INTERNATIONAL JOURNAL OF PEST MANAGEMENT, 2016
http://dx.doi.org/10.1080/09670874.2016.1252478

Taylor & Francis
Taylor & Francis Group

Suppression of seed-borne *Alternaria arborescens* and growth enhancement of wheat with biorational fungicides

Analía Perelló[a], Gladys Lampugnani[b], Cecilia Abramoff[b], Alan Slusarenko[c] and Gustavo Dal Bello[d]

[a]CIDEFI-CONICET (Centro de Investigaciones de Fitopatología)-Facultad de Ciencias Agrarias y Forestales de la Universidad Nacional de La Plata, Buenos Aires, Argentina; [b]Curso de Terapéutica Vegetal; [c]Department of Plant Physiology (BioIII), RWTH Aachen University, Aachen, Germany; [d]CIDEFI-CIC FCAyF, UNLP

ABSTRACT

Alternaria spp. are among the major fungal contaminants of wheat grain under postharvest and storage conditions, where *A. arborescens* was recently detected as a new member of this complex in Argentina causing black point. The aim of this study was to assess the potential of some biorational agents to control *A. arborescens* and their plant growth promoting of wheat. Seed treatments with spore suspensions of *Trichoderma harzianum* and *Eppicoccum nigrum*, extracts from *Lippia alba* and garlic, sodium bicarbonate, salicylic acid (SA), potassium chloride and dibasic sodium phosphate (SP) were applied to grains of wheat cultivar BIOINTA 1004 before their inoculation with the pathogen. After 7 days, seed germination and infection, necrotic symptoms on emerged seedlings and fresh weight were evaluated. Remarkable results were obtained with *L. alba*, SA and SP treatments that reduced symptoms markedly compared with the control. Interestingly, necrosis of radicles was significantly reduced by the application of all treatments tested. Moreover, fresh weight of seedlings was significantly increased with the application of the two antagonists, diluted garlic juice and the three tested salts in comparison with controls. Therefore, a positive role as growth promoters can be elucidated. It is concluded that compounds here tested have potential as ecofriendly alternatives to control seed-borne *Alternaria* fungi of wheat.

ARTICLE HISTORY
Received 1 April 2016
Accepted 19 October 2016

KEYWORDS
Wheat; *Alternaria*; seed-borne-fungi; biofungicides

Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop in Argentina grown extensively in different parts of Buenos Aires Province. From seed germination to harvest, wheat is attacked by a great number of fungi, which under certain climatic conditions significantly reduce the yield and quality of the crop. Some of the fungal diseases are seed-borne and transmitted through seeds (Mathur & Cunfer 1993; Agrios 2005; Fakhrunnisa et al. 2006). Of the various fungal organisms associated with wheat, members of *Alternaria* complex reduce germination and vigor of wheat seed and cause seedling blight disease in Argentina (Perelló & Larrán 2013; Perelló et al. 2015). *Alternaria arborescens* is a ubiquitous fungus that can be found in many kinds of plants such as wheat (Patriarca et al. 2007). Inoculations on wheat grains in Argentina showed the pathogenicity of isolates and symptoms of discoloration, blight and spots on seedlings leaves, radicular necrosis and weakness of emerged plants (Perellò et al. 2015). Moreover, *A. arborescens* can produce several mycotoxins, such as alternariol, alternariol monomethyl ether, tenuazonic acid and phytotoxins, such as the AAL toxins (Gargouri-Kammoun et al. 2014; Vaquera et al. 2014).

Planting cereal seeds free of seed-borne pathogens is the primary means of limiting the introduction of pathogens into a field. Planting infected seed may also result in widespread distribution of disease within the crop, and allows for an increased number of initial infection sites from which the disease can spread. Management approaches of seed-borne fungi have been mainly focused on the use of synthetic fungicides. However, their efficacy can be limited by the rapid development of resistance of the pathogen to these compounds. Chemicals applied as either seed dressing or spray, such as Abendazim, Benomyl, Benomyl + copper sulfate, probenazole, thiabendazole and pyroquilon fungicides have been used in different rice growing countries to manage the disease. However, such chemicals are expensive, not easily available to small-scale farmers and have detrimental effects on the environment, farmer and consumer health, beneficial predators and parasitoids. In order to use integrated pest and disease management in the protection of cereals, new strategies are considered that match the growing concern about the consequences of the use of fungicides on both health and environment (Deverall 1995; Arslan et al. 2009). Efforts have, therefore, been made to allow the use of generally recognized as safe (GRAS) compounds (natural plant products or chemicals), and microbial

CONTACT Analía Perelló aeperello@agro.unlp.edu.ar

antagonists as alternative fungicides to manage plant diseases (Dionisio et al. 2004; Arya & Mónaco 2007; Arya & Perelló 2010).

Plant extracts have been known for their medicinal and antimicrobial properties since ancient times (Lalhita et al. 2010). Gurjar et al. (2012) and Amadioha (2000) reported that plant extracts from *Azadirachta indica* A. Juss., *Allium sativum* L., *Eucalyptus globulus* L., *Curcuma longa* L., *Nicotiana tabacum* L., and *Zingiber officinale* Rosc inhibited growth of pathogens such as *Alternaria alternata*. Natural chemicals from plants offer a greater scope than synthetic chemicals as they are relatively safe, easily biodegradable and eco-friendly (Dal Bello & Sisterna 2010). Furthermore, botanicals are cheap, readily available and cost effective in developing countries where synthetic fungicides are scarce and expensive for resource-poor farmers (Mossini et al. 2004; Hasan et al. 2005). The use of some non-hazardous chemicals is another potential means for protecting crop plants from fungal diseases. Inorganic salts including bicarbonate and phosphate have widely been used in the food industry as preservatives, pH regulators, and antimicrobial agents and are known to have low mammalian toxicity (Olivier et al. 1998; Mecteau et al. 2002; Palou et al. 2002; Mills et al. 2004; Mecteau et al. 2008; Arslan et al. 2009). These salts are generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA) (FDA 2009). In addition, several of them were reported to have broad inhibitory effect against a range of fungal plant pathogens (Montville & Shih 1991; Olivier et al. 1998; Türkkan & Erper 2014). Mann et al. (2004) found 50% reduction in the leaf area of winter wheat plants affected by *Septoria tritici* leaf blotch after foliar application of potassium chloride, compared with untreated controls. Similarly, it is known that exogenous application of salicylic acid would activate systemic acquired resistance (SAR) against phytopathogens. For instance, Mandal et al. (2009) demonstrated that exogenous application of salicylic acid through root feeding and foliar spray could induce resistance against *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Salicylic acid added at the stem base of tobacco plants resulted in significant protection against blue mold caused by *Peronospora tabacina* (Zhang et al. 2002). Earlier work has shown that challenge inoculation of salicylic acid-treated tomato plants using conidia of *Alternaria solani* resulted in a higher reduction of lesions per leaf and the blighted leaf area as compared with control plants not receiving salicylic acid (Spletzer & Enyedi 1999).

Regarding biological control of plant diseases, the use of microbial antagonist is currently receiving increased research effort to enhance the sustainability of agricultural production systems and to reduce the use of chemical pesticides. Consequently, the possibility of controlling pathogenic fungi by antagonistic microorganisms has been widely explored (Zhou & Reeleder 1990; Biggs & Alm 1991; Larena et al. 2004). In previous studies in Argentina, various fungal microbes have shown potential as biocontrol agents in the management of foliar wheat diseases. Among them, species of *Trichoderma* and *Epicoccum* are highly efficient antagonists and could become good biocontrol agents (Perelló & Mónaco 2007).

Accordingly, this investigation examines: (1) the potential of some antagonists (*Trichoderma harzianum* Rifai and *Epicoccum nigrum* Link (syn. *Epicoccum purpurascens* Ehrenb. ex Schlecht.), botanicals (*Lippia alba* and *A. sativum* extracts) and substances categorized as GRAS (sodium bicarbonate, salicylic acid, potassium chloride and dibasic sodium phosphate) for the control of *A. arborescens* on wheat seeds; and (2) the effect of the above agents on seed germination, plant biomass and growth.

Materials and methods

Seed material

Wheat cultivar BioINTA 1004 was used throughout in this study. The field-collected seed samples were transported to the Centro de Investigaciones de Fitopatología (CIDEFI) laboratory at Facultad de Agronomía de la Universidad Nacional de La Plata (UNLP) and stored at 4 °C for further testing.

Selected fungal isolates and culture conditions

The culture of *A. arborescens* Aa 2113 used in this study was previously characterized by its morphobiometrical and molecular features (Perelló et al. 2015) and stored at 5 °C in the refrigerator at the culture collection of CIDEFI-FCAyF UNLP, Argentina. The fungus was transferred to Petri dishes containing potato carrot agar (PCA) (20 g potato, 20 g carrot, agar powder and 800 mL of distilled water). The Petri dishes were sealed and incubated at 22 °C–24 °C for 5 days in alternating cycles of 16 h light and 8 h darkness to induce growth of *A. arborescens* and were kept upside down. The pure cultures of the isolate were grown on V8 agar (3 g calcium carbonate, 200 mL V-8 juice and 20 g agar in 800 mL of distilled water) for 14 days to induce sporulation. The PCA was used for producing *A. arborescens* conidia. The Petri dishes containing *A. arborescens* inoculum were stored in the refrigerator at 5 °C for further use.

Cultures of the antagonists *T. harzianum* (Th 56) and *E. nigrum* (En 0314) were obtained from wheat grains micoflora and maintained on potato dextrose agar (PDA) at the culture collection of CIDEFI-FCAyF UNLP at 5 °C.

Before treatments, fungal isolates were grown on PCA (*A. arborescens*) and PDA (*T. harzianum* and *E. nigrum*) in a growth chamber at 22 °C–24 °C for

14 days in alternating cycles of 16 h light and 8 h darkness under fluorescent and near ultra violet (NUV) (365 nm) lighting. Then, 5 mL distilled water containing 0.0125% Tween 80 were added to sporulating cultures, and the surface was gently rubbed with a rubber scraper. The conidial suspensions were vortexed for 30 sec and filtered through cheesecloth to remove mycelial debris. Spore concentration was counted using a hemocytometer and adjusted to 1×10^6 (*A. arborescens*) or 1×10^8 (antagonists) conidia/mL by the addition of sterilized distilled water.

Plant extracts

Plants species used as sources of extracts in this study were *L. alba* and *A. sativum* (garlic). Essential oils were extracted from leaves of *L. alba* chemotype carvone collected from fields located in La Plata, Province of Buenos Aires, Argentina during March 2014 to June 2014. The leaves were dried in absence of sunlight at room temperature (25 ± 5 °C) and grounded in a domestic mixer. The dried powdered material was hydro-distilled in Clevenger apparatus to yield essential oils and then dehydrated with anhydrous sodium sulfate. *L. alba* essence concentration was 5% formulated in distilled water with 5% polyethyleneglicol oleate as emulsifier. Garlic extract was obtained from fresh garlic bulbs desegmented and deskinned. The segments were sterilized by washing with 3% sodium hypochlorite for 2 min followed by five to six washings with distilled water. They were then ground in aseptic pestle and mortar with 100 mL of distilled water. After that, the juice was filtered through Whatman No. paper and then sterilized with Millipore filters of 0.45 mμ for use in the treatments (Alcalá de Marcano et al. 2005). The extracts were then kept at 4 °C for 2 days until their use.

GRAS compounds

GRAS substances used in this study (concentrations related to previous studies) included sodium bicarbonate (84 mg/L), salicylic acid (45.6 mg/L), potassium chloride (3.7 g/L) and dibasic sodium phosphate (7.2 g/L) diluted in distilled water. Aliquot (50 mL) of each solution was placed into individual small beakers just before the seeds were treated.

Treatment of seeds

Four hundred seeds of the wheat variety BioINTA 1004 were surface disinfected by inmersion in 1% sodium hypochlorite (NaOCl) solution for 5 min and washed three times with distilled water. For screenings, disinfected seeds were treated with the bioprotectants prior to be artificially infested with the pathogen. Botanicals (*Lippia* and garlic extracts), chemical compounds (salicylic acid, sodium bicarbonate, potassium chloride and sodium phosphate) and the two antagonists (*T. harzianum* and *E. nigrum*) were applied to wheat seeds by the submersion technique during 30 min. After treating, the grains were air dried at 20 °C–22 °C for 12 h under a laminar flow hood. Subsequently, the seeds were submersed in 20 mL spore suspension (1×10^6) of *A. arborescens* for 10 min, excess liquid was poured off and the coated seeds were dried for 2 h under a stream of sterile air provided by a laminar air flow bench. The treatments consisted of wheat grain treated with *A. arborescens*; *A. arborescens* + *L. alba*; *L. alba*; *A. arborescens* + *E. nigrum*; *E. nigrum*; *A. arborescens* + *T. harzianum*; *T. harzianum*; *A. arborescens* + salicylic acid; salicylic acid; *A. arborescens* + garlic; garlic; *A. arborescens* + sodium bicarbonate; sodium bicarbonate; *A. arborescens* + potassium chloride; potassium chloride; *A. arborescens* + sodium phosphate; sodium phosphate; and sterile distilled water. Wheat seeds soaked in distilled water and *A. arborescens* alone were included in the experiments as negative and positive controls, respectively.

The grains thus treated were placed in plastic trays following ISTA rules (1993, 2008). Two hundred seeds in four replicates (50 seeds per replicate) were tested per each extract/antagonist/chemical. Treated seeds were put into plastic trays and kept under screen house conditions (22 °C–25 °C). The effect of treatments on seed germination was evaluated after 7 days of incubation by counting the number of normal seedlings, abnormal seedlings and dead seeds as recommended by International Seed Testing Association Seedling Evaluation Handbook (Mathur & Kongsdal 2004). Also, spotted grains, necrosis of radicles and coleoptiles, and fresh weight of emerged seedlings were analyzed in order to evaluate the effect of the extracts on seedling growth and vigor

Germination was considered present when the radical protrudes reached 2–4 mm. Seedlings were monitored for appearance of symptoms during two weeks. Fourteen days after sowing, the condition (visual appearance) of seedlings in each treatment was recorded (Shafique et al. 2007). Seedlings emergence and seedling mortality were evaluated and the percent of emerged seedlings was calculated. The presence of visible symptoms (seed rot, germination failure and infection or death of emerged seedlings) caused by the pathogen was registered by examining the seeds under stereo-binocular microscope.

Fresh weight was recorded based on individual plants carefully removed from each tray 14 days after treatment. Fresh weight measurements were made on a laboratory scale (Mettler AE 163, Switzerland).

Data analysis

Experiments were arranged in a completely randomized design and repeated twice with similar results. All data collected were analyzed based on analysis of

variance (ANOVA) model using GenStat statistical software. Means were separated by least significant difference (LSD) test at $P = 0.05$. Analysis of data of the two experimental runs invariably resulted in treatment effects in the same significance classes. Similarity among experimental runs allowed combining of data for ANOVA.

Results

The germination of BioINTA seeds inoculated with *A. arborescens* was of 86.6% (Figure 1). This value was not statistically different from the obtained from grains treated only with distilled water (80%). According to these results, the fungus does not affect the germination of wheat grains. Two treatments, salicylic acid and *Lippia* were phytotoxic to the concentration tested (100% grains without emergence). Interestingly, *E. nigrum* applied to grain, significantly increased the germination percentage in comparison with the control (treatment with distilled water) (Figure 1).

Grains of the cultivar BioINTA 1004 inoculated with *A. arborescens* reached almost 100% infection 7 days after inoculation with the fungus (Figure 2). Seedlings that emerged from the seeds inoculated with *A. arborescens* showed weakness, chlorosis of coleoptiles or reduced length. Necrotic symptoms were consistent with typical symptoms descriptions as "black point" of wheat. The necrosis observed in the grains was partial or total in some cases. Abundant mycelium of *A. arborescens*, light gray or dark in color, cottony, was observed onto the surface of infected seeds. There were significant differences ($P \leq 0.05$) between the control and all the treatments applied. These results indicate that the antagonists, plant extracts and salts tested against *A. arborescens* had a positive effect in inhibiting infection by the fungus. Considering the necrotic symptoms observed, all treatments significantly reduced symptoms. Previous assays using garlic extract to reduce seed borne fungi of wheat grains in agreement with the present results (Perelló et al. 2013a, 2013b). The interest in the replacement of synthetic fungicides with, e.g. plant-derived products, is especially related to the biodegradability of natural products. Extracts showed antifungal activity and a documented effect was shown against plant pathogens *in situ* (Tripathi & Dubey 2004; Yeni 2011). Remarkable results were obtained with *L. alba*, salicylic acid

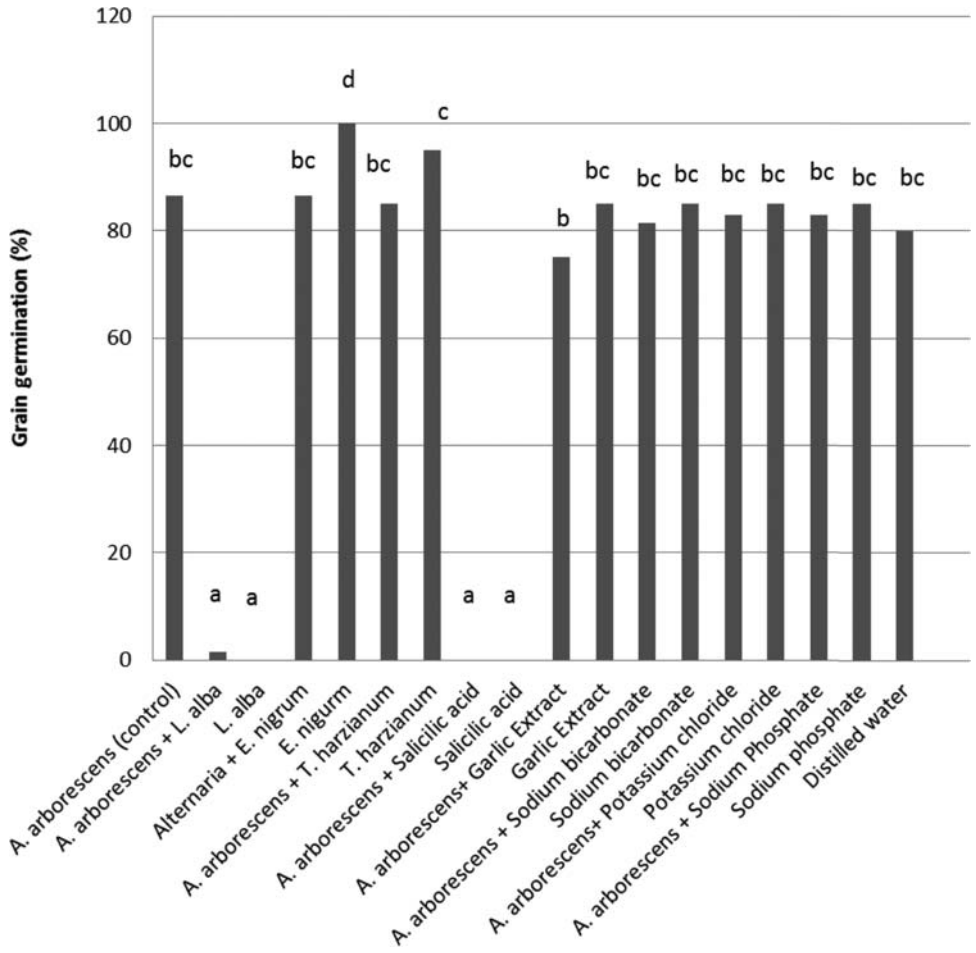

Figure 1. Grain germination (%) of wheat cv BioINTA 1004 inoculated with *Alternaria arborescens* alone (control) and in combination with *Lippia alba*, *Epicoccum nigrum*, *Trichoderma harzianum*, salicylic acid, garlic extract, sodium bicarbonate, potassium chloride, sodium phosphate and distilled water. Columns denoted by a different letter indicate significantly different values at $P \leq 0.05$ in one-way ANOVA.

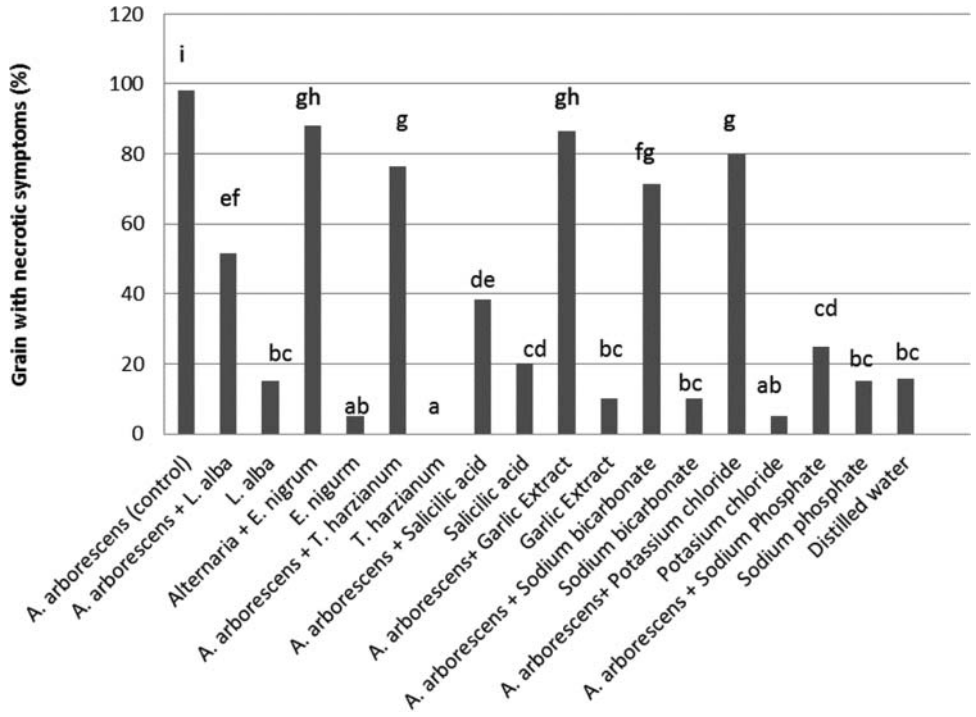

Figure 2. Grain with necrotic symptoms of cv BioINTA 1004 inoculated with *A. arborescens* alone (control) and in combination with *L. alba, E. nigrum, T. harzianum*, salicylic acid, garlic extract, sodium bicarbonate, potassium chloride, sodium phosphate and distilled water. Columns denoted by a different letter indicate significantly different values at $P \leq 0.05$ in one-way ANOVA.

and sodium phosphate treatments that in combination with *Alternaria*, reduced more than 50%. Also noteworthy is the effect of *T. harzianum* and potassium chloride improving the health of naturally infected grains. On the other hand, necrosis, rot, and shortening of radicles from the emerged infected seedlings were observed after 7 days of inoculation with the pathogen. These symptoms were observed in the 33.3% of grains in the control treatment (*Alternaria*). This percentage was significantly reduced with the application of the two antagonists (*Epicoccum* and *Trichoderma*), plant extracts (*L. alba* and garlic) and the tested salts (sodium bicarbonate, potassium chloride, sodium phosphate). Fresh weight of 14 days old seedlings was increased significantly with the application of the two potential antagonists (*Epicoccum* and *Trichoderma*) and the three tested salts (sodium bicarbonate, potassium chloride and sodium phosphate) applied in combination with *Alternaria* (Figure 3). These substances increased the seedling growth (leaf and root fresh weight/plant) in applications alone when compared with the value of grains treated with distilled water only (Figure 3). Therefore, a positive role as growth promoters can be elucidated.

Discussion and conclusion

Among the antagonists tested, *E. nigrum* is an ubiquitous hyphomycete which grows saprophytically on many substrates. *E. nigrum* produces many secondary metabolites such as antibiotics (Tuttobello et al. 1969; Deffieux et al. 1976; Baute et al. 1978) and carotenoid pigments (Foppen & Gribanovski-Sassu 1968). The quantity of the exuded carotenoid pigments, as well as the dynamics of the growth of *E. nigrum* (Tuttobello et al. 1969) are diverse, and as such they affect growth and development of other fungi. *E nigrum* has been described as an organism antagonistic to several fungal pathogens, such as *Fusarium* spp. (Ogórek & Plaskowska 2011), *Sclerotinia sclerotiorum* (Zhou & Reeleder 1989; Pieckenstain et al. 2001), *Colletotrichum gloeosporioides* (Pandey et al. 1993), *Colletotrichum kahawae* (Guerra-Guimarães et al. 2007), *Botrytis cinerea*, *Monilinia laxa* (Larena et al. 2004) and to some bacteria (Punja 1997). Similarly, a significant increase in seedling growth parameters (seedling length and fresh and dry weights) was previously observed in seedlings treated with *E. purpurascens* compared to pathogen-treated seedlings (Koubt & Ali 2010). On the other hand, it is well known that *Trichoderma* species can be used as biocontrol and plant growth promoting agents. In this study, *T. harzianum* isolates evaluated for their growth promotion effects on wheat in greenhouse experiments increased wheat length, root dry weight and shoot dry weight (Kukuc 2014).

Inorganic salts, such as bicarbonates, chlorides and phosphates, have been shown to have activity against certain pathogens (Reuveni et al. 1993; Aharoni et al. 1997; Heckman 1998; Reuveni et al. 1998). Foliar-applied sodium bicarbonate was shown to inhibit the germination of powdery mildew *Podosphaera xanthii* on cucumbers and to cause abnormal conidial formation with reduced pathogenicity (Homma et al. 1981). Reuveni et al. (1998) showed that pre and

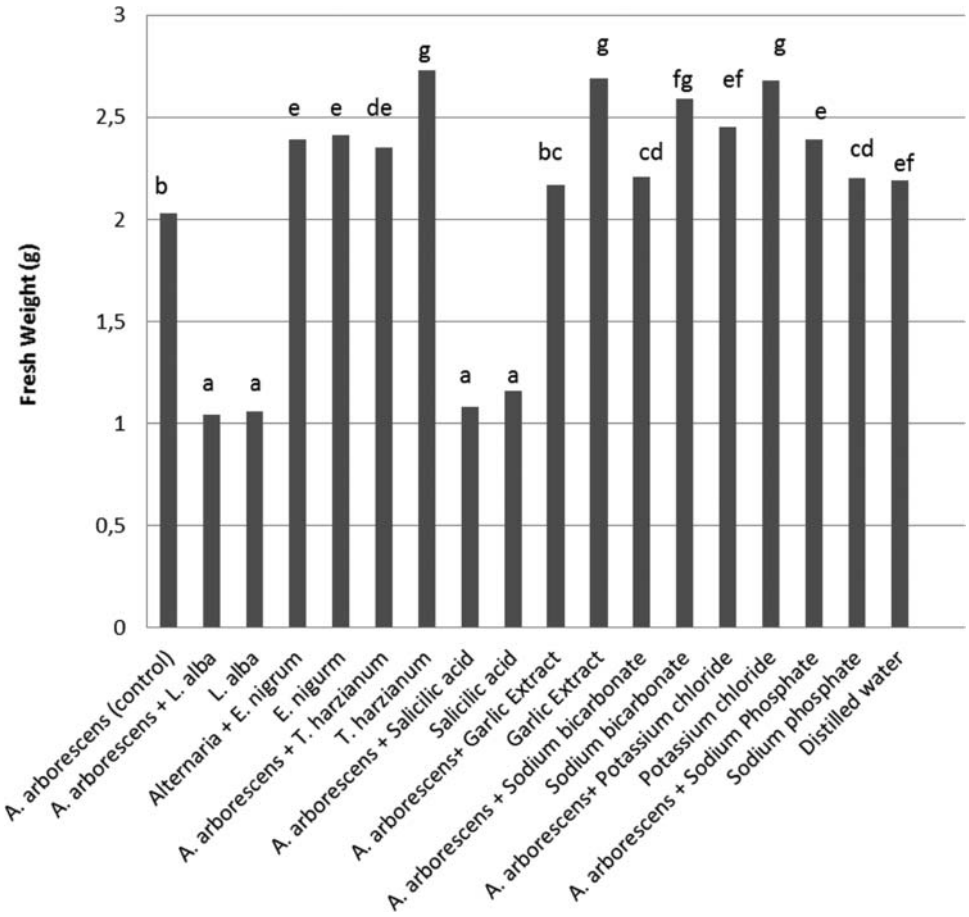

Figure 3. Fresh weight (g) of 14 days old seedlings of wheat cv BioINTA 1004 inoculated with A. arborescens alone (control) and in combination with *L. alba, E. nigrum, T. harzianum*, salicylic acid, garlic extract, sodium bicarbonate, potassium chloride, sodium phosphate and distilled water. Columns denoted by a different letter indicate significantly different values at $P \leq 0.05$ in one-way ANOVA.

postinoculation treatments with monopotassium phosphate inhibited powdery mildew (*Leveillula taurica*) on peppers, significantly reducing sporulation 18 days after inoculation compared with untreated control plants. Cook et al. (1993) showed foliar-applied potassium chloride to be effective against powdery mildew [*Blumeria graminis* (syn. *Erysiphe graminis*)] and *S. tritici* in a field experiment. Similarly, the efficacy of sodium salts as possible alternatives to synthetic fungicides for the control of a broad group of phytopathogens was demonstrated including many species of *Alternaria* like *A. cucumerina,* (Ziv & Zitter 1992), *A. alternata,* (Mills et al. 2004), and *A. solani,* (Abdel-Kader et al. 2012). In this study, salicylic acid significantly reduced the symptoms caused by *A. arborescens* on infected wheat seeds. Similarly, the population of all fungi present in naturally infected seed samples of *Momordica charantia* (bitter gourd) was reduced by using salicylic acid (Shakoor et al. 2011). There have been several reports on the efficacy of salycilic acid as protectant against fungal diseases, including soilborne fungi, attributed to its central role in the SAR. (Yalpani et al. 1991; Nie 2006; Mandal et al. 2009). Accordingly, our results might be a case of salicylic acid-dependent systemic acquired resistance. However, salycilic acid at the concentration used in this research (0.33 mM), inhibited seed germination. The exogenous application of salycilic acid to plants results in a range of physiological responses including phytotoxicity symptoms (War et al. 2011; Durango et al. 2013) and inhibition of both seed germination (Leslie & Romani 1988) and plant growth (Raskin 1995). It was reported that salicylic acid inhibits seed germination in maize and *Arabidopsis* (Khan & Ungar 1986; Guan & Scandalios 1995). Xie et al. (2007) showed that salicylic acid also blocks barley seed germination and post-germination growth in a dosage dependent manner. Salicylic acid might exert its suppressing activity on seed germination through induction of a WRKY gene repressor which blocks gibberellic acid induction of α-amylase expression. The production of this enzyme in aleurone layers is believed to be essential for seed germination in cereal grains (Xie et al. 2007). Our study showed that aqueous extracts of *L. alba* inhibits seed germination of wheat. It could be related to the phytotoxic activity of carvone, the main component of the chemotype here tested. Phytotoxicity effects of some naturally occurring monoterpenes, such as carvone on cultivated plants including wheat seeds and other cereals, have been observed (Ibrahim et al. 2001).

The current findings suggest that the tested substances can be used as seed treatments for the control of *A. arborescens* in wheat seeds and for improving wheat seedling growth. Moreover, they could be safe and eco-friendly fungicides compared to synthetic chemicals. More studies are therefore, needed to determine the most effective formulation against *A. arborescens*. Identification and characterization of the active compounds from currently tested plant extracts and their role in wheat seedborne diseases control are also needed. Further research on the range of activity of GRAS substances, plant extracts and antagonists for control of other wheat pathogens is also recommended.

It is concluded that compounds here tested, particularly the two antagonists – *T. harzianum* and *E. nigrum* –, diluted garlic juice and the tested salts sodium bicarbonate, potassium chloride and dibasic sodium phosphate have potential as biofertilizers and eco-friendly alternatives to conventional fungicide treatments to control seed-borne fungi of wheat. These results generally indicated that, salicylic acid and *L. alba* extract used can be phytotoxic to wheat seedlings depending on the concentration assayed. More studies are, therefore, needed to confirm the current findings and to determine the most effective formulation against *A. arborescens*.

Acknowledgements

The authors wish to thank UNLP Project 11A 224 and CONICET for financial support for this work. Also thanks to Cynthia Henning (Fitoquímica-FCAyF UNLP) for providing the *L. alba* extract.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by CONICET and UNLP.

References

Abdel-Kader MM, El-Mougy NS, El-Gammal NG, Abd-El-Kareem F, Abd-Alla MA. 2012. Laboratory evaluation of some chemicals affecting pathogenic fungal growth. J Appl Sci Res. 8:523–530.

Agrios G. 2005. Plant pathology. 5th ed. San Diego (CA): Academic Press. Elsevier Science Publishing Co Inc.

Aharoni Y, Fallik E, Copel A, Gil M, Grinberg S, Klein JD. 1997. Sodium bicarbonate reduces postharvest decay development on melons. Postharvest Biol Technol. 10:201–202.

Alcalá de Marcano D, Vargas N, Pire A. 2005. Efecto de extractos vegetales y fungicidas sintéticos sobre el crecimiento micelial *in vitro* de *Sclerotium rolfsii* y *Thielaviopsis basicola* [Effect of plant extracts and syntetic fungicides on the in vitro growth of Sclerotium rolfsii and Thielaviopsis basicola sis basicola]. Rev. Fac Agron (LUZ). 22:315–323.

Amadioha AC. 2000. Controlling rice blast in vitro and in vivo with extracts of *Azadirachta indica*. J Crop Prot. 19:287–290.

Arslan U, Kadır I, Vardar C, Karabulut OA. 2009. Evaluation of antifungal activity of food additives against soilborne phytopathogenic fungi. World J Microbiol Biotechnol. 25:537–543.

Arya A, Mónaco C. 2007. Seed borne diseases: eccofriendly management. New Dehli: Scientist Publishers Journal Dept.

Arya A, Perelló A. 2010. Management of fungal plant pathogens. London: CAB International;.

Baute MA, Deffieux G, Baute R, Neveu A. 1978. New antibiotics from the fungus *Epicoccum nigrum*. I. Fermentation, isolation and antibacterial properties. J Antibiot. 31:1099–1101.

Biggs AR, Alm GR. 1991. Response of peach bark tissues to inoculation with epiphytic fungi alone and in combination with *Leucostoma cincta*. Can J Bot. 70:186–191.

Cook JW, Kettlewell PS, Parry DW. 1993. Control of *Erysiphe graminis* and *Septoria tritici* on wheat with foliar-applied potassium chloride. J Sci Food Agric. 63:126.

Dal Bello G, Sisterna M. 2010. Use of plant extracts as natural fungicides in the management of seedborne diseases. In: Arya A, Perelló A, Editors. Management of fungal plant pathogens. London: Cabi; p. 51–66.

Deffieux G, Baute MA, Baute R, Filleau MJ. 1976. New antibiotics from the fungus *Epicoccum nigrum*. II. Epicorazine A: structure elucidation and absolute configuration. J Antibiot. XXXI:1102–1105.

Deverall BJ. 1995. Plant protection using natural defense systems of plants. In: Hammerschmidt R, Kuc J., editors. Advances in plant pathology. Vol. 11. Sydney: Academic Press; p. 211–228.

Dionisio G, Alvindia DG, Kobayashi T, Natsuaki KT, Tanda S. 2004. Inhibitory influence of inorganic salts on banana postharvest pathogens and preliminary application to control crown rot. J Gen Plant Pathol. 70:61–65.

Durango D, Pulgarin N, Echeverri F, Escobar G, Quiñones W. 2013. Effect of salicylic acid and structurally related compounds in the accumulation of phytoalexins in cotyledons of common bean (*Phaseolus vulgaris* L.) cultivars. Molecules. 18:10609–10628.

Fakhrunnisa MH, Hashmi M, Ghaffar A. 2006. Seed-borne mycoflora of wheat, sorghum and barley. Pak J Bot. 38:185–192.

FDA. 2009. Código de alimentos [Food code]. Recomendaciones de la administración de medicamentos y alimentos del servicio de salud pública de los estados unidos 2009 [Recommendations and Drug Administration food public health service of the United States 2009]. College Park (MD): Departamento de salud y servicios humanos de los Estados Unidos. Administración de Medicamentos y Alimentos; p. 20740.

Foppen FH, Grivanovski-Sassu O. 1968. Lipids produced by *Epicoccum nigrum* in submerged culture. Biochem J. 106:97–100.

Gargouri-Kammoun L, Bensassi F, Mnari-Hattab M, Rhouma A, Bacha H, Hajlaoui MR 2014. Identification of *Alternaria* species recovered from stored durum wheat kernels in Tunisia. Tunisian J Plant Prot. 9:119–129.

Guan L, Scandalios JG. 1995. Developmentally related responses of maize catalase genes to salicylic acid. Proc Natl Acad Sci USA. 92:5930–5934.

Guerra-Guimarães L, Azinheira HG, Martins AC, Silva MC, Gichuru EK, Varzea V, Bertrand B. 2007. Antagonistic interaction between *Epicoccum nigrum* and *Colletotrchum kahawae*, the causal agent of coffee berry disease. 21st International Conference on Coffee Science; 2006 Sep 11; Montpellier. p. 1284–1290.

Gurjar MS, Ali S, Akhtar M, Singh KS. 2012. Efficacy of plant extracts in plant disease management. J Agric Sci. 3:425–433.

Hasan MM, Chowdhury SP, Alam S, Hossain B, Alam MS. 2005. Antifungal effects of plant extracts on seed-borne fungi of wheat seed regarding seed germination, seedling healthy and vigor index. J Biol Sci. 8:1284–1289.

Heckman JR. 1998. Cornstalk rot suppression and grain yield response to chloride. J Plant Nutr. 21:149–155.

Homma Y, Arimoto Y, Misato T. 1981. Effect of sodium bicarbonate on each growth stage of cucumber powdery mildew fungus (*Sphaerotheca fuliginea*) in its life cycle. J Pestic Sci. 6:201–209.

Ibrahim MA, Kainulainen P, Aflatuni A, Tiilikkala K, Holopainen JK. 2001. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. Agric Food Sci. 10:243–259.

[ISTA] International Seed Testing Association. 2008. International Rules for Seed Testing. Secretariat, Zürichstrasse, Bassersdorf. Available from: http://www.seedtest.org

ISTA. 1993. International rules for seed treating. Proc Int Seed Testing Assoc. 13:200–520.

Khan MA, Ungar IA. 1986. Inhibition of germination in *Atriplex triangularis* seeds by application of phenols reversal of inhibition by growth regulators. Botanical Gazette. 147:148–151.

Koubt M, Ali E. 2010. Potential of *Epicoccum purpurascens* strain 5615 as a biocontrol agent of *Pythium irregulare* Root Rot in three leguminous plants. Mycobiology. 2010 38:286–294.

Kucuk C. 2014. Enhanced root and shoot growth of wheat (*Triticum aestivum* L.) by *Trichoderma harzianum* from Turkey. Pak J Biol Sci. 17:122–125.

Lalitha V, Raveesha KA, Kiran B. 2010. Antimicrobial Activity of *Solanum torvum* Swart. against important seed borne pathogens of Paddy. Iranica J Energy & Environ. 1:160–164.

Larena I, De Cal A, Melgarejo P. 2004. Solid substrate production of *Epicoccum nigrum* conidia for biological control of brown rot on stone fruits. Int J Food Microbiol. 94:161–167.

Leslie CA, Romaani RJ. 1988. Inhibition of the ethylene biosynthesis by salycilic acid. Plant Physiol. 88:833–837.

Mandal S, Mallick N, Mitra A. 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp.*lycopersici* in tomato. Plant Physiol Biochem. 47:642–649.

Mann Rl, Kettlewell PS, Jenkinson P. 2004. Effect of foliar-applied potassium chloride on *septoria* leaf blotch of winter wheat. Plant Pathology. 53:353–359.

Mathur SB, Cunfer BM. 1993. Seedborne diseases and seed health testing of wheat. Hellerup: Danish Government Institute of Seed Pathology for Developing Countries.

Mathur SB, Kongsdal O. 2004. Common laboratory seed healthy testing methods for detecting Fungi. 2nd ed. Bassersdorf: International Seed Testing Association (ISTA).

Mecteau MR, Arul J, Tweddell RJ. 2002. Effect of organic and inorganic salts on the growth and development of *Fusarium sambucinum*, a causal agent of potato dry rot. Mycol Res. 106:688–696.

Mecteau MR, Arul J, Tweddell RJ. 2008. Effect of different salts on the development of *Fusarium solani var. coeruleum*, a causal agent of potato dry rot. Phytoprotection. 89:1–6.

Mılls AAS, Platt HW, Hurta RAR. (2004). Effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens. Postharvest Biol Technol. 34:341–350.

Montvılle TJ, Shıh PL. 1991. Inhibition of mycotoxigenic fungi in corn by ammonium and sodium bicarbonate. J Food Prot. 54:295–297.

Mossini SAG, de Oliveira KP, Kemmelmeier C. 2004. Inhibition of patulin production by *Penicillium expansum* cultured with neem (*Azadirachta indica*) leaf extracts. Basic Microbiol. 44 :106–113.

Nie X. 2006. Salicylic acid suppresses potato virus Y isolate N:O-induced symptoms in tobacco plants. Phytopathology. 96:255–263.

Ogórek R, Plaskowska E. 2011. *Epicoccum nigrum* for biocontrol agents in vitro of plant fungal pathogens. Comm Appl Biol Sci. 76:671–697.

Olıvıer HDE, Halsth DE, Mızubutı ESG, Loria R. 1998. Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant Dis. 82:213–217.

Palou L, Usall J, Smılanıck JL, Aguılar MJ, Vınas I. 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Manag Sci. 58:459–466.

Pandey RR, Arora DK, Dubey RC. 1993. Antagonistic interactions between fungal pathogens and phylloplane fungi of guava. Mycopathologia. 124:31–39.

Patriarca A, Azcarate M, Terminiello L, Fernández Pinto V. 2007. Mycotoxin production by *Alternaria* strains isolated from Argentinean wheat. Int J Food Microbiol. 119:219–222.

Perelló A, Aulicino M, Martinelli C, Regueira M, Moreno V, Steinglein S. 2015. Caracterización morfocultural de nuevos grupos taxonómicos de *Alternaria* asociados a enfermedades del trigo en Argentina [Morphocultural characterizaion of new taxonomic groups of Alternaria associated to wheat diseases in Argentina]. Revista Ciencias Morfológicas. 17:1–15.

Perelló A, Gruhlke M, Slusarenko A. 2013. Effect of garlic juice on seed-borne fungi of wheat: seed germination, seedling health and vigor. J Plant Prot Res. 53:317–323.

Perelló A, Larrán S. 2013. Nature and effect of *Alternaria* spp complex from wheat grain on germination and diseases transmission. Pak J Bot. 45:1817–1824.

Perelló A, Mónaco C. 2007. Status and progress of biological control of wheat (*Triticum aestivum*) foliar diseases in Argentina. Fitosanidad. 11:85–105.

Perelló A, Noll U, Slusarenko, A. 2013. Efficacy in vitro of garlic extract to control fungal pathogens of wheat. J Med Plant Res. 7:1809–1817.

Pieckenstain FL, Bazzalo ME, Roberts AMI, Ugalde RA. 2001. *Epicoccum purpurascens* for biocontrol of *Sclerotinia* head rot of sunfower. Mycol Res. 105:77–84.

Punja ZK. 1997. Comparative efficacy of bacteria, fungi, and yeasts as biological control agents for diseases of vegetable crops. Can J Plant Pathol. 19:315–323.

Raskin I. 1995. Salicylic acid. In: Davies PJ, editor. Plant hormones and their role in plant growth and development. 2nd ed. Dordrecht: Kluwer Academic Publishers; p. 188–205.

Reuveni M, Agapov V, Reuveni R. 1993. Induction of systemic resistance to powdery mildew and growth increase in cucumber by phosphates. Biol Agric Hortic. 9:305–315.

Reuveni R, Dor G, Reuveni M. 1998. Local and systemic control of powdery mildew (*Leveillula taurica*) on pepper plants by foliar spray of mono-potassium phosphate. Crop Prot. 17:703–709.

Shafique S, Javaid A, Bajwa R, Sjafique S. 2007. Effect of aqueous leaf extracts of allelopathic trees on germination and seed-borne mycoflora of wheat. Pak J Bot. 39:2619–2624.

Shakoor S, Chohan S, Riaz A, Perveen R, Naz S, Abid Mehmood M, Saleem Haider M, Shakeel Ahmad S. 2011. Screening of systemic fungicides and biochemicals against seed borne mycoflora associated with *Momordica charantia*. Afr J Biotechnol. 10:6933–6940.

Spletzer ME, Enyedi AJ. 1999. Salicylic acid induces resistance to *Alternaria solani* in hydroponically grown tomato. Phytopathology. 89:722–727.

Tripathi P, Dubey NK. 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biol & Tech. 32:235–245.

Türkkan M, Erper I. 2014. Evaluation of antifungal activity of sodium salts against onion basal rot caused by *Fusarium oxysporum* f.sp. *cepae*. Plant Prot Sci. 50:19–25.

Tuttobello l, Foppen FH, Carilli A. 1969. Growth and pigmentation of *Epicoccum nigrum* in submerged culture. Appl Microbiol. 17:847–852.

Vaquera S, Patriarca A, Fernández Pinto V. 2014. Water activity and temperature effects on growth of *Alternaria arborescens* on tomato. Int J Food Microbiol. 185:136–139.

War AR, Paulraj MG, War MY, Ignacimuthu S. 2011. Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). Plant Signal Behav. 6:1787–1792.

Xie Z, Zhang ZL, Hanzlik S, Cook E. Shen QJ. 2007. Salicylic acid inhibits gibberellin-induced alpha-amylase expression and seed germination via a pathway involving an abscisic-acid-inducible WRKY gene. Plant Mol Biol. 64:293–303.

Yalpani N, Silverman P, Wilson TMA, Kleier DA, Raskin I. 1991. Salicylic acid is a systemic signal and an inducer of pathogenesis related proteins in virus-infected tobacco. Plant Cell. 3:809–818.

Yeni IJ. 2011. Evaluation of antifungal effects of extracts of *Allium sativum* and *Nicotiana tabacum* against soft rot of yam (*Dioscorea alata*). J Agric Res. 3:1–5.

Zhang Y, Tian Z, Xi R, Gao H, Qu P. 2002. Effect of salicylic acid on phenolics metabolization of Yali pear growing fruits. J Agric Univ Hebei. 25:33–36.

Zhou T, Reeleder RD. 1989. Aplication of *Epicoccum purpurascens* spores to control white mold of snap bean. Plant Dis. 73:639–642.

Zhou T, Reeleder RD. 1990. Selection of strains of *Epicoccum purpurascens* for tolerance to fungicides and improved biocontrol of *Sclerotinia sclerotiorum*. Can J Microbiol. 36:754–759.

Zıv O, Zıtter TA. 1992. Effects of bicarbonate and film-forming polymers on cucurbit foliar diseases. Plant Dis. 76:513–517.