

## Botanical fungicides in the control of soybean leaf diseases

### Fungicidas botânicos no controle de doenças foliares na soja

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**ABSTRACT**

The soybean cultivation (*Glycine max* (L.) Merrill) is responsible for the highest pesticides use in agriculture in Brazil. There is an environmental and social need to reduce the use of these substances in crops. The alternative products applied in agriculture such as plant extracts and essential oils, becomes necessary and indispensable, mainly in disease control. Among the plants studied, the Noni (*Morinda citrifolia* L.), has stood out in some studies, where relevant fungitoxic results have been demonstrated, however, there are still few works that prove its viability in the diseases management in field. Thus, this work aimed to evaluate the soybean diseases alternative control through the aqueous extracts and noni essential oil application, in plantings high and low disease pressure. Two field experiments were implemented, with soybean culture, evaluating leaf, fruit aqueous extract and noni essential oil as a fungicidal action. Foliar application of noni extracts and essential oil did not differ from fungicide in Asian Rust and Anthracnose control, in the 2016/17 crop, in both experiments. Soybean productivity was similar in treatments that received leaf extract (1748,8 Kg ha<sup>-1</sup>), essential oil (1762,5 Kg ha<sup>-1</sup>) and fungicides (2031,7 Kg ha<sup>-1</sup>). Where there was no large disease pressure all agronomic characteristics were equivalent, regardless of treatment.

**Keywords:** Biological control, *Colletotrichum truncatum*, *Glycine max*, *Morinda citrifolia*, *Phakopsora pachyrhizi*.

**RESUMO**

A cultura da soja (*Glycine max* (L.) Merrill) é responsável pelo maior consumo de agrotóxicos no Brasil. Existe a necessidade ambiental e social de diminuir a utilização dessas substâncias nas lavouras. O uso de produtos alternativos na agricultura, como extratos e óleos essenciais de plantas, torna-se necessário e imprescindível, principalmente no controle de doenças. Entre as plantas estudadas, o Noni (*Morinda citrifolia* L.), tem se destacado em alguns estudos realizados, onde tem sido demonstrado resultados fungitóxicos relevantes, porém, ainda são escassos trabalhos que comprovem a sua viabilidade no manejo de doenças em campo. Desta forma, este trabalho teve como objetivo avaliar o controle alternativo de doenças da soja por meio da aplicação de extratos aquosos e óleo essencial de noni, em plantios de alta e baixa pressão de doenças. Foram implantados dois experimentos a campo, com a cultura da soja, avaliando extrato aquoso da folha, do fruto e o óleo essencial de noni como ação fungicida. A aplicação foliar de extratos e óleo essencial de noni não diferiram do fungicida no controle da Ferrugem Asiática e Antracnose, na safra 2016/17, nos dois experimentos. A produtividade da cultura foi semelhante nos tratamentos que receberam extrato da folha (1748,8 Kg ha<sup>-1</sup>), óleo essencial (1762,5 Kg ha<sup>-1</sup>) e fungicidas (2031,7 Kg ha<sup>-1</sup>). Onde não houve grande pressão de doenças todas as características agrônômicas foram equivalentes, independente do tratamento.

**Palavras chave:** *Colletotrichum truncatum*, Controle biológico, *Glycine max*, *Morinda citrifolia*, *Phakopsora pachyrhizi*.

## 1 INTRODUCTION

The soybean (*Glycine max* (L.) Merrill) is the main agricultural crop in Brazil, with production approximately 117 millions tons in crop 2017/2018, occupying a 35.1 million hectares planted area (CONAB, 2018). How crops are grown in all geographical country regions, plants are subject to various diseases that occur due adopted management, cultivate, climate, stress, causing direct plants damage, affecting grain quality and decreasing productivity. Annual production losses due to diseases are estimated at around 15 to 20% (EMBRAPA, 2013). Asian rust, caused by the fungus *Phakopsora pachyrhizi* Sydow & P. Sydow, can cause yield loss above 50%, under favorable environmental conditions for the disease development (Nascimento et al., 2018). Anthracnose, caused by the fungus *Colletotrichum truncatum* (Schw.) Andrus & Moore, is another very important disease that concerns producers for causing significant losses (Pesqueira et al., 2016).

As the soybean cultivation normally done in large areas, these diseases control have been accomplished with fungicides. Pignati et al. (2017), report that soybean is responsible for more than half of the pesticides used in Brazilian agriculture. Is therefore, it is necessary seek new antifungal agents that are profitable, non-polluting, and that eliminate or reduce the diseases incidence (Simonetti et al., 2015). An alternative that is being studied by researchers is the application of extracts and essential oils from plants. They are plant origin volatile components naturally occurring and have strong antimicrobial and antioxidant potential (Prakash et al., 2015). The phytopathogenic fungi control with essential oils has been showing efficiency in studies carried out in several cultures, such as maize (Mourão et al., 2017), melon (Dalcin et al., 2017), bean plant (Hillen et al., 2012), tomato plant (Santos Neto et al., 2016), among others.

Among the works carried out, can stand out the studies by Dalcin et al. (2017), Silva et al. (2017) and Osorio et al. (2018) with noni (*M. citrifolia* L.). The plant contains phytochemicals with antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory and immunological effects. Fruits, seeds, peels, leaves and flowers are also used for nutritional and therapeutic purposes, however, it is considered that the fruit contains most valuable chemical compounds (Assi et al. 2017).

Several researchers worldwide seeking the technical and economic feasibility of using essential oils, which will only be possible, through studies in controlled conditions

and in the field, to prove its effectiveness. It was verified in the literature that there are many works *in vitro*, but there are few experiments carried out in the field mainly with soybean culture, aiming at disease control. Thus, this work aimed to evaluate the control of Asian Rust and Anthracnose in soybean by applying aqueous extract and noni essential oil.

## 2 MATERIAL AND METHODS

### 2.1 OBTAINING EXTRACTS

The extracts were obtained from ripe noni fruits and leaves dehydrated in an oven with air circulation at 40 ° C for 48 hours. The vegetable material was collected in crop 2016/2017, in the Gurupi municipality, Tocantins, Brazil. Materials were grinded by adding water, in the 1/10 (m / v) ratio, in a food processor. The solution was filtered through a paper filter and from it, dilutions are made for use in experiments.

### 2.2 EXTRACTS ANALYSIS IN HPLC

The analyzes was developed at the Natural Products Research Laboratory, localized at the Federal University of Tocantins -Palmas Campus. The HPLC system (High performance liquid chromatography) (Shimadzu, Tokyo, Japan) chromatography consists (LC-10Avp series) equipped with a pump (LC-10 AD), a degasser (DGU-14A) to pump the mobile phase, manual rheodyne injector (20 µL loop) and class integrator (LC-10A), a UV-vis detector (SPD-10A) and a column oven (CTO 10A). The HELn solutions and standards were prepared with methanol and filtered through a Millipores® membrane (0.22 µm pore size). The separation was accomplished by a gradient system, using a Phenomenex Luna 5 mm C18 (2) reverse phase (250x4.6 mm<sup>2</sup>) column with Phenomenex C18 direct connection safety cartridges (4x3.0 mm<sup>2</sup>) filled with material similar to the main column. Mobile phase A was 0.1% phosphoric acid in Milli-Q water and the mobile phase B was 0.1% phosphoric acid in Milli-Q water / acetonitrile / methanol (54:35:11). Program gradient: 0 to 0.01 min, 0% B; 0.01-5 min, 0% B, 5-10 min, 30% B, 10-20 min 40% B, 20-29 min, 40% B, 29-30 min 50% B, 30 min 50% B, 50-80 min, 100% B. Flow quotient: 1 mL / min, temperature: 22 °C. UV detection was performed at 280 nm. The compounds were identified by comparing the samples retention times with the authentic standards (Sigma®) (Oliveira et al., 2015).

### 2.3 ESSENTIAL OIL EXTRACTION

For noni essential oil extraction were used ripe fruits, and was carried out by the hydrodistillation method using Clevenger apparatus, for two hours. After this process, the oil was collected and stored in a sealed amber bottle and kept refrigerated (4°C) (Clevenger, 1928).

### 2.4 ESSENTIAL OILS CHROMATOGRAPHIC ANALYSIS

Qualitative and quantitative essential oil analyzes were performed by gas chromatography coupled to CG-EM mass spectrometry. The chromatograph used was the Shimadzu GC-210 model equipped with a selective mass detector model QP2010 Plus, the equipment was operated under the following conditions: fused silica capillary column RTX-5MS (30 m x 0.25 mm x 0.25 µm film thickness); with the following temperature programming in the column: 60 – 240 °C (3 °C / min); injector temperature: 220 °C; helium carrier gas; splitless injection with injected 1 µL of a 1: 1000 solution in hexane volume. For the mass spectrometer (MS), the following conditions were used: 70 eV impact energy; ion source and interface temperature: 200 °C. The obtained spectra were compared with the Nist and Wiley 229 library database and the retention index, calculated for each constituent, it was compared with the tabulate, according to Adams (2007) and the compounds contents quantification was expressed in percentage. The aqueous extracts were liquid chromatography (HPLC) subjected, while the essential oil was evaluated in a gas phase coupled to mass spectrometry.

### 2.5 PHYTOTOXICITY TEST

The phytotoxicity test was performed with *M. citrifolia* essential oil on soybean plants using five different concentrations (0.05; 0.1; 0.25; 0.5; 0.75; 1.0; 1.25% v/v). A stock solution was prepared with distilled water and Tween 80 (1.0%), which was used in essential oil dilutions. After homogenization, the solutions were applied to the plants, using manual sprayers to runoff on the leaves. Were kept in the laboratory at 25 °C for a 24 hours period, and then the evaluation was performed through a phytotoxicity scale adapted from Dequech et al. (2008), Freitas et al. (2009) and Cogliatti et al. (2011), where: 1-25% = low necrosis on the leaves or low chlorosis of the plant; 26-50% = moderate leaf necrosis or moderate plant chlorosis; 51-75% = high leaf necrosis or high plant chlorosis; ; 76-100% = wilt and plant dryness.

## 2.6 FIELD EXPERIMENTS

Two experiments were implemented, in the Gurupi municipality, Tocantins, Brazil, with soybean culture, in the 2016/17 agricultural crop. The first, in the traditional commercial area, considered as high diseases pressure, due to soybean planting, in monoculture for over ten years, privately owned, located in the countryside (48°53'34"W; 11°44'29"S). The other, at the experimental station, at the Federal University of Tocantins (49°38'96" W; 11°44'45" S), in a low inoculum pressure area, due to the great diversity of small plant cultivations from different families botanicals, such as vegetables, grasses, etc.

The soil preparation was realized in a conventional manner, using a plow harrow. Fertilization (N-P-K) was done according to the soil analysis results. The seeds were treated with the insecticides imidacloprid + thiodicarb (Cropstar<sup>®</sup>) and subsequently the peat-based inoculation (Masterfix<sup>®</sup>) was performed, containing the bacteria *Bradyrhizobium elkani* (Strain Semia 5019) and *Bradyrhizobium japonicum* (Strain Semia 5079).

The cultivar used was TMG 1288 RR, maturation group 8.8, with a determined growth habit and susceptible to diseases. The plant population was 240 000 ha<sup>-1</sup>, with a 0.5 m between rows spacing. The experiment was implemented using a randomized block design, with 4 replications. Each parcel was 20 m<sup>2</sup> in size. Five treatments were used, being: control (without alternative products or fungicides application), noni leaves aqueous extract, noni fruit aqueous extract, noni fruit essential oil and fungicides.

## 2.7 FIELD TREATMENTS APPLICATION

The extracts and essential oil doses applied to plants in the field were made based on the phytotoxicity tests results. The fungicides used were: pyraclostrobin (133 g L<sup>-1</sup>) + epoxiconazole (50 g L<sup>-1</sup>) 0.5 L ha<sup>-1</sup>, an application made in V6 (fifth open trefoil); mancozeb (750 g Kg<sup>-1</sup>) 2 Kg ha<sup>-1</sup> with two applications made in R1 (flowering beginning) and R 5.3 (pod filling 25 to 50%). The applications were performed using a manual backpack sprayer with a full cone type nozzle. A spray mix volume corresponding to 150 L ha<sup>-1</sup> was used.

## 2.8 AGRONOMIC EVALUATION

The agronomic characteristics evaluated were: total plant tissue mass (TTM), first pod insertion height (FPIH), plant height (PH), pods number per plant (PNP), thousand

grains mass (TGM) and productivity ( $\text{Kg ha}^{-1}$ ). For sampling, 10 plants were removed from each plot.

## 2.9 DISEASE ASSESSMENT

The disease assessment was carried out jointly, measured by the total area affected in the plants, with notes scale proposed by Finoto et al. (2011), where: 0 (zero) for disease absence, 1 (one) for severity between 1 and 10%, 2 (two) for severity between 11 and 25%, 3 (three) for severity between 26 and 50%, 4 (four) for severity between 51 and 75% and 5 (five) for severity between 76 and 100%. The notes were given based on the leaf area, stem and pods affected in all plants in the useful parcel. The note values were converted into an Area Under the Disease Progress Curve (AUDPC), according to the formula proposed by Shaner & Finney (1977).

During the experiments conduct, agrochemical applications were realized to control ants, caterpillars, bed bugs and weeds, with products recommended by MAPA (Brasil, 2016).

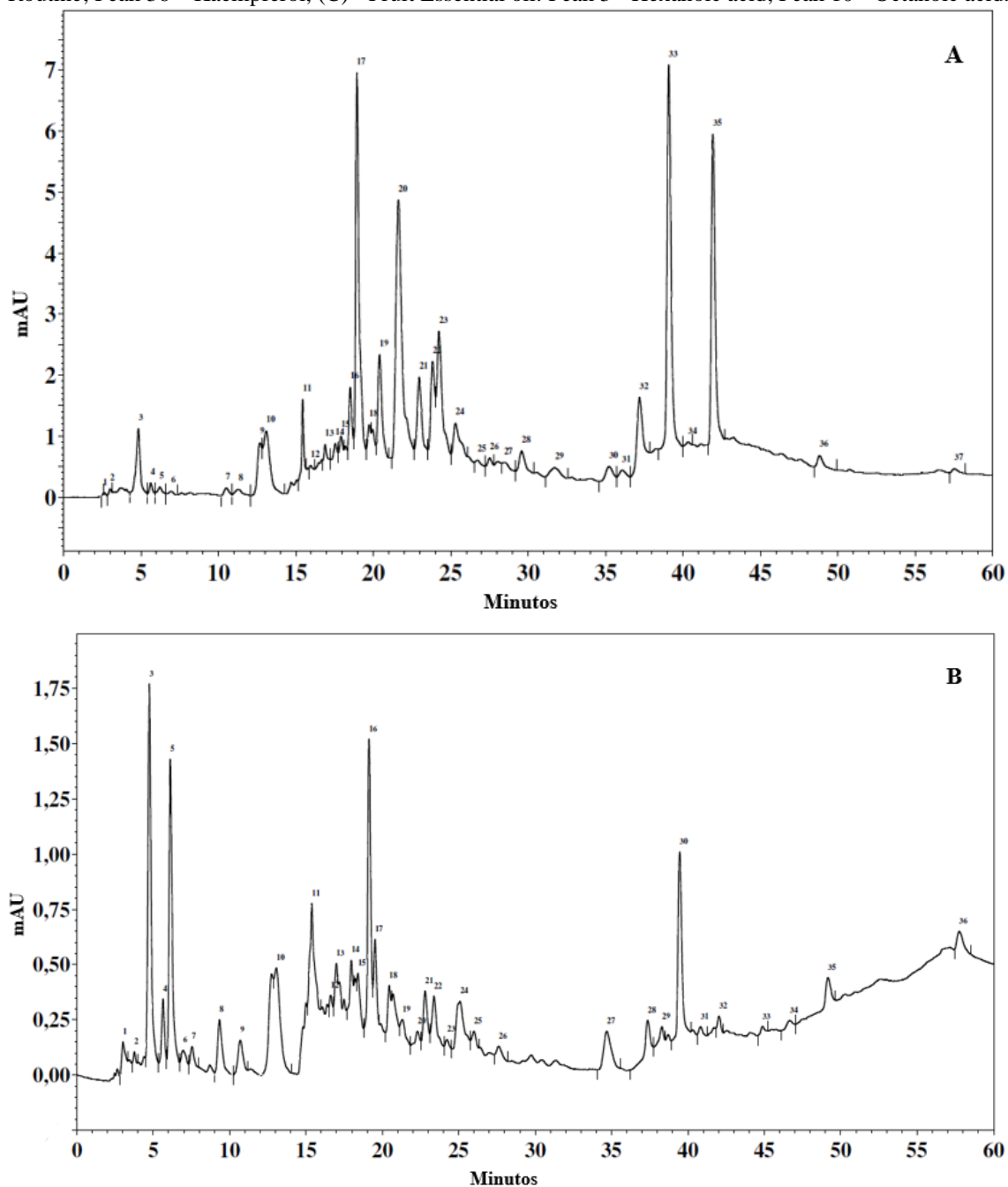
## 2.10 STATISTICAL ANALYSIS

Variance analysis was performed for all characteristics and mathematical models were adjusted to the quantitative treatments and tests for compare the means to the qualitative ones. All analyzes were performed using the Sisvar software (Ferreira, 2014).

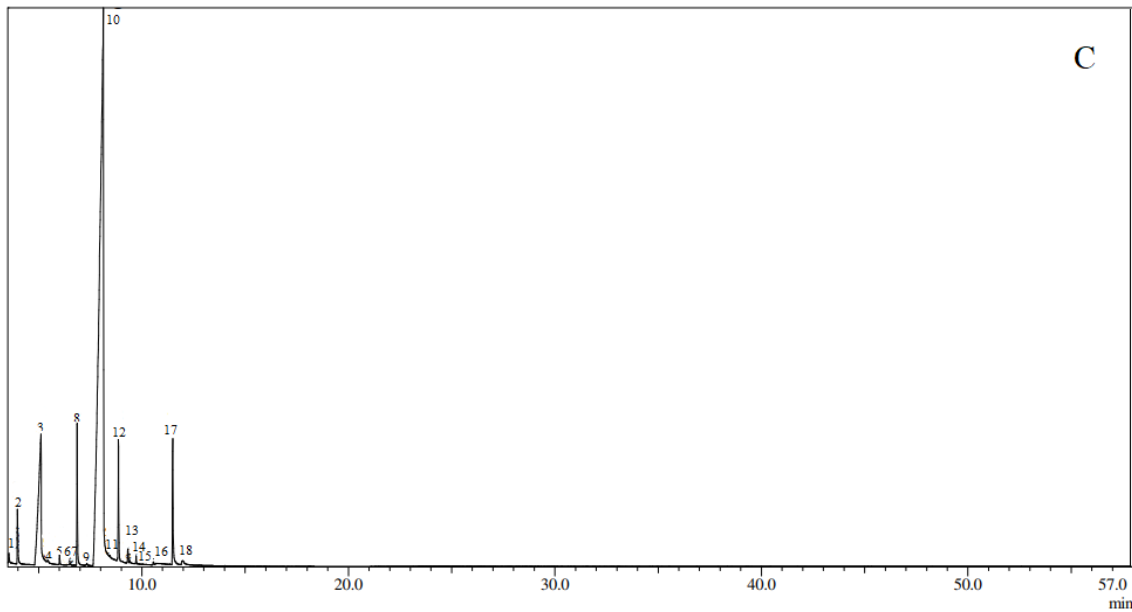
## 3 RESULTS

It can be seen that the compounds that constitute the extracts (Figure 1), are mostly potent antimicrobials. Figures 1A and 1B show the aqueous extract constituents of the leaf and noni fruit, respectively. In the composition study, compounds present in the extract was verified with retention time in minutes, being demonstrated as follows: Gallic acid (16.89); Hydroxybenzoic acid (23.37); Catechin (23.82); Vanylic acid (24,24); Cyrinic acid (26.73); Epigallocatechin gallate (28.44); P-cumaric acid (35.22); Rutin (40.33); Kaempferol (57.51). In figure 1C, the essential oil compounds have been quantified and their two main constituents are hexanoic acid (5.11) and octanoic acid (8.13).

Fig. 1. Chromatograms of the alternative treatments used in the soybean test (mAU - Milli-Absorbance Units). (A) - *Morinda citrifolia* L. leaf aqueous extract. Compounds: Peak 13 - Gallic acid; Peak 22 - Catechin; Peak 23 - Vanylic acid; Peak 25 - Surgical acid; Peak 27 - Epigallocatechin gallate; Peak 30 - p-cumaric acid; Peak 34 - Rutin; Peak 37 - Kaempferol, (B) - Aqueous fruit extract. Compounds: Peak 12 - Gallic acid; Peak 22 - Hydroxybenzoic acid; Peak 23 - Vanylic acid; Peak 27 - p-cumaric acid; Peak 31 - Routine; Peak 36 - Kaempferol, (C) - Fruit Essential oil: Peak 3 - Hexanoic acid; Peak 10 - Octanoic acid.



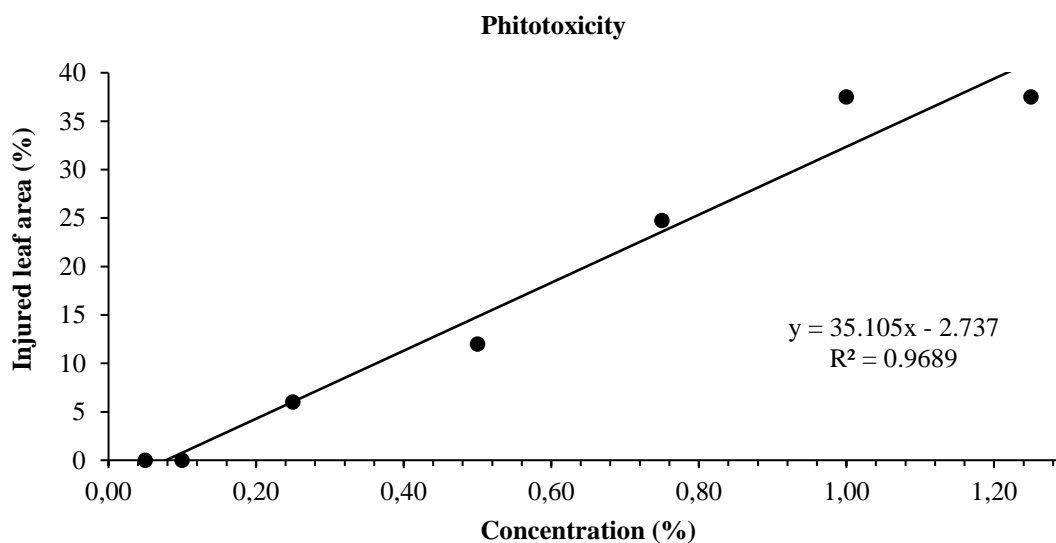




The greater compounds diversity in the extracts compared to essential oil was predictable, because the latter is a purified product obtained from a hydro-distillation method by steam drag. Only some the brute extract constituents remain in the oil, making it a more concentrated product. Thus, it needs a greater dilution so that it can be used in application on plants.

Figure 2 shows the phytotoxicity test result with noni essential oil application in cultivar TMG 1288 soybean plants, with aim to use as an antifungal agent in field experiments.

Fig. 2. Injured leaf area (phytotoxicity) observed in soybean leaves (cultivar TMG 1288), depending on the *Morinda citrifolia* L. essential oil different concentrations application.



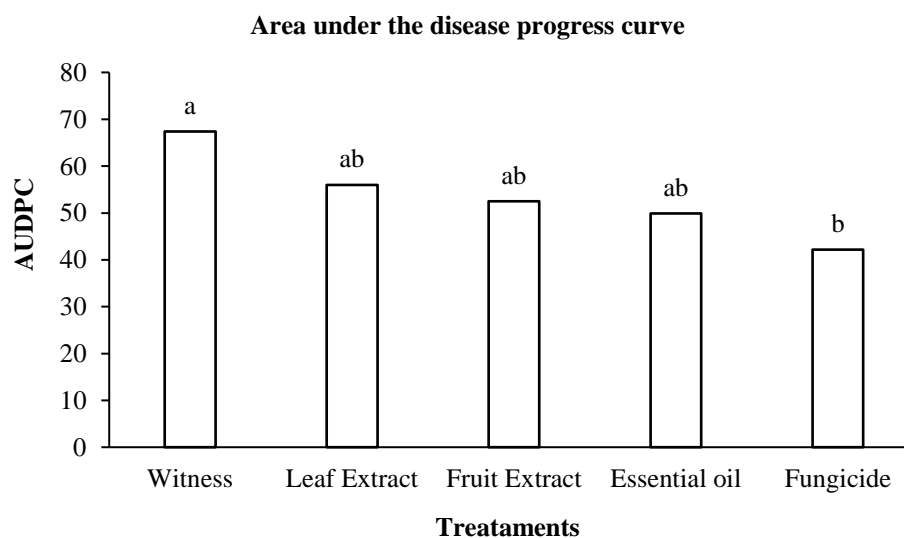
The increase essential oil concentration increased the injured leaf area (ILA) to plants in a linear manner. The effects on soybean leaves ranged from “no phytotoxicity”, when the plants received applications at 0.05% and 0.1% concentrations, ILA phytotoxicity up to 37.5%, for concentrations application 1.0% and 1.25%. The 0.25% concentration was the highest under 10% of ILA, an estimated value so that there would be no significant damage to the plants.

The aqueous extracts were also subjected to the phytotoxicity test on soybean plants (data not shown). However, even at the highest concentration (stock solution 1/10 m/v) no lesions were observed on the leaves. For field applications, a 25% the stock solution was stipulated so that the experiments were feasible technically.

With preliminary evaluations of the compounds from the noni plant, it was possible to establish two field experiments, designated as the Farm and UFT trial, in order to assess the diseases severity and soybean crops agronomic characteristics.

In the Farm area, there was an anthracnose incidence in soybean plants, caused by *Colletotrichum truncatum* and Asian rust, caused by *Phakopsora pachyrhizi*. The disease severity quantification that affected the experimental soybean crop plots was expressed through the Area under the disease progress curve (AUDPC), as shown in Figure 3.

Fig. 3. Area under the disease progress curve (AUDPC), for Anthracnose and Asian Rust in soybean cultivar TMG 1288 RR, submitted to leaf, fruit aqueous extract foliar applications, *Morinda citrifolia* L. essential oil and fungicides. Essay Farm, in Gurupi, diseases in a high-pressure area, Tocantins State. Same letters in the figure above each column do not differ statistically from each other, by Tukey's test, at 5% probability.



It is observed that the only treatment that differed statistically from the control was with fungicide recommended for soybean culture (Figure 3). The reduction in AUDPC among them resulted in approximately 38%. This treatment did not differ from noni extracts and essential oil treatments, which showed intermediate values, 56 for leaf extract, 52.5 for fruit and 49.9 for essential oil. In the present work it was verified that the diseases anthracnose and Asian rust caused high severity in the plants. Thus, there was a greater protecting effect the treatments and reducing the treated plants severity, allowing significant results to be observed in the soybeans agronomic characteristics (Table 1).

Table 1. Soybean cultivar TMG 1288 RR agronomic characteristics, submitted to leaf, fruit aqueous extract foliar applications, *Morinda citrifolia* L. essential oil and fungicides.

Treatment	TTM (Kg)	FPIH (m)	PH (m)	PNP	TGM (kg)	PROD (Kg ha <sup>-1</sup> )
Witness	1.9c	0.118 <sup>ns</sup>	0.472 <sup>ns</sup>	36.2b	0.0918 <sup>ns</sup>	1159.2b
Leaf Ext.	2.8abc	0.134	0.513	41.4ab	0.0929	1748.8a
Fruit Ext.	2.3bc	0.093	0.470	36.2b	0.0939	1185.8b
Essential Oil	3.3a	0.123	0.509	46.8ab	0.0986	1762.5a
Fungicide	3.1ab	0.092	0.512	52.8a	0.0947	2031.7a
V.C. (%)	16.02	20.08	10.90	13.26	3.86	13.58

Same letters in the column do not differ by Tukey's test at 5% probability.

TTM = total tissue mass; FPIH = first pod insertion height; PH = plant height; PNP = pods number per plant; TGM = thousand grain mass; PROD = productivity

ns- not significant

The results observed in the total tissue mass (Table 1) demonstrate how the high rust severity was able to cause damage to plants, represented by tissue necrosis and significant leaf abscission. This rapid leaf loss reflected directly in the decrease in the total plants tissue mass. Anthracnose was also responsible for severe lesions on leaves, pods and also on the plants stem, directly affecting grain quality and productivity. The treatments with noni fruit essential oil and with the fungicides application differed significantly from the control, presenting the highest production values. This result is related to the AUDPC values, where it was found that the lower the disease severity, the more vigorous the plant vegetative part.

The first pod insertion height agronomic characteristics, plants height and thousand grains mass did not show significant difference between treatments (Table 1).

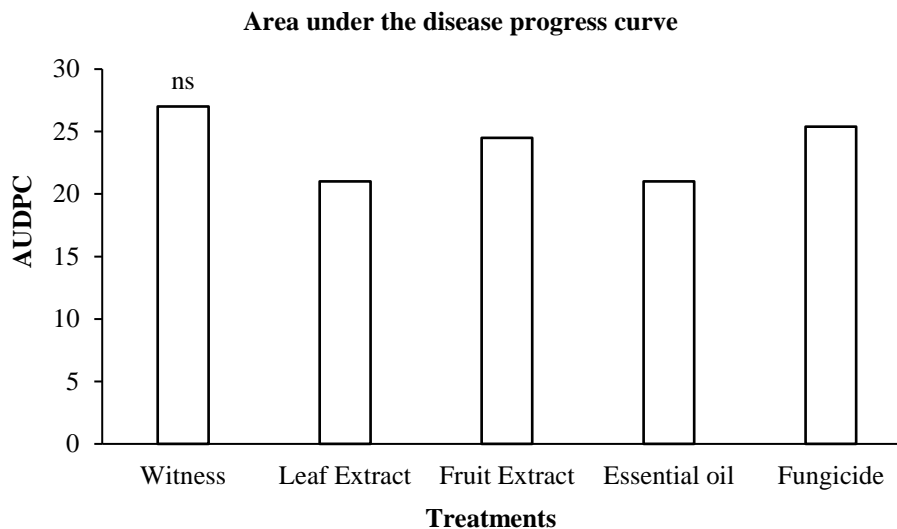
Regarding the pods number per plant (Table 1), some differences were observed. The soybean experimental plots treated with the leaf aqueous extract, fruit essential oil and fungicides had a pods greater amount in each plant. However, only fungicides application differed statistically from the control and the fruit extract, showing an average of 52.8, 36.2 and 36.2, respectively.

The pods number per plant was directly proportional to the culture productivity (Table 1). The treatments with fungicides, essential oil and leaf extract obtained the following productivity in Kg ha<sup>-1</sup>, respectively: 2031.7; 1762.5; 1748.8. The three differed statistically from the fruit extract (1185.8 kg ha<sup>-1</sup>) and control (1159.2 kg ha<sup>-1</sup>). In general, productivity was considered low due to the diseases attack severity on soybean (cultivar TMG 1288 RR).

In the trial conducted under low disease pressure, at the Federal University of Tocantins experimental station, Campus de Gurupi (Figure 4), the disease levels, expressed by the AUDPC, are observed depending on the treatments.

Fig. 4. Area under the disease progress curve (AUDPC), for Anthracnose and Asian Rust in soybean cultivar TMG 1288 RR, submitted to leaf, fruit aqueous extract foliar applications, *Morinda citrifolia* L. essential oil and fungicides. Essay UFT, in Gurupi, State of Tocantins.

<sup>ns</sup> = means do not differ significantly by Tukey's test at 5% probability.



Unlike the previous test, the disease severity values did not differ statistically between treatments. On average, the disease severity values were 50% lower than the farm test, affecting plants less. This was probably due to the lower pathogens inoculum density present at the site, since the trial area has no frequent history soy plantations, in addition to the previous crops diversity, including corn, melon, pastures.

The low leaf diseases level in the field (Figure 4) also did not allow significant differences in plants to be verified for Total Tissue Mass, Pod and Plant Height, Pods number, and productivity (Table 2).

Table 2. Soybean cultivar TMG 1288 RR Agronomic characteristics, submitted to leaf, fruit aqueous extract foliar applications, *Morinda citrifolia* L. essential oil and fungicides recommended for the crop (UFT test).

Treatment	TTM (Kg)	FPIH (cm)	PH (cm)	PNP	TGM (g)	PROD (Kg ha <sup>-1</sup> )
Witness	3.5 <sup>ns</sup>	16.2 <sup>ns</sup>	62.1 <sup>ns</sup>	36.2 <sup>ns</sup>	90.9 <sup>ns</sup>	2090.8 <sup>ns</sup>
Leaf Ext.	3.2	12.5	55.2	39.3	95.0	2348.8
Fruit Ext.	3.2	13.9	57.1	50.8	88.6	2286.7
Essential Oil	3.5	13.4	59.9	39.3	89.8	2578.3
Fungicide	3.4	13.6	62.8	53.1	94.0	2638.3
V.C. (%)	9.02	17.69	8.09	36.34	4.06	14.60

TTM = total tissue mass; FPIH = first pod insertion height; PH = plant height; PNP = pods number per plant; TGM = thousand grain mass; PROD = productivity  
<sup>ns</sup> = means do not differ significantly by Tukey's test at 5% probability.

These results demonstrate that the treatments applied did not significantly stimulate other plant productive characteristics, when under low pathogens inoculum pressure.

#### 4 DISCUSSION

The compounds found in the noni tissues chromatographic analysis are mostly antimicrobial agents. Other studies with different species plants have already demonstrated the antimicrobial compounds presence, as well as its effects on phytopathogenic structures. Nguyen et al. (2013) proved that gallic acid has a high antifungal activity against *Fusarium solani*, and this activity was dose dependent. The hyphae were collapsed and shrunk after incubation per 24 h. Peyer et al. (2016) observed the ability to inhibit spore germination in another *Fusarium* species by carboxylic acids (vinyllic acid; p-coumaric acid). Rutin has also been described as having antifungal potential for several phytopathogenic fungi (Lupascu et al., 2017).

There are few studies related to alternative control using noni against phytopathogens. Most of the studies are aimed at use in diseases that affect humans (Tintino et al., 2015; Assi et al., 2017). However, some recent studies demonstrate a significant noni essential oil fungicidal capacity in controlling diseases caused by the

fungi *Didymella bryoniae*, *Olivea neotectonae* and *Exserohilum turcicum* (Dalcin et al., 2017; Osorio et al., 2018; Silva et al., 2017, respectively), but these studies were carried out under greenhouse conditions and were not tested in the field. Thus, the present work contributed to the search for answers on the alternative treatments behavior when applied in adverse field conditions, under the natural inoculum influence and under the bad weather action, such as sun, heat and humidity.

Yet, other alternative products have already been evaluated in a greenhouse to control soybean rust. Medice et al. (2007) found the *Phakopsora pachyrhizi* uredinospores germination inhibition and delayed disease evolution through the *Corymbia citriodora* (eucalyptus citriodora), *Cymbopogon nardus* (citronella) *Azadirachta indica* (nim) and *Thymus vulgaris* L. (thyme) essential oils application. Bigaton et al. (2013) working with extract and peppermint (*Schinus terebinthifolius*) essential oil observed an increase in the disease intensity. This indicates that not every alternative product has the fungitoxic capacity.

In this work, there was a statistical equivalence of the products from the noni plant to the fungicides (Figure 3), even in an adverse situation, with high disease pressure, expressed by the high severity in the plants (AUDPC). In the Anthracnose control in soy, it has already been verified by Pesqueira et al. (2016), that fungicides application provided a reduction in defoliation, a diseased petioles lower percentage, a pods greater number, greater plant height and greater productivity. In the present work, under high inoculum potential, in the commercial area test, this effect could also be seen, both by the fungicides application and also in the treatment with noni essential oil.

It was demonstrated that the treatments impact on disease control in soybean crops was verified only under high inoculum potential. This fact was evident when comparing the two planting areas results. In the experiment at the commercial farm area, there was a strong inoculum pressure, probably due to frequent cultivation in soybeans monoculture extensive areas both on site and in surrounding areas. The test in an experimental area is always done with crop rotation, with different species plants, including melon, pastures, corn, among others. The soybean plantations, when implanted in these experimental areas, are installed in small plots and it is important to note that the surrounding areas were composed by cerrado native reserves, and pastures. Cultivation with non-host species, breaks the pathogen cycle and decreases its survival. Crop rotation can reduce the phytopathogens inoculum density through two mechanisms: food suppression food for fungus metabolism and increased antagonistic microorganisms activity in the soil

(Reis et al., 2011). This increase in microbial diversity with phytopathogenic characteristics in crop rotation systems is described in detail in a study by Peralta et al. (2018). Andreu et al. (2018) evaluated plant extracts and obtained significant results for *Plasmopara viticola* spores inhibition. However, additional field trials have shown no effect on disease control *in vivo*. The potential sensitivity of the studied extracts to biodegradation and UV rays could explain the loss activity observed. Thus, a future compounds formulation study with other products is necessary to evaluate their activity in real conditions.

Therefore, it was demonstrated in the present work that the use to natural compounds applied so protective in plants grown in large areas can promote the necessary protection against phytopathogens. Though, new studies seeking to improve residual power, as well as to develop technologies that reduce these compounds degradation under field conditions are necessary.

#### 4 CONCLUSION

Under strong inoculum pressure the best treatments were leaf extract and noni essential oil and fungicides, which differed from the witness and had the productivity highest levels. Noni extracts and essential oil also had a similar effect to fungicide treatment in Asian rust and soybean anthracnose control.

The trial under low disease pressure (experimental station) did not allow a significant difference verification between treatments.

## REFERENCES

- Adams, R.P. (2007). *Identification of essential oil components by gas chromatography/mass spectroscopy*. (4th ed.) Carol Stream: Allured Publishing Corporation, 804 pp.
- Andreu, V., Levert, A., Amiot, A., Cousin, A., Aveline, N., & Bertrand, C. (2018). **Chemical composition and antifungal activity of plant extracts traditionally used in organic and biodynamic farming**. *Environmental Science and Pollution Research*, 25(30), 29971–29982. DOI: 10.1007/s11356-018-1320-z
- Assi, R.A., Darwis, Y., Abdulbaqi, I.M., Khan, A.A., Vuanghao, L., & Laghari, & M.H. (2017). **Morinda citrifolia (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials**. *Arabian Journal of Chemistry*, 10(5), 691-707. DOI: <https://doi.org/10.1016/j.arabjc.2015.06.018>
- Bigaton, D., Bacchi, L.M.D., Formagio, A.S.N., Gavassoni, W.L., & Zanella, C.S. (2013). **Evaluation of the extracts and essential oils fungicidal activity on Asian soybean rust**. *Agronomic Science Magazin*, 44(4), 757-76. DOI: <https://doi.org/10.1590/S1806-66902013000400012> (In Portuguese).
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. (2016). **Agrofit: sistema de agrotóxicos fitossanitários**. Available on: [http://agrofit.agricultura.gov.br/agrofit\\_cons/principal\\_agrofit\\_cons](http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons) . [Accessed on: 10 Dez. 2016]
- Clevenger, J.F. (1928). **Apparatus for the Determination of Volatile Oil\***. *The Journal of the American Pharmaceutical Association (1912)*, 17(4), 345-349. DOI: <https://doi.org/10.1002/jps.3080170407>
- Cogliatti, M., Juan, V.F., Bongiorno, F., Da lla Valle, H., & Rogers, W.J. (2011). **Control of grassy weeds in annual canary grass**. *Crop Protection*, 30(2), 125-129. DOI: <https://doi.org/10.1016/j.cropro.2010.11.002>
- CONAB. **Companhia Nacional de abastecimento**. (2018). Safra Brasileira de Grãos. Brasília-DF. Available on: <https://www.conab.gov.br/info-agro/safras/graos> [Accessed on: 21 Set. 2018]
- Dalcin, M.S., Café-Filho, A.C., Sarmiento, R.A., Nascimento, I.R., Ferreira, T.P.S., Aguiar, R.W.S., & Santos G.R. (2017). **Evaluation of essential oils for preventive or curative management of melon gummy stem blight and plant toxicity**. *Journal of Medicinal Plants Research*, 11 (26), 426-432. DOI: <https://doi.org/10.5897/JMPR2017.6405>
- Dequech, S.T.B., Ribeiro, L.P., Sausen, C.D., Egewarth, R., & Kruse, N.D. (2008). **Phytoxicity caused by botanical insects in beans (*Phaseolus vulgaris* L.)** FZVA Magazine 15 (1):71-80. (In Portuguese).



Embrapa. **Tecnologias de produção de soja - Região Central do Brasil**. 2013 Londrina: Embrapa Soja, 265 pp.

Ferreira, D.F. (2014). **Sisvar: a Guide for its Bootstrap procedures in multiple comparisons**. *Ciência e Agrotecnologia [online]*, 38(2), 109-112. DOI: <https://doi.org/10.1590/S1413-70542014000200001>

Finoto, E.L., Carrega, W.C., Sedyama, T., Albuquerque, J.A.A., Cecon, P.R., & Reis, M.S. (2011). **Effect of fungicide application on agronomic traits and severity of end-of-cycle diseases in soybean crop**. *Revista Agroambiente On-line*, 5(1), 44-49. DOI: <http://dx.doi.org/10.18227/1982-8470ragro.v5i1.418> (In Portuguese).

Freitas, S.P., Moreira, J.G., Freitas, I.L.J., Freitas Júnior, S.P., Amaral Júnior, A.T., & Silva, V.Q.R. (2009). **Phytotoxicity of herbicides in different popcorn cultivars**. *Planta Daninha*, 27, 1095-1103. DOI: <https://doi.org/10.1590/S0100-83582009000500023>

Hillen, T., Schwan-Estrada, K.R.F., Mesquini, R.M., Cruz, M.E.S., Stangarlin, J.R., & Nozaki M. (2012). **Antimicrobial activity of essential oils in the control of some fungal phytopathogens in vitro and in seed treatment**. *Revista Brasileira de Plantas Mediciniais*, 14(3), 439-445. DOI: <https://doi.org/10.1590/S1516-05722012000300003> (In Portuguese).

Lupaşcu, D., Tuchiluş, C., Lupaşcu, F., Constantin, S., Apotrosoaci, M., Vasincu, I. M., ... Profire, L. (2017). **Rutin semisynthetic derivatives with antifungal properties**. *Farmacia*, 65 (2), 184-188

Medice, R., Alves, E., Assis, R.T., Magno Júnior, R.G., & Lopes, E.A.G.L. 2007. **Essential oils to control Asian soybean rust *Phakopsora pachyrhizi* (Syd. & P. Syd)**. *Ciência e Agrotecnologia*, 31(1), 83-90. DOI: <https://doi.org/10.1590/S1413-70542007000100013> (In Portuguese).

Mourão, D.S.C., Ferreira, T.P.S., Souza, D.J., Chagas Júnior, A.F., Dalcin, M.S., Veloso, R.A., ... Santos G.R. (2017). **Essential oil of *Cymbopogon citratus* on the control of the *Curvularia* Leaf spot disease on maize**. *Medicines*, 4(62), 1-11. DOI: <https://doi.org/10.3390/medicines4030062>

Nascimento, J.M., Gavassoni, W.L., Bacchi, L.M.A., Oliveira, J.L., Laborde, M.C., Pontim, B.C.A., & Mendes, M.P. (2018). **Management of Asian soybean rust with fungicide applications initiated in the detection of the pathogen or later**. *Agrarian*, 11(39), 42-49. DOI: <https://doi.org/10.30612/agrarian.v11i39.4396> (In Portuguese).

Nguyen, D.M., Seo, D.J., Lee, H.B., Kim, I.S., Kim, K.Y., Park, R.D., & Jung, W.J. (2013). **Antifungal activity of gallic acid purified from *Terminalia nigrovenulosa* bark against *Fusarium solani***. *Microbial Pathogenesis*, 56, 8-15. DOI: <https://doi.org/10.1016/j.micpath.2013.01.001>

Oliveira, D.M., Melo, F.G., Balogun, S.O., Flach, A., Souza, E.C., Souza, G.P., ... Martins, D.T.O. (2015). **Antibacterial mode of action of the hydroethanolic extract of**

***Leonotis nepetifolia* (L.) R. Br. Involves bacterial membrane perturbations.** *Journal of Ethnopharmacology*, 172, 356-63. DOI: <https://doi.org/10.1016/j.jep.2015.06.027>

Osorio, P.R.A., Leão, E.U., Veloso, R.A., Mourão, D.S. C., & Santos, G.R. (2018). **Essential oils for alternative teak rust control.** *Floresta e Ambiente [online]*, 25(2). DOI: <https://doi.org/10.1590/2179-8087.039116>

Peralta, A.L., Sun, Y., McDaniel, M.D., & Lennon, J.T. (2018). **Crop rotational diversity increases disease suppressive capacity of soil microbiomes.** *Ecosphere*, 9(5), 1-16. DOI: <https://doi.org/10.1002/ecs2.2235>

Pesqueira, A.S., Bacchi, L.M.A., & Gavassoni, W.L. (2016). **Fungicide association in the control of soy anthracnose in Mato Grosso do Sul.** *Revista Ciência Agronômica*, 47(1), 203-212. DOI: <https://doi.org/10.5935/1806-6690.20160024> (In Portuguese)

Peyer, L.C., Axel, C., Lynch, K.M., Zannini, E., Jacob, F., & Arendt, E.K. (2016). **Inhibition of *Fusarium culmorum* by carboxylic acids released from lactic acid bacteria in a barley malt substrate.** *Food Control*, 69, 227-236. DOI: <https://doi.org/10.1016/j.foodcont.2016.05.010>

Pignati, W.A., Lima, F.A.N.S., Lara, S.S., Correa, M.L.M., Barbosa, J.R., Leão, L.H.C., & Pignatti, M.G. (2017). **Spatial distribution of pesticide use in Brazil: a tool for health surveillance.** *Ciência & Saúde Coletiva*, 22(10), 3281-3293. DOI: <https://doi.org/10.1590/1413-812320172210.17742017> (In Portuguese).

Prakash, B., Kedia, A., Mishra, P.K., & Dubey, N.K. (2015). **Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities e potentials and challenges.** *Food Control*, 47, 381-391. DOI: <https://doi.org/10.1016/j.foodcont.2014.07.023>

Reis, E.M., Casa, R.T., & Bianchin, V. (2011). **Control of plant diseases by crop rotation.** *Summa Phytopathologica*, 37(3), 85-91. DOI: <https://doi.org/10.1590/S0100-54052011000300001> (In Portuguese).

Santos Neto, J., Schwan-Estrada, K.R.F., Temporal, W.M., Andrade, L.M.A., & Sena, J.O.A. (2016). **Lemongrass by-products to control septoriosiis in tomatoes grown in an organic production system.** *Revista Brasileira de Agroecologia*, 11(1), 35-44. (In Portuguese).

Shaner, G., & Finney, R.E. (1977). **The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat.** *Phytopathology*, 67(8), 1051-1056.

Silva, J.C., Mourão, D.S.C., Lima, F.S.O., Sarmiento, R.A., Dalcin, M.S., Aguiar, R.W.S., & Santos, G.R. (2017). **The efficiency of noni (*Morinda citrifolia* L.) essential oil on the control of leaf spot caused by *Exserohilum turcicum* in maize culture.** *Medicines*, 4(60), 1-10. DOI: <https://doi.org/10.3390/medicines4030060>

Simonetti, E., Viso, N.P., Montecchia, M., Zilli, C., Balestrasse, K., & Carmona, M. (2015). **Evaluation of native bacteria and manganese phosphite for alternative**

**control of charcoal root rot of soybean.** *Microbiological Research*, 180, 40–48. DOI: <https://doi.org/10.1016/j.micres.2015.07.004>

Tintino, S.R., Neto, A.A.C., Menezes, I.R.A., Oliveira, C.D., & Coutinho, H.D.M. (2015). **Antimicrobial activity and combined effects on antifungal and antibacterial drugs the fruit of *Morinda citrifolia* L.** *Acta Biologica Colombiana*, 20(3), 193-200. DOI: <http://dx.doi.org/10.15446/abc.v20n3.45601> (In Portuguese)