springtails are recorded as crop pests around the world (5). The family Hypogastruridae is common and widespread and has a global distribution having nearly 656 species around the world in about 40 genera (24). This family is represented by 9 genera in Australia, one of these genera of which is Hypogastrura (14). The family Hypogastruridae has several species regarded as pests and is reported yearly in Australia (5 and 8). The damage is caused by springtails that move up from the ground level to the top of plants, eating plant leaves, starting from the underside of foliage, and leaving behind a thin transparent layer of leaf membrane (26). For the pre-harvest control of springtails, some studies showed that different use formulations of pesticides, such as granules (G) and wettable granules (WG) added to the soil and seed treatment, caused high mortality of springtails (6).

Chemical fumigation is a low-cost and simple tool for postharvest disinfestations; however, it sometimes has phytotoxic effects on fresh horticultural products. Alternatives to chemical fumigants have been investigated, including physical control methods such as heat and cold treatments (28).

Generally, chemical pesticides have been used to control field pests, which are essential for managing different pests before harvest; however, pest management after harvest should be done to reduce or kill any remaining insects. Postharvest treatments, such as fumigation treatment and insecticidal dips, have successfully reduced insect pests (16).

Cold treatments are widely used in postharvest disinfestation of horticultural products (25). Many insect pests cannot survive prolonged exposure to low temperatures, so they are regularly used in quarantine treatments (31). Cold storage is a commonly used procedure for guaranteeing that vegetables do not contain any insect pests. Cold disinfestation is established by several of Australia's trading partners (e.g., Japan, China, Taiwan, USA) for products by Horticulture Innovation Australia using the vegetable industry.

Cold treatment can kill insect pests and is widely used along with pest management techniques. Low-temperature treatments have successfully controlled insect populations in exportation industries for several pests. Cold disinfestation can be done in transit, with data loggers used to provide proof of treatment. Cold treatment is already registered as quarantine in Australia for many insects, such as fruit flies (9). It has been shown to be useful in the control of Mediterranean fruit flies (Ceratitis capitata) and Queensland fruit fly (Bactrocera tryoni) in 14-day treatments (10 and 19).

Thus, springtail (Hypogastrura vernalis) (Collembola: Hypogastruridae) contaminates celery bunches, and they live in large numbers inside them. This experiment will investigate the use of essential oils as a dip treatment and the use of cold treatment on celery to evaluate its impact on the mortality of springtails.

Materials and Methods

Celery and Target Pest: Celery (*Apium graveolens* var. *dulce*) bunches infested with springtails (*Hypogastrura vernalis* Carl) were obtained from the Mandogalup Celery Farm (Sumich Group) located around 23 km south of Perth, Western Australia (lat. 32.20ºS, long. 115.84ºE) (fig. 1). The species of springtails found in Western Australia were identified taxonomically by (22), and current species have been confirmed by the Department of Primary Industries and Regional Development (DPIRD) using the Austral Entomology classification key (13). Celery samples were stored in a cold room at 15ºC at Murdoch University for 1-2 days before being prepared for experimentation.

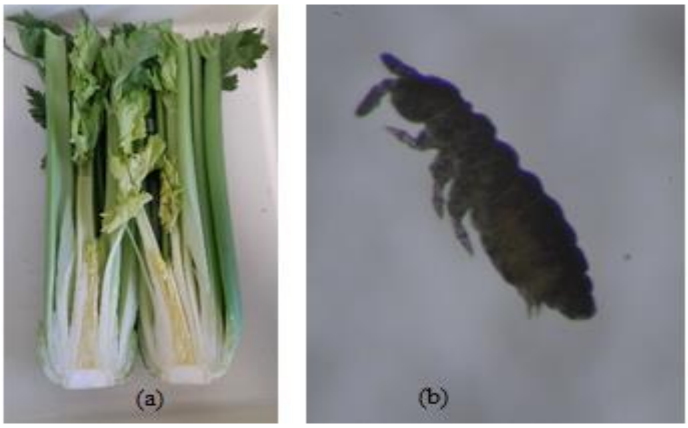

Figure 1 Celery infested with springtails (a) celery bunch (b) springtail *Hypogastrura vernalis* (Collembola: Hypogastruridae).

Dip treatment with eucalyptus and rosemary essential oils: Eucalyptus essential oil *Eucalyptus globulus* (Family: Myrtaceae) and rosemary essential oil *Rosmarinus officinalis* (Family: Lamiaceae) 100% pure essential oils were purchased from the Australian market from Range Products Company (Perth, Australia). Eucalyptus essential oil was extracted from wood and leaves of eucalyptus, while rosemary essential oil was extracted from herbs by steam distillation (3).

For dip treatment, a ten-liter solution of eucalyptus, rosemary essential oils, and the combination of eucalyptus and rosemary (ER) with water and tween 20% at the concentration of 5% were prepared separately. Fresh harvest celery bunches were dipped in the prepared solution for 10 mins before being stored, and treated celery bunches were dried by room air for 30 mins. After that, the treated bunches were stored after drying in the cooling room at 3.5°C for 24 h, and then the mortality of springtails was counted. The experiment used three replicates and three celery bunches per replicate. The observations of the springtail population inside each celery check were recorded after 24 houses.

Evaluation of Low Temperature on Springtails:Constant temperature-humidity laboratory incubators 350L (HWS series, Ningbo, China) and cool rooms in the Veterinary and Life Sciences School, Murdoch University (Murdoch, Perth, Western Australia) were used to treat cold and store celery samples. Fresh celery bunches naturally infested with springtails were tested at three different low temperatures 3, 5, and 10°C at 75-80% relative humidity (RH) for 3, 5, 7, and 14 days of exposure. These were compared with celery stored at 15°C as the control.

Three replicates were used for each treatment. Three bunches of celery were placed in a white plastic tray and covered with aluminum foil for each replicate. For the 10 and 15°C treatments, trays containing bunches were placed in the HWS incubator; for the 3 and 5°C treatments, trays were placed in the cold room.

Mortality assessment of Springtails: For both treatments, springtail mortality was assessed on each bunch in each replicate by dismantling all leaves and carefully transferring dead and live springtails into a 9 cm glass petri dish lined with white Whatman filter paper. A magnifying glass was used to distinguish between dead and live springtails. The mortality rate was corrected according to Abbot's 1925 equation.

Evaluation of essential oils and cold treatment damage: Essential oils and cold damage were assessed on each bunch of celery in each replicate by observation of color change, spots on leaves, and wilting compared with the control.

Statistical analysis: A one-way analysis of variance (ANOVA) using SPSS software (version 24.0, IBM Crop, Armonk, NY) at the least significant difference of 5% was used to analyze data in all experiments.

Results and Discussion

Results from mortality assessments of eucalyptus and rosemary essential oils and their combination show that the high mortality was recorded in the rosemary essential oil and caused 21.66%, followed by the combination of eucalyptus and rosemary (ER) and caused 18.66% mortality of springtails. At the same time, eucalyptus essential oil caused 15.33% mortality compared with rosemary and the combination of ER. The statistical analysis shows no significant differences between essential oils and the combination of ER compared with the control treatment (*p-value*=0.176) (Table 1 and Figure 1).

Table 1 The effect of eucalyptus and rosemary essential oils and their combination against springtails infested celery bunches after 24 h.

Treatments	N	Mortality (%)	SEM	95% CI
Control	3	1.66a	0.33	0.23-3.10
Eucalyptus	3	15.33b	1.33	9.59-21.07
Rosemary	3	21.66b	1.76	14.07-29.25
Mix ER	3	18.66b	3.17	4.98-32.34

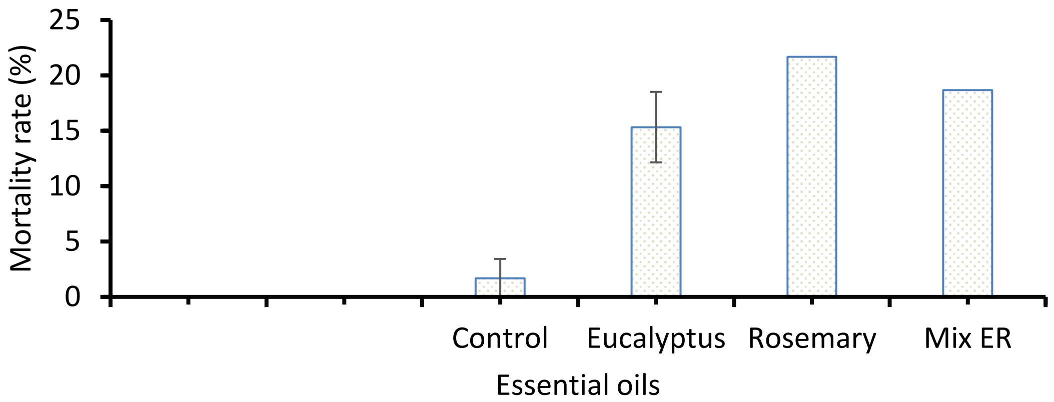

Figure 2 Mortality of springtails *Hypogastrura vernalis* that naturally infested fresh celery *Apium graveolens* in different essential oils.

However, the results show that the low temperature at 3, 5, and 10°C and control treatments 15°C on celery bunches were naturally infested with between 56 and 146 springtails (Table 2 and Figure 3). There was no significant difference in springtail mortality across low-temperature treatments for 3, 5, 7, and 14-day exposure periods (Table 2 and Figure 3). The relationship between concentration and time was assessed (percentage of variance accounted for R2=0.621 and adjusted R2=0.267). Data on the percentage mortality of springtails at each cold temperature degree (exposure period in days) showed that the mortality rate could increase with the time of celery storage.

The mortality of springtails was less than 16% at 3°C for 3 days of low-temperature storage. The results indicated that the percentage of mortality increased with increasing the time of celery storage, such as the mortality at 3°C was 21.59, 27.46, 29.87 for 5, 7, and 14 days, respectively of storage. Whereas, at the 5°C treatment, the percentage of springtail mortality was 20.55, 8.45, 11.99, and 12.69% for 3, 5, 7, and 14 days, respectively. The number of insect mortality at the 10 days storage was 14.06, 2.60, 8.96 and 20.81%, respectively, while celery stored at 15°C caused 17.73, 8.36, 6.72 and 11.51% for 3, 5, 7, and 14 days of exposure time. The efficacy of springtail mortality low-temperature treatments was compared by using celery store at 15°C. The number of springtails varied in each treatment because of the natural infestation.

Table 2 Mortality of Springtails *Hypogastrura Vernalis* in Celery *Apium Graveolens*. Data are the Average Number of Springtails Obtained from Three Replicates in the % Mortality for Each Exposure Time (Days) at 3, 5, 10, and 15°C for 3, 5, 7, and 14 Days.

Low Temp (°C)	Time (day)	N [a]	Mortality (%)	SEM [b]	95% CI [c]
3	3	91	15.64	3.40	8.95–22.33
	5	91	21.59	5.90	9.98–33.20
	7	80	27.46	0.43	26.60–28.3
	14	104	29.87	3.45	23.07–36.6
5	3	94	20.55	9.44	1.98–39.12
	5	107	8.45	4.75	0.88–17.79
	7	144	11.99	7.05	1.87–25.86
	14	56	12.69	2.69	7.39–17.98
10	3	84	14.06	9.18	3.99–32.13
	5	114	2.60	0.78	4.14–4.14
	7	146	8.96	4.75	0.38–18.32
	14	86	20.81	6.10	32.82–32.8
15	3	93	17.73	10.83	3.57–39.03
	5	145	8.36	0.86	10.06–10.0
	7	122	6.72	5.13	0.46–12.98
	14	70	11.51	3.18	5.25–17.78
LSD [d]			6.384		

[a] refers to the total number of springtails; [b] SEM refers to the standard error of the mean; [c] 95% CI refers to the confidence interval; [d] $LSD_{0.05}$ refers to the least significant difference.

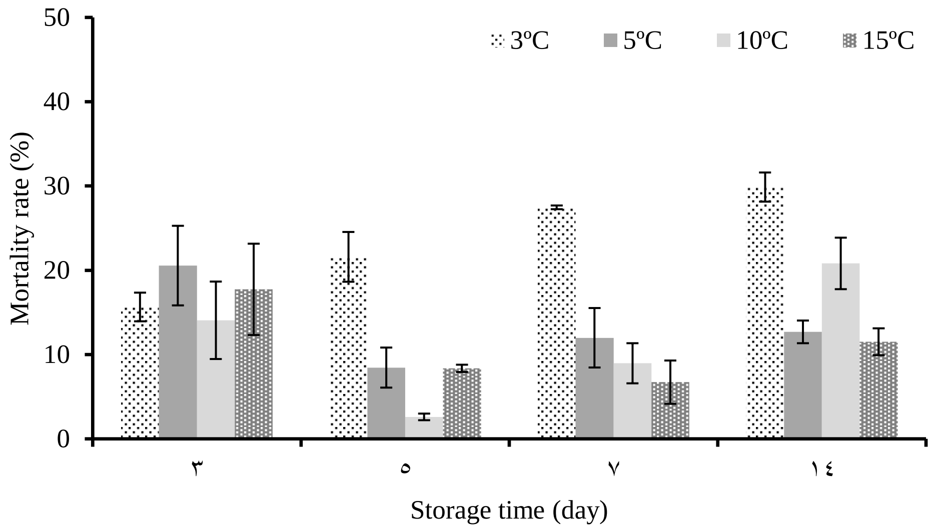

Figure 3 Mortality of springtails *Hypogastrura vernalis* that naturally infested fresh celery *Apium graveolens* in different exposure 3, 5, 7, and 14 days at 3, 5, 10, and 15°C.

The quality of celery after using essential oils was in good case, and the phytotoxicity was absent. These results confirmed the results of (30), who used dip treatment against banana aphids for postharvest treatments. At the same time, the cold treatment was significantly different from the control. Celery exposed to 14-day low-temperature treatments at 3 and 5°C showed signs of cold damage (F=0.951; ns). Discoloration and yellowing, cold-burn spots on external leaves, wilting on internal leaves, and decay of the bunch were seen in these treatments. In 3, 5, and 7 days of low-temperature treatments, damage was not seen on celery bunches. The weight of each bunch was varied and ranged between 686 – 979 grams, and the average of total leaves per bunch was 20.5, 19, and 21.5, and the damage was zero at 3, 5, and 10°C for the first week of exposure time to low temperature, then the damage started to appear after the second week of storage. The yellowing and the wilting started from the outside of the leaves.

Cold injury can occur when vegetables are stored at low temperatures. Symptoms of cold treatment damage were pitting, water loss, rots, yellowing and internal browning spot color on the leaves and stem, and wilting. Cold damage is very commonly a problem for leafy vegetables. A study (12) from Horticulture Innovation Australia showed that celery could be storage 5 – 7 weeks at close to 2°C; however, at 5°C storage life can be reduced shelf life to half, and the internal leaves can keep fresh if celery bunches stored at more than 4°C with the quality of celery can be reduced; the estimated storage life for celery can be 2 – 4 weeks at 2°C with 100 relative humidity as well as low of respiration rate (12). The same author indicated that the celery has a high susceptibility to freezing with a moderate rate of water loss during storage.

Traditional quality standards for fresh products have included appearance, texture, flavor, and nutritional content. However, safety (chemical, toxicological, and pests) and traceability are becoming more crucial for all producers involved in the supply chain, from the farm to the consumer. Since fresh vegetables are frequently consumed directly or with little processing, insect pest infestation might increase the danger of pest outbreaks in the commodities. Postharvest treatments are essential to minimize insect pests and reduce pest contamination of exporting fresh commodities. To preserve fresh vegetable-like quality with excellent nutritional content and meet safety requirements for fresh produce, a variety of postharvest treatments, such as physical, chemical, and gaseous, may be used on fresh products (21). These postharvest procedures are frequently used in association with suitable temperature control for storage.

The use of biopesticides in dipping treatments is a highly effective method of getting rid of insect pests in postharvest treatment (4). The results of tested essential oils caused high mortality on springtails in the treated celery bunches compared with the control treatment but were not reached 100% mortality. The reasons for these results with the essential oils may explain that the essential oil solution did not reach the inside of the celery bunches. These results are consistent with the previous study, which showed 92% mortality on mealybugs *Pseudococcus longispinus* by dipping for 1 min using limonene essential oil solution (20). Furthermore, a 5 mins dip with insecticidal soap kills the remaining insect pest infestation on red ginger (16).

From the results, we found that springtails can tolerate tests with low-temperature treatments; this tolerance might happen because of springtails' living conditions. Furthermore, springtails prefer moisture and organic content media condition for living because they are among the most primitive insects and require damp, moist, or very humid environments (13). In this experiment, the low-temperature treatment caused less springtail mortality than expected. High mortality rates have been observed with low-temperature treatments on fruit and vegetables for several other insect species, including Queensland fruit fly 16, 18, 22-23; 100% mortality has been seen for maize weevil (*Sitophilus zeamais*) in 6°C low-temperature treatments (27). To be viable, low-temperature treatment must not damage the quality of the commodity. This experiment supports the findings of other literature stating that fruit and grain are more tolerant of cold treatment than leafy vegetables (15 and 17).

Conclusions: Postharvest treatment is essential for pest disinfestation in vegetables and their international trade. The results from this study indicated that eucalyptus and rosemary essential oils and their combination ER had not affected springtails inside celery bunches 100%. In comparison, cold temperature treatment of springtails in celery bunches was inefficient. The advantage of the essential oils and cold temperature treatment is that it is easy to apply and has no residues. However, in the case of springtail infestations on celery, it does not appear to be effective and increases the risk of essential oils and cold temperature injury.

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