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Noni essential oil associated with adjuvants in the production of phytoalexins and in the control of soybean anthracnosis

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Amongst the most important agricultural products in Brazil, the soybean stands out, mainly as an export good. One of the end-of-cycle diseases, Anthracnose, caused by the fungus *Colletotrichum truncatum* (Schwein.) leads to severe damage to grain quality and yield. The use of natural products as a substitute for conventional pesticides for plant diseases control is an alternative found to reduce food contamination and environmental impacts. However, despite the progress made, much work still needs to be done to enable the safe and effective use of alternative products. Thus, this study aimed to test efficacy of noni essential oil (EO), combined with adjuvants, in the control of anthracnose in soybean plants. Gas chromatography was performed to identify the chemical profile of the EO. *In vitro* mycelial growth inhibition tests and analyzes on the stimulation of phytoalexin production were also performed. In the field trial, two soybean cultivars submitted to EO were used combined with orange and mineral oils as adjuvants. Octanoic acid (OA) was identified as the major component of EO. There was efficacy in the control of *C. truncatum* by EO and OA *in vitro*, as well as the induction of the production of the phytoalexin gliceolin in soybean seedlings with an increase of almost 80% with OA but with no impact on the growth of *Trichoderma* sp. Between the soybean cultivars tested, Coodetec 2728 IPRO presented lower severity to anthracnose symptoms, however, the cultivar Brasmax Foco demonstrated good tolerance to pathogen's attack maintaining high productivity. Application of fungicides on the cultivar Coodetec 2728 IPRO resulted in a detrimental effect on yield possibly due to induction of the green stem syndrome formation in plants and a harvest delay of 15 days.

Key words: *Morinda citrifolia* L., *Glycine max* L.; *Colletotrichum truncatum*, *Trichoderma* sp, alternative control.

INTRODUCTION

A growing world population requires an increase in the quality and quantity of food production. Among the commodities that stand out in agricultural production is soybean and Brazil is its largest exporter in the world.

The 2018/2019 harvest totaled approximately 120 million tons, constituting the country's production record (CONAB, 2019).

Soy is affected by several diseases that interfere with the capacity of obtaining high levels of productivity and approximately 40 diseases caused by fungi, bacteria, nematodes, and viruses have already been identified in Brazil by the latest research performed by the state's agricultural research corporation; EMBRAPA (Henning et al., 2014; Saran, 2014). Among the predominant diseases in the culture is anthracnose, caused by the fungus *Colletotrichum truncatum* (Schwein.), plants show black spots on the leaf veins, stems, and pods (Godoy et al., 2014). Field losses due to biotic stresses are currently estimated between 10 and 20% worldwide. The risk of resistance and strict pesticide legislation require innovative agronomic practices to properly protect crops in the future, such as the identification of new substances with new modes of action (Gillmeister et al., 2019).

The use of natural products originating mainly from plants, such as extracts and essential oils (EO), have positive characteristics as replacement of pesticides in the control of pests and diseases in the fields (Sevindik et al., 2017; Mohammed et al., 2020). Several studies have been carried out to prove the fungitoxic efficacy of EO and also to describe their mechanisms of action against fungi, such as disruption of endomembranes, including the plasma membrane and mitochondria, specifically inhibiting ergosterol synthesis, mitochondrial ATPase, malate dehydrogenase, and succinate dehydrogenase (MA et al., 2016; El ouadi et al., 2017; HU et al., 2017; Mohammed et al., 2021). In addition to fungi development inhibition, EO are responsible for activating the production of substances that are part of the plant's defense mechanism, the phytoalexins (Oliveira et al., 2017).

Between the difficulties encountered regarding the effectiveness of the EO´s use, there is the high degradation of its constituents when exposed to high temperature and solar radiation (Chang et al., 2021; Guimarães et al., 2008). Therefore, these compounds need to penetrate the plant as fast as possible. The addition of adjuvants/surfactants can assist in this process, as they help to overcome the layer of epicuticular wax present in the leaf (Kovalchuk and Simmons, 2021; Räsch et al., 2018; Steurbaut et al., 1989).

Due to these described characteristics, EO tends to be less toxic to nature, and their use is more environmentally friendly when replacing synthetic pesticides. Among the plants that produce EO, noni or Indian mulberry (*Morinda citrifolia* L.), from the Rubiaceae family, is considered to have great potential for effectiveness in phytopathogenic control (Dalcin et al., 2017; Osorio et al., 2018). Noni is a traditional plant and is been used for over 2000 years for its nutritional value but also for its therapeutical properties (Gupta et al., 2020; Wang et al., 2021, 2002). Although most of the early work done on Noni shows the depth of the anti-bacterial properties, it is suggested that it contains multiple pharmacologically active substances that still need to be isolated (Coutinho de Sousa et al., 2017; Leach et al, 1988; MA et al, 2013; Yilmazer et al., 2016).

Other studies have shown that, besides its anti-bacterial properties, it may also control fungi (Dos Santos et al., 2021). There is no significant amount of research work using EO as fungicidal agents in fields exposed to the climatic conditions of the place (Abou Assi et al., 2017). Due to this factor, the present study aimed to test the efficacy of noni EO, combined with adjuvants, in the control of anthracnose in soybean plants.

MATERIALS AND METHODS

Essential oil extraction and chemical analysis

The EO was obtained from ripe noni fruits (*M. citrifolia* L.) extracted by hydrodistillation for a period of two hours. In a round-bottom flask with a capacity of 1000 ml, 0.02 kg of the material was deposited for extraction, adding 500 ml of distilled water and then coupled in a Clevenger-type apparatus. At the end of the extraction period, the EO was collected in the form of a supernatant, stored in an amber flask, identified and kept in a refrigerator at 4°C until the moment of implantation of the bioassays.

Qualitative analyzes of the main EO components were performed by gas chromatography coupled to GC-MS spectrometry using a Shimadzu GC-210 equipped with a QP 2010 Plus mass selective detector. The equipment was operated under the following conditions: RTX-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm thick of film); with the following column temperature program: 60 - 240°C (3°C/min); injector temperature: 220°C; helium carrier gas; spitless injection with an injected volume of 1 μl of a 1:1000 solution in hexane. For the mass spectrometer (MS), the following conditions were used: impact energy of 70 eV; ion source and interface temperature: 200°C.

The constituents were identified by comparing their mass spectra with those in the databases of the NIST and Wiley 229 libraries. Some compounds had their identities confirmed by comparing their retention rates calculated with those present in the NIST webbook and with the literature (Adams, 2007). The quantification of compound contents, expressed as a percentage obtained by injection of a mixture of standards containing a homologous series of C7-C30 alkanes and the Kovats index, calculated for each constituent, was compared with the tabulated according to Adams (2007).

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In vitro tests

With the identification of the major component of the EO, an *in vitro* analysis was carried out to compare the fungi control effectiveness, between noni´s EO and the major compound. The causal agent of anthracnose in soy, *C. truncatum*, combined with the antagonist fungus *Trichoderma* sp., was used to measure mycelial growth.

The *in vitro* bioassays were designed in Petri dishes (90 mm in diameter), testing different concentrations (0.1, 0.5, 1, 2 and 4%) of EO, major compound, and fungicide as a control. A completely randomized design with four replicas was used. The different concentrations of EO were diluted in a solution of sterile water with Tween 80 (1.0%). Subsequently, 200 µl of different concentrations were spread on the surface of a commercial Potato-Dextrose-Agar (PDA) culture media, with the aid of a Drigalsky handle, and then, in the center of each Petri dish, a disc containing mycelia of *C. truncatum* and *Trichoderma* sp*.* was placed (4 mm). The plates were sealed, identified, and kept in an incubation chamber at 25°C for ten days. The fungi mycelial growth was measured using a digital caliper.

Phytoalexin induction in soybean cotyledons

Soybean seeds were disinfected in 1% sodium hypochlorite and washed in distilled water. Subsequently, sown in trays containing autoclaved sand (121°C and 1 atm for 20 min). They were kept in a greenhouse for 10 days and the cotyledons were detached for the tests. The cotyledons were placed in Petri dishes, where each dish contained 3 cotyledons and 2 sheets of sterile filter paper moistened with sterile distilled water. Each cotyledon was cut into small fragments, which were treated with 100 µl of the solutions to be tested. The plates were incubated at 25 ± 1°C, in the dark, for 20 h. Then, the cotyledons were weighed and submerged in 10 ml of sterile distilled water and left under orbital agitation (150 rpm) for 1 h to extract the pigments. Finally, the supernatant absorbance was read on a spectrophotometer (BioSpectro model SP-220) at 285 nm (Meinerz et al., 2008). The treatments used were: seeds inoculated with *C. truncatum*, potassium phosphite (Yantra® - K2O: 26% and P_2O_4: 33.6%), different concentrations of EO and OA (0.10; 0.25; 0.50; 0.75; 1.00) and water.

In vivo tests

The soybean cultivars used were Brasmax Foco 74i77RSF IPRO, maturation group 7.4, indeterminate growth habit, moderately resistant to diseases and recommended planting density of 380000 plants ha^{-1}; Coodetec 2728 IPRO, maturation group 7.2, indeterminate growth habit, moderately susceptible to diseases, and density of 400000 plants ha^{-1}. During planting, the spacing of 0.5 m between rows was adopted for both cultivars.

The experiment was placed using a randomized block design, with 3 replicas. Each plot was 10 m² in size. Four applications were made in the phenological stages of V6 (fifth open trefoil), R1 (beginning of flowering), R3 (end of flowering), and R5.3 (25 to 50% of pod filling). For each cultivar, five treatments were used: control (without application of EO or fungicides), noni´s fruit EO (0.25% in solution of 150 L ha^{-1}), noni´s fruit EO (0.25% in solution of 150 L ha^{-1}) + orange oil adjuvant (Orobor N1 200 ml ha^{-1}), noni´s fruit EO (0.25% in solution of 150 L ha^{-1}) + mineral oil adjuvant (Aterbane® BR 0.5 L ha^{-1}) and fungicides: pyraclostrobin (133 g L^{-1}) + epoxiconazole (50 g L^{-1}) 0.5 L ha^{-1} (V6), azoxystrobin (200 g L^{-1}) + cyproconazole (80 g L^{-1}) 0.3 L ha^{-1} (R3) mancozeb (750 g Kg^{-1}) 2 Kg ha^{-1} (R1 and R5.3). The applications were carried out using a manual backpack sprayer with a full-cone type nozzle.

The agronomic characteristics evaluated were: total tissue mass (TTM), first pod insertion height (FPIH), plant height (PH), number of pods per plant (NPP), thousand-grain mass (TGM), and productivity (kg ha^{-1}). 10 plants were sampled from each plot. For the disease evaluation, an adapted rating scale proposed by Finoto et al. (2011) was used, where: 0 (zero) for the absence of disease, 1 (one) for severity between 1 and 10%, two for severity between 11 to 25%, three for severity between 26 and 50%, four for severity between 51 and 75%, and five for severity between 76 and 100%. The grades were assigned accordingly to the aspects of the stem and pods affected and taking into account all plants in the plot. Then, the grade values were converted into an Area Under the Disease Progress Curve (AUDPC), according to the proposed formula for Shaner and Finney (1977).

Statistical analysis

The variance analysis was performed for all characteristics and mathematical models were adjusted for the quantitative treatments and tests to compare the means to the qualitative ones using the Tukey test at a 5% probability, since there was a low number of variables to be compared. All analyzes were performed using the Sisvar software (Ferreira, 2014).

RESULTS

The results of the chromatographic analysis of the noni EO demonstrate its main components (Table 1). There is a major presence of octanoic acid (OA) in the EO, with 75.77%. The second main component was hexanoic acid present in 12.75% of the sample. All other components are divided into smaller fractions, where most do not have a representation greater than 1% and summed only represent 1.61%.

The expressed results demonstrate the efficacy in the control of the causal agent of soy Anthracnose (*C. truncatum*) by the action of the oil and the major compound OA (Figure 1). OA was effective at the dose of 1%, where there was a mycelial growth of the phytopathogen of only 41.52%, when compared to its growth in the control treatment (PDA). In this same concentration, the essential oil of noni allowed the mycelial growth of 62.7%. The inhibition of mycelial growth was total for both at the concentration of 2%. However, there was no influence on the growth of *Trichoderma* sp. For neither of the compounds even in the highest concentration (4%). This result demonstrated great potential for the use of substances in the field application because, in addition to proving the effectiveness in the control of the phytopathogen, it also demonstrated selectivity to the antagonist fungus, *Trichoderma*, important in biological control. Another important characteristic evaluated as effect of the application of these compounds is the production of substances responsible for the plant's defense mechanism. In this case, the induced production of gliceolin, a phytoalexin produced by the soybean plant against the attack of phytopathogens, by the application

Table 1. Relative percentage (Area %) of the compounds identified in the noni´s essential oil (*M. citrifol*ia) by gas chromatography coupled with mass spectrometry. [1]Retention index calculated on retention times compared to a series of n-alkanes. [2] Kovats Index calculated for compounds

Name	Retention time[1]	Indexing time[2]	Area (%)
Hexanoic acid, methyl ester	3.973	3.933	1.27
Hexanoic acid	5.113	4.800	12.75
Octanoic acid, methyl ester	6.863	6.825	2.91
Octanoic acid	8.130	7.625	75.77
Hexanoic acid, 4-pentenyl ester	8.861	8.767	2.57
Isobutyl pent-4-enyl carbonate	11.490	11.433	3.12
Other	-	-	1.61

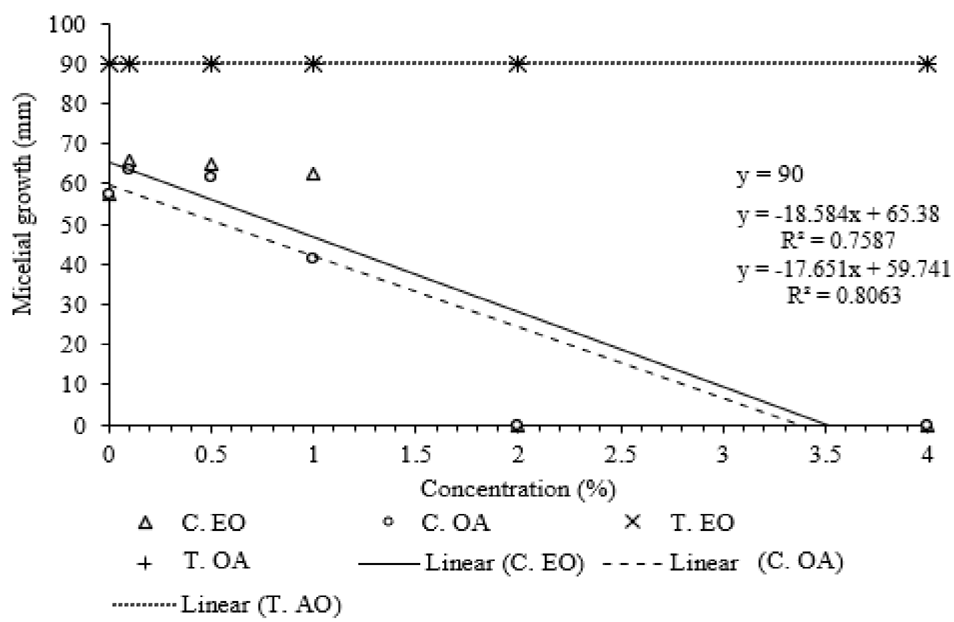

Figure 1. Mycelial growth of *C. truncatum* (C.) and T*richoderma* sp (T.) in PDA media with different concentrations of noni essential oil (EO) and its major component octanoic acid (OA), after ten days of incubation.

of EO of noni and OA was tested. It was demonstrated that the production of gliceoline was higher in cotyledons treated with OA (Figure 2).

The highest absorbance, 13.3, was obtained at the concentration of 0.75% of this substance, which had a decrease in the production of phytoalexin in the concentration at 1%. EO concentrations showed an increasing linear trend in the production of gliceolin. In the end, the concentration of 1% obtained the value of 6.2 in the absorbance per gram of cotyledons. These results showed potential in the ability to activate the defense mechanism in soybean plants against attacks of phytopathogens when treated with these substances, especially OA.

In order to prove the production effectiveness of phytoalexins by the treatments used, the most effective concentrations of EO (1%) and OA (0.75%) were compared with potassium phosphite (plant stimulant) and also the effect of the application of *C. truncatum* conidia in soy cotyledons was verified (Figure 3). Among the inducing treatments tested, OA was the major promoter of gliceolin production stimulation in seedlings, differing significantly from the control and potassium phosphite, which presented low stimulus to the production of phytoalexin. The activation of the seedling defense mechanisms triggered by the phytopathogen that causes anthracnose (*C. truncatum*) and EO were equivalent. These results demonstrate that the application of alternative treatments can be effective in inducing the production of soy phytoalexin, making the plant more

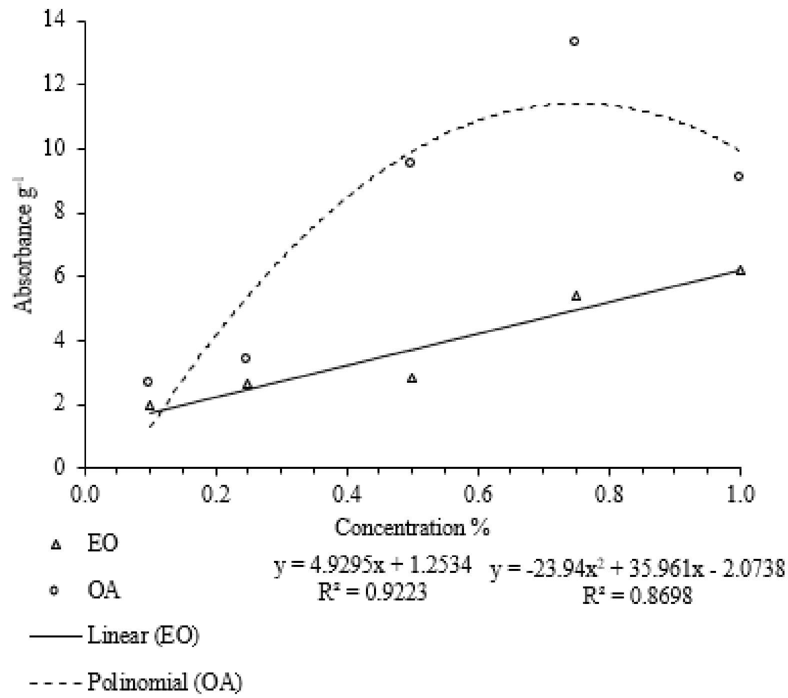

Figure 2. Induction of phytoalexin gliceolin in cotyledons of soybean seedlings treated with noni essential oil (EO) and octanoic acid (OA).

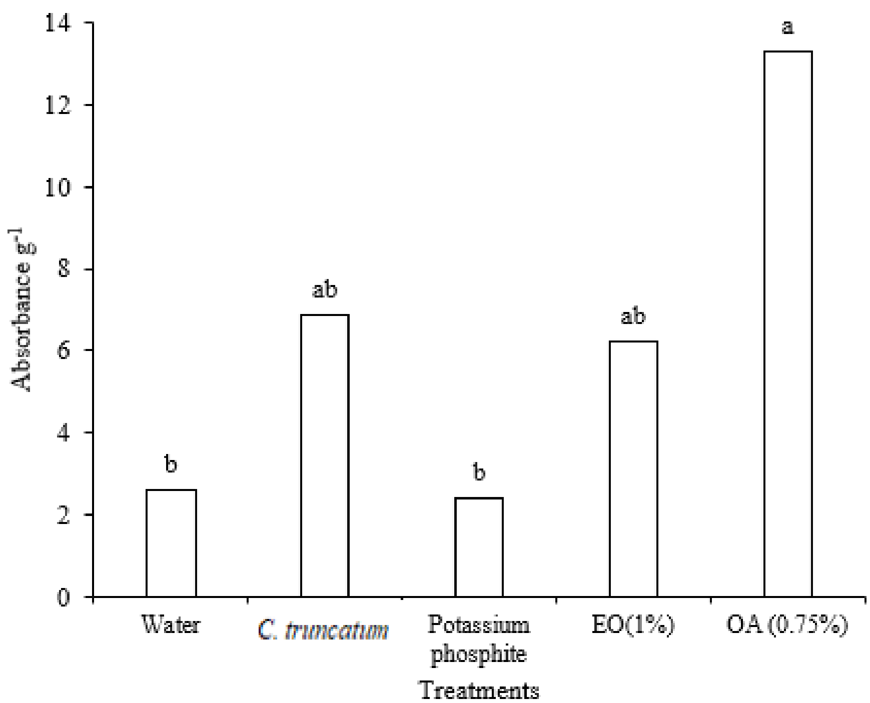

Figure 3. Induction of phytoalexin gliceolin production in soy cotyledons treated with different stimulating agents of the biochemical defense mechanism of plants. -Equal lower-case letters show no significance between treatments and different lower-case letters represent significant deference by the Tukey test at 5% probability.

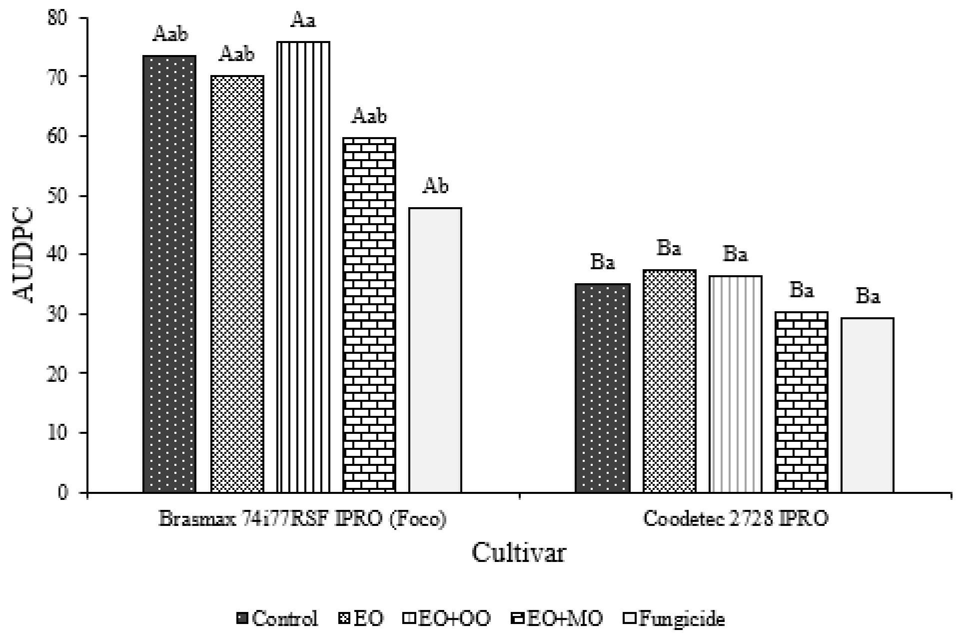

Figure 4. Area under the disease progress curve (AUDPC) of Anthracnose in soybean cultivars Brasmax 74i77RSF IPRO (Foco) and Coodetec 2728 IPRO, submitted to treatments: control (water application), essential oil (EO) of the noni fruit (*M. citrifolia*), essential oil (EO) from noni fruit + orange oil adjuvant (OO), essential oil (EO) from noni fruit + mineral oil adjuvant (MO) and fungicides (pyraclostrobin (133 g L^{-1}) + epoxiconazole (50 g L^{-1}) 0.5 L ha^{-1}, azoxystrobin (200 g L^{-1}) + cyproconazole (80 g L^{-1}) 0.3 L ha^{-1}, mancozeb (750 g Kg^{-1}) 2 Kg ha^{-1}). -Equal lower-case letters between cultivars and upper-case letters between treatments do not differ significantly from each other by the Tukey test at 5% probability

resistant against pathogen attack.

In the field trial, which sought to make the use of noni essential oil and adjuvants feasible, the incidence of anthracnose was observed in the stems and plant pods (Figure 4), on cultivars Brasmax Foco and Coodetec 2728, submitted to different treatments.

There was a statistically significant difference between cultivars in all treatments. The values decreased by almost 50% when the level of disease expressed by the AUDPC value of the cultivar Brasmax Foco was compared with Coodetec 2728. Thus, it was possible to verify the greater susceptibility of the cultivar Brasmax Foco to Anthracnose.

Regarding the comparison of treatments within each cultivar, it was found that the combination of noni EO + orange oil showed the highest value of AUDPC (75.8), differing statistically from fungicides (47.8). The other treatments used to the plants obtained median results between the two, with AUDPC values of 73.5 for the control, 70 for EO and 59.5 for EO + mineral oil. The AUDPC values between the treatments in the cultivar Coodetec 2728 did not differ statistically, varying from 29.2 for the treatment submitted to the fungicides, up to 37.3 for the EO. The agronomic characteristics of total tissue mass, height of insertion of first pod, and plant height of cultivars in the 2017/2018 crop can be seen in Table 2.

The Brasmax Foco cultivar differed statistically from Coodetec 2728 in the characteristic of total tissue mass in the treatments with EO (2.5 and 3.7 Kg) and fungicides (3.2 and 5.0 Kg), where the last cultivar represents higher values in both treatments. Comparing treatments within each cultivar, the only difference was observed by Coodetec 2728 when treated with fungicides, differing significantly from the rest. In this treatment, the syndrome of green soybean stem and leaf retention was observed, probably as a physiological response of the plant to fungicides. This fact delayed the plot’s harvest by 15 days when compared to the other treatments.

For the height of insertion of the first pod, there was a significant difference between cultivars, but not between treatments. The values, on average, doubled between cultivars, with an average of 12.3 cm for treatments at Brasmax and 32.2 cm for Coodetec. The plants differed in relation to height, with the cultivar Brasmax Foco pH showing the lowest values. Only in the treatment with fungicides, there was no difference between the two materials, with 69.4 cm for the first and 73.9 cm for Coodetec 2728. For the latter, differences between treatments were observed. The control (84.1 cm) and

Table 2. Total tissue mass (TTM), average first pod insertion height (FPIH), average plant height (PH) of the soybean cultivars Brasmax Foco and Coodetec 2728, submitted to treatments: control, noni fruit essential oil (EO) (*M. citrifolia*), noni fruit essential oil (EO) + orange oil adjuvant (OO), noni fruit essential oil (EO) + mineral oil adjuvant (MO), and fungicides.

Treatment	TTM (Kg)		FPIH (cm)		PH (cm)	
	Foco	**2728**	**Foco**	**2728**	**Foco**	**2728**
Control	2.9^{a}	3.5^{aB}	11.5^{b}	21.7^{a}	68.7^{b}	84.1^{aA}
EO	2.5^{b}	3.7^{aB}	11.7^{b}	22.0^{a}	66.1^{b}	77.6^{aAB}
EO+OO	2.6^{a}	3.3^{aB}	13.1^{b}	23.1^{a}	69.1^{b}	81.1^{aA}
EO+MO	2.7^{a}	3.0^{aB}	13.4^{b}	22.7^{a}	66.1^{b}	79.7^{aAB}
Fungicide	3.2^{b}	5.0^{aA}	11.9^{b}	26.0^{a}	69.4^{a}	73.9^{aB}
C.V (%)	13.85		14.14		3.81	

-Equal lower-case letters between cultivars and upper-case letters between treatments do not differ significantly from each other by the Tukey test at 5% probability.

Table 3. Pods number per plant (PNP), thousand grains mass (TGM), productivity (PROD) of the soybean cultivars Brasmax Foco and Coodetec 2728, submitted to treatments: control, noni fruit essential oil (EO), noni fruit essential oil (EO) + orange oil adjuvant (OO), noni fruit essential oil (EO) + mineral oil adjuvant (MO), and fungicides.

Treatment	PNP		TGM		PROD (kg ha^{-1})	
	Foco	**2728**	**Foco**	**2728**	**Foco**	**2728**
Control	28.7	34.5	119.1^{b}	136.3^{aAB}	3964.4^{aBC}	3966.7^{aA}
EO	31.9	37.6	$122^{a}6^{a}$	134.3^{aB}	3871.1^{aBC}	4124.4^{aA}
EO+OO	31.9	37.3	118.5^{b}	135.2^{aB}	3342.2^{aC}	3584.4^{aA}
EO+MO	37.2	42.1	120.9^{b}	137.2^{aAB}	4315.6^{aAB}	3711.1^{bA}
Fungicide	34.8	34.7	133.2^{b}	153.5^{aA}	4646.6^{aA}	2483.7^{bB}
V. C. (%)	13.45		5.34		6.91	

Equal lower-case letters between cultivars and upper-case letters between treatments do not differ significantly from each other by the Tukey test at 5% probability

noni fruit EO + orange oil (81.1 cm) differed from the fungicides (73.9 cm). The height shown by the plants submitted to spraying of the pesticides was the smallest, contrasting with their total plant mass. However, in addition to the leaf retention provided by the green stem syndrome, a significant increase in the stem diameter of the plants was observed (data not presented). The values of number of pods per plant, mass of a thousand grains, and productivity of soybean cultivars are shown in Table 3.

There were no observed significant differences for the number of pods per plant, either between cultivars or between treatments. The values ranged from 28.7 and 34.5 in the control to 37.2 and 42.1 for the noni EO + mineral oil for Brasmax Foco and Coodetec 2728, respectively. The cultivars differed statistically for the mass characteristic of a thousand grains, except in the treatment with EO, with 122.6 g for Brasmax Foco and 134.3 for Coodetec 2728. For the second cultivar, there was a difference in fungicide (153.5 g) for EO (134.3 g) and EO + orange oil (135.2 g). The productivity of the Brasmax Foco cultivar varied significantly between treatments. Fungicide spray provided 4646.6 kg ha^{-1}, differing from the control (3964.4 kg ha^{-1}), EO (3871.1 kg ha^{-1}) and EO + orange oil (3342.2 kg ha^{-1}). For Coodetec 2728, the fungicide treatment obtained the lowest productivity (2483.7 kg ha^{-1}), differing significantly from the others. This decrease may be related to the green stem syndrome that affected the plants. The treatments with fungicides and essential oil + mineral oil differed between cultivars.

DISCUSSION

The use of gas chromatography as a tool for the identification and quantification of constituents of essential oils is common. The variation of the constituents of the EO depends on multiple factors, such as plant nutrition, maturation stage, geographic location among other morphophysiological characteristics that can affect the plants (Chrysargyris et al., 2016; Djerrad et al., 2015; Moghaddam et al., 2015). However, the characterization of the noni´s fruit EO is still scarce in the literature compared to other plants, such as *Lippia* sp. One of the few studies available is where Silva et al. (2017), found a value of 82% for the OA in its composition. This corroborates with the present study, which obtained 75.77% of this substance in its constitution.

Fungi *in vitro* mycelial growth test is the first step to verify the capacity of pathogenic suppression of EO or any other substances that are proposed for this purpose (Balouiri et al., 2015). The antifungal capacity of the compounds was proven in the present work through the *in vitro* assay against *C. truncatum*. The work with noni EO against phytopathogens is innovative. Dalcin et al. (2017) and Osorio et al. (2018) observed inhibition of *Stagonosporopsis cucurbitacearum* and *Olivea neotectonae*, respectively, by the action of EO. OA also demonstrated inhibitory activity against phytopathogens in studies conducted by Liu et al. (2008). The action mechanism of these compounds has not yet been completely clarified. However, it is possible to state that, in their majority, they act in increasing the permeability of the cell membrane, due to its lipophilic nature, causing its content to overflow (Pohl et al., 2011; Connell et al., 2013; Silva et al., 2014).

In this work, the selectivity of compounds to fungi of the *Trichoderma* genus was also verified. Abdel-Kader et al. (2011) obtained effective fungi control of *Fusarium solani* (Mart.) Sacc. 1881, *Rhizoctonia solani* J.G. Kühn 1858, *Sclerotium rolfsii* Sacc. 1911, and *Macrophomina phaseolina* (Tassi) Goid. 1947 through the seed treatment associating EOs with *Trichoderma harzianum* Rifai 1969, and the oil did not prevent its development.

One of the characteristics attributed to fungitoxic compounds from plants (extracts; EOs) is the induction of defense mechanisms activation. The production of phytoalexins may be a viable alternative for resistance induction and in the control of diseases in some cultivated plants. Results obtained by Matiello et al. (2016) and Ferreira et al. (2018) demonstrated that crude aqueous extracts and essential oils are efficient in inducing phytoalexins. These added factors strengthen and support the results obtained in the tests of this present work, where it was observed that the OA promoted induction of glyceoline superior to the plant stimulants' and the action of the phytopathogen itself.

In the field experiment, where only noni EO was used as an alternative control, it was observed that surfactants play an important part in the processes of penetration and transport in the different barriers of the plant: the epicuticular wax layer, the cuticle, and the cell membranes. The surfactants part in cell membrane permeability is limited, although it can be shown that they cause severe rupture of the cell membrane and thus influence the penetration of fungicides into plants (Tot et al., 2020; Steurbaut et al., 1989).

The difference between the addition of orange oil and mineral oil did not obtain significant results when AUDPC was observed between treatments. Coradini et al. (2016) when using orange oil and mineral oil in conjunction with fungicides also did not observe significant differences in the same product without the addition of adjuvants.

A significant difference was found when comparing the level of severity of anthracnose between the two cultivars tested. This result demonstrated that the cultivar choice to be planted in the region is of great importance in the crop´s health. However, a peculiar feature emerged from the treatment with fungicides in the cultivar Coodetec 2728: the green stem syndrome. The term green stem has been generally used and refers to plants with non-senescent stems or with delayed senescence. However, it also refers to plants with or without adhered leaves, and may or may not be associated with yield loss (Harbach et al., 2016). Green stalk in soybeans is a syndrome that keeps the primary and secondary stems of soybeans green, even after the physiological maturation of the seed. There is leaf retention and the maintenance of green leaves for a longer period, after maturation of the seed. Its occurrence in soybean culture has been attributed to the use of some fungicides used to control the complex of leaf blight in soybeans, mainly rust and end-of-cycle blossoms (Silva et al., 2013).

Some authors also suggest that the cause of this disturbance in the plant is related to other factors that stress the plant, such as water deficit, insect attack, and soil-related problems (Meyer et al., 2017; Ranulfi et al., 2018). However, the only factor that distinguished treatments was the use of fungicides. It can be safely said that the spray of these fungicides used in the experiment can cause the green stem syndrome in soybean culture. With this, it is necessary to research the behavior of the cultivar to be sown for the use of the active ingredients of fungicides so that the producer does not have harvest losses.

Conclusion

There are still not many studies been developed on fungi control using noni and the majority of those experiments use its extract, not separating compounds to investigate which of the metabolite is responsible for the observed result. The major compound of noni essential oil is octanoic acid with around 75% in its constitution. In the *in vitro* control of *C. truncatum* the two compounds were efficient and did not affect the development of the antagonist fungus *Trichoderma* sp. They have been shown to be inducers in the production of gliceolin, soy phytoalexin, mainly octanoic acid, thus giving a greater resistance of the treated plants against pathogens attack.

The soybean cultivar Coodetec 2728 IPRO presented less severity to anthracnose, however, the cultivar Brasmax Foco showed tolerance to the attack of the pathogen maintaining high productivity, especially when comparing the treatment with essential oil + mineral oil between cultivars. The commercial fungicides application evaluation in the cultivar Coodetec 2728 IPRO resulted in a detrimental effect on productivity as it causes green stem syndrome in plants. Allowing to prove that biological controls are promising in disease control and as inducing the plants' immune system, also standing out for the non-harmful effects such as those caused by commercial fungicides available on the market.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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