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Activity of Neem (*Azadirachta Indica* A. Juss) Oil against Phytopathogenic Fungi

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***Abstract* – The potential of biofungicides such as plant extracts and essential oils is being assessed to minimize the use of chemicals, which are harmful to humans and the environment. This work aimed to evaluate the *in vitro* activity of commercially available neem oil on the mycelial growth of the phytopathogenic fungi *Corynespora cassiicola*, *Colletotrichum gloeosporioides*, and *Fusarium* spp. The evaluation was performed using four different concentrations (5000, 2500, 1250, and 625 ppm) of the commercial product. The results showed a decrease in the radial size of the colonies with increasing concentrations of neem oil. In *Corynespora cassiicola*, a reduction in mycelial growth was observed using the 2500 ppm concentration, whereas in *Fusarium* spp. and *Colletotrichum gloeosporioides*, a reduction was observed using the 625 ppm concentration.**

***Keywords* – Neem, Antifungal Activity, Phytopathogen.**

I. Introduction

Synthetic chemical compounds have been indiscriminately used to control agricultural pests. Despite their significant contribution to agriculture, many of these products are toxic to humans, contaminate the environment, and favor the appearance of secondary pests [13]. The potential of biofungicides such as plant extracts and essential oils, especially neem oil (*Azadirachta indica*), is currently being studied to minimize the use of chemicals that are harmful to humans and the environment [3]. *A. indica*, popularly known as neem, belongs to the Meliaceae family, grows in tropical and subtropical regions, can reach 30 meters in height, and lives for 200 years. This tree has been characterized as the world's most important botanical insecticide [7]. It produces over 40 terpenoids, which are effective in controlling insects and have low toxicity to vertebrates [14]. According to [17] and [16], active compounds can be extracted from different parts of the plant, and among these, four compounds, namely azadirachtin, salannin, melantriol, and nimbidin, exhibit pesticide activity. The compound azadirachtin has been most extensively used in research because it imparts more selective effects on insects than using all the other compounds together. In addition, [10] reported that among the botanical insecticides currently used, neem oil has the lowest toxicity to humans. Reference [8] stated that in addition to its insecticidal potential, neem also has the capacity to control the proliferation of phytopathogens. However, [9] has previously shown that the effectiveness of neem oil on fungi depends on several factors, including the species of the target pathogen. Several studies have reported the use of neem in the control of fungi such as *Alternaria alternata*, *Macrophomina phaseolina* [5], *Fusarium* spp. [6], *Plasmopara viticola* [19], and *Pyricularia oryzae* [1], which are responsible for major losses and diseases in crops. Based on these characteristics, neem has been extensively used to control pests, mainly in organic agriculture [9]. However, consumers are currently more concerned about the health risks of consuming conventionally produced food [15]. In view of the inhibitory property of vegetable oils on the development of pathogenic fungi and the importance of the fungi *Corynespora cassiicola*, *Colletotrichum gloeosporioides*, and *Fusarium* spp. as causative agents of diseases in crops of economic interest, the present study aimed to evaluate the *in vitro* effect of commercial neem oil on the mycelial growth of fungi as a possible alternative for its control in agriculture.

II. Materials and Methodos

The experiment was conducted at the Laboratory of Phytopathology, Department of Agronomy, State University of Maringá in Paraná, Brazil. Three fungal isolates, obtained from different hosts [*Corynespora cassiicola* (soybean leaves), *Colletotrichum gloeosporioides* (papaya fruit), and *Fusarium* sp. (wheat seeds)] were used. The isolates were cultured in Petri dishes containing potato-dextrose-agar (PDA) medium and placed in a Heated incubator chamber with biochemical oxygen demand (BOD), with an average temperature of 24 ± 1°C, and in the dark for 8 days.

Effect of neem oil on phytopathogenic fungi:

The commercial emulsified neem oil, with a brand name of Neenmax®, was used at a concentration of 1.20 g i.a./L azadirachtin (information obtained from the product label, 2012).

The effect of neem oil on phytopathogenic fungi was evaluated using four different concentrations, namely 5000, 2500, 1250, and 625 ppm per liter of PDA.

For the preparation of the solutions using serial dilutions, an Erlenmeyer flask containing 0.48 L of PDA

medium, which was sterilized by autoclaving for 20 min at a temperature of 120°C and a pressure of 1.5 atm, was used. When the medium reached a temperature between 45.0 and 50.0°C, 2.4 mL of neem oil was added to obtain a concentration of 5000 ppm c.p./L of culture medium. Thereafter, the medium was stirred vigorously for 2 min for homogenization of the oil in the culture medium, and 120-mL aliquots were poured into 12 Petri dishes (20 mL/plate). To the remaining 120 mL of culture medium, 120 mL of PDA were added to obtain a concentration of 2500 ppm, which was then poured into 12 Petri dishes (20 mL/plate). Again, to the remaining 120 mL, 120 mL of PDA was added, thereby obtaining a concentration of 1250 ppm, which was poured into 12 Petri plates. Finally, the procedure was repeated by adding 120 mL of PDA to the remaining culture medium; this solution was then poured into 12 Petri plates, thus obtaining the desired final concentration of 625 ppm (Figure 1). As a control treatment, Petri dishes containing only PDA were used.

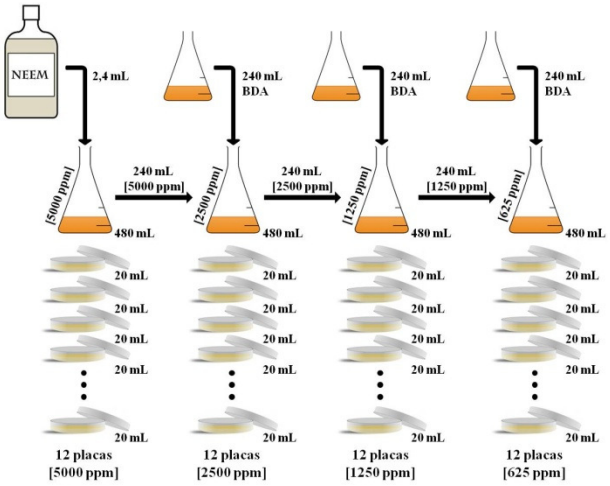

Fig.1. Schematic diagram of the preparation of the treatments using different concentrations (5000, 2500, 1250, and 625 ppm) of neem oil.

The inoculum from each isolate comprised of an 8-day monosporic culture. Mycelial discs were cut from the edges of the colonies using a pourer stopper with a diameter of 8 mm. Subsequently, with the aid of a straight-tip handle, the mycelial discs were transferred to the center of a Petri plate containing the PDA medium plus neem oil at various concentrations. The Petri dishes were incubated in a semi-climatized BOD incubator.

The variables evaluated in this study were the pattern of the colony and mycelial growth. The assessment of the mycelial growth was performed when the diameter of the colony reached the edge of the Petri dish or at the cessation of growth. For this, the mycelial growth of each isolate and replicates at different concentrations were quantified using a millimeter ruler, by measuring the diameter of the colonies in diametrically opposite directions. The diameter of the colony was expressed by the arithmetic mean of the diameters.

The original data were subjected to an analysis of variance and the means of the treatments were grouped using the Scott-Knott test at 5% probability.

III. Results and Discussion

Various concentrations of neem oil showed antifungal activity against the mycelial growth of *Corynespora cassiicola*, *Fusarium* spp. and *Colletotrichum gloeosporioides* compared to the control. The assessment of mycelial growth of the fungi showed a reduction in the radial size of the colonies with increasing concentrations of neem oil.

In *C. cassiicola*, the reduction in mycelial growth was observed starting from the neem oil concentration of 2500 ppm. The diameter of the colonies was 6.62 and 5.65 cm, and did not significantly differ from each other, showing a 26.48 and 37.22% reduction, respectively, after treatment using neem oil concentrations of 2500 and 5000 ppm compared to that observed in the control, which showed a growth of 9.0 cm (Table 1).

In contrast, in *Fusarium* spp., the effect of neem oil was observed starting at the concentration of 625 ppm, with a reduction in mycelial growth with increasing concentrations of neem oil. The most significant result was observed using the highest concentration of oil (5000 ppm). The results showed a 3.73, 3.68, 3.40, and 2.60 cm inhibition of mycelial growth, representing a 22.54, 23.58, 29.46, and 46.06% reduction for the concentrations of 625, 1250, 2500, and 5000 ppm, respectively (Table 1).

Similar to that observed in *Fusarium* spp., the reduction in the mycelial growth of *Colletotrichum gloeosporioides* was identified using neem oil at a concentration of 625 ppm, and the effect followed a dose-dependent fashion. The treatments at the two highest concentrations, 2500 and 5000 ppm, were statistically higher, representing a mycelial growth of 3.17 and 2.73 cm, with a growth reduction of 64.81 and 69.63% respectively (Table 1).

The results of this study differ from those reported by [2], who analyzed the effect of neem on *Corynespora cassiicola* and *Colletrotrichum acutatum*, and detected no inhibitory action on the mycelial growth of these pathogens.

On the other hand, [12] observed the efficiency of commercially available neem oil in reducing the mycelial growth of *Colletotrichum gloeosporioides*. The same was observed by [4] who observed a significant growth reduction of *Colletotrichum acutatum* by using a dosage of 5000 ppm of the neem oil commercial product. Reference [11] reported that 4.0% neem oil significantly inhibited the growth of *Fusarium oxysporum in vitro*, confirming the results obtained in this study.

According to Locke, as cited in [15], the variation in the results reported in the literature can be attributed to origin, quality, refinement, and formulation of the oils used in the studies.

The inhibition of fungal growth due to the application of neem oil may be attributed to its constituent compounds such as azadirachtin, which possibly inhibits the production of fungal enzymes, thereby reducing mycelial growth and spore germination [18].

Table 1. *In vitro* effect of neem oil (*Azadirachta indica* A. Juss.*)* on mycelial growth of various pathogenic fungal species.

Óleo	Concentração (ppm do p.c/litro de BDA)	Diâmetro médio da colônia (cm)[1]/Redução no crescimento (%)[2]					
		Corynespora cassiicola	Redução (%)	*Fusarium* sp.	Redução (%)	*Colletotrichum gloeosporioides*	Redução (%)
Neem	0,0	9,00 b[3]	---	4,82 c	---	9,00 c	---
	625,0	9,00 b	0,00	3,73 b	22,54	3,70 b	58,89
	1250,0	9,00 b	0,00	3,68 b	23,58	3,67 b	59,26
	2500,0	6,62 a	26,48	3,40 b	29,46	3,17 a	64,81
	5000,0	5,65 a	37,22	2,60 a	46,06	2,73 a	69,63
CV (%)	---	11,23	---	7,08	---	8,61	---

[1/]Mean diameter of colonies measured in diametrically opposite directions (cm); [2/]Reduction in mycelial growth in percentage (%) after the application of different concentrations of neem oil supplemented to a potato dextrose agar (PDA) medium; [3/]Means followed by the same letter in the columns do not differ among themselves, according to the Scott-Knott test at 5% significance

Based on the results of this study, we confirm the direct effect of neem oil on the inhibition of mycelial growth of the pathogens *Corynespora cassiicola*, *Fusarium* spp., and *Colletotrichum gloeosporioides*.

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