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Bioactivity of *Artemisia Herba alba* essential oil against plant pathogenic fungi

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ABSTRACT

This work is part of the chemical composition's study, of the essential oil's and antifungal activity. This oil is extracted, from an aromatic and medicinal plant of the Algerian flora, in order to find new metabolite products, which are characterized by a biological activity. The investigations and research on the essential oil; extracted from the dried aerial part of Artemisia Herba alba, which was harvested in the region of Djelfa (South of Algeria); and separated by gas chromatography coupled by a mass spectrometry (GC/MS); resulted in obtaining Thirty-three constituents, representing 97.54% of the essential oil of Artemisia Herba alba. The main compounds identified are Davanone (42.8%), Camphor (15.96%) and Thujone (9.63%). The antifungal activity of oils was tested using the direct contact method against the growth of Fusarium moniliforme, Fusarium solani, Fusarium oxysporum and Stemphyliumsolani. Our results clearly demonstrate that the essential oil could supply a valid alternative to chemical treatments on the basis of their efficacy on different types of plant pathogens and their flexibility of use.

K**eywords:** *Artemisia Herba alba*; Antifungal Activity; Biopesticides; Chemical composition; Essential oil; GC/MS;

INTRODUCTION

The economic importance represents the aromatic and medicinal plants related to their biological properties, therapeutic, fragrant ..., their impact in the environment and their multiple uses in various industries, arouses a growing interest in biology and even organic chemistry.

Currently, several questions are raised regarding the safety of synthetic chemicals used in medicine or in food industry. Indeed, the use of chemical pesticides for the protection and conservation of plants in areas agriculture against bacteria and fungi cause very serious side effects for the environment and health.

For this, the search for new alternatives to chemical pesticides has become indispensable. Among the alternatives considered, biological control that aims to control and kill pathogens by biopesticides.

For this purpose, the essential oil extract from the plants begin to have great interest as a potential source of bioactive natural molecules. Essential oils are used in aromatherapy, pharmacy, perfumery and cosmetic products due to their wealth of active ingredients that are loaded by a vital energy of natural origin. Also, they are used in

biological control as biopesticides due to their inhibitory action on growth and toxinogenesis of several bacteria and fungi.

Artemisia herba alba Asso (chih), belonging to the family "Asteraseae"; This is among the most medicinal plants used by the local population because of their medicinal properties, as well as a flavoring in tea and coffee [1]. The plant known as the "wormwood' is characteristic of the steppes of the Middle East and North Africa. It is extensively used to treat stomach disorders, hepatic, in addition to a wide variety of ailments and against certain forms of poisoning, also as antitumor agent, antispasmodic, antiseptic, antigenotoxic, antidiabetic, antibacterial and antioxidant [1-4].

This work was focus on the study of the antifungal activity of essential oil of *Artemisia Herba alba*. It was conducted to illustrate the use of these essential oil as a natural alternative to chemical fertilizers (biopesticide).

MATERIALS AND METHODS

2.1 Vegetal Material

Aerial part of *Artemisia Herba alba* were collected in April 2013 from Mount of Boukhil in Djelfa region (Algeria) coordinates (N 36 ° 52'18 .011 "E 6 ° 53'14 .786 ') and then dried in the shade for 10 days. The plant was identified by Dr. Abdelmadjid Chahmaa botanist in Biology department, Ouargla University, Algeria, a specimen was deposited at the herbarium of the University under the number GO2013-3.

2.2 Extraction of essential oil

The extraction of essential oil was carried by steam distillation, in a Clevenger apparatus by immersing 100g of dry leaves in a flask of 1000 ml of water for 3 hours. The obtained essential oil was dried with MgSO4 and stored in the dark at 4°C.

2.3 Gas chromatography–mass spectrometry essential oil analysis

The essential oil analysis of *Artemisia Herba alba* was performed at the INRAP (National Institute of Research and physico-chemical analysis) of Tunisia, the gas chromatograph used is an Agilent 6890, coupled to a mass spectrometer type Agilent 5975B with a quadrupole ionization voltage of 70 eV. The column used is a HP-5MS; 5% Phenyl Methyl Siloxane with a length of 30 m and an internal diameter equal to 0.25 mm. The wire thickness being 0.25 µm.

The operating conditions are:

- The temperature of the injector (1:50 split mode): 250°C.
- The temperature programming: from 50°C to 300°C at a rate of 2°C/min.
- The vector gas used is helium with a flow rate of 0.8 ml / min.

The temperatures of the quadrupole source are fixed, respectively, at 230°C and 150°C. The Linear retention indices (RI) for all the compounds were determined using n-alkanes as standards. Identification of individual compounds was performed by matching their mass spectral fragmentation patterns with corresponding data (NIST 05 and Wiley7 libraries), and by the laboratory database.

2.4 Antifungal Activity:

2.4.1 Origin of fungal strains:

The fungi used in this study were isolated from tomato leaves, peppers and wheat leaves. These are 03 species within the genus Fusarium (*Fusarium moniliforme, Fusarium solani, Fusarium oxysporum*) and a specie belonging to the genus *Stemphylium solani* they entail considerable loss of production in several varieties of plant. The species were identified by Mr. Messaoud Bensaci, Ecosystems Protection in Arid and Semi-Aridareas Laboratory. University of Ouargla, Algeria.

2.4.2 Direct contact method:

The evaluation of the antifungal activity of essential oils is adopted by the direct contact method where four concentrations are obtained by addition of 30, 150, 300, and 450 µl of essential oils upon 60ml warm PDA in a vial with adding drops of tween 20. The technique involves adding the oil at different concentrations (0.05%, 0.25%, 0.5% and 0.75%) in the middle of still liquid culture followed by 5 minutes of stirring in order to homogenize the medium PDA with essential oil. After shaking the vials, the mixture (PDA + HE + Tween 20) is poured into petri dishes.

The inoculation is done under the hood where depositing at the center of the box a mycelial disc of 0.6 cm in diameter.; The Witnesses (fungal strains + PDA + Tween 20) are made in the same conditions without essential oil and the measure is taken after 72 hours of incubation. These boxes (control and test) are incubated at 25 ± 2 ° C for 7 days respectively [5].All tests are repeated three times.

2.4.3 Inhibition rate (% TI):
The calculation of inhibition percent of growth compared to the control allows to evaluate the effect of oil concentrations on fungal growth. The technique consists of measuring the diameters of the various fungal colonies after the required incubation time [6].

$$\boldsymbol{TI}(\%) = \mathbf{100} \times (\boldsymbol{dC} - \boldsymbol{dE})/\boldsymbol{dC}$$

TI (%) = Inhibition rate expressed as a percentage
dC = Diameter of settlements in the dishes "positive control"
dE = Diameter of colonies in the dishes containing the plant extract

2.4.4 Determining the mycelial growth rate (VC):
According[7],the rate of mycelial growth of each concentration is determined by the formula:

$$\boldsymbol{VC} = [\boldsymbol{D1}/\boldsymbol{Te1}] + [(\boldsymbol{D2} - \boldsymbol{D1})/\boldsymbol{Te2}] + [(\boldsymbol{D3} - \boldsymbol{D2})/\boldsymbol{Te3}] + \cdots + [(\boldsymbol{Dn} - \boldsymbol{D_{n-1}})/\boldsymbol{Ten}]$$

D: Diameter of the growth zone of each day.
Te: Incubation time.

RESULTS AND DISCUSSION

3.1 Gas chromatography–mass spectrometry analysis of essential oil
Essential oils yields have been calculated based on the dry plant material of the aerial part of the plant. *Artemisia Herba alba* is provided a rate of 0.54%.The chromatographic analysis of essential oil has identified Thirty-three compounds, representing about 97.54% of the whole harvested plant HE (**Table 1**).

Table 1: Chemical composition of the essential oils of *Artimisia Herba alba*

Compounds	RT	Aera %
Tricyclene	715	0,12
1R-α-Pinene	762	0,45
Camphene	823	2,91
Sabinene	929	0,37
L-β-Pinene	939	0,21
β-myrcene	1020	0,94
β-Cymene	1184	0,25
Eucalyptol	1219	6,5
γ-Terpinene	1365	0,17
Thujone	1650	**9,63**
Trans-8-hydroxylinalool	1681	0,35
β-Thujone	1708	1,52
1,2,5,5-Tetramethyl-1,3-cyclopentadiene	1780	6,56
Camphor	1893	**15,96**
Pinocarvone	1983	0,3
2-Nonyne	2013	1,36
L-4-terpineol	2085	0,53
trans-Chrisanthenylacetate	2617	0,64
L-bornylacetate	2764	0,24
α-Terpinene	3172	0,29
cis-Jasmone	3483	0,35
β-Cubebene	3946	1,24
Bicyclogermacrene	4036	0,35
Davanaether	4176	0,94
Caryophylleneoxide	4524	0,65
Davanone	4564	**42,8**
nerolidol	4684	0,17
β-Dihydroagarofuran	4698	0,39
Cyclohexane ketone	4767	0,21
α-Pineneoxide	4828	0,33
α-Himachalene	4884	0,28
Lilacalcohol	4944	0,53
Total		97,54

RT : retention index

Artemisia Herba alba essential oil of South Algeria is composed mainly by Davanon (42.8%), Camphor (15.96%) and Thujone (9.63%), which represent 68.39% of our oil's total.

Generally, the oil has been widely reported to be mainly composed of oxygenated monoterpenoids, such as 1,8-Cineole, Chrysanthenone, Chrysanthenol (and its acetate), α / β-Thujone and Camphor [1, 2, 8, 9].However, another investigation from Spain showed the sesquiterpene Davanone to be the principal component of the oil, which was also dominated by the *p*-Menthane and Pinane skeletons [10].

It is learned that the variation in the chemical composition of essential oils could be attributed to the geographical origin of the plant, the extraction technique, the time of harvest and climatic factors [11, 12].

3.2 Antifungal Activity:

3.2.1 Kinetics of mycelial growth:

The mycelial growth kinetics was evaluated every 24 hours by measuring the mean of three perpendicular diameters passing through the middle of the puck. This reading is always performed in comparison with control cultures that they are started on the same day under the same conditions. Any even slight growth of each fungus will be considered negative action that the essential oil in question does not have any inhibitory action against fungal growth. The Figure 01 summarizes the results of mycelial growth (cm) of the fungal strains as a function of incubation time and the concentration of essential oil of *Artemisia Herba alba*.

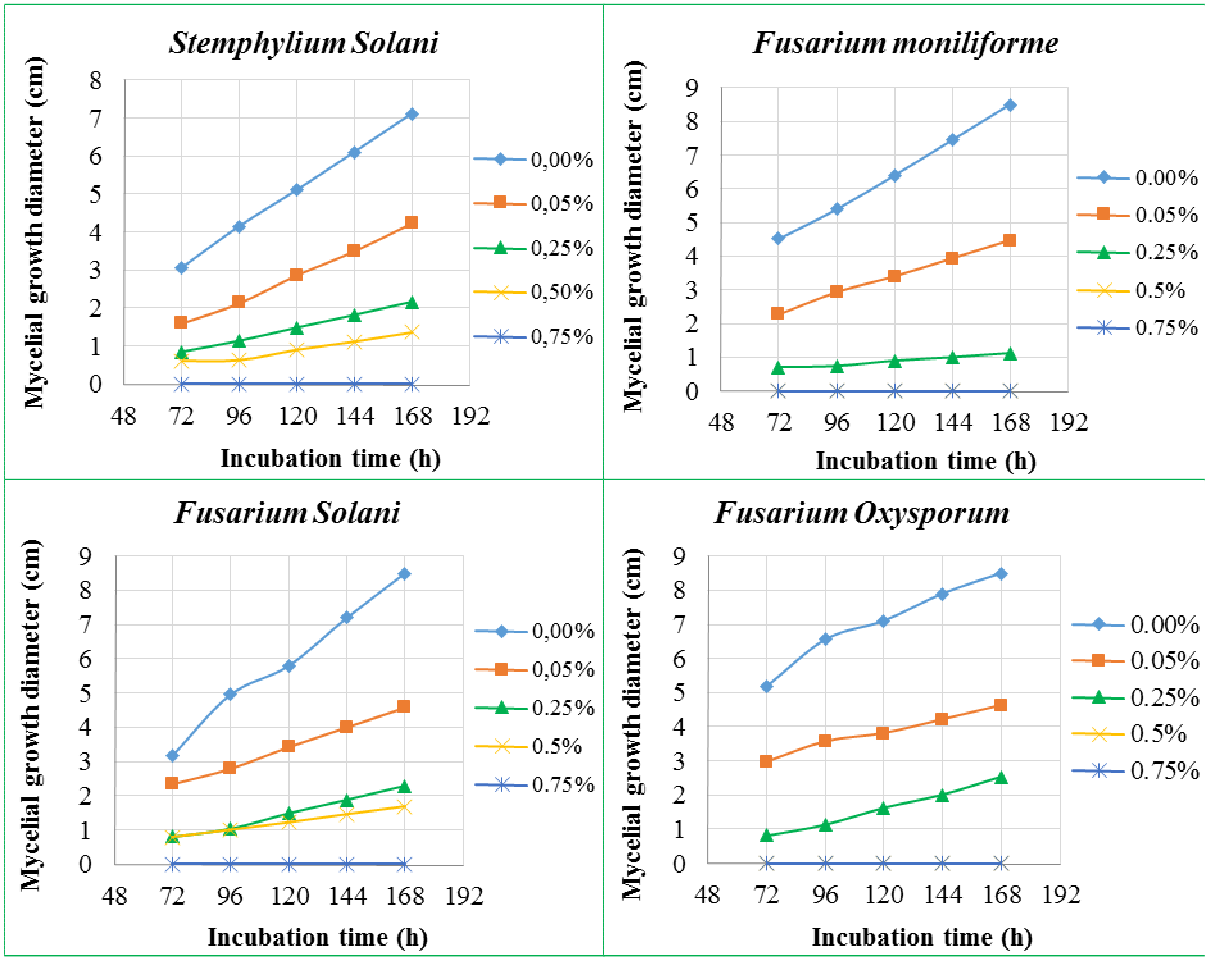

Figure 01: Kinetics of mycelial growth as a function of time and concentration of essential oils from *Artemisia Herba alba*

The essential oil of *Artemisia Herba alba* effect (Figure 01) shows an increase in mycelial growth with the incubation time except the concentration of 7.5 µl/ml (0.75%) that exhibits no mycelial growth for all the strains. However, a reduction in mycelial growth with increasing concentration of *Artemisia* oil is noted.

3.2.2 Mycelial growth:

Antifungal activity is revealed by the absence or presence of mycelial growth. The results of diameters of the essential oil antifungal activity of *Artemisia Herba alba* are shown in figure 02, *Artemisia Herba alba* effect on mycelial growth was sign in the concentrations that corresponds of the absence of essential oil (control).

The results showed that mycelial growth is notable to witness. We also observe the effect of different doses of oil on the fungal strains namely 0.05%, 0.25% which indicates mycelial decrease on all strains. Against 0.5% concentration, no mycelial growth for strains of *Fusarium moniliforme*and *Fusarium oxysporum* observed. A concentration of 0.75% report no mycelial growth for all strains.

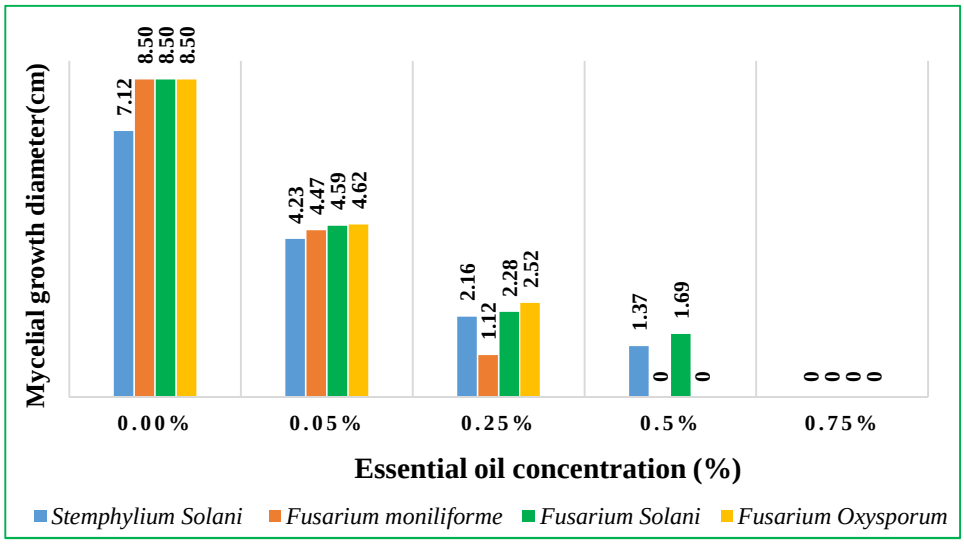

Figure02: Effect of essential oil of *Artemisia Herba alba*on fungal strains

3.2.3 Antifungal Index:

Inhibition rates of essential oils studied are given in Figure 03

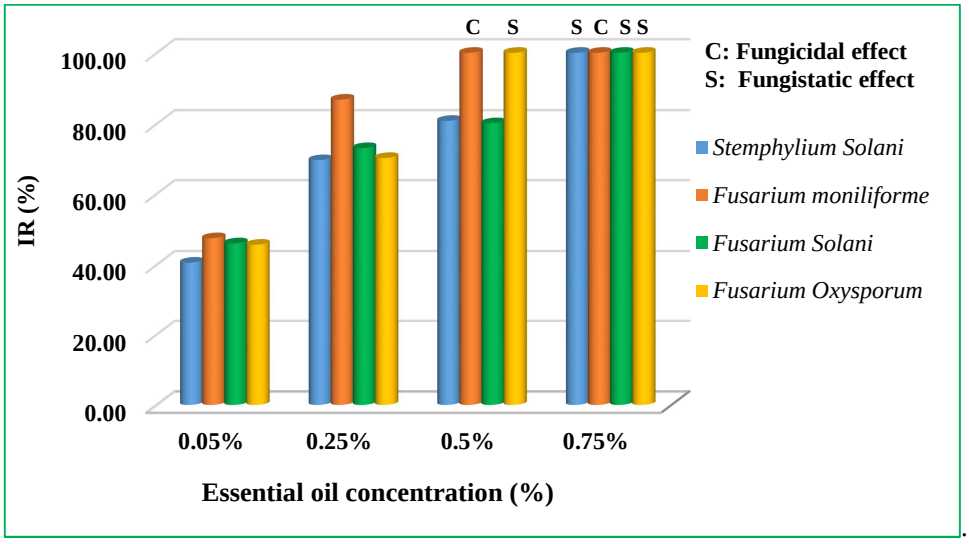

Figure 03: strains inhibition rate as a function of the concentration of *Artemisia Herba alba* essential oil

We note that according to Figure 03 all concentrations of essential oils applied were partially inhibited the growth of fungal strains tested. Figure 03 show that the inhibition rate is increased with increase in concentration of oils, and the minimal inhibitory concentration (MIC) is in the order with a good antifungal effectiveness shown by the essential oil.

Indeed, for *Artemisia Herba alba*, the MIC is 0.5% for *Fusarium moniliforme* strains with a fungistatic effect and *Fusarium oxysporum* with a fungicidal effect. For the *Stemphylium solani* and *Fusarium solani*, the MIC is 0.75%.

3.2.4 Speed of mycelial growth:

The speed of the mycelial growth of the four fungal strains as a function of the concentration of *Artemisia Herba alba*essential oils is shown in Figure 04.

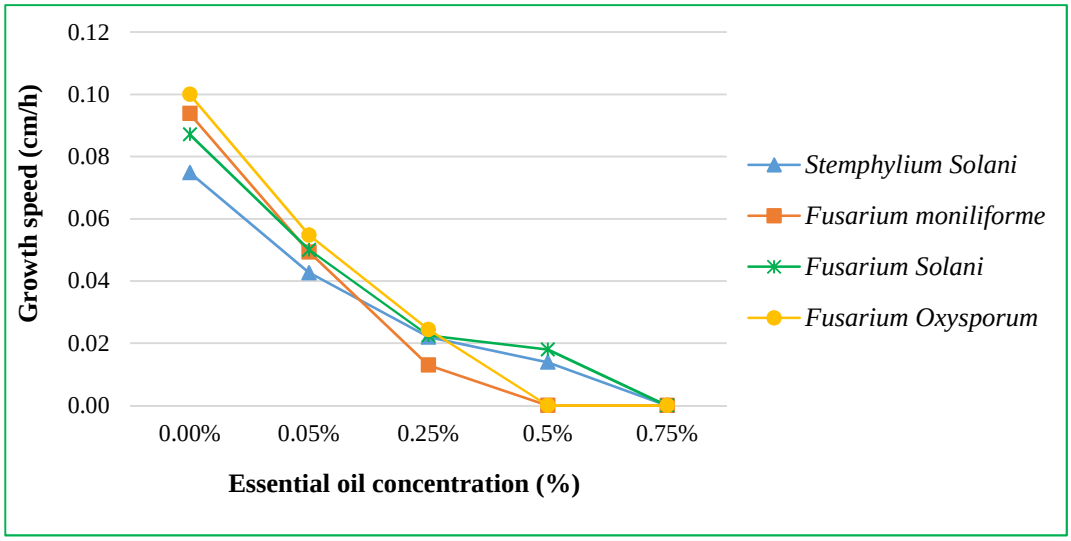

Figure 04: mycelial growth speed under the effect of increasing concentration of *Artemisia Herba alba* essential oil
According to the results of Figure 04, there is a decreasing speed of mycelial growth by increasing the concentration of essential oil. The speed of the strains is decrease to total inhibition (0 cm/h) in 0.5% concentration of essential oil to *Fusarium moniliforme, Fusarium oxysporum* and 0.75% for other strains

The direct contact technique involves contacting the essential oil and microorganisms, and observing the growth of the latter. The essential oil of *Artemisia Herba alba* exerted a significant inhibitory activity vis-a-vis the fungus tested. The diameters, the speed and the antifungal index of the mycelium growth decreases each time it increases the concentration of essential oil to the non germination on disc at determined MIC.

Substances and extracts isolated from different natural resources especially plants have always been a rich arsenal for controlling the fungal infections and spoilage.

Indeed, biological control through the use of natural alternatives gave a lot of interest in this moment. Many Researchers noted that the possibility of using the extract from plants as an effective natural alternative.

The effect of the essential oils of *Artemisia herba-alba* and *Oreganum* against spore germination, mycelial elongation and sporulation were studied in three fungi. The results obtained showed that all three stages of fungal asexual reproduction were affected but mycelium growth was the most sensitive, followed by spore germination and then sporulation of the three fungi studied. *Zygorrhynchus* sp. was found to be the most sensitive followed by *Aspergillus niger* and then *Penicillium italicum*. *Artemisia herba-alba* was less active on the three phenomena studied than the *Oreganum* oil [13].

KOLAI et al studied the inhibitory effect of essential oil of *Artemisia herba alba* on two strains of *Fusarium oxysporum*, the results showed that the antifungal activity is mainly due to substances contained in the essential oil extracted from the plant.[14]

In another study performed on the *Artemisia Herba alba* essential oils against *Penicillium citrinum* and *Mucora rouxii* found that extract plant which is rich in Carvone and piperitone has great power over the mushrooms tested [15].

According Chami, Prasad et al, the difficulty of developing an antifungal molecule is linked to the ultrastructure of fungal cell which presents three barriers: the cell wall, chitin, membrane ergosterol and eukaryotic nucleus in firstly; and secondly, antifungal molecules themselves that can lead to resistance.[16, 17]

The antifungal potency of essential oils studied could be attributed by the presence of components has an antifungal activity cause severe membrane damage and loss of homeostasis in which cell death or total inhibition. The majority of our essential oils constituents: Davanone, Camphor and Thujone proved by several researchers that have power antifungal[18-21]

Moreover, the antifungal activity of essential oils can be explained by the synergistic effect between the different essential oil compounds. However, as the majority compounds are often responsible for the antifungal activity. More, these minor components can contribute significantly to the activity of essential oil.

CONCLUSION

This work has been devoted to the study of the antifungal activity of a very abundant medicinal plant in the Algerian flora, *Artemisia herba-alba*. The chemical characterization of the essential oil has been determined in order to contribute to valorization and to redefine as their better exploitation. The chemical profiles of the investigated oil were highlighted by Davanone, Camphor and Thujone as major compounds. Bioassays conformed the effectiveness of essential oils against microorganisms studied. This results clearly demonstrate that the essential oil could supply a valid alternative to chemical treatments on the basis of their efficacy on different types of plant pathogens and their flexibility of use.

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