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Efficacy of plant extracts for anthracnose control in bell pepper fruits under controlled conditions

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ABSTRACT

Anthracnose is an important disease of bell pepper (*Capsicum annuum*) in Brazil and worldwide. This research investigated the efficacy of plant extracts for control of this disease in bell pepper fruits caused by *Colletotrichum acutatum* under controlled conditions and analyzed the best treatments in relation to different extract and inoculum concentrations, pathogen isolates, and temperatures. In the preliminary screening, among aqueous or ethanolic extracts of 16 plant species evaluated, the best were 6% aqueous garlic, mallow, and ginger extracts, which reduced disease severity by more than 97%. Garlic extract required the highest concentrations to reduce severity by 75% (CL_{75}) when compared to ginger and mallow extracts, but exhibited the best efficacy in relation to different inoculum concentrations and isolates of *C. acutatum*. It was also able to maintain greater than 90% efficacy at 20-30°C. The garlic aqueous extract effectively controlled anthracnose caused by *C. acutatum* in bell pepper fruits under controlled conditions.

Keywords: *Capsicum annuum*, *Colletotrichum acutatum*, plant disease control, garlic extract.

RESUMO

Eficácia de extratos de plantas no controle da antracnose em frutos de pimentão sob condições controladas

A antracnose é uma importante doença do pimentão (*Capsicum annuum*) no Brasil e no mundo. Esta pesquisa investigou a eficácia de extratos de plantas no controle dessa doença em frutos de pimentão causada por *Colletotrichum acutatum* sob condições controladas e analisou os melhores tratamentos em relação a diferentes concentrações de extrato e inóculo, isolados do patógeno e temperaturas. Na seleção preliminar, entre os extratos aquosos ou etanólicos de 16 espécies de plantas avaliadas, os melhores foram extratos aquosos a 6% de alho, gengibre e malva, que reduziram a severidade da doença em mais de 97%. O extrato de alho necessitou a maior concentração para reduzir a severidade em 75% (CL_{75}) quando comparado aos extratos de gengibre e malva, mas exibiu a maior eficácia em relação a diferentes concentrações de inoculo e isolados de *C. acutatum*. Esse também manteve mais de 90% de eficácia a 20-30°C. O extrato aquoso de alho controlou eficazmente a antracnose causada por *C. acutatum* em frutos de pimentão sob condições controladas.

Palavras-chave: *Capsicum annuum*, *Colletotrichum acutatum*, controle de doença de planta, extrato de alho.

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Anthracnose is one of the most important diseases of bell pepper (*Capsicum annuum*) in tropical and subtropical regions (Koike *et al*., 2006; Than *et al*., 2008; Sahitya *et al*., 2014), including Brazil (Azevedo *et al*., 2006; Pereira *et al*., 2011). In mild to warm climates and rainy seasons, fruit production losses can reach as high as 100% when adequate control measures are not applied (Kurozawa *et al*., 2005; Azevedo *et al*., 2006). Disease symptoms may occur either during the crop development in the field or after harvest, but only the fruit exhibits symptoms. Infection begins with the appearance of small, circular, depressed lesions that rapidly expand and have no defined diameter. When relative humidity is high, the formation of a pink or orange conidial mass might be observed (Lopes & Ávila, 2003).

Anthracnose of bell pepper is caused by a complex of *Colletotrichum* species (Than *et al*., 2008). Studies conducted in Brazil revealed that *C. acutatum*, *C. gloeosporiodides*, *C. coccodes*, *C. boninense* and *C. capsici* were associated with anthracnose on bell pepper. However, *C. acutatum* was identified as the predominant species, accounting for more than 70% of cases of this disease in this country (Tozze Junior, 2007).

Damage caused by anthracnose in bell pepper can be reduced when integrated management measures are adopted, including the use of healthy seeds of known origin and application of registered fungicides. Nonetheless, it is not always possible to avoid epidemics and the loss in efficacy of various active principles has been reported (Fernandes *et al*., 2002; Azevedo *et al*., 2006). As there is no commercial bell pepper variety or hybrid in the world that exhibits good levels of resistance to anthracnose (Pereira *et al*., 2011), there is a need for the investigation of new control alternatives for the establishment of an integrated disease management program.

The need for safer, more efficacy, economical, and non-polluting methods has stimulated the search for alternative methods to control plant diseases instead of chemical products (Stangarlin *et al*., 2011). Empirically, plant extracts have been used in non-conventional agriculture production systems for the control of plant diseases. Such extracts are often produced in a homemade fashion from materials available on the property that are sprayed on the crops (Silva *et al*., 2005). Plant extracts that possess secondary compounds have been investigated with regard to their potential for controlling phytopathogens and some have proven to have antimicrobial properties that affect fungal development both *in vitro* and *in vivo* (Stangarlin *et al*., 2008, 2011).

A number of studies have been carried out to assess the efficacy of plant extracts in the control of anthracnose in bell pepper fruits caused by *Colletotrichum* species (Carvalho *et al*., 2008; Koppula *et al*., 2010; Johnny *et al*., 2011; Ranasingh *et al*. 2011; Saravanakumar *et al*., 2011; Ajith *et al*., 2012; Song *et al*., 2012), but no study have yet been carried out considering *C. acutatum*. Importantly, *Colletotrichum* species respond differently to various control measures and use of the predominant species in the studies may lead to more effective disease control (Than *et al*., 2008).

The objective of this study was to assess the efficacy of plant extracts for control of anthracnose in bell pepper fruits caused by *C. acutatum* and to analyze the consistency of the best treatments in relation to different extract and inoculum concentrations, pathogen isolates, and temperatures.

MATERIAL AND METHODS

Plant species - Extracts of 16 plant species were used. Plants were selected based on folk remedies as well as the scientific reports concerning potential antimicrobial activity against phytopathogens. The following species were used as aqueous extracts: lemon balm (*Melissa officinalis*), marjoram (*Origanum majorana*), basil (*Ocimum basilicum*), mallow (*Malva* sp.), wormseed (*Chenopodium ambrosioides*), wormwood (*Artemisia absinthium)*, garlic (*Allium sativum*), ginger (*Zingiber officinale*), and clove (*Syzygium aromaticum*). The species used as hydroalcoholic extracts were: croton selowii (*Croton selowii*), croton micans (*Croton micans*), Brazilian vernonia (*Vernonia herbacea*), pereiro-do-sertão (*Aspidosperma pyrifolium*), leucaena (*Leucaena leucocephala*), protium (*Protium bahianum*), and calabura (*Muntigia calabura*); there were 18 treatments in total. The plant parts used were bulb, bark, stem, leaf, inflorescence, and rhizome. The first seven species were collected from an organic production area (Chã Grande, Pernambuco State, Brazil), the three subsequent species were obtained from the Distribution Center of Pernambuco, and the others were obtained from the Laboratory of Natural Bioactive Products (Department of Chemistry) of the Universidade Federal Rural de Pernambuco.

Preparation of aqueous and hydroalcoholic extracts - In order to obtain the aqueous extracts, the collected plant material was submitted to defoliation and the leaves were distributed among trays for moisture content reduction. After five days at the temperature of 30±2°C and 55±5% relative humidity, the leaves were ground in an industrial blender for 5 min. To obtain the aqueous extract at the desired concentration, 200 g of ground leaves from each species were used. Samples were immersed in 1000 mL of sterile distilled water and infused for three days, sheltered from light. The extract was then filtered through a double layer of cheesecloth, placed into glass flasks wrapped in aluminum foil, and kept for 30 days at a temperature of 25±2°C.

In order to obtain garlic, ginger, and clove extracts, 200 g of bulbs, rhizome, and inflorescence were used, respectively, which were weighed, shredded in an industrial blender and then immersed in 1000 mL of water. Samples were then filtered through a double layer of cheesecloth. These extracts were prepared moments before each bioassay.

To obtain the hydroalcoholic extract, leaves, bark, and stems of each plant species were previously dried in a ventilated hothouse (45ºC), then ground, weighed, and placed into a recipient containing ethanol. Contact time of the ground material with the solvent (ethanol) was 24 h. To obtain the desired concentration of 1% of the hydroalcoholic solution, 1 mg of raw extract was weighed, dissolved in 1 mL of ethanol, and added to 100 mL of water.

Phytopathogen and inoculum preparation - Monosporic cultures of *C. acutatum* isolates were used in all experiments. The isolates were obtained from bell peppers with symptoms of anthracnose that were collected from commercial fields in the state of Pernambuco. The isolates were characterized and identified based on culture characteristics and the morphological characteristics of the conidia (Sutton, 1992), as well as polymerase chain reaction (PCR) analysis with specific primers to amplify the internal transcribed spacer (ITS) region (Cgint/ITS4 and Caint2/ITS4) (Adaskaveg & Hartin, 1997) and the β-tubulin gene (TBCG/TB5 and TBCA/TB5) (Talhinhas *et al*., 2005). Stock cultures were stored in potato-dextrose-agar (PDA) media slants at 5ºC in the dark and were maintained at the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" in Recife. The fungal inoculum was produced in Petri dishes containing PDA, which were incubated for 10 days at 25±2°C under alternating light (12 h light/12 h dark) in biochemical oxygen demand (BOD) incubators. Spore suspensions were prepared adding 20 mL of sterile distilled water to the surface of the cultures, brushing with a soft bristle brush, and filtering through a double layer of cheesecloth. Spore concentration was determined using a hemacytometer and adjusted to the desired value with sterile water.

Preliminary screening of plant extracts for anthracnose control in bell pepper - Healthy bell pepper fruits (cv. Atlante) in the stage of commercial maturity were washed and disinfested by immersion in 1.0% NaClO for 5 min and dried in an aseptic chamber. Since non-wounded treatment caused no lesions of *Colletotrichum* (unpublished data), after drying, the epidermis of each fruit was marked on the surface at four equidistant points, where four lesions of approximately 3 mm in depth were made with a disinfected pin.

Atomization was done with 20 μL of plant extract solution at a concentration of 20% for aqueous extracts and 1% for ethanolic extracts. Each plant extract solution was supplemented with 0.05% Tween 20. Two hours after the extract atomization, the lesions were inoculated with 20 μL of a conidial suspension ($2x10^5$ conidia/mL) of *C. acutatum* (CMM–729 isolate), supplemented with 0.05% Tween 20. The control was sprayed with sterile distilled water, also supplemented with Tween 20, and later inoculated with the pathogen conidial suspension. Following inoculation, the peppers were kept at high relative humidity (≥90%) for 24 h in moist chambers made from plastic trays containing four layers of paper towels moistened with 100 mL of sterile distilled water and placed in polyethylene bags. Contact between the peppers and water was avoided by placing each pepper on the cover of a sterile Petri dish. The moist chamber trays were kept at 25±2°C under alternating light (12 h light/12 h darkness) in BOD incubators. The experimental design was completely randomized, with six replicates, each including three pepper fruits.

Seven days after inoculation, the evaluation of the anthracnose severity at each inoculated point was made. The external affected area was determined by measuring the length of the lesion in two diametrically opposed directions. Using the disease severity data in the control plants (SDC) and the treatments with the application of the aqueous and hydroalcoholic extracts (STR), the efficacy in anthracnose control (AC) was calculated using the following equation: AC (%) = {(SDC–STR)/SDC}x100.

Efficacy of concentrations of garlic, ginger, and mallow aqueous extracts on control of bell pepper anthracnose - The efficacy of the concentration of aqueous garlic, ginger, and mallow extracts, which showed greater efficacy in the control of anthracnose in the preliminary screening, was assessed in two independent experiments. In the first experiment, the extracts were applied at concentrations of 6%, 10%, 16%, and 20%. The latter concentration was previously used in the preliminary selection. Since no differences were observed between maximum and minimum concentrations with regard to the efficacy of anthracnose control, the lowest dosage was used as the basis for the second experiment, in which the extracts were applied at concentrations of 0.01%, 0.05%, 0.1%, 0.5%, 1%, 3%, and 6%. In both the experiments, the pathogen isolate, bell pepper cultivar and the procedures utilized were the same as in the preliminary screening. The experimental design was completely randomized in both the experiments, with six replicates, each including three pepper fruits. The first experiment was carried out in a 3x4 factorial arrangement and the second in a 3x7 factorial arrangement.

Efficacy of garlic, ginger, and mallow aqueous extracts on control of bell pepper anthracnose induced by different concentrations of pathogen inoculum - In order to evaluate the efficacy of *C. acutatum* inoculum concentration on the efficacy of anthracnose control in bell pepper, the 6% aqueous extracts of garlic, ginger, and mallow were tested against five concentrations of the pathogen ($1x10^1$, $1x10^2$, $1x10^4$, $1x10^5$ and $1x10^6$ conidia/mL), following the same procedures and using the same pathogen isolate and bell pepper cultivar as in the preliminary screening. The experimental design was completely randomized in a 3x5 factorial arrangement with six replicates, each including three pepper fruits.

Efficacy of garlic, ginger, and mallow aqueous extracts on control of bell pepper anthracnose induced by different isolates of *Colletotrichum acutatum* - The aqueous garlic, ginger and mallow extracts were assessed with regard to the control of anthracnose induced by seven *C. acutatum* isolates, namely: CMM–727, CMM–729, CMM–730, CMM–905, CMM–1070, CMM–1073, and CMM–1076. The extracts were applied at a concentration of 6% and the fungal isolates were inoculated at a concentration of $2x10^5$ conidia/mL. The remaining procedures were the same as those performed in the preliminary screening. The experimental design was entirely randomized in a 3x7 factorial arrangement with six replicates, each including three pepper fruits.

Efficacy of garlic, ginger, and mallow aqueous extracts on control of bell pepper anthracnose under different temperatures - The aqueous garlic, ginger, and mallow extracts were assessed for their ability to control anthracnose under the temperatures of 10, 15, 20, 25, 30 and 35°C. The extracts were applied in a concentration of 6% and the pathogen isolates were inoculated at a concentration of $2x10^5$ conidia/mL. The moist chambers containing the bell pepper were kept in BOD incubators at the different temperatures and under alternating light (12 h light/12 h dark). The remaining procedures, pathogen isolate and bell pepper cultivar were the same as in the preliminary screening. The experimental design was entirely randomized in a 3x6 factorial arrangement with six replicates, each including three pepper fruits.

Statistical analysis - All experiments were repeated twice. In all cases, analysis of variance (ANOVA) indicated that the data between the two repetitions were similar ($p>0.05$), thus data of all variables from both experiments were combined. Anthracnose control (AC) data obtained in the second experiment of the extract concentration were used to estimate the effective extract concentration (%) to reduce 75% of disease severity (EC_{75}) for each plant extract by Probit regression analysis, with the log_{10} transformation of extract concentration as the independent variable and AC as the dependent variable. In the experiment of pathogen inoculum concentration, a regression analysis with cubic polynomial model was performed to assess the relationship of AC to inoculum concentration. When the regression analysis was used, the significance of regressions and their parameters was checked by F test ($p \leq 0.05$). The AC data from preliminary screening and the EC_{75} data were subjected to one-way ANOVA, while the AC data from pathogen isolates and temperature experiments were subjected to two-way ANOVA. In all situations, Duncan's multiple range tests ($p \leq 0.05$) was applied when ANOVA revealed significant differences. The statistical analyses were performed with the Statistix® 9.0 software (Analytical Software, Tallahassee, USA).

RESULTS AND DISCUSSION

Preliminary screening of plant extracts for anthracnose control in bell pepper - There were significant differences ($p \leq 0.05$) between the raw plant extracts with regard to their efficacy in the control of anthracnose in bell pepper. Among the 18 treatments evaluated in the preliminary screening, the aqueous garlic, mallow, and ginger extracts were the most efficient and provided more than 97% reduction of anthracnose severity. The aqueous clove extract exhibited the lowest efficacy, with a 30.5% reduction of disease severity. The other aqueous and hydroalcoholic extracts exhibited intermediate levels of control, with efficacy ranging from 41.5% (croton selowii) to 87.7% (wormwood) (Figure1). Overall, aqueous exacts showed greater efficacy in the control of anthracnose in bell pepper than hydroalcoholic extracts.

This is the first study in which the efficacy of plant extracts on the control of anthracnose caused by *C. acutatum* in bell pepper has been determined. In the studies previously conducted on anthracnose control in bell pepper by plant extracts were used other plant species and species of *Colletotrichum* (Carvalho *et al*., 2008; Koppula *et al*., 2010; Johnny *et al*., 2011; Ranasingh *et al*. 2011; Saravanakumar *et al*., 2011; Ajith *et al*., 2012; Song *et al*., 2012; Sahitya *et al*., 2014).

The results indicate that aqueous garlic, mallow and ginger extracts

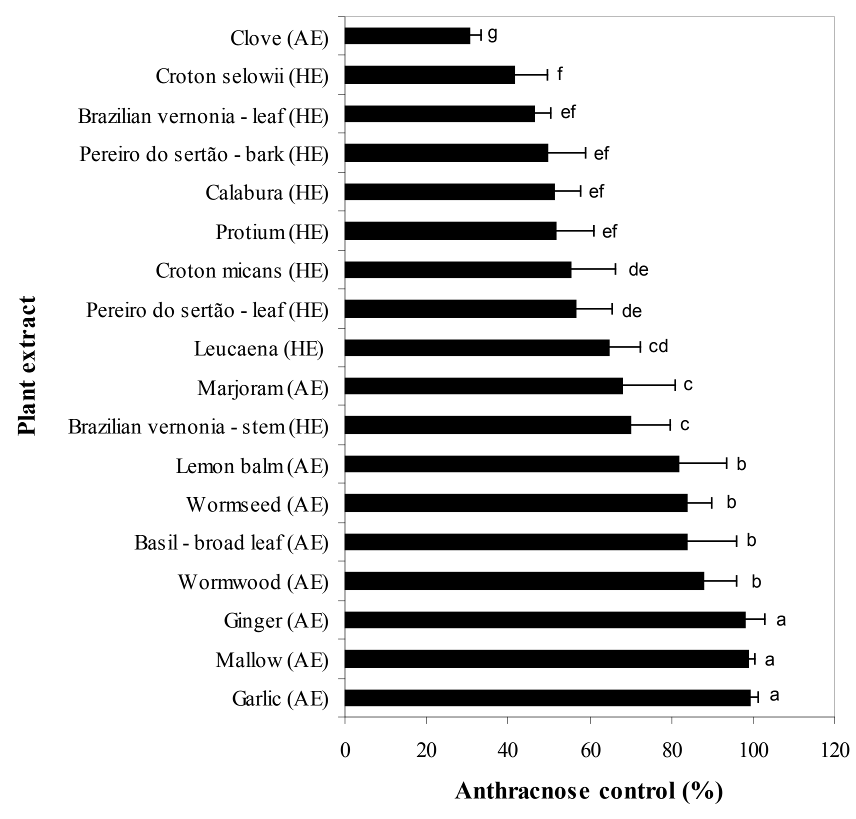

Figure 1. Efficacy of 20% aqueous extracts (AE) and 1% hydroalcoholic extracts (HE) of the tested plants in the control of anthracnose in bell pepper fruits cv. Atlante. Error bars represent the standard deviations of means. Means followed by the same letter do not differ significantly by Duncan's multiple range test ($p \leq 0.05$) {eficácia de extratos aquosos a 20% (AE) e extratos alcoólicos a 1% (HE) das plantas testadas no controle da antracnose em frutos de pimentão cv. Atlante. Barras de erros representam os desvios padrões das médias. Médias seguidas pela mesma letra não diferem significativamente pelo teste de Duncan ($p \leq 0.05$)}. Recife, UFRPE, 2010.

Table 1. Influence of *Colletotrichum acutatum* isolates on anthracnose control in bell pepper fruits by 6% aqueous extracts of garlic, ginger, and mallow (influência de isolados de *Colletotrichum acutatum* no controle da antracnose em frutos de pimentão por extratos aquosos a 6% de alho, gengibre e malva). Recife, UFRPE, 2010.

***Colletotrichum* isolate**	**Anthracnose control (%)**		
	Garlic	**Ginger**	**Mallow**
CMM-727	80.6 abcA	79.9 aA	69.7 bB
CMM-729	94.3 aA	91.1 aA	86.0 aA
CMM-730	82.2 abcA	48.1 cB	19.9 deC
CMM-905	71.8 cA	64.6 bA	27.5 deB
CMM-1070	91.1 aA	83.6 aA	6.4 eB
CMM-1073	83.7 abcA	87.5 aA	11.2 eB
CMM-1076	83.1 abcA	84.6 aA	51.7 cB
CV (%) = 14.8			

Means followed by the same lowercase letter within columns and capital letter on the line do not differ significantly by Duncan's multiple range test ($p \leq 0.05$) {médias seguidas pela mesma letra minuscula na coluna e maiúscula na linha não diferem significativamente pelo teste de Duncan ($p \leq 0.05$)}.

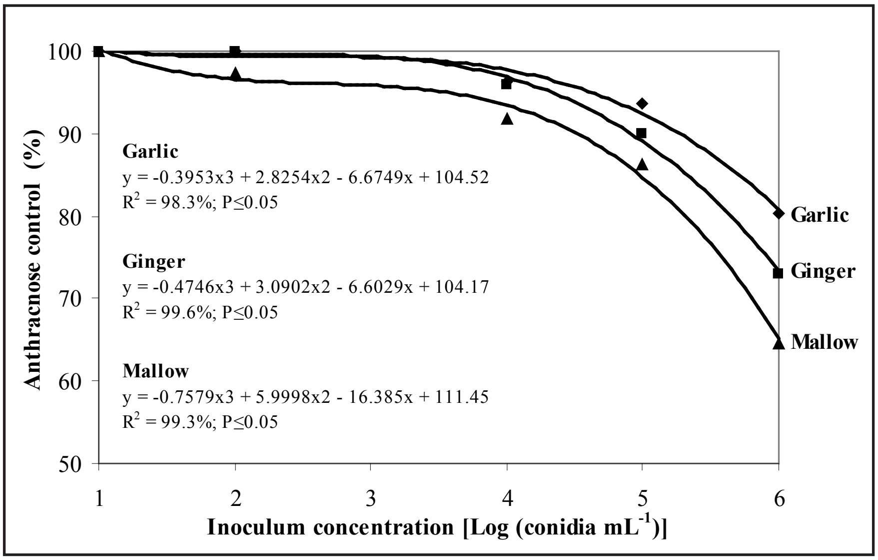

Figure 2. Influence of *Colletotrichum acutatum* inoculum concentration on anthracnose control in bell pepper fruits by 6% aqueous extracts of garlic, ginger, and mallow. Inoculum concentration and anthracnose control were related by regression analysis, using cubic polynomial model (influência da concentração do inóculo de *Colletotrichum acutatum* no controle da antracnose em frutos de pimentão por extratos aquosos a 6% de alho, gengibre e malva. Concentração de inoculo e controle da antracnose foram relacionados por análise de regressão, utilizando o modelo polinomial cúbico). Recife, UFRPE, 2010.

Table 2. Influence of temperature on anthracnose control in bell pepper fruits by 6% aqueous extracts of garlic, ginger, and mallow (influência da temperatura no controle da antracnose em frutos de pimentão por extratos aquosos a 6% de alho, gengibre e malva). Recife, UFRPE, 2010.

Temperature (°C)	Anthracnose control (%)		
	Garlic	Ginger	Mallow
20	94.6 aA	95.3 aA	72.0 bB
25	94.7 aA	93.0 aA	91.3 aA
30	100.0 aA	95.7 aA	96.0 aA
CV (%) = 10.8			

Means followed by the same lowercase letter within columns and capital letter on the line do not differ significantly by Duncan's multiple range test ($p \leq 0.05$) {médias seguidas pela mesma letra minúscula na coluna e maiúscula na linha não diferem significativamente pelo teste de Duncan ($p \leq 0.05$)}.

can efficaciously reduce the disease severity in bell pepper. Aqueous garlic extract incorporated into PDA at a concentration of 200 ppm, resulted in the inhibition of mycelial growth of *C. gloeosporioides* (Ribeiro & Bedendo, 1999). Additionally, spore germination was reduced by 78.5% when spores were incubated with a 7.5% garlic extract (Venkataravanappa & Nargund, 2007). Important is the fact that no harmful effects of garlic on humans or environment have been reported. Furthermore, the preparation of the extract is simple and does not require sophisticated equipment (Obagwu & Korsten, 2003).

No studies were found dealing with control of anthracnose with mallow extract, but ginger extract has been found to have an inhibitory effect on mycelial growth of *C. gloeosporioides* (Rozwalka *et al.*, 2008). The low efficacy of the clove extract in the control of anthracnose in bell pepper observed in the present study contrasts with results obtained in the control of anthracnose in guava (Rozwalka *et al.*, 2008), which achieved 100% inhibition of *C. gloeosporioides* growth.

Influence of concentrations of garlic, ginger, and mallow aqueous extracts on anthracnose control in bell pepper - There were significant differences ($p \leq 0.05$) among garlic, ginger, and mallow extracts in the efficacy of anthracnose control when applied at different concentrations. The garlic extract achieved the highest CL_{75} (concentration that reduced the anthracnose severity by 75%) value (3.5%), indicating that a higher concentration of this extract is required to achieve this degree of control, compared to the other extracts. The lowest CL_{75} value was achieved with the ginger extract, which was able to reduce the severity disease by 75% at a concentration of 1.4%. The mallow extract achieved the intermediate CL_{75} value (2.1%), differing from the extreme values observed with garlic and ginger extracts.

Influence of *Colletotrichum acutatum* inoculum concentration on anthracnose control in bell pepper by garlic, ginger, and mallow aqueous extracts - The inoculum concentration of *C. acutatum* significantly ($p \leq 0.05$) influenced the anthracnose severity in bell pepper fruits when the garlic, ginger, and mallow extracts were used, and disease control reduced with increasing inoculum concentration. All extracts were completely effective in controlling the disease when the inoculum concentration was 1×10^1 conidia/mL (Figure 2). However, at higher inoculum concentration (1×10^6 conidia/mL), the garlic extract showed the highest efficacy, with 80.4% disease control, while mallow extract showed 64.6%. The cubic polynomial model ($y = a + bx + cx^2 + dx^3$) provided excellent fit curves of the anthracnose severity progress with respect to inoculum concentrations of *C. acutatum*, with coefficients of determination (R^2) from 98.3% and 99.6% (Figure 2). The garlic extract showed the lowest estimated control efficacy reduction rate of anthracnose (-0.3953) with an increase in the pathogen inoculum, whereas the mallow extract achieved the highest (-0.7579) (Figure 2), when the regression parameter was compared by T-test using the confidence interval.

The efficacy of garlic extract, with regard to the concentration of the pathogen inoculum, is an essential aspect in the management of anthracnose in bell peppers, indicating that this extract may be effective at different degrees of inoculum pressure. Furthermore, the highest CL_{75} value associated with the lowest control efficacy reduction rate value indicates that the treatment needing the least amount of extract does not always exhibit the best efficacy in the control of the disease at different concentrations of pathogen inoculum.

Influence of *Colletotrichum acutatum* isolates on anthracnose control in bell pepper by garlic, ginger, and mallow aqueous extracts - The anthracnose control was significantly ($p \leq 0.05$) influenced by the interaction between plant extract type and *C. acutatum* isolates. All the extracts exhibited variable efficacy in anthracnose control with regard to different pathogen isolates. The garlic extract exhibited the lowest variation in control efficacy (71.8% to 94.3%) in comparison to the ginger extract (48.1% to 91.1%) and the mallow extract (6.4% to 86.0%). When considering each isolate separately, the garlic and ginger extracts exhibited the highest degrees of efficacy for most of the isolates, with no significant differences ($p > 0.05$) between the two extracts, whereas the mallow extract showed the lowest degree of efficacy, except with regard to the isolate CMM–729 (Table 1).

The differences in the degree of control of the disease induced by different isolates, when a single extract is applied, indicate the possibility of variability among the pathogen isolates. The lesser variation in the degree of efficacy exhibited by the garlic extract in relation to the different *C. acutatum* isolates is important, as it indicates a greater potential for consistency of the control of different pathogen populations.

Influence of temperature on anthracnose control in bell pepper by garlic, ginger, and mallow aqueous extracts - There was no development of anthracnose symptoms in the bell pepper at temperatures of 10, 15, and 35ºC. Thus, only temperatures of 20, 25, and 30ºC were considered. The interaction between this range of temperatures and extracts was significant ($p \leq 0.05$). No differences in efficacy were observed between the garlic and ginger extracts, as both maintained control levels above 90% at temperatures of 20, 25, and 30ºC. However, the mallow extract exhibited lower efficacy at 20ºC, differing from the other temperatures as well as from the other extracts at this same temperature (Table 2).

Temperatures between 25 and 28ºC are considered more favorable to *C. acutatum* growth (Tozze Junior *et al*., 2006) as well as to the induction of anthracnose in Solanaceae fruits (Koike *et al*., 2006). The lesser efficacy of the mallow extract at 20ºC may indicate the need for a higher temperature in order for its antimicrobial action to be activated, whereas the other two extracts are stable at the different temperatures that are favorable to the induction and development of the disease.

The most promising raw extracts for the control of anthracnose in bell peppers were selected in the present study and the influence of various factors on the stability of the efficacy of disease control was assessed. The garlic aqueous extract effectively controlled anthracnose caused by *C. acutatum* in bell pepper fruits under controlled conditions. These results provide evidence of the potential use of the garlic aqueous extract in control of anthracnose in field conditions, since anthracnose in bell pepper has significant importance in this phase compared to postharvest. However, the metabolites responsible for the suppression of the disease were not investigated. Furthermore, homemade extracts present certain limitations, such as the lack of quality control, the low stability of the organic compounds in the solutions, and the inability to monitor possible toxic substances on the plants or those resulting from the decomposition of the products during handling (Silva *et al*., 2005). Therefore, more in-depth investigations of plant extracts are required, along with the development of products with a higher technological level, so that both producers and consumers can have security in the use of raw extracts.

REFERENCES

ADASKAVEG JE; HARTIN RJ. 1997. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. *Phytopathology* 87: 979-987.

AJITH OS; LAKSHMESHA KK; MURTHY SM; LAKSHMIDEVI N. 2012. Botanicals for control of anthracnose of bell peppers. *The Journal of Plant Protection Sciences* 4: 13-19.

AZEVEDO CP; CAFÉ FILHO AC; HENZ GP; REIS A. 2006. *Recomendações de manejo da antracnose do pimentão e das pimentas*. Brasília: Embrapa Hortaliças. 4p.

CARVALHO JB; SCHWAN-ESTRADA KRF; BONALDO SM; CRUZ MES; CARLOS MM; STANGARLIN JR. 2008. Fungitoxicity of *Cymbopogon citratus* and *Cymbopogon martinii* on *Colletotrichum gloeosporioides* in bell peppers fruits. *Revista Brasileira de Plantas Medicinais* 10: 88-93.

FERNANDES MCA; SANTOS AS; RIBEIRO RLD. 2002. Adaptação patogênica de isolados de *Colletotrichum gloeosporioides* obtidos de frutos de jiloeiro, pimentão e berinjela. *Summa Phytopathologica* 28: 325-330.

JOHNNY L; YUSUF UK; NULIT R. 2011. Antifungal activity of selected plant leaves crude extracts against a pepper anthracnose fungus, *Colletotrichum capsici* (Sydow) Butler and Bisby (Ascomycota: Phyllachorales). *African Journal of Biotechnology* 10: 4157-4165.

KOIKE ST; GLADDERS P; PAULUS AO. 2006. *Vegetable diseases*: a color handbook. San Diego: Academic Press. 448p.

KOPPULA S; AMMANI K; BOBBARALA V. 2010. Selected plant extracts as biocontrol agents against the management of chilli (*Capsicum annuum*) diseases. *Journal of Pharmacy Research* 3: 3143-3146.

KUROZAWA C; PAVAN MA; KRAUSE-SAKATE R. 2005. Doenças das solanáceas. In: KIMATI H; AMORIM L; BERGAMIN FILHO A; CAMARGO LEA; REZENDE JAM (coords). *Manual de Fitopatologia: doenças das plantas cultivadas*. 4. ed. São Paulo: Agronômica Ceres. p.589-596.

LOPES CA; ÁVILA AC. 2003. *Doenças do pimentão*: diagnose e controle. Brasília: Embrapa Hortaliças. 96p.

OBAGWU J; KORSTEN L. 2003. Control of citrus green and blue molds with garlic extracts. *European Journal of Plant Pathology* 109: 221-225.

PEREIRA MJZ; MASSOLA JUNIOR NS; SUSSEL AAB; SALA FC; COSTA CP; BOITEUX LS. 2011. Reação de acessos de *Capsicum* e de progênies de cruzamentos interespecíficos a isolados de *Colletotrichum acutatum*. *Horticultura Brasileira* 29: 569-576.

RANASINGH N; BEURA SK; DAS R; PANDA S. 2011. Effect of different botanicals against *Colletotrichum capsici* causing anthracnose/die-back of chilli. *Environment and Ecology* 29: 1853-1855.

RIBEIRO LF; BEDENDO IP. 1999. Efeito inibitório de extratos vegetais sobre *Colletotrichum gloeosporioides* - agente causal da podridão de frutos de mamoeiro. *Scientia Agrícola* 56: 1267-1271.

ROZWALKA LC; LIMA MLRZC; MIO LLM;

NAKASHIMA T. 2008. Extratos, decoctos e óleos essenciais de plantas medicinais e aromáticas na inibição de *Glomerella cingulata* e *Colletotrichum gloeosporioides* de frutos de goiaba. *Ciência Rural* 38: 301-307.

SAHITYA UL; DEEPTHI RS; KASIM DP; SUNEETHA P; KRISHNA MSR. 2014. Anthracnose, a prevalent disease in *Capsicum*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 5: 1583-1604.

SARAVANAKUMAR P; KARTHIKEYAN V; PATHARAJAN S; KANNABIRAN B. 2011. Antifungal activity of *Plumbago* species against anthracnose fungus *Colletotrichum gloeosporioides* of chilli. *Archives of Phytopathology and Plant Protection* 44: 287-297.

SILVA MB; ROSA MB; BRASILEIRO BG; ALMEIDA V; SILVA CA. 2005. Desenvolvimento de produtos à base de extratos de plantas para o controle de doenças de plantas. In: VENEZON M; PAULA JR. TJ; PALLINI A. (eds). *Controle alternativo de pragas e doenças*. Viçosa: EPAMIG/CTZM. p. 221-246.

SONG CH; CHUNG JB; JEONG BR; PARK SY; LEE YS. 2012. Antifungal activity of crude extract compound from *Rhus verniciflua* against anthracnose fungi (*Collectotrichum* spp.) of red-pepper. *Korean Journal of Environmental Agriculture* 31: 60-67.

STANGARLIN JR; KUHN OJ; ASSI L; SCHWAN-ESTRADA KRF. 2011. Control of plant diseases using extracts from medicinal plants and fungi. In: MÉNDEZ-VILAS A. (ed). *Science against microbial pathogens: communicating current research and technological advances*. Badajoz: Formatex. v. 2, p.1033-1042.

STANGARLIN JR; KUHN OJ; SCHWAN-ESTRADA KRF. 2008. Control of plant diseases by plant extracts. *Revisão Anual de Patologia de Plantas* 16: 265-304.

SUTTON B. 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. In: BAILEY JA; JEGER MJ (eds). *Colletotrichum: biology, pathology and control*. Wallingford: CAB International. p. 1-26.

TALHINHAS P; SREENIVASAPRASAD S; NEVES-MARTINS J; OLIVEIRA H. 2005. Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Applied and Environmental Microbiology* 71: 2987-2998.

THAN PP; PRIHASTUTI H; PHOULIVONG S; TAYLOR PWJ; HYDE KD. 2008. Chilli anthracnose disease caused by *Colletotrichum* species. *Journal of Zhejiang University - Science B* 9: 764-778.

TOZZE JUNIOR HJ. 2007. *Caracterização e identificação de espécies de Colletotrichum associadas à antracnose do pimentão (Capsicum annuum) no Brasil*. Piracicaba: ESALQ/USP. 81p (Tese mestrado).

TOZZE JUNIOR HJ; MELLO MBA; MASSOLA JR. NS. 2006. Caracterização morfológica e fisiológica de isolados de *Colletotrichum* sp. causadores de antracnose em solanáceas. *Summa Phytopathologica* 32: 71-79.

VENKATARAVANAPPA V; NARGUND VP. 2007. Fungitoxic properties of some medicinal and aromatic plants against *Colletotrichum gloeosporioides*. *Annals of Plant Protection Science* 15: 513-514.