БИОТЕХНОЛОГИЯ ПРОДУКТОВ ПИТАНИЯ И БИОЛОГИЧЕСКИХ АКТИВНЫХ ВЕЩЕСТВ / BIOTECHNOLOGY OF FOOD AND BIOLOGICAL ACTIVE SUBSTANCES

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INDUCTION OF DEFENSE RESPONSE IN POTATO TUBERS AGAINST *ALTERNARIA ALTERNATA BY LAVANDULA ANGUSTIFOLIA ESSENTIAL OIL*

Research article

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Abstract

One of the main potato diseases during storage is Alternaria tuber rot (early blight), caused by the fungus *Alternaria alternata*, resulting in significant economic losses. Plant essential oils could be an eco-friendly alternative to synthetic fungicides for the control of postharvest diseases on agricultural produces. The objective of this study was to evaluate the antifungal activity, the control of Alternaria tuber rot, and the activity of defense enzymes in potato tubers treated with lavender essential oil (LAO) of *Lavandula angustifolia*. Application of 0.0-10 g/l LAO significantly inhibited mycelial growth and spore germination of *A. alternata* in vitro, with the greatest inhibitory effect observed at 10.0 g/l. LAO at 10 g/l showed an effective reduction in disease severity of 61.4% of *A. alternata* after 21 days of storage at 25°C. An increase in peroxidase (POD) and phenylalanine ammonia-lyase (PAL) activities were observed in the potato tubers. LAO can be an alternative for the control of Alternaria tuber rot.

Keywords: lavender, alternaria, potato, essential oils, defense enzymes, antifungal, Lavandula angustifolia.

ИНДУКЦИЯ ЗАЩИТНОЙ РЕАКЦИИ КЛУБНЕЙ КАРТОФЕЛЯ ПРОТИВ *ALTERNARIA ALTERNATA* ЭФИРНЫМ МАСЛОМ *LAVANDULA ANGUSTIFOLIA*

Научная статья

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Аннотация

Одним из основных заболеваний картофеля при хранении является альтернариозная клубневая гниль, вызываемая грибом *Alternaria alternata*, приводящим к значительным экономическим потерям. Эфирные масла растений могли бы стать экологически чистой альтернативой синтетическим фунгицидам для борьбы с послеуборочными болезнями сельскохозяйственной продукции. Целью этого исследования была оценка противогрибковых активность, контроля клубневой гнили Alternaria и активности защитных ферментов в клубнях картофеля, обработанных эфирным маслом лаванды (LAO) *Lavandula angustifolia*. Применение 0,0–10,0 г/л LAO значительно ингибировало рост мицелия и прорастание спор *A. alternata* in vitro, причем наибольший ингибирующий эффект наблюдался при 10,0 г/л в отношении прорастания спор и роста мицелия соответственно. LAO при 10,0 г/л показал эффективное снижение индекса поражения *A. alternata* на 61,4% после 21 дня хранения при 25°C. В клубнях картофеля наблюдалось повышение активности пероксидазы (POD) и фенилаланин-аммиачлиазы (PAL). LAO может быть альтернативой для борьбы с альтернариозной клубневой гнилью.

Ключевые слова: лаванда, альтернариоз, картофель, эфирные масла, защитные ферменты, противогрибковые, Lavandula angustifolia.

Introduction

Potato is a widely grown agricultural crop, used both fresh and for processing into potato products such as chips, French fries, mashed potatoes, starch, alcohol, and fodder. Potato has rich vitamins, and antioxidants contents [1].

Early blight (EB) represents a devastating potato disease in most potato-growing countries [2]. The phytopathogen of EB contains varied species of Alternaria genus, these species vary dependent on geographical cultivation regions. Many researchers recorded specific Alternaria species on potato, for example, *A. solani* Sorauer, *A. tenuissima*, *A. grandis* Simmons, *A. protenta*, *A. alternata*. The symptoms appear during vegetation, and the tubers can be infected, leading to dry rot during storage [3]. In different countries, EB causes annual yield losses up to 58% [4], and losses during storage reached up to 30% [5]. Pesticides application is a primary management option in controlling potato diseases. Although, because of public health concerns, and preservation of the environment, alternative methods are needed to combat these phytopathogens.

Chemicals can be replaced by botanical-derived products like plant-based extracts and essential oils [6]. These environmentally friendly substances are less harmful to the environment, do not contaminate food with pesticides, reducing the human poisoning risk, and limiting the spread of resistant plant diseases [7]. Because essential oils EOs are sources of physiologically active molecules in various patho-systems, their diversity allows them to regulate plant pathogens by direct fungitoxic activity, and resistance induction [8]. Many EOs were employed in the agricultural sector to prevent post-harvest losses on various crops [9] Lavender's essential oil has an antimicrobial effect on different plant pathogens, for example in apricots, nectarines, plums, and apples [10], [22].

The research objective is investigating LAO's fungicidal action on Alternaria tuber rot during potato storage.

Materials and methods

Pathogen and Essential Oil

Alternaria alternata isolated from diseased tubers from a previous study was used in current research. LAO was manufactured by NRC, Egypt, by steam distillation extraction technique.

In vitro experiments

The agar dilution method (contact phase) was used to test LAO's fungicidal effectiveness on mycelial growth of *A. alternata* [11]. LAO in various quantities was mixed in sterile PDA media containing 0.05% (v/v) Tween-80 as an emulsion immediately before it was poured into petri dishes (90 mm diameter) to obtain final oil concentrations (0.0-10 g/l). Control traits contain medium amended with Tween-80, but without oil. Petri plates inoculated with a five-millimeter plug of the pathogen. After 7-days incubation at 25 C, the percentage of mycelial growth inhibition was assessed according to [12], and calculating as follows:

$$\text{Mycelial growth inhibition (\%)} = [(C-T)/C] \times 100$$

Where C and T are the radial growth (mm) of fungal colony in the control and treatments, respectively.

For spore viability assay: 500 µl aliquots of the spore suspension (containing 104 spores ml-1) were placed in 2 ml sterilized Eppendorf tubes containing PDB media with the above-mentioned concentrations of LAO with 0.05% Tween-80 as emulsifying agent, then incubated at 25°C/12 hr. Germination was regarded when the germ tube length exceeds the spore diameter [13], and calculated using the formula given by [14] as follows:

$$\text{Spore germination \%} = [(\text{No. of germinated spores})/(\text{Total No. of examined spores})] \times 100$$

In vivo experiments

Disease severity on potato tubers. Tubers of cultivar "Rivera" were used to validate the in vitro results. After rinsing the tubers with tap water, and sterilizing with sodium hypochlorite (2%) for three minutes, then washed by sterilized water. Tubers were wounded with a 5×5 mm (diameter × depth) using a sterile cork borer, then tubers were treated 2 hours before infection with *A. alternata* by spraying the whole tuber with 8.0 or 10.0 g/l LAO, after that tubers inoculated by deposing in the occasioned hole a 5 mm-agar plug of *A. alternata* removed from an active culture at 25°C. The control traits (infected tubers) received sterilized distilled water (with Tween-80). All treatments were kept at 25°C and moderately high humidity for 21 days. Each treatment had ten tubers with four replicates [15]. Tubers were sliced through the inoculation site and the depth and width of the rot region after storage, and tubers' length and width were measured. The disease index (X) according to [16] calculated by the formula:

$$X=[dh/DH] \times 100, \text{ where:}$$

d – lesion width (mm);
h – lesion depth (mm);
D – tuber length (mm);
H – tuber width (mm).

Defense enzymes assay

LAO activity on the defense enzymes, peroxidase POD, and phenylalanine ammonia-lyase PAL was measured in potato tuber tissues samples collected (0-4 days) after treatments (using 10.0 g/l of LAO, which was the concentration that showed the total inhibition of mycelial growth and spore germination). The crude enzyme was extracted according to [17] (0-4 days) after treatments. POD, and PAL activities were determined as described by [18], [19]. The total protein content was measured by Bradford method (1976) using bovine serum albumin as a standard [20].

The results were presented as the mean (±SD). A one-way analysis of variance (ANOVA) was performed on the data. LSD test was used to separate means, using (CoStat 6.45 software package), significance was defined as $P < 0.05$.

Results and discussion

In vitro experiments

The results of LAO fungicidal activities are shown in Table 1. The results show that different LAO concentrations significantly inhibited mycelial growth (14.5-100%) and spore germination (39.7-100%). When compared to the control, the highest concentration 10.0 g/l inhibited mycelial growth, and spore germination completely, however, there wasn't a significant effect between 8.0 and 10g/l concentrations against spore germination. These findings are partly in harmony with that obtained by [21] who found that LAO at concentration of 1000 µL/L showed 50.0-70.1% mycelial growth inhibition of *B. cinerea*, *Colletotrichum* spp,.and *Alternaria* spp.

Table 1 - Effect of LAO different concentrations on mycelial growth and spore germination of *A. alternata*

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Concentration, g/l	*A. alternate*			
	Mycelial growth,	Inhibition, %	Spore	Reduction, %

	мм		germination, %	
0.0 (control)	85.0[a]	0.0	96.6[a]	0.0
2.0	72.6[b]	14.5	58.2[b]	39.7
4.0	43.2[c]	49.17	35.0[c]	63.7
8.0	19.0[d]	77.6	11.6[d]	87.9
10.0	0.0[e]	100	0.0[d]	100

Note: values followed by different letters are significantly different according to LSD test at P < 0.05

In vivo experiments

Disease severity on potato tubers. Figure 1 shows LAO treatment effect on the disease severity in tubers after 21 days /25°C. The disease severity decreased gradually throughout increasing LAO concentrations. In tubers treated with LAO at 8.0, and 10 g/l disease severity reduced by 26.5, and 61.4%. [22] found that *Lavandula officinalis* oil at 1.0-10% reduced the disease severity of *B. cinerea* and *P. expansum* rots on apples, but concentration 10% was phytotoxic on fruits.

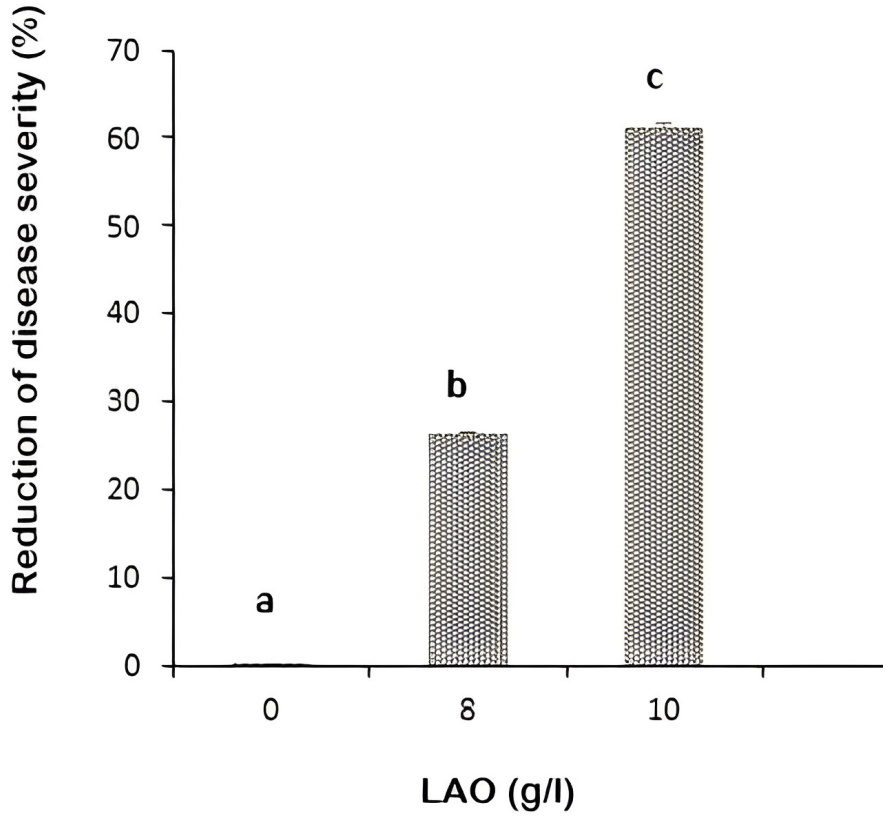

Figure 1 - Effect of LAO different concentrations on disease severity in potato tubers inoculated with *A. alternata* mycelium
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Note: the columns marked with different letters are significantly different P < 0.05

Defense enzymes assay

When we treated potato tubers with LAO, we noticed that the activity of POD reached its highest value during the fourth day in comparison to the control (Fig. 2A). PAL activity kept going up for two days. It was about 1.3 times higher than the control group. But then on the third day, it started to go down (Fig. 2B). However, the enzyme activity of POD and PAL in the treated group stayed higher than the control group for the whole time of the experiment (Fig. 2A, B).

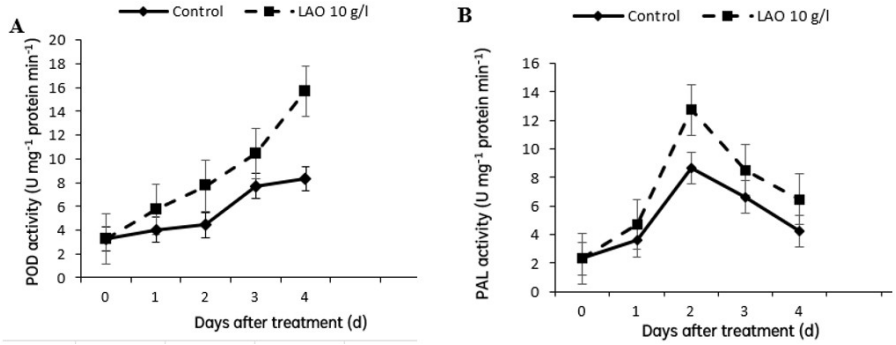

Figure 2 - Effect of LAO (10 g/l) on the activity of POD (A), and PAL (B) in potato tubers inoculated with *A. alternata* mycelium during (0-4 days) after treatment

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Note: values followed by different letters are significantly different at P < 0.05

The recent study also showed that LAO can effectively manage Alternaria rot in potato tubers by induction of defense-related enzymes such as POD ad PAL. [23] observed an increase in ascorbate peroxidase and catalase activities in the apricot fruit treated with LAO at 1000 ppm. The lignin biosynthesis by the phenylpropanoid pathway depends on specific enzymes including POD and PAL as well as the participation of H_2O_2 [24]. Thus, a resistance inducer action can make it difficult for the pathogen to penetrate the cell wall, promoting greater resistance against the pathogens' toxins [25].

Conclusion

LAO inhibited *A. alternata* and reduced early blight (Alternaria rot) in potato tubers, which may be due to direct fungitoxic action and activation of defense responses in potato tubers, including the defense enzymes PAL and POD.

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Конфликт интересов

Не указан.

Рецензия

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

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Conflict of Interest

None declared.

Review

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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