

## Fumigation of Peach Fruits with Essential Oils to Control Postharvest Decay

<sup>1</sup>El-Sayed H.E. Ziedan and <sup>2</sup>Eman S.H. Farrag

<sup>1</sup>Plant Pathology Department, National Research Centre, Dokki, Cairo , Egypt.

<sup>2</sup>Agricultural Botany Department (Plant Pathology),  
Faculty of Agriculture, Quina, South Valley University, Egypt.

**Abstract:** In a survey of decayed fruit samples of peach showing yellowish, curl, brown blotch, white and soft rots syndromes were collected from local markets at Gharbeia, Cairo, Giza and Assuit governorates, Egypt. Rotuine isolation yielded 3 fungi i.e., *Rhizopus stolonifer* (Ehrenb., Fr.) Vuill (56.5 %) , *Monilinia fructicola* (Wint.) Hony (17.1 %) and *Aspergillus niger* Vantighm (7.5 %). Pathogenicity trails were carried out on wounded ripe peach fruits, fungal isolates were differ to cause rot of peach fruits . Fungal reisolated from damaged fruit tissue and Kock's pastulated were completed. Peppermint and sweet basil volatile oils extracted from leaves were tested as a natural biocide to control postharvest decay of peach. *In vitro*, vapors crude oils of peppermint and sweet basil was found to be antifungal against fungal pathogens . The antifungal activity of oils increased with an increase the concentrations. The results showed that *R. stolonifer* and *M. fructicola* could be completely inhibited by crude oils at dose of 30µl/400cm<sup>3</sup> air in a closed system. Vapor essential crude oils and blends of the major individual constituents ratio similar to their concentrations in the original oil inhibited the elongation rate of the fungal pathogens (mentone and menthol) was found to be the antifungal properties of peppermint oil, mentone had moderately effect at 10 µl/400cm<sup>3</sup> air. In case of sweet basil oil, linalool and eugenol showed antifungal activity in 20 µl/400cm<sup>3</sup>. Mixing two components for each oil was found to enhance the antifungal properties (in lower dose, indicating as synergistic effect. Fungal hyphae not re-growth after exposure at 20ml/400 cm<sup>3</sup> of vapor to oils crude and two major components tested before for each two essential oils. The optimal oil dose treatment to reduce decay and maintaining fruit quality after prolonged storage and marketing simulation at the rate 3 ml/box. This findings strengthen the possibility of using crude of peppermint and sweet basil oils as an alternative components to chemicals for preservations stored fruits.

**Key words:** Fumigation, peach, (*Prunus persica*) *Rhizopus stolonifer*, *Monilinia fructicola* and essential oil.

### INTRODUCTION

Postharvest losses of perishable crops has been estimated can reach 50% or more of the harvest. Even in production areas with most advanced technologies available, postharvest food losses are still substantial<sup>[8,9]</sup>. Although, it is difficult to determine precisely the full extent of postharvest losses due to diseases, conservative estimates consider that over 20% of the fruits and vegetables are lost, in developed countries, due to postharvest diseases. This value strongly increased in third world countries because the lack of adequate refrigeration facilities and poor sanitation<sup>[22]</sup>. Postharvest losses of peaches are mainly due to brown rot and *Rhizopus* rot, caused by *Monilinia fructicola* and *Rhizopus stolonifer*,

respectively<sup>[28,29,32,12,11,33,14,24,8,14]</sup>. Fungicides are primary means of controlling postharvest diseases of most fruits<sup>[8,14]</sup>. Public awareness of pesticides residues in food has increased and government regulatory agencies are responding by re evaluating the use of many pesticides. Concomitant development of fungicide resistance by postharvest decay fungi<sup>[9]</sup> has stimulated efforts to develop alternative systems of disease control for agricultural products<sup>[30]</sup>. Application of essential oils presents an alternative method for control postharvest diseases of fruit vegetables and grains<sup>[25,16,19,17]</sup>.

The objective of this study aimed to determine the effectiveness of essential oils from peppermint and sweet basil, against decay of peach to extending shelf-life of peach fruits postharvest.

## MATERIALS AND METHODS

**Isolation and Identification Fungal Pathogens:** Peach fruits with different rotting symptoms, curl, yellowish, softness, black powdery and light brown discoloration were collected periodically from markets at Gharbeia, Cairo, Giza and Assuit governorates, Egypt. were subjected to repeated isolations. The isolation was done from rotten tissue, using the methods described by Gamagae *et al.*<sup>[15]</sup>. Pieces were surface sterilized with sodium hypochlorite 1% for 2 minutes than rinsed several times in sterile distilled water. Pieces were plated out on acidified potato dextrose agar (PDA). Plates were incubated at 25 °C for 5 days and checked regularly from fungal growth. The fungi isolated were purified and identified according to<sup>[5,21]</sup>. Inocula of *M.fructicola* and *R.stolonifer* were prepared by growing on potato dextrose agar (PDA) for 1-2 weeks at 20-23 °C. Spores were harvested by washing a sporulating culture with 5-10 ml sterile distilled water into a screw cap test tube. Contents were thoroughly mixed prior to counting with a haemocytometer<sup>[29]</sup>.

**Pathogenicity Test:** The isolated fungi were used for pathogenicity test. Five apparent healthy fruits were used as a replicates for each particular treatment. The fruits were surface sterilized with sodium hypochlorite (2%), then rinsed by sterilized distilled water and left to dry at room temperature. Each fruit wounded by small scratch in two sides. Wounds were inoculated by spore suspension ( $1 \times 10^6$  spores / ml) from 7 days old cultures. The results were obtained by using the decay index severity of infection according to Chastanger and Ogawa,<sup>[7]</sup>.

**Essential Oil Extraction and Analysis:** Two hundred and fifty grams of the leaves of peppermint (*Agonis flexuosa*) and sweet basil (*Ocimum basilicum*) was put in a round bottom flask. 1000 ml of distilled water was added and subjected to hydrodistillation in a Clevenger-type apparatus for 2.5h<sup>[4]</sup>. The oil recovered was dried over by anhydrous sodium sulfate. Oil was stored in dark vials kept at refrigerator until biological evaluation. The crude essential oils were analysis by GC-MS analysis for identity of the unknown compounds of oils as conducted in previous work<sup>[10]</sup>. Authentic aroma components (major oil constituents), menthol (99%); menthone (90%); linalool (97%) and eugenol (99%), were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

**Determination of Vapor Minimum Inhibitory Concentration (MIC):** The antifungal activity of volatiles essential oils of peppermint and sweet basil

oils were tested against to *R. stolonifer* and *M. fructicola* according to<sup>[26]</sup>. The presence of volatile inhibitory compounds was tested in small petri dishes (90 x 15 mm) containing PDA medium without cover. Plates were inoculated with the tested fungi and placed together inside a large Petri-dishes (140 x 23 mm) (400 mm<sup>3</sup> volume) containing the oils tested ( $\mu$ l). The large dishes were sealed and kept at 25°C. The reduction in linear growth was observed and interpreted as minimal inhibitory concentration (MIC) of the tested oils.

**Effect of Oil Vapors on the Elongation Rate of Fungal Hyphae:** A few number of conidia of *Rhizopus stolonifer* and *Monilinia fructicola* were inoculated in PDA medium (1 ml) in a small Petri-dish (7 cm x 1 cm), then incubated for 5 h or 1 day at 30 °C for germination. At least three hyphae were selected and their elongation rates were determined microscopically at 30 °C by using ocular and stage micrometer method. After confirmation the uniform rate of elongation of each hyphae, the Petri-dish lid was exchanged with another lid containing a filter paper impregnated with a definite amount of peppermint and sweet basil essential oils or its components, then the dishes were sealed. The elongation rate was measured continuously until no more change occurred. After exposure time (50 min at 30 °C), the lid containing essential oil was again replaced with the essential oil-free lid and the dishes were incubated at 30 °C for 24 h without sealing. During incubation, the hyphae were grown to form colonies and the size of colony was measured to determine hyphal regrowth. At least three colonies were selected for measurement.

**Fumigation Peach Fruits with Essential Oils Against Postharvest Decay:** These experiments were to test the efficiency of peppermint and sweet basil crude oils to control postharvest decay of peach fruits caused by *Rhizopus stolonifer* and *Monilinia fructicola*. Fresh peach fruits (Cv. Early grand) apparently free of physical damage and diseases were used in this experiment. Fruits were surface disinfest by immersion in sodium hypochlorite 0.5% for 3 m, then rinsed with sterile water and allowed to air dry. Before fumigation treatments, peach were wounded (2 mm depth) twice with sterile needle. Inoculation of fruits was carried out by dipping in spore suspension  $1 \times 10^6$  spore/ml of either *R. stolonifer* or *Monilinia fructicola*. Then allowed to air dry at room temperature. Peppermint and sweet basil oil were applied by fumigation in carton boxes (52 x 27 x 13 cm). Inoculated fruits were placed into each box with filter paper impregnated with a definite amount of oil or water as control. Four concentrations were replicated twice. All treatments were performed at 27 °C. Finally, fruits quality was evaluated for decay, sugar expressed

as total soluble (TSS) and general appearance as described by Aharoni *et al.*,<sup>[1]</sup>. Results were analysed using Duncan's multiple range test at  $P \geq 0.05$  according to Snedecor and Cochran<sup>[27]</sup>.

## RESULTS AND DISCUSSION

**Symptomatology of Peach Fruits Decayed:** During survey of decayed peach fruits at different markets in Gharbeia, Cairo, Giza and Assuit Governorate. Different pictures of decayed peach fruits were observed *i.e.* Rhizopus rot, brown blotch, soft rot, Aspergillus rot and white mould as well as yellowish and curl symptoms Fig (1) and Table (1). Rhizopus rot commonly begins from puncture wounds or wounds caused by insects. Lesions are circular, soft and watery and are soon covered with masses of shiny, erect sporangiophores that appear at break in the fruit skin Fig (2). On the other hand, brown firstly appear and then cottony appearance covers the whole fruit soon after infection by *Monilinia fructicola* Fig. (2). Mycelia grew at a linear rate of 8.0 mm per day on PDA at 22°C, forming a grayish colony with heavy sporulation showing concentric rings.

**Fungi Associated with Peach Decayed Fruits:** Data presented in Table (1) indicated that, three fungi were isolated from rotted fruits. The most dominant fungi that isolated from symptomatic fruits, *Rhizopus stolonifer* (56.50%), *Monilinia fructicola* (17.35%) and *Aspergillus niger* (7.1%). Furthermore, *R.stolonifer* was isolated only from peach fruits appeared healthy.

**Pathogenicity Test of Isolated Fungi:** Data presented in Table (2) pathogenicity test of *Rhizopus stolonifer*, *Monilinia fructicola* and *A.niger* on peach fruits revealed that *R. stolonifer* produced highest infected area (28.83 mm<sup>2</sup>) and decay percent (100%) followed by *Monilinia fructicola* (19.94 mm<sup>2</sup>) and (70%) respectively. On the other hand, *A.niger* produced lowest infected area and decay percent compare the control.

**Oil Analysis:** From Table (3), it is evident that linalool and eugenol represent the major oil constituents of sweet basil oil (55.78 and 12.15%, respectively). On the other hand, menthone and menthol were the major oil constituents of peppermint oil (33.34 and 29.53%, respectively).

**Minimum Inhibitory Concentration (MIC) of Fungal Pathogens:** Data in Table(4) it is clear that sweet basil and peppermint oils manifested its antifungal activity at a minimum inhibitory dose of 30.0 µl/400ml air. The *R. stolonifer* and *M.fructicola* fungus failed to re-store growth even after fungal disks were transferred to free-oil vapors conditions.

**Effect of Essential Oil and Active Ingredient on Apical Growth of Fungal Pathogens:** The vapor of peppermint oil and two its of major constituents (menthol and menthone) and sweet basil oil and two of its major constituents (linalool and engenol) were tested against fungal pathogens hyphae elongation by rate similar in crude oils . Data in Table (5) revealed that increasing oil crude concentration and its major constituents from 10 ml to 20 ml/400 cm<sup>3</sup> was increasing harmful effect of mycelial of *R.stolonifer* and *Monilina fructicola* . Peppermint oil and two major components more effective than sweet basil oil and its two components tested before. Also, oils and their two components more effective of *Monilina fructicola* than *Rhizopus stolonifer* . In addition fungal hyphae not re growth after exposure at 20 ul/400 cm<sup>3</sup> of crud oils and mixed of two of major component for each oil and also, the same result showed of *M.fructicola* when exposur to vapor of two crud or each and mixed two major compounds tested before.

**Effect of Essential Oil Fumigation on Decay Incidence and Peach Fruits Quality:** Storage peach fruits for 22 days under fumigation by essential oils (peppermint and sweet basil) at different concentrations *i.e.*, 1,2,3 and 4 ml/box. Data in Table (6) indicate that vapor peach fruits by different rates significantly supress Rhizopus and brown rots than the control. Increasing oil concentration was significantly supress rots incidence of peach fruits. peppermint oil more effective than sweet basil on Rhizopus and brown rots incidence of peach fruits with no significantly. Based on general appearance, the marketability of oil-treated fruits was significantly better than that of untreated fruits until using 3 ml/box. For example in Fig (3) fumigation peach fruits by 3 ml of sweet basil / box under artificial infestation by *R.stolonifer* appearance of fruits and marking values were higher than of peach fruits left without treatment as a control . Increasing the dose to 4 ml/box, some phytotoxicity was noticed as light brown spots or yellowish spots and curl of peach fruits services. So that, the marketability decreased when 4 ml/box was applied. No differences were observed in TSS among the treatments.

**Discussion:** Rhizopus and brown rots are the most important post harvest diseases of peach fruits and can be responsible for heavy losses especially in peaches. These diseases caused by *Rhizopus* spp. and *M.fructicola* (Wint.) Honey which are widespread in Europe, America, Asia and Africa<sup>[8,28,32,11,12,21,33,14,24]</sup>.

In this study vapor essential oil of peppermint and sweet basil were used as crude oils and two major components for each oils showed that inhibition growth of *R.stolonifer* and *M.fructicola* due to menthol and menthone of peppermint and linalool and eugenol of

**Table 1:** Frequency of fungal associated with decayed peach fruits

symptoms	Frequency %		
	<i>R. stolonifer</i>	<i>M. fructicola</i>	<i>A. niger</i>
Soft rot	17.75	10.43	3.55
Brown rot	14.20	7.10	3.55
Curl and yellowish	14.20	0.00	0.00
Symptomless	10.65	0.00	0.00
Total	56.50	17.53	7.10

Values followed by the same letter are not significantly different at  $P \geq 0.05$  according to Duncan's multiple test.

**Table 2:** Pathogenicity test of fungi isolated from decayed peach fruits.

Isolated fungi	Infected area (mm) <sup>2</sup>	Infection(%)
<i>Rhizopus stolonifer</i>	28.83a	100.0a
<i>Monilinia fructicola</i>	19.94a	70.0b
<i>Aspergillus niger</i>	1.21b	15.0c
Control	0.0c	0.0d

Values followed by the same letter are not significantly different at  $P \geq 0.05$  according to Duncan's multiple test.

**Table 3:** Chemical constitution ((w/w %) of sweet basil and peppermint essential oil.

Components	Sweet basil	Peppermint
α-pinene	0.53	1.11
β-pinene	1.13	1.4
Myrcene	1.12	0.38
Limonene	0.54	2
1,8-cineol	10.26	6.99
Trans-sapinin hydrate	-	1.01
Linalool	55.78	0.5
Eugenol	12.15	-
Menthone	-	33.34
Methyle eugenol	3.79	-
Menthofurane	-	1.63
Isomenthone	-	7.58
Menthol	-	29.53
Menthyle acetate	-	3.17
B-caryophyllene	-0.12	2.34
Caryophyllene oxide	-	1.76

**Table 4:** Effect of crud essential oils of sweet basil and peppermint on fungal growth.

Essential oil (μL)	Reduction of linear growth (%)			
	Sweet basil		Peppermint	
	<i>R. stolonifer</i>	<i>M. fructicola</i>	<i>R. stolonifer</i>	<i>M. fructicola</i>
0	0.0c	0.0b	0.0c	0.0c
5	0.0c	0.0b	0.0c	0.0c
10	0.0c	80.0a	68.88b	58.88b
20	17.77b	100.0a	100.0a	100.0a
30	100.0a	100.0a	100.0a	100.0a

Values followed by the same letter are not significantly different at  $P \geq 0.05$  according to Duncan's multiple test.

**Table 5:** Inhibitory effect of crude vaporized essential oils and its components on apical growth of *R.stolonifer* and *M. fructicola*

Essential oil	Apical growth at concentration of				Regrowth after exposure to 20µL/dish	
	10 µL/dish*		20µL/dish**		<i>R.stolonifer</i>	<i>M. fructicola</i>
	<i>R.stolonifer</i>	<i>M. fructicola</i>	<i>R.stolonifer</i>	<i>M. fructicola</i>		
Sweet basil	+	±	±	-	-	-
Linalool	+	+	±	-	+	+
Eugenol	+	+	±	-	+	-
Linalool+eugenol	-	-	-	-	-	-
Peppermint	+	±	-	-	-	-
Mentone	+	-	-	-	+	-
Menthole	-	-	-	-	+	-
Mentone+menthole	-	-	-	-	-	-

- = no growth ; ± = doubtful growth; + = normal growth

\* Amount of linalool / or eugenol present in 10 µl of sweet basil is 5.74 and 1.44 µl. Also, Amount of mentone / or menthole present in 10 µl of peppermint is 3.7 and 2.9 µl.

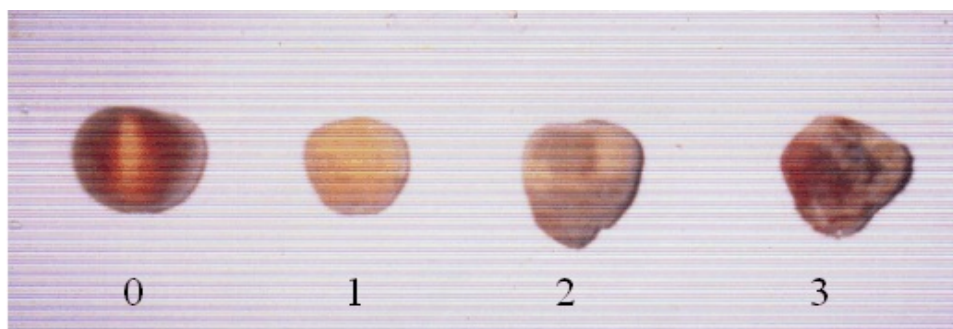
\*\* Amount of linalool / or eugenol present in 20 µl of sweet basil is 11.84 and 2.88 µl. Also, Amount of mentone / or menthole present in 20 µl of peppermint is 7.4 and 5.95 µl.

**Table 6:** Effect of essential oil fumigation on decay incidence and their effect on quality on peach fruits

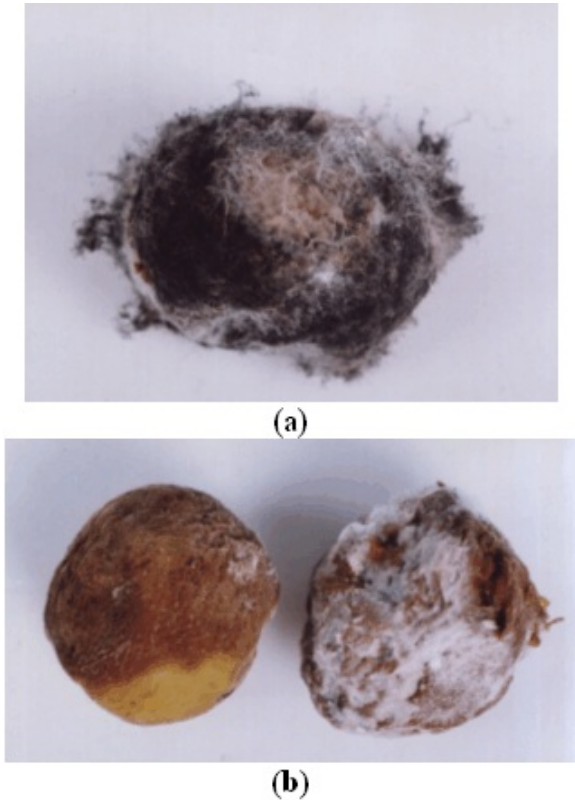
Oil (ml/box)	<i>Rhizopus stolonifer</i>			<i>Monilinia fructicola</i>		
	Decay %	TSS %	General* appearance	Decay %	TSS %	General appearance
Peppermint						
1	28.0b	14a	1.5d	19.0b	15a	1.7c
2	6.0c	14a	2.5b	3.0c	14a	2.5b
3	0.0d	15a	3.1a	0.0c	14a	3.5a
4	0.0d	14a	2.0c	0.0c	15a	1.0d
Sweet basil						
1	37.0b	14a	1.1b	22.0b	14a	1.6c
2	11.0c	15a	3.0a	1.0c	14a	2.5b
3	2.0d	15a	2.0b	0.0c	14a	2.5b
4	0.0d	15a	1.5d	0.0c	14a	1.5c
Control	100.0a	15a	0.41e	87.0a	15a	0.37e

\*General appearance: 1= poor; 2= moderate , 3= good , 4= very good and 5= excellent quality. Fruits evaluated at less than 2.5 were considered unfit for marketing as described by Aharoni *et al.*, (1997).

Values followed by the same letter are not significantly different at  $P \geq 0.05$  according to Duncan's multiple test.



**Fig. 1:** Different symptoms of peach fruits 0=healthy, (symptomless) 1-3 =decayed fruits 1=yellowish 2=brown rot and 3 =soft rot of peach fruits



**Fig. 2:** Symptoms induced in peach fruits after infection by *R. stolonifer* (a) and *Monilinia fructicola* (b)



**Fig. 3:** Vapor effect by a sweet basil oil 3 ml/box under infestation by *R.stolonifer* 1= without treatment (control) 2 = treatment by sweet basil

sweet basil by vapor phase. Their effect was increased by increasing the concentration up to 20 ul/400cm<sup>3</sup>. Various essential oils have been found to possess varied degrees of fungal toxicity against different pathogens<sup>[2,4,6,7,16,23,25,26,10,24]</sup>.

The antifungal mechanisms of essential oil is the synthetic inhibition of DNA, RNA, Protein and polysaccharides<sup>[31,16]</sup>. Also, Horberg<sup>[18]</sup> stated that vapor of the essential oils caraway (*Carum carvi*), spearmint (*Mentha spicata*), thyme (*Thymus vulgaris*), basil (*Ocimum basilicum*) and garlic showed as antifungal properties against 3 carrots, pathogens on storage i.e *Mycocentrospora acerina*, *Rhizoctonia carotae* and *Sclerotinia sclerotiorum*. These 3 pathogens also, was completely inhibited by the vapours from garlic oil in amounts from 10-80 ppm

Fumigation of peach fruits with peppermint and sweet basil crude oil under artificial infestation by *Rhizopus stolonifer* and *M.fructicola* was studied. Results obtained indicated the all different concentrations of peppermint and sweet basil essential oils as vapor treatment of peach fruits were significantly suppress decay of peach fruits caused by *R.stolonifer* and *M.fructicola*. Increasing oils concentrations were also increased oils efficiency for control *Rhizopus* and brown rots of peach fruits. Oils at different concentrations more effective of brown rot incidence than of *Rhizopus* rot incidence of peach fruits. These results are similar to the results obtained with tomato, where Ragab *et al.*<sup>[26]</sup> found that the vapor of lemon grass oil at 6 ml/L significantly suppress postharvest decay of tomato fruits caused by *F.oxysporum*. Schlech emend Snyder Hans, *Alternaria alternata* (Fr) keissler, *Botrytis cinerea* Pers., a Vantigham and *Rhizopus stolonifer* (Ehrenb., Fr.) Vuill. Kinokitiol, a natural biocide containing volatile substances, provides effective protection for eggplant and red papper fruits against the two main storage fruits *B.cinerea* and *A.alternata*. This results in additional chemical residues on fruits, thereby presenting a possible health hazard. Shelf-life of peach fruits is limited due to the development of postharvest decay i.e brown rot caused by *Monilinia fructicola* and *Rhizopus* rot caused by *Rhizopus* spp.<sup>[20,28,29,11,12,33,24]</sup>.

Natural plant products with low mammalian toxicity are promising alternatives to synthetic fungicides for postharvest decay control of fruits and vegetables i.e, benzaldehyde,<sup>[3]</sup> Hinokitiol extract, obtained by the steam distillation of the root of an *Hiba arborvitae*, of Hinoki (*Japanese cypress*)<sup>[28,13]</sup>.

Peppermint and sweet basil were offers promise as natural products should be given high priority control postharvest decay of fruits and vegetables .

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