Adv. Hort. Sci., 2022 36(1): 43-52 DOI: 10.36253/ahsc-10449

AHS
Advances in Horticultural Science

Phytoprotective film for resistance induction, growth, and yield of organic strawberries

I. Dos Santos Pereira (*), F. Grecco da Silva Porto, L.E. Corrêa Antunes, Â. Diniz Campos
Brazilian Agricultural Research Corporation, EMBRAPA, Brasília, Brazil.

Key words: *Colletotrichum* spp., enzymatic activity, *Fragaria* x *ananassa*, Induced systemic resistance, K_2HPO_4.

(*) **Corresponding author:**
ivanspereira@gmail.com

Citation:
DOS SANTOS PEREIRA I., GRECCO DA SILVA PORTO F., CORRÊA ANTUNES L.E., DINIZ CAMPOS Â., 2022 - *Phytoprotective film for resistance induction, growth, and yield of organic strawberries.* - Adv. Hort. Sci., 36(1): 43-52.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 10 February 2021
Accepted for publication 25 February 2022

Abstract: **The objective of this work was to evaluate a phytoprotective film of chitosan-pyroligneous extract in promoting growth, productivity, induction of systemic resistance in strawberry cultivars managed in an organic production system. Treatments consisted of rates (0, 25, 50, and 100 mL L^{-1}) of Chi-Pyro-Film and a reference resistance inducer (dipotassium hydrogen phosphate - K_2HPO_4), evaluated in three strawberry cultivars ('Albion', 'San Andreas' and 'Portola'). Growth, yield, anthracnose incidence, and enzymatic activity were evaluated. The experimental design was a randomized block design with four replications. Chi-Pyro-Film increases the growth, yield, and anthracnose resistance of strawberry plants. The best concentration of Chi-Pyro-Film varies between 50 and 60 mL L^{-1}, according to strawberry cultivar.**

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is the most planted red fruit in Brazil. The high demand is due to its sensory characteristics such as color, texture, aroma, and taste (Ventura-Aguilar *et al*., 2018).

However, according to a report by the Brazilian Health Surveillance Agency (ANVISA) of 2016, 26% of strawberry samples collected between 2013 and 2015 presented nonconformities with pesticide residues. Pesticides detected not authorized for the crop, captan stood out, detected in 20.4% of the samples analyzed, among others such as dithiocarbamates, pyrimethanil, carbendazim, tebuconazole, iprodione, and azoxystrobin (ANVISA, 2016). Most of these fungicides aim to control or prevent anthracnose (*Colletotrichum* spp.), a disease that is considered main in strawberry fields in Brazil (Kososki *et al.*, 2001; Capobiango *et al*., 2016).

Currently, there is a search for technologies, which make agriculture sustainable and "smart", with practices and ways to minimize the excessive use of chemicals (Grewal *et al*., 2018). In this context, the use of resistance inducing products activates the plant's natural defenses, enabling disease control in the organic production system.

The resistance is associated with various defense responses, such as protein and phytoalexin synthesis, cell wall changes, and increased activity of various enzymes defense-related (Durrant and Dong, 2004). Responses that are associated with changes in the activity of various enzymes such as peroxidase (POX, EC 1.11.1), polyphenol oxidase (PPO, EC 1.14.18.1), and phenylalanine ammonia-lyase (PAL, EC 4.1.3.5) (Prasannath *et al*., 2014; Prasannath, 2017).

POXs have been implicated in many defense processes, such as hypersensitive response, lignification, phenolic and glycoprotein cross-linking, suberization, and phytoalexin production (Thakker *et al*., 2012; Prasannath, 2017). PPOs are a group of enzymes that catalyze the oxidation of hydroxyphenols to their quinone derivatives, which have antimicrobial properties (Prasannath, 2017). PAL (E.C.4.1.3.5) is the major enzyme in the phenylpropanoid pathway and acts in the synthesis of defense-related secondary compounds such as phenols and lignins (Hemm *et al*., 2004; Vanitha *et al*., 2009).

Among the compounds that have activating properties of defense mechanisms in plants are the chitosan and pyroligneous extract (Di Piero and Garda, 2008; Grewal *et al*., 2018; Souza *et al*., 2018).

Chitosan is a polycationic β-1,4 polymer bound to D-glucosamine chemically derived from crustaceans and soluble in organic acids and known to be a natural elicitor and triggers various physiological and biochemical responses in plants that act in the growth, production, and protection against disease (Chandra *et al*., 2015; Katiyar *et al*., 2015; Pichyangkura and Chadchawan, 2015). Chitosan has several characteristics that make this polymer advantageous for many applications: (1) has a defined chemical structure; (2) may be chemically and enzymatically modified; (3) is physically and biologically functional; (4) is biodegradable and biocompatible with many organs, tissues and cells; (5) can be processed into various products including flakes, powders, membranes, fibers and films (Badawy, 2012; van den Broek *et al*., 2015; Porto *et al*., 2019).

The pyroligneous extract is a liquor with strong smoke flavors, is a crude and acid condensate produced from the distillation of the smoke generated in the carbonization of wood. It consists of a complex mixture of compounds derived from the chemical decomposition of wood components through the condensation of vapors and gases generated during pyrolysis in a low oxygen concentration (Campos, 2018; Pimenta *et al*., 2018).

The pyroligneous extract is composed of water (80-90%) and more than 200 species of organic compounds (10-20%) (Theapparat *et al.*, 2018). The presence of phenolic compounds in the pyroligneous extract confers growth-promoting and antifungal properties, as found in the literature on *Helminthosporium sativum*, *Cochliobolus sativus*, Waltz, *Colletotrichum orbiculare*, *Alternaria mali* (Jung, 2007; Baimark and Niamsa, 2009; Wei *et al.*, 2010 a; Grewal *et al*., 2018).

In this context, the cationic character of chitosan in acidic conditions offers the possibility to establish electrostatic interactions with other negatively charged compounds, for example with the pyroligneous extract, considered a raw material obtained from renewable sources, and is a good solvent for chitosan (Campos *et al*., 2012; van den Broek *et al*., 2015; Porto *et al*., 2019).

The phytoprotective film of chitosan-pyroligneous extract (Chi-Pyro-Film) consists of the chitosan diluted in the pyroligneous extract, and its characteristics are the formation of a film with photoprotection capability against radiation (UV-B and UV-C), fungi toxic action, and inducing systemic resistance in plants (Campos *et al*., 2012).

The objective of the present work was to evaluate the effect of different concentrations of phytoprotective film formulated with chitosan and pyroligneous extract (Chi-Pyro-Film) and dibasic potassium phosphate (K_2HPO_4) on growth promotion, resistance induction to anthracnose, yield, and defense enzyme activity in strawberry cultivars in the organic production system.

2. Materials and Methods

The study was carried under field conditions, at Embrapa Temperate Climate Experimental Station, in Pelotas city, Rio Grande do Sul state, Brazil (31°40'49 "S 52°26'18" O, at 60 m altitude) (Fig. 1 a). The climate of the region, according to the Köppen classification is Cfa type, temperate and humid, with hot summers.

Raw materials

The pyroligneous extract was obtained through an extraction procedure of *Eucalyptus grandis* proposed by Campos (2018) and the distillation process was performed according to that described by Porto *et al.*

(2019). Chitosan was supplied by Nutrifarm™, with a 97% degree of deacetylation, determined by proton magnetic resonance (Porto, 2011).

Plant material

The seedlings of neutral-day strawberry cultivars, 'Albion', 'San Andreas' and 'Portola', were planted in May and were grown under a low tunnel system with mulching and drip irrigation. The spacing between lines and between plants was 0.30 m, with three lines per bed. The area had a history of many years with severe anthracnose incidence in the strawberry plants (Fig. 1b, 1c). Liming and fertilization were performed according to the recommendation for strawberry organic production. Climatic data from the experiment period are shown in figure 1d.

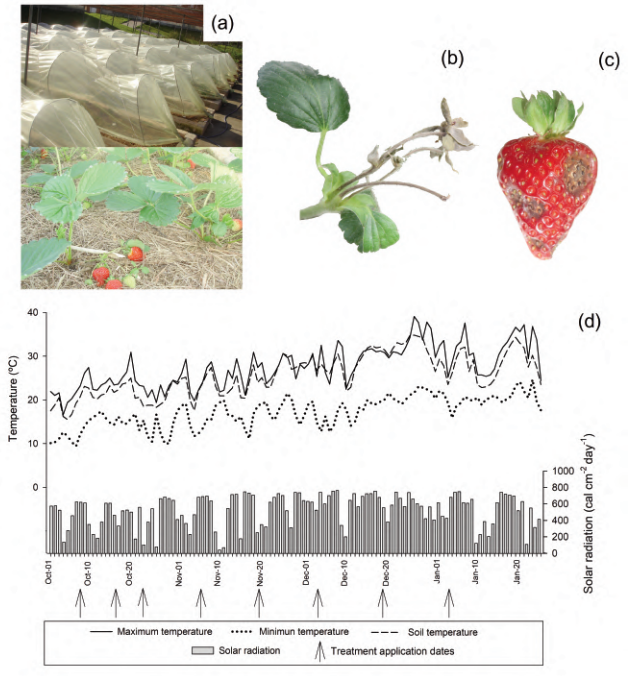

Fig. 1 - Experiment under field conditions (a), anthracnose symptoms in strawberry plants (b) and fruits (c), and climatic data from the experiment period (d).

Treatments

The treatments of induction of systemic resistance consisted of four concentrations of the Chi-Pyro-Film and a reference treatment with dibasic potassium phosphate (K_2HPO_4). The Chi-Pyro-Film was registered in the field of Green Chemistry at the National Institute of Intellectual Property in Brazil (PCT/BR2013/000597), United States (US201503 36854A1) and Germany (DE112013006230T5) as a phytoprotective for agriculture use.

The Chi-Pyro-Film with a concentration of 30 g L^{-1} (Fig. 2) was diluted with distilled water in different concentrations (0, 25, 50, and 100 mL L^{-1}). Treatment with K_2HPO_4 was applied at a concentration of 50 mM (Orober *et al*., 2002; Aleandri *et al*., 2010). This compound has shown efficacy against e.g. powdery mildew on barley, cucumber, pepper, and tomato (*Blumeria graminis* f. sp. hordei, *Sphaerotheca fuliginea*, *Leveillula taurica,* and *Erysiphe oronti*, respectively), anthracnose (*Colletotrichum lagenarium*) on cucumber, rust (*Puccinia sorgi*) and leaf blight (*Exserohilum turcicum*) on maize and rice blast (*Pyricularia oryzae*), mildew (*Sphaerotheca fuliginea*) (Reuveni *et al*., 1996; Reuveni and Reuveni, 1998; Manandhar *et al*., 1998; Reuveni *et al*., 2000; Ehret *et al*., 2002; Orober *et al*., 2002; Hamza *et al.,* 2017).

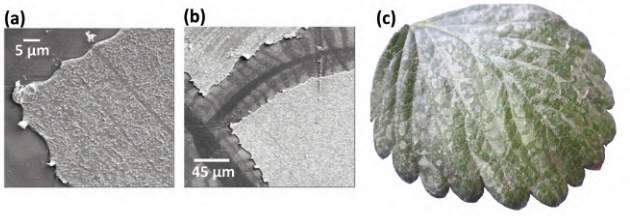

Fig. 2 - Electron micrograph of the phytoprotective film of Chi-Pyro-Film, after spraying on a smooth surface at a temperature of 18 to 25°C (a and b). Chitosan strawberry leaf covered with a film formed by Chi-Pyro-Film (c).

The spraying of treatments started at the beginning of fruiting, and the application dates were indicated in figure 1d. The spray volume was 4.5 mL per plant (500 liters per hectare), applied through conical jet nozzles, observing a total coverage of the plants until close to the runoff point.

Analysis of plant growth, damage by anthracnose, and fruit yield

Vegetative growth evaluations consisted of dry mass (g $plant^{-1}$) of crown, root, and leaf, by drying to constant weight (65°C) of three plants collected in each experimental unit at the end of the production cycle, 256 days after seedlings transplanting. The productive variables measured were fruit yield (g $plant^{-1}$) and fruit weight (g $fruit^{-1}$), obtained by evaluating the total fruits harvested in each experimental unit. The percentage of fruits with anthracnose was obtained by counting the fruits with symptoms at each harvest (Fig. 1c). The harvests were carried out three times a week between October and January.

Enzymatic activity analysis

Biochemical evaluations were realized by determination of the specific activity of peroxidase (POX),

polyphenoloxidase (PPO), and phenylalanine ammonia-lyase (PAL) enzymes, in strawberry leaf samples collected immediately before application (BA) and 48 hours after application (AA) of Chi-Pyro-Film concentrations and reference treatment (K_2HPO_4). For the extraction of POX and PPO, was used 500 mg of ground tissue below 4°C in 10 mL of 0.05 M phosphate buffer (pH 7.0) containing 1 mg of polyvinylpyrrolidone-10. Subsequently, centrifugation was performed at 4,000 *g* for 30 minutes under refrigeration. The supernatant was preserved on ice and used for determinations according to Campos *et al*. (2003). The POX and PPO extraction was carried out by grinding the leaves with 20 mg polyvinylpyrrolidone (Sigma-Aldrich). The enzyme extract obtained after filtration (Whatman 1) and centrifugation (5,600 g*n*, 15min) were used to test the activity.

POX activity was determined in the enzyme extract mix with a phosphate-citrate buffer composed of 0.2 M sodium phosphate solution and 0.1 M citric acid (pH 5.0).

The mixture was homogenized in vortexed for 15 seconds. POX activity was determined according to Campos *et al*. (2004).

PPO activity was determined in the enzyme extract with 3.6 mL of 0.05 M phosphate buffer (pH 6.0) and 0.1 mL of 0.1 M catechol. PPO activity was determined according to Campos *et al*. (2004).

PAL activity was determined in crude leaf extracts according to the methods described by Hyodo and Yang (1971) and Hyodo *et al*. (1978) modified by Campos *et al*. (2003).

For extraction, 500 mg of tissue was macerated (below 4°C) with 8 mL of 50 mM sodium borate buffer (pH 8.5) containing 25 g L^{-1} of polyvinylpyrrolidone-10 and 4 mL L^{-1} of mercaptoethanol. The protein extract obtained after filtration (Whatman 1) and centrifuged (5,600 g_n, 30 min) under refrigeration (below 4°C). was used to assay the PAL activity. Protein in the extracts was determined by the Bradford method (Bradford, 1976).

Experimental design and statistical analyses

The experimental design was randomized blocks with four replications of nine plants. The results were submitted to variance analysis and the means of the variables with significant effect were compared using the Tukey test (cultivars) or regression analysis (concentrations) at 5% error probability.

3. Results

Chi-Pyro-Film concentrations had a significant effect on the growth, yield, anthracnose resistance induction, and enzymatic activity of strawberry plants.

Growth and development

The dry mass of the plant showed factors interaction. The three cultivars showed a quadratic response to Chi-Pyro-Film concentrations (Fig. 3), but the highest efficiency concentration was different. 'Albion' and 'Portola' had the highest efficiency concentration estimated at 60 mL L^{-1} of Chi-Pyro-Film, while for 'San Andreas' the concentration was 50 mL L^{-1} (Fig. 3). The treatment with reference resistance inducer, K_2HPO_4, had also different according to cultivars. In 'Albion', it provided a leaf mass slightly higher than the control treatment and similar to the 100 mL L^{-1} Chi-Pyro-Film concentration, but it was lower than 25 and 50 mL L^{-1} (Fig. 3).

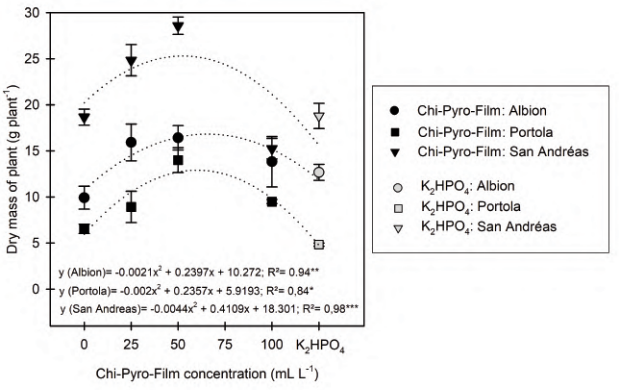

Fig. 3 - Dry mass of leaves of Albion, Portola and San Andreas cultivars, in response to Chi-Pyro-Film concentrations and reference treatment (K_2HPO_4, 50 mM). Interaction effect between factors (cultivar and film concentration). *, **, ***, significant at p<0,05, p<0,01, p<0,001, respectively.

The yield also was significantly influenced by Chi-Pyro-Film, with a quadratic response to isolated effect of the film concentration factor (Fig. 4). Regardless of cultivar, the highest efficiency concentration was estimated at 60 mL L^{-1}. Regarding the reference treatment (K_2HPO_4), it was observed that it had a performance similar to 100 Chi-Pyro-Film and higher than control (0 mL L^{-1} of Chi-Pyro-Film), but less than the 50 mL L^{-1} (Fig. 4). Between cultivars

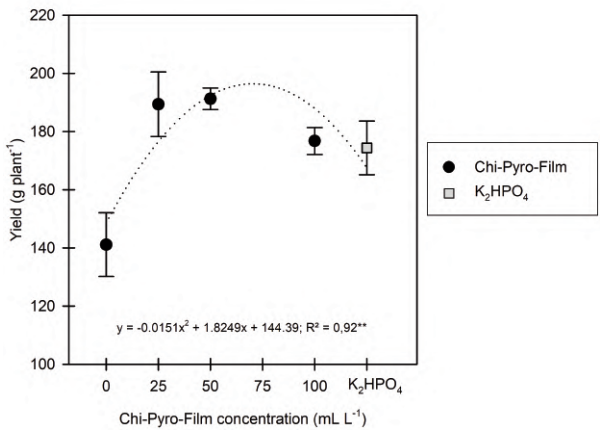

Fig. 4 - Yield of strawberry, in response to Chi-Pyro-Film concentrations and reference treatment (K_2HPO_4, 50 mM). Isolated effect of film concentration (average of cultivars responses). *, **, ***, significant at p<0,05, p<0,01, p<0,001, respectively.

effect, 'Portola' showed the highest yield than 'Albion' and 'San Andreas' (Table 1). Results similar to those observed by Carini *et al*. (2015) in a study of evaluation of strawberry cultivars in an organic system, in which 'Portola' was also more productive than 'Albion' and 'San Andreas'.

The fruit weight was not influenced by Chi-Pyro-Film treatments. However, there was an effect of the cultivar factor, with 'San Andreas' producing fruits of a higher mass (Table 1). Corroborating with Carini *et al.* (2015), which evaluated the same three cultivars, found a higher fruit weight of 'San Andreas', but with the same weight that 'Albion'.

The variable fruits with anthracnose showed an isolated effect of the factor Chi-Pyro-Film concentrations, that is, all cultivars had the same response to the application of Chi-Pyro-Film, with a quadratic reduction in the number of fruits attacked, with the maximum efficiency concentration estimated at 60 mL L^{-1} Chi-Pyro-Film (Fig. 5). The reference treatment with K_2HPO_4 was similar to the 100 mL L^{-1} of Chi-Pyro-Film, but lower than the 25 mL L^{-1} and 50 mL L^{-1} (Fig. 5). Among the cultivars, 'Portola' was the most sensitive to the occurrence of anthracnose in fruits, with no difference between 'Albion' and 'San Andréas' (Table 1).

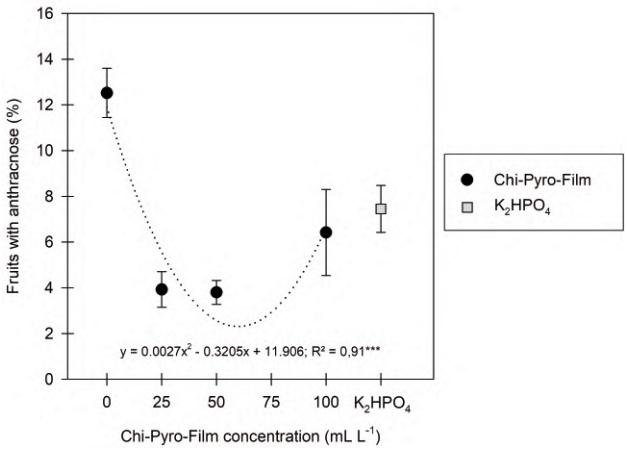

Fig. 5 - Percentage of fruits with anthracnose in response to Chi-Pyro-Film concentrations and reference treatment (K_2HPO_4, 50 mM). Isolated effect of film concentration (average of cultivars responses). *, **, ***, significant at p<0,05, p<0,01, p<0,001, respectively.

Enzymatic activity

In the present study, there was a significant effect of Chi-Pyro-Film concentrations on the activity of POX, PPO, and PAL enzymes (Figs. 6 and 7). An interaction effect between film concentration and sampling time indicated that 48 hours after application, there was a quadratic effect of film concentrations on the activity of the three enzymes studied. In the case of POX and PPO, an activity reduction effect was obtained up to the estimated concentrations of 54 mL L^{-1} and 50 mL L^{-1}, respectively, followed by an increase (Fig. 6a and 6b). However, PAL activity increased until the estimated concentration of 36 mL L^{-1}, with a subsequent reduction (Fig. 6c). About the

Table 1 - Yield, fruit weight and fruits with anthracnose in Albion, Portola and San Andreas cultivars ([z])

Variables	Cultivar			C.V. (%)
	Albion	Portola	San Andreas	
Yield (g $plant^{-1}$)	131.90 ± 7.93 c	221.35 ± 12.87 a	170.4 5± 9.30 b	20.18
Fruit weight (g $fruit^{-1}$)	8.60 ± 0.67 b	8.50 ± 0.57 b	13.33 ± 1.09 a	72.71
Fruits with anthracnose (%)	5.29 ± 1.71 b	9.13 ± 1.53 a	5.83 ± 1.49 b	107.57

[z] Means followed by different lowercase letters in the row, differ by Tukey test at 5% error probability. The averages correspond to the isolated effect of cultivar factor to studied variables.

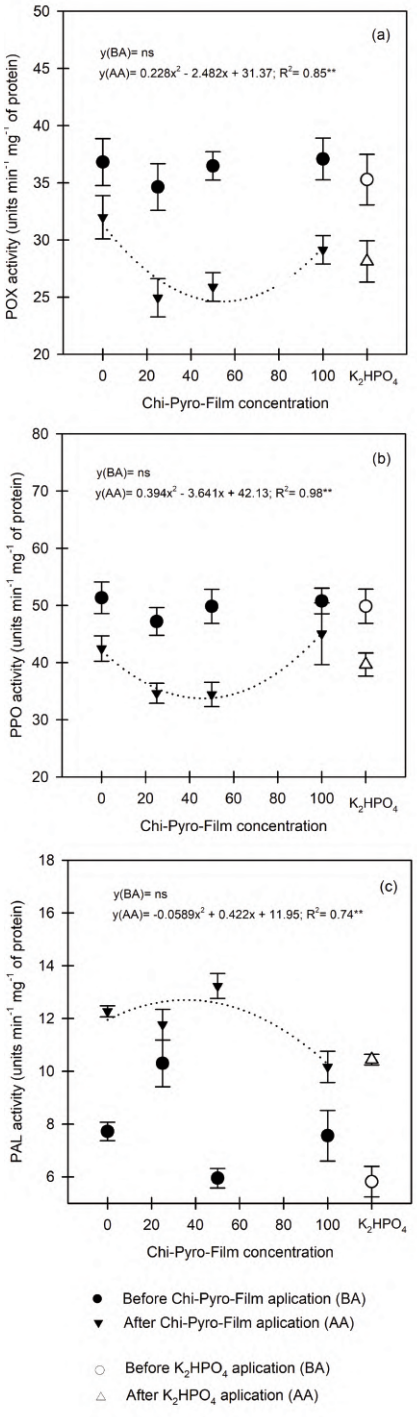

Fig. 6 - Specified activity of peroxidase-POX (a), polyphenoloxidase-PPO (b) and phenylalanine ammonia lyase-PAL (c) in the leaves, before (BA) and after application (AA) of Chi-Pyro-Film concentrations and reference treatment (K_2HPO_4, 50 mM). Interaction effect between film concentration and sampling time (before and 48 hours after application). *, **, ***, significant at p<0,05, p<0,01, p<0,001, respectively.

reference treatment, with K_2PHO_4, it induced activities similar to Chi-Pyro-Film in the concentration of 100 mL L^{-1} for POX, PPO, and PAL.

The enzymatic activity also had an interaction effect between treatments of resistance induction and strawberry cultivars. POX activity decreased to concentrations of 49 mL L^{-1} and 57 mL L^{-1} in Albion and Portola, respectively (Fig. 7a). For San Andreas, although there was a similar trend, it was not significant (Fig. 7a).

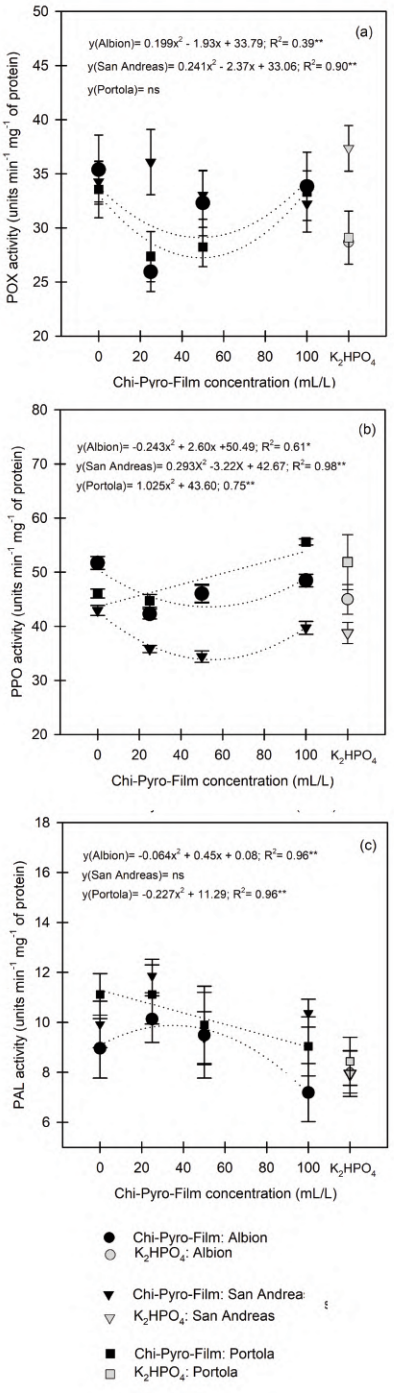

Fig. 7 - Specified activity of peroxidase-POX (a), polyphenoloxidase-PPO (b) and phenylalanine ammonia lyase-PAL (c) in leaves of Albion, Portola and San Andreas, in response to Chi-Pyro-Film concentrations and reference treatment (K_2HPO_4, 50 mM). Interaction effect between film concentration and cultivars. *, **, ***, significant at p<0,05, p<0,01, e p<0,001, respectively.

The cultivars Albion and San Andreas also had a quadratic response to Chi-Pyro-Film, with a decrease in PPO activity up to the estimated concentrations of 54 mL L^{-1} and 55 mL L^{-1} (Fig. 7b). Concerning Portola, there was a linear increase up to a 100 mL L^{-1} concentration of Chi-Pyro-Film (Fig. 7b).

Concerning PAL, there was a quadratic response in Albion, with an increase up to the 38 mL L^{-1} rate of Chi-Pyro-Film, as well as a linear reduction in Portola (Fig. 7c).

The reference treatment with K_2HPO_4, showed a response similar to the 100 mL L^{-1} film concentration for the enzymes PPO and PAL in the three cultivars studied (Figs. 7b and 7c). For the POX in Albion and San Andreas, the reference treatment was similar to the 25 mL L^{-1} concentration, while in Portola, it was similar to the 50 mL L^{-1} film (Fig. 7a).

4. Discussion and Conclusions

In general, the results indicate that Chi-Pyro-Film contributed to the increase of vegetative growth and yield, as well as, to the anthracnose damage reduction in strawberry fruits. Results that are in agreement with those found in the literature, where it is observed an increase in vegetative, productive, and health variables, such as height, number of leaves, leaf area, yield and reduction of incidence of diseases in plants that received chitosan or pyroligneous extract (El-Miniawy *et al*., 2013; Masum *et al*., 2013; Mungkunkamchao *et al*., 2013; Mukta *et al*., 2017; Kumaraswamy *et al*., 2018; Ventura-Aguilar *et al*., 2018).

The effect of Chi-Pyro-Film on strawberry plant growth can be attributed to the role of chitosan as a non-toxic and biodegradable plant growth promoter (Salachna and Zawadzińska, 2014; Ahmed *et al.*, 2020). Some authors suggest that foliar application of chitosan enhances the endogenous concentration of phytohormone such as gibberellic acid and auxin (Uthairatanakij *et al*., 2007; Ahmed *et al*., 2016). But the increase in macro and micronutrient accumulation and improved the content of photosynthetic pigments, provided by chitosan are also related to its influence on plant growth (Shehata *et al*., 2012; Ahmed *et al*., 2016). On the other hand, pyroligneous extract contributes to plant growth by its phytoprotective effect against pathogens, especially fungi. Was reported antipathogenic effects of the pyroligneous extract on plant pathogenic fungi like *Helminthosporium sativum*, *Cochliobolus sativus*, *Valsa mali*, *Colletotrichum orbiculare*, and *Alternaria mali* (Jung 2007; Wei *et al.*, 2010 a). This antifungal activity has been related to the presence of furaldehydes and phenols in pyroligneous extract (Grewal *et al.*, 2018).

The efficiency of spraying with Chi-Pyro-Film can be attributed to the effects of pyroligneous extract and chitosan individually, and to an interaction effect between both. According to Porto *et al.* (2019), who studied physicochemical properties of Chi-Pyro-Film, the film showed a semicrystalline structure, which is smooth and stable up to 50°C, being persistent in environmental conditions; it is permeable to water vapor and has high hygroscopicity, in addition to being able to efficiently block incident UVB and UVC radiation. The coverage presented by the Chi-Pyro-Film, as well as its persistence on the leaf surface (Fig. 2), probably provide several days of action, perhaps a large part of the application interval (15 days).

The main effect of chitosan and the pyroligneous extract is attributed to resistance induction by increased defense enzyme activity and accumulation of phenolic compounds acting on reactive oxygen species (ROS) (Wei *et al*., 2010 b; Katiyar *et al*., 2015; Pichyangkura and Chadchawan, 2015; Grewal *et al*., 2018). This effect is in line with the behavior verified by the PAL activity in this study (Figs. 6c, 7c). However, the activity of the POX and PPO enzymes responded differently, with a reduction in activity in the concentrations estimated between 50 and 60 mL L^{-1}, 48 hours after application of treatments (Figs. 6a 6b), especially in the Albion and San Andreas cultivars (Figs. 7a, 7b). This film rate (50-60 mL L^{-1}) had a better performance in the dry mass accumulation, yield, and incidence of anthracnose.

The results indicate that in addition to the increase in systemic resistance, suggested by the PAL response, there is a direct effect of phytoprotection and a reduction of stress condition. Some aspects that can be associated with this second aspect, maybe the block that the film exerts concerning UVA and UVB radiation, as well as its potential action on pathogens, a hypothesis that corroborates the results obtained by Porto *et al.* (2019).

In this way, it can be suggested that Chi-Pyro-Film has a complex performance, acting both on metabolism with increased plant resistance and reducing cellular damage caused by physical (radiation) and biological stress (pathogens), as well as forming a phytoprotective film that inhibits the direct action of stress

agents, such as UVA and UVB radiation and inhibiting the attack of pathogens.

In general, Chi-Pyro-Film showed greater efficiency in promoting growth, yield, and resistance to anthracnose than the reference treatment (K_2HPO_4), mainly in concentrations between 25 and 50 mL L^{-1}. K_2HPO_4 performed similarly to the 100 mL L^{-1} of the film. Different studies indicate the effect of K_2HPO_4 in the resistance induction of several species (Reuveni and Reuveni, 1998; Kashiap and Dhiman, 2009; Aleandri *et al*., 2010; Hamza *et al*., 2017; El-Tanany *et al*., 2018). According to Orober *et al*. (2002), foliar application of K_2HPO_4 results in the activation of systemic resistance mechanisms. The positive effect of K_2HPO_4 is associated with salicylic acid involved in triggering plant cell defense and sensitization responses for a faster and stronger response to subsequent pathogen attack (Mauch-Mani and Métraux, 1998; Orober *et al.*, 2002).

The 100 mL L^{-1} concentration of Chi-Pyro-Film may be high for the strawberry crop. Probably the increase in film thickness, which according to Porto *et al*. (2019), can significantly reduce the film's permeability to water vapor. An aspect that can make some physiological processes such as the flowing water, the absorption of nutrients, and photosynthesis less efficient.

This study provides indicates that the phytoprotective film (Chi-Pyro-Film) is effective as a growth promoter and inducer of systemic resistance to anthracnose, resulting in increased growth and fruit production in strawberry plants of different cultivars. The optimal concentration of Chi-Pyro-Film ranges between 50 and 60 mL L^{-1}, depending on the strawberry cultivar.

We believe that the tested film can be an important tool for production systems, especially agroecological and organic systems, which require alternatives for disease control. But also, due to its physical-chemical properties and great stability, we believe that the film can be combined with other components, such as nutrients, biostimulants, and plant hormones, to enhance its effect. However, such combinations need to be studied.

Acknowledgements

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and by the project "Avaliação de biofilmes estabilizados a base de quitosana associada a diferentes moléculas, para uso em sistemas de produção orgânico".

References

AHMED A.H.H., NESIEM M.R.A.E., ALLAM H.A., EL-WAKIL A.F., 2016 - *Effect of preharvest chitosan foliar application on growth, yield and chemical composition of Washington navel orange trees grown in two different regions*. - Afr. J. Biomed. Res., 10: 59-69.

AHMED K.B.M., KHAN M.M.A., SIDDIQUI H., JAHAN A., 2020 - *Chitosan and its oligosaccharides, a promising option for sustainable crop production - a review*. - Carbohydr. Polym., 227: 115331.

ALEANDRI M.P., REDA R., TAGLIAVENTO V., MAGRO P., CHILOSI G., 2010 - *Effect of chemical resistance inducers on the control of* Monosporascus *root rot and vine decline of melon*. - Phytopathol. Mediterr., 49: 18-26.

ANVISA, 2016 - *Relatório das análises de amostras monitoradas no período de 2013 a 2015*. - Agência Nacional de Vigilância Sanitária, Portugal, pp. 246. (In Portuguese).

BADAWY A., 2012 - *New rapid and sensitive spectrophotometric method for determination of a biopolymer chitosan*. - Int. J. Carbohydr. Chem., 139328, pp. 7.

BAIMARK Y., NIAMSA N., 2009 - *Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets*. - Biomass Bioenerg., 33: 994-998.

BRADFORD M.M., 1976 - *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*. - Anal. Biochem., 72: 248-256.

CAMPOS A.D., 2018 - *Processo de Coleta e Produção do Extrato Pirolenhoso para Uso Agrícola*. - Embrapa Clima Temperado, Circular Técnica 178, Pelotas, pp. 4. (In Portuguese).

CAMPOS A.D., FERREIRA A.G., HAMPE M.M.V., ANTUNES I.F., BRANCÃO N., SILVEIRA E.P., SILVA J.B., OSÓRIO V.A., 2003 - Induction of chalcone synthase and phenylalanine ammonia-lyase by salicylic acid and *Colletotrichum lindemuthianum* in common bean. - Braz. J. Plant Physiol., 15: 129-134.

CAMPOS A.D., FERREIRA A.G., HAMPE M.M.V., ANTUNES I.F., BRANCÃO N., SILVEIRA E.P., OSÓRIO V.A., AUGUSTIN E., 2004 - *Atividade de peroxidase e polifenoloxidase na resistência do feijão à antracnose*. - Pesqui. Agropecu. Bras., 39: 637-643.

CAMPOS A.D., UENO B., PORTO F.G.S., ANTUNES I.F., GARCIA I.T.S, PEREIRA J.F.M., CASTRO L.A.S., SCIVITTARO W.B., 2012 - *Processo de obtenção de formulação com capacidade fertilizante e fitoprotetora, Formulação com capacidade fertilizante e fitoprotetora, Uso da for-*

mulação com capacidade fertilizante e fitoprotetora. - Registered at Instituto Nacional de Propriedade Intelectual in Brazil (PCT/BR2013/000597), In United States (US20150336854 A1) and in Germany (DE112013006230T5).

CAPOBIANGO N.P., PINHO D.B., ZAMBOLIM L., PEREIRA O.L., LOPES U.P., 2016 - *Anthracnose on strawberry fruits caused by* Colletotrichum siamense *in Brazil*. - Plant Dis., 100: 859-859.

CARINI F., NORMBERG A.V., FONSECA F.D., SCHWENGBER J.E., 2015 - *Performance of strawberry cultivars in ecologically-based system*. - Cadernos de Agroecologia, 10: 5.

CHANDRA S., CHAKRABORTY N., DASGUPTA A., SARKAR J., PANDA K., ACHARYA K., 2015 - *Chitosan nanoparticles: A positive modulator of innate immune responses in plants*. Sci. Rep., 5: 15195-15195.

DI PIERO R.M., GARDA M.V., 2008 - *Quitosana reduz a severidade da antracnose e aumenta a atividade de glucanase em feijoeiro-comum.* - Pesqui. Agropecu. Bras., 43: 1121-1128.

DURRANT W.E., DONG X., 2004 - *Systemic acquired resistance*. - Annual Review of Phytopathology, 42: 185-209.

EHRET D.L., MENZIES J.G., BOGDANOFF C., UTKHEDE R.S., FREY B., 2002 - *Foliar applications of fertilizer salts inhibit powdery mildew on tomato.* - Can. J. Plant Pathol., 24: 437-444.

EL-MINIAWY S.M., RAGAB M.E., YOUSSEF S.M., METWALLY A.A., 2013 - *Response of strawberry plants to foliar spraying of chitosan*. - Res. J. Agric. & Biol. Sci., 9: 366-372.

EL-TANANY M.M., HAFEZ M.A., AHMED G.A., ABD EL-MAGEED M.H., 2018 - *Efficiency of biotic and abiotic inducers for controlling tomato early blight disease*. - Middle East J. Agric. Res., 7: 650-670.

GREWAL A., ABBEY L., GUNUPURU L.R., 2018 - *Production, prospects and potential application of pyroligneous acid in agriculture*. - J. Anal. Appl. Pyrol., 135: 152-159.

HAMZA A., MOHAMED A., DERBALAH A., 2017 - *Chemical inducers for resistance induction against powdery mildew of cucumber under greenhouse conditions*. - Acta Phytopathol. Entomol. Hung., 52: 49-60.

HEMM M.R., RIDER S.D., OGAS J., MURRY D.J., CHAPPLE C., 2004 - *Light induces phenylpropanoid metabolism in* Arabidopsis *roots*. - Plant J., 38: 765-778.

HYODO H., KURODA H., YANG S.H., 1978 - *Introduction of PAL and increase in phenolics in lettuce in relation the development*. - Plant Physiol., 62: 31-35.

HYODO H., YANG S.F., 1971 - *Ethylene-enhanced synthesis of phenylalanine ammonia lyase in pea seedlings*. - Plant Physiol., 47: 765-770.

JUNG K.H., 2007 - *Growth inhibition effect of pyroligneous acid on pathogenic fungus,* Alternaria mali, *the agent of* Alternaria blotch *of apple*. - Biotechnol. Bioproc. E., 12: 318-322.

KASHIAP P.L., DHIMAN J.S., 2009 - *Induction of resistance in cauliflower against alternaria blight using potassium and phosphonic salts*. - AAJBB, 3: 66-70.

KATIYAR D., HEMANTARANJAN A., SINGH B., 2015 - *Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review*. - Indian J. Plant Physiol., 20: 1-9.

KOSOSKI R.M., FURLANETTO C., TOMITA C.K., CAFÉ FILHO A.C., 2001 - *Efeito de fungicidas em Colletotrichum acutatum e controle da antracnose do morangueiro*. - Fitopatol. Bras., 26: 662-666.

KUMARASWAMY R.V., KUMARI S., CHOUDHARY R.C., PAL A., RALIYA R., BISWAS P., SAHARAN V., 2018 - *Engineered chitosan based nanomaterials: Bioactivity, mechanisms and perspectives in plant protection and growth.* - Int. J. Biol. Macromol., 113: 494-506.

MANANDHAR H.K., LYNGS J.H.J., MATHUR S.B., SMEDEGAARD-PETERSEN V., 1998 - *Resistance to rice blast induced by ferric chloride, di-potassium hydrogen phosphate and salicylic acid*. - Crop Prot., 17: 323-329.

MASUM S., MALEK M., MANDAL M., HAQUE M., AKTHER Z., 2013 - *Influence of plant extracted pyroligneous acid on transplanted aman Rice*. - J. Expt. Biosci., 4: 31-34.

MAUCH-MANI B., MÉTRAUX J.P., 1998 - *Salicylic acid and systemic acquired resistance to pathogen attack*. - Ann. Bot., 82: 535-540.

MUKTA J.A., RAHMAN M., SABIR A.A., GUPTA D.R., SUROVY M.Z., RAHMAN M., ISLAM M.T., 2017 - *Chitosan and plant probiotics application enhance growth and yield of strawberry*. - Biocatal Agric. Biotechnol., 11: 9-18.

MUNGKUNKAMCHAO T., KESMALA T., PIMRATCH S., TOOMSAN B., JOTHITYANGKOON D., 2013 - *Wood vinegar and fermented bioextracts: natural products to enhance growth and yield of tomato* (Solanum lycopersicum *L.).* - Sci. Hortic., 154: 66-72.

OROBER M., SIEGRIST J., BUCHENAUER H., 2002 - *Mechanisms of phosphate-induced disease resistance in cucumber*. - Eur. J. Plant Pathol., 108: 345-353.

PICHYANGKURA R., CHADCHAWAN S., 2015 - *Biostimulant activity of chitosan in horticulture*. - Sci. Hortic., 196: 49-65.

PIMENTA A.S., FASCIOTTI M., MONTEIRO T.V.C., LIMA K.M.G., 2018 - *Chemical composition of pyroligneous acid obtained from* Eucalyptus *GG100 clone*. - Molecules, 23: 1-12.

PORTO F.G.S., 2011 - *Caracterização de quitosana em ácido pirolenhoso destilado com potencial uso como coberturas protetoras*. - Dissertação, Universidade Federal de Pelotas (in Portuguese).

PORTO F.G.S., CAMPOS A.D., GARCIA I.T.S., 2019 - *Distilled pyroligneous liquor obtained from* Eucalyptus grandis *and chitosan: physicochemical properties of the solution and films*. - Environ. Sci. Pollut. R., 26: 672-683.

PRASANNATH K., 2017 - *Plant defense-related enzymes against pathogens: A Review*. - Int. J. Agric. Sci., 11:

38-48.

PRASANNATH K., DHARMADASA K.N.P., DE COSTA D.M., HEMACHANDRA K.S., 2014 - *Variations of incidence, types of virus diseases and insect vector populations of tomato (*Solanum lycopersicum *L.), grown in different agroecological regions of Sri Lanka under two crop management systems*. - J. Sustain. Trop. Agric. Res., 25: 376-395.

REUVENI R., DOR G., RAVIV M., REUVENI M., TUZUN S., 2000 - *Systemic resistance against* Sphaerotheca fuliginea *in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate*. - Crop Prot., 19: 355-361.

REUVENI R., REUVENI M., 1998 - *Foliar-fertilizer therapy-a concept in integrated pest management*. - Crop Prot., 17: 11-118.

REUVENI R., REUVENI M., AGAPOV V., 1996 - *Foliar sprays of NPK fertilizers induce systemic protection against* Puccinia sorghi *and* Exserohilum turcicum *and growth response in maize.* - Eur. J. Plant. Pathol., 102: 339-348.

SALACHNA P., ZAWADZIŃSKA A., 2014 - *Effect of chitosan on plant growth, flowering and corms yield of potted freesia*. - J. Ecol. Eng., 15: 97-102.

SHEHATA S.A., FAWZY Z.F., EL-RAMADY H.R., 2012 - *Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions*. - Aust. J. Basic Appl. Sci., 6: 63-71.

SOUZA J.L.S., SILVA V.B., CAMPOS G.A.D., LUN R.G., 2018 - *Antimicrobial potential of pyroligneous extracts - asystematic review and technological prospecting*. - Braz. J. Microbiol., 49: 128-139.

THAKKER J.N., PATEL S., DHANDHUKIA P.C., 2012 - *Induction of defense-related enzymes in banana plants: effect of live and dead pathogenic strain of* Fusarium oxysporum *f. sp. Cubense*. - ISRN Biotechnol., 601303, pp. 6.

THEAPPARAT Y., CHANDUMPAI A., FAROONGSARNG D., 2018 - *Physicochemistry and utilization of wood vinegar from carbonization of tropical biomass waste*. - In: SUDARSHANA P., M. NAGESWARA-RAO, and J.R. SONEJI (eds.) *Tropical Forests.* IntechOpen Linited, London, UK.

UTHAIRATANAKIJ A., SILVA J.A.T, OBSUWAN K., 2007 - *Chitosan for improving orchid production and quality*. - Orchid Sci. Biotech., 1: 1-5.

VAN DEN BROEK L.A.M., KNOOP R.J.I., KAPPEN F.H.J., BOERIU C.G., 2015 - *Chitosan films and blends for packaging material*. - Carbohydr. Polym., 116: 237-242.

VANITHA S.C., NIRANJANA S.R., UMESHA S., 2009 - *Role of phenylalanine ammonia lyase and polyphenol oxidase in host resistance to bacterial wilt of tomato*. - J. Phytopathol., 157: 552-557.

VENTURA-AGUILAR R.I., BAUTISTA-BAÑOS S., FLORES-GARCÍA G., ZAVALETA-AVEJAR L., 2018 - *Impact of chitosan based edible coatings functionalized with natural compounds on* Colletotrichum fragariae *development and the quality of strawberries*. - Food Chem., 262: 142-149.

WEI Q., MA X., DONG J., 2010 a - *Preparation, chemical constituents and antimicrobial activity of pyroligneous acids from walnut tree branches*. - J. Anal. Appl. Pyrol., 87: 24-28.

WEI Q., MA X., ZHAO Z., ZHANG S., LIU S., 2010 b - *Antioxidant activities and chemical profiles of pyroligneous acids from walnut Shell.* - J. Anal. Appl. Pyrol., 88: 149-154.