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Synergistic *in vitro* and *in situ* effects of combinations of *Psychotria pedoncularis* and *Cupressus lusitanica* extracts on the development of two morphotypes of *Fusarium oxysporum* associated with tomato fruit rots in the highlands of West Cameroon

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Abstract

Fusariosis is one of the main causes of post-harvest losses in tomatoes. This disease is caused by several fungal species, one of the most important being *Fusarium oxysporum*. While it is possible to use synthetic fungicides in the field to limit losses, post-harvest producers, traders and consumers are virtually powerless to deal with this problem. Medicinal plants are known for their antimicrobial properties and non-toxicity. However, previous studies have shown that although plant extracts can be an alternative to fungicides, some fungal species have developed resistance to these plant extracts when administered individually. It is therefore possible to use the extracts in combination to increase the spectrum of activity of the bioactive molecules and thus maximise the desired antifungal effect. Thus, the present work aimed at evaluating the efficacy of the combination of *Cupressus lusitanica* and *Psychotria pedoncularis* extracts on the development of *F. oxysporum* morphotypes resistant to its extracts administered separately. To achieve this objective, a test was carried out to evaluate the combination activity of different concentrations of *P. pedoncularis* and *C. lusitanica* extracts on the development of *F. oxysporum* Fo_1 and Fo_2 morphotypes. The results showed that the combination of the different concentrations of the aqueous and ethanolic extracts of these plants on the Fo_1 and Fo_2 morphotypes is synergistic according to the 5% Duncan test. Their combination effect index (CEI) was significantly higher than 2. These CEIs had values that varied between 2.07 and

2.46. Under *in situ* conditions, the first seven combinations of the aqueous and ethanolic extracts of these two plants tested on the Fo_1 and Fo_2 morphotypes are synergistic according to the 5% Duncan test. Their combination effect index (CEI) values were less than 1 (CEI <1). However, the last two combinations (Mixed 8 and Mixed 9) showed indifference towards the different morphotypes. Their CEI values were greater than 1 (CEI > 1). The results of this study suggest the possibility of using combinations of *C. lusitanica* and *P. pedoncularis* extracts for the preservation of tomato fruits against postharvest contamination by *F. oxysporum.*

Keywords: Antifungal activity, plant extracts, *Fusarium oxysporum*, synergism, tomato fruits.

Introduction

Species of the genus Fusarium, especially *Fusarium oxysporum*, are among the fungi most involved in tomato fruit rot (Abu *et al*., 2013; Mugao, 2015; Micah *et al*., 2018; Hassan et al., 2020; Ayele et al., 2021) in the different production basins in West Cameroon (Njimah *et al*., 2021). But in addition to their impact on post-harvest losses, tomatoes contaminated by this fungal species are dangerous to human health, as they produce mycotoxins such as lycomarasmines and fusarium acids responsible for cell permeability and degradation of the plant product (Nelson *et al*., 1990; Maja *et al*., 2012; Heit, 2015).

Furthermore, the application of fungicides is a common preventive strategy to control post-harvest losses due to this fungal species, but their application on tomato fruits presents risks for the consumer and the environment, in addition to the development of resistant fungal strains (Alavanja *et al*., 2004; Andreotti *et al*., 2009).

Plant extracts are used to make biofungicides, which are environmentally friendly and have little or no toxic effects on humans (Anjum *et al*., 2016). They can be credible alternatives to synthetic fungicides. Indeed, plants are an important source of a wide range of bioactive secondary metabolites: tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds, which have antifungal properties (Arif *et al*., 2009; Murtaza *et al*., 2015). Thus, *Cupressus lusitanica* contains a variety of monoterpenes, sesquiterpenes and diterpenes that have antifungal properties (Florisvaldo, 2011; Teke *et al*., 2013). Indole, quinoline and benzoquinolizidine alkaloids, terpenoids, steroids, phenolic and aliphatic compounds have been isolated from species of the genus Psychotria with good antimicrobial, antiviral and antiparasitic activities (Yang *et al*., 2016).

However, several cases of fungal species resistant to biofungicides have been reported in numerous works. For example, those of Njimah *et al*. (2021) showed that *Fusarium oxysporum* Fo_1 and Fo_2 morphotypes were the most resistant to aqueous and ethanolic extracts of *C. lusitanica*, *P. pedoncularis, C. maniana* and *C. citratus*. In contrast, *F. oxysporum* morphotypes Fo_3, Fo_4, Fo_5, Fo_6, Fo_7 and *F. solani* morphotypes Fs_1 and Fs_2 were the most sensitive to extracts of four plants administered separately.

This resistance of phytopathogenic fungi to individually administered plant extracts is of concern to the scientific community and calls for the search for a new technique of administration of these plant extracts. This new method can be done by combining extracts, as plants are used either separately or more often in combination in order to increase the spectrum of activity of the bioactive molecules and thus maximise the desired positive effect.

It is on this basis that the present study is carried out, which aims to evaluate the activity of the combination of *C. pedoncularis* and *P. pedoncularis* extracts on the development of the most resistant morphotypes of *F. oxysporium* Fo_1 and Fo_2 to these two extracts administered separately.

Materials and Methods

Preparation of the stock extract solutions

The plant extracts were prepared according to the method described by Njimah *et al* (2021). The different stock solutions of these extracts were prepared to obtain the final concentrations to be tested. For the ethanolic extract, 1.17 g, 585.04 and 292.52 mg of extract were taken and dissolved in 1 ml of DMSO and then made up to 30.07 ml with distilled water for final stock solution concentrations of 38.91, 19.46 and 9.73 mg/ml. The effect of DMSO was tested beforehand to ensure that it did not influence the development of the different morphotypes. For the aqueous solutions, 9.37, 4.685 and 2.3425 g of extract were taken and dissolved in 1 ml of DMSO and then made up to 30.1 ml with distilled water for final stock solution concentrations of 311.296; 155.648 and 77.824 mg/ml.

In vitro activity of combinations of different concentrations of aqueous and ethanolic extracts of *P. pedoncularis and C. lusitanica* on the development of morphotypes *of Fusarium oxysporum*

The combination effect between the extracts of the two plants was evaluated by the solid-state diffusion method such as described by Njimah *et al*.(2021). 1 ml of *P. pedoncularis* stock solution was combined with 1 ml of *C. lusitanica* stock solution in the ratio 1: 1 and then shaken and dispersed in 17 ml of PDA culture medium, for concentrations of the test solutions of 2.048, 1.024 and 0 .512 mg/ml for the ethanolic extract and 16.384; 8.192; 4.096 for the aqueous extract. After solidification of the medium, an explant of the most resistant mycelium was taken from the growth front of a 10-day-old pure culture using a 5 mm diameter punch, then aseptically placed in the middle of each Petri dish supplemented or not with extracts. The plates were then sealed with para-film and incubated at 24-20°C. Petri dishes supplemented with one type of plant extract were used as negative controls and those supplemented with Mancozeb at the manufacturer's dose were used as positive controls. After 7 days of incubation, the growth diameters of the different morphotypes were measured using a graduated ruler (mm) and transformed into percentage inhibition. The combination effect index (CEI) was used to analyse the interactions in this study according to the formula of Soraya *et al*. (2019) below:

$$CEI = \frac{\%\ I \text{ of extract A in combination}}{\%\ I \text{ of extract A alone}} + \frac{\%\ I \text{ of extract B in combination}}{\%\ I \text{ of extract B alone}}$$

With % I: Percentage of inhibition ;

The antifungal effects of the combinations of these extracts were assessed as follows according to the modified method of Soraya *et al*. (2019).

- Synergistic: when CEI> 1; the effect is significantly greater than the sum of each extract studied in isolation: (A + B) > effect A + effect B.
- Additive: when 1< CEI< 2; the effect of the combination of extracts is equal to the sum of the effects of each extract studied in isolation: (A + B) = effect A + effect B.
- Indifferent: when CEI = 1; the activity of one extract is not affected by the other: (A + B) = effect A or effect B.
- Antagonists: when CEI< 1; the combination decreases the activity. It is less than the sum of the effects of each extract taken separately: (A+B) < effect A or effect B.

Combination activity of *C. lusitanica* and *P. pedoncularis* extracts on the development of different morphotypes of *Fusarium oxysporum* inoculated on tomato fruits

Apparently healthy tomato fruits collected from gardeners were washed with tap water and then dried and superficially disinfected with 70% alcohol for 1 minute (Wamalwa *et al*., 2018). Each tomato fruit received 1ml of the combination of *C. lusitanica* and *P. pedoncularis* extracts. These tomato fruits were then sprayed

with 50 µl of inoculum of each *F. oxysporum* morphotype using a sprayer (Lee *et al*., 2005). Fruits that received only unassociated extract (extract alone) and inoculum, and those that received inoculum and mancozeb at the manufacturer's recommended dose (1mg/ml) served as negative and positive controls, respectively. All these fruits were placed in crystallizers where humidity was maintained by cotton soaked with distilled water and the whole was incubated at room temperature. After 7 days of incubation, the area of Fusarium rot developed by the different morphotypes of F. oxysporum on the different fruits was measured using graph paper. The experiment was repeated three times. The combination effect index (CEI) was used to analyse the interactions in this study according to the modified formula of Soraya *et al*. (2019) below:

$$CEI = \frac{\text{Lesion area of extract A in combination}}{\text{Lesion area of extract A alone}} + \frac{\text{Lesion area of extract B in combination}}{\text{Lesion area of extract B alone}}$$

The antifungal effects of the combinations of these extracts were assessed according to the modified method of Saffidine (2015).

- Synergistic: when CEI <1
- Additive: when CEI=1
- Indifferent: when 1<CEI<2
- Antagonists: when CEI >2

Results

In vitro activity of plant extract combinations on different morphotypes of *Fusarium oxysporum*

Tables 1 and 2 show the in vitro combination effect indices (CEIs) of aqueous and ethanolic extracts of *P. pedoncularis* and *C. lusitanica* on the development of *F. oxysporum* Fo_1 and Fo_2 morphotypes isolated from tomato fruits from different production basins in West Cameroon.

In vitro activity of combinations of aqueous extracts

The percentages of inhibition of the tested morphotypes by the different combinations of extracts are compared to the percentages of inhibition of the extracts tested in isolation. The aqueous extracts of *C. lusitanica* and *P.pedoncularis* applied separately showed some efficacy on all the morphotypes tested. This efficacy increased with the combination of the extracts of both plants. The first five (mixed 1, mixed 2, mixed 3, mixed 4, mixed 5) and the seventh combination (mixed 7) of the extracts of both plants were the most effective on the Fo1 and Fo_2 morphotypes inducing a percentage of inhibition of 100%. The other combinations significantly reduced the growth of the same morphotypes compared to the extracts tested individually according to Duncan's 5% test.

The combination effect of the aqueous extracts of the different concentrations of these two plants tested on the $Fo_1$and Fo_2 morphotypes is synergistic according to the Duncan 5% test. Indeed, their combination effect index (CEI) was significantly higher than 2. These CEIs had values that varied between 2.13 and 2.46. However, no indifference or addition effect, let alone antagonism, of the combination of the different concentrations of the two extracts was obtained on the different morphotypes tested (Table 1).

Table 1: *In vitro* effect of combinations of different concentrations of aqueous extracts of *C. lusitanica* and *P. pedoncularis* on the development of the most resistant morphotypes of *F. oxysporum* to the extracts tested individually.

Morphotype code	Treatments	Concentration (mg/ml)	Percentage of inhibition (%)	Combination Effect Index (CEI)
	C. lusitanica	C1	93.96±00.27^{c}*	/
		C2	91.39±00.54^{d}	/
		C3	89.59± 00.51^{e}	/
	P. pedoncularis	C1	93.92±00.47^{c}	/
		C2	91.57±00.24^{d}	/
		C3	90.30±00.33^{e}	/
Fo_1	Combination of *C. lusitanica* and *P. pedoncularis*	Mixed 1	100.00±00.00^{a}	2.13±00.01^{c}(S)
		Mixed 2	99.96±00.03^{a}	2.16±00.00^{abc}(S)
		Mixed 3	100.00±00.00^{a}	2.17±00.01^{ab}(S)
		Mixed 4	100.00±00.00^{a}	2.16±00.00^{abc}(S)
		Mixed 5	99.67±00.58^{a}	2.18±00.03^{a}(S)
		Mixed 6	97.30±00.52^{b}	2.14±00.02^{bc}(S)
		Mixed 7	99.33±01.15^{a}	2.17±00.03^{ab}(S)
		Mixed 8	97.27±00.38^{b}	2.15±00.02^{bc}(S)
		Mixed 9	96.90±00.01^{b}	2.15±00.00^{abc}(S)
Fo_2	*C. lusitanica*	C1	90.34±00.63^{bc}	/
		C2	74.76±01.64^{e}	/
		C3	73,50± 02.05^{e}	/
	P. pedoncularis	C1	90.61±00.03^{c}	/
		C2	90.17±00.07^{bc}	/
		C3	85.56±08.03^{d}	/
	Combination of *C. lusitanica* and *P. pedoncularis*	Mixed 1	100.00±00. 00^{a}	2.21±00. 01^{c}(S)
		Mixed 2	99.98±00.03^{a}	2.22±00. 01^{c}(S)
		Mixed 3	99.74±00.39^{a}	2.28±00. 10^{bc}(S)
		Mixed 4	99.83±00.29^{a}	2.43±00. 04^{ab}(S)
		Mixed 5	99.67±00.58^{a}	2.44±00. 03^{ab}(S)
		Mixed 6	100.00±00.00^{a}	2.39±00. 09^{abc}(S)
		Mixed 7	100.00±00.00^{a}	2.46±00. 04^{a}(S)
		Mixed 8	94.87±00.68^{b}	2.34±00. 05^{abc}(S)
		Mixed 9	94.86±00.60^{b}	2.33±00. 23^{abc}(S)

**a, b, c, d, e and f: comparison of the percentages of growth inhibition of the different F. oxysporum morphotypes by the plant extracts; means assigned the same letter in the same column are not significantly different according to the Duncan test at P ≤0. 05 and (S): synergism; T-: negative control, C1: 16.384 mg/ml, C2: 8.192 mg/ml and C3: 4. 096 mg/ml, Mixed 1: C1+C1, Mixed 2: C1+C2, Mixed 3: C1+C3, Mixed 4: C2+C1, Mixed 5: C2+C2, Mixed 6: C2+C3, Mixed 7: C3+C1, Mixed 8: C3+C2, and Mixed 9: C3+C3. /: no CEI*

In vitro activity of ethanolic extract combinations

Ethanolic extracts of *C. lusitanica* and *P. pedoncularis* applied separately showed some efficacy on all morphotypes tested. The most marked efficacy was observed with the combination of extracts of both plants. The first five (mixed 1, mixed 2, mixed 3, mixed 4, mixed 5) and the seventh combination (mixed 7) of the extracts of both plants were the most effective on the Fo_1 morphotype inducing a percentage of inhibition of 100%. The other combinations significantly reduced the growth of the same morphotype compared to the *C. lusitanica* extracts tested at 4.096; 2.048 and 1.024 mg/ml and the *P. pedoncularis* extracts tested at 2.048 and 1.024 mg/ml. For the Fo_2 morphotype,

all combinations except combination 9 (mixed 9) completely (100%) inhibited the growth of this morphotype.

Thus, all the different concentrations of the ethanolic extracts of these two plants had synergistic effects on the Fo_1 and Fo_2 morphotypes according to the Duncan's test at 5%. Their combination effect index (CEI) was significantly higher than 2. These CEIs had values that varied between 2.07 and 2.23. However, no indifference, addition or antagonism effect of the combination of the different concentrations of these two extracts was observed towards these different morphotypes tested (Table 2).

Tabe 2 : *In vitro* effect of combinations of different concentrations of ethanolic extracts of *C. lusitanica* and *P. pedoncularis* on the development of the most resistant morphotypes of *F. oxysporum* to the extracts tested individually.

Morphotype code	Treatments	Concentration (mg/ml)	Percentage of inhibition (%)	Combination Effect Index (CEI)
Fo_1	*C. lusitanica*	C1	96.02±00. 60^{d}*	/
		C2	93.76±00.02^{e}	/
		C3	93.73± 00.68^{e}	/
	P. pedoncularis	C1	96.47±00.01^{b}	/
		C2	94.29±00.01^{e}	/
		C3	91.06±00.61^{f}	/
	Combination of *C. lusitanica* and *P. pedoncularis*	Mixed 1	100.00±00.00^{a}	02.08±00.01^{d}(S)
		Mixed 2	100.00±00.00^{a}	02.10±00.01^{bcd}(S)
		Mixed 3	100.00±00.00^{a}	02.14±00.01^{a}(S)
		Mixed 4	100.00±00.00^{a}	02.10±00.00^{bcd}(S)
		Mixed 5	100.00±00.00^{a}	02.13±00.00^{bc}(S)
		Mixed 6	99.00±01.00^{b}	02.14±00.03^{a}(S)
		Mixed 7	100,00±00.00^{a}	02.10±00.01^{bcd}(S)
		Mixed 8	98.33±00.58^{b}	02.09±00. 02^{cd}(S)
		Mixed 9	97.57±00.51^{c}	02.11±00.02^{bc}(S)
Fo_2	*C. lusitanica*	C1	95.98±00.80^{c}	/
		C2	92.45±00.01^{d}	/
		C3	90.47± 00.01^{e}	/
	P. pedoncularis	C1	96.80±00.58^{c}	/
		C2	91.29±00.00^{e}	/
		C3	87.04±01.26^{f}	/
	Combinaison of *C. lusitanica* and *P. pedoncularis*	Mixed 1	100.00±00.00^{a}	02.07±00.01^{d}(S)
		Mixed 2	100.00±00.00^{a}	02.14±00.01^{c}(S)
		Mixed 3	99.99±00.02^{a}	02.19±00.02^{b}(S)
		Mixed 4	100.00±00.00^{a}	02.13±00.01^{c}(S)
		Mixed 5	100.00±00.00^{a}	02.18±00.01^{b}(S)
		Mixed 6	99.99±00.58^{a}	02.23±00.02^{a}(S)
		Mixed 7	100.00±00.00^{a}	02.14±00.01^{c}(S)
		Mixed 8	100.00±00.00^{a}	02.20±00.00^{b}(S)
		Mixed 9	98.67±01.15^{b}	02.22±00.04^{ab}(S)

**a, b, c, d, e and f: comparison of the percentages of growth inhibition of the different F. oxysporum morphotypes by the plant extracts; means assigned the same letter in the same column are not significantly different according to the Duncan test at P ≤0. 05 and (S): synergism; T-: negative control, C1: 16.384 mg/ml, C2: 8.192 mg/ml and C3: 4. 096 mg/ml, Mixed 1: C1+C1, Mixed 2: C1+C2, Mixed 3: C1+C3, Mixed 4: C2+C1, Mixed 5: C2+C2, Mixed 6: C2+C3, Mixed 7: C3+C1, Mixed 8: C3+C2, and Mixed 9: C3+C3. /: no CEI*

Activity of plant extract combinations on the development of different morphotypes of *Fusarium oxysporum* inoculated on tomato fruits

Tables 3 and 4 show the *in situ* effect of combining aqueous and ethanolic extracts of *C. lusitanica* and *P. pedoncularis* on the development of *F. oxysporum* morphotypes

Combination activity of aqueous extracts of two plants on the development of the most resistant morphotypes inoculated on tomato fruits

Fruits treated with combined extracts showed no decay except those treated with combinations 6, 8 and 9 which showed decay areas varying between 20 and 93 mm^2. The highest areas of decay were obtained with fruits treated in isolation with *C. lusitanica* and *P. pedoncularis* extracts. These lesion areas ranged from 100, 33 and 105 mm^2.

The first seven combinations of the ethanolic extracts of these two plants showed a synergistic effect on the Fo1 and Fo2 morphotypes according to the 5% Duncan test. Their combination effect index (CEI) values were less than 1 (CEI <1). These values ranged from 0 to 0.39. However, the last two combinations (mixed 8 and mixed 9) showed cases of indifference to the different morphotypes. Their CEI values were higher than 1 (CEI > 1). Furthermore, no addition or antagonism effect of the combination of the two extracts was obtained with the different morphotypes tested (Table 3).

Table 3: Combination effect of different concentrations of aqueous extracts of *C. lusitanica* and *P. pedoncularis* on lesion area (mm^2) of *Fusarium oxysporum* morphotypes least susceptible to the extracts tested individually

Morphotype Code	Treatments	Concentration (mg/ml)	Lesion areas (mm^2)	Combination Effect Index (CEI)
Fo_1	*C. lusitaniaca*	C1	93.33±00.58^{c}*	/
		C2	102.17±01.04^{ab}	/
		C3	104.00± 01.00^{ab}	/
	P. pedoncularis	C1	91.67±02.89^{c}	/
		C2	103.00±01.00^{ab}	/
		C3	104.33±02.52^{a}	/
	Combinaison of *C. lusitaniaca* and *P. pedoncularis*	Mixed 1	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 2	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 3	01.70±00.61^{f}	00.03±00.01^{c} (S)
		Mixed 4	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 5	00.00±00.00^{f}	00,00±00.00^{d} (S)
		Mixed 6	29.30±00.75^{e}	00,06±00.02^{b} (S)
		Mixed 7	00.00±00.00^{f}	00,00±00.00^{d} (S)
		Mixed 8	81.00±00.00^{d}	01,57±00.00^{a} (I)
		Mixed 9	82.50±00.50^{d}	01,58±00.02^{a} (I)
Fo_2	*C. lusitaniaca*	C1	90.67±01.15^{c}	/
		C2	100.33±00.58^{b}	/
		C3	102.83± 04.48^{ab}	/
	P. pedoncularis	C1	89.00±00.03^{c}	/
		C2	101.83±02. 08^{ab}	/
		C3	105.00±02.00^{a}	/
	Combinaison of *C. lusitaniaca* and *P. pedoncularis*	Mixed 1	00.00±00.00^{a}	00.00±00.00^{d} (S)
		Mixed 2	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 3	01.66±00.58^{f}	00.03±00.01^{d} (S)

Mixed 4	00.00±00.00^{f}	00.00±00.00^{d} (S)
Mixed 5	00.00±00.00^{f}	00.00±00.00^{d}(S)
Mixed 6	20.00±01.00^{e}	00.39±00.02^{c} (S)
Mixed 7	00.00±00.00^{f}	00.04±00.00^{d} (S)
Mixed 8	66.33±04.73^{d}	01.30±00.01^{b} (I)
Mixed 9	93.00±06.08^{c}	01.79±00.08^{a} (I)

**a, b, c, d, e and f: comparison of the percentages of growth inhibition of the different F. oxysporum morphotypes by the plant extracts; means assigned the same letter in the same column are not significantly different according to the Duncan test at P ≤0. 05 and (S): synergism; T-: negative control, C1: 16.384 mg/ml, C2: 8.192 mg/ml and C3: 4. 096 mg/ml, Mixed 1: C1+C1, Mixed 2: C1+C2, Mixed 3: C1+C3, Mixed 4: C2+C1, Mixed 5: C2+C2, Mixed 6: C2+C3, Mixed 7: C3+C1, Mixed 8: C3+C2, and Mixed 9: C3+C3. /: no CEI*

Combination activity of ethanolic extracts of two plants on the development of the most resistant morphotypes inoculated on tomato fruits

Table 4 shows the combination activity of the ethanolic plant extracts on the lesion area developed by the different morphotypes of *Fusarium oxysporum* on apparently healthy tomato fruits. Fruits treated with the combined extracts showed no decay caused by the Fo_1 morphotype except those treated with combinations 6, 8 and 9 which showed decay areas varying between 79.33 and 81 mm^2. The highest areas of decay were obtained with fruits treated in isolation with extracts of *C. lusitanica* at a concentration of 2.048 mg/ml and *P. pedoncularis* at concentrations of 2.048 and 1.024 mg/ml. These lesion areas ranged from 102.33 to 103.33 mm^2. For the Fo_2 morphotype, all fruits treated with combinations 1, 2, 3, 4, 5 and 7 developed almost no lesions according to the 5% Duncan test. However, tomato fruits treated with combinations 8 and 9 (mixed 8 and 9) still showed lesion areas but significantly lower than those of control fruits (treated with plant extracts separately). These lesion areas ranged from 69 to 93 mm^2.

The evaluation of the activity of these two plants on the development of the Fo_1 morphotype is synergistic with the combinations 1, 2, 3, 4, 5 and 7 according to the Duncan 5% test. Their combination effect index (CEI) values were equal to 0 (CEI <1). However, lesion areas developed on fruits sprayed with combinations 6, 8 and 9 (mixed 6, 8 and 9) showed indifference to the same morphotype. Their IEC values were higher than 1 (CEI > 1). Similarly, this synergistic effect was also observed with the first seven combinations (mixed 1, 2, 3, 4, 5, 6, 7) on the Fo_2 morphotype. Their CEI values were lower than 1 (CEI<1). On the other hand, combinations 8 and 9 (mixed 8 and 9) were indifferent towards the same morphotype with combination effect indices higher than 1 (CEI > 1). As with the Fo_1 morphotype, no addition, let alone antagonism, was observed (Table 4).

Table 4: Combination effect of ethanolic extracts of *C. lusitanica* and *P. pedoncularis* on lesion area (mm^2) of *F. oxysporum* morphotypes

Morphotype code	Treatments	Concentration (mg/ml)	Lesion areas (mm^2)	Combination Effect Index (CEI)
Fo_1	*C. lusitanica*	C1	79.67±00.58^{b}*	/
		C2	70.38±00.58^{c}	/
		C3	103.00± 01.73^{a}	/
	P. pedoncularis	C1	78.67±01.15^{b}	/
		C2	102.33±01.53^{a}	/
		C3	103.33±00.58^{a}	/
	Combination of *C. lusitaniaca* and *P. pedoncularis*	Mixed 1	00.00±00.00^{f}	00.00±00.00^{c} (S)
		Mixed 2	00.00±00.00^{f}	00.00±00.00^{c} (S)
		Mixed 3	00.00±00.00^{e}	00.00±00.00^{c} (S)
		Mixed 4	00.00±00.00^{e}	00.00±00.00^{c} (S)
		Mixed 5	00.00±00.00^{f}	00.00±00.00^{c} (S)
		Mixed 6	45.67±04.93^{d}	01.09±00.11^{b} (I)
		Mixed 7	00.00±00.00^{f}	00.00±00.00^{c} (S)
		Mixed 8	79.33±01.15^{b}	01.54±00.03^{a} (I)
		Mixed 9	81.00±01.73^{b}	01.58±00.02^{a} (I)
Fo_2	*C. lusitanica*	C1	89.00±00.00^{c}	/
		C2	99.67±00.58^{a}	/
		C3	101.00± 00.00^{a}	/
	P. pedoncularis	C1	70.00±00.58^{d}	/
		C2	100.00±00.00^{a}	/
		C3	102.67±00.58^{a}	/
	Combination of *C. lusitanica* and *P. pedoncularis*	Mixed 1	00.00±00.00^{f}	00,00±00.00^{d} (S)
		Mixed 2	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 3	01.33±00.58^{f}	00.03±00.01^{d} (S)
		Mixed 4	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 5	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 6	40.00±01.00^{e}	00.96±00.02^{c} (S)
		Mixte 7	00.00±00.00^{f}	00.00±00.00^{d} (S)
		Mixed 8	69.00±01.00^{d}	01.37±00.02^{b} (I)
		Mixed 9	93.00±06.08^{b}	01.83±00.12^{a} (I)

**a, b, c, d, e and f: comparison of the percentages of growth inhibition of the different F. oxysporum morphotypes by the plant extracts; means assigned the same letter in the same column are not significantly different according to the Duncan test at P ≤0. 05 and (S): synergism; T-: negative control, C1: 4.096 mg/ml, C2: 2.048 mg/ml and C3: 1. 024 mg/ml, Mixed 1: C1+C1, Mixed 2: C1+C2, Mixed 3: C1+C3, Mixed 4: C2+C1, Mixed 5: C2+C2, Mixed 6: C2+C3, Mixed 7: C3+C1, Mixed 8: C3+C2, and Mixed 9: C3+C3. /: no CEI.*

Discussion

The plants are used separately or most often in combination in order to increase the spectrum of activity of the bioactive molecules and thus maximise the desired positive effect.

In general, the combination activity of *C. lusitanica* and *P. pedoncularis* on all morphotypes was synergistic. The synergistic activity of these extracts of the two plants may be due to the complementarity between the components of the two extracts, or it may be due to the formation of a complex between the antifungal agents contained in these extracts, which becomes effective in destroying a particular species of microorganism, probably by acting on the cell wall or by causing their lysis or death. Some authors believe that it is a combined effect on the

permeability of the cytoplasmic membrane of the germs, facilitating the influx of the active compounds (Sibanda, 2007); or the inhibition of β-lactamases (Kusuda *et al*., 2006; Eumkeb *et al*., 2010). Several works carried out on the associative antimicrobial activity of plant extracts have shown good synergy towards the tested strains (Adwan *et al*., 2009; Sanjeev Ranjan *et al*., 2012; Akinbobola *et al*., 2014). The work of Saffidine (2015) also reported that the combination of *Plantago major* and *Carthamus caeruleus* extracts has a synergistic effect on several microbial species tested. The work of Soraya *et al*. (2019) on the hidden synergistic effects of plant extract combinations against plant pathogenic fungi showed that the different plant combinations had both synergistic, additive, indifferent and even antagonistic effects against *Alternaria brassicicola*, *Colletotrichum capsici* and *F. oxysporum* f.sp.cubense

The combination of two extracts that was effective *invitro* on all tested morphotypes was also active in the in situ test condition. The combination of these two plant extracts that showed a synergistic effect *in vitro* was also effective in situ with the same effect. This trend leads us to believe that under *in vitro* test conditions, on the culture medium, the effect of the active molecules against the different morphotypes would not have been masked. These observations are in contrast to those of Djeugap *et a*l. (2011) and similar to those of Keuete *et al*. (2015). Indeed, the work of Djeugap *et al.* (2011) reported that the *S. aromaticum* extract, which was effective in vitro, was less active under *in vivo* test conditions and that of *C. viminalis*, which was less effective *in vitro*, was the most effective *in vivo*. On the other hand, those of Keuete *et al* (2015) on the antifungal activity of some plant extracts on three post-harvest fungi showed that the extracts of *Cupressus lusitanica*, *Erigeron floribundus*and *Euphorbia hirta* effective *in vitro* were also effective in the conditions. The similarity of the present results with those of Keuete *et al*. (2015) could be due to the fact that the *Cupressus lusitanica* extract used in his work was the same one used by Keuete *et al*. (2015) in their work. Similarly, the work of Nguefack *et al*. (2012) on the synergistic action of the essential oil fractions of *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against *Penicillium expansum* showed that all these plants had synergistic activity against this fungal species.

The synergistic effect of the plant extracts can be exploited to minimise the effects of the most resistant morphotypes of *F. oxysporum* to the extracts tested separately.

Conclusion

In order to contribute to the control of resistance problems developed by some fungal species associated with tomato fruits in the major production areas of the West Cameroon region against individually administered plant extracts, the combination activity of *Cupressus lusitanica* and *Psychotria pedoncularis* extracts on the development of *F. oxysporum* morphotypes resistant to its separately administered extracts was evaluated in this study. The results showed that under both in vitro and in situ conditions, the first seven combinations of the concentrations of the aqueous and ethanolic extracts of these two plants tested showed synergistic activity on the Fo_1 and Fo_2 morphotypes. This study suggests that these two extracts can be combined in the control of fungal species resistant to extracts administered separately. However, this study will have to be continued by the bioguided fractionation of these two extracts in order to identify the fraction possessing the active molecules and then isolate them and formulate biofongicides.

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