Halocnemum strobilaceum AGAINST DATE PALM FUNGI (ALGERIA)

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Abstract: Since years, many plants contain components that have antifungal effect against plant pathogens. As a biological control, several plant extracts, belonging to the family of Amaranthaceae, have been tested against some phytopathogenic fungi from different crops. For this, an antifungal test of an indigenous plant extracts from the south eastern Algeria was evaluated for its inhibitory effect on phytopathogenic fungicausing date palmdiseases, *Alternaria* sp., *Fusarium* sp., *Phytophthora* sp. and *Cladosporium* sp.The aqueous extract of this plant showed the maximum inhibition with *Phytophthora* sp.which recorded 90,6 % at 100% and 83,0 % at 50%, followed by *Cladosporium* sp. (D2: 89,02%; D3: 89,10%; D4: 86,15%). Statistical analysis confirms that all the doses of *H. strobilaceum* had a significant effect, comparatively with the control, of inhibition against the tested phytopathogenic fungi mentioned before with $p < 0,05$; $p = 0,0087$. High inhibition was detected against *Phytophthora* sp. and *Cladosporium* sp. with all the doses, unlike, *Fusarium* sp. was exhibited only in the fourth dose and *Alternaria* sp. that was revealed a low rate in the fourth dose. These effective plant extracts may contribute to development of potentially effective and environmentally safer alternative fungicide to control date palm diseases caused by these phytopathogenic fungi.

Keywords: Antifungal activity; Biological control; Date palm; *Halocnemum strobilaceum;* Algeria.

***Halocnemum strobilaceum*contre les champignons du palmier dattier**

Résumé : Depuis longtemps, nombreuses plantes contiennent des composants qui ont un effet antifongique contre les agents phytopathogènes. En lutte biologique, plusieurs extraits de plantes, appartenant à la famille des Amaranthaceae, ont été testés contre certains champignons phytopathogènes de différentes cultures. Pour cela, un test antifongique des extraits d'une plante indigène du sud-est algérien, *Halocnemumstrobilaceum*Pall., a été évaluée par son effet inhibiteur contre les champignons phytopathogènes causants des maladies fongiques au palmier dattier, *Alternaria*sp., *Fusarium* sp., *Phytophthora* sp. et *Cladosporium*sp. L'extrait aqueux de cette plante a induit l'inhibition maximale avec *Phytophthora* sp., avec des taux d'inhibition de 90,6 % à 100% et 83,0% à 50%, suivi par *Cladosporium*sp. (D2: 89,02%; D3: 89,10%; D4: 86,15%). Les analyses statistiques confirment que toutes les doses d'*H. strobilaceum* ont un effet significatif, pour l'inhibition contre les champignons phytopathogènes mentionnés, en comparaison avec le témoin ($p< 0,05$;$p = 0,0087$). Forte inhibition a été détectée contre *Phytophthora* sp. Et *Cladosporium* sp., avec toutes les doses, par contre, *Fusarium* sp. est décelé en4^{ème} dose et *Alternaria*sp. a révélé un faible taux pour cette dernière dose. Ces extraits de plantes efficaces peuvent contribuer au développement de fongicides alternatifs potentiellement efficaces et sans danger pour l'environnement. Ceci permettra d'exploiter ces extraits pour lutter contre certaines maladies du palmier dattier, causées par ces champignons phytopathogènes.

Mots clés: Activité antifongique, lutte biologique, palmier dattier, *Halocnemum strobilaceum,* Algérie.

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Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops of the tropical and subtropical regions of the world [1]. It considered a symbol of life in the desert, because it tolerates high temperatures, drought and salinity more than many other fruit crop plant species. In the southeastern Algeria, date palm is a patrimony plant that needs to be preserved.

The most important pathogens of root rot (decline) disease in date palm and offshoots (*Phoenix dactylifera* L.) were *Fusarium oxysporum*; *Alternaria* sp.; *Cladosporium* sp.; *Rhizoctonia solani* [2]. Date palm can be subjected to many diseases or complexes of diseases, among which some, which are serious such as *Diplodia phoenicum, Thielaviopsis paradoxa, Phytophthora* sp., *Helminthosporium* sp., *Stemphyium botryosum, Alternaria* sp., *Cladosporium* sp*., Penicillium, Stemphylium* [3]. Roots of date palm are liable to attack by many pathogenic soil-borne fungi, that causing serious diseases.

In order to look for other alternatives of biological control against aggressive pathogenic fungi of date palm, several studies regarding the action of plant extracts against some phytopathogenic fungi have been performed. Several authors have pointed out the importance of halophyte species [4].

Most of the plants contain several components with properties for protection against pathogenic agents. *Halocnemum strobilaceum* Pall is typical of vast areas of saline flats with high salinity and high ground-water level, both inland saline and littoral marshes. In the peripheries of most of the inland salines it forms a pioneer halophytic community [5, 6, 7]. Mediterranean basin and middle Asia is representatives with fungicidal and insecticidal activity such as Chenopodiaceae (*Halocnemum strobilaceum* Pall.) [8]. The aim of this study was to evaluate the antifungal activity of an aqueous extract from a native plant (used in traditional medicine) against deferent phytopathogenic fungi of date palm. It contributes to sustainable crop production, organic agriculture, and eco-friendly pest management that was essentially required and applicable.

Material and methods

Study area

This study was conducted in the locality of Sidi Mehdi, which is an area located in the Southeastern Algeria. It is a Saharan locality with one dry period throughout the year. This very low region is located at an altitude of 69 m at 06°4’ E and 33°7’N. This area is approximately 7 km of Touggourt on the road leading to the airport [9].

Isolation of phytopathogenic fungi

Samples were brought separately from different palm groves of Sidi Mehdi. Theirinfected vascular tissues were collected from stem; leaflet and root parts of date palm cultivars that were showing symptoms with cryptogamic diseases(Figure1).

Samples were brought to the laboratory of plant pathology in the INRAA station forseeding and purification of date palm fungi. Fragments of reached parts from 5 to 10 mm presenting of the typical symptoms were cut out then planted in a suitable culture medium after disinfection,

rinsing with sterile distilled water, and then drying (Figure2). Incubation took place at a temperature between 24-26°C [10].

Plant material

Fresh healthy plant parts ofan indigenous plant from the southeastern Algeria, *Halocnemum strobilaceum*,was collected from the experimental station of the National Institute of Agronomic Research (INRAA) in Sidi Mehdi region. It's a halophytic plant, depend to several old man in our region, it is used to treat diarrhea. According to [11], they indicated that *H.strobilaceum*was correlated positively with salt-affected and alkaline soils. In addition, by using the PCA analysis, [12] has noted that *Halocnemumstrobilaceum*was related with soil salinity, pH, lime, and loam. However, it is known that the Chenopodiaceae family includes several Taxa (such as *H.strobilaceum*) which possess some mechanisms facilitating their installation in the saline environments [13].

Preparation of aqueous plant extract and powder

Samples were washed with tap water and were sterilized with sodium hypochlorite solution (0.1%) by dipping them for two minutes. After they were washed with distilled water and dried at room temperature (25° C) for about a week on a laboratory desk. Samples were covered with clean sheets of paper to avoid any deposition of dust. A quantity of 25 g of this latter was added to 150 ml of distilled water [14]. The resulting mixture was then placed in a stirrer (GFL 3040; 21 rmp per min) for 30 min and passed through a fine muslin cloth to have the crude solution, which had been diluted to obtain different doses *i.e.* 100%, 50%, 25% and 10% of the aqueous extracts. The antifungal effect was assessed by using the poisoned food technique adopted by Grover and Moore [15], which conducted to add 0.01 ml of the extract to the PDA medium.

In vitro bioassay of the antifungal activity

The assay was conducted in the laboratory of plant protection in the experimental station of INRAA; six species of fungi: *Alternaria* sp., *Fusarium* sp., *Phytophthora* sp., *Cladosporium* sp., *Aspergillus niger* and *Pinicillium*sp. were isolated from date palm trees and were used as tested fungi for antifungal activity assay.

The aqueous extract of *H. strobilaceum* was evaluated for its activity against pathogenic fungi of date palm; Disc of any fungi (5mm) was remove from the edge colonies of active cultures and placed on the centerof Petri dishes containing the aqueous extract spreadon thePotato Dextrose Agar (PDA) medium that was flowed in the Petri dishes 24 h after this operation. Similar dishes of each pathogenic fungus isolates grown in the same manner were placed on different Petri disheswithfive replicatesand PDA without plant extract served as control. Cultures were observed daily and recorded for antifungal activity of *H. strobilaceum* against pathogenic fungi. Incubation was continued for 7 days in a temperature of 28°C.

The evolution of mycelia growth is performed every 24 hours by measuring the diameter of the colony of the pathogen. The valuation of inhibition by the aqueous extractwas estimated by

calculating the percentage inhibition of mycelia growth using the following formula [16]:

$$I\ \% = \left[\frac{Cn}{Co}\right] \times 100$$

Cn:pathogen average diameter in the presence of extract.
Co:control average diameter.

Statistical analyses

For the *in vitro* (on Petri dishes) assays, the data were collected as mean colony diameter (efficiency of aqueous extract on pathogenic fungi) values in each replication. These analyses were calculated by statistical software (STATISTICA V.8.0.725.0). The device is held in total uni-factorial randomization by Kruskal-Wallis test.

Results

Antifungal activity

The present study tested the antifungal activity of medicinal plant (*Halocnemumstrobilaceum*) extracts and its respective dilutions against four pathogenic fungi that were isolated from date palm trees (*Alternaria* sp., *Fusarium* sp., *Phytophthora* sp. And*Cladosporium* sp.), this medicinal plant was chosen based on traditional usage.

According to the data showed in the table below, all the fungi were inhibited by this plant extract.

The inhibitory activities of extracts of *H. strobilaceum* on thefungal species tested are shown in the last table. Generally,the inhibition percentage increased with increase in concentrationof extract. Throughout the experimentation, we observed that all the Petri dishes which contain the aqueous extract have released an oily substance that decreased the development of all the fungi.

The aqueous extract of *H. strobilaceum*inhibited the mycelium growth of*Fusarium* sp. and let this fungus changed its aspect and color from pinkish white to canary yellow; after six days of incubationwe had an inhibition percentage 76,34%, 68,21%, 66,19%for 100%, 50% and 25%, respectively (Table 1), and the last dose (10%) the inhibition increased to 58.99%. All the Petri dishes of this test showed a high difference in the development of *Fusarium* sp. with the deformation of spores and the changes of color (Figure3A).

For *Phytophthora* sp.it was inhibited by the aqueous extract of *H. strobilaceum* in the four concentrations. The crud of this extract exhibited a percentage of 90.6% and the concentration of (50%) showed a rate of 82.98% (Table 1), the 3rdconcentration of this aqueous extract (25%); represented an inhibition percentage about 79.6 % and the concentration of (10%) showed a rate of 73,22% (Table 1; Figure3 B).

Similar results were noted for the *Cladosporium* sp., the extract has inhibited its growth of with a percentage of 86,15%, 89,10% for 100% and 50% (Table 1), like the concentration of (25%) with 89,02%; and the (10%) which represent an inhibition percentage less than the others with 80,61% (Table 1; Figure3C).

At last, we have the inhibition of *Alternaria* sp. by the aqueous extract of *H. strobilaceum*(Table 1; Figure3D). This extract inhibited the mycelia growth of *Alternaria* sp. in the first concentration (100%) with a rate of 68,92% and the second concentration (50%) with 71,64%, like the third one with 71,2% and the last

concentration of extract (10%) with an inhibition percentage of 67,15% (Table 1). As a comparison of the growth inhibition by the crude extracts and their respective dilutions that showed a strong dependent effect on extract concentrations. In general, the antifungal activity of extract dilutions is weaker than the crude extract. These results revealed that an antifungal activity of the crude extracts was enhanced by increasing the concentration of the extracts, in effect; the inhibition activity of the extracts was dependent to the concentration.

The aqueous extract of *H. strobilaceum* showed a fort inhibition against these fungi which were isolated from date palm trees (Figure 4). The greatestinhibition percentage was detected against *Phytophthora*sp.(D4:90.6%) followed by *Cladosporium*sp. (D2: 89.02%, D3: 89.10%). In conclusion, the fungal species used in this study showed a variable sensitivity to extracts of *Halocnemumstrobelaceum*.

The statistical analyses showed that all treatment tests on pathogenic fungi of date palm affected significantly. These results confirm that there is a significant inhibition, comparatively with the control, caused by the four doses of the used aqueous extract of this plant (*Halocnemumstrobilaceum*) against these four pathogenic fungi with *P*-value $p<$ 0,05; p = 0.0087. High inhibition was detected against *Phytophthora* sp. and *Cladosporium* sp. with all the doses, unlike, *Fusarium* sp. was exhibited only in the fourth dose and *Alternaria* sp. that was revealed a low rate in the fourth dose.

Discussion

Most of plants contain several components that have antifungal properties for protection against aggressor agents, especially microorganisms. As a biological control, several plant extracts were testified in antifungal activities against many pathogenic fungi of different cultures and many species belonging to the family of Chenopodiaceae, which have been used in traditional medicine for many centuries in several regions of the world, especially in our region were tested like: *Cornulaca monacannta* Del that was used in liver diseases; *Haloxylon articulatum* Boiss. against indigestion, scorpion bites, skin diseases and back pain; *Traganum nudatum* Del. against diarrhea, fever sore and skin diseases [14, 6]. No reports are available by using *H. strobilaceum* plant in controlling pathogenic fungi.

Halocnemum strobilaceum has a very high content of Na and S (more than 50000 mg / Kg and 10000 mg / kg respectively). Theratios of Na / K+Na / Ca and S / P are 8,5 –26,0 and about 1 in the other plant species. The ratio of Ca / Mg is 1,0 – 1,6. The seasonal changes of 6 elements in *H. strobilaceum* indicate that N has a similar change with S but an opposite pattern with Ca and Mg. The high contents of CI^- and $S0^{-2}$ are found in the water extract from *Halocnemum strobilaceum* whose pH is 6,66–6,84. So we recommend utility this plant extracts as effective eco-friendly agents for an antifungal control.

Halocnemumstrobilaceum extracts have been identified to possess medically and pharmaceutically interesting properties due to being a source of fatty acids, triterpenoids and flavonoids [17].

According to [18], five flavonoids were isolated from the epigeal parts of *H.strobilaceum*such as: isoquercitrin, isorhamnetin 3-*O*-β-D- glucopyranoside, isorhamnetin, rhamnazin, and 3,4',5–trihydroxy-3'–methoxyflavone 7-*O*-β-D-glucosaminopyranoside.

The obtained data from our study showed that the aqueous extracts of *H. strobilaceum*were potent and exhibited an antifungal activity against these pathogenic fungi that are isolated from date palm.

According to several studies of the antifungal activity of different plant extracts against pathogenic fungi of numerous cultures around the world. The antifungal activity of ethanol extract of *Lowsoniainermis*and *Psidium guajava*against *Fusarium* wilt in tomato was assessed. All the extracts inhibited mycelia growth at various levels. Among them the superior inhibition (100%) was found in 15% concentration [19].

The results of [20] revealed that plants extract of *Azadirachtaindica*and *Jatropha curca*s had a strong antifungal activity with significant inhibition on the growth of the all tested fungi (*Aspergillus flavus, Alternaria alternate, Fusarium oxisporium, Rhizopus stolinifier*and *Cladosporium herbaru*). Extracts of *Azadirachtaindica*and *Jatropha curca*s were the most effective to inhibit the growth of the tested fungi.

According to [21], the garlic (*Allium sativum*) extracts with different concentrations have exhibited a significant antifungal activity in the protection of potato plant against *Phytophthora infestans*.

Conclusion

This study suggested that the aqueous extracts of *H.strobelacium* would be helpful in treating diseases in date palm trees caused by these fungi. In conclusion, the findings of this experiment confirmed that plant extracts can be used as natural fungitoxicant to control the growth of date palm pathogenic fungi and thus reduce the dependence on the synthetic fungicides.

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Table 1. - Inhibition percentagesof*H. strobilaceum* against the isolated fungi from date palm

	Dose 1	**Dose 2**	**Dose 3**	**Dose 4**
***Fusarium* sp.**	58,99%	66,19%	68,21%	76,34%
***Phytophthora* sp.**	73,22%	79,61%	82,98%	90,60%
***Cladosporium* sp.**	80,61%	89,02%	89,10%	86,15%
***Alternaria* sp.**	67,15%	71,20%	71,64%	68,92%

Figure 1. Diseases symptoms in date palms

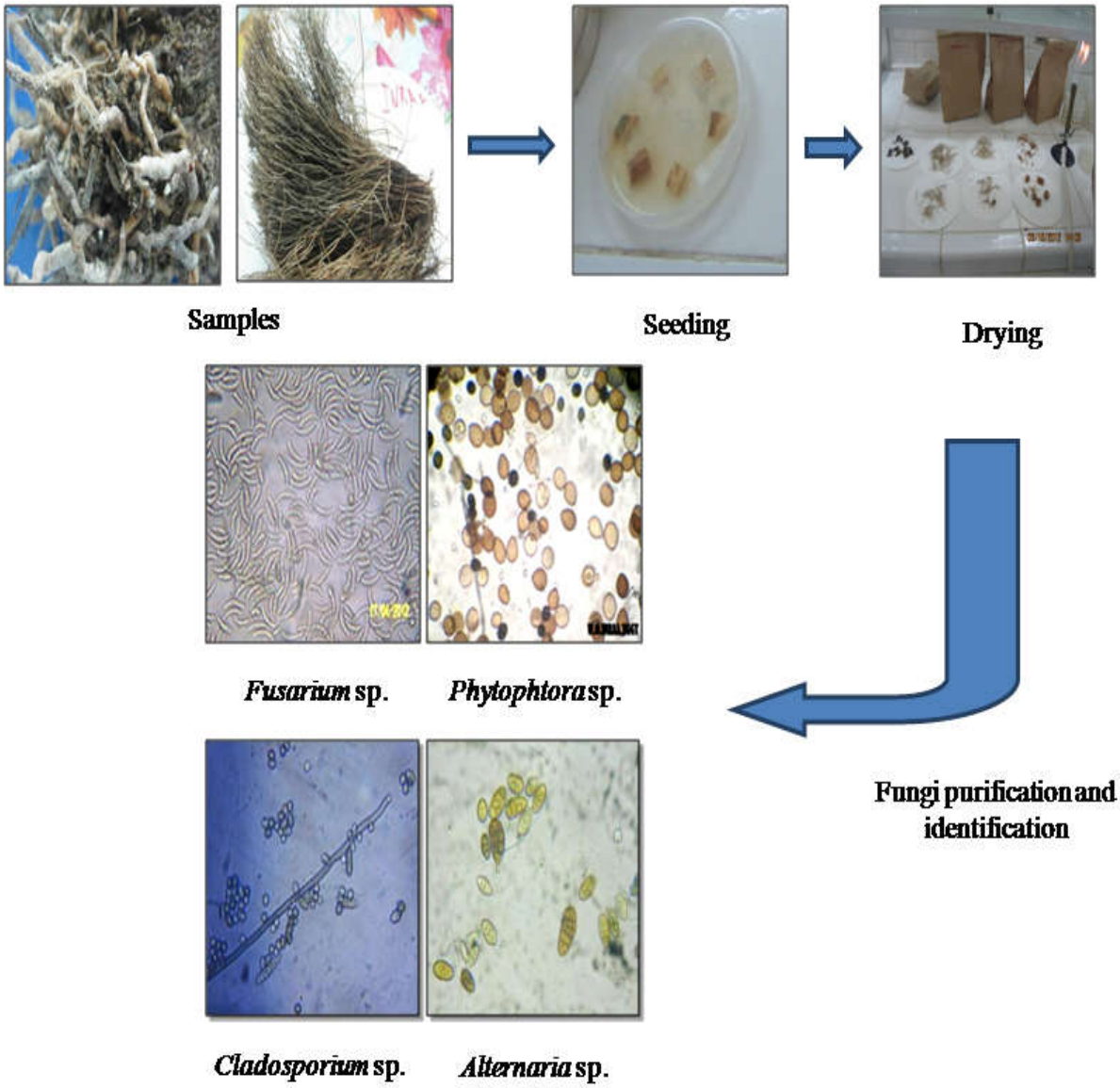

Figure 2. Isolation and identification of fungi

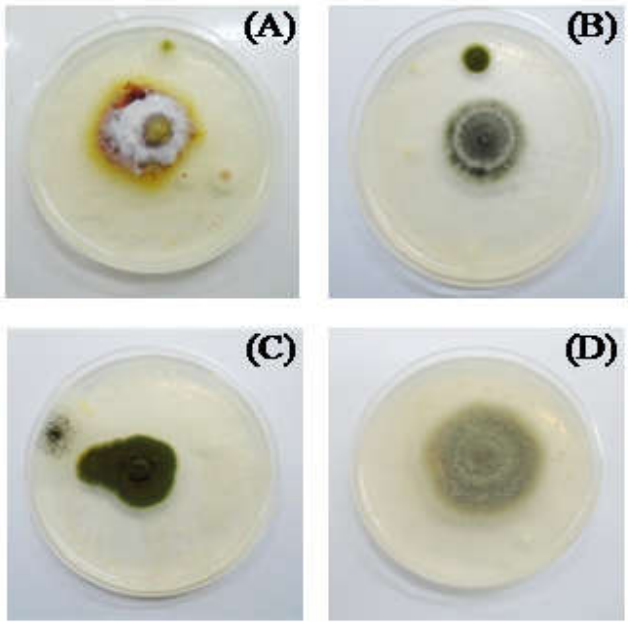

Figure 3.- Antifungal activity of *H. strobilaceum* against studied fungi

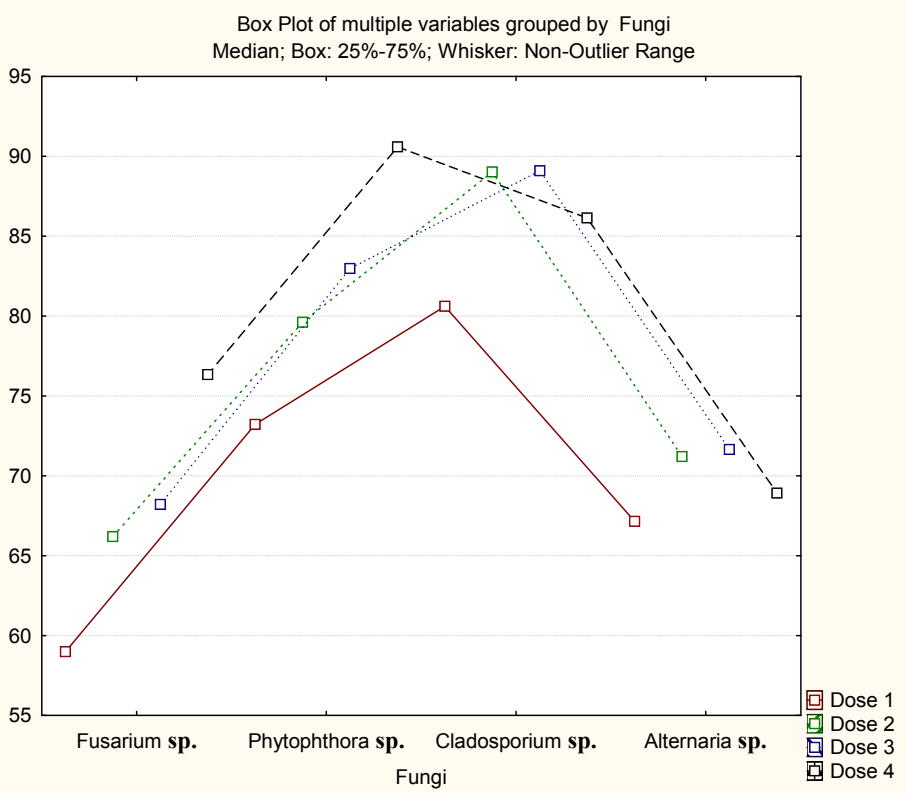

Figure 4.- Efficiency of *H. strobilacium* doses on the studie